Current Trends In Mycorrhizal Research

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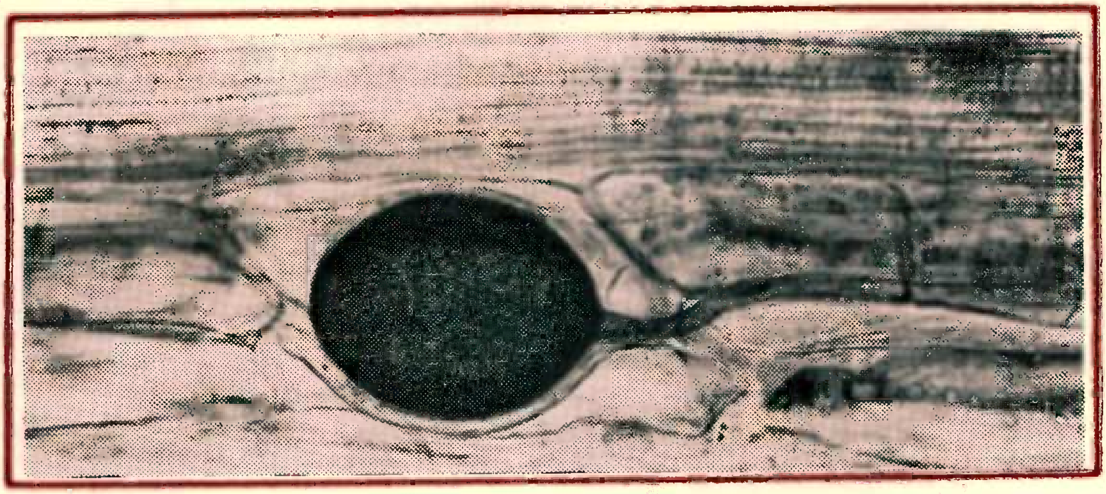

TATA ENERGY RESEARCH INSTITUTE
DEPARTMENT OF BIOTECHNOLOGY
DEPARTMENT OF SCIENCE AND TECHNOLOGY
AND
HARYANA AGRICULTURAL UNIVERSITY

CURRENT TRENDS IN MYCORRHIZAL RESEARCH

PROCEEDINGS OF THE

NATIONAL CONFERENCE ON MYCORRHIZA

At Haryana Agricultural University, Hisar

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EDITORS

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PREFACE

To him who devotes his life to science, nothing can give more happiness than making discoveries, but his cup of joy is full only when the results of his studies find practical application.

—Louis Pasteur

This dictum is relevent even today, particularly so in the field of mycorrhizal research. No doubt, considerable progress has been made, over the years, in furthering knowledge on various dimensions of mycorrhizas. Inoculation of agricultural crop plants and forest trees with mycorrhizal fungi has been convincingly demonstrated to stimulate their growth and development. Such probiotic effects are particularly striking in nutritionally poor soils, which abound in large areas of the arid and semi-arid tropics. The significant impact of mycorrhiza can easily be realised from the fact that almost all plant species of economic importance growing under diverse agro-climatic conditions are able to be infected; most form vesicular-arbuscular mycorrhiza, and several tree species form ectomycorrhiza. Most of the experimental work have amply demonstrated the physiological benefits that are conferred by mycorrhizal endophyte on their potential host plants, and such effects have been explained in terms of measurable enhancement of phosphate transport. Another dimension of immediate relevance is their role in induced suppression of soil/root-borne pathogens,-a group of diseases which are otherwise difficult to control by conventional fungicidal applications. No less important is the part they play in conferring resistance to water stress, as well as decreased leakage of electrolytes from cells of diseased plants.

Substantial quantum of information has been generated on these and other aspects at various research centres of the world. Realising the importance of this microbial model system in enhancing crop productivity, concerted efforts have been made in India and other Asian countries to strengthen and mobilise the research endeavours in a cohesive manner.

A step forward in this direction has been the establishment of Mycorrhiza Network Asia Project at TERI, launched recently to strengthen research, encourage cooperation and promote exchange of information in this important field. In its very first meeting held in January, 1989, the Technical Advisory Committee of this Network decided to organise the 'National Conference on Mycorrhiza' at Haryana Agricultural University, Hisar.

The primary objective of this conference was to bring together active mycorrhiza researchers to highlight the state of art in this thrust area, review progress, project future goals and to identify constraints, so as to help give momentum to this field of research. Keeping the Asian scenario in view, the scientific programme was tailored to five technical sessions comprising of : Ecology, Physiology/Biochemistry, Biological Interactions, Biocides, and Soil-Plant Relationships.

To sustain the impact of this conference, the Organising Committee decided to make this publication available at the time of conference rather than deferring it to a later date. Thus, the present publication entitled "Current Trends in Mycorrhizal Research", embodies the invited as well as contributed papers, submitted in the form of 'extended abstracts'.

The mission has been to ascertain where we stand today, and then to look forward to the problems that may challenge us in decades ahead. This publication, hopefully, will highlight the different directions that mycorrhizal research has taken in recent years in India and other Asian countries. This should eventually help to strengthen our efforts in launching newer areas of investigation in the years to come.

Time has come when we must concentrate more mycorrhizal research on "Does it work in the field" rather than "How does it work in the laboratory", so as to harness its utility and applicability in promoting economic gains.

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A WORD OF GRATITUDE

I am deeply indebted to the members of the Steering Committee and the Organising Committee for their valuable advice and guidance in the smooth conductance of the conference. Equally important was the liberal support and cooperation received from Tata Energy Research Institute, Department of Biotechnology, and Department of Science & Technology, Government of India.

I count myself fortunate to have had such an understanding and ever-helpful circle of dedicated faculty of plant pathologists at H. A. U., who have put in lot of effort for the success of this conference. Sincere thanks to the authors who contributed to this volume; obviously, their timely contributions have made this publication possible.

I owe a great deal to each of them. If any credit is due, it is to them. Criticisms that are surely to arise are mine to endure.

—**Bushan L. Jalali**
Conference Coordinator

NATIONAL CONFERENCE ON MYCORRHIZA

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Vesicular arbuscular mycorrhiza in aquatics

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Hydrophytes were regarded non-mycorrhizal until the last decade (Gerdemann, 1975). They could become mycorhizal under a change from wet to drier conditions (Read *et al.*, 1976). There are only a few reports on the occurrence of VA mycorrhizal endophytes in natural aquatic conditions. Rayner (1927) observed VAM fungi in bogs. Dowding (1959) noted VAM fungi in roots of four swamp plants and swamp mud. Khan (1974) observed that none of the hydrophytes under study colonized VAM fungi though a low frequency of spores occurred in their rhizosphere. Read *et al.* (1976) found mycorrhizal colonization almost neglegible in marsh plants. Sondergaard and Laegaard (1977) observed five out of seven temperate aquatic plants mycorrhizal.

Bagyaraj *et al.* (1979) were the first to report VA mycorhiza in non-root part of any hydrophyte (modified leaves of *Salvinia cucullata*).

The present survey was conducted to observe the VA mycorrhizal status of some common aquatics in ponds and marshes of the Punjab.

Mycorrhizal status of the common aquatics namely *Azolla pinnata* R. Br., *Chara* sp., *Ceratophyllum demersun* Linn., *Cyperus eleusinoides* Kunth., *Eicchornia crassipes* (Mart.) Solms., *Equisetum arvense* Linn., *Hydrilla verticillata* Royle, *Lemna polyrrhiza* Linn., *L. paucicostata* Hegelm., *Marsilea quadrifoliata* Linn., *Najas major* All., *Nelumbium nelumbo* (Linn.) Druce, *Nymphaea lotus* Linn., *N. stellata* Willd., *Phragmites kirka* Linn., *Pistia stratiotes* Linn., *Polygonum barbatum* Linn., *Potamogeton crispus* Linn., *P. pectinatus* Linn., *Ranunculus aquatilis* Linn., *Sagittaria guayanensis* Kunth., *Salvinia cucullata* Roxb., *Trapa bispinosa* Roxb., *Typha angustata* Chaub & Bory., *Vallisneria spiralis* Linn. and *Zannichellia palustris* Linn. was observed. VA mycorrhizal colonization was observed in rhizoids of *Chara* sp. (Iqbal *et al.*, 1988) and roots of *Azolla pinnata*, *Eicchornia crassipes*, *Equisetum arvense*, *Marsilea quadrifoliata*, *Phragmites kirka* and *Ranunculus aquatilis*. In non-root plant parts mycorrhizal colonization was observed in leaves of *Azolla pinnata* (Iqbal and Firdaus-e-Bareen, 1989); senescing leaves and stem of *Ceratophyllum demersum*; dried scales on leaves of *Eicchornia crassipes*; senescing leaves of *Equisetum arvense* (Nasim *et al*; 1987), *Lemna* spp., *Nymphaea lotus*, *Typha angustata*; and modified leaves of *Salvinia cucullata* (Iqbal and Firdaus-e-Bareen, 1989) and *Trapa bispinosa*.

Multiple VA infections occurred in most cases as many kinds of vesicles were observed. Oil droplets characteristics of the aquatic environment could be observed in most plants (Bagyaraj, *et al.*, 1979). Arbuscules were totally absent.

A low frequency of endogonaceous spores was found in the rhizosphere of most plants. Many species of *Glomus* and *Acaulospora* sp. were actually observed causing infections in non-root plant parts in the aquatic environment.

The occurrence of mycorrhiza in aquatic conditions indicates that it must be playing some role in nutrient absorption especially in plants growing in nutritionally poor sediments. The special shapes of vesicles with prominant oil droplets shows the adaptation of VA mycorrhizal symbionts according to the environment. The occurrence of mycorrhiza or its absence in the same plant in ecologically different conditions indicates that the ecological and environmental conditions (both qualitative and quantitative) seem to have a great influence on the colonization of plants. The variation in colonization from place to place could also be due to several other environmental factors.

The plants tend to acquire mycorrhizal colonization in drier conditions (Iqbal and Firdaus-e-Bareen, 1989). This is obvious as greater colonization was observed in semi-aquatics or aquatics in marshy places. Once the VAM endophyte causes infection in a plant, aquatic conditions can not reduce the intensity of colonization (Bagyaraj *et al.*, 1979). Most probably the lesser colonization in aquatic conditions is because of low availability of spores or other types of efficient inocula. Low availability of spores in aquatic or water-logged conditions could be directly attributed to soil aeration (Saif, 1981). The colonization of non-root parts of aquatics show that VAM endophytes have greatly expanded and safer ecological niches. The decaying organic matter formed by leaves of common aquatics in marshes harboured VA endophytes. In our irrigation system in the Punjab this could become an efficient way of dispersal for VA endophytes and enhance fertility of soil as well. The decaying thalli of *Azolla* in dried rice fields acquire mycorrhizal infection. Methods could be formulated for artificially inducing VAM endophytes in biofertilizers like *Azolla pinnata* which forms dual symbiotic associations with *Anabaena azollae* and VA endophytes (Iqbal and Firdaus-e-Bareem, 1989). Under water-logged conditions in the paddy-fields it fixes nitrogen and under drier conditions it could fix phosphates.

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Vesicular arbuscular mycorrhiza in portions other than root

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Vesicular arbuscular mycorrhiza is the most common type of all the mycorrhizae. This association not only facilitate the nutrient uptake, its effects on plant growth have also been revealed.

The occurrence of VA mycorrhiza in roots has been reported from an exceptionally wide range of plants. However, the first report on its presence in portions other than roots i. e. modified submerged leaves of vascular aquatic plants in tropical conditions was by Bagyaraj *et al.*(1979). They reported the presence of a mycorrhiza fungus in the root-like leaves of *Salvinia*, a rootless aquatic fern. Park & Linderman (1980) reported the occurrence of spores of VA mycorrhizal fungi in senescent leaves of a moss (*Fumaria hygromatrica* Sibth.). *Glomus* spores were observed in decaying peanut leaves in the field in Texas by Taber and Trappe (1982). The pegs of peanut plant have also been reported to harbour VA mycorrhiza (Graw and Rehm, 1977). Taber & Trappe (1982), for the first time reported the presence of VA mycorrhizal fungi in the vascular system of rhizomatous tissues and in scale like leaves of *Zingiber officinale.*

The present study is focused to review the presence, nature and structure of VA mycorrhizal fungi associated with portions other than roots. This study is based on VA mycorrhizal investigations of herbaceous plants many of which were perennials.

The plants studied for the presence of vesicular arbuscular mycorrhizal infections were *Agremonia eupatorium*, *Allocasia indica*, *Arisaema* spp., *Cana indica*, *Cala aetheopica*, *Colocasia antiquorum*, *C. gigantea*, *Curcuma longa*, *C. zoedoria*, *Cyprus rotundus*, *Elettaria cardamomum*, *Musa paradisiaca*, *Oxalis corniculata*, *O. corymbosa*, *Plantago lanceolata*, *Polygonum* sp., *Primula* sp., *Saxifraga stracheyi*, *Senseviierea trifaciata*, *Sorghum halepense* and *viola* spp. Some non-angiospermic plants were different spp. of ferns, Mosses, Bryophytes, *Selaginella* and *Equisetum.*

The roots of almost all the plants studied had VA infections. Vesicles as well as arbuscules were present in many of the specimens but a few of them lacked arbuscules. Scale like leaves as well as epidermis of rhizomes of almost all the plants had VA infections. Vesicles were universally occurring while arbuscules were totally lacking (Iqbal *et al.*, 1988; Nasim *et al.*, 1989). In most of the cases the infections were multiple. Mostly the infections were caused by *Glomus* spp. It was indicated by the presence of Chlamydospores which are formed at a hyphal tip usually one per tip. In the scales were also observed spores mostly of the genus *Glomus*, borne singly on the hyphal tips e. g. in *Colocasia antiquorum*, *Musa paradiasica* (Iqbal and Nasim, 1986). Azygospores of *Gigaspora* spp. were observed in scale of rhizome of *Curcuma longa* (Nasim, 1985) and *Aresaema* sp. The azygospores bud from the bulbous suspensor like tip of a hypha. Various types of Endogonaceous spores were found in the rhizospheres of *Cana indica*, *Elettaria cardamomum* and *Curcuma longa* (Nasim, 1985). No arbuscules were found in the scales but highly branched and septate mycelium was very common inside as well as outside the cells. Septate mycelium and small endospore like structures were very common in the scale cells of rhizome of almost all the plants. Scales of the rhizomes of *Cana indica*, *Colocasia antiquorum*, *Curcuma longa*, *Elettaria cardamomum*, *Musa paradiasica* and *Sansenieria trifaciata* had very heavy (80-100%) vesicular infections (Nasim, 1985). The scale leaves of the rhizomes of *Zingiber officinale*, *Colocasia gigantia*, *Oxalis* spp. and of *Sorghum halepense* had moderate VA infections while those of the rhizomes of *Allocasia indica*, *Cala aethiopica*, *Aresaema* sp., *Plantago lanceolata*, *Saxifraga stracheyi*, *Viola* spp., *Polygonum* sp., *Agrimonia eupatorium* had lower VA infections (Nasim, 1985). Scales in many cases had multiple VA infections as is indicated by the vesicles size and width of the hyphae which were markedly different.

This study extends the list of mycorrhizal plants consumed as food or used as medicines (Nasim *et al.*, 1989). Mycorrhizal status of non-root portions of many plants has been reported for the very first time. Non-root portions had heavy vesicular infections and lacked arbuscular infections, instead the VAM mycelium became septated and filled the cell lumen at various instances. These septate hyphae probably functioned like arbuscules increasing the absorptive area (Iqbal *et al.*, 1988; Nasim *et al.*, 1989). Extramatrical spore formation was also observed at various places (Nasim *et al.*, 1989). Occurrence of VA mycorrhizal fungi in this scale like portions which are probably dead suggests the saprophytic way of living of these fungi as indicated by Warner and Mosse (1980). The rhizomes corms and root stocks remain viable even after years and can be used as seeds for vegetative propagation. These underground portions are thus said to carry the VA mycorrhizal inoculum because when they are sown the VAM hyphae revive and the spore germinate (Iqbal and Nasim, 1986). Presence of these fungi in medicinally important plants suggests that these fungi are resistant to the active principal of these medicinally important plants (Iqbal *et al.*, 1988). The term "Mycorrhiza" was regarded inappropriate by Taber and Trappe (1982). They proposed the term "Mycophyllon" for leaf association and "Mycorrhizome" for rhizomatal associations (Iqbal and Nasim, 1986).

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Mycorrhizal relations of successional stages of mangrove vegetation at the Ganges river estuary in India

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Estuarine or maritime salt marshes are one of the most productive ecosystems of the world (odum, 1971). The tropical or sub-tropical parts such as saline wetlands are inhabited by mangroves, a climax formation of hydrohalophytes belonging to several families. Besides for primary production of biomass, mangroves are also important as contributors to the complex geo-aquatic food web of the estuarine salt marsh ecosystems.

Large areas of mangrove and other salt marsh centres of the world have been reclaimed aud replaced by diverse agro-ecosystems. Plant performance in these saline wetlands is generally poor due to salinity and inundation stress and also limitaiions of nutrients (Valiela and Teal, 1979). Knowledge regarding ecosytemic adaptations of mangroves may help in the conservation of such and other saline wastelands through plant culture. In the context of our studies on microbiological aspects of ecosystemic adaptations of mangroves, mycorrhizal relations of the successional stages of mangrove vegetation at the terminal part of the Ganges river estuary (Sundarban) were examined.

Based on floristic ecology and physiography macrophytic ecosystem of Sundarban was divided into four well distinct stages : I. Formative mangrove swamps on river beds, II. Tidally inundated well developed mangroves on raised beds, III. Declining mangroves on inter-tidal ridges, and, IV. Declined mangroves on embankment protected highlands where saline agriculture and non-littoral tree forestry is practiced. The highly saline, clayey soils of the successional stages had very low nitrogen and phosphorus content. Organic matter content of particularly the well developed mangrove beds and protected agricultural lands were high. Fiftythree plant species belonging to twentyfive families were present in these successional stages as true mangroves, mangrove associates and non-littoral, introduced but naturalised macrophytic flora.

Root samples of plants collected from nine different locations representing the successional stages when examined by standard methods, revealed the presence of VA-mycorrhizal association in twentyfour mangroves, ten mangrove associates and eighteen non-littoral species. There were differences among the successional stages in root infection intensities of predominant plant colonizers. In general, root infection intensities of mangroves at the early successional stages were less than that of similar mangroves at the late successional stages. Within the latter, however, declined mangroves at the protected stage showed less infection than the declining mangroves at the inter-tidal ridges. Non-littorals including the common agricultural crops at the protected stage were also infected by VA-mycorrhiza. At the highly saline, inundation prone IInd and IIIrd successional stages non-mangroves showed less root infection than the true mangroves. At the protected stage root infection intensities of declined mangroves and all non-littorals considered togethrr were comparable. But, at any of these stages non-mangrove trees were less infected than the tree mangroves. VA-mycorrhizal inoculum and spore densities of the successional stage rhizosphere soils were inversely related with levels of soil water salinity and at only the protected stage were comparable to that of the common non-saline alluvial soils.

Evidences obtained on natural root colonization, spore and inoculum densities and VAM infection of a large number of common herbs in transported successional stage soils were circumstantial for involvement of VA-mycorrhiza in plant colonization of the ecosystem. Edaphic and physiographic differences between esturaine and maritime mangrove habitats would explain some of the previous report about common absence of VA-mycorrhiza in mangrove habitats (Kannan and Lakshminarayan, 1989). Two ecologically variable VAM infection determining factors were operating in the ecosystem. As all other soil microorganisms, VA-mycorrhiza were also sensitive to salinity and inundation stress and ecologically variable soil physical-chemical factors appeared to be the primary determinants of VAM infection in the ecosystem. Plant genetic variations were signtficant only within a successional stage. Data further showed that within the mangrove ecosystem considerable adjustment exists between the natural plant colonizers and their mycorrhizal partners and ecosystemic changes not conducive to the natural plant colonizers might adversely alter the mycorrhizal relations. Exotic trees or plant species unnatural for the stress habitat may need time for adjustment with the existing VAM inoculum.

Nine different Endogonaceous spore types were isolated from the soils of the ecosystem of which five were identified as belonging to the more common species of VA-mycorrhiza. Distribution of these spore types to the successional stages showed that there was probably no VA-mycorrhizal species specific for either the ecosystem or for any plant species. Histological and plant growth response data have shown that besides the typical VA-mycorrhiza, some atypical endophytes may also have mycorrhizal functions for such stress adapted plants of the ecosystem as has been reported for some other ecosystems also (Read and Haselwandter, 1981).

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Spore populations of VAM fungi in foxtail millet fields of Anantapur district, Andhra Pradesh

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Numerous studies have indicated that vesicular-arbuscular mycorrihzas (VAM) are nearly ubiquitous for the majority of agricultural crops. The impact of VAM in tropical agriculture will be greater than in temperate regions, and the beneficial effects are dependent on their population density as well as species composition in the field soils. Foxtail millet is one of the major rain-fed crops being cultivated in the semi-arid soils of Anantapur district in Andhra Pradesh. No information is available on the occurrence of VAM fungi in such vast agricultural fields. The present study represents an attempt to establish the occurrence of spore populations of VAM fungi in the fields planted to foxtail millet.

The five different places, within a radius of 50 km from Anantapur town, selected for the survey included Atmakur, Gangulagunta, Jangalapalli, Kondapuram and Miduthur. The agricultural fields in these localities consisted soils with varying physico-chemical characteristics. Root and rhizosphere soil samples were collected every 15 days after sowing the millet and were examined for the mycorrhizal spore populations. Foxtail millet raised in all different agricultural fields developed extensive mycorrhizas. The root systems as well as the rhizosphere soil samples showed varied populations of VAM fungi. Three species of *Sclerocystis* viz., *S. sinuosa*, *S. clavispora* and *S. pakistanica* were found to occur consistently at all places. The species of *Glomus* that most commonly occurred in heavy black soils were *G. deserticola*, *G. etunicatum*, *G. fecundisporum*, *G. geosporum*, *G. invermaium*, *G. intraradices* and *G. tortuosum* besides several unidentified isolates. In fields with alluvial soils, species of *Gigaspora* and *Scutellospora* were observed, in particular, along with the species of *Glomus*.

The results of the present study indicate that VA mycorrhizas formed by a greater diversity of the endophytes, are quite extensive in fields of foxtail millet. Attempts are being made to exploit these biofertilizers by clearly appreciating the impact of the most abundant species of *Glomus* on growth, yield potential and drought-resistance of foxtail millet.

Response of a shola plant *Rhododendron nilgricans* to endomycorrhizal inoculation

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The natural vegetation of the upper region of Nilgiris consists of vast stretches of grassland interrupted with numerous isolated compact sharply defined broad leaved woods called sholas or montane evergreen forests. These shola forests occupy roughly 20 per cent of the vegetation leaving the rest occupied by the grasses. These forests are mainly located in hill slopes or between mountain cliffs wherever soil moisture is high. Considerable destruction of sholas took place since the early days of human settlement. Among the shola vegetation, *Rhododendron nilgricans* is the most wide-spread plant. The root systems of several shola plants including *R. nilgricans* were reported to be associated with endomycorrhizae (Rangarajan *et al.*, 1987). Experiments in unsterile soil frequently showed that introduced VAM-fungi can stimulate more nutrient uptake than the indigenous mycorrhizal fungi. However, the studies on the effect of VAM fungi in influencing the biomass production and nutrient uptake of the shola plants are scanty. Hence investigations were carried out to study the interactlon of VAM fungi with *Rhododendron nilgricans* at Ooty.

The soil used in the present study was an acid (pH 5.7) phosphorus deficient soil with 15 ppm of extractable P. Pots of 16.5×19.0 cm were filled with unsterile soil (3 kg/pot), which had endomycorrhizal population of 1.4 spores/g of soil.

The soil-sand inoculum of eight VAM fungi were prepared by using maize (*Zea mays*) as host. The eight VAM-fungi included *Glomus versicolor*, *G. mossae*, *Gigaspora margarita* and *Acaulospora laevis* from University of Agricultural Sciences, Bangalore, *Glomus fasciculatum*, *G. epigaeum* and *Acaulospora morroweae* from Horticultural Research Station, Ooty and *Glomus mosseae* from Tamil Nadu Agricultural University, Coimbatore. The inoculum consisted of hyphae and spores of VAM fungi, infected root pieces of maize and soil having spore density of 35 spores/g of soil. A quantity of 10 g of inoculum was inoculated at the time of transplanting by placing the inoculum adjacent to the root zone (Schenck, 1982). Seedlings of *R. nilgricans* grown in natural sholas, were uprooted and planted in pots which were used for inoculation.

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After the establishment of the seedlings in pots, the initial heights were recorded. Six months after the planting, the heights of the plants were again recorded. Percentage of increase in height as compared to the control was assessed to find out the efficiency of inoculation with individual VAM-fungus.

Rhododendron nilgricans responded well to the inoculation with all eight VAM-fungi. The increase in height due to inoculation ranged from 15.85 to 39.02 per cent over control with maximum increase by *Glomus mosseae* from Bangalore. Effect of inoculation varied depending upon the fungus. While *Glomus fasciculatum*, *Gigaspora margarita* and *Acaulospora laevis* reported similar increase of 32.93 per cent over control, *Glomus versicolor* and *G. epigaeum* recorded 26.82 and 25.61 per cent increase only. Less response to inoculation was observed with *Acaulospora morroweae* (19.51) and *Glomus mosseae* from Coimbatore (15.85).

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Ectomycorrhizal fungi associated with different forest trees of Himachal Pradesh

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Ectomycorrhizae are symbiotic associations between the hyphae of certain fungi and the roots of most forest trees including all members of pinaceae. The importance of ectomycorrhizae in the nutrition of most vascular plants and the health of forest ecosystems has been overwhelmingly demonstrated in recent decades (Marks and Kozlowski, 1973; Trappe and Fogel, 1977).

Our present knowledge of the functions of ectomycorrhizae has come mainly from researches directed towards solving practical problems in forestry. Repeated failures in establishing exotic pine plantations in various parts of the world, where ectomycorrhizal hosts did not naturally occur, clearly demonstrated the dependence of these trees on their fungal symbionts (Mikola, 1970). A root system strongly mycorrhizal with proper beneficial fungi can vastly improve reforestation success with containerized nursery seedlings.

There has been marked decrease in forest cover in Himachal Pradesh due to urbanization, construction, tree felling for fuel, timber and boxes for fruit packaging and forest fires. The process can be reversed by launching a massive reforestation programme with appropriate species often in denuded and adverse, stressful sites. It has been observed that nursery grown seedlings without mycorrhizae generally fail to establish in such sites. Thus, to achieve success in reforestation programme mycorrhizal inoculation at nursery stage with the appropriate fungal symbiont is essential. The present study was therefore undertaken to gather information on ectomycorrhizal fungi associated with different tree species in healthy forest sites in different altitudinal ranges of Himachal Pradesh.

Periodic surveys were conducted during the monsoon season in 1987 and 1988 to collect information on their distribution and host specificity. Species of higher fungi which were frequently found associated with particular type of host were preserved, dried and identified. Efforts were also made to bring in culture such species for their further evaluation in relation to different host species in containerized seedlings under controlled environmental conditions.

The vegetation of Himachal Prahesh comprises purely coniferous, angiospermous or mixed forests as well as pastures in tropical, subtropical, temperate and alpine regions. An account of the ectomycorrhizal species found frequently associated with the forest trees in various zones of the state is given below :

Pure formations of *Pinus roxburghii* are common in tropical zone at an altitude of about 700 m. The important ectomycorrhizal fungi collected from this zone were *Scleroderma verrucosum*, *S. areolatum*, *S. dictyosporum*, *Rhizopogon* spp. *Suillus* spp., *Astreus hygrometricus*, *Amanita vaginata*, *Laccaria laccata* and *Lactarius sanguiflus*.

The important vegetational elements of subtropical zone are *Pinus roxburghii*, *P. wallichiana*, *Quercus glauca*, *Q. incana*, *Acer oblongum* and *Ficus palmata*. At some places, at an altitude of around 1800 m, *Cedrus deodara* forms an important component of vegetation. Mycorrhizal symbiosis appears to be highly pronounced in the mixed forests of 'deodar' and 'oaks'. The important fungal species found growing in association with *Pinus roxburghii* in this zone are mainly the species o' *Amanita*, *Lepista nuda*, *Lactarius sanguiflus*, *Suillus sibricus*, *Boletus edulis*, *Cantharellus cibarius* *Scleroderma texense*, *Rhizopogon rubescense*, *Astreus hygrometricus* and *Thelephora terrestris*. The important mycorrhizal symbionts of *Cedrus deodara* are several species of *Amanita* and *Russula* as well as *Clitocybe infundibuliformis*, *Lactarius deliciosus*, *L. zonarius*, *Leucopaxillus giganteus*, *Strobilomyces floccopus*, *Boletus edulis*, *B. erythropus*, *Thelephora terrestris*, *Canthrellus cibarius* and *Laccaria laccata*. In the forests dominated by oaks, several species of genus *Russula* are very common. The other species in oak forests are *Agaricus augustus*, *Amanita muscaria* var. *flavivolvata*, *Collybia* spp. *Lactarius piperatus*, *L. zonarius*, *L. deliciosus*, *L. camphoratus*, *Stropharia rugosoannulata*, *Boletus edulis*, *Thelephora terrestris*, *Ramaria* spp. and *Scleroderma dictyosporum*.

The greater part of forests in temperate zone comprises oaks with rhododendrons among the hard woods and blue pine, deodar, spruce and silver fir among the conifers in different belts according to altitude. The distribution of hardwoods and conifer trees in relation to altitude is as follows :

Alt. in m	Hard woods	Conifers
3000	Rhododendrons	*Abies pindrow*
2500	*Quereus semicarpifolia*	*A. pindrow*
2200	*Q. dilatata*	*A. pindrow*
		P. smithiana
2000	*Q. incana*	*Cedrus deodara*
		Pinus wallichiana

Ectomycorrhizal fungi are particularly abundant. Most of the species which occur in association with deodar and oaks in the sub-tropical zone are also present in this zone. However, some species like *Clitocybe nebularis*, *Pluteus cervinus*, *Oudmansiella redicata*, *Lactarius detrinum*, *Gomphus floccosum*, *Helvella atra* and

Sparssis crispa have been frequently found growing in association with deodar. Oak forests mixed with rhododendron also form an important component in this zone. This combination of vegetational elements has ectomycorrhizal species in the genera *Ramaria* and *Russula*.

Abies pindrow and *Picea smithiana* form important components of forests in the higher ranges. Pure formation of these species has ectomycorrhizal species like *Amanita pantherina*, *A. vaginata*, *Clitocybe gibba*, *Melanogaster durissimus* and *Scleroderma tenerum* for *A. pindrow* and *Hygrophorus pudorinus*, *H. chrysodon*, *Leucopaxillus amarus*, *Lactarius deliciosus*, *Suillus sibricus*, *Melanogaster* spp. and *Scleroderma* spp. for *P. smithiana*.

Forests are few in alpine zone and these are confined to lower zone of this belt. The important tree found in the lower and intermediate zones are *Quercus semicarpifolia*, *Rhododendron lepidotum* and *Salix tetrasperma* showing stunted growth and rarely exceeding 10 m in height. Ectomycorrhizal fungi are rare in this zone. However, *Paxillus involutus*, *Lepista nuda*, *Leucopaxillus* sp. and *Scleroderma areolatum* have been found growing in association with *Querus semicarpifolia* and *Salix tetrasperma*.

From the foregoing account on distribution of various ectomycorrhizal fungi in different forest types of Himachal Pradesh, it can be seen that many fungal species associate with a wide range of host plants e. g. *Amanita pantherina*, *Lactarius sanguiflus*, *Boletus edulis*, *Laccaria laccata*, *Russula delica*, *Cantharellus cibarius* and *Thelephora terrestris* to mention a few. Other species are more restricted in their distribution. For example species of *Suillus* and *Rhizopogon* mostly fruit under conifers particularly *Pinus* spp. However, true nature of host specificity is not clear. It is not understood whether a host-specific fungus forms mycorrhizae only with the host under which it fruits, or can it form mycorrhizae with many hosts but fruit only in association with certain ones. To understand the host specificity of ectamycorrhizal fungi data on experimentation with these spp. in nursery conditions is highly desirable.

Species with wider host-specificity offer greater opportunity to evaluate their potential of forming symbiotic association with different host species in nursery conditions and their subsequent survival and establishment at the site of planting.

From the foregoing account it is apparent that mycorrhizal symbiosis is more pronounced and varied in the temperate zone.

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Survey of native VAM fungi of saline soils of Haryana State

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Members of Endogonaceae are distributed widely in agriculture and forest soils throughout the world. However, no effort seems to have been directed to isolate and identify the VAM fungi inhabiting wastelands, degraded soils and soils of high salinity. Isolation, and multiplication of salinity tolerant efficient strains of VAM fungi is important for mass inoculum production and for tailoring roots of tree seedlings suitable for rehabilitation and reclamation of these soils. With this objective, a survey was conducted to isolate and identify genera and species of Endogonaceae which form a normal component of soil microflora in saline soils of Haryana State under barren aud vegetation conditions.

Soil samples were collected from Revar rectangle 74, Saraswati Range, Kurukshetra Forest Division and Kawani, Dhandh, Kirdhan and Bhanawali Panchayat areas of Hisar Forest Division. The soils at Saraswati are silty loam, hard and deficient in organic matter. The soil pH ranges between 9 to 10 at Saraswati and 7.5-8.1 at Hisar. Due to their poor physical condition and adverse chemical composition the forest cover is normally very scanty or entirely absent. Among the different species tried in the past, *Acacia nilotica*, *Albizia procera* and *Prosopis juliflora* are able to survive and grow on such lands.

Soil samples were collected randomly from 30 cm. deep pits from different locations of trials plots (150 m^2) laid down by F. R. I. at Saraswati covering a total area of 70,000 m^2. The area was planted during July, 87 with 10 different hard wood species. The soil was collected by scraping vertically from top to the bottom of the pit and thoroughly mixed to form a composite sample. Five hundred gm. soil from each sample in 5 different lots of 100 g each was assayed by wet sieving and decanting technique (Gerdemann and Nicolson, 1963) and flotation method (Ohms, 1957) to collect spores and sporocarps of VAM fungi. The spores were cleared and mounted in lectophenol and examined under low and high magnifications.

In all 10 species of Endogonaceous fungi were isolated of which 7 belong to *Glomus*, one to *Gigaspora* and two to *Sclerocystis* as listed below :

1. *Glomus caladonius* (Nicol. and Gerd.) Trappe and Gord.
2. *G. albidum* Walker and Rhodes

3. *G. fasciculatus* (Thex.) Gerd. and Trappe
4. *G. macrocarpus* Tul. and Tul.
5. *G. microcarpus* Tul. and Tul.
6. *G. multicaulis* Gerd. and Bakshi
7. *G. reticulatus* Bhattacharjee and Mukerji
8. *Gigaspora nigra* Nicolson and Schenck
9. *Sclerocystis coremoides* Berk. and Broome.
10. *Sclerocystis sinuosa* Gerd. and Bakshi

The frequency of VAM fungi varied qualitatively in different locations and under different vegetation cover. *Glomus* spp. were most common of which *Glomus macrocarpus* was most abundant in the study sites distantly located from each other. Next to *Glomus* spp. *Sclerocystis coremoides* was common whereas *Gigaspora* sp. occurred rarely. The lowest frequency was of *Gigaspora* spp. followed by *G. reticulatus*, *G. multicaulis* and *G. fasiculatus*. According to Mosse (1973) *Gigaspora* and *Acaulospora* are more tolerant to acidity whereas *Glomus* spp. favour neutral and alkaline soils. The difference in species may be attributed to adaphic factors and host plant interactions at the particular site. Higher spore frequencies under *Acacia* (*A. nilotica*), *Prosopis* (*P. juliflora*) and *Delbergia* (*D. sissoo*) may be due to high crop density, pure composition of tree crop, age and host species compatibitity with VAM fungi.

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Impact of season on the distribution of VAM fungi associated with sesame

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In India, fat and edible oils are mostly derived from oilseed crops like groundnut, sunflower and sesame. Most of these crops are grown in phosphorus-deficient soils which are also usually deficient in other nutrients including moisture in varying degrees. The need to improve the efficiency of oilseed production is therefore obvious. In this context, the role of vesicular-arbuscular mycorrhizal fungi is receiving great attention in recent times in view of their beneficial effects on plant growth. The information on VAM association with sesame is scanty (Vijayalakshmi and Rao, 1988). Recently an exhaustive range of studies has been carried out on VAM in relation to sesame (Sulochana, 1989).

VA mycorrhizae are ubiquitous. They are present in the soil in the form of chlamydospores, zygospores and azygospores, VAM symbiosis directly helps in the uptake of phosphorus and other nutrients (Mosse, 1981). However, seasonal variations can have a remarkable influence on the occurrence of the VAM spores (Nicolson and Johnston, 1979). Environmental conditions, besides the host plant, have significant effects on the distribution, density and composition of VA mycorrhizae (Daniels and Trappe, 1980).

The occurrence and number of VAM fungal propagules in the rhizosphere soils supporting six cultivars of *Sesamum indicum* L. in kharif and rabi seasons of two years have been worked out. Altogether eleven VAM fungi belonging to *Acaulospora*, *Glomus* and *Gigaspora* genera were found associated in both the seasons. Out of the six cultivars tested, Gowri cultivar showed maximum VAM association in rabi as well as kharif seasons.

VAM propagule number varied considerably among individual species and all the cultivars have shown at least five VAM species during their growth period. The quantitative data clearly indicate that, in all the seasons studied, the Gowri cultivar, followed by T-4 and E-8 were the best colonized and the propagule number was in direct correlation with the age of the crop plant. Among the VAM fungal species isolated, *Gigaspora margarita*, *Glomus fasciculatum*, *G. epigeaum*, *G. constrictum* and

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G. monosporum are of predominant occurrence over others. Characteristically, Gowri cultivar was found harbouring all the eleven VAM fungal species in rabi and kharif seasons.

The VAM propagule number was more in the rhizosphere soils of kharif crop than in rabi crop. The kharif season experiences good precipitation, moisture, average temperature and requisite quantities of nutrients. Accordingly the cultivars of sesame were found colonized heavily by VAM fungi both quantitatively and qualitatively in the kharif crop.

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Density of vesicular arbuscular mycorrhizal fungi in different crops grown under black soil

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The group of fungi that form vesicular-arbuscular (VA) mycorrhiza are among the most common soil fungi and probably colonize more plant tissue than any other fungal group. They significantly benefit the crop in better nutrition. Native population of VA mycorrhizal fungi may or may not be effective in stimulating the growth of a crop in a particular soil. The relationship between VA mycorrhizal fungi and chemical factors have been studied little in tropical soils.

VA mycorrhizae occur in almost all perennial crops of economic importance in the tropics. Wide variations in the VAM infections of trees growing under dry conditions (Diem *et al.*, 1981). Very little information is available regarding the effect of different crops on native mycorrhizal propagules in tropical black soils. The present survey was done to know VA mycorrhizal population density in tropical black soils under different crop stands.

Soil and root samples collected from three Agricultural Research Stations (ARS) located at Hagari, Siruguppa and Gulbarga were analysed for population of VA mycorrhizal fungi. The soils have a pH of 7.8 to 8.5 and are low in nitrogen and available phosphorus, but fairly adequate in available potassium. Fifteen soil and root samples were collected from rhizosphere of each plant stand. Percentage mycorrhizal colonization in the root was assessed after staining with tryphan blue (Phillips and Hayman, 1970). Extra matrical chlamydospores in 100 g soil were determined by wet seiving and decantation procedure as outlined by Gerdemann and Nicolson (1963). The predominent spores were identified using the synoptic keys as outlined by Trappe (1982).

The soils of ARS, Hagari are of two types. Majority of the area consists of black soils with clay to sandy clay in texture. Sand dunes are formed near the river bank, neem trees have been grown to stabilize the sand dunes. The major crops grown in black soils are : rabi sorghum *(Sorghum vulgare)*; safflower (*Carthemus tinctorius*); wheat (*Triticum aestivum*) and cotton (*Gossypium hirsutum*). Some plantation crops like guava (*Psidium guayava*), mulberry (*Morus alba*) and subabul (*Leucana leucocephaula*) are also grown in black soils. Neem and guava are the main plantations in sand dues and sandy loam soils. The per cent root colonization of VA mycorrhizal fungus varied from 18 to 20 and spore number from 170 to 175 per 100 g soil. Among the field crops tested, safflower recorded the highest root colonization (30%) and spore number (320/100 g soil) where as, the lowest spore count was observed under plantation trees and the per cent root colonization in sorghum crop. The spore population in Newzeland soil varied from 6 to 1590 with an average spore number of 196 per 100 g of black soil.

The soils of ARS, Siruguppa are deep black (vertisol) with silty clay texture. The major crops grown are wheat, maize (*Zea mays*), groundnut (*Arachis hypogaea*), cotton and safflower under protective irrigation. The per cent root colonization varied from 10 to 28 and spore number from 78 to 290 per 100 g soil. Highest per cent root colonization and spore count was observed in safflower and the lowest in maize.

The soils of ARS, Gulbarga consisted of black soil with clay texture. Sorghum and redgram (*Cajanus cajan*) are the major crops grown in this region. The per cent root colonization of VA mycorrhizal fungus varied from 6 to 10 and spore count from 76 to 80 per 100 g soil.

The predominent VA mycorrhizal fungal spores isolated from black soils belonged to genus *Glomus*. The spores isolated from the soils of arid and semi-arid region belonged to three genera viz., *Glomus, Gigaspora and Sclerocystis.*

The native VA mycorrhizal population was less in black soil (vertisol) as compared to red soil (alfisol). The black soils with sandy loam texture exhibited higher mycorrhizal activity than those with clay texture. The clay content in soil was also found to influence the VA mycorrhizal population. The host plant also influenced the VA mycorrhizal population. The highest mycorrhizal population was noticed associated with safflower, while the lowest was with sorghum. Wheat, maize, groundnut, cotton and redgram had intermediate population. The distribution of VA mycorrhizal population in black soils was mainly dependent on soil properties and mycorrhizal dependancy of host plant.

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Mycorrhizal investigations in some orchids

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Wahrlich (1986) reported 500 orchid species which show fungi inside their roots. Present study was made to investigate the mycorrhizal association in the four genera i. e. *Epipactis*, *Calanthe*, *Aerides* and *Vanda*. The former two are terrestrial and latter two are epiphytic. All genera bear mycorrhizal associations with their roots of different intensity depending upon the different environmental conditions. Bernard (1909) considerd mycorrhiza as parasitic interaction in orchids. Rayner (1926) suggested that mycorrhiza are beneficial to host plant due to the absorption of mineral nutrients from the soil.

A number of authors have studied nature, mode of infection and penetration of the mycorrhizal fungus into the host. Parasitic pathogenic nature of mycorrhiza and relation of mycorrhizal fungi was studied by Curtis (1939).

In all four taxa mycorrhizal endophyte was found to be present in the roots. In the middle zone of root cortex more intensity of peletons was found in comparison to epidermal or central V. B. region. In case of epiphytic orchids as *Aerides multiflora* and *Vanda Cristata* the infection mostly takes place at the base of the attachment of the root to the substratum while in terrestrial taxa as *Calanthe plantagineae* and *Vanda parviflora* it appears all around in the cortical region.

The size of peletons was also measured and found that infection was more in *Epipactis latifolia* (64-176 um) in comparision to *Calanthe plantagineae* (16-19 um) in terrestrials while in case of epiphytic taxa infection was more i.e. 40-110 um in *Aerides multiflora* in comparision to *Vanda cristata* (17-22 um).

Penetration of hypha were observed through the epiblamal cells while in terrestrial taxa entry of fungus was observed through root hairs.

The fungal endophyte of the above mentioned four orchid species were isolated and identified on the basis of their growth character, size of hypha, spore cells etc. The isolated mycorrhizal species are :

1. *Rhizotonia sps.* :- Mycelium branched, moniliform hyphae, brownish in colour, 3–3.5 um. Sexual reproductive bodies are not formed.

Isolated from—*Epipactis latifolia*

2. *Rhizotonia dichotoma* :- Mycelium branched, white, septate, moniliform. Vegetative hyphae of much elongated cylindrical cells 120—176×6—8 um. Spore cells are very few in number ranging 32—48×9.6—12.8 um.

Isolated from—*Calanthe plantagineae*

3. *Rhizotonia repens* :- Mycelium white differentiated into main and aerial hyphae which are elongated ranging from 6-8 um. Sexual reproductive bodies/ sporodochia were not observed.

Isolated from—*Aerides multiflora*

4. *Rhizotonia dichotoma* :- Mycelium white, elongated, brached with septate hypha, 5-6 um thin of intertwined moniliform cells 18-20 um×7-11 um in size. Sexual reproductive bodies were not observed.

Isolated from—*Vanda cristata*

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Incidence of vesicular arbuscular mycorrhizal fungi in Raipur soils

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Occurrence of the symbiotic VAM fungi has been reported in soils of several places in India. It is now well known that these organisms are ubiquitous in distribution and perform important roles in uptake of phosphorus, sulphur, water and other ions for their macrosymbiont host plants, besides being a source of nutrition in certain cases. The functional attributes of VAM fungi assign them a great priority in modern researches in microbial biotechnology. This study is an outcome of such a consideration that has been initiated in Chhattisgarh region of M.P. for the first time ever.

The isolation of VAM spores (Gerdemann and Nicolson, 1983) from these soils has shown incidence of eight species of *Glomus* and only one of *Sclerocystis*. *Glomus fasciculatus*, *G. mosseae* and *G. macrocarpum* were distributed widely here, with almost an equally wide incidence of *Glomus* sp. I. However, *G. reticulatum*, *G. constrictum*, *G. monosporus*, *Glomus* sp. 2 and *Sclerocystis rubricolla* were restricted in their distribution only to certain soil samples. It appeared that in the occurrence of the latter species, the host plant distribution had some relationship. The unidentified species of *Glomus* appear to be undescribed in the literature so far.

Root association of VAM (Phillips and Hayman, 1970) has so far been recorded in 19 plants belonging to 10 families that grow here. In the plants of Leguminaceae and Euphorbiaceae root colonization was relatively better in respect of mycelial and vesicular growth. VAM association found in *Amaranthus spinosus* and *Eclipta alba* was recorded in this study. Gerdemann, (1968) has reported that the family Amaranthaceae is non-mycorrhizal. Similarly, *Eclipta alba* has been considered non-mycorrhizal (Manoharachary *et al.*, 1987). It suggests that the mycorrhizal associations might be governed by microhabitat conditions.

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Mycorrhizal reproduction as influenced by moisture stress

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The effect of changing water availability on the vesicular arbuscular (VA) mycorrhizal fungi has been sorely ignored barring few stray reports. Manson (1964) pointed out that *Endogone* spore numbers in soils increased after mild and dry winter than cold and wet winter. Redhead (1975) reported that the amount of water which was optimal for the growth of plant also resulted in the greatest production of fungal spores. Nelson and Safir (1982) reported that drought stress reduced spore production by *Glomus fasciculatum*. Bethlenfalvay *et al.* (1988) reported that colonization of soybean roots by *Glomus mosseae* did not vary with stress but the biomass and length of extracellular mycelium was greater in severely stressed than non-stressed plants. The present experiment was conducted to understand the effect of moisture stress on mycorrhizal root colonization, and production of spore and infective propagule numbers by *Glomus fasciculatum*.

Cowpea plants were grown with *G. fasciculatum* inoculum in sterilised and unsterilised soil with different levels of moisture stress imposed after 6 weeks of sowing viz. i) No stress–A) watering daily to field capacity (FC). ii) Mild stress–B) watering once in 3 days to FC. C) Maintained continuously at 75% FC. iii) Severe stress – D) watering once in 5 days to FC – E) Maintained continuously at 50% FC. Observations on plant biomass, root dry weight, per cent mycorrhizal root colonization, spore numbers and infective propagule numbers were recorded after 3 weeks of imposing treatments.

Maximum plant biomass was recorded in plants watered daily to FC. Root growth was reduced markedly by increased moisture stress. In sterilised soil the per cent mycorrhizal root colonization ranged from 73.6 to 78.8 while in unsterilised soil it ranged from 72.4 to 80.4. Maximum mycorrhizal colonization was observed under severe stress where plants were watered once in 5 days to FC.

The number of spores and infective propagules were highest in pots watered daily to FC and least in pots maintained continuously at 50% FC. But the per cent reduction in spore numbers was always more than the per cent reduction in the number of infective propagules suggesting that there is more hyphal growth and less sporulation during moisture stress. The milder and severely stressed pots which were watered back to FC (Treatments B and D respectively) had more number of spores and infective propagules than the pots maintained continuously under similar stresses (Treatment C and E respectively). Under similar stress condition, the number of spores and infective propagules were more in unsterilised soil than the sterilised soil.

Severe stress condition of watering once in 5 days to FC encouraged mycorrhizal root colonization, while the severe stress condition of maintaining pots continuously at 50% FC suppressed the number of spores and infective propagules. Watering to FC once in 3 or 5 days favoured sporulation better than maintaining pots continuously at 50 or 75% FC.

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Studies on thc mycorrhiza of Apple (*Malus domestica* borkh.)

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No work on the mycorrhiza of apple plants has been done so far in any part of India. The present studies are the first on characterization and identification of mycorrhiza and on the mycorrhizosphere of Royle delicious var. of apple plants.

Soil samples of apple plant of different ages (1 yr, 2 yr, 3 yr, 4yr and mature fruit bearing plants) were taken at monthly intervals beginning with December 1988 to May 1989.

For characterizing the mycorrhizal types, morpho anatomical studies were conducted following Zak (1971) and for ascertaining the VAM infection, the whole roots were stained with trypan blue as given by Philips and Hayman (1970). For isolating VAM from soil samples, wet sieving and decanting method of Gerdemann and Nicolson (1963) was followed.

For isolation of fungi from the mycorrhizosphere, the dilution plate method (Waksman, 1927) was employed. Martin's Czapekdox and Potato Dextrose Agar medium were used for raising cultures. The petriplates were incubated at $22 \pm 2^{\circ}C$ and examined at regular intervals.

Moisture content and pH of soil was recorded by standard procedures. The mycorrhizal root activity was measured following Hervey *et al.* (1976).

Mycorrhizal roots were observed to be without any surrounding fungal hyphae or rhizomorphs but their tips were swollen near the apices. These roots were creamish white in colour.

The mycorrhizal roots showed a typical ectoendomycorrhizal anatomy. The sections showed both inter and intracellular infection. T. S. of 1 yr old roots showed hyphal penetration in the entire cortex region surrounded by Hartig net but the stelar portion had little infection and the pith region had no infection at all. Sections of two yr old roots showed in addition vesicle like structure in the cortical cells, and the hyphal infection extended up to stelar portion but pith was still without infection. Three yr old plants showed infection in the stelar and pith region as well. Four yr old plants showed variable development of the Hartig net. The hyphal penetration was both inter and intracellular. The whole cortical region was infected and each cortical showed the presence of many vesicles per cell compared to the 2–4 per cell vesicles in 1–3 yr old plants. The sections from mature tree bearing fruits showed heavy vesicular infection of endomycorrhiza in the cortex, stele and pith region, each cell having many vesicles per cell. Because of secondary growth, the primary cortex and hence the Hartig net was greatly reduced. Instead the cork cells formed a definitive layer.

In the younger plant roots the Hartig net was well developed and easily discernible conforming to ectoendomycorrhizal type, while in older plant roots, Hartig net was not clearly visible due to the secondary growth. The VAM infection was confirmed by the presence of large number of hyphae running in the cortical and stelar portion, and these hyphae forming vesicles. Finger like projections or arbuscules were also observed arising from the hyphae, confirming hypal and vesicular infection of VAM.

In the studies on mycorrhizosphere, twenty species of fungi, 4 belonging to zygomycotina, 16 to Deuteromycotina and one form of sterile mycelium have been isolated from the mycorrhizosphere. *Aspergillus niger* and *A. fumigatus* were most predominant and the frequency distribution of *A. niger*, *A. fumigatus* and *Mucor heimalis* was recorded to be higher than the other fungi isolated. *Rhizopus nigricans* exhibited abundant growth in the months of January, March, April and May; *Fusarium moniliforme* in the months of December, January, March, April and May; *F. oxysporum* in December, January and March. Trichoderma sp. was found only in the month of December, *Curvularia lunata* in March and *Phoma* sp. was abundant in April and May. *Alternaria alternata* in December, January and February; *Mucor* sp. in the months of January, March and May and *Rhizopus* sp. in February and March. Sterile mycelial form was recorded in all the six samplings but was more predominant in the months of March, April and May.

VAM studies showed six fungal spore types, i. e. *Glomus mosseae G. candidus, G. fasciculatum, G. macrosporum, Endogone increseta* and *Gigaspora* sp. Unidentified perithecia of an ascomycete were also present in abundance in all the soil samples. The root activity was observed to be less in December, January and February and more in April and May.

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Occurrence and distribution of vesicular-arbuscular mycorrhizal fungi in acid soils of North Eastern India

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The intrinsic interest in the study of vesicular-arbuscular mycorrhizal fungi is due to their beneficial role in the growth of the plants. Prior to exploiting the biofertilizer potential of these organisms, it is necessary to examine the occurrence and distribution of the VA mycorrhizal fungi in soils. Although several studies hove been conducted in other parts of the country on these fungi (Bagyaraj *et al.*, 1979), their distribution in the soils of N.E. India has not been studied. The qualitative and quantitative distribution of the VA mycorrhizal spores in soils of North Estern India are described.

Soil samples from different areas in Assam, Arunachal Pradesh, Manipur, Mizoram and Nagaland were collected, air dried and sieved through 2.0 mm mesh to remove coarse debris and root bits. Each soil sample was thoroughly mixed before examining them for spores. For measurment of pH, soil was mixed with two volumes of distilled water, stirred for 30 minutes and the supernatant was collected. For isolating spores, 100g soil of each sample was stirred in excess quantity of water and decanted into sieves (Gerdemann and Nicolson, 1963). The fractions collected between 300–90 μm mesh were examined for spores. Four replicates were screened for each sample.

VA mycorrhizal spores were abundant in samples of Assam as compared to those of hilly regions viz. Arunachal Pradesh, Kohima, Nagaland, Manipur, Mizoram. Highest number of spores was recorded in samples from Golaghat followed by Titabor, Jorhat and Halflong in Assam. The VA mycorrhizal spores were present in all the samples except that from Mizoram. The low count in hill land samples may be due to factors like soil erosion or poor vegetational cover.

Scutellospora nigra (*Gigaspora nigra*) Walkers and Sanders Comb. nov. *Sclerocystis rubiformis*, *Glomus macrocarpus* were commonly found in plain soils of Assam. Other species infrequently isolated were *Gigaspora decipiens*, Hall and Abbot, *Glomus heterosporum* Smith and Schenck and *Glomus leptotichum*, Schenck and Smith. In hilly regions large spore types were absent but screenings from 150–90 μm consistently revealed individual spores of *Glomus* sp. A hitherto undescribed species of *Gigaspora* was isolated from several areas in and around Jorhat. The spores occurred singly in soil which were globose or ellipsoidal. Older spores were dark brown to black with pale brown suspensor cell attached to the base of the cell. Spore wall with multiple layers was dark brown to black with separable inner hyaline wall layer. Accessory vesicles were borne on coiled hyphae they had yellow outer wall, smooth vesicles (6–8, 33.2 μm in diam). The new species has been tentatively named as *Gigaspora echinulata*.

Spores provide a reliable index to the existence of these fungi in a particular soil. They do not give an absolute index to the infectivity of soil because spore forming capacity varies from species to species and is an inherent species characteristic (Gerdemann, 1975). Nevertheless the preponderance of VA mycorrhizal spores in plains as compared to those in hilly areas indicate that hill land soil will be more amenable and responsive to VAM fungal inoculation trials. As VA mycorrhizal fungi have wide host range in native soils where their prevalence is more, these native fungi are likely to mask the efficiency of the inoculant fungi. This would render inoculation trials with VA mycorrhizal fungi difficult and unsatisfactory. It is felt that in hill land soils the persistency of the species diversity among VA mycorrhizal fungi is a field of investigation which needs to be evaluated before any meaningful field trials could be conducted. From the study it appears that in hilly areas, inoculation trials with VAM fungi will be successful in highly susceptible crops like chillies, soybean and maize.

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Preliminary survey of mycorrhizal fungi in some weeds and cultivated plants in Meerut

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VAM fungi are usually associated with most plants and are important in agriculture, horticulture and forestry. VAM are obligate symbionts formed by nonseptate phycomycetous fungi belonging to the genera *Glomus*, *Gigaspora*, *Acaulospora*, *Sclerocystis* and *Endogone*, in the family Endogonaceae of the order Mucorales. Endotrophic VA Mycarrhizae are well known to increase nutrient uptake, resistance to drought and salinity and tolerance to pathogens. They seem to have a potential as biofertilizers (Jalali, 1989). The limited Indian work on VAM reviewed by Bagyaraj (1986), showed that the hosts plants can influence the qualitative and quantitative nature of the spores of VAM fungi. If this is so, weeds growing on fallow agricultural lands would greatly influence the activities of VAM fungi and the health of the crops grown on such lands. As VAM fungi associated with weeds have been surveyed by few workers from Delhi, Allahabad, Hyderabad and Jorhat and no such work has been undertaken in Meerut area, the present investigation was taken up.

One cm root segments of plants under reference were cleared in 10% KOH solution and stained in cotton blue. Under the light microscope characteristic fungal structures were seen. The data are presented in Table 1.

Table 1. Mycorrhizal association of weeds and cultivated plants.

Host		Mycorrhizal Association
1.	*Abutilon indicum*	+
2.	*Achyranthes aspera*	+
3.	*Ageratum conyzoides*	+
4.	*Argemone maxicana*	+
5.	*Cassia glauca*	—
6.	*Cassia occidentalis*	—
7.	*Cassia sophera*	—
8.	*Cannabis sativa*	+

	Host	Mycorrhizal Association
9.	*Croton bonplandianum*	—
10.	*Convolvulus arvensis*	+
11.	*Convolvulus pluricaulis*	+
12.	*Commelina benghalensis*	+
13.	*Eclipta alba*	—
14.	*Euphorbia hirta*	+
15.	*Euphorbia microphyla*	+
16.	*Euphorbia thymifolia*	—
17.	*Heliotropium indicum*	+
18.	*Justicia gendusassa*	—
19.	*Nicotiana plumbaginifolia*	+
20.	*Parthenium hysterophorus*	+
21.	*Peristrophe bicalyculata*	+
22.	*Phyllanthus niruri*	+
23.	*Peperomia pellucide*	+
24.	*Portulaca grandiflora*	—
25.	*Sida cordifolia*	+
26.	*Xanthium strumarium*	+
27.	*Catharanthus roseus*	+
28.	*Dalbergia sissoo*	+
29.	*Ficus krishnae*	+
30.	*Ginkgo biloba*	—

+ = Present, — = Absent

Maximum percentage infection in decreasing order was found in *Peperomia pellucide* (66.1%), *Abutilon indicum* (63.7%), *Euphorbia hirta* (58.0%), *Nicotiana plumbaginifolia* (56.5%), *Argemona mexicana* (51.5%), which was followed by *Parthenium* and *Phyllanthus*.

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Extraction of vesicular–arbuscular mycorrhizae spores and effect of fertilizers on their population in oilseeds cropped soils

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Several methods have been developed from time to time for extraction of vesicular–arbuscular mycorrhizae (VAM) spores from soil (Hayman, 1970; Sutton and Barron, 1972). The floatation and adhesion technique of Sutton and Barron (1972) is claimed to be the most efficient technique for recovery of VAM spores from soil. The present paper reports a modification of the technique and is most suitable for survey studies.

Information pertaining to VAM associated with oilseed plants in Jabalpur is lacking. Qualitative and quantitative examination for distribution of major genera of VAM associated with oilseed crops was undertaken and the influence of fertilizers on VAM population was also investigated.

The modified method is less time consuming as it takes only 15 minutes whereas the method of Sutton and Barron (1972) requires 2 hours. White polyster cloth with a pore size less than 10 μm is most ideal for retention of all types of VAM spores. This cloth can be reused after washing and hence is economical. Further, separatory funnels are not required. Maximum spores can be mounred at a time and recovery on adhesive tape is excellent and the spores are clearly discernible.

The effect of nitrogen on VAM population with regards to oilseed crops (Sesamum, Sunflower and Mustard) revealed a unique adverse effect of increasing levels of nitrogen. From the initial low level itself, the population decreased and continued gradually till zero at high levels indicating high sensitivity of VAM to increasing levels of nitrogen in oilseed cropped soils.

A declining trend of VAM population with increase in phosphorus levels has been observed in Sesamum, Suflower and Mustard crops. High phosphorus levels in soil are deterimental for the symbiotic establishment of VAM with these oilseed roots.

In oilseed crops, there is an adverse effect of potash as its level is increased. In oilseeds, it seems that there is inverse relationship between VAM spore population and potash level.

In all soil samples analysed, only one genus i. e., *Glomus* spp. was encountered.

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Suitable source and level of nitrogen for mass production of the VA mycorrhizal fungus *Glomus fasciculatum*

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Vesicular-arbuscular mycorrhizal (VAM) fungi being obligate symbionts have not been cultured on artificial media. Recent studies have shown that large scale production of VAM inoculum is technically feasible through pot culture using an appropriate host, substrate and nutrients. Nitrogen is one of the important nutrients required by plants for growth and development. Yet, the information available on the effect of different nitrogen sources on VA mycorrhiza is scanty. It appears that hightest concentration of nitrogen fertilizers will be inhibitory to VAM development. Some workers found that ammonium source encourages VAM development compared to nitrate source (Davis and Young, 1985), while few others recorded the reverse to be true (Chambers *et al.*, 1979). When N-source was applied as 50% ammonium+50% nitrate, maximum root colonization was observed in careals (Thompson, 1986). The present investigation was undertaken to find out the best source and level of N for mass production of the VAM fungus, *Glomus fasciculatum*.

Our earlier studies on mass production of *G. fasciculatum* brought out that Rhodes grass (*Chloris gayana Kunth*) and perlits : soilrite mix (1:1 by volume) to be the best host and substrate respectively for mass production of *G. fasciculatum* (Sreenivasa, 1986). Hence the same host and substrate were used in the present study. Three sources of N were used in the study : Nitrate as calcium nitrate with 15.5% N, ammonium as urea with 45% N and ammonium nitrate as calcium ammonium nitrate with 20.5% N at 0, 10, 20, 40, 80 and 120 ppm N.

The mycorrhial parameters viz., percentage root colonization, extramatrical chlamydospore number and number of infective propagules per unit weight of inoculum increased upto 10 ppm N in both the sources. When N was used as a mixture of ammonium and nitrate, all the three mycorrhizal parameters increased with increase in N-level upto 80 ppm but decreased significantly at 120 ppm N level. The inoculum potential was maximum at 80 ppm N applied as ammonium nitrate which was the best level and source of N for mass production of *G. fasciculatum*. A significant increase in shoot and root dry weight was ecorded with increasing levels of N applied as any source except ammonium nitrate at 120 ppm N level. Maximum shoot and root biomass was recorded at 80 ppm N applied as ammonium nitrate. This suggests that the source and level of N supporting maximum mycorrhization also supports maximum plant growth. Thus the results of the present study clearly bring out that nitrogen applied as ammonium nitrate at 80 ppm N level, is the best for mass production of *G. fasciculatum* with maximum inoculum potential.

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Nutrient release from litter and the development of mycorrhizae in more disturbed and less disturbed forest communities

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Forest litter is a unique component of the forest biogeoneocoenosis and one of its major indicators of energy transfer. Equally significant is the role of forest litter in soil formation and its evolution. Litter fall and decomposition are important functional aspects of a forest ecosystem. The litter on the soil surface acts as an input-output system and is important in the nutrition of wood-lands, particularly of those on soils of low nutrient availability, where the trees rely to a great extent upon the efficient recycling of nutrients.

Slash-burn agriculture, which is locally called as 'Jhum' is the predominant type of agriculture in North-Eastern India. In recent times, owing to population explosion and urbanization the jhum cycle has been reduced from a more favourable 20-30 years to a short period of 5 years. It has adversely affected the yield, the soil environments in terms of physical structure, soil fertility and vegetation cover.

The maintenance of soil fertility in hot, humid high rainfall area is a serious problem and is more severe when the jhum cycle becomes short. Heavy losses of carbon, nitrogen, phosphorus and sulphur occur due to volatilization during burn, perculation and runoff. This nutrient stress condition causes a variety of reproductive and growth strategies in successional species. It is evident that majority of plants occuring in natural stressed environments are normally mycorrhizal (Reeves *et al.*, 1979).

The aim of the study was to evaluate the probable reasons of disturbance on nutrients release and their effect on VAM development. The study was carried out at Byrnihat located in East Khasi Hills of Meghalaya at 26'ON latitude 95'O" longitude at an altitude of 100m MSL. The climate of the study area was hot and humid with an average rainfall of 220cm per annum.

Decomposition of litter of different plant species showed a great variation. The microbial degradation of the herbaceous woody litter on highly disturbed site was faster than the same in case of less disturbed ones. The release of nutrients like nitrogen and phosphorus also showed variations. *Ageratum* sp. a common weed harboured more nitrogen content than woody *Mallotus* sp. In both the cases nitrogen was more in the beginning and it decreased afterwords, which was again increased in the later part of decomposition.

In case of less disturbed forest stand, the rates of decomposition of *Vitex* and *Holarohena* sp. leaf litters were slow. These two species too had initial high nitrogen content, which decreased after that with the onset of decomposition. The least amount of nitrogen was detected in July. An increase in nitrogen content was observed again in the later part of decomposition. The phosphorous content of the leaf litter was more in the beginning which decreased in the following months. An increase in P-contents was observed in May and in later part of decomposition.

The soil samples from less distrurbed site contained high nutrient concentration as compared to its more disturbed counterpart. During dry winter months the soil contained less nitrogen as compared to wet rainy months. The maximum available P was estimated more in May and less in winter months.

The plant species growing in more degraded site had higher mycorrhizal infection than those in less disturbed site. No marked seasonality was observed in mycorrhizal infection. In general, there was heavy infection in spring and rainy months. The endogonaceous spore population was high in less disturbed site than in more disturbed one. Maximum spores were counted in winter months.

From the results, in was evident that disturbance of forest stand has affected the mycorrhizal establishment. The less no. of endogonaceous spores in more disturbed site may be due to the high degree of disturbance which led to the reduction and possibly elimination of mycorrhizal propagules (Reeves *et al.*, 1979). On the other hand, the high intensity of mycorrhizal infection was attributed to low nutrient status of soil. It seemed that under nutrient stressed condition and under favourable climatic conditions the mycorrhizal fungi spread better in the fast growing host species and formed chlamydospores in nutrtient rich and slow growing tree species. Infection was high in winter months when less N and P were estimated from soil, where as the low infection level in wet rainy months were positively correlated with moderate nutrient status of soil due to rapid release of nutrients from litter after increased microbial activity. The soil disturbance may affect P-mobilization due to effect on vesicular-arbuscular mycorrhizal infection (Fairchild and Miller, 1988). A rapid recovery in P-uptake and VAM infection with time may take place. The growth advantages attributed to VAM are believed to be associated with an increase in the nutritional status of plants brought about by increased P-uptake from degraded soil (Mosse, 1973) and water transport (Safir *et al.*, 1972).

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Spore dispersal of endogonaceae by worms, wasps, dung rollers and Indian domestic fowls

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Endogone, the most common fungus component of vesicular arbuscular mycorrhizae is non specialized in host range. In association with the host *Endogone* produces in internal mycelium in the cortex and a loose external mycelium bearing chlamydospores and zygospores in the rhizosphere and soil. These spores are the means of surviral of *Endogone* the absence of living hosts. Chlamydospores suggest that they may be able to survive passage through the digestive system of rodents, worms and other animals inhabitans of soil (Dowding, 1959). A number of other vectors of VAM fungal spores have been described as early as 1922. Endogonaceae spores were observed in the digestive tracts of mullipedes (Thaxter, 1922).

Present studies reported here deal with the occurrence and dispersal of species of Engogonoceae by worms, wasps, dung rollers and Indian domestic fowls.

Samples of earthworm casting, mud nests of wasps, dung rollers and guana of fowls samples were individually collected in different days and placed in polythene bags. The contamination of the samples with underlying soil was avoided. Soil samples from 5-10 cms depth profile were taken with the help of a 2 cm diameter soil sampling tube in the areas immediately adjacent to the worm cast mouls, mud nests, dung rollers, and near guana of fowls in the fields. Spores were extracted with care using procedure of Gerdemann and Nicolson (1963). The sample of earthworms, dung roller and wasps were sacrified by immersion in hot water and then they were dissected and intestinal contents were examined for presence of spores. Fowls were killed and dissected. Their undigested storage organs were examined for the presence of Endogonaceae spores.

Ten grams of crushed air dried sample material was mixed with 300 g. of red loam and placed in plastic containers. The loam soil had been treated previously with aerated steam at 65°C for 30 min. to kill any Engogonaceae spores present in the soil. The sample materials including worm casts, mud nests, balls of dung and fowls samples consisted of steamed soil without additional material. Sunflower seeds were planted in the prepared soil and thinned to three plants per container after emergence. The plants were grown in garden for 4 weeks. The root systems were then carefully washed free of soil and 25 root bits of each 1 cm long were boiled in

10% NaoH and cleaned (Phillips and Hayman 1970). The root bits were stained with trypanblue in lactophenol and mounted on microscope slides and the percentage of root infection by vesicular arbuscular (VA) Mycorrhizae was determined acording to the method of Hayman (1970).

Spores were found in all samples examined. In general more were extracted from the worms casts and mud nests and relatively a few sporse were recovered from dung balls.

VA Mycorrhiza did not develop in the roots of sunflower plants grown in steam treated check soil. However, in soils amended with air dried worm cast, mud pots, sun flower roots contain 2.4% VAM infection on root infection basis. Dung balls and fowls guana soil treatment developed 3% and 3.5% root infection respectively.

The large amount of soil which is constantly being mixed through earth worm activity is probably important means of distribution of Endogonaceae spores within the soil. The importance of fowls in India, mud nests dung balls in dispersal of the fungus is probably rather limited, however, the distance and speed aspects of these agents should not be over-looked. It appears to us that earth worms bring Endoganacae spores to the surface. These organisms movements may result in some horizontal dispersion.

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Survey of Indian arid zone tree species for the occurrence of VAM infections

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Vesicular arbuscular mycorrhizal associations with plants are widely distributed and are geographically ubiquitous. But there is not much information available on the occurrence of mycorrhizae in desert plant species. VAM fungi, a group of important soil microorganisms, are known to improve the plant growth through better uptake of nutrients and water, resistance to drought and increased tolerance or resistance to root pathogens. Water stress and nutrient deficiencies are the common constrains that the desert ecosystem experiences. The role of mycorrhizae in the building and improving the soil properties is well recognized (Koske *et al.*, 1975). The frequency and density of VAM infections vary widely depending on the plant species and soil type (Rani and Mukherjee, 1987). However, knowledge on the occurrence of mycorrhizae with tree species of Indian Arid Zone is scanty. The present investigation reports the extent of occurrence of VAM infections in different tree species growing in different localities of Indian arid zone.

Twelve plant species from nine environmentally harsh sites in Rajasthan were surveyed for the presence of VAM infections. The data indicated that varied types of tree species growing at different arid regions were found to have VAM infections with their roots. VAM infections were present in all the species examined in different locations except in the roots of *Salvodora oleiodes* collected from Chandan where no infection was observed. The intensity of infection varied among the different plant species collected from the same place as well as within the same plant species collected ftom different locations. For example, VAM infection in the roots of *Parkinsonia aculeata* varies from 25+ (Pokran) to 100+++ (Bikaner); similarly, infection in the roots of *Prosopis cineraria* varies from 35+ (Dubla) to 95+++ (Lathi). In general infection was more under Lathi (78.3%) and Bikaner (78.1%) soils, irrespective of plant species, whereas least infection was observed under Nagaur soils (35%). Among the plant species, irrespective of locations, the maximum infection was observed under *Azadirachta indica* (85.8%) followed by *Parkinsonia aculeata* (76.7%) and *zizyphus marutiana* (73.6%). Of-course plant samples of *Punica granatum* collected only from Bikaner gave 100% infection.

However, percentage of VAM infections do not vary with plant age. Samples collected from Lathi as well as Chandan showed that per cent infection in the roots of *Albizzia lebbek* aged from 3 to 12 months varies only 5-10% whereas no differences was observed under *Zizyphus marutiana*. The denisity of infection did not vary with plant age but the young roots mostly carried mycelia with a small number of vesicles spores and vesicles were found in matured roots.

An assessment of the mycorrhizal associations in tree species collected from different sites of Indian arid zone was made. The per cent as well as density of infection varied with the plant species and the locations. Even the plants of same family differed in the intensity of mycorrhizal association in a particular soil. Similar differences were recorded in plants of same species collected from different soils. The infection rate, in general, is independent of plant age. Most of the VAM fungi belong to *Gigaspora, Glomus, Acaulospora* and *Endogone*.

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VAM fungi from the rhizospheres of desert cacti

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The endomycorrhizae of vesicular-arbuscular type develop most commonly in majority of the cultivated and wild plant species of arid and semi-arid regions (Singh and Varma, 1980; Srivastava *et al.*, 1989) of western India (a part of Indian Thar Desert). The native endophytes play an important role in nutrient uptake and biomass production in the region (Slarikis, 1974). The variability in the fungal isolates towards root colonization, phosphorus uptake and biomass production have been well documented (Khan, 1974). However, no serious effort has yet been made to screen the endomycorrhizal isolates to know their role in drought regulation and the water use. The desert VAM fungi differ in their ecological requirements and adaptability. Little is known about the ecology of such isolates. Very few studies have been made on the members of family Cactaceae for mycorrhizal presence (Rose, 1981; Bloss and Walker, 1987) but to author's knowledge, no systematic study has been done on cacti roots and VAM association in India. Cacti are important because if spineless, they can be used as supplementary fodder during dry season in in deserts (Shankarnarayan and Shankar, 1986). Mycorrhizal fungi help in the formation of or preservation of soil structure, in the uptake of bound soil water and increased internal nutrition of their host plants (Sieverding, 1981). Kirkun *et al.*, (1987), Singh and Varma (1988) and Neeraj *et al.*, (1989) have studied the mycorrhizal associations with plants in stressed environments and found it of great biological significance. The aim of this study was to screen and select species of VAM fungi to determine host-fungus combinations under stressed soil and climatic conditions to explore their possible applications in plant-VAM associations.

Root and rhizosphere soil samples of *Opuntia coccinlillifera*, *O. cylindrica*, *O. ficus indica*, *O. santarita*, *O. vulgaris* and *Opuntia* sp. were collected from Jodhpur district of Rajasthan in the months of December 1988 and March 1989. Temperature of the soil-30 cm below the surface was noted at the time of sample collection. The roots of the respective plants were cut into 1.0 cm long segments, preserved in FAA and brought to the laboratory. These were cleared and stained with Trypan Blue. Presence of mycorrhizal infection in 100 root segments was observed using light microscope.

Soil moisture and pH was determined immediately after bringing the soil samples to the laboratory. The soil samples were air dried and stored at 4°C in air tight polythene bags. Wet sieving and decanting method (Gerdemann and Nicolson, 1963) followed by sucrose centrifugation (Smith and Skipper, 1979) was used for isolating the VAM spores from 50 gm air dried soil. The spores were mounted in PVLG for observations and identified with the help of the key of Hall and Fish (1979) and the Manual of the Manual of Schenck and Perez (1987).

Most of the Cactus plants studied were mycorrhizal but there was a great deal of variations in their percentage of root infection. *O. coccinlillifera* had maximum infection (35.6%) and *Opuntia* sp. the minimum (12%). The former had high infection density followed by *O. cylindrica.* Significantly, no arbuscules were seen. The spore number in rhizospheres varied with the season, host species and the physical properties of the soil.

In winters the number of VAM spores in the rhizospheres of *Opuntia* sp. and *O. coccinlillifera* was 240 ($\pm$6) and 328 ($\pm$8) respectively, wherease in spring *Opuntia* sp. had 332 spores and *O. vulgaris* 1142. Strikingly, *Glomus macrocarpum* not only dominated the spore population but also was common to all the six hosts studied. The other common species was *Sclerocystis sinuosa* associated with *O. ficus, O. cylindrica* and *O. vulgaris.* Spores of *G. fasciculatum, G. feugianum, G. geosporum, G. mosseae, G. reticulatum, Gigaspora candida* and *Entrophospora infreguens* were the other type species isolated from the rhizosphere soils. *E. infreguens* was observed only with the *O. ficus.* Its spores were orange brown, 96 μm dia., subglobose or ellipsoidal, contents oily, globular, vesicle subglobose to ellipsoid 128×153 μm and attached to the spore. Sporocarps of *S. sinuosa* were 175–235 $\times$ 187–257.5 μm, light brown to golden brown and usually tuberculate, enclosing obovate, elliptical, fusiform or clavate, 37.5–80 $\times$ 32.5–45 μm and chlamydospores in a peridium were made up of interwoven sinuous hyphae. Chlamydospores were arranged radially around a plexus of hyphae.

Absence of the arbuscules in the root system may indicate the nonfunctional mycorrhizal associations or non-symbiotic colonization by hyphae from a mycorrhizal plant growing close by. Even if the plants are widely spaced, colonization from a neighbouring plant may occur. At Peresent it can not be stated with certainty whether the mycorrhizae of the species are viable or structural remnants from a once-functional association (Hirrel *et al*; 1978).

The number of spores produced in the spring was higher than in the winter. This observation is in agreement with Steffeldt and Vogt (1975). Rose (1981) reported the presence of mycorrhiza in Cardo cactus (*Pachycereus pringlei*) and *Opuntia* sp. roots but no spores were encountered in the rhizosperes whereas *Machaerocereus gummosus* had VA fungi neither in the roots nor in rhizosphere soils. In such cases the questions raised by Hirrel *et al.* (1978) may have weightage. We have found both the VAM hyphal system in the roots as well as the VA spores in rhizosphere soils irrespective of the host species and their geographical distribution. This strongly suggests that the Cacti studied were truely mycorrhizal and spores heavily colonized the plant root system.

Low moisture content (3.5% in winter and 2.0% in spring) and pH value ranging from 8.3 to 8.96 indicate that these fungi can induce severe water stress conditions and survive well in alkaline soil conditions. The average temperature recorded at mid-day was 30°C which is usually favourable for fungal growth.

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Occurrence of VA-mycorrhizal associations with fruit and ornamental plants

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The importance of VA-mycorrhizal associations to agricultural and forest plants have been well documented. However, meagre information is available about the occurrence of VA-mycorrhizal associations with fruit plants in general and temperate fruits in particular. Similarly, VAM fungal associations in ornamental plants too, have received very little attention. Mycorrhizae are important in maximizing ornamental and fruit plant's productivity. The understanding of mycorrhizal associations is necessary for wise management of tnese plants. A preliminary report on the distribution of VA-mycorrhizae in some fields of the country highlights the necessity and importance of such survey (Phillips and Hayman, 1970).

Keeping in view, the information regarding the distribution and occurrence of VA-mycorrhizal associations with ornamental and temperate fruit plants grown in Himachal Pradesh–the horticulture state of the country, it will be of immense help to the state, to improve its production and quality of fruits and flowers. A part of our investigations is reported in the present communication, which includes the occurrence of VAM fungal associations in some temperate fruit and ornamental plants.

During June, 1989 roots of temperate fruits (Apple, Peach, Wild apricot and Kainth) and ornamental plants (Chrysanthemum, Sylvia, Zinnia, Balsam and Marigold) were collected from different nurseries of the university. Roots were collected from five plants of each species. Samples of thin lateral roots were excised from the main laterals of each plant and stored in sealed container until analysis.

To assess mycorrhizal fungal colonization, 50 fine root segments, measuring 1 cm in length excised from the lateral root specimens were washed, cleaned and differentially stained following the procedure of Philips and Hayman (1970) with some modifications. The per cent mycorrhizal colonization was determined by using the systematic slide method (Hayman, 1970).

Four fruit and 5 ornamental (flowering) plants were surveyed and observed for VA-mycorrhizal associations from Solan. All the fruit as well as ornamental

*Department of Fruit Culture and Orchard Management plant's species were VA-mycorrhizal, with vesicles or arbuscules and intracellular hyphae in the root cortical cells. The per cent colonization varied with the plant species. The VA-mycorrhizal colonization in the fruit plants ranged from 8.51–18.05%, highest being in *Prunus armeniaca* and lowest in *Pyrus pashia*. Vesicle formation was noticed in all the four fruit plants the number of which ranged from 0.09 to 2.24 cm^{-1} of root tissue. The highest number of vesicles were registered in *Prunus armeniaca*. The mycorrhizal colonization in ornamental plants ranged from 5.72 to 16.46%. Highest mycorrhizal colonization was recorded in *Chrysanthemum marifalium* whereas lowest in the *Zinnia elegans*. No arbuscules and vesicles formation was noticed in *Zinnia elegans* and *Impatiens balsamina*.

The per cent mycorrhizal colonization was related to the number of vesicles formed in root tissues. Vesicles being a reproductive structure, in addition to carbohydrate containing organ, it could have helped in multiplication of VA-mycorrhizal and subsequently in increased colonization. As the natural mycorrhizal colonization in both fruit as well as ornamental plants was found to be poor, there is a immense need to exploit the mycorrhizal associations with these plants by using artificial inoculum, which may prove significantly beneficial in better management of these crops.

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Growth and nutrient uptake of *Eucalyptus camaldulensis* and *Pinus caribaea* var. *hondurensis* seedlings grown on old tin-mined soils after *Pisolithus tinctorius* ectomycorrhizal inoculation and manure fertilization

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After 6 months of trial by using *Pisolithus tinctorius* (Pers.) Coker and Couch ectomycorrhizal inoculation and manure fertilization with *Eucalyptus camaldulensis* Dehn. and *Pinus caribaea* More. var. *hondurensis* Barr. and Golf. seedlings grown on tin-mined soils contained in polythene bags in nursery at Central Forest Research Laboratory and Training Center, Royal Forest Department, Bangkok, Thailand during February to September, 1986 was experimented by a split-split plot in completely randomized design comprising uninoculated ectomycorrhizal and non-manure fertilized treatments as control.

Results showed that plant height, growth (diameter at root collar) and total biomass (stem, foliage and root) of test seedlings were significantly increased than uninoculated ectomycorrhizal and non-manure fertilized treatments. Growth performance of *E. camaldulensis* was closely prominent in correlation with manure fertilization, followed by ectomycorrhizal inoculation and manure fertilization plus ectomycorrhizal inoculation, respectively. The growth response of *P. caribaea* var. *hondurensis* was also obviously correlated with manure fertilization plus ectomycorrhizal inoculation and merely ectomycorrhizal inoculation, respectively. Nutrient uptake in shoot (foliage plus stem) of *E. camaldulensis* seedlings was exhibited at a larger amount than those of control treatment by Mg (40.8%), Ca (32.1%), P (9.7%), N (3.5%) and K (1.8%), respectively; whereas *P. caribaea* var. *hondurensis* was indicated by Ca (35.7%), Mg (26.5%), K (21.4%), P (13.3%) and N (1.3%), respectively. Distinctinctively more consumption of nutrients in *P. caribaea* var. *hondurensis* seedlings after 6 months of experiment seemed to be ascertained than those of *E. camaldulensis* seedlings.

This finding suggests that ectomycorrhizal inoculation and manure fertilization promote vigour, durability and survival of prepared seedlings before transplanting to reforest in the old tin-mined areas. Remarkedly, manure fertilization in cooperation with *P. tinctorius* ectomycorrhizal inoculation to seedlings should be applied with care, because manure actually affected the increase of soil pH and stunted the growth of *P. tinclorius* ectomycorrhizal fungus. It is also evident that *P. tinctorins* revealed more symbiotic association with *P. caribaea* var. *hondurensis* than *E. camaldulensis.*

Photoassimilate partitioning and translocation in mycorrhizal sorghum

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Vesicular-arbuscular mycorrhizal (VAM) fungi derive carbon compounds from their host plants and use them as source of energy. This affects host carbon metabolism. Increased rate of CO_2 assimilation and higher amount of carbon translocation from shoot to root of mycorrhizal plants are some of the reported adaptations by which plants respond to the increased demand for carbon by the mycosymbiont (Harold, 1980). Increase in rate of CO_2 assimilation in mycorrhizal plants has mainly been ascribed to increased assimilatory area associated with lowering of dry matter in leaves, rather than to higher rates of photosynthesis (Pang and Paul, 1980). Such a differential partitioning of assimilated carbon to plant parts is, however controversial (Harris *et al.*, 1985). In the present study translocation and partitioning of assimilated carbon was followed using radioactive carbon dioxide in mycorrhizal and non-mycorrizal sorghum plants.

Mycorrhizal and non mycorrhizal plants of sorghum were grown in pots for six weeks. Non-mycorrhizal plants received phosphorus to achieve P nutrient status similar to that of mycorrhizal plants. The shoot dry weight, root : shoot ratio and shoot phosphorus concentration were similar, while leaf area, specific leaf area and leaf area ratio were 33, 39 and 40 per cent higher in mycorrhizal than non-mycorrhizal plants, respectively. These plants were used for labelling with $^{14}CO_2$. The ^{14}C assimilates were transported from labelled leaf to other leaves, stem and roots of both mycorrhizal and non-mycorrhizal plants within 24 hr of chase period. In labelled leaf, only 37 and 43 per cent of the total activity was retained in mycorrhizal and non-mycorrhizal plants. There were no differences in the amounts of activity present in stem and leaves of both types of plants. However, roots infected with *Glomus versiforme* contained 20 per cent as compared with that of 14 per cent present in the roots of non-mycorrhizal sorghum plants.

The ethanol soluble fraction contained 62 and 46 per cent of the total activity present in the leaves of mycorrhizal and non mycorrhizal plants. Acid digested fraction of labelled leaf from mycorrhizal plants showed 34 per cent compared with that of 49 per cent of activity in non-mycorrhizal plants. In the lipid fraction, there was no much variation between mycorrhizal and non-mycorrhizal plants.

In the roots, the amount of activity present in ethanol soluble fraction was 51 per cent and 62 per cent in mycorrhizal and non mycorrhizal plants. Acid digested fraction contained 46 and 36 per cent of the total activity present in mycorrhizal and non-mycorrhizal plants.

The distribution of ^{14}C-assimilated carbon to plant parts indicates that about 6 per cent more of the total carbon was translocated from shoot to root of mycorrhizal than non-mycorrhizal plants within 24 hr. of the chase period. This additional amount of carbon was supplied by the labelled leaf of mycorrhizal plants without affecting the amount of assimilates being distributed to other tissues. This observation substantiate that higher amounts of photosynthates are translocated from shoot to root of mycorrhizal than non-mycorrhizal plants without decreasing the leaf area (Pang and Paul, 1980). The specific leaf area, therefore showed an increase of 40 per cent.

Development of a carbon sink in the plant normally influence upon partitioning and distribution of assimilated carbon. Our results also indicates that establishment of VAM in sorghum altered the distribution of C assimilates into ethanol soluble and acid digested fraction of leaves and roots. Higher amount of the activity recovered from the ethanol fraction of the labelled leaf suggested that carbon in greater amount is being transfered from shoot to root of mycorrhizal plants.

The variations observed in partitioning of assimilated carbon into different chemical fractions of roots may be attributed to the altered metabolic activities of mycorrhizal than non-mycorrhizal plants (Kucey and Paul, 1982).

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Sporulation in *Glomus mosseae* under *in vitro* conditions

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Fungi which form vesicular arbuscular mycorrhizae (VAM) have not been cultivated *in vitro* and so it is difficult to produce large quantity of fungal biomass for biochemical and genetical analysis. The collection of extramatrical mycelium/vesicles/spores is time consuming and cause physical damage to the fungus. These difficulties have restricted the progress of fundamental research with these fungi.

There have been numerous studies on the axenic growth of VAM fungi in medium. Hepper (1983) showed the development of hyphae from germinating resting spores of *Glomus caledonius* which were viable even after detachment from their parent spore. Louis and Lim (1988) showed the formation of extramatrical vesicles in *Glomus clarum*. Recently Burggraff and Beringer (1989) showed the formation of secondary spores in *in vitro* culturing of *Glomus caledonius* which when subcultured yielded immatured spores.

One of the essentials of axenic propagation of VAM endophyte is good spore germination followed by proliferation of mycelium and sporulation. The present investigation has examined the requirements for axenic spore germination in *Glomus mosseae*.

Spores of *Glomus mosseae* (Nicol and Gerd.) Gerd. and Trappe were isolated by wet sieving and decanting method (Gerdemann and Nicolson, 1963). The spores were then purified from root pieces and debris by sucrose gradient centrifugation by the method of Jenkins (1984) modified as follows : The spore suspension was layered onto a 5M sucrose solution and centrifuged at 400 'g' for 3 minutes at 4°C in a swing bucket rotor. The concentrated spores (with less amount of debris) formed a layer in the upper half of the gradient. These spores were subjected to sucrose density gradient centrifugation four times for getting purified spores free from debris. The concentrated spores were then washed repeatedly with water to remove sucrose. These purified spores were surface sterilized with sodium hypochlorite (2.5% V/V) for 30 minutes and washed repeatedly with sterile distilled water to remove traces of sodium hypochlorite. These spores were then used for germination studies.

Germination of *G. mosseae* observed with different combinations of agar, sucrose and soil extract showed very marginal differences. The addition of root extract either from maize or mung bean, soil extract or sucrose (2%W/V) respectively did not enhance hyphal proliferation and spore formation as compared to agar (0.8% Himedia W/V) alone. Thus in all further experiments agar medium was used.

The effect of light, moisture and aeration was studied on spore germination. The spores incubated in dark, germinated and showed hyphal elongation while in the presence of light they failed to do so. To study the effect of moisture and aeration, the Petri dishes were wrapped with nescofilm just after inoculation. In another set nescofilm was not wrapped. It was observed that the application of nescofilm resulted in extended hyphal proliferation (up to three weeks) whereas the proliferation of hyphae in Petri plates without nescofilm stopped after two weeks. During this experiment it was also observed that freshly prepared Petri plates showed improved spore germination and proliferation of hyphal as compared to plates which were more than three day old at room temperature. Thus in all further experiments freshly prepared Petri dishes were incubated after inoculation in dark and wrapped with nescofilm.

The spores when incubated on the simple water agar medium formed germ tube after 48 hrs. These hyphae emerged either from the hyphal attachment point or directly through the spore wall. Proliferation af these hyphae was observed and after 6 days of incubation small globular structures were visualized. These were single walled hyaline in appearance and after another 10–12 days double walled dark globular structures were observed. Hyphal proliferation was observed only up to three weeks after spore germination. These secondary spores did not germinate even after 6 weeks of incubation. The secondary spores so obtained were transferred to fresh medium but no germination was observed. This clearly indicated that environmental conditions were not a limiting factor for spore germination.

The secondary spores were tested for their viability and infectivity. Positive infectivity was observed for these spores thus indicating their infective capability.

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Enhanced phosphatase activity in mycorrhizal papaya (*Carica papaya* cv. Coorg Honey Dew) roots

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The presence of active phosphatases on the surface of beech mycorrhizas catalyzing hydrolysis of complex phosphates have been demonstrated by Bartlett and Lewis (1973). Capaccio and Callow (1982) studied the specific phosphatases of mycorrhizal onion roots responsible for the synthesis and degradation of polyphosphates. Krishna *et al.* (1983) further demonstrated the correlation between phosphate uptake and non-specific phosphatase activity in roots of mycorrhizal *Arachis hypogea* L. Studies conducted in our laboratory revealed that papaya (*Carica papaya* cv. Coorg Honey Dew) responded excellently to VAM inoculation and phosphorus content in the leaves increased by 30-35% in inoculated plants compared to controls (Sukhada, 1988). Hence, it was thought worthwhile to study the activity of acid and alkaline phosphatases in papaya roots which might have been the potential agents causing phosphate increase in leaves.

Experiments were conducted with 3 month old papaya plants grown in pots (containing sand : soil mixture 1 : 1, pH 6.0, available P 63 ppm (Olson) and inoculated with cultures of *Glomus mosseae* and *G. fasciculatum*.

Root surface activity was tested by incubating five 1 mm unbranched root tips in P-nitrophenol phosphate (1.5 ml of 1 mg/ml solution) and 0.5 ml. 0.25 M sodium acetate buffer pH 6.0 (for acid phosphatase) or Tris-Hcl buffer (for alkaline phosphatase) and thereafter stopping the reaction with 5 ml of 0.1 N NaOH and recording absorbance at 410 nm. Similarly 0.2 ml enzyme extract (obtained by macerating the root tissue in 0.1 M phosphate buffer) and 1 g of root surrounding the root region of the plants were tested for acid and alkaline phosphatase activity which was expressed as n moles of P-nitrophenol released per gram fresh weight of tissue or soil per hour.

Results revealed that there was an enhanced activity of acid and alkaline phosphatase (ranging from 25 to 114%) on the root surface in the enzyme extract from the root and soil surrounding the root region of mycorrhizal plants over uninoculated plants. Acid phosphatase activity was considerably more than alkaline phosphatase. *G. mosseae* inoculated plants showed better activity than *G. fasciculatum* inoculated plants.

As there was a relation between the mycorrhizal infection, phosphorus content in the leaves and dry matter accumulation in the plant it is concluded that mycorrhizal infection helped in efficient utilization of phosphate by enhancing phosphatase activity in the roots which breaks down complex phosphates and increases availability of phosphates to the plant. Further work on specific phosphatases involved in the process is interesting to pursue.

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Characterization of mycorrhiza-specific alkaline phosphatase from french bean

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French bean (*Phaseolus vulgaris* L.) is one of the most important legume crops. In tarai region of Uttar Pradesh (India) it forms poor nodulation and requires very high nitrogen fertilization (Smith and Daft, 1977). Phosphorus is by far the most important nutrient, because it not only affects growth response of plants, but also influences VAM infection and development (Gianinazzi and Gianinazzi, 1978). Improved P nutrition in VAM associated plants results from an increased efficiency of P uptake from the soil (Sanders and Tinker, 1973).

The increased transfer of P from soil to the mycorrhizal plant appears to be due to a result of absorption, transport and release of available P by the symbiont to the host root rather that a specific stimulation if ion uptake by infected or uninfected host cells (Hayman, 1983). Relatively little is known concerning the mechanisms and Pathway of P metabolism within the external hyphae of the endophyte although its release in the host is an active process (Hayman, 1983).

Alkaline phosphatase (pH optimum 9-10) acts on phosphoric esters, with the liberation of inorganic phosphate. The available literature on the activity of alkaline phosphatase suggests that the enzyme is localized in the vacuoles of the mycorrhizal fungus. It appears with the proliferation of the hyphae, but becomes particularly intense in mature arbuscules and intercellular hyphae, polyphosphate like granules have been indentified within these vacuoles in *Glomus caledonicus* which are thought to be involved in phosphate transport mechanism (Hayman, 1983). The vacuole is regarded as an active system which plays an important role in the transport mechanism.

But little efforts have been made to examine the make up of alkaline phosphatase as influenced in plants inoculated with mycorrhizal spores.

Alkaline phosphatase was purified from NM and M treated plants on 45th day. The enzyme preparation from NM and mycorrhizal french beans was purified using sephadex gel chromatography and DEAE 52 to achieve 71,103 and 73,121 fold purification.

The purified preparations gave a single band on 5 to 10% gradient PACE. A single protein peak was observed by UV spectrophotometry with an absorption maxima at 275 nm. The Vmax and Km differed slightly for the two preparations but they had similar pH (8.5) and temperature (40°C) optima. The molecular weight of the alkaline phosphatase from non-mycorrhizal french beans was found to be 139,000 daltons. Whereas subunit molecular was 67,000. This enzyme preparation showed a wide substrate specificity as compared to the alkaline phoshatase purified from plants infected with VA mycorrhiza. Both the enzyme preparations showed end product inhibition towards pi and mycorrhizal association showed modification of alkaline phosphase.

Mycorrhizae are known to enhance the phosphate uptake by enhancing phosphatases activity. Phosphatases play an important role in the solubilization of phosphorus from non-available phosphorus sources. Alkaline phosphatase play an important role in catalyzing the hydrolysis of complex phosphate esters. Alkaline phosphatase is known to help in the solubilization of phosphorus sources and thus it can provide additional phosphorus for important metabolic processes which lead to improved production and yield.

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Nitrate reductase activity of vesicular-arbuscular mycorrhizal fungi

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Capability of reducing or utilizing the nitrate ions by several ectomycorrhizal fungi has already been reported by various workers (Plassard *et al.*, 1987). Endomycorrhizal fungal species like *Glomus macrocarpus* and *G. mosseae* have also been known to reduce nitrate ions (Ho and Trappe, 1975). However, so far there is no systematic report available on nitrate reducing ability of different members of endomycorrhiza. The present paper embodies the results on the nitrate reducing capacity of the spores of different vesicular-arbuscular mycorrhizal fungi.

Extramatrical chlamydospores of *Glomus fasciculatum*, *G. mosseae*, *G. intraradices*, *G. caledonicum*, *Gigaspora margareta*, *G. calospora*, *Endogone duscii* and *Acaulospora* sp. were collected from single species pot cultures maintained with *Cenchrus cilliaris* using wet sieving and decanting technique (Gerdemann and Nicolson, 1963). Ten samples of 200 spores each were surface sterilized with 1.0% chloramine T for 20 minutes and washed three times with sterile distilled water to get rid of the traces of sterilizing agent. Each sample was put in a Thunberg tube to which was added 3 ml of phosphate buffer (pH–7.0), 1.0 ml of 0.1 M succinic acid and a drop of 0.003% streptomycin in order to arrest the bacterial growth. Five samples received 1 ml of 0.1M KNO_3 each. The other five samples served as control which received 1.0ml sterilized distilled water instead of KNO_3. The samples were checked for bacterial contamination by plating them on nutrient agar medium and incubated at 30°C. No growth was noticed even after 3 days of incubation. The tubes were sealed under oxygen free conditions and incubated at 30°C for 24 hr. Nitrate reductase activity was assessed and was expressed as μ moles nitrate formed per sample.

The results revealed that all the vesicular-arbuscular mycorrhizal fungal spores exhibited the property of nitrate reducing ability and it varied from 1.5 μ moles to 3.8 μ moles/tube 24 hr. The maximum nitrate reductase activity was noticed with *Glomus caledonicum* and the lowest activity was recorded with *Acaulospora* sp. The other endomycorrhizal fungi recorded intermediate values. No activity was detected in any control treatment, indicating thereby that there was no contribution of nitrite to the solutions from the spores or extraneous sources. Occurrence of nitrogen fixing *Azospirilla* from the surface sterilized spore of VA-mycorrhizal has been reported (Tilak *et al.*, 1987). It is likely that these diazotrophs present inside the chlamydospores might have resulted in nitrate reduction.

The results in the present investigation suggest that with a capacity for reducing nitrate it is likely that the symbiotic effectiveness of the vesicular-arbuscular mycorrhizal fungi is enhanced in terms of nitrogen assimilation and translocation to the host plant. However, further physiological and biochemical studies on the interaction of host and endomycorrhizae should lead to a better understanding of the mechanism of nitrate reduction by vesicular-arbuscular mycorrhizal spores.

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The response of mycorrhizal maize plants to variations in water potentials

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Plant and water stress is considered a major limiting factor in crop productivity. Water stress can have profound metabolic influence on plant resulting not only in impaired gas exchange but also in considerable alteration of physiological processes (Hsiao, 1973). Vesicular Arbuscular mycorrhizal association in plants contributes towards the development of resistance to water stress. It may be attributed to imoroved phosphate nutrition or reduced stomatal resistance or its influence on the root/shoot hormonal balance (Nelson and Safir, 1982). However, the mechanism of resistance is not clear. Present study, therefore, was carried out to find out the response of maize inoculated with VAM fungus *Glomus caledonius* under various osmotic stresses.

Five surface sterilized Seeds of maize (*Zea mays* L. var. HS 123) were planted per pot filled with 2 kg of sand : Phosphorus deficient soil (3.5 ppm available P) 1 : 1 mixture. Potting mixture was autoclaved on three alternate days. Thinning was done to maintain three seedlings per pot. Parallel experiments were carried out with unsterile soil. An inoculum containing approximately 400 spores of *G. caledonius* (Nicol. and Gerd.) Gerdemann and Trappe, obtained from the Rothamsted Experimental Station, UK was used per pot. Plants were grown in a glass house with day temperature ranging 24 to 32 °C and were watered daily. Under sterile soil, five week old VAM plants exhibited 48% infection while under non-sterile soil it was about 38%.

Five week old non-VAM and VAM plants were exposed to osmotic potentials of 0, –2, –5 and –10 bar (Goyal and Gupta, 1985). After 8 hours of exposure, leaf water potential using Pressure Chamber Instrument (PSM Instrument Company, Oregon, USA) was measured. They were about –3, –5, –8 and –12 bar under varying osmotic potentials in the case of both non-VAM and VAM plants. Net photosynthesis ($^{14}CO_2$ dpm/cm^2) was found to increase insignificantly at 0, –2 and –5 osmotic potentials. But at very low osmotic potential (–10 bar), there was a significant increase in the assimilation of $^{14}CO_2$ by the VAM plants (10.36%). On lowering the the water potentials, the rate of decrease in the assimilation of CO_2 in VAM plants was very little as compared to the rate of non-VAM plants. Among the VAM plants and non-VAM plants, the difference in total chlorophyll content was nonsignificant at all low osmotic potentials.

The ability and efficiency of VAM plants to survive under drought stress was studied. Stomata begin to close earlier if leaf water potential reaches the threshold level for closure (Allen and Boosalis, 1983). The result of present study showed that there was no change in the leaf water potentials suggesting the mycorrhizal influence on stomatal regulation. Net assimilation rate was increased significantly only at low water potential. No change had been observed in the levels of total chlorophyll content among them. A difference in the ability to adjust osmotically if present, could account for the difference between non-VAM and VAM plants in stomatal and photosynthetic behaviour. The subject of VAM associated physiological changes is to be emphasized as it is essential for proper assessment of the potential benefits of inoculating crops with VAM fnngi.

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Effect of soil degradation on soil microbes, symbionts and their activities

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Soil degradation is defined as any change in physical and chemical properties of soil, which reduces the productivity of site. Soil of the N.E. region has very poor nutritional status. Most of the nutrient capital of site is concentrated in the organic horizon, which takes years to accumulate and enrich the upper soil layers. Besides, soil organic matter also acts as rooting substrata and supports natural regeneration. It also provides energy source for microorganisms that contribute to release of nutrients. So, any change in this organic layer may lead to the formation of fragile ecosystem. In N.E. region of India, where slash burn agriculture is rhe most prevalent form of agriculture, it desrroys the organic layer and thus slows down the process of succession.

Soil is considered to be the most dynamic site of biological interactions. Microbial population and their activities in soil can be regulated by its physicochemical characters (Tiwari *et al.*, 1987). Climate and vegetation are other two parameters which can affect the population and activity of soil microbes (Mishra and Sharma, 1977). Wohlrab *et al.* (1963) demonstrated that there is a shift in the species composition of soil microbes which parallels pioneer vegetational succession. There are, however, few studies on effect of disturbance on soil micro-fungal population (Donald and Whittingham, 1978) but the effect of same on microbial activities is yet to be studied. This study aims to analyse the changes in the microbial population and their activities due to soil degradation.

For this study two forest stands, showing different stages of disturbances were selected at Byrnihat (100m MSL, latitude, 26°00″ N, longitude 91°50″) about 80km from Shillong. Soil was collected randomly from five different places from a depth of 0–10cm from each site separately. Soil fungi and bacteria were isolated by dilution plate method where as for the isolation of endomycorrhizal spores Gerdemann and Nicolson's (1963) wet seiving and decanting method was followed. The intensity of mycorrhizal infection was assessed by the morphometric technique. Dehydrogenase activity was measured by TTC reduction technique of Casida (1977).

The soil fungal population showed an almost similar seasonal trend in both the forest stands. However, the less degraded site harboured more population than more degraded one. The peak was observed in May in both the cases. In case of bacteria, two peaks i. e. in May and September were recorded. The minimum bacterial population was observed in July. The less degraded forest stand had more bacterial population than its more degraded counterparts. Altogether 25 fungal species were isolated. In general, almost all the fungal species were isolated from both the forest stands except few species present only in less degraded site. *Penicillium chrysogenum* was dominant in both the cases.

The plant species inhabitating more degraded site were more mycotrophic than those on less degraded ones. No marked seasonality was observed in the intensity of infection. However, the less degraded site harboured more spores than more degraded ones. Maximum spores were counted during winter months while less during summer.

The dehydrogenase activity too was more in less degraded forest stand than in more degraded one. Maximum dehydrogenase activity was measured in May.

The marked seasonality in microbial may be attributed to soil organic matter content, moisture, temperature and pH (Tiwari *et al.*, 1987). Low microbial counts in July may be due to washing away of soil microflora. Maximum dehydrogenase activity in May may be attributed to higher bacterial number and increased moisture level (Baruah and Mishra, 1984). From the results it was evident that soil degradation had an adverse effect on microbial population and their activities. High microbial population in less degraded site may be attributed to high organic carbon added by high litter fall and high moisture content owing to closed canopy with trees and herbaceous species which have helped in conservation of soil moisture and less penetration of light to ground. Thus it might have also prevented the loss of organic matter. The extensive degraded site was dominated by the herbaceous weedy pioneer species with sparse canopy. The change in population and activities may also be due to change in resource quality of litter added by different species composition of sites. More mycotrophy in plants growing at more degraded site may be explained in terms of the fibrous root nature of the weedy species, low nutrient status of soil and availability of more light required for photosynthesis and increased root contacts. Low VAM count in more degraded site can be attributed to soil disturbance which reduced the mycorrhizal propagules (Reeves *et al.*, 1979).

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Effect of pH on the growth of ectomycorrhizal fungi in vitro

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Ectomycorrhizal association is important for the establishment and growth of pine seedlings. Several criteria have been used in selection of ectomycorrhizal fungi for their inoculation in tree nurseries (Trappe, 1977). Some experiments on pH for ectomycorrhizal fungi indicate that optimum growth of most of the fungi occurred between the pH range 4.5 to 5.5, but these can be affected by other factors like; duration of growth (Modess, 1941; Norkrans, 1950); nitrogen source (How, 1940) and salts (Norkrans, 1950).

Information on the behaviour of mycorrhizal fungi at different soil reactions is very less (Hung and Trappe, 1983). Therefore, the present experiment was carried out to assess the growth of ectomycorrhizal fungi in response to different pH.

The ectomycorrhizal fungi namely, *Laccaria laccata*, *Collybia radicata*, *Rhizopogon luteolus* and *Pisolithus tinctorius* were isolated from sporocarps and roots of *Pinus kesiya* in earlier studise (Sharma, 1981). These fungi were grown on modified Melin Norkran's (MMN) medium. Five levels of pH i.e. 3, 5, 6, 7 and 8 were maintained on MMN solid and liquid media with the help of .1N HCl and .1N NaOH solutions.

L. laccata showed maximum colony growth at pH-5, while its dry weight was maximum at pH-7. *C. radicata* and *R. luteolus* produced maximum growth at pH-6. *P. tinctorius* exhibited better colony spread at pH-7 and dry weight at pH-6. Colony growth of *L. laccata* and *G. radicata* was lowest in highly acidic condition (pH-3) whereas, *R. luteolus* was advearsely affected by a slight acidic condition (pH-5). No growth was obtained by *P. tinctorius* at pH-3. Production of dry weight by *L. laccata*, *R. luteolus* and *P. tinctorius* was less in highly acidic condition and by *C. radicata* in alkaline condition. Significant increased growth of *L. laccata* was observed at pH-5 but showed very poor growth in alkaline conditions compared to other mycorrhizal fungi. In case of *C. radicata* and *R. luteolus* maximum growth was obtained at pH-6 while, *P. tinctorius* showed better growth in alkaline condition.

Interspecific variation in growth was noticed in all the pH conditions. *R. luteolus* exhibited a broad tolerance range for pH, while *L. laccata* was with narrow ecological amplitude. *P. tinctorius* showed some affinity with alkaline condition.

It has been observed that most of the fungi grew better at pH range of 5-6. Melin (1924) and Modess (1941) had also advocated the acidophilic nature of ectomycorrhizal fungi. *P. tinctorius* grew well at higher pH. It indicated that the fungi was able to grow in alkaline condition. Bokar (1959) has reported growth of ectomycorrhizal fungi at pH 8.3. In contrast *R. luteolus* showed its growth in extreme acidic condition. The selective ion uptake and production of organic acids by the mycelium may account to their variability in growth at different pH (Hung and Trappe, 1983). The organic acids of fungi may help in increasing the uptake of phosphorus either through chelating the metals or increasing the phosphatase activity. However, growth of ectomycorrhizal fungi may also be assessed on the contents of nitrogen, phosphorus, temperature and soil moisture (Safir and Duniway, 1982). Therefore, specificity of the strain to different pH may depend on its physiological demand for the ions in medium.

Based on the study, it may be suggested that *R. luteolus* may be used as inoculum for pine under highly acidic condition, *L. laccata* and *C. radicata* in medium acidic condition and *P. tinctorius* in alkaline condition to get their maximum benefit.

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Phosphate response curve of *Leucaena* inoculated with *Gigaspora margarita*

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Vesicular-arbuscular (VA) mycorrhizal fungi improve plant growth mainly through increased uptake of phosphorus from soil. Plants lacking root hairs are more dependent on mycorrhizae. *Leucaena leucocephala* (Lam.) de wit. has virtually no root hairs and is strongly mycorrhizal (Munns and Mosse, 1980). Recently it was found that *Gigaspora margarita* is one of the efficient VA mycorrhizal fungus for inoculating *Leucaena* (Bagyaraj *et al*., 1989). Hall (1978) stressed that mycorrhizal inoculation experiments should be carried out using a series of phosphate fertilizer levels. It should then be possible to select the fertilizer level at which the responses to inoculation can be optimised (Menge *et al*., 1978). These kinds of experiments suggest the amount of P fertilizer that can be saved with mycorrhizal inoculation to produce the same amount of biomass. Therefore an experiment was carried out to determine the response of *Leucaena*, uninoculated and inoculated with *Gigaspora margarita* using super-phosphate and Mussorie rock phosphate at different levels (0, 5, 10, 15, 20, 30 and 40 kg per hactare). The plants were harvested 75 days after sowing.

The recommended level af phosphatic fertilizer for *Leucaena* is 20kg P/ha. The observations revealed that of the two sources of P, superphosphate is a better source producing higher shoot biomass compared to Mussorie rock phosphate. The results also showed that the shoot biomass production with the recommended level of P (when Mussorie rock phosphate was added as the source of P) was 7.22 g/plant. Nearly the same amount of shoot biomass was produced with half the recommended level of P when the plants were inoculated with *Gigaspora margarita*. When superphosphate was used as the source of P, the observations revealed that nearly 75% of recommended P can be saved through inoculation with *Gigaspora margarita*.

Nearly 50 to 75% of the phosphatic fertilizer application can be saved by inoculating *Leucaena* with the efficient VA mycorrhizal fungus, *Gigaspora margarita*.

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Response of different cultivars of sorghum (*Sorghum vulgare*) to inoculation with *Glomus versiforme*

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Recent advances in agricultural technology particularly with the development of high yielding nutrient-responsive varieties of crops, have opened new vistas in crop productivity leading to green revolution in India. However, the yield of cereals per unit area remain low and variable because of many constraints including inadequate fertilization. Among the plant nutrients, phosphorus is the essential nutrient next to nitrogen in increasing crop production. Biological agents like vesicular-arbuscular (VA) mycorrhizal fungi if improve the efficiency of phosphorus uptake by plants, the increase in crop production could be possible without application of high levels of phosphate fertilizer. The beneficial response of wheat, barley, onion, maize and other crops to inoculation with VAM fungi have been well documented (Fitter, 1985).

The selection of efficient VAM fungal species, which can help in enhancing the phosphorus supply and the choice of genotype which can get maximum benefits of the VAM association are the two vital factors to improve the existing VAM-plant association. The present study deals with the response of different genotypes of sorghum (*Sorghum vulgare* L.) to inoculation with *Glomus versiforme*.

A pot culture experiment was conducted using steam sterilized sandy-loam soil to evaluate the mycorrhizal dependency of five different sorghum genotypes viz. CSH-9, CSH-5, PC-9, PC-6 and PC-23. There were two treatments for each variety-un-incoulated control and inoculated with *Glomus versiforme* (Daniels and Trappe). The experiment was laidout in a complete randomized block design with six replications. Uniform basal dose of nitrogen was applied in all pots in the form of urea at the rate of 60 kg N ha^{-1}.

Inoculation with VAM fungus was done by the layering method (Jackson *et al.*, 1972). The inoculum (20 g pot^{-1}) consisting of infected root segments (50–70%) and chlamydospores (100-150 spores per 10 g soil) was placed at a depth of 3-5 cm in the pot. Sorghum seeds were sown just above the inoculum layer. The uninoculated control pots received the washings of inoculum soil sieved through 45μ filter to assure similar microbial population in all treatments.

The plants were cut at 60 days of growth just 2-3 cm above the base and allowed to exude for 3 min. The exudate was collected with a syringe in aid washed tubes which were kept in an icebath. The phosphorus in the xylem sap was determined using ascorbic acid as reducing agent (Chen *et al.*, 1956).

The mycorrhizal efficacy was calculated using the following formula :

$$\text{Mycorrhizal efficacy} : 100\left(1 - \frac{\text{Non-mycorrhizal plant weight}}{\text{Mycorrhizal plant weight}}\right)$$

Response of different genotypes of sorghum to inoculation with *G. veriforme* was highly variable. The interaction between the endosymbiont and plant cultivars was significant. Among the 5 genotypes, the growth promoting ability of *G. versiforme*, measured in terms of mycorrhizal efficacy varied from 24-43 per cent. The maximum increase was observed in variety PC 23, followed by CSH-5, CSH-9, PC-9 and PC-6 varieties of sorghum.

Total phosphorus uptake by the mycorrhizal plants of different genotypes was significantly higher than the control plants. The phosphorus uptake by plants due to inoculation with *G. versiforme* among the various cultivars varied from 45-136 per cent, the maximum increase being observed in cultivar PC-9, although this cultivar did not register maximum mycorrhizal efficiency. Mycorrhizal infection has been reported to alter biochemical and physiological activity of plants (Carling aod Brown, 1980) and these processes are more likely to be controlled by the host. Thus mycorrhizal efficiency in terms of phosphorus uptake and its utilization may be under the influence of host genome. The results are in agreement with the observations made in wheat (Azcon and Ocampo, 1980) and pearlmillet (Krishna *et al.*, 1985).

Total phosphorus concentration in the xylem sap of mycorrhizal plants was usually higher than the non-mycorrhizal plants. However, in the case of PC-23, the concentration of P in the xylem sap of both mycorrhizal and non-mycorrhizal plants was similar. Inoculation with *G. versiforme* brought in significant increase in the concentration of inorganic phosphorus of xylem sap collected from varieties CSH-9, CSH-5 and PC-9. However, similar increase was noticed in PC-6 and PC-23 varieties. The organic P concentrations in xylem sap of different cultivars was not significantly affected due to mycorrhizal inoculation in case of FC-9.

Growth and phosphorus uptake of sorghum (*Sorghum bicolor*) on steam-sterilized, phosphorus deficient soil was improved by the soil inoculation with VAM fungus, *Glomus versiforme*. Among the 5 sorghum genotypes viz. CSH-9, CSH-5, PC-9, PC-6 and PC-23, tested for their mycorrhizal dependency, PC-23 produced maximum mycorrhizal efficiency and phosphorus uptake with *G. versiforme*.

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Influence of vesicular arbuscular mycorrhizae on the photo synthesis and photo respiration of sweet potato (*Ipomoea batatas*)

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Role of VAM on the photosynthesis and photo respiration in sweet potato was studied under pot culture condition. The infected cuttings of sweet potato (Kanjangad) were maintained under three phosphorus regimes. Under low and medium phosphorus levels growth of mycorrhizal plants were higher than that of non-mycorrhizal. The rate of CO_2 fixed by the mycorrhizal plant was to the tune of 22.27 ppm CO_2. $Sqm.^{-1}$ $Sec.^{-1}$ which was 33.3% increase over control at half recommended dose of phosphorus and was significantly higher than that of non-mycorrhizal and mycorrhizal plants receiving higher phosphorus level. The study showed that root to tuber ratio was narrow when compared to non-mycorrhizal and mycorrhizal plants receiving higher dose of phosphorus. There was an increase in leaf area and dry matter to the tune of 6% and 17.5% respectively in mycorrhizal plants. High rate of CO_2 fixation in the leaves of mycorrhizal plants might be an inherent mechanism of the host plant to compensate the carbon drain from the plant to mycorrhiza in the initial stages and ultimately resulting into the changed root to tuber ratio, in favour of plant.

Effect of foliar application of urea on nitrogen metabolism of mycorrhizal moong plants under varying phosphorus levels

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Supplementary N provided by foliar spray has been shown to be rapidly absorbed and assimilated by leaves resulting in increased growth of seedlings and high seed protein of mature barley plants (Turley and Ching, 1986 . The present investigations were carried out to study the effect of foliar sprayed urea during pod filling on nitrate reductase and glutamine synthetase activities and content of urea, ammonia, amino acids, soluble proteins, sugars and chlorophyll in leaves of mycorrhizal and non-mycorrhizal moong plants grown under different P levels in a pot experiment.

Moong (*Vigna radiata* L. cv. ML-131) seeds were sown in the pots each containing 5 kg sterilized soil. The P treatments in the absence of mycorrhizal inoculation were : Control - P_0, 10μg P g^{-1} soil - P_{10}, 20μg P g^{-1} soil - P_{20} and MP_0, MP_{10}, MP_{20} in the presence of mycorrhizal inoculation. Potassium dihydrogen phosphate was used as the P source. All the pots received 10 μg KNO_3–N g^{-1} soil at the sowing time along with *Rhizobium* (strain MA 7) inoculation to seeds. For mycorrhizal inoculation, the spore suspension prepared from the soil containing 275–300 spores of *Glomus fasciculatum* per 100 g, was mixed 5 cm below the soil surface. Four plants per pot were maintained. Sixty three days after sowing, the leaves were sprayed with 100 μg ml^{-1} urea-N in 0.1% Triton X–100. The plants which received only 0.1% Triton X–100 spray, served as control. The plants were harvested at 4 DAA for the analysis of various biochemical parameters in fully expanded young leaves.

In vivo NR activity and GS activity were assayed as described earlier (Sekhon *et al.*, 1987). In the enzyme extract prepared for GS, soluble proteins were estimated by the method of Lowry *et al.* (1951). The proteins in the above extract were precipitated using 20% TCA. After neutralization with NaOH, in this extract sugars, total amino acids, ammonia and urea concentrations were estimated (Dubois *et al.*, 1956; Lee and Takahashi, 1966; Watt and Crisp, 1954; yuen and Pollard, 1982). Leaf chlorophyll was extracted with 96% (v/v) ethanol and determined (Johnston *et al.*, 1984). The data represent the mean of triplicate analysis.

From the studies it can be concluded that the increase at P_0 and P_{10} levels in leaf nitrate reductase, glutamine synthetase activities and ammonia concentration in mycorrhizal plants is equivalent to P_{20} level in non-mycorrhizal plants.

The increased glutamine synthetase activity and total amino acid concentration suggest that foliar applied urea gets assimilated into the amino acids.

Mycorrhizal plants had lesser concentration of sugars but more chlorophyll compared to nonmycorrhizal plants, due the foliar urea spray.

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Biological interactions between VA mycorrhizal fungi and other beneficial soil organisms

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Of the various microorganisms colonizing the rhizosphere, vesicular arbuscular mycorrhizal (VAM) fungi occupy an unique ecological position as they are partly inside the host and party outside the host. The present position of VAM research pointed out that more investigations are needed on the biological interactions between VAM fungi and beneficial soil organisms, and their effects on plant growth (Linderman, 1988). In this paper the different aspects of biological interactions between VAM fungi and other beneficial soil organisms are considered.

Most of the studies on VAM-*Rhizobium* interaction suggest that colonization with efficient endophytes significantly improve P nutrition and consequently nodulation and nitrogen fixation (Hayman, 1986). While the principal effect of mycorrhiza on nodulation is undoubtedly phosphate mediated, mycorrhiza may have other secondary effects. Such potentially limiting factors may include supply of photosynthate, trace elements and plant hormones. Recent field studies have shown great advantages of dual inoculation by the two symbionts. The method of application of inoculum would also pose a problem as most of the grain and forage legumes are directly sown in the field. Perhaps an immediate application could be in forestry as some leguminous tree species like *Acacia*, *Robinia*, *Leucaena* and many others could be preinoculated with selected rhizobia and mycorrhiza in the nursery to produce nodulated mycorrhizal seedlings before planting out in the field during the afforestation programmes.

Bagyaraj and Menge (1978) studied the interaction between *Azotobacter chroococcum* and the VAM fungus *Glomus fasciculatum* in tomato and found a synergistic effect on plant growth. Mycorrhizal colonization increased the *A. chroococcum* population in the rhizosphere which was maintained at a high level for a longer time and *A. chroococcum* enhanced colonization and spore production by the mycorrhizal fungus. Similar interactions have also been observed between other free living nitrogen fixers and VAM by other workers. Sometimes the beneficial effect on plant growth from free living nitrogen fixing organisms was attributed to hormone production rather than, or in addition to, nitrogen fixation.

Many soil microorganisms solubilize unavailable forms of P and these bacteria, called 'phosphobacteria' have been used as 'bacterial fertilizers'. Interaction studies showed that phosphate solubilizing bacteria survived for a longer period in the rhizosphere of mycorrhizal roots (Linderman, 1988). The phosphate solubilizing bacteria rendered more P soluble, while mycorrhiza enhanced P uptake thus with combined inoculation there was a synergistic effect on P supply and dry matter production. Phosphate solubilizing bacteria also produce hormones and vitamins.

Krishna *et al.* (1982) studied the interaction between the VAM fungus *G. fasciculatum* and the actinomycete *Streptomyces cinnamomeus* introduced into the rhizosphere of finger millet. Simultaneous inoculation with both the organisms had an antagonistic effect on each other each suppressing the growth and multiplication of the other in the rhizosphere. Interaction between the actinomycete *Frankia* and VA mycorrhiza was found to be synergistic with consequential benefit on plant growth (Gardner, 1986).

Certain 'companion fungi' live in close association with VAM fungi. In a field trial with pasture in a low P soil, one isolate of the companion fungus gave a 3-fold increase in dry matter, equivalent to an application of 250 kg/ha superphosphate. These observations question whether the improved growth obtained by inoculating plants with roots and soil from a pot culture of VA endophytes is due to VAM fungus alone or a cumulative effect of the mycorrhizal fungus and the companion fungus.

Epiphytic associations of an *Azotobacter* sp. with spores of *G. fasciculatum* have been observed. *Pseudomonas* sp. was found to be a common associate with VAM spores, and helped the mycorrhizal fungi in infecting the roots. The bacteria associated with different endophytes were even found to have a stimulatory effect on plant growth (Mosse, 1972).

It should not be forgotten that rhizosphere is a complex region in the soil-plant interface with high micorobial activity. However, the results obtained so far with biological interaction studies between VAM fungi and other beneficial soil organisms are encouraging and indicate the need for strengthening research in this area.

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Interactions of mycorrhizal fungi with root pathogen of cocoa

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This study evaluates the feasibility of using the mycorrhizal fungi as a biological control agent against one root pathogen of cocoa, *Ganoderma pseudoferreum* which causes the "Red root" disease. This disease, though not as destructive as the vascular streak dieback, is nevertheless rampant in most cocoa plantations of Malaysia.

Three-week old cocoa seedlings from the Sabah hybrid were subjected to the following treatments : (1) pre-inoculation with VAM fungi at age 3 weeks followed by inoculation with the pathogen at age 11 weeks, (+M......+P); (2) simultaneous inoculation with both VAM and pathogen at age 11 weeks (+M+P); and (3) inoculation with pathogen only at age 11 weeks, (+P); and (4) without any inoculation (−M−P).

The effect of the mycorrhizal fungi on host-pathogen relationship is an indirect one (Dehne, 1982). This is achieved by physiological alterarion of the host or by competition for space or host resou:ces (Schenck, 1981). Such an effect is evident from the present study, where preinoculation of the cocoa seedlings with the VAM fungi (+M......+P), significantly reduced the pathogenic fungal infection of the cocoa roots. Ten per cent of the roots of the +P seedlings succumbed to *Ganoderma* infection, while in the +M+P seedlings, infection was reduced to 5%. The −M−P plants did not show any infection.

Tissue analysis done on the seedlings from the various treatments, indicated significant difference in the levels of Ca, K and P. The concentrations of these elements, in decending order are as follows : (+M......+P) > (+M+P) > (−M−P) > (+P). Increase in P content of mycorrhizal roots had been shown to be responsible for reduced membrane permeability, with a resultant decrease in exudation out of roots (Graham, 1988). This simultaneously resulted in reduced soil borne diseases. Presence of high Ca^{+} ion content in mycorrhizal tissues seemed to inhibit the pectolytic activity of the pathogen (Marschner, 1986).

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Biotechnology for mass production of VA mycorrhiza inocula

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Biotechnology encompasses many facets of the management and manipulations of the biological systems. It embodies recent research in the field of Cell Biology. Recombinant DNA, Molecular Genetics and the related fields such as life science appears to be laying the groundwork for important technological development. The resulting technological take-off stage is being launched with all possible efforts and sincerity from Government of India in all the sphere of Science and Technolgy. These are likely to have a major impact on varieties of economic activities involving food, biochemistry, pharmaceuticals, biology, energy and environment. There are also likely to be significant industrial application through new products, new processes and methodologies. This type of direct participation in assessing the implication of 'new-biology' for it's programmes and priorities for taking-up biotechnological development programmes in the field of land management. Infusion of the management science with skill to manage the "LAND CARRYING CAPACITY" for food production and to sustain threat for environment and ecology. Thus in this regard 'biofertilizer' is getting importance as widely accepted low cost input in agriculture, agro-forestry, wasteland development and ecology. Scientists, Extension Workers and Planners are looking to this for exploitation for increasing crop production in agriculture. Scientists are unfolding the mysterious useful characteristics of microbes in the rhizosphere soil. Vesicular Arbuslar Mycorrhiza (VAM) is one to it's effect.

In India agriculture and agro-forestry constitute potential rosources in making rural economy suitable for development. In agriculture, continuous cropping practiced on land is resulting into depletion of major soil nutrients such as Nitrogen, Phosphorous and Potash. These major nutrients need to be replenished to avoid any adverse effect on plant growth and subsequently on yield of the crops.

Phosphate is one of the major nutrients required for plant growth. However, lack of phosphate in many tropical soils is one of the most serious constraint in plant growth.

Dr. B. Mosse in 1957 demonstrated for the first time the importance of Vesicular Arbuscular Mycorrhiza in phosphate uptake. It is also established fact that zinc, copper and sulphur uptake is also improved in the pesence of Vesicular Arbuscular Mycorrhiza. In addition to above, most important role of VAM is in increasing plant resistance to drought conditions, protection against soil borne diseases, improving plant establishment and positive effect on soil aggregation thus contributing to soil erosion.

Incorporation of these useful micro-organisms as biofertilizers to supply essential nutrients to cultivated crops especially under-intensive cropping conditions and unassured rainfall areas can help in successful sustainance type of agriculture primarily because they are low cost inputs. About 70 to 75% of the cultivated area in agriculture comes under rainfed conditicns, of this neary 50% is under unassured rainfall conditions. Crops such as legumes are grown mostly undor unassured rainfall conditions with limited agronomical practices and no organic nutrient supply. *Rhizobium* biofertilizers have specific impɔrtant role in increasing crop productivity in legume crops.

Deforestation and degradation of forest areas have seriously affected atmosphere and ecology. Due to this nearly 1200 crore tonnes of top soil is washed away every year having potential to produce 40 to 50 metric tons of food grains. As per recent surveys hardly 10 to 11% land has now remained under worthy forests. Trees on 1.5 M. hectares of area of land are being illegally cut down every year resulting into ecological imbalance.

Vesicular Arbuscular Mycorrhiza is specifically important for plantation crops of commercial value and free species important in agro-forestry, wasteland development programmes. Here usefulness of VA mycorrhiza is successful in plant establishment on topical soils which are deficient in phosphate and characterized by high inbuilt temperature due to low moiture, no irrigation and erosion problems.

Thus, owing to these prolems in increasing productivity in agriculture development of biofertilizers making use of the beneficial organisms such as *Rhizobium* and VA mycorrhiza is timely and just.

BAIF is a development research foundation engaged in socio-economic transformation of rural masses and laying emphasis on the development and application of appropriate teconologies for harnessing benefits through increased productivity. It's approach to rural development embodies efforts at improving the existing natural resource as available to the rural population and thereby creating means of gainful self-employment at the grassroot level. Among the various instruments which can come handy for such measures the use of beneficial micro-organisms in increasing productivity in agriculture stands out importantly.

By virtue of the experience of working in rural areas, BAIF has recognized the need for creating a perennial source of income to farm families. Low productivity in agriculture is due to the in-appropriate management to the natural resources such as land, water, vegetation and livestock available to the farmer. In view of this, BAIF has devised suitable models for optimum utilization of the land which is important among these resources.

Agro-forestry is one to its effect which promises to be a vital land-use alternative for our rural economy. As Agro-forestry ensures income accruing from both agricultural and forest commodities where the prices of the products enhanced by agro-forestry are gradually on the rise comparcd to the prices of agricultural commodities.

BAIF is operating agro-forestry and wasteland development programmes in rural areas. The comparative advantage of these programmes are for the marginal and rainfed land areas where productivity is low and cultivation is at the risk of the vagaries of the monsoon. As such these lands are deficient in the phosphate content, which is one of the major nutrients required in successful establishment and growth of the plant. Being marginally productive replenishment of the phosphate to the soil through chemical fertilizers is also not been done adequately as it is not an economically feasible proposition. Owing to these factors, the soils are causing serious constraints for plant growth.

However, it has been the experience at BAIF that on arid, shallow and unproductive soils plantation of agro-forestry tree species such as subabul (*Leucaena leucocephala*) can also be established successfully to produce fodder and fuel while in the process of carrying out soil amoelioration. In establishing such successful plantation a vital supporting role was provided by *Rhizobium* cultures. BAIF has taken-up a research programme on isolation, characterization and mass production of efficient strains of nitrogen fixing bacteria in relation to important multi-purpose tree species suitable for agro-forestry and wasteland development.

In view of the experience, BAIF believes that biofertilizer technology constitutes a complentary and supplementary source of nitrogen nutrient supply in the input-intensive agriculture. This technology benefits small and marginal farmers who stand in immediate need of the low cost input to increase productivity in grains, legumes, forages, forestry and horticultural crops. Accordingly, BAIF has been involved in largescale production and distribution of adequately standardized cultures of micro-organisms, such as 'Rhizobia' which constitutes biofertilizers suitable for selected leguminous crops. BAIF is now exploring mycorrhizal potentials which is an important and responsive part of environment for plant nutrition, growth improvement, successful afforestation and land reclamation programmes. However, lack of massive inoculum is a bottleneck in the technological transfer of knowledge to grass-root level. Hence, at this stage, the standardization of largescale production technology and distribution of viable inoculum at the field level in the tropical countries is timely and just.

BAIF has undertaken a project on Development and Standardization Production Technology of VA Mycorrhiza Inocula.

General objective of the project is to develop the mass production technology for VA Mycorrhiza and it's production methodology and delivery system.

VA Mycorrhiza inoculum, it's mass production and application in the field for successful establishment of agricultural crops and forest tree species is gaining much importance. Considering the need of this lowcost agricultural input in cultivated crops, agro-forestry and wasteland development programmes, the lack of massive inoculum is an obstacle coming in the way of largescale production of VA mycorrhizal fungi. The development of large quantities of pure inoculum free from pathogen and with high infective propagule numbers is situated at the cutting edge of the advances of knowledge on the use of biofertilizers. Achievement of goal of mass production of pathogen free endomycorrhizal inoculum will make the core of a network permitting transfer of technology to farmers, foresters, research and extension workers at the nursery and the farm level.

It is more likely that climatic and edaphic factor rather than host specificity will determine successful introduction of VAM fungal species into a given agricultural situation. In the selection of an efficient strain, problem in comparing performance of fungal strain lies in the lack of standardization of experimental inocula. Various parameters are used in comparing different VAM fungi which are as initial inoculum-concentration, spore viability, or contaminate micro-organisms in the pot cultures rather than true differences in the efficiency of mycorrhiza formation and function of individual fungi. In this regard, we have screened few mycorrhizal fungi obtained from different parts of Maharashtra for inoculating their host plant which have shown encouraging results. *Glomus* species have proved efficient in the experimental trials undertaken on onion and sunflower crops.

Mycorrhizal fungi are maintained and mass produced at pot cultures on suitable host plants, plant growth period of 2-3 months can give a large crop of mycorrhizal fungal spores to produce sizeable amount of inoculum. In the selection of the host plant important criteria followed are as under—

—Host plant species suitable to agro-climatic conditions of the area

—Host plants with thick root system for sizeable sporulation and infection

—Annual in growth habitat

—Adaptability to glasshouse conditions

—Crops important as staple food, cash crop, forage crops etc.

In the present studies considering the agro-climatic aspect, host plant species such as maize, sorghum, bajra, bahia grass, rhodes grass, anjan grass and varieties of guinea grass as forage crops are tried for root infection in the inoculum production. These hosts being annual, densely populated and producing thick root system are much suitable and can be easily grown under glasshouse condition. All the host plants have shown positive results in the sporulation and infection to the root system. However, percentage of root infection in bahia grass is considerably high (98.5%). Hence, bahia grass could be a possible host useful in largescale production of the inoculum.

Selection of substratum is important from the point of view of its suitability as material enhancing growth of the host plants in the mass production process and as a carrier in effective delivery of viable undiminished count of spores, sporocarps and infected root segment upto the user's end. However, certain criterias need to be followed in the selection of substratum in mass scale production of the inculum. We have considered following criteria in the selection of suitable substate.

—Substrate non-organic in nature to avoid deterimental effect on the inoculum

—Light weight to ensure economy in transportation cost

—Good water holding capacity

—Less leaching of essential nutrients

—Easy to be removed from roof surface

Accordingly, in cur present studies following substrates are used in different proportions with each other (by volumes)

—Soil : Sand (1:3)

—Soilrite : Sand (1:3)

—Vermiculite : Peat (4.3:1) by weight

—Perlite : Soilrite (1:1)

The host plants mentioned earlier are grown under laboratory conditions on the substrates using above combinations. In this study vermiculite and peat (4.3:1) and Perile and soilrite (1:1) have proved to be the best substrates considering growth of plant, sporulation, percentage infection and viability of the inoculum. Our results are comparable with the findings of the studies undertaken by Bagyaraj *et al.* at the University of Agricultural Sciences Bangalore on this aspect of selection of substrate.

The handling of the inoculum for mass production and evaluation under greenhouse conditions for it's potentials will depend on the form of inoculum selected and production methodology standardized accordingly. Literature describing inoculum required especially for largescale field use is rare however, most of the inoculations are carried out with inoculum prepared in the form of—

—Granular, structure where VAM inoculum is produced in polyprophylene basins containing plant roots, mycorrhizal spores and substrate

—Pellets containing mycorrhizal inoculum and sedimentary clay

We in our present studies have tried combinations of VA mycorrhizal spores, infected root segments and substrates of different kind such as sand, soil, soilrite, perlite, peat and vermiculite in different proportions by volume as mentioned above. Infected root segments of the host plant used to produce the inoculum are highly infective and can be preserved long under cold room (4°C) conditions and at pot culture, seedlings and field level to keep their infectivity. However, among the three types of VA mycorrhizal inoculum, namely pure fungal cultures, infected roots and infested soil/substrate, in the largescale production and delivery at field level, use of fungal culture will be difficult. Accordingly, production of large quantity of infected root segments of the host plants free of substrate can be effective providing more rapid growth stimulation than the spores.

Freeze dried cultures could be one of the possible avenues in the standardization of forms of inoculum. BAIF has standardized freeze drying technology in 30 ml. glass vial of cultures, which prolongs shelflife and economises transportation cost. Work at BAIF on freeze drying of biofertilizers incorporating requisite cryo-protectives have given encouraging results in preservation of viability of organisms for a long period. The freeze dried material has been used to inoculate field grown plants.

We have initiated work on lypholisation of infected root segments spray dried and packaged in autoclavable polypropylene bottles, adding sucrose and skimmed milk powder as cryoprotective. Freeze drying technology will also prove to be an effective methodology for the lypholised Inocula with micronutrient as reconstituted buffer application to locally available substrate and hence inoculation to the sub-soil in desired manner. Experiments are going and MPRC will be able to come out with this inoculum in near future after successful pot culture and field trials.

In the process of mass production of the inoculum candidate, mycorrhizal strain selected on the basis of its performance in glass and plastic containers and with due quality control measures is used for preparing secondary inoculum.

—These inoculums are prepared in plastic or concrete basins of size $0.5m^2 \times 25$ cm. deep which can be easily sterilized.

—Secondary inoculum is produced using candidate strain with suitable host plant and subtrate of non-organic nature. Several plants are seeded over a layer in the basins.

—The whole subtrate including spores and infected roots is used to prepare a composite inoculum after due quality control tests.

—Pilot scale mass production is being undertaken in the growth room or production unit which will provide necessary arrangements for plants growth under controlled conditions, these include—

—Light intensity of 15 Klux on the plants using fluorescent and incondescent lights

—Photometer to measure light intensity

—Filtered air to maintain aseptic conditions

—Maintenance of the humidity of the air not under 60% and not over 80% Temperature control to ± 10°C providing 25-28°C temperature for plant growth

—Autoclavable plastic containers for production of the inoculum, arranged on racks in production room

Considering magnitude of problem of low productivity in agriculture BAIF has devised suitable models for appropriate use of land in rural areas to generate gainful self-employment and increasing productivity in agriculture. Having developed an innovative technology of largescale production of *Rhizobium* biofertilizers, its effective system of packaging and field delivery, BAIF has now undertaken and gradually progressing wtth the objectives of this Project on Development and Standardization of Technology for Mass Production of VA Mycorrhiza Inocula.

Application of mass production of inocula biotechnology, its proper handling and distribution of viable inoculum at the farmer's level through networking of strong field force will support and give impetous to programme of agro-forestry, wasteland development and energy plantation in rural areas.

VAM biotechnology facets are to be sudied unravelled considering the aspects of the physiology, plant and soil interaction, phosphate mobilisation by this fungus etc. which will benefit research workers, planners, decision makers, extension workers, technology transfer, linkmen and ultimate farmers of the country for betterment of ecology and environment of earth.

Therefore mass production of VAM inocula at MPRC at BAIF, Pune is receiving and will continue to receive the feed back for effective application, economic benefits, practical problems and corrective measures in wider acceptance of this technology at grassroot level can use this information for making improvements at various stages of research and production of inocula.

Interaction of VA-mycorrhizae with beneficial soil microorganisms

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Root penetration by VAM fungi is affected by several factors of different origin. Among which soil bacteria appear to be involved (Mosse, 1981). Such an enhancement of VAM formation, by selected microorganisms inoculated from pure cultures has also been reviewed (Barea and Azcon-Aquilar, 1982).

Microbial interactions in the mycorrhizosphere of VA-mycorrhizae have been reviewed (Linderman, 1988). Interactions related to nutrient cycling have been described for nitrogen fixing bacteria, nitrifying bacteria, phosphate solubilizing bacteria and many other microorganisms.

Bagyaraj and Menge (1978) found that inoculation with *Azotobacter chroococcum* or P-solubilizing bacteria in addition to a VAM fungus resulted in a synergistic host response. They concluded that the production of phyto hormone or growth regulators by these microbes might have had a greater effect on plant growth than small increase in N or P availability. Brown and Carr (1984) also reported improved growth and yield of lettuce plants in both sterile and unsterile soils when they were simultaneously inoculated with VAM endophytes and *Azotobacter chroococcum*. For cereal roots infected with *Azospirillum* and a VAM fungus, both endophytes are present in the same cortical region of the root making it possible to have direct interactions between the three symbionts which could range from enhancement of growth to competition for photosynthates. Interactions between *Azospirillum* and VAM fungus relative to the growth response of sorghum have been examined (Pacovsky and Fuller, 1985). Synergistic effect of VAM and *Azospirillum brasilense* on the growth of barley and pearlmillet have also been reported (Subba Rao *et al.*, 1985). Interactions between *A. brasilense* and the mycorrhizal fungus, *Glomus mosseae* in relation to their effects on the growth and nutrition of C_3 and C_4 plants showed that although *Azospirillum* exhibited C_2H_4 reduction activity, no significant effect of inoculation on N concentration in plant was found. *Azospirillum* and N behaved similarly in enhancing the growth and nutrition of mycorrhizal maize plants (Barea *et al.*, 1983).

Response of a plant to colonization by mycorrhizae depends on many biotic and environmental factors. Plant available P is considered to influence the degree of relative growth inhibition or enhancement in mycorrhizal symbiosis (Bethlenfalvay *et al.*, 1982).

The study of *Azospirillum*-mycorrhiza-host interactions under various P levels should allow the *Azospirillum* or the VAM fungus to be highly competitive for photosynthate and hence influence the growth and nutrition of this tripartite association.

The response of legumes to inoculation with *Rhizobium* and mycorrhizae have been assessed in various pot and field trials (Subba Rao *et al.* 1986; Tilak, 1985). However, the inoculation with a correct fungal species is as important as the selection of *Rhizobium* species for better growth and development of these plants (Green *et al.*, 1983). By simultaneous inoculations with *Rhizobium* sp. and mycorrhiza, legumes can receive growth benefits because of improved P and N supplies. The dual symbiosis in legumes not only reduce the input of synthetic fertilizers, thereby saving energy but also appear to reduce the cost of the system itself in terms of photosynthate drain (Kucey and Paul, 1982).

Distinct responses of lentil to dual symbiosis on a P-deficient soil suggested its dependency on *Rhizobium* and *Glomus* for N_2 fixation, N and P nutrition and growth (Singh *et al.*, 1984). To derive maximum benefit from dual inoculation with *Rhizobium* and VAM, addition of small amounts of P is also required. Inoculation with *Rhizobium* and *Glomus fasciculatum* improved the nodulation, mycorrhizal colonization, dry weight, N and P content of *Leucaena* plants compared to single inoculation with either organisms (Manjunath *et al.*, 1984). There is, however, no direct interaction between the VAM fungus and the N_2 fixing bacteria as reported by Carling *et al.* (1978). They showed that nodulating soybean plants showed increase in total dry weight, nodule dry weight as well as in N_2-ase and NR activities over single or non-infected plants. But, when P was substituted for mycorrhizal infection, similar growth and enzyme activity increase were observed which suggested the absence of direct interaction between VAM and the N_2-fixing bacterium-*Rhizobium japonicum.*

Rapid colonization by VAM fungus would result in an an enhanced P status, but would lower the level of carbohydrate in the roots. *Bradyrhizobium* strains unable to store P but capable of stroring C and poly-B-hydroxybutyrate could have a competitive advantage under these conditions (Pacovsky *et al.*, 1986).

It, however, appears that dual inoculation with a suitable species of *Rhizobium* and mycorrhizal fungi not only enhances the nutrient content in the above ground plant material but also seems to provide a nutrient supply that is well balanced. Using labelled ammonium sulphate fertilizer, Subba Rao *et al.* (1986) reported that dual inoculation of *Rhizobium* sp. and *Glomus fasciculatum* brought significant increase in N_2 fixation in straw and grain of chickpea over that of the individual organism(s) in a sandy-loam alluvial soil.

Waidyanatha *et al.* (1979) reported increased N_2-ase activity in *Pueraria* sp. when the growth phosphate response curve became asymptotic and suggested that nodule formation may be preferentially stimulated by mycorrhizal infection, which make the phosphate directly available to plants. Because there is no contact between the mycorrhizal fungi and *Rhizobium* bacteroids, any P supply must pass through the host cells. The role played by the VAM specific phosphatases in the arbuscules developed inside root cells adjacent to nodules was suggested as being of great importance in phosphate transfer to bacteroids (Asimi *et al.*, 1980).

VA-mycorrhizal fungi are omnipresent and are being studied extensively throughout the globe. In spite of many unanswered questions concerning their use, researchers have an obligation, for socio-economic reasons to continue to explore means of utilizing these microorganisms. Mycorrhizae and nitrogen-fixers can, therefore, be regarded as alternative means for more rational agricultural programmes in economising the use of chemical nitrogenous and phosphatic fertilizers.

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Mycorrhiza induced resistance, a mechanism for management of crop diseases

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Plant Physiologists and Agronomists have exploited the mycorrhizal fungi to help plants to acquire mineral nutrients from the soil, especially immobile elements such as P, Zn and Cu and also more mobile ions such as S, Ca, K, Fe, Mg, Mn, Cl, Br and N. As these fungi alter the nutrition and development of host plants, the plants either become more resistant or more susceptible depending upon the host and pathogen. In fact better-developed mycorrhizal plant becomes more susceptible to pathogens than the poorly grown nonmycorrhizal one (Davis *et al.*, 1978). Several published reports indicate greater damage of mycorrhizal plants by soilborne fungi. In some cases host-parasite relationships lead to less disease incidence on mycorrhizal plants mostly because of increased vigour of the plants to tolerate the diseases (Graham and Menge, 1982). In such cases high inoculum levels of the pathogen decrease the efficacy of mycorrhizal fungi in reducing the disease.

Mycorrhizae can be exploited in another fascinating way also. Since mycorrhizal fungi also infect the roots, the principle of cross protection with avirulent or less virulent isolates can be utilized to manage the soil-borne diseases. The degree of success of induced resistance may depend upon the potential of the mycorrhizal fungus to induce resistance (Chakravarty and Unestan, 1987). Some of the mycorrhizal fungi do not infect the plants when abundant phosphorus is available in the soil and such fungi will be of no use in affording cross protection (Graham, 1988). Prior inoculation with mycorrhizal fungi adapted to infect plants in soils fertilized with phosphate-based fertilizers gave effective protection against nematode infection in tamarilla (Cooper and Grandison, 1987). Pea root rot caused by *Aphanomyces enteiches* was reduced when the plants were inoculated with *Glomus fasciculatum* two weeks prior to the inoculation of the pathogen. The induced resistance was systemic as when root systems were split into two halves, one with mycorrhiza and another without the mycorrhiza. The oospore production was reduced in both root systems (Rosendahl, 1985). *Glomus intrardices* controls crown and root rot caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* in tomato and phosphorus nutrition does not have any role in the disease incidence (Caron *et al.*, 1986).

Glomus mosseae protected tomato plants against *Erwinia carotovora* when inoculated simultaneously along with the pathogen (Duchesne *et al.*, 1988).

For effective management of crop diseases with mycorrhizal fungi, avirulent strain of mycorrhizal fungi may have to be selected. The successful mycorrhizal fungus which colonizes the roots in a 'susceptibte interaction manner' may not give much protection like incompatible interaction. Extensive screening should be taken up to identify effective strains to induce resistance. Such a success has been reported recently. Two fungi, *Glomus mosseae* and *Gigaspora margarita*, neither isolated from nor as nutritionally efficient as others on citrus, conferred resistance to *Phytophthora parasitica* in sweet orange. The efficient mycorrhizal fungi like G. *fasciculatus* strains which induced growth of the sweet orange plants did not control the disease effectively (Davis and Menge, 1981).

Most plant cells are capable of elaborating inhibitory substances during their quinones, metabolic response to pathogen attack. Phenols, quinones, phytoalexins and numerous other compounds have been found in tissues of a variety of plants during pathogenesis (Vidhyasekaran, 1988; 1989). Plant cells exposed to symbiotic parasitism have also been reported to respond by production of substances inhibitory to the fungal symbiont (Duchesne *et al.*, 1987). Accumulation of toxic phenols and phytoalexins in mycorrhizal roots has been reported (Krishna and Bagyaraj, 1986; Morandi *et al.*, 1984). The most efficient strains which may increase the synthesis of inhibitory substances in the roots should be selected for successful biological control of diseases using mycorrhizal fungi.

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Interaction Study of *Glomus mosseae* and *Rhizoctonia solani*

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Because of the interaction among microorganisms in soil, the effect of VAM fungi on soil-borne plant pathogens is inevitable (Schenek and Kellam, 1978). VA mycorrhizae may influence the microbial population of the rhizosphere and vice-versa (Menge *et al.*, 1978). Many authors have reported the interaction between VAM fungus and soil-borne plant pathogen. Most of the studies on the biological control with VA mycorrhizae do not clarify whether or not it is effect of nutritional mediated or direct interaction effect. The split-root method (Menge *et al.*, 1978) is suitable for the study of the interaction between VAM fungus and plant pathogen to differentiate nutritional mediated effect from direct interaction effect.

In the study, corn (*Zea mays* L.) var. IPB 1 was used to study the interaction between *Glomus mosseae* and *Rhizoctonia solani.*

A reduction in the root growth was observed in *R. solani* inoculation. *Rhizoctonia* inoculated corn had 29% less root dry weight compared with that of the uninoculated check. The plant inoculated with *G. mosseae* alone gave highest total dry root weight. When *R. solani* was inoculated on the mycorrhizal corn, either on the same root portion or separately, there was no significant reduction in root weight.

R. solani multiplied less in the mycorrhizal root than in the nonmycorrhizal root. The number of sclerotia recovered form soil with the mycorrhizal root was less and viability of sclerotia was reduced significantly. When *R. solani* was inoculated in the mycorrhizal root 35 days after *G. mosseae* inoculation, there was a reduction in root colonization by *G. mosseae*. The presence of *R. solani* in the same portion of the root significatly reduced the root colonization and chlamydospore production by *G. mosseae*. Besides total root weight reduction in the roots inoculated with *R. solani* was consistently less in the split root portion inoculated with the pathogen. The addition of phosphorus in separate root portion did not change the multiplication of *R. soloni.* Thus phosphorus factor only may not be the resistance mechanism of the plant inoculated with *G. mosseae.* There is clear indication of direct interaction between the two organisms. The result implies that the effectiveness of *G. mosseae* may depend on the population of *R. solani*. High inoculum dose may be needed to counteract the pathogen population. On the other hand *G. mosseae* may be useful in the management of *Rhizoctonia* diseases.

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Mechanism of resistance of mycorrhizal tomato against root-knot nematode

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Vesicular-arbuscular (VA) mycorrhizal plants have been shown to react to disease differently than non-mycorrhizal plants, however, the mechanisms for interaction are poorly understood (Dehne, 1982; Hussey and Roncadori, 1982). The antagonistic effects of the fungus on nematode may be either physical or physiological in nature. Based on these assumptions, three hypotheses have been propounded to understand mechanism of resistance of mycorrhizal plants. These include role of mycorrhiza in improving plant vigour and growth to off set yield loss, physiological alteration or reduction of root exudates responsible for chemotactic attraction of nematode and lastly, direct role of mycorrhiza in retarding the development and reproduction of nematode within root tissue. These mechanisms may operate singularly or in combination to make mycorrhizal plant resistant against invasion of the plant pathogens.

Attempts were made to determine if VA mycorrhiza, *Glomus fasiculatum* can bring any direct or indirect change in the nature of the Pusa Ruby tomato plants which turned it resistant to *Meloidogyne javanica*. First step towards these investigations was to know whether difference of attraction for nematodes, if any, existed between mycorrhizal and nonmycorrhizal roots or their exudates. Subsequently, penetration, development and reproduction of root-knot nematode was studied in mycorrhizal and nonmycorrhizal roots. Various biochemical changes including lignins, total phenolics, proteins, total sugars and reducing sugars were measured in the roots of mycorrhizal as well as nonmycorrhizal plants.

No correlation was observed between migration of nematodes and their penetration of roots, thereby, no direct role of the root exudates of *G. fasciculatum* infected plants in altering the attraction of root-knot nematode for roots could be established.

The nematode penetration was reduced significantly in pre-inoculated mycorrhizal roots. Moreover, gall, a manifestation of feeding site of *M. javanica*, developed less on mycorrhizal roots. Also their size was smaller on such roots. The smaller size of galls may be the outcome of delayed development of the nematode in mycorrhizal plants.

Reproduction of nematode or recovery of second stage juveniles from soil supporting mycorrhizal plants was significantly less than soil containing nonmycorrhizal plants. This difference could be justified on the basis of higher counts of females without eggs or less number of eggs per egg-sac in mycorrhizal roots.

Lignins and phenols were found significantly more in the mycorrhizal roots. Both the chemicals are known for their role in host resistance (Bhatia *et al.*, 1972; Krishna and Bagyaraj, 1984) especially in influencing the penetration of roots by the nematode (Dehne and Schonbeck, 1979). Contrary to it, proteins and sugars were less in endophyte inoculated roots.

The increased resistance of mycorrhizal roots to nematodes can be elicited by specific alteration in the physiology of the host plants due to microbial metabolism of the endophyte (Dehne, 1982) and its presence in the plant roots. Pre-occupation of roots by *G. fasciculatum* coupled with subsequent biochemical changes appear to play a vital role influencing the various events of infection process of *M. javanica*, thereby, making Pusa Ruby tomatoes resistant to root-knot nematode.

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Antagonism of ectomycorrhizal fungi to some common root pathogens

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A possible beneficial role of ectomycorrhizae in the tree growth and development is that the mycorrhizal fungus protects unsuberized roots from attack by parasitic fungi. Zak (1964) postulated that mycorrhizal fungi may conceivably afford protection to the root by i) Utilizing root carbohydrates and other chemicals which would be attractive to pathogens, ii) Providing a physical barrier to pathogens in the form of fungus mantle, iii) Secreting antibiotics which inhibit or kill the pathogens and iv) Supporting a protective rhizosphere population of other microorganisms.

In common with many soil fungi, some mycorrhizal symbionts are capable of antibiotic action. Several workers have reported the production of antibacterial and antifungal compounds by mycorrhizal fungi in pure culture (Marx, 1971; Pratt, 1971).

In the present study the antagonistic effect of some of the ectomycorrhizal fungi occuring in *Pinus patula* and eucalypt plantations in the Nilgiri Hills in Southern India against some common root pathogens was studied. *Amantia muscaria*, *Laccaria laccata*, *L. fraterna* and *Suillus brevipes* were, tested against six root pathogens viz., *Armillaria mellea*, *Cylindrocladium parvum*, *C. scoparium*, *Fusarium oxysporum*, *F. solani* and *Rhizoctonia solani*.

The results indicate that *Suillus brevipes* inhibited all the fungi tested. It was most effective in the case of *Fusarium solani* and *Cylindrocladium parvum* and least against *Rhizoctonia solani*. *Laccaria laccata* inhibited the growth of *Armillaria mellea*, *C. parvum*, *C. scoparium* and *F. oxysporum*. The percentage inhibition was high in *C. parvum* and it was least against *C. scoparium*. It did not have any effect against *F. solani* and *R. solani*. In the case of *Amanita muscaria* the inhibitory effect was found only against *R. solani* and against all other fungi tested there was no inhibitory effect. *Laccaria fraterna* did not have inhibitory effect against any of the fungi tested.

Of the four fungi tested *Suillus brevipes* inhibited all the root pathogens tested. But Marx (1969) demonstrated that *Suillus luteus* has no effect on *Armillaria mellea* while it inhibited the growth of *F. oxysporum* and *Rhizoctonia* spp. He also found that *Laccaria laccata* inhibited half the number of fungi among 48 different pathogens tested which include *R. solani* but no inhibition was noted against *A. mellea*, *C.*

scoparium and *F. oxysporum*. In the present study the reverse case occurred i.e., *Laccaria laccata* inhibited *A. mellea*, *C. parvum*, *C. scoparium* and *F. oxysporum* and showed no inhibition on *F. solani* and *R. solani*. But other studies have shown that *Laccaria laccata* to be highly antagonistic to *F. oxysporum* in natural conditions (Sinclair *et al.*, 1982; Sylvia and Sinclair, 1983).

Hyppel (1968) has shown that *Amanita muscaria* produced antifungal compounds which inhibited the growth of pathogens but in the present study *A. muscaria* weakly inhibited only *R. solani* among the six test pathogens. *L. fraterna* showed no inhibition against any of these pathogens.

Results of antagonism test between mycorrhizal fungi and root pathogens conducted on nutrient agar medium can only be regarded as suggestive relative to natural conditions. There is further scope to assess to value of these pine mycorrhizal isolates against the various root pathogens in the nursery trials.

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Influence of VA mycorrhizal colonization on root-knot nematode infestation in *Piper nigrum* L.

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The role cf vesicular–arbuscular mycorrhizae (VAM) in reducing the harmful effect of root infestation by many parasitic nematodes in crop plants is now well recognized (Hussey and Rancadori, 1982). In the present investigation the specific effect of inoculation of pepper (*Piper nigrum* L.) vines with VA mycorrhizae in the presence of root-knot nematode, *Meloidogyne incognita* was studied.

The cuttings of pepper cultivar Panniyur 1 were raised in pots filled with 10 kg of *M. incognita* free red sandy loam soil (pH 5.2, 4.0 mg kg^{-1} available P) preinoculated with cultures of VAM (sand : soil mixture containing 320 spores) wherever necessary. Nematode inoculation was done artificially with second stage infective larvae of *M. incognita* (1 g^{-1} soil) on 90th day of plant growth. There were six treatments, viz., control, inoculated with *Glomus fasciculatum* alone, *G. etunicatum* alone, *M. incognita* alone, *G. fasciculatum* along with *M. incognita* and *G. etunicatum* along with *M. incognita*. Experiment was conducted in CRD with four replications each. Observations were recorded on root-knot index, nematode population in root and soil, mycorrhizal colonization (Phillips and Hayman, 1970), plant height and shoot and root dry weights on 18th day of plant growth.

There was significant reduction in root-knot index in plants preinoculated with either culture of VAM. The reduction in root-knot index was to the extent of 32.4% with *G. fasciculatum* and 36% with *G. etunicatum*. This indicated that preinoculation of pepper vines with VA mycorrhiza will be highly useful in reducing the degree of root infestation by *M. incognita*. One of the possible mechanisms of VAM interaction with nematode is that when both are present at the same time in the soil, it will induce certain degree of competition between them for root colonization in which often the mycorrhizal association of beneficial nature is preferred by the host plant (Sikora and Scho'nbeck, 1975). Further, it will be possible for the VAM to establish well earlier in the root system becauce of the preinoculation and rapid rate of root colonization. This may result in the alteration of root physiology of host plant (Sikora, 1978) by way of increasing wall thickness and changing the chemical composition of root and root exudates. Thus this sort of preferential and earlier root colonization by VAM in pepper will lead to a type of host restriction for subsequent root infestation by *M. incognita*. It was further observed that even the root and soil population of this nematode is considerably reduced when plants are mycorrhizal. This reduction in population was to the order of 53.3 and 47.5% with *G. fasciculatum* and 40 and 34.2% with *G. etunicatum* inoculation in root and soil respectively.

The above beneficial effect of VAM had a favourable effect on plant growth as well. The extent of mycorrhizal association was significantly high in inoculated plants. Presence of *M. incognita* did not affect the mycorrhizal colonization.

Proper VA mycorrhizal association in crop plants has already been reported to induce better plant growth (Mosse and Hayman, 1971). This was evident from the improved growth parameters observed in pepper under inoculation with VAM. The plant height of 122 and 113 cm, shoot and root dry weight of 12.1 and 11.6 g and 1.3 and 1.5 g were recorded for *G. fasciculatum* and *G. etunicatum* respectively, which was significantly higher than the control. However, more interesting was the better growth of pepper vines inoculated with VAM even in the presence of the root-knot nematode, *M. incognita*. The increase in root dry weight due to *G. fasciculatum* (77.1%) and *G. etunicatum* (54.2%) and shoot dry weight due to *G. etunicatum* (67.4%), were significantly high when compared to plants inoculated with *M. incognita* alone.

Thus the present investigation clearly showed that it will be highly beneficial to have mycorrhizal pepper cuttings for field cultivation especially in areas where root-knot nematode is an endemic problem.

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The role of VA mycorrhiza in controlling certain root diseases of tobacco

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Studies on the interaction between root-knot nematode and mycorrhizal fungus *Glomus fasciculatum* alone showed highest gall index whereas plants treated with both mycorrhizal fungus and nematode showed moderate gall index of 3 and control recorded the gall index of 4.

Interaction between mycorrhizal fungus *G. fasciculatum* and *Orobanche* with treatments : control, mycorrhiza alone, *Orobanche* alone and mycorrhiza plus *Orobanche* revealed that the plants treated with both mycorrhiza and *Orobanche* also showed the emergence of *Orobanche* shoots and their growth is almost equal to that of plants treated with *Orobanche* alone. Similar study has been conducted in *Orobanche* sick soil and CTRI farm with 4 mycorrhizal cultures viz. *G. fasciculatum*, *G. constrictum*, *G. mosseae* and *Acaulospora* along with the control. Emergence of *Orobanche* was observed in all the 4 cultures showing high intensity of infection as that of control.

Studies on the interaction between *Pythium* and mycorrhiza in tobacco nurseries revealed that non-mycorrhizal plots were wiped off by *Pythium* attack whereas mycorrhizal plots after inoculation of *Pythium* also continued to produce healthy, transplantable seedlings out of 4 cultures of mycorrhiza, *G. fasciculatum*, *G. constrictum*, *G. mosseae* and *Acaulospora* tested for *Pythium* resistance, of these *G. fasciculatum* was found to be promising.

Prevalence of native VAM fungi and their relative performance in infectivity of local crops

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Use of VAM fungi for the improvement of crop productivity requires selection of an efficient and appropriate fungus (Menge, 1983), the right type of soil and the identification of crop (Krikun *et al.*, 1987). An effective mycorrhizal association is required for every crop for P uptake in nearly all soils. Two of the criteria suggested for appropriateness are infectivity and efficacy (Haas and Krikun, 1985) besides, the root colonization ability and the ability of the fungus to survive in soil. The plant growth response usually is dependent upon the quantity of VAM formed ea-ly in the growth season of rop. Since, different VAM fungi differ in their ability to form efficient VAM with different crop plants and sometimes indigenous endophytes were less efficient than introduced (Manjunath and Bagyaraj, 1980). Studies were undertaken (a) to isolate native VAM and (b) to study their relative performance on local crops.

The VAM spores were recovered from local soil samples of Gulbarga by wetsieving and and decanting method (Gerdemann and Nicolson, 1963). Different spore types recognized were purified and multiplied by raising single spore cultures. The spores thus isolated were identified.

Four local crops, chickpea (cv. Annigeri), pigeonpea (GSI), sorghum (cv. Neerjola) and onion (cv. Desi) were raised in pots containing infested loamy sand soil (spore concentration 45 ± 5/gm soil) with five VAM fungi (3 native +2 introduced) seperately examined after 35 days, for % root colonization (Giovanetti and Mosse, 1980) after clearing and staining (Phillips and Hayman, 1970).

Three VAM fungal species, all belonging to the genus *Glomus* viz. *G. constrictum*, *G. fasciculatum* and *G. aggregatum* were recorded in the present survey of native soils. All the four local crops responded to infectivity by all five VAM fungi tested with characteristic vesicles and arbuscles, *G. aggregatum*, though infected sufficiently, the formation of vesicles and arbuscles were scarcely seen. The % root colonization differed with all VAM fungi tested with respect to different crops tested confirming the earlier findings (Manjunath and Bagyaraj, 1980). High mycorrhizal root infection was recorded in all the crops except sorghum in soil infested with *G. fasciculatum* irrespective of the source followed by *G. aggregatum*. The response of the crops to mycorrhization with *G. constrictum* and *Gigaspora margarita* was considerably less irrespective of the crop and did not show any appreciable degree of infection in the early development. Many legumes are poor forgers for soil P and in the present study both pigeonpea and chickpea showed significantly higher VAM formation than onion and sorghum crops showing their higher dependency on VAM associations as evidenced already in pigeonpea which responded less to P fertilizer application than sorghum. Since the plant growth response usually is dependent upon the quantity of VAM formed early in the growth season and the efficiency of VAM symbiosis is the sum of interaction between, the fungal endophyte, the host and the soil (Hayman, 1983), the present study helps for an early detection and selection of VAM inoculant which is compatible with local soils.

In the present survey of soils of Gulbarga for native VAM fungi 3 species viz. *Glomus fasciculatum, G. aggregatum, G. constrictum* were recorded and tested on 4 local crops along with two other VAM fungi *G. fasciculatum* and *Gigaspora margarita* (introduced) by pot culture experiments using local soil. Variability was ovserved in infectivity and % mycorrhization in all the crops. *G. fasciculatum* (irrespective of the source) proved better inoculant on all the crops as its % root colonization was significantly higher when compared to the rest. *G. aggregatum* was the next best while the other did not show any appreciable degree of infection.

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Role of VA mycorrhizae, phosphate solubilising bacteria and their interactions on growth and uptake of nutrients by wheat crop

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Role of phosphate solubilising bacteria, VA mycorrhizae and their interactions on growth, yields and uptake of nutrients by *Triticum aestivum* was studied in sandy loam alluvial soil in pot culture condition without and with application of chemical nitrogen at 60 kg and 80 kg N ha^{-1} as urea and phosphorus and 60 kg P_2O_5 as mussoorie rock phosphate ha^{-1}. Phosphate solubilising bacteria such as *Pseudomonas striata* and *Agrobacterium radiobacter* and VA mycorrhizae *(Glomus fasciculatum* and *Gigaspora margarita)* were used. Wheat seeds were inoculated with either of PSB and soil was inoculated with either of VA mycorrhizae singly and in combinations. Each treatment was replicated 9 times, 3 pots each were kept to study dry matter and grain and straw yields and 6 pots were kept for the rhizosphere study of PSB, nutrient uptake and dry matter yields.

The results showed that inoculation with phosphate solubilising bacteria and VA mycorrhizae improved dry matter yield at various growth periods. The use of nitrogenous and phosphatic fertilizers augmented the dry matter yields significantly over the control and microbial inoculation treatments. *Pseudomonas striata* and *Glomus fasciculatum* when used together were found more effective than either of them but the treatment, *P. striata* and *G. fasciculatum* with chemical fertilizers (N_{60} and P_{80}) gave the highest yield which was significant to all the treatments. Similarly *Agrobacterium radiobacter* with either *G. fasciculatum* or *Gigaspora margarita* and soil amended with chemical fertilizers also gave significant increases.

Grain yield was significantly augmented with simple inculation of *P. striata* alone. The other microbial treatments were at par with the control. The application of N_{120} P_{80} gave the highest yield which was significantly superior to all microbial inoculants used singly except *P. striata* which was at par with this treatment. The combinations of either *P. striata* was at par with this treatment. Likewise *A. radiobacter* and *G. fasciculatum/Gigaspora margarita* gave increased yields over single inoculations. Phosphorus uptake was maximum with *P striata* alone, chemical

nitrogenous and phosphatic fertilizers and combined inoculation of *P. striata* + *G. fasciculatum/Gigaspora margarita*. The effect of *A. radiobacter* and VAM combinations were less marked but the results were statistically at par. The rhizosphere counts of PSB and percent infection by VAM showed that the inoculation of PSB help in their establishment in the soil. Single inoculation gave their higher numbers in the rhizosphere of the plant. The establishment of *A. radiobacter* was less marked ar compared to *P. striata*. The percent root infection by VAM was enhanced due to its inoculation of soil.

Interaction of vesicular-arbuscular mycorrhiza (*Glomus etunicatum* and *Rhizobium* in cowpea

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The beneficial effect of vesicular-arbuscular mycorrhizae on host plants is well known (Jalali and Thareja, 1982). Hayman and Mosse (1979) reported the effect of VA mycorrhiza on legumes. But studies on the interaction of VA mycorrhiza and *Rhizobium* are very few. Since 'P' is essential for biological nitrogen fixation and VA mycorrhiza helps to increase the uptake of 'P', the present study was taken up to investigate the interaction effect of *Glomus etunicatum* and *Rhizobium* in cowpea *(Vigna unguiculata)*. The effects of *G. etunicatum* alone and along with *Rhizobium* besides *Rhizobium* alone were studied.

Both shoot and root weights were on par in *G. etunicatum* inoculated plants, *G. etunicatum*+*Rhizobium* inoculated plants and *Rhizobium* inoculated plants in sterilized soil. But these treatments were significantly superior to untreated plants. The grain yield also was on par in the VAM inoculated plants, *Rhizobium* inoculated plants and in plants with dual inoculation of VAM+*Rhizobium*. But they were all significantly superior than plants without any treatment. The nodule number was significantly more in the dual inoculation of VAM+*Rhizobium* than either with VAM alone or *Rhizobium* alone which were in turn better than uninoculated plants. The percent colonization with VAM was similar in VAM inoculation alone and in dual inoeulation of VAM and *Rhizobium*.

In unsterilized soil, the shoot weight and grain yield were on par in the plants inoculated with VAM, *Rhizobium* and dual inoculation. But they were significantly superior to uninoculated plants. The root weight in the plants with dual inoculation was significantly better than other treatments. The nodule number was significantly more in plants with dual inoculation of VAM and *Rhizobium* than either VAM or *Rhizobium* alone.

In both sterilized and unsterilized soil conditions, dual inoculation with VA mycorrhiza and *Rhizobium* increased the rhizobial nodules and mycorrhizal colonization and consequantly the biomass than either of them alone. The increase in nodulation may be due to the enhancement of 'P' uptake by VAM. Similar observations were made by Smith and Daft (1977) and Islam *et al.* (1980).

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Interaction between *Glomus versiforme* and *Azospirillum brasilense* in barley

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Vesicular-arbuscular mycorrhizal (VAM) associations are formed with a large number of graminaceous plants. Recent researches have also established that some of these plant species which are able to form VAM are also mutualistically associated with nitrogen-fixing procaryotes. Nitrogen fixing bacteria, particularly of genus *Azospirillum* are able to associate closely with the roots of gramineae and increase crop production (Neyra and Dobereiner, 1977). Improvement in yield due to inoculation with VAM and *Azospirillum* on barley and pearlmillet has also been reported (Subba Rao *et al.*, 1977). The present study deals with the aspects of plant improvement in terms of nutrient (nitrogen and phosphorus) uptake and nitrogen fixation in barley in the presence of VAM fungus, *Glomus versiforme* and N_2-fixing *Azospirrillum brasilense* under varying levels of nitrogen and phosphorus.

A pot culture experiment was conducted with sandy-loam soil which was deficient at available phosphorus (4, 5 kg ha^{-1}) using barley (*Hordeum vulgare* L.) var. 62–65–7–4–36 as host plant. A mixed culture of the most efficient strains of *Azospirillum brasilense* (B-1 and B-2) was used. Soil and sand mixture containing extramatrical chlamydospores and root segments of *Cenchrus ciliaris* infected with *Glomus versiforme* (Daniels and Trappe) grown for 90 days which contained 250-300 spores per 100 g soil served as inoculum. Nitrogen was applied as labelled ammonium sulphate with 5% 15_N atom excess at the rate of 50 kg N ha^{-1}. While phosphorus was applied as single superphosphate at the rate of 30 kg P_2O_5 ha^{-1}. The plants were harvested at different stages of plant growth and were subjected to N and P analysis following standard methods (Jackson, 1967). The atom percent ^{15}N in plant samples was determined by an emission spectrophotometer and the N fixed by *Azospirillum* per plant was calculated as under :

N fixed by *Azospirillum*/plant =
(Soii and atmospheric N uptake by inoculated plant)—(Soil and atmospheric N uptake by uninoculated plant)

In shoots in the presence of combined inoculation and N and P application, the phosphorus uptake, as high as 39.9 mg/plant was recorded. Inoculation with mycorrhiza alone had no significant effect on P uptake by roots as well as by shoots. In grains also dual inoculation resulted in maximum uptake (120.2 mg plant^{-1}) in the presencc of both N and P application. The higher uptake in plants in the presence of both N and P due to combined inoculation highlights the importance of VAM in conjunction with *Azospirillum*. The presence of *Azospirillum* may possibly increase N mineralisation in soil (Pacovski and Fuller, 1985). Better translocation rates of phosphorus within and transfer out of the hyphae in the presence of both the endophytes resulted in increased P uptake in roots and shoots.

Plant inoculated with both VAM and *Azospirillum* resulted in more nitrogen fixation than that in singly inoculated ones. A maximum of 335.3mg N fixed plant^{-1} at 120 days in dually inoculated N_1P_0 treatment. In case of grains, however, a maximum of 575.4 mg N was fixed per plant in N_1P_1 treatment as against 315.4 mg N fixed N_1P_0 treatment in plants inoculated with mycorrhiza alone. The observed effect has been attributed to a summation of seperate mycorrhizal and *Azospirilluu* effects. The effect was, however, more pronounced in the presence of phosphorus supporting the fact that phosphorus is ruquired for nitrogen fixation. The phosphorus made available by the VA-mycorrhizal fungus might have contributed towards enhanced N_2-fixation by *Azospirillum*.

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Vesicular arbuscular mycorrhizal associations and root colonization in some important tree species

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Many commercially important hardwood forest trees are naturally infected with vesicular-arbuscular endophytes. However, little work has been done to improve seedling quality in forest tree nurseries by manipulation of these fungi and understand, levels of root colonization before transplanting. Adequate root colonization by VAM fungi in natural and artificially inoculated soils is of paramount importance in improving seedling vigour and it proves a useful index to predict the performance of seedlings in artificial regeneration programmes in different stresses and agriculturally unproductive sites. Seedling development of hardwood species that normally form VAM has not been satisfactory in forest nurseries and consequently seedling quality remains a major problem in artificial establishment of hardwood plantations of sweet gum (*Liquidamber styraciflua*) in United States (Kormanik *et al.*, 1982). Keeping in view the importance of root colonization by VAM, the present study was undertaken to evaluate the frequency and level of cortical infection in nursery seedlings belonging to 19 genera and 14 families of angiosperms.

A quantitative evaluation of the intensity of VA mycorrhizal infetion in nursery seedlings of 20 important hardwood tree spp. belonging to 19 genera of 14 families was conducted in nurseries at New Forest, Dehra Dun.

The frequency of VAM infection and percentage of infection in roots among species within a family and among different host genera were found to be variable. Highest level of root colonization (92%) was recorded in *Michelia champaca* and *Toona ciliata* whereas very low (1 to 6%) levels were recorded in *Albizia lebbek*, *Grewia robusta* and *Madhuce longifolia*. The percentage infection was almost same in *Terminalia arjuna*, *Dendrocalamus strictus*, *Dalbergia sissoo*, *Acacia auriculiformis*, *Emblica officinalis*, *Olea glandulifera*, *Acacia nilotica* and *Serraca asoca* (<40%). Species of *Glomus macrocarpus* and *Sclerocystis coremioides* were found in the rhizosphere of infected roots. Heavy arbuscular infection was found in *Madhuca longifolia*, extramatrical spores in *Acacia auriculiformis* and heavy vesicular infection in *Acacia auriculiformis*, *Dalbergia sissoo* and *Delonix regia*. The highest frequency of VAM infection was recorded in *Toona ciliata* (83.8%), followed by *Michelia champaca* (79.54%), *Jackranda minosifolia* (67.25%) and *Dendrocalamus strictus* (65.5%) while it was lowest in *Albizia lebbeck* (8.33%). Low frequency levels of VAM infection in nursery seedling are indicative of the need of inoculation of nursery stocks with efficient VAM endophytes for better nursery and field performance.

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Interaction between *Glomus fasciculatum* and two phosphate solubilizing fungi in finger millet

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Research in the last three decades has established that plants inoculated with vesicular-arbuscular (VA) mycorrhizal fungi grow better through increased uptake of phosphorous and other mineral nutrients especially in soils of low fertility (Jeffries, 1987). Gain in plant dry matter has been demonstrated with increase in phosphorous availability caused by the P-solubilizing bacteria inoculated to soil (Gaur and Ostwal, 1972). Synergistic interactions between VA mycorrhizal fungi and P-solubilizing bacteria with consequential improvement in plant growth has been reported earlier (Raj *et al.* 1981). The objective of the present study is to examine the interaction between two phosphate solubilizing fungi *Penicillium funiculosum* and *Aspergillus niger*, and the VA mycorrhizal fungus, *Glomus fasiculatum* in the rhizosphere and its effect on growth and mineral content of finger millet. After 50 days of transplanting, plant and soil samples were collected for various analyses.

Dual inoculation with *G. fasciculatum* and *P. funiculosum* recorded the highest mycorrhizal spore count in soil. But there was not much difference in per cent root colonization between mycorrhiza inoculated plants with and without phosphate solubilizing fungi. However, *G. fasciculatum* plus *P. funiculosum* treatment exhibited slightly more root colonization. The populations of introduced phosphate solubilzing fungi multiplied and remained in significantly higher numbers in the rhizosphere for a longer period.

Plants inoculated with both mycorrhiza and phosphate solubilizing fungi took up more P and K and grew better. Treatment with *G. fasciculatum* plus *A. niger* recorded the highest Zn concentration. Dual inoculation failed to increase Mn uptake.

Dual inoculation with *Glomus fasciculatum* and P-solubilizing fungi *Penicillium funiculosum* or *Aspergillus niger* had synergistic interaction with each other with consequential improvement on growth and nutrient uptake of finger millet.

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Infection by a fungal endophyte, *Balansia sclerotica* enhances vesicular-arbuscular mycorrhizal (VAM) association in lemongrass

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Lemongrass (*Cymbopogon flexuosus* (Steud.) Watts) is an important essential oil bearing plant commercially cultivated in India. The plant is the source of the essential oil, lemongrass oil, the main constituent of which is citral (75–85%). Citral is a basic raw material for the synthesis of β-ionones, a precursor for the synthesis of a number of aromatic compounds and vitamin A. Citral is also used extensively in perfumery, soap and cosmetic industry (Anonymous, 1950). A variety of lemongrass (Kerala local) introduced for experimental cultivation was found to produce abnormal and deformed inflorescences. The malformations resulted in grassy-shoot or phyllody appearance. Observations revealed that these plants were infected with *Balansia sclerotica* (Pat.) Hohnel (Janardhanan *et al.*, 1989). The disease is systemic. *Balansia sclerotica* infected plants produced significantly higher tillering and biomass than the healthy plants.

Vesicular-arbuscular mycorrhizae (VAM) impart significant beneficial effect to plant growth (Mosse, 1973). These symbiotic associations are therefore, important in crop and biomass production.

An attempt was made to find out the effect of *B. sclerotica* infection on the VAM-association in lemongrass. Healthy and *B. sclerotica* infected plants were examined for VAM-association. Both infected and healthy plants were found to be associated with three *Glomus* species. Level of VAM infection in *B. sclerotica* infected roots was 89.6%, while it was 44.6% in healthy plants. Rhizosphere soil of *B. sclerotica* infected plants showed greater VAM spore population (1211/100 g soil) than healthy plants (549/100 g soil). Hence, VAM-association was significantly higher in plants infected with the fungal endophyte, *B. sclerotica*. The examination of roots and the rhizosphere soils of healthy and *B. sclerotica* infected plants supports this conclusion.

The results of the investigation indicate that endophytic infection of *B. sclerotica* had significant influence on the VAM-association. Enhanced VAM-association can be one of the contributing factors for better growth performances and biomass production of endophyte infected grasses. It is now recognised that one of the benefits of VAM-association might be the reduction in disease expression due to pathogenic microorganisms (Schenck, 1981). The present finding, however, is contradictory to this concept. The VAM-association appears to support the growth of endophyte infected grasses. This is the first attempt to study the impact of a fungal endophyte on VAM-association in plants.

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Endophytic mycorrhiza in different varieties of *Litchi chinensis* Sonn. and its effect on rhizosphere-microbial population

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The species of *Rhizophagus* belonging to the vesicular-arbuscular group of phycomycetous endophytes have been observed to be in association with the roots of different varieties of *Litchi chinensis* Sonn. Rhizosphere of Kasva, Deshi and Purbi varieties of mycorrhizal litchi had higher population of bacteria than the other varieties studied. The protozoal population were also higher in these varieties as well as in the late Bedana variety. There was also a marked difference in the frequencies of Gram negative and Gram positive bacteria in the non-rhizosphere soil as compared to mycorrhizal rhizosphere of different litchi varieties. However, there was a clear difference in the bacterial population in the rhizosphere of different litchi varieties. Results indicated that the rhizosphere population types differ not only with non-rhizosphere soils, but also with mycorrhizal formation in different varieties of litchi, though the differences in the later case were not marked.

Interaction of VA mycorrhizal fungus and *Tylenchulus semipenetrans* on citrus

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The growth of citrus is greatly influenced by vesicular arbuscular mycorrhizal (VAM) fungi (Nemec, 1978). *Tylenchulus semipenetrans* is known for causing slow decline, or die back disease in this major fruit crop. The concomitant incidence of nematode and VAM fungi influences the host growth as a result of varying interaction, antagonistic or beneficial to each other (Fox and Spasoff, 1972). Presence of both the organisms, VAM fungus (Baghel, 1985) and *T. semipenetrans* (Baghel *et al.*, 1980) in Haryana, on commonly grown citrus root stock 'Jatti Khatti' (*Citrus jambhiri*) created interest in their interaction studies. The present investigation was carried out to observe the individual and combined effect of VAM fungus *Glomus mosseae* and *Tylenchulus semipenetrans* on the growth of citrus root stock (*Citrus jambhiri*) seedling and their effect on development of each other.

Surface sterilized seeds of 'Jatti Khatti' (*C. jambhiri*) were sown in earthen pots containing autoclaved soil. Two month old seedlings were transplanted @ one seedling per pot containing 1 kg sterilized soil. Two weeks after transplanting, inoculation of nematode *T. semipenetrans* @ 5000 larvae/pot and VAM fungus *G. mosseae* infested soil (20 spore/g) @ 100 g/pot were given in following combinations : i) nematode alone, ii) VAM fungus alone, iii) nematode+VAM fungus simultaneously, iv) nematode 20 days prior to VAM fungus, v) VAM fungus 20 days prior to nematode, vi) no inoculum (control). With five replicates of each treatment, the experiment was terminated six months after inoculation.

Growth parameters, viz,, length, fresh and dry weights of root, shoot and stem diameter of the seedlings inoculated with *G. mosseae* were greater than seedlings inoculated with nematode(*T. semipenetrans*) alone, with different combinations of nematode and VAM fungus and non-inoculated control. Seedlings with simultaneous inoculation of nematode and fungus had better growth than seedlings inoculated with nematode alone, nematode inoculated 20 days prior to fungus and uninoculated control.

Multiplication of *T. semipenetrans* as indicated by population build up in roots and soil was highest in pots containing seedlings inoculated with nematode alone. In combined inoculation of nematode and fungus, higher population was recorded in pots containing seedlings having nematode inoculation 20 days prior to VAM fungus than fungus inoculated 20 days prior to nematode and simultaneous inoculation. Mycorrhizal density of *G. mosseae* was statistically at par in seedlings inoculated with fungus alone, fungus inoculated 20 days prior to nematode and simultaneous inoculation of both organisms. Seedlings inoculated with nematode 20 days prior to fungus had lowest mycorrhizal density.

On the basis of growth pattern it is apparent that *G. mosseae* had stimulatory effect on the growth of *C. jambhiri* seedlings, while *T. semipenetrans* had suppressive effect. In simultaneous inoculation of nematode and fungus, the adverse effect of nematode was partly neutralised and fungal symbiont limited the development of nematode. Similar finding were earlier reported by O'Bannon *et al.* (1979). While studying the effect of *G. mosseae* and *T. semipeuetrans* on *Citrus limon* seedlings. This is evident from the fact that low population build up of nematode was observed in the seedlings receiving simultaneous inoculation of nematode and fungus. This seemed to be due to quick penetration and colonization of fungus in roots as compared to nematode penetration and establishment of feeding sites. Nematodes inoculated 20 days prior to fungus got enough time to penetrate and establish on the root system leaving little space for fungus to spread mycelium within root tissues. In such cases fungal capability to enhance growth was subsided by nematode as is evident from low mycorrhizal colonization and high nematode population build up. This phenomenon was inversed when fungus was inoculated 20 days prior to nematode. Once fungus penetrated roots and established itself in root systcm, perhaps it becomes difficult for nematode to multiply fast and exhibit the adverse effect on growth. Fox and Spasoff (1972) reported similar observations while working on interaction of *Heterodera solanacearum* and *Endogone gigantea* on tobacco.

From the present study it can be concluded that VAM fungus *G. mosseae* can provide substantial check on citrus nematode infesting citrus root stock. However, field application of fungus for the control of citrus nematode needs to be worked out in detail.

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Responses of dual inoculation with *Bradyrhizobium* and VA mycorrhiza or phosphate solubilizers on soybean in mollisol

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Inoculation with VA mycorrhizal fungi (Singh and Varma, 1988), efficient strains of *Rhizobium* (Chandra and Pareek, 1985) and phosphate solubilizing microorganisms (Gaur, 1985) separately and in dual form (Singh and Singh, 1988) have increased nodulation, dry matter accumulation in plant, nitrogen fixation and grain yield through better uptake of nutrients because of increased activities of soil microorganisms in the rhizosphere region. The present study was, therefore, undertaken to find out inoculation responses in terms of nodulation, plant growth and grain yield of soybean in a Mollisol using inoculants of *Pseudomonas striata*, *Bacillus polymyxa*, indigenous VA mycorrhiza, *Glomus fasciculatum*, *Bradyrhizobium japonicum* and their few main dual combinations.

As a result of increased activities of microorganisms in the rhizosphere region either directly or indirectly, inoculation with *Bradyrhizobium*, VAM and phosphate solubilizers individually increased the number and dry weight of root nodules and organic matter accumulation in plant. Increase in the grain yield due to individual inoculation with *Bradyrhizobium*, VAM and phosphate solubilizers ranged 8.66 to 13.82%, 0.0 to 46.55% and 0.0 to 41.38% respectively. Dual inoculation with *Bradyrhizobium japonicum* and VA mycorrhiza or phosphate solubilizers did not show significant increase in any of the parameters studied. The efficacy of multi strains inoculum of phosphate solubilizers was almost similar to the single-strain inoculum.

Thus, based on the results, it may be concluded that, even in high phosphate soils, inoculation with single-strain inoculum of *Bradyrhizobium* or VAM or phosphate solubilizers is an essential technique for the successful cultivation of soybean.

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Clonal selectivity in *Populus deltoides* for vesicular arbuscular mycorrhizal association

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In view of the ever increasing demand for wood, fast growing tree species like poplars have attracted worldwide attention. Poplars belong to the genus *Populus* of the family Salicaceae. Their wood is in much demand for paper, pulp, plywood, veneer, matchwood, packing cases, fibreboard and light construction timber all over the world. Attempts are being made by the Uttar Pardesh Forest Department to select suitable clones for sites below 28 latitude. Several *Populus* clones are under trial on partially reclaimed alkaline soil site at Banthra Research Station of National Botanical Research Institute, Lucknow.

Endomycorrhizae often improve the quality of the nursery seedlings. Since *Populus* is multiplied by cuttings raised in nursery, association of vesicular-arbuscular mycorrhizal fungi can improve the growth and performance of the nursery grown cuttings. There have been reports of formation of endomycorrhizae in *Populus* species in Iowa (USA) soils (Walker, 1980). However, there has been no systematic study of VAM association in *Populus deltoides* particularly on marginal soils in India.

Nine clones of *Populus deltoides* (59-101, ST-148, 15-29, 55-264, D-121, S7C15, S7C20, G-48 and AST-242) growing at Banthra Research Station of the institute were screened for association of VAM fungi. These clones were initially obtained from U.P. Forest Department nursery at Lalkua (elevation 256m). Soils where cuttings were grown are alkaline (pH=8.53 and EC=0.24 mmhos/cm) with moisture content ranging from 7.5 to 18.2%.

Only three clones (15-29, ST-148 and 59-101) out of the nine clones investigated were found to form endomycorrhizae. Two VAM species were found to colonize the roots of these three clones. These fungi were identified as *Glomus intraradices* Schenck and Smith and *Scutellospora gigantea* (Nicol. and Gerd.) Gerd. and Trappe (Schenck and Perez, 1987; Trappe, 1982). Though all the soil samples had spores of *Glomus intraradices* and *Scutellospora gigantea* only these three clones formed mycorrhizae. Out of the three mycorrhizal clones ST-148 which is a hybrid of *Populus deltoides*×*P. tricocarpa* had the least root infection

(30%) and only 8.5% of the cortex area was infested. On the other hand clones 59-101 and 15-29 had 70% and 40% infestation, respectively.

S7C15 and S7C20 have been reported to be fairly promising at lalkua nursery (Personal communication). However, these clones didnot form mycorrhizae with the reported VAM species and thus no correlation between performance and VAM association could be established.

The study revealed that there is a preference in mycorrhizal infection amongst the 9 clones of *Populus deltoides* grown at alkaline sites. Even amongst the three mycorrhizal clones there is a variation in the root infection even when relatively abundant spore population of the two VAM species was available in the soil. Certain clones where no VAM association was observed cannot be regarded as non mycorrhizal until a controlled experimentation by inoculating other potential mycorrhizal species is tried.

Selectivity in mycorrhizal formation in other *Populus* hybrids being cultivated in USA has also been reported by Schultz *ct al.* (1984). This selectivity should be identified for the most important clones available so that proper inoculum can be used for producing healthy propagules. Although some hybrids seem to show autotrophic growth without mycorrhizal development, potential for mycorrhizal formation should be established for all hybrids. Selection of appropriate VAM fungi and regulation of cultural practices such as site preparation and application of herbicides for weed control are important consideration in order to improve the quality of the seedlings and promote the growth of poplars.

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Interactions between isolates of ectomyrrhizal *Laccaria* spp. and root rot fungi of coniffers

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The protection of roots by ectomycorrhizal fungi against soil borne diseases has received considerable attention in recent years. *Laccaria* spp. are ubiquitous fungi occurring in diverse forest habitats forming ectomycorrhizal association with many forest trees (Shaw and Molina 1980). Isolates of *Laccaria* sp. protected young seedlings of Norway spruce (*Picea abies* Karst) and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) from *Fusarium oxysporum* Schlecht., the causal agent of damping off and root rot of conifers (Sampangiramaiah *et al.*, 1985). In the forest nurseries, other than *F. oxysporum, Pythium* spp. and Rh*izoctonia solani* are the universal soil borne pathogens co-existing in several forest nurseries (Sampangiramaiah and Perrina, 1988).

Isolate variability in inoculum effectiveness of ectomycorrhizal fungi is reported with species and strains within the same species differing in their beneficial effects and root protection (Trappe, 1977). This necessitates the need for evaluating several strains or ecotypes of each fungus before selecting the performing isolates.

The present study investigates the variability *in-vitro* by three isolates of *Laccaria* sp. and one isolate of *L. bicolor* in the suppressive influence on an isolate each of *Fusarium oxysporum*, *Rhizoctonia solani* and *Pythium* sp., the principal root pathogens of conifer nurseries.

In the paired culture tests on agar plates, all the four isolates of *Laccaria* sp. did not show any substantial growth inhibition of *F. oxysporum.* Mycelial inhibition was comparatively pronounced with *Pythium* sp. and *R. solani* (15-28%) especially with *L. bicolor* on *Pythium* sp. In general there was no substantial inhibition of hyphal elongation of these pathogens.

The cell free sterile extracts of all the *Laccaria* sp. showed appreciable reduction in the mycelial dry weight of *F. oxysporum* (30%) but not so with *Pythium* sp. and *R. solani.* Extracellular extracts of individual mycorrhizal isolates had varied effects with few having stimulatory effect on mycelial growth of *Pythium* sp. and *R. solani.* Extracellular metabolites of *Laccaria* sp. had an inhibitory effect on the germination of chlamydospores of *F. oxysporum* (30 to 35%) although few isolates had no effect or slight stimulatory effect.

Commercial preparations of two volatile organic constituents (terpenes in ectomycorrhizal root systems of conifers), L-pinen and Limonene were evaluated *in-vitro* on mycelial growth inhibition of the root pathogens. Both the terpenes in their gas phase had inhibitory effect on growth of *F. oxysporum*, *Pythium* sp. and *R. solani* when used at conc. of 0.005 and 0.05%. The inhibition ranged from 5 to 40% with the pathogens differing in their tolerance to individual compounds. *Pythium* sp. was very sensitive to both the compounds (36 to 38% inhibition), while *R. solani* being least sensitive (13 to 16%) but, *F. oxysporum* was intermediate in its response (15 to 20% inhibition).

The suppressive influence of four isolates of *Laccaria* sp. on principal forest nursery pathogens, *Fusarium oxysporum*, *Pythium* sp. and *Rhizoctonia solani* were investigated. The mycelial growth of these pathogens and chlamydospore germination of *F. oxysporum* was partially inhibited by the metabolites liberated into the culture filtrate by isolates of *Laccaria* sp. Isolate variability in suppressive influence was observed with few cell free extracts stimulating mycelial growth. Thus, extracellular metabolites are considered un-important in the mechanisms of suppression. Commercial preparations of the terpenoid constituents of conifer root system (L-pine and Limonene) gave substantial inhibition of hyphal elongation of these pathogens. Induced host response through production of toxic metabolites (terpenes) in root system (Sampangiramaiah and Perrina, 1989) are well known to inhibit both mycorrhizal and root pathogenic fungi and this may be the chemical basis of root protection by *Laccaria* spp.

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Studies on the growth of certain ectomycorrhizal fungi in culture media and in the host under axenic conditions

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For understanding the physiology of ectomycorrhizal association with *Pinus* species, it is essential to know the growth requirements of the fungi under *in vitro* conditions as influenced by different media. Similarly it is also an important prerequisite before certain fungus is recommended for artificial inoculation, to find out the behaviour of different ectomycorrhizae on a *Pinus* species under axenic conditions. Although several studies have been made on the growth and nutritional requirements of ectomycorrhizae such as *Scleroderma bovista* and *Cenococcum geophyllum* (Thaper, 1989), growth studies *in vitro* and *in vivo* of certain fungi such as *Amanita muscaria*, *Rhizopogon* sp., *Laccaria laccata* etc. as occurring in Nilgiris region has not been made. Hence studies were carried out and the results reported herein.

The ectomycorrhizal fungi isolated from *Pinus* roots were cultured in 3 different media for studying their characters. The three media which were used in the study include : Modified melin Norkran's medium (MMN), Norkan's medium (NM) and Hegem's medium (HM).

The fungi were cultured in agar as well as liquid media and the growth was assessed in terms of fungal dry weight and colony diameter.

The pure culture synthesis techniques of Molina and Palmer (1982) were adopted using glass synthesis tubes of 38×300 mm filled with 110 ml of vermiculite and 10ml of peat moss, which were autoclaved. Test fungus, grown for 3 weeks was inoculated. Uninoculated set served as control.

Typical ectomycorrhizal synthesis was completed at 4 to 6 months after inoculation depending on the growth rates of fungus and host. During the synthesis test a small bit of substrate was aseptically removed from the vessel, transferred into nutrient agar, incubated and checked for contamination and reisolation of the original fungus. The seedling was removed intact from the synthesis vessel and its roots gently washed free of substrate with tap water. The entire root system was than placed under water in a Petri dish and observed with a stereomicroscope for ectomycorrhizal colonization.

The different ectomycorrhizal fungi such as *Amanita muscaria, Hebeloma crustuliniforme, Laccaria laccata, Rhizopogon luteolus* and *Suillus brovinus* and *S. granulatus* were tested for their growth characteristics in three different media with a view to assess the suitability of an appropriate medium for mass multiplication. The results revealed that among the three media tested, MMN was found to be the best in terms of colony diameter and dry weight of fungi produced when compared to NM and HM media.

Pure culture synthesis studies were carried out to understand the effect of different ectomycorrhizal fungi viz., *Amanita muscaria, Rhizopogon, Laccaria laccata, Scleroderma verrucosus* and *Pisolithus tinctorius* on the growth of *Pinus caribaea* under axenic conditions. The results revealed that in control plants where mycorrhizae were not inoculated, 5 plants out of 6 died and the remaining one also recorded comparatively lesser growth. Nearly 80% of plants, on the other hand, inoculated with ectomycorrhizal fungi survived indicating thereby the essentiality of the fungus in the establishment of *Pinus*. Among the different fungi, *Pisolithus tinctorius* recorded maximum shoot and root length and the biomass followed by *Rhizopogon*.

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The mycorrhizal association of morels in N. W. Himalayas

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Morels had been so far regarded either parasitic (Robert, 1985) and or saprophytic (Heim, 1984). Only recently they have been reported to enter into mycorrhizal relationship with some plant species (Buscot and Roux, 1987). These observations are extremely important in understanding the nutritional relationships of morels and the whole question needs to be reinvestigated giving due consideration to the mycorrhizal partner. The present studies were undertaken to study the mycorrhizal association of different morel species, collected from N. W. Himalayas, which were seen to enter into such an association.

Three species of morels, viz. *Morchella deliciosa* Fries, *M. esculenta* (L.) Pers. and *M. conica* Pers. were observed to enter into mycorrhizal relationship with different plant species. Mycorrhizal connections were established by digging the soil and tracing the roots of the plants to the fructifications following Young (1940) and Zak (1973). The roots of associated plants were thoroughly washed in tap water, fixed in formalin, acetic acid and alcohol (70%) in the ratio of 5:5:90 for 24 hours and then preserved in 70% alcohol. In order to confirm the formation of mycorrhiza, both stained and unstained sections were prepared and examined microscopically.

The ovservations for the mycorrhizal link between the sporocarps of the tree morel species and the roots of associated plants i.e. strawberry, grasses and fern rhizomes, were made visually. The sporocarps were taken out carefully and freed from soil particles by a gentle stream of tap water. The subterranean portion of the carpophore was seen to form a loosely woven cord near its base, which gradually transformed into a more compact and shapely cord, producing some short roots on way, and tapering at the distal end, and there getting connected to the farther end of the roots of these plants, forming a 'mycorrhizal bridge'.

Anatomical observations made on sections stained with cotton blue show that morel hyphae penetrate into all the tissues of the root except xylem. The hyphae penetrate into the cells where they mostly grow near the cell wall but they also sometimes grow deeply within the lumen. The root hairs were seen in very few sections. The typical fungus mantle was absent. The hyphae have not been seen to grow intercellularly. In longisections of the root, the hyphae were seen to form a loose weft on the root periphery; some of them penetrating directly into the cell lumens.

Short roots also showed a similar structure and they were almost evenly distributed all along the long root and its branches.

Buscot and Roux (1987) reported *M. rotunda* (Per.) Boud. to from mycorrhiza with many tree species and herbaceous plants. During the present study none of the species collected has been seen to enter into mycorrhizal relationship with any of the tree species, although they were all collected from the forested areas. As discovered in the present study, Buscot and Roux (1987) also had observed the ascocarps of *M. rotunda* to be joined by subterranean hyphal systems, the "mycelial "muffs", surrounding living roots of various plants. They also mention the presence of conspicuous "mycelial muffs" on the subterranean parts of stems of young trees, not seen in the present study. They attach great importance to these mycelial muffs and according to them the organs to which the "muffs" are attached always belong to the living plant, roots contain sap and their absorptive extremities are always functional. They record that the hyphae constituting the muffs are more compacted than in the connective mycelium and that the mycelial muffs do not induce morphological modification in the roots or stem which it surrounds. They further emphasize that this together with the localization of the muffs on parts of roots that are non absorptive, suggests that the association is not truely mycorrhizal. However, the association that we have observed during the present studies seems to be doubtlessly truely mycorrhizal as the fungus has been seen to be associated with young absorbing roots. But the significance of the "muffs" needs to be ascertained in relation to nutrition and ascocarp formation. May be the clue for artificial cultivation of these fungi is revealed by such studies.

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Relative efficacy of VAM isolates for green gram under water stress conditions

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Low level of soil moisture may reduce the phosphate diffusion in plants (Bieleski, 1976). VA mycorrhiza may improve the water relations in plants and ensure a better phosphate nutrition (Puppi *et al.*, 1988). Several authors have reported the mycorrhizal association in plants in relation to soil moisture level (Allen and Bossalis, 1983; Ponder, 1983). VAM fungi can adapt to a wide range of soil water regimes and may thus be exploited for improving the performance of crops under drier areas. The purpose of present study was to select efficient endophytes which could be exploited for improving the performance of green gram under drier conditions.

A number of VA mycorrhizal fungi collected from local fields under legume cultivation (GM, NS, 4S, IWC, 7MY, OBS, ST, 1S) were compared with *Glomus fasciculatum* (Thaxter sensu Gerd.). Gerd. and Trappe and *G. macrocarpum* Tull. and Tull. for their efficacy in improving the performance of two cultivars of green gram (T-44 and Pusa Baisakhi) under normal (20% soil moisture) and strained (5% soil moisture) water schedules.

The crops were raised under greenhouse conditions in earthen pots in unsterilized field soil separately supplemented with different VAM fungi. Different water schedules were followed to maintain 20% and 5% soil moisture in the pots. Soil and root samples were collected at regular intervals and processed for estimating the mycorrhization. Data on nodulation, root/shoot biomass and yield were also collected and analysed statistically.

Out of 10 VAM fungi evaluated, only 4 including *G. fasciculatum* and three of the local isolates (GM, NS, and OBS) caused an improvement in the mycorrhization in cvr. T-44 raised under normal supply of water. Out of these only two viz. *G. fasciculatum* and isolate OBS showed alround verstality in improving the performance of the cultivar in terms of yield, nodulation and root/shoot biomass. Isolate NS caused an improvement in the yield and nodulation but failed to make an appreciable change in root/shoot biomass. Further, isolate GM raised the mycorrhization status of the crop but failed to make a positive effect on its performance. Interestingly, inspite of its failure to raise mycorrhizal status or nodulation in the crop, isolate 1S caused an improvement in yield and root biomass.

The mycorrhization status of other cultivar raised under normal level of water supply was improved by only isolate IWC. An improvement in yield was also recorded due to inoculation of this fungus. Inspite of their failure to raise the mycorrhization status, *G. fasciculatum* as well as isolates NS and OBS caused an overall improvement in the performance of the cultivar. The behaviour of isolate 7MY was interesting since it caused an improvement in yield but failed to improve either mycorrhization or nodulation.

The response of both the cultivars to various VAM fungi under water stress condition also varied with the isolates. T-44 responded positively to isolate NS showed improved mycorrhization and performance in terms of yield, nodulation and root/shoot biomass. *G. fasciculatum* and isolate OBS improved the overall performance but failed to improve the mycorrhizal status. Isolates GM, IWC and 7MY also failed to improve the mycorrhization status, however, two of them (GM and IWC) caused an improvement in the yield and nodulation while the third one in yield and root/shoot biomass.

In all, 5 isolates including *G. fasciculatum* caused an improvement in the mycorrhization in Pusa Baisakhi raised under water stress conditions. However, only *G. fasciculatum* and isolate OBS showed an alround verstality in improving the performance of the cultivar. Isolate 7MY caused improvement in yield and root biomass, isolate ST only in yield while isolate GM only in root biomass. Inspite of its failure to raise the mycorrhization status of the cultivar, isolate NS also proved efficacious in improving its overall performance.

Superiority of introduced VAM fungi in comparison to indigenous ones in improving the performance of crops has been reported earlier (Powell, 1976).

Variations in the response of different isoiates to the crop under a particular water schedule were evident in our study. Similar variations have been earlier reported (Carling and Brown, 1980; Raja *et al.*, 1987). Such variations could be safely attributed to the intrinsic ability of the isolates to explore more soil area, plant-fungal compatibility and interaction between the endophyte and soil environment.

Improvement in the performance of the crop due to introduced VAM isolates was coupled with an improvement in its mycorrhization status. However, in certain cases, an isolate improved the performance but failed to cause an improvement in mycorrhization status. At the same time, in certain other cases, an isolate failed to improve the performance of the crop, inspite of its ability to raise its mycorrhization status.

The present study has revealed that out of 10 isolates evaluated, three isolates including *G. fasciculatum* and two locals (NS and OBS) were uniformally efficacious for both the cultivars under both the water schedules. They may be exploited for improving the performance of green gram under irrigated and rainfed regions. However, before employing them for practical application, it is necessary to establish their efficacy under field conditions.

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Effect of interaction between VA-mycorrhizae and graded levels of phosphorus on the growth of papaya (*Carica papaya*)

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In many tropical soils, lack of phosphate is the most important constraint to plant growth. Research in the past years has proved that VA-mycorrhizae (VAM) can improve the plant growth through increased uptake of phosphorus and other mineral nutrients especially in low fertile soils. It is said that nearly 80-85 per cent of P we apply is made unavailable to plants because of their inaccessibility, fixation and immobilization. There has been a keen interest to show that VAM inoculation can increase the recovery of phosphatic fertilizer from soil by plants. It is suggested that the mycorrhiza are able to explore the soil more thoroughly and hence are able to locate and use the point source of P. Experiments were conducted to find out the optimum level of P to which VAM can be combined to get maximum growth of papaya (*Carica papaya*) plants.

Pots of size 30 × 30 cm were filled with soil (red loam; pH 7.0; EC 0.61 m. mhos/cm^2; available N 250.5 kg/ha; available P 16.1 kg/ha) and one hundred gram of mixed inoculum of VAM (*Glomus mosseae*+*G. fasciculatum*+*Gigaspora margarita*) was placed 2.5 cm below the seeds which contained a spore load of 350 per 100 g of soil. The following treatments were imposed in a completely randomized block design with twenty replications : (i) control (no VAM; no fertilizer), (ii) VAM alone (iii) 100% of recommended level of P (iv) 100% of recommended level of P+VAM (v) 75% of recommended level of P (vi) 75% of recommended level of P+VAM (vii) 50% of recommended level of P and (viii) 50% of recommended level of P+VAM.

The recommended doses of N and K (250 : 200 g/pot) were applied to all pots in the form of urea and muriate of potash, respectively. The recommended (200 g/plant) or graded levels of P was applied as basal in the form of super phosphate.

Four seeds per pot were sown, after applying the VAM inoculum. Usual agronomic practices like watering, weeding, thinning etc., were carried out. The biometric observations like plant height, number of leaves, shoot and root dry weight were taken on 90th day after sowing. The VAM colonization in roots, VAM spore population in soil, total nitrogen and phosphorus content of plant samples were determined.

The results revealed that among the different levels of P, 75 per cent of the recommended level of P+VAM significantly enhanced the growth of papaya than the other P levels, thus indicating the optimum dose for the crop. For instance, there was an increase in height of plants by 25.47 per cent by application of 75% of P along with VAM over its respective control (75 per cent P alone). The biomass production and number of leaves were also increased by VAM application and the increase was maximum when VAM was inoculated along with 75 per cent of the recommended level of P. The increase in N and P contents of plants was higher by VAM application along with 75 per cent P and was least with 100 per cent P. The N content of plants inoculated with VAM at 75 per cent of recommended level of P was increased by 23.32 per cent on 90th day over that of the plants applied with 75 per cent P alone. By application of VAM along with 100 per cent P there was only 4.65 per cent increase in N content over that of the plants applied with 100 per cent P alone. In plant P content also, the maximum increase was recorded by VAM application with 75 per cent P (30.85 per cent increase). Thus the present study indicated that application of 75 per cent recommended level of P+VAM was equivalent to 100 per cent P in enhancing the growth of the plant, thus saving a fertilizer input of 25 per cent.

It was also observed that there was a decrease in the intensity of VAM colonization when large amount of P was added to papaya seedlings. The per cent colonization in roots of plants inoculated with VAM along with 75 per cent of recommended level of P was 41.67 as against 35.0 by inoculation with VAM+100% of recommended level of P. This confirms the earlier reports of many workers (Stribley *et al.*, 1980; Mosse and Phillips 1971; Krishna and Dart, 1984).

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Influence of VA-mycorrhizal inoculation on growth and development of rapeseed

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Various field and laboratory experiments conducted at different locations have demonstrated that VA-mycorrhizal inoculation can improve health and nutrition of crop plants (Mosse, 1973; Lin and Hao, 1988). This symbiotic relationship with plants also leads to biological supression of soil-borne plant pathogens (Jalali and Hari Chand, 1988). Investigations initiated earlier indicated positive correlations between VA-mycorrhizal colonization and development in mustard (*Brassica juncea*), when such plants were subjected to mycorrhizal inoculation (Jalali, 1984). The aim of the present study was to quantify the impact of VA-mycorrhizae on growth and development of rapeseed (*B. campestris* var. Toria cv PT-303).

The procedures adopted by Phillips and Hayman (1970) and Jalali and Domsch (1975) for root-clearing & staining and assessment of mycorrhizal colonization respectively, were employed. The physico-chemical analysis of soil was carried out in mycorrhizal as well as non-mycorrhizal-inoculated soils at two stages, viz., at pre and post planting time.

Available N, P, K, electrical conductivity, C.E.C. and organic carbon content were less in the samples taken at the time of harvesting as compared to samples taken before planting. Of these parameters, most significant change was observed in electrical conductivity. VA-mycorrhizal colonization exhibited significant increase in mean shoot and branch length, total dry matter production, total pod weight as well as yield per plant, as compared to uninoculated controls.

Nitrogen, phosphorus and potassium contents of root and shoot increased significantly in mycorrhizal-inoculated plants. Among these major nutrients, maximum response was observed in the transport of phosphorus.

These results suggest that mycorrhizae either were themselves responsible for higher survival and growth of mycorrhizal inoculated plants of rapeseed or influenced growth by modifying chemical composition of plants.

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Role of endomycorrhizae in fuelwood plantation nurseries for alkaline soil sites

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Throughout the developing world, substandard soil sites have become relevant on account of paucity of good arable land. Nearly 7 million hectares of saline and usar land has been regarded unfit for agriculture on account of high concentration of soluble salts and exchangeable sodium (Khoshoo, 1987). Fuelwood plantation can meet the challange as the nation faces an acute firewood shortage. Many experiments to raise fuelwood plantations on degraded soils fail due to high mortality and poor establishment. Healthy and quality seedlings, though difficult to grow are a prerequisite to the successful establishment of hardwood plants particularly for usar type substandard soil sites. Consistant nursery production of such seedlings is a major obstacle in implementation of fuel wood production programme on degraded soils.

Besides rhizobia, endomycorrhizae improve the quality of seedlings in tree nurseries (Kormanic, 1980). Data from earlier studies show that when root systems are tailored in nursery with vesicular-arbuscular mycorrhizal fungi prior to planting the tree survival and growth improves significantly. Performance of hardwood tree species such as sycamore on kaolin has been improved by specific endomycorrhizal fungi (Marx, 1977). Only a limited research has been done on the importance of endomycorrhiza to plant growth. These symbionts improve the nutrient uptake, facilitate the uptake of moisture in plants. There is a selective ion absorption and accumulation especially of phosphorus and other micronutrients like Zn and Cu (Moawad, 1986). These fungi also increase the longivity of feeder root function and they persist longer on the root system than the normal roots.

With the exception of a few species of *Acacia* and *Leucaena* VAM affinity of tropical tree legumes is not fully recorded. Also there have been very few studies on association of ecologically adapted VAM fungi with tropical trees on degraded soil sites. In the tropics, where P fertilizers are expensive and where soils are often P deficient, VAM fungi can play an important role in improving tree productivity.

Eight nitrogen fixing tree species : *Acacia nilotica*, *A. nilotica var. cupressiformis*, *A. auriculiformis*, *Cassia siamea*, *Leucaena leucocephala*, *Parkinsonia aculata*, *Prosopis juliflora* and *Tamarindus indica*, and certain non-leguminous tree species like *Casuarina* *glauca*, *C. obessa*, *C. equisetifolia* and *Populs deltoides* under biomass trial at Banthra Research Station of National Botanical Research Institute, Lucknow have been screened for mycorrhizal association. Dominant VAM species like *Glomus fasiculatum*, *G. intraradices*, *G. dimorphicum*, *Scutellospora calosopora* and *S. gigantea* have been isolated. Their effect in promoting the growth of nursery seedlings is being investigated. Initial experimentation has suggested that these fungi are not only beneficial to growth of the seedlings, but promote the survival and growth of transplanting stock (Sidhu and Behl, Unpublished). On the basis of our study the following considerations are significant for a research programme related to the role of endomycorrhiza in nurseries of hardwood tree species particularly for alkaline soil sites.

A particular tree species may enter into mycorrhizal association with one or many different species of mycorrhizal fungi at a given time. Some mycorrhizal fungi have a broad tree host range whereas others have a very narrow host range. Many plant species like *Populus deltoides* have shown selectivity and preference for a particular species. Dominant taxa of VAM fungi should be identified for a particular soil site. Some species of mycorrhizal fungi are more beneficial to nursery stocks than others. Certain mycorrhizal fungi are more ecologically adapted to certain sites than other fungi. There is an interaction between rhizobia and endomycorrhizae or Frankia and endomycorrhiza. It has been observed in certain cases that the growth of tree seedling is better with only N fixing microbe inoculum or endomycorrhiza but when there is a synergistic effect the growth is relatively less pronounced. Hence a study of interaction of N fixing bacteria or Frankia and endomycorrhiza is essential. Degraded soils like usar soil sites don't have a rich flora of VAM fungi. Hence VAM inoculated nursery seedling is an important technology for better survival and establishment of tree species in usar soils. Soil fumigation often destroys the mycorrhizal flora. Once these symbiotic fungi have been eradicated from soil, reinfestation is slow. Only limited work has been done on the role of endomycorrhiza for fuelwood plantation and practically none on usar type degraded soils. It should be regarded as a thrust area of research.

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A strategy for selection and application of VAM fungi for *Glycine max*

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Vesiculars arbuscular mycorrhizal (VAM) system has three components : plant, fungal endophyte and soil. The practical significance of this is manifested in screening procedures to select optimum combinations of all the three components as a guide to field inoculation trials. For the economical utilization of VAM on large scale, it is necessary to (i) characterize and identify VAM fungi, (ii) define more precisely VAM specificities and preferences with different host plants and (iii) amend soil conditions for best results. In view of the above facts the present work was carried out with the objective to determine growth responses of soybean inoculated with selected VAM fungi.

Scanning Electron Microscopic (SEM) studies were carried out to taxonomically characterize the VAM fungi (*G. fasciculatum*, *G. macrocarpum*, *G. multisubstensum*) for their proper application and selection as inoculant fungi for soybean (*Glycine max*). These species were identified according to Trappe (1982). The major distinguishing characters as revealed by SEM were the wall layers and hyphal attachments. Spores of *G. multisubstensum* showed two inseparable wall layers and the wide attachment of subtending hyphae (2-4 in number) at one end of the spore; apart from the characters observed by light microscopy. Spores of *G. macrocarpum* and *G. fasciculatum* showed two distinct separable layers, but in former the inner wall was laminate whereas spores of *G. fasciculatum* showed thickened inward projections in the inner thicker wall.

The effect of five VAM fungi (*G. fasciculatum*, *G. macrocarpum*, *G. fuegianum*, *G. multisubstensum* and *G. mosseae*) was observed on plant growth in terms of dry weight and total phosphorus. *G. macrocarpum* significantly influenced growth of soybean as compared to other inoculated VAM fungi. In terms of dry weights fungal effectiveness was in the order : *G. macrocarpum* > *G. fasciculatum* > *G. fuegianum* > *G. multisubstensum* > *G. mosseae*. Mycorrhizal colonization was maximum (85%) in plants inoculated with *G. macrocarpum* and minimum (48%) in plant inoculated with *G. mosseae*. Colonization was observed throughout the growing season, however intensity of colonization increased during final harvest. Dry weight, total P and colonization data clearly shows that *G. macrocarpum* was the most efficient mycosymbiont for soybean, whereas *G. mosseae* was least effective. On the basis of the above data *G. macrocarpum* and *G. fasciculatum* were selected for further studies.

The effect of different phosphorus levels and mycorrhizal inoculation was observed on growth of soybean in terms of dry weight and total P with the intention to find out the most suitable phosphorus level for this association. Plants showed varied response to phosphorus application (0, 0.2, 0.4, 0.6, 1.0, 1.4, 2.0, 2.8 mg/pot) and mycorrhizal inoculation of *G. macrocarpum* and *G. fasciculatum* individually. At the highest level of phosphorus, growth of both endophytes was 2.8 mg/pot. Plants showed greater response to mycorrhizal inoculation at intermediate levels of applied phosphorus. Mycorrhizal colonization decreased approximately three folds at higher levels of phosphorus. Total P in the tissues was maximum (6.08 mg/pot) in plants inoculated with *G. macrocarpum* as compared to other inoculated treatments. The total P in the tissues of mycorrhizal plants was much more than that of control plants. There was higher phosphorus content in the plant tissues inoculated with *G. macrocarpum* than inoculated with *G. fasciculatum* and in uninoculated controls.

G. macrocarpum was efficient VAM fungus for soybean over other VAM species i. e. *G. mosseae*, *G. fuegianum*, *G. multisubstensum*. *G. macrocarpum* was superior over *G. fasciculatum* at intermediate levels of applied phosphrus. SEM can be used as an aid in taxonomic characterization of VAM fungi.

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Effect of different VAM fungi under varying levels of phosphorus on growth and nutrition uptake of pigeon pea (*Cajanus cajan*)

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Mycorrhizal plants grow better in infertile soil, largely because of increased uptake of nutrients especially phosphorus. Mycorrhizal fungi enhance water transport in plants (Safir *et al.*, 1971), help plants to withstand high temperature (Marx and Bryan, 1971), promote establishment of plants in wasteland (Marx and Artman, 1979). Pigeon pea (*Cajanus cajan*) plants have short and few root hairs and it will depend more on mycorrhiza as the volume of permeated soil is much greater with the hyphae of a mycorrhizal fungus than with plants root hairs, and for this reason many plants with short or rudimentary hairs depend more on mycorrhizae than do plants with finely branched roots and long and abundant hairs (St. John, 1980). These attributes of mycorrhizae are being considered important in modern agriculture. Looking to these facts the present study was undertaken to test relative performance of pigeon pea to inoculation with different mycorrhizal fungi at varying levels of phosphatic fertilization in pots.

Sandy loam soil which was deficient in phosphorus (3 mg available P/kg of soil extracted with NH_4F+HCL), with pH 7.8 was used. Pots of 30 cm diameter were filled with 5.0 kg sterilized soil. Soil was sterilized by autoclaving at 1.1 kg cm^{-2} pressure for 2 h. The VAM fungi *Glomus fasciculatum* (Thaxt) Gerd and Trappe, *Glomus constrictum* Trappe and *Gigaspora calospora* (Nicol. and Gerd.) Gerd. and Trappe used as inocula were maintained on *Cenchrus ciliaris* a perennial host grown in sterile soil for a period of 90 days. For VAM inoculation extramatrical chlamydospores and infected root pieces of *Cenchrus ciliaris* of the particular fungus (50 ml soil/pot) were layered 2 cm below the soil surface before sowing to produce mycorrhizal plants. Control plants did not receive any inoculum. Four different levels of phosphorus (0 kg P/ha, 25 kg P/ha, 50 kg P/ha and 75 kg P/ha) in the form of single super phosphate were applied in all the pots before sowing. Two seeds of pigeon pea were sown in each pot. The plants were allowed to grow in each pot, after 15 days they were thinned to one per pot. Plants were raised in a wire-mesh house receiving sunlight for 12 h each day and were irrigated with sterilized water. The experiment was laid out in a randomised block design consisting of 4 VAM and 4 levels of phosphorus. Thus, in all there were 16 treatments and each treatment consisted 4 replications.

Plants were harvested after 60 days. Dry weight of shoot and root were recorded. Per cent root colonization by mycorrhizal fungi was determined according to Phillips and Hayman (1970). Mycorrhizal spores in the root zone soil were estimated by wet sieving and decanting technique (Gerdemann and Nicolson, 1963). Shoot and root phosphorus was estimated by the vanadomolybdate yellow colour method (Jackson, 1971). The nitrogen content of the shoot and root was analysed by microkjeldhal method (Bremner, 1960).

The overall growth of mycorrhizal plants was superior to non mycorrhizal plants at all levels of added P and plant shoot and root dry weight were increased. Maximum plant growth improvement was noticed at 50 kg P/ha. Among the three VAM fungi *Glomus constrictum* was superior to *Glomus fasciculatum* and *Gigaspora calospora* in enhancing plant dry weight at all levels of P except at 75 kg P/ha, where *Gigaspora calospora* proved to be more effective than *Glomus constrictum.* Significant increase in shoot and root phosphorus and nitrogen uptake was observed in all the three VAM fungi at all levels of P application. However, highest uptake of phosphorus and nitrogen was observed by *Glomus constrictum* at 50 kg P/ha. Increase in phosphorus application resulted in reduction in the intensity of mycorrhizal infection as well as number of extramatrical chlamydospores in the soil. Maximum number of spores and per cent infection was recorded in *Gigaspora calospora* when no phosphorus was applied.

Pigeon pea plants responded to all the three VA mycorrhizal inoculation, the response being prominent at low levels of P. The results of the present study revealed that high concentration of phosphorus is detrimental to proliferation of the fungal symbiont and subsequent spore production. Excess phosphorus is known to reduce infection and spore production by *Glomus* in *Abelmoscus esculentus* (Krishna and Bagyaraj, 1982). Maximum beneficial effect of mycorrhizal symbiosis was achieved at lower levels of soil fertility. The mycorrhizal dependency is generally high at low levels of added phosphorus. Similar observations were made by Krishna and Dart (1984) in pearl millet. In pigeon pea the increase in plant dry weight and nutrient uptake was so pronounced that this treatment should be adopted for substitution of chemical fertilizer.

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Effect of superphosphate, rock phosphate as sources of phosphorus in combination with *Glomus fasciculatum* on root colonization, growth and chemical composition of blackgram

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Legumes play a major role in agriculture by providing high protein grain and in improving soil fertility (Manjunath and Bagyaraj, 1984). Phosphorus deficiency is probably the major limitation in Indian soils which have high capacity to fix phosphorus. Vesicular arbuscular mycorrhizae which occur widely under various environmental conditions are implicated to enhance phosphorus uptake and growth of legumes. Therefore, there has been increasing interest on the use of vesicular-arbuscular mycorrhiza to improve crop productivity in legumes.

Blackgram (*Vigna mungo* L. Hepper) is an important pulse crop in Andhra Pradesh and is grown widely in rice fallows. Very little information is available about the blackgram and mycorrhizal symbiosis. Therefore, in this paper the effect of vesicular arbuscular mycorrhizal inoculation with added phosphorus on root colonization, plant growth and nutrition of blackgram (cv. LBG-20) is reported. Superphosphate and rock phosphate at three different levels viz., 0, 20 and 40 kg P_2O_5/ha were applied to the pots. The studies were conducted in unsterilized black cotton soil.

Vesicular arbuscular mycorrhizal inoculated blackgram plants recorded higher per cent root colonization over uninoculated plants although the plants were supplemented with phosphorus irrespective of its source and dosage. However, the per cent colonization decreased with corresponding increase in the concentration of phosphorus. Among the two sources of phosphorus, rock phosphate was found to encourage greater root colonization compared to superphosphate at 20 kg/ha. Earlier, Lim and Cole (1984) reported that mycorrhizal root colonization decreased with increase in phophorus levels in legumes.

Mycorrhizal inoculated plants also recorded higher dry weights of root and shoot, total chlorophyll content compared to plants supplied with phosphorus alone. With the increase in the level of phosphorus a corresponding increase in the dry weight of shoot and root was noticed in mycorrhizal inoculated and uninoculated plants when phosphorus was supplied in the form of superphosphate. This is in confirmity with the observation of Mardch *et al.* (1967) in maize.

Application of phosphorus in combination with *Glomus fasciculatum* had increased effect in enhancing nitrogen, phosphorus, potassium, calcium and magnesium content in blackgram plants compared to uninoculated plants supplemented with phosphorus alone. Krishna (1984) also reported identical observation in peanut. Super phosphate as a phosphorus source enhanced nitrogen, potassium, calcium and magnesium content to a greater extent compared to rock phosphate irrespective of its levels. But the phosphorus concentration was more in the plants supplemented with rock phosphate. Murdoch *et al.* (1967) also observed similar results. However, phosphorus when applied at 40 kg P_2O_5/ha enhanced the per cent nitrogen, phosphorus, potassium, calcium and magnesium irrespective of phosphorus sources in mycorrhizal inoculated as well as uninoculated plants.

Inoculation with *Glomus fasciculatum* in combination with phosphorus increased per cent mycorrhizal root colonization, dry weight of root and shoot, total chlorophyll content and chemical contents of blackgram plants over uninoculated plants supplied with phosphorus alone. However, increased application of phosphorus decreased the per cent root colonization. Both VA mycorrhizal inoculated and uninoculated blackgram plants derived maximum benefit when phosphorus was supplied in the form of superphosphate than as rock phosphate.

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Response of brinjal genotypes in terms of dry weight and phosphorus uptake as influenced by VAM inoculation

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The host genotype dependence for response to VAM inoculation has been shown to exist in many crops. However, the information on genotypical response of brinjal to VAM inoculation is lacking. A pot culture experiment was, therefore, conducted during rainy season (August-November) of 1988 in FCRD with three replications to study the response of some brinjal genotypes to VAM inoculation under unsterile soil conditions. The mycorrhizal seedlings of eleven genotypes viz., Vaishali, Manjri Gota, Pragati, Annamalai, PS-8, Dorli, Borgaon-1, Ruchira, Krishnakathi, P.P. Long and *Solanum writti* were raised by inoculating the nursery bed (1.0×3.0 m) with 5 kg inoculum of *Glomus fasciculatum* consisting of extramatrical chlamydospores (415 spores 50^{-1} ml), infected guinea grass roots and the soil. The seedlings raised from the beds applied with uninfected soil+sand (1:1) mixture served as control. The 45 day old seedlings were transplanted to the pots (one seedling pot^{-1}) containing 8 kg P deficient unsterile soil (Olsen P=3.00 ppm). The plants were fertilized with N, P_2O_5 and K_2O @ 100, 50 and 50 kg ha^{-1} respectively. The plants were harvested at 50 days after transplanting (flowering stage). The observations on shoot and root dry weights were recorded and the P uptake was determined by vanadomolybdate yellow colour method (Jackson, 1971). The mycorrhizal dependency was worked out and the VAM root colonization was determined by following the root slide technique (Nicolson, 1960) after clearing the roots with KOH and staining with trypan blue (Phillips and Hayman, 1970).

The results revealed that the per cent VAM colonization in roots of genotypes inoculated with *G. fasciculatum* varied from 48.0 to 65.33 which reflected in varied response of the genotypes towards the dry matter and P uptake. The shoot dry weights differred significantly for the genotypes and the inoculation but the interactions were non-significant. The genotypes, P.P. Long (7.17 g $plant^{-1}$) and Ruchira (7.0 g $plant^{-1}$) recorded significantly higher shoot dry weight than Borgaon-1, *S. writti*, Vaishali, Dorli, Krishnakathi and Annamalai. The mycorrhizal plants across the genotypes recorded significantly higher mean shoot dry weights (6.57 g $plant^{-1}$) than the non-mycorrhizal ones (4.20 g $plant^{-1}$). The root dry weights differred significantly only for the inoculation treatments. Although the genotypes exhibited the non-significant differences, Borgaon-1 recorded the highest root dry weight (1.9 g plant^{-1}). The mycorrhizal inoculation across different genotypes recorded significantly superior mean root dry weight (1.93 g plant^{-1}) over the non-mycorrhizal ones (1.35 g plant^{-1}). The P uptake also differred significantly only for the inoculation treatments. The mycorrhizal plants across the genotypes recorded significantly higher mean P uptake (62.01 mg plant^{-1}) than the non-mycorrhizal ones (32.16 mg plant^{-1}).

The genotypes displayed varying VAM dependency as revealed through their shoot and root dry weights and the uptake of phosphorus. The shoot and root dry weights in mycorrhizal plants of various genotypes ranged from 1.20 to 3.83 times and 1.03 to 1.78 times respectively that of non-mycorrhizal plants. The mycorrhizal dependency of the genotypes ranged from 117.20 to 309.91 per cent. An increase in P uptake by eleven genotypes due to mycorrhizal inoculation ranged from 1.27 to 4.30 times that of comparable controls. The results, in general, indicated a genotype-dependent variation in dry matter and P uptake as influenced by VAM inoculation.

Thus the brinjal genotypes could exhibit different degrees of VAM colonization and differential response to inoculation in terms of their dry matter and P uptake. The genotypes viz., *S. writti*, PS-8, Dorli, Pragati and Borgaon-1 were found to be better VAM responsive than the others and indicated the possibility of their use in the plant breeding programmes. The genotypic variation in colonization and response to VAM inoculation could be due to an interaction between the host genotypes and the VAM strain preferences. The number of infection sites on the roots could also be a factor and different levels of colonization amongst the genotypes could arise from differences in the rate of growth of the fungus through the root cortex (Smith and Walker, 1981). The study also threw light on the need for rigorous screening of the available brinjal germplasm to search for the lines with high levels of VAM colonization for further utilization in the plant breeding programmes for enhanced yields.

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Improved yields in potato through mycorrhizal inoculations

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Growth responses to mycorrhizal inoculations have been demonstrated in many crops and this is attributed to the increased phosphate uptake from soil (Hayman, 1980). As other crops, potato also responds to mycorrhiza in terms of increased vegetative growth and tuber yield (Black and Tinker, 1977). In view of this, field experiments were carried out at Patna for two consecutive years, 1987-88 and 1988-89 to assess the role of mycorrhiza on two potato cultivars, Kufri Sindhuri and Kufri Lalima.

The inocula of *Glomus masseae* and *G. fasciculatum* were obtained and multiplied on Ragi plants grown in infertile soils. The test inoculum consisted of mycorrhizal roots and spores and the spore-count was determined before inoculation in the field by plate method (Smith and Skipper, 1979). With a view to have less fertile soil, fields were selected which did not receive phosphatic fertilisers during previous two crop seasons. The soil analysis indicated the level of Organic carbon, available P_2O_5 and available K_2O as : 0.35%, 27.5 and 185.0 kg/ha, respectively.

In the RBD experiment, the mycorrhizal inoculum (8 spores/g soil) was applied in furrows before the placement of tubers followed by ridge formation. Normal irrigations were given during the crop period. At the maturity of the crop, the root samples were drawn and processed (Phillips and Hayman, 1970) and the yields were also recorded. The quantification of mycorrhization in terms of per cent root infection and per cent root length area infected were calculated (Biermann and Linderman, 1981).

In cv. Kufri Sindhuri, the per cent root infection and per cent root length area infected were 48.4 and 2.74, respectively in inoculated plants whereas these were 28.0 and 0.20%, respectively in non-inoculated plants. Similarly in cv. Kufri Lalima, the per cent root infection and per cent root length area infected in inoculated and non-inoculated plants were 87.5 & 4.63 and 60.0 & 1.73, respectively. These results clearly indicated the increased level of mycorrhization in the inoculated plants over the noninoculated plants, however, the mycorrhization in non-inoculated plants is attributed to the native population of mpcorrhiza in the test field.

The data on tuber yield were also comparable in inoculated and non-inoculated plants of both cultivars. In cv. Kufri Sindhuri, the yields were 99.01 and 93.77

q/ha in inoculated and non-inoculated plants. In cv. Kufri Lalima, the yields (q/ha) in inoculated and non-inoculated plants were 96.22 and 92.26, respectively. Thus the mycorrhizal inoculations resulted in the increased yields in both cultivars to the tune of 5.5 and 4.2%, respectively. Although these increases appeared to be marginal but statistically significant.

The present study has revealed that mycorrhizal applications result in the increased yields leading to a marginal benefit. However, there still remains a scope for use of selective efficient strains of mycorrhizal fungi which may in turn prove to be more beneficial in term of substantial increased yields. Their effects are likely to be more pronounced in lesser fertile or infertile soils.

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Studies on vesicular-Arbuscular (VA) mycorrhizal impact on growth and development of cowpea (*Vigna unguiculata* (L.) Walp)

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The wide spread occurrence of VA-mycorrhiza in nature and their importance in mineral nutrition of almost all plants have sufficiently been documented. Several field, laboratory as well as green house experiments have demonstrated that VA-mycorrhizal colonization can greatly improve growth and nutrition of host plants (Mosse, 1973; Sanders, 1977; Jalali and Thareja, 1985) and can also induce biological suppression of soil-borne pathogens effectively (Jalali and Thareja, 1981). Although VA-mycorrhizal endophytes are associated symbiotically with most crop plants, little is known of their role in the utilization of less or unavailable sources of phosphorus for plant growth. The aim of the present study was to investigate the impact of VA-mycorrhizal inoculation on growth and development, and nutrient-contents (N, P and K) of cowpea (*Vigna unguiculata*) grown in nutrient-deficient soil.

In all these studies, nutrient-deficient soil (collected from Rawalvas, Haryana) was used. Procedures adopted by Phillips and Hayman (1970) and Jalali and Domsch (1975) for root clearing and staining, and assessment of mycorhizal colonization respectively, were employed.

VA-mycorrhizal inoculation induced significant increase in height of the plants as compared to uninoculated controls. Mycorrhizal infection resulted in significant increase in total dry matter production of root and shoot. VA-mycorrhizal endophyte developed extensively (53.78% colonization) in root system of cowpea.

N, P and K content of plants also increased significantly in mycorrhizal inoculated plants as compared to control. Of these nutrients, the pronounced Increase was observed in the uptake of P.

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Vesicular-arbuscular (VA) mycorrhiza in presence of *Rhizobium* sp. enhances nodulation, N_2 fixation, N utilization of pigeon pea (*Cajanus cajan*) as assessed with a ^{15}N technique

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Vesicular-arbuscular mycorrhizae (VAM) fungi are now known to enhance nutrient uptake, especially in P-deficient soil. However, N_2 fixation of various crops such as soybean, chickpea etc. has been reported to be enhanced in presence of VAM inoculation, as assessed by ^{15}N isotopes dilution technique (Barea *et al.*, 1987; Subba Rao *et al.*, 1986). Pigeon pea (*C. cajan*) forms very poor nodule with native *Rhizobium* sp. under field condition and life of the nodule is also very short, thus reflect on N_2 fixation. Therefore it was essential to study the role of VAM fungi inoculation, in association with *Rhizobium* sp. on symbiotic process, especially on N_2 fixation, of this legume, using ^{15}N isotopes dilution technique under soil potted condition.

Root colonization (%) of pigeon pea (*C. cajan*) was increased due to soil inoculation of *G. fasciculatus* (VAM). However, higher root colonization was recorded with combined inoculation of *Rhizobium*+VAM than soil inoculated with VAM alone, at various levels of nitrogenous and phosphatic fertilization. Fertilization with 20 kg P_2O_5 ha^{-1} brought perceptible increase in mycorrhizal infection in root over control. However, combination of nitrogen and phosphorus fertilizer (20 kg N ha^{-1} and 50 kg P_2O_5 ha^{-1}) in presence of VAM resulted in maximum mycorrhizal root infection.

Improvement in nodulation was observed due to soil inoculation with VAM alone and it was scored more in presence of 20 kg N ha^{-1} and 50 kg P_2O_5 ha^{-1}. The increase in nodulation with VAM, in presence of N and P, was almost equivalent to the effects of seed inoculation with *Rhizobium* sp. alone. Another interesting observation could be made from the result that incorporation of 20 kg N ha^{-1} did not influence nodule number. However, application of N and P (20 kg N ha^{-1} and 50 kg P_2O_5 ha^{-1}) resulted in significant increase in nodulation over 0 kg P_2O_5 ha^{-1} level, especially with dual inoculation of *Rhizobium* sp.+VAM. Significant increase in nodule number was also recorded with *Rhizobium* sp. inoculation at all levels of N and P fertilization of soil.

Grain and shoot yield of pigeon pea was significantly increased due to *Rhizobium* sp. and VAM inoculation, over uninoculated control, at all levels of N and P fertilization. The increase in grain and shoot due to VAM inoculation was almost equivalent to yield recorded with *Rhizobium* sp. inoculation alone and combined inoculation with the organisms significantly enhanced the yield (grain and shoot), especially in the presence of 20 kg N ha^{-1}. The result also indicate that pigeon pea crop was more benefited due to P and N (50 kg P ha^{-1} and 20 kg N ha^{-1}) application, as far as yield was concerned especially when the two endophytes are inoculated together.

In this experiment no generalization can be made with the increase in % nitrogen of grain and shoot. However, total N yield in grain and shoot as calculated on % N and total yield (grain and shoot, respectively) was recorded maximum with combined inoculation of *Rhizobium* sp. and VAM as compared to the increase brought due to inoculation of individual organism. The total N yield increase of grain and shoot tissue was more pronounced at P and N application (50 kg P_2O_5 ha^{-1} and 20 kg N ha^{-1}, respectively). It was recorded more with VAM than *Rhizobium* sp. inoculation, even in presence of P and N fertilization. It is also worth to point out that total N yield was recorded higher in shoot portion of the plant than grain.

In general % ^{15}N in the grain and shoot tissue of the plant was higher in absence of P and it was even more higher in the presence of *Rhizobium* sp.+VAM inoculation. There was a higher amount of ^{15}N in grain and shoot tissue of VAM inoculated plant as compared to *Rhizobium* sp. treated plant. Percent ^{15}N atomic excess was estimated higher in straw than grain tissue.

Fertilizer N uptake from soil solution to the tissues of grain and shoot was significantly higher with VAM inoculation and it was more with the combined inoculation of *Rhizobium* sp.+VAM, at both P levels. However, significant increase in the fertilizer N uptake was also recorded even at N and P application (20 kg N ha^{-1} and 50 kg P_2O_5 ha^{-1}) with VAM inoculation, as compared to corresponding control. There was a higher uptake of fertilizer N_2 uptake in shoot than grain tissues.

Utilization of applied N $(^{15}NH_4)_2SO_4$ was significantly increased with VAM inoculated plant. Results have also indicated that combined inoculation (*Rhizobium* sp. +VAM) effect on fertilizer utilization was recorded higher than the plant inoculated with individual endophytes. Application of P did not adversely effect the fertilizer utilization by pigeon pea crop. The utilization of fertilizer N was estimated higher in shoot than grain.

In general biological N_2 fixed in grain and straw was higher at 50 kg P ha^{-1} and 20 kg N ha^{-1} fertilizer application either due to inoculation of *Rhizobium* sp., VAM and/or combination of *Rhizobium* sp.+VAM. Biologically fixed N of *C. cajan* (grain and shoot) was recorded more with VAM as compared to *Rhizobium* sp. inoculation treatment. However, it was estimated maximum in presence of two endophytes (*Rhizobium* sp.+VAM). It is worth to mention here that fixed N (biologically) was recroded more in shoot than grain (as estimated by total N yield-total N assimilated in tissue from $(^{15}NH_4)_2SO_4$).

Nodulation and grain yield of pigeon pea (*C. cajan*) was significantly increased due to *Rhizobium* sp. and *Glomus fasciculatus* (VAM) inoculation especially at 50 kg P_2O_5 ha^{-1} application. VAM alone enhanced the nodulation of pigeon pea. Using ^{15}N dilution technique $(^{15}N\ H_4)\ SO_4$ total N yield of the crop, utilization of soil nitrogen and biologically N_2 fixation was more with VAM inoculation, which was at par with *Rhizobium* sp. inoculation. N_2 fixed in grain and straw was higher due to combined effect of *Rhizobium* sp. and VAM, as compared to their individual effect. It was more pronounced in presence of 50 kg P_2O_5 ha^{-1} and 20 kg N ha^{-1} in the form of super phosphate and ammonium sulphate respectively.

Therefore it can be inferred that increase in total N yield of shoot and grain could be due to increase in translocation of soil nitrogen to plant mediated by VAM, resulted in improvement in nutrition of the crop plant. The higher amount of translocation of soil N and P is positively correlated with the intensity of VAM root colonization and symbiotic parameters. Thus VAM has good potential in crop nutrition, in general.

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Effect of interaction between *Rhizobium* and VA mycorrhizal fungi inoculation on the growth of groundnut applied with different levels of gypsum

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The nutritional requirements of legumes are in no way different from other plants, except their potential for symbiotic assimilation of dinitorgen, creating special demands, notably for molybedenum, cobalt and also for phosphate, calcium and zinc. Any nutritional disorder may limit nitrogen fixation for the symbiotic system on the growth of plants (Munns and Mosse, 1980).

It is well documented that groundnut is associated with VA-mycorrhizae (Graw and Rehme, 1977). It has been reported that VA-mycorrhizae help in increasing the absorption of nutrients such as calcium and zinc from soil (Copper and Tinker, 1978). In Tamil Nadu gypsum is recommended to get the increased yield of groundnut. There seems to be no information available on the effect of dual inoculation of *Rhizobium* and VA-mycorrhizae with different levels of gypsum on the growth ot groundnut. The objective of this study was to determine the levels of of gypsum for getting maximum benefit while using dual inoculation of *Rhizobium* and VA-mycorrhizal fungi for groundnut. The experiment consisted, of sixteen treatments (4 levels of gypsum × control; *Rhizobium*, VA-Mycorrhizae, *Rhizobium*+ VA-mycorrhizae) with three replications, Plant dry weight, VA-mycorrhizal colonization, P content of plant and calcium content of plant were recorded at 30, 60 and 90th days after sowing.

In general, dual inoculation was found to be superior over individual inoculants or control at 30th and 90th day. Significant interactions were observed between gypsum levels and inoculants on 60th and 90th day so far as dry weight of plant was concerned.

Colonization of root by VA-mycorrhizal fungi significantly increased due to inoculation of VAM fungi alone or combination of *Rhizobium*+VAM over uninolculated control at three stages of growth. There was an increse in colonization of VA mycorrhizal fungi up to 150 kg/ha rate with a decrease in VA-mycorrhizal colonization with further increase up to 450 kg./ha of gypsum. There was gypsum levels and inoculants interaction on 60th and 90th day.

With regard to phosphorus content of plant, significant interactions were observed between the two variables at any sampling time. Dual inoculation of *Rhizobium* and VA-mycorrizal fungi was on par with individual inoculation of VAM fungi on increase of shoot and root P content of groundnut.

So far as calcium content of plant was concerned, there was a large and highly significant response due to dual inoculation with 150 kg./ha of gypsum level with no further response to 450 kg/ha. A significant interaction between inoculation and gypsum levels on shoot calcium content (30th day) and root calcium content (30th and 60th day) was observed.

The present study brings out clearly that magnitude of increase of growth, VA-mycorrhizal colonization and nutrient content in groundnut plant were maximum due to dual inoculation as compared to individual inoculants at 150 kg/ha. P content of groundnut plant was maximum due to dual inoculation at 150 kg/ha. presumably due to the synergistic effect o both the symbionts. However, at 300 kg ha^{-1} or more there was a reduction in VA-mycorrhizal colonization due to VAM application with or without *Rhizobium*. Although information on the influence of gypsum on VAM colonization is scarce, a similar type of work employing calcium (a constituent of gypsum) indicated that higher concentration of calcium greatly inhibited colonization of *Glomus mosseae* (Elmes and Mosse, 1984). Such an apparent change in VAM colonization due to gypsum addition may be due to change in the soil pH (7.5 to 8.6) caused by the addition of gypsum. The present study adds that dual inoculation help grundnut in a large way.

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Yield and nutrient uptake by brinjal as influenced by *Azospirillum brasilense* and/or *Glomus fasciculatum* inoculations under graded phosphorus levels

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VAM inoculations to vegetables have increased the yield through increased uptake of P and other nutrients particularly in low fertility soils (Ramachandra and Rai, 1987). *Azospirillum brasilense* inoculation has also improved the yield and nutrient uptake by vegetables (Patil *et al.*, 1989). Recently, the synergistic interactions of VAM fungi and *Azospirillum* improved the growth, yield and nutrient uptake in various crops (Subba Rao *et al.*, 1985). However, the information on the effect of *A. brasilense* and or *Glomus fasciculatum* inoculations at various levels of applied phosphorus is scanty. A field experiment was, therefore, conducted on brinjal cv. Pragati on a P deficient soil (Olsen P=3.6 ppm; 75 chlamydospores 50^{-1}ml soil) during the rainy season (June-December) of 1988 in a split plot design with four replications. The five P_2O_5 levels (0, 12.5, 25, 37.5 and 50 kg ha^{-1} designated as P_0, P_1, P_2, P_3 and P_4 respectively) were the main treatments whereas the four inoculation treatments (control, *A. brasilense*, *G. fasciculatum* and *A. brasilense*+*G. fasciculatum*) served as the sub-treatments. The mycorrhizal seedlings were raised by inoculating the nursery bed (1.0×3.0 m) with 5 kg inoculum of *G. fasciculatum* (soil+sand (1:1) mixture; 415 chlamydospores 50^{-1} ml). The non-mycorrhizal seedlings were obtained from the bed applied with equal quantity of uninfected mixture. For *A. brasilense* inoculation, the roots of 48 d old seedlings were dipped in the suspension of carrier-based inoculum (250g lit^{-1} water) for half an hour before transplanting to the field plots. Nitrogen (100 kg ha^{-1}), phosphorus (as per main treatments) and potassium (50 kg ha^{-1}) were applied through urea, single superphosphate and muriate of potash respectively. The total fruit yields per plot were recorded in nine pickings. Nitrogen and phosphorus content of fruits of the fifth picking were determined by Micro-Kjeldahl's digestion and distillation and vanadomolybdate yellow colour method (Jackson, 1971).

The results in general revealed that the yield and the uptake of N and P by the fruits differred significantly for the P_2O_5 levels, inoculations and their interactions. The yield was significantly improved with an increase in dose of P_2O_5 upto P_3 level. The yeeld at P_3 level (27.58 t ha^{-1}) was at par with that recorded at P_4 level (27.80 t ha^{-1}). The individual inoculations improved the yield significantly over the control but a synergistic effect was noticed after coinoculation which recorded the highest yield (25.80 t ha^{-1}). The interaction P_3 level × dual inoculation registered the highest yield (30.21 t ha^{-1}) and was significantly superior to all other combinations. The N uptake by fruits at P_4 level was although the highest (61.07 kg ha^{-1}), it was at par with that recorded by P_3 level (60.31 kg ha^{-1}). Both the endo-symbionts significantly enhanced the N uptake over the control but were at par with each other. Combined inoculalion, however, registered the highest N uptake (60.14 kg ha^{-1}). Amongst the interactions, dual inoculation at P_3 level registered the highest N uptake (71.81 kg ha^{-1}) and was significantly superior to all other combinations barring dual inoculation at P_4 level (69.83 kg ha^{-1}). The P_3 level recorded the highest P uptake (13.46 kg ha^{-1}) by fruits and was significantly superior to all other P levels. The combined inoculation registered the highest P uptake (11.61 kg ha^{-1}) followed by *G. fasciculatum* (10.26 kg ha^{-1}) and *A. brasilense* (8.84 kg ha^{-1}). The interaction P_3 level × dual inoculation recorded the highest P uptake (17.10 kg ha^{-1}) and was significantly superior to all other combinations.

Amongst the various P_2O_5 levels, P_3 (75% of recommended dose) coupled with inoculation treatments recorded the almost equal fruit yield (27.58 t ha^{-1}), N uptake (60.31 kg ha^{-1}) and a significantly higher P uptake (13.46 kg ha^{-1}) to that recorded by P_4 level conjugated with inoculations. This may be attributed to the efficient VA fungal activity either alone or in combination with *A. brasilense* at moderate P level. The best mutualistic relations of VAM fungi with the plants have been observed at moderate P levels (Bethlenfalvay *et al.*, 1983). *Azospirillum brasilense* singly could enhance the yield and the uptake of N and P as reported earlier (Palil *et al.*, 1989). *Glomus fasciculatum* inoculation also improved the yield and uptake of N and P (Ramachandra and Rai, 1987). However, their combination resulted in a synergistic interaction which recorded the significant improvement in the yield and uptake of N and P by the fruits (Subba Rao, 1985). Dual inoculation at P_3 level appeared to be the most superior interaction recording the highest yield and uptake of N and P followed by the dual inoculation at P_4 level. Best performance of dual inoculation of pearl millet with *Azospirillum* and *Glomus* at moderate P_2O_5 level has been reported. (Santhanakrishnan and oblisami, 1987). Similar results have also been obtained by coinoculation of brinjal with *Azotobacter chroococcum* and *Glomus fasciculatum* at 50% recommended phosphorus (Ramachandra and Rai, 1987). Thus, it could be concluded that 25% of the recommended phosphorus can be saved if dual inoculation is used for brinjal.

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Mycorrhizal status of some desert plants and their physiological significance

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Vesicular arbuscular mycorrhizae (VAM) is world wide in angiosperms (McGee, 1986). VAM are involved in uptake of essential mineral nutrients and water (Mikola, 1987). The importance of VAM to agricultural crops has been well documented. Studies on sand dunes plants have indicated that VAM plays a significant role in the growth of desert plants (Bergen and Koske, 1984) and stablization of sand dunes. Even though agriculrural and forest lands have been extensively studied for mycorrhizae, there are only a few reports on the significance of VAM under extreme desert conditions. Singh and Varma (1981) have reported the occurrence and importance of VAM in the metabolic functions of Indian xerophytic plants. This study reports the mycorrhizal associations and their significance on plants growing under stress conditions from arid and semi-arid regions.

Plant roots and rhizospheric soils were collected from five regions of Rajasthan (Jodhpur, Ossian, Balotra, Bhikomkor and Shergarh). Except Jodhpur, all were sand dunes areas. The root samples preserved in FAA were cleaned, stained and mounted in lactophenol for examination. The percentage of mycorrhizal infection was determined nonsystematically. The spores were recovered from soil (Varma *et al.*, 1981) and mounted in PVL and observed under compound microscope. The spores were identified following the Manual of Trappe (1982) and Schenck and Perez (1987).

Twenty four plant species from arid regions of Rajasthan belonging to eight families were examined.

Amaranthus caudatus was the most mycorrhized host and *Indigofera cordifolia* did not possess any symbiosis. All spores belonging to cactii (*Opuntia ficusindica, O. vulgaris* and several others) were mycorrhizal.

Fourteen species of different VA mycorrhizal fungal spores were isolated from the rhizospheric soils. They were grouped in six genera : *Endogone* (2spp.), *Gigaspora* (2 spp.), *Glomus* (6 spp.), *Sclerocystis* (2 spp.), *Scutellospora* (1 sp.), *Achylospora* (1 sp.). Most dominant species was *Glomus macrocarpum* which was uniformly present in all the rhizosphere samples. Several spores were seen to be new as they did not resemble with any of the type species. The characteristics of chlamydospores of *Glomus fasciculatum* were : spore size 52—116.2×112.6—118 μm, wall 7.6 μm thick, 3 layered, outer layer 2 μm thick, hyaline to yellow, middle 5 μm thick, yellow to brown and innermost very thin and membranous. Diameter of subtending hypha 12.6 μm. The Sporocarp of *Sclerocystis sinuosa* was brown, 253 μm in diameter. Peridia 12.2 μm, tightly enclosing sporocarp comosed of thick walled sinuous hyphae. Chlamydospores 44.0—115.5×33.0—81.0 μm, ovate, elliptical, fusiform elliptical, wall oi chlamydospore brown, 1.3—4.9 μm thick. The *Glomus macrocarpum* spore was slightly longer than wide, light yellow to golden brown to brown, 143-165-177 μm in diameter, wall 16.8 μm thick, double layered, outer spore wall wrinkled, attachment rarely seen. Spore number was maximum during winter season (1260/50g air dried soil) and remarkably declined in summer months. The pH of the soil samples obtained from the arid zones were alkaline and in general the spore counts were low as compared to semi-arid zones where pH was nearly neutral. This suggests that the growth and spore production is inhibited by alkaline pH. The moisture content varied between 2.5-9.5 per cent.

The spore count was higher when moisture content was above 6 per cent. However, no definite correlation could be established between spore count and mycorrhizal root infection. For example roots of *Aerva javanica* had only 8.6 per cent root infection with 1050 spores per 50 g rhizospheres soil whereas *Amaranthus caudatus* had 185 spores with 65.6 per cent infection. Invariably the rhizosphere soils from the cultivated field showed higher spore counts but the extent of root infection was relatively low. This could be ascribed due to either influence of nutrients in the soil (Singh and Varma, 1981) or water logging or fungal specificity (Khan, 1974). Root sample of cactii showed mycelia and vesicles but no arbuscules, this is in confirmity with the earliar observation of Rose (1981) on a cactus species *Pachycereus pringeli*.

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Distribution and intensity of native VAM in Maharashtra region

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Three dimensional system of soil, VA mycorrhiza and plant, if properly managed may substantially improve the production potential of nutritionally deficient soils and help in conserving the costly fertilizers reserves. However, maximum benefit from the system may be achieved only when a full information regarding plant species, their specific nutritional requirement, their ability to extract the nutrients from the soil, nutrient status of the soil, potentiality of the VA-endomycorrhizae, including their ability of adaptation to a specific soil, climate and plant species is in hand. A part of our findings is included in the present paper, which reports the distribution of VAM in soils at and around Wagholi (Pune).

Rhizosphere samples containing roots and soil were collected from different sites in polythene bags. The roots were cleared and stained bv the procedure of Phillips and Hayman (1970). The VAM propagules were recovered from soil by sieving and decanting (Gerdemann and Nicolson, 1963). Their population was recorded in terms of number per gram oven dry soil.

All the plants studied, showed the presence of VA mycorrhizae, but the extent of colonization varied. A variety of spores were recovered from the rhizosphere soils. They mainly belonged to the genus *Glomus*, however, azygospores of *Acaulospora* or *Gigaspora* were also recovered but very rarely. The higher number of spores (15 spores/g soil) was recorded in cultivated soils than the non-cultivated soils (10 spores/g soil).

The population of spores in the rhizosphere varied with the plant species as well as soils. The average population in the rhizosphere of plants from non-cultivated soil ranged from 5 to 10 spores/g dry soil, the lowest being in the rhizosphere of *Dalbergia sisoo* and highest in that of *Prosopis juliflora.*

In the rhizosphere of plants from cultivated fields, the highest spore population was recorded from millets and forage crops (Gramineae) i. e. 15 and 12 spores/g dry soil respectively. In millets, lowest population (6 spores/g dry soil) was in the rhizosphere of *Triticum aestivum*, while the highest (15 spores/g dry soil) in that of *Sorghum vulgare*. In forage crops, the range of population was from 8 to 12 spores/g dry soil with minimum in the rhizosphere of *Panicum maximum* and maximum in that of *Paspalum notatum*. The population in the rhizosphere of pulses ranged from 6 to 10 spores/g dry soil, the lowest being in the rhizosphere of *Vigna synensis* and highest in that of *Vigna radiata*. The spore population of vegetable plant rhizosphere soils ranged from 2 to 9 spores/g dry soil, the lowest being in the rhizosphere of *Solanum melongena* and highest in that of *Allium cepa*. In the rhizosphere soils of oil seed crops, the spore population was more or less similar and was 11 spores/g dry soil.

The VA mycorrhizal infection consisted of hyphal, vesicles and arbuscules. The percentage infection varied with the soils and plant species. The infection in the plants from non-cultivated fields ranged from 15 to 91%, lowest in *D. sisoo* and highest in *P. juliflora*. The range of infection in the plants from cultivated field was from 0 to 99%, there was no infection in *Solanum melongena* and highest in *Paspalum notatum*. In millets the range of infection was 61–96%, the lowest being in *Pennisetum typhoidium* while highest in *Sorghum vulgare*. The range of infection in pulses varied from 36-85%, the lowest infection was in *Vigna mungo*, while highest in *V. radiata*. In vegetable crops the infetion ranged from 0–89%, no infection was found in *Solanum melongena* where as it was highest in *Allium cepa*. The oil seed crops *Arachis hypogea* and *Helianthus annus* exhibited 92 and 94% infection respectively.

In the Present study rhizosphere soil samples were collected from two different soils. Their plants showed different range of spore population/root infection in different soils. This may be attributed to the differences in the phsico-chemical and biological, characteristics. In all the host plants studied *Paspalum notatum* showed the highest percentage of root infection and considerably high population of spores. This plant is selected as a host plant for studying further aspects in the mass production.

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The occurrence of vesicular-arbuscular-mycorrhizal fungi in arable soils of Konkan region of Maharashtra

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A survey was conducted to quantify spore density and to assess the relation between available phosphorus, total phosphorus, total nitrogen and spore count. Soil samples were collected from five places in the Konkan region of Maharashtra viz. Madban, Dhamnas Ganpatiphule, Chiplun, Sangameshwar and Khed in the first week of June 1989, where the crops grown are generally rice and ragi. The soils are mostly acidic in this region, the pH ranging from 5.01 to 5.77. Spore density ranged from 30 to 715 per 50 gms air dried soil. The wet sieving and decanting technique of Gerdmann and Nicolson (1963) was used for isolating the spores. Species of *Glomus* and *Acaulospora* dominated the soils with few other VAM genera. It is concluded from the survey that there exists no significant relationship between the available phosphorus and spore density in this region.

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Interaction between *Rhizobium*, mycorrhiza, nitrogen and phosphorus and their effect on growth and symbiotic behaviour of *Leucaena leucocephala*

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Symbiotic nitrogen fixation in legumes decreases under P-deficient soils (Mosse, *et al.*, 1976). Under such conditions, application of VA mycorrhizal fungi capable of uptaking phosphorus away from the phosphorus depleted zones enhances symbiotic nitrogen fixation as has been seen in case of arhar and peas (Gupta *et al.*, 1987; Bhandal *et al.*, 1989). However, very little information is available on the role of *Rhizobium* in association with VA mycorrhiza on the growth and symbiotic behaviour of *L. leucocephala*, which is known to be the best forest tree for its quick growth and multiple uses such as fuel, fodder etc. The present investigation deals with the study of interaction of *Rhizobium* isolates and VA mycorrhiza and their effect on growth and symbiotic behaviour of *L. leucocephala* in presence and absence of nitrogen and phosphorus fertilizers.

Rhizobium strains used were isolated from nodules of *L. leucocephala* grown in Ludhiana district. VA mycorrhizal fungus, *Glomus fasciculatum* grown on lentil roots in sterilized soil was used.

Pot experiment was conducted under sterile conditions in two sets. In the first set, four surface-sterilized seeds treated with *Rhizobium* cultures (10^8 c. f. u./g) were sown in pots containing different levels of nitrogen (CAN) and phosphorus (superphosphate) which were later thined to one plant per pot. In the second set of experiment, the pots were inoculated with VAM spore suspension (200 spores/pot) after 10 days of growth.

Observations were made for plant height, plant dry weight, nodule number, nodule dry weight and nitrogenase activity (Hardy *et al.*, 1968); and phosphorus (Jackson, 1983) and nitrogen (McKenzie and Wallace, 1954) concentrations in stem and leaves after 60 days of growth.

Rhizobium, nitrogen and phosphorus interaction was found significant for plant dry weight, nodule dry weight and nitrogenase activity both in absence and presence of mycorrhiza. The best combinations were found to be $P_1N_1\ M^+\ R_4$,

P_1N_0 M^+R_5 and P_1N_1 M^+R_5 for plant dry weight, nodule dry weight and nitrogenase activity respectively.

All the interactions, viz., *Rhizobium*, nitrogen and phosphorus, nitrogen and *Rhizobium* as well as *Rhizobium* and phosphorus significantly affected the accumulation of phosphorus and nitrogen in stem and leaves both in presence and absence of mycorrhiza.

Relatively low values of all parameters at P_0 level suggests the need of phosphorus application for the establishment of *Leucaena* plants. The improvement in ancillary characters and nitrogenase activity by mycorrhizal application suggests positive role of mycorrhiza in plant productivity.

Phosphorus is a critical limiting factor in case of legumes, translocation of phosphorus by mycorrhiza not only improves the growth of host but also helps in nodulation and nitrogen fixation.

Dual inoculation with *Rhizobium* and mycorrhizal fungi not only enhances the nutrient content in the above grand plant material but also seems to provide a well-balanced and regulated nutrient supply, consequently the biosynthetic processes taking place in these adequately established legume. *Rhizobium* mycorrhizal association can lead to better productivity of *Leucaena leucocephala*.

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Vesicular-arbuscular mycorrhizal associations in *Glycine max* (L.) Merrill, improves the symbiotic nitrogen fixation under water stress

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Water is prerequisite for the growth of any plant. Plants growing in a natural environments are rarely free from water stress. Low availability of water exerts a controlling influence on crop distribution and productivity (Fischer and Turner, 1978). Interaction between plant and bacteria determines the efficacy of symbiotic nitrogen fixation in leguminous plants and it can be affected by both the aerial and below ground environments. The moisture stress has detrimental effect on the process of nitrogen fixation. This effect has been well documented for numerous crop species (Sprent, 1971; Minchin and Pate, 1975).

Under low moisture levels, water absorption capacity of roots might be enhanced by mycorrhizae. The first systematic examination of mycorrhizal influence on plant-water relations was conducted by Safir *et al.* (1972) on soybeen plants.

The main aim of this research was to investigate the enhancement of growth and nitrogen fixation in response to dual infection of *Glycine max* and to study the effects of VA-mycorrhizal formation on nitrogen fixation and growth, in nodulated and non-nodulated soybean under water stress.

In order to evaluate the effect of different VA-mycorrhizal fungi on biological nitrogen fixation in soybean (cv. Lee) root nodules under moisture stress, the system developed by Khanna-Chopra *et al.* (1984) was adopted with some modifications. Pots (30 × 30 cm.) were filled with sandy-loam soil (pH 8.2 and available P–3.2 kg ha^{-1}) up to 18 cm. from the bottom, and 4 cm. layer of gravel (0.5–1.5 cm. in size) was provided above the soil. The portion above the gravel (8.0) cm.) was filled wiih sandy-loam soil. A plastic pipe of 3.0 cm. diameter with a plastic container (200 ml.) at top was kept vertically on the surface of gravel for providing water to the lower part of the pot by drip action. The set up ensured deficit in the soil zone containing nodules but maintaining adequate water supply to the root system. The assembly without plastic pipe and container were steam sterilized before use.

Soil-sand mixture containing extramatrical chlamydospores and infected root segments of *Cenchrus ciliaris*, infected by either *Glomus fasciculatum* Thaxter Sensu

Gerd., *G. mosseae* Nicol. and Gerd. and *Gigaspora margarita* Becker and Hall, grown for 100 days served as the inoculum. The inoculum contained 200 chlamydospores per 100 g of inoculum. A thin layer of inoculum (200 ml) was placed 2-3 cm. below the soil surface in pots before sowing to obtain mycorrhizal plants. The control treatments received sterilized VAM fungal inoculum. The seeds of soybean (*Glycine max*, cv. Lee) were sown in the pots. Four plants were allowed to grow in each pot. The experiment was laidout in a randomized block design consisting five treatments and each was replicated thrice. A basal dose of nitrogen at the rate of 25 kg ha^{-1} and phosphorus at the rate of 50 kg P_2O_5 ha^{-1} in the form of urea and super phosphate, respectively, were applied to each treatment.

All the pots received uniform irrigation with 300 ml of water each in upper and lower soil zone for the first thirty-five days. Thereafter, the pots were divided into following three sets :

Set I_1 : 300 ml of water day^{-1} was given to the top soil and 300 ml of water day^{-1} was given through the container attached to the pipe, which served as irrigated control.

Set I_2 : A total of 600 ml. of water (200 ml each thrice a day) was given to the lower soil soil zone through the pipe, which created stress in the upper zone.

Set I_3 : Lower zone was watered with half the quantity provided to Set I_2.

Colonization of roots with VA-mycorrhizal fungi was detected by following the method of Phillips and Hayman (1970) with some modifications. The tertiary root segments taken in vials containing 8 per cent KOH were allowed to clear overnight at room temperature. After decanting the KOH solution, excess alkali was neutralized with 1 per cent HCl and stained with trypan blue for 12 hrs. The per cent mycorrhizal colonization was determined by using the systematic slide method (Hayman, 1970). Nitrogenase activity in terms of ARA levels in intact root nodules was estimated following the method of Hardy *et al.* (1968). Plant biomass, after drying at 60°C till constant weight, was recorded. Observations for all the parameters were recorded at the early pod formation stage,

Moisture stress in the upper zone of soil had detrimental effects which resulted in reduced number of nodules, dry weight of nodules, plant biomass and nitrogenase activity as compared to the plants grown under normal conditions.

In the set I_2, the maximum per cent of mycorrhizal colonization of 26.50 was observed in the plants inoculated with *G. margarita*-SB 113 together. The intensities of reduction in number of nodules, dry weight of nodules and nitrogenase activity, under water stress, varied due to inoculation with different VA-mycorrhizal fungi. The maximum per cent of reduction (147%) in the number of nodules occurred in the plants inoculated with SB 113 alone, whereas, in the presence of various VAM fungi along with bradyrhizobia it ranged between 41-59 per cent. Reduction in dry weight of nodules of the plants grown in the set I_2 ranged between 7-65 per cent as compared to plants grown in Set I_1. Although minimum reduction (7%) was noticed in the plants receiving dual inoculum of *G. mosseae* and bradyrhizobia, the highest dry weight of nodules (335 mg plant^{-1}) was registered in the treatments receiving dual inoculum of *G. margarita* and bradyrhizobia. Average reduction in nitrogenase activity in nodules of the plants grown in set I_2 as compared to set I_1, ranged from 8-29 per cent. In general, nitrogenase activity was improved significantly due to various VA-mycorrhizal fungi. In set I_2 highest increase in nitrogenase activity was registered (185%) due to dual inoculation with *G. margarita* and bradyrhizobia over the inoculation with bradyrhizobia alone.

In the nodules obtained from plants stressed in the upper zone, reduced nitrogenase activity was observed (Khann-Chopra *et al.*, 1984) in comparison to in nodules obtained from fully irrigated plants. Nitrogen fixation has been shown to be sensitive to reduction in soil water availability for numerous crops (Engin and Sprent, 1973; Minchin and Pate, 1975). Finn and Brun (1980) have suggested that water stress reduces nitrogen fixation by the inability of stressed leaves to supply photosynthates to nodules. Total plant biomass also responded significantly to the dual inoculation with VA-mycorrhizal fungi and bradyrhizobia under normal as well as stress conditions.

Beneficial effects of VAM towards increasing various parameters including nitrogenase activity under water stress condition could be attributed to :

i) Fungal hyphae extending out into the soil, accounting for the increased ability in water uptake

ii) The hyphae could enhance nutrient uptake, which in turn, could decrease the resistance to water transport with in the roots

iii) The hyphae, which penetrate the root cortex to the endodermis could provide a low resistance pathway for water movement across the root (Safir *et al.*, 1971).

Nodular water probably flows through the root vascular tissue before entering the nodule (Sprent, 1972). So, mycorrhizal hyphae could enhance the flow of water to the nodule through the root vascular tissue, by tapping the water sources available outside the vicinity of rhizosphere and prevent the nodule from dessication.

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Effect of VAM on mulberry cultivation : New avenues of VAM application

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Mulberry is widely used for sericulture in India, as well as other silk producing countries of the world. The occurrence of vesicular arbuscular mycorrhiza (VAM) in mulberry is of great significance, as it is efficient in absorbing nutrients from soils and enhancing the root and shoot system for fixing phosphorus in phosphorus-deficient soils. It also increases the water absorbing capacity of the roots.

VAM isolated from the roots of field collected *Morus alba* (LK_2 variety) were inoculated in sterile soil in which the susceptible sorghum seeds were planted. Sorghum has been reported to be a good host for the multiplication of VAM. Hence studies were undertaken to utilise the VAM rich bed for replantation of mulberry cuttings.

Microscopic examinations of the roots revealed that these were heavily infested with VAM. Studies on the sprouting, growth rates in the root system and the multiplication of VAM will be discussed. This has great potential for dry land farming, where there is low soils and less nutrients.

Side-effects of pesticides on mycorrhizal system-an overview

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Conceptually, pesticides are tailored to react with living cells. Although these toxicants are directed against target pathogenic organisms, a good number of these biocides have deleterious influences on non-target organisms including symbiotic relationships involved in mycorrhizal model systems. While quantifying the impact of soil fungitoxicants on the growth and development of VA mycorrhiza in wheat, Jalali (1979) observed that soil application of four toxicants adversely affected the colonization of the mycorrhizal fungi in host roots, and such effects were most pronouced in PCNB-treated soil. The ability for increased phosphate uptake by mycorrhizal root was also lost when soils were treated with thiram or PCNB. Earlier, Jalali and Domsch (1975) observed that seed, as well as foliar applications, with conventional and systemic fungitoxicants restricted the development of mycorrhizal endophytes on host roots. They postulated that since foliarly-applied pesticides may not be translocated intact to the roots, the side effects on mycorrhizae may be brought about by changes in the spectrum of root exudates as a result of the stress exerted by the pesticides. In further tests the systemic fungicides triforine and tridemorph applied to the host foliage changed the pattern of amino acid exudation (Jalali and Domsch, 1977).

Formation of mycorrhiza in clover roots was prevented by soil drenches of benomyl and thiophenate methyl, and the spread of established infections was halted (Boatman *et al.*, 1978). Immersion of fungal inoculum in suspensions of the fungicides reduced infectivity. However, clover plants grown in benomyl-treated soil did not retain enough fungicide to affect the amount of infection after transplanting into benomyl-free soil. Similarly, while studying the effect of five fungicides on VA mycorrhizal symbiosis in onion grown in phosphate-deficient soils, Manjunath & Bagyaraj (1984) showed that except for captan, all other test fungicides applied even at lower concentrations reduced plant growth and phosphate uptake.

The foliar application of the symplastic fungicide fosetyl-al at different concentrations to mycorrhizal leek (*Alium porrum*) plants significantly increased colonization by *Glomus intraradices*, the number of intra-matrical vesicles and plant growth, compared to inoculated but untreated plants (Jabaji-Hare and Kendrik, 1987) and these effects did not diminish with time. The mechanism by which fosetyl-al produces this effect seems to be related to its role in altering root exudation. This fungicide causes a significant increase in exudation of soluble sugar from mycorrhizal roots, especially during the first few days after treatment.

Population dynamics of mycorrhiza fungi in arable fields are inversely affected more by fungicides than by herbicides, nematicides and insecticides. These chemicals usually decrease mycorrhizal infection and spore numbers, similar to the effects of benomyl applications. Benomyl, which decomposes in soil to yield carbendazim and butyl isocyanate, has been shown to reduce percentage mycorrhizal infection in root samples (Tommerup and Briggs, 1981). Benomyl suspensions are toxic to several mycorrhizal endophytes on direct immersion and when mixed with irradiated or infested soil. This probably offers positive indications that soil drenched with benomyl is toxic to external mycelium from established infections so that new roots remain uninfected. Ectomycorrhizal development by artificially-introduced *Pisolithus tinctorius* and naturally occurring fungi was significantly inhibited by three to four foliar applications with triadmefon applied with the initial objective of controlling fusiform rust on loblolly seedlings (Marx *et al.*, 1986). Basidiocarp production by ectomycorrhizal fungi in fungicide-treated plots was 3 to 10 times less than in the control.

Several investigators have demonstrated that several commonly used herbicides drastically affect mycorrhizal fungi. However, Kelley and South (1980) observed that with few exceptions, herbicide concentration necessary to affect fungal growth were much higher than recommended doses. Furthermore, several herbicides are reported to stimulate growth of some ectomycorrhizal fungi in axenic cultures, usually at low concentrations. However, this response does not correlate with any specific group of toxicants. Chlorotoluron, under certain conditions, has resulted in increased spore populations of mycorrhizal fungi in soil, but mycorrhizal formation in hosts remains either unaffected or suppressed (Nemec and Tucker, 1983). Schwab *et al.* (1982) postulated that mycorrhizal formation was promoted by a simazine-induced increase in the root exudation of sugars and amino acids. In nurseries, herbicides that tend to promote mycorrhizal diversity might be preferred under certain conditions, but in others it might be possible to control weeds with a toxicant that at the same time is able to promote growth of specific, inoculated mycorrhizal fungi.

By and large, the systemic pesticides as a group appear more damaging to mycorrhizal symbiosis than non-systemic ones. Such toxicants affect spore germination and ultimately colonization of the mycorrhizal endophyte within the host root system. Since translocation is primarily upwards, systemics would be more damaging to mycorrhizal fungi when applied as soil drenches. These findings indicate that investigations on the impact of a pesticide on mycorrhizal colonization should consider whether infection level attained under pesticide use will benefit the host.

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Interaction between vesicular arbuscular mycorrhizal fungi and fungicides

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The importance of vesicular arbuscular mycorrhiza (VAM) fungi in plant growth, mineral nutrition and biological suppression of soil-borne plant pathogens has been demonstrated (Jalali, 1989). VAM are formed by *Glomus* species with roots of many cultivated crops (Gerdemann and Trappe, 1974). The use of fungicides for the control of some plant diseases is indispensible. The review examines some of the interactions between fungicides and VAM fungi.

A. Interactions :

Diverse groups of fungicides are primarily used to reduce soil and seed-borne pathogens invariably and their application, however, results in increase (Jabaji Hare and Kendrick, 1985) or no effect (Nemec, 1980) or in reduction or delaying VAM infection (Nesheim and Linn, 1969) but rarely eliminate them Menge, 1982). The subject has been reviewed recently (Vyas, 1988). The adverse effect of fungicides such as botran, PCNB, vapam, vorlex, mylon, lanstan in corn (Nesheim and Linn, 1969), benomyl and thiophanate in clover, onion and strawberries (Clark, 1978), dichlofluanid, ethirimol, chloraniformethan, thiabendazole, triforine, and triademofon in wheat (Jalali and Domsch, 1975), benomyl in soybean and red clover (Bailly and Safir, 1978; Hale and Sanders, 1982) and dicloran, captan, benomyl, dazomet and PCNB in several crops (Menge, 1982; Nemec, 1980) have been observed.

Application time has significant effect on the interaction of VAM and fungicides. Chlorothalonil, PCNB, benomyl, triademefon, chloroneb and iprodione reduced mycorrhizal development of bentgrass when applied 4 to 8 weeks after seeding and inoculated with *G. fasciculatum*. However, when they are applied 16-20 weeks after seeding did not have adverse effect (Rhodes and Larson, 1981).

Some fungicides such as pyroxychlor and prothiocarb, selective to oomycetes, appeared to have no effect on the VAM development (Poget *et al.*, 1976; Stewart and Pfeger, 1977). Similarly captan at certain concentrations also had no significant effect on infection (Nemec, 1980). Chloroneb did not reduce VAM infection in bentgrass (Rhodes and Larson, 1981).

On the other hand, some fungicides increased root colonization and increased VAM activity and stimulated infection. An increase in VAM infection by soil application of captan in beans (Sutton and Sheppard, 1976) and dibromopropane in cotton (Bird *et al.*, 1974) was observed. Terrazolc significantly increased root colonization and spore production by *G. fasciculatum* in sorghum (Menge, 1982) while metalaxyl in maize (Groth and Martinson, 1983). Foliar application of systemic symplastic and anti-oomycetes fungicide, fosetyl-Al to leek plants significantly increased VAM colonization by *Glomus* sp. (Jabaji-Hare and Kendrick, 1985). Earlier ro this Clark (1978) reprted 10 per cent increase in colonization by fosetyl-Al by *Glomus microcarpum* and two other unidentified *Glomus* spp. in lettuce by folair application.

B. Mechanisms of fungitoxicity and stimulation :

1. Fungitoxicity : Fungicides are *per se* toxic to the VAM spores and mycelium. Benomyl was toxic to VAM in a 3:1 soil and sand mixture (Sutton and Sheppard, 1976), although other group of zygomycetes are innocuous (Edgington *et al.*, 1974).

2. Stimulatton : The increase in VAM activity in leek after folair application was due to root exudation of soluble sugars in mycorrhizal plant (M) in higher concetration than in nonmycorrhizal plants (NM); there was a significant increase in total lipid also in M roots. However, this increase was not observed in NM plants (Janaji-Hare and Kendrick, 1985). Fosetyl-Al directly or indirectly influenced the physiology of both host plant and VAM fungus.

C. Effect of fungicides on phosphate accumulation by mycorrhiza :

VAM fungi in plants have been demonstrated to increase plant growth by utilizing less available form of phosphorus in the soil. A 16-fold reduction of ^{32}P uptake was observed in 12 week-old M onions when PCNB was applied 48 hrs before application of labelled phosphorus (Gray and Gerdemann, 1969). Similar effects ware observed in maize and also in onion (Hirrel and Gerdemann, 1979). Soil drenches reduced phosphate uptake by benomyl and thiophanate in onion and strawberries (Clark, 1978) and PCNB and thiram at 100 ppm each in wheat (Jalali, 1979).

Studies on VAM at the root surface are of vital interest because of their potential biofertilizer effects and biocontrol effects (Clark, 1978; Jalali, 1979). The repeated use of fungicides in farming systems merits careful consideration. It is suggested that the fungicides which posses narrow-spectrum, non-volatile, fungistatis and that are relatively nonspecific for VAM should be incorporated in the crop productivity schedule.

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Interaction of dual inoculation of VA-mycorrhiza and *Rhizobium* with pesticides treated chickpea plants

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Several reviews have compiled information on various factors, including microorganisms, which affect parasitism of plant roots by vesicular-arbuscular (VA) mycorrhiza. Informations have also been generated on side-effects of pesticides on this microbial model system. However, data available on interaction of dual inoculation of VA-mycorrhizal endophyte and *Rhizobium* with pesticides on crop plants, are fragmentary. Inoculation of chickpea plants with VA-mycorrhiza+*Rhizobium* was found to have synergistic effect on nodulation, plant growth, dry matter production, nitrogen fixation and phosphorus uptake (Jalali and Thareja, 1982). Several legume crops inoculated with VA-mycorrhizal fungi and *Rhizobium* usually had a beneficial effect on plant growth, nodulation and nitrogen assimilation (Bagyaraj *et al.*, 1979; Gueyo *et al.*, 1988). The effect of dual inoculation of VA-mycorrhiza and *Rhizobium* with captan on field beans revealed its adverse effect on plant growth and nitrogen fixation (Kuccey and Bonetti, 1988). Several other studies clearly revealed that an effective VA-mycorrhizal fungus and *Rhizobium* could contribute to the efficiency of such a system, especially in nutrient-deficient soils.

In the present studies, interaction of dual inoculation of VA-mycorrhizal fungus (*Glomus fasciculatum*) and *Rhizobium* with pesticide-treated chickpea *(Cicer arietinum)* plants grown in nutrient-deficient soil were assessed. The test pesticides used were : bavistin and aldrin (as seed treatment) and basalin (as soil application). Inoculation with *G. fasciculatum* and *Rhizobium* sp. (strain no. Ca 181) were carried out after the chickpea seeds (cv. H 75-35) were treated with bavistin and aldrin. In case of soil application, basalin was thoroughly mixed with the test soil. Procedures adopted by Phillips and Hayman (1970) & Jalali and Domsch (1975) for root clearing and staining, and assessment of mycorrhizal infection respectively were employed.

Among the pesticides used as seed treatment, aldrin had most inhibitory effect on dual application (*G. fasciculatum*+*Rhizobium*) followed by bavistin. These pesticides significantly altered the plant height, total dry matter production of root and shoot, number of nodules and pods/plant, population dynamics of mycorrhizal sporocarps, as well as grain weight, as compared to uninoculated and inoculated plants with either *G. fasciculatum* or *Rhizobium*. N, P and K contents were also significantly reduced by the application of these pesticides as compared to controls. The soil application of basalin resulted in most inhibitory effect on mycorrhizal colonization, total dry matter production with all recorded parameters, as compared to other pesticidal applications. Most potent effect was, however, expressed in case of number of nodules/plant and nutrient uptake.

Studies are in progress to quantify the biochemical nature of root exudates in mycorrhizal as well as non-mycorrhizal host plants under the influence of these pesticides.

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Effects of fungicides on vesicular-arbuscular mycorrhizal association and plant growth response of citrus seedlings

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The use of agricultural chemicals is essential for crop production in present era. It is also apparent that various chemicals used in plant protection affect non-target organisms for which these are not formulated and ultimately alter the microbial balance in a particular environment (Nemec, 1980). The vesicular-arbuscular (VA) mycorrhizal fungi are cosmopolitan in nature and occur with most of plant, species (Gerdemann, 1968; Harley and Smith, 1983; Hayman, 1982). Its population is also affected by the application of pesticides (Trappe *et al.*, 1984). The intensity of effect may vary with specific combination of host and symbiont. To grow healthy citrus seedling in nursery, different types of fungicides are used as soil or seed treatments. Hence, the experiments were conducted to observe the effect of various fungicides on VA mycorrhizal fungi in relation to rough lemon seedlings.

The effects of soil treatment with ten fungicides, i. e. Bavistin (0.1%), Blitox (0.2%), Brassicol (0.25%), Captaf (0.2%), Captafol (0.2%), Copper sulphate (0.2%), Emisan (0.1%), Mancozeb (0.25%), Sulfex (0.25%) and Vitavax (0.15%) were noted on root colonization and spore population of VA mycorrhizal fungus in rhizosphere soil of rough lemon seedlings.

The treatment of three fungicides, Mancozeb, Sulfex and Vitavax significantly reduced the root colonization and spore population. The minimum colonization, 50.4% was observed in Mancozeb treated soil followed by 52.6% in Sulfex and 52.8% in Vitavax against 76.2% in control. The numer of spores were also less in soil treated with these fungicides. There were 362, 370 and 379 spores/50 g. soil in Mancozeb, Vitavax and Sulfex treated soil respectively as compared to 501 spores/50g untreated soil. Other fungicides also suppressed the colonization and spores population of VA mycorrhizal fungi upto some extent, but the differences were non-significant. There were 60.2, 60.7, 65.7, 70.1, 60.4, 59.6 and 58.3% mycorrhizal colonization and 420, 452, 475, 498, 441, 428 and 426 spores/50 g of soil treated with Bavistin, Blitox, Brassicol, Captaf, Captafol, Copper sulphate and Emisan respectively.

The fungicides affect the establishment of mycorrhizae which ultimately influenced the root, shoot growth, number of leaves and their dry matter in rough lemon seedlings. On average, the root length 8.2, 8.1 and 7.9 cm; the shoot length 7.1, 6.9 and 7.4 cm along with 16, 14 and 15 number of leaves were recorded in seedlings grown in Mancozeb, Vitavax and Sulfex treated soil respectively as compared to 18.7 cm root, 17.8 cm shoot length and 29 leaves per mycorrhizal plant in non-fungicidal treated soil. Similarly, 12.9, 13.2, 13.6, 15, 12.9, 12.7 and 13 cm root length; 11.3, 14.3, 11.5, 14.7, 12.8, 11.4 and 11.2 cm shoot length as well as 22, 25, 24, 28, 22, 23 and 25 number of leaves were observed per plant in Bavistin, Blitox, Brassicol, Captaf, Captafol, Copper sulphate and Emisan treated soil respectively.

Dry matter of various plant parts of rough lemon seedling was affected due to imbalance of mycorrhizal colonization. It was significantly reduced in the seedlings grown in soil treated with three fungicides i.e. Mancozeb, Sulfex and Vitavax. On average, 0.6, 0.9 and 0.7 g dry weight of root, 0.7, 0.8 and 0.9 g dry weight of shoot as well as 0.9, 1.0 and 1.1 g dry weight of leaves per seedling were observed in Mancozeb, Vitavax and Sulfex treated soil respectively. However, the dry weights of root, shoot and leaves of mycorrhizal seedlings grown in untreated soil were 1.9, 2.1 and 2.9 g respectively. The difference in dry matter production of the seedlings grown in soil treated with other fungicides were non-significant. The 1.3, 1.5, 1.7, 1.2, 1.2, 1.6 and 1.6 g dry matter of root; 1.7, 1.9, 2.0, 1.8, 1.6, 1.4 and 1.3 g dry matter of shoot and 2.2, 2.5, 2.7, 2.1, 2.1, 2.0 and 2.2 g dry matter of leaves/seedling were observed in Bavistin, Blitox, Brassicol, Captaf, Captafol, Copper sulphate and Emisan treated soil respectively.

It was concluded that out of ten fungicides tested, Manozeb, Sulfex and Vitavax significantly reduced spore population and VA mycorrhizal colonization of rough lemon seedling which resulted in decrease in root, shoot growth, number of leaves and their respective dry matter.

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Effect of methyl isocyanate on vesicular arbuscular mycorrhizal fungi

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Mycorrhizal fungi are cosmopolitan and are associated with the roots of most crops, helping in improved mineral nutrition. The use of fungicides and fumigants to control soil-borne pathogens is a common practice. Recently, concern has developed among agriculturists about the effects of pesticide usage upon vesicular arbuscular mycorrhizal (VAM) fungi. The biocidal fumigants such as mylone, vapam and vorlex all decompose into methyl isocyanate (MIC), which for these fumigants is the active fungicidal compound in soil. MIC released into the soil consistently reduces mycorrhizal infection, both in the field and in the green house (Menge, 1982). Many of the observations of pesticides upon mycorrhizal fungi have taken place in the field and no attempt has been made to identify the fungal symbiont involved. In this study, methyl isocyanate, a basic ingredient in the production of various pesticides was tested for activity against *Glomus fasciculatum*.

Fresh *G. fasciculatum* inocula from onion plant rhizospheres were used to evaluate their survival at various concentrations of MIC and their subsequent infection in onion plants. Each of the soil inocula contained 560 surface sterlized spores per 100g sterilized soil and was used as inoculum for each of the pots containing sterilized soil, after exposing to 500, 1000 and 2500 ppm of MIC. Onion bulbs were planted in the pots and suitable controls were maintained. Mean plant dry matter production and infection data were recorded every 5 days for 45 days.

Mycorrhizal plants showed a good phytomass production than non-mycorrhizal plants. Dry weight of plants grown in soil with inocula exposed to 500 ppm and 1000 ppm was lesser than the inoculated check, but was higher than the ones inoculated with 2500 ppm treated soil inoculum. Mycorrhizal control plants showed 57 to 90% infection between 15 to 45 days. From 35 days onwards, 500 ppm treated soil showed mycorrhizal activity and the rate of infection was 45 per cent at 45 days. Onion plants grown in 500 and 1000 ppm treated soil developed infection only after 30 days, but in the plants with 2500ppm treated soil infection was observed only on the 45th day, which was 3 per cent and purely hyphal.

These tests showed that VAM has a range of sensitivity to the different concentrations of MIC. Differences were reflected in plant growth and extent of infection. The negative effect of MIC on VAM fungal population indirectly suppressed the plant growth. Inoculum treated with 500 ppm of MIC did not alter much of the growth metabolism of onion. But 1000 and 2500 ppm of MIC proved to be toxic to VAM fungi, by reducing the plant growth. These results coincided with the studies on the effect of carbamate pesticides on plants. Nesheim and Linn (1969) noted reduced infection in corn fumigated with vapam. In citrus also vapam reduced VA infection significantly (Timmer and Leyden, 1978). Very poor infection was noted in corn and citrus roots due to the application of vorlex (Nesheim and Linn, 1969; Schenck and Tucker, 1974) and in corn and bean by the application of mylone (Nesheim and Linn, 1969; McEven *et al.*, 1973). Sodium azide which is used in the preparation of MIC significantly reduced vesicle formation in citrus seedlings when used at a rate of 98% at 28, 84 and 147 kg/ha (Nemec and O'Bannon, 1979). Clearly, the composition of MIC in different pesticides must be standardized for the field applications. Results from such studies may predict the limits for field use of these chemicals in pest management programs.

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Effect of dichlone on *Nostoc* and VAM

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A very complex relationship exists amongst plants, microbes and soil due to which many microbial activities governing relationship between plants and microbes are not fully explored. On one hand, the micropopulation present in the soil exert a decisive influence on the physiological activities of the plants (Subba Rao, 1977) and on the other, plants also supply their nutrients in the form of residues and excretions and affect them either on the root surface or in the vicinity of the roots. Many soil microflora such as fungi and algae show well known examples of symbiosis. Symbiosis involving cyanophycean microflora is of wide spread occurrence just like symbiotic mycorrhizal association between roots of higher plants and fungi.

Although fungicides are normally used to control plant pathogens, their major portion is deposited on the surface of soil and might affect adversely the cyanobacterial and mycorrhizal species. Herein a preliminary attempt has been made to study the effect of fungicide dichlone (2,3-dichloro 1,4 naphthoquinone) on the survival of mycorrihza and cyanobacterium *Nostoc* sp.

Both VAM (mostly *Glomus* sp.) and *Nostoc* sp. were isolated from garden soil. Survival was monitored on agar plates following application of graded concentrations of dichlone (ranges from 0.1 to 5.0 ppm).

Being a fungicide, dichlone (commonly marketed as Phygon XL) is having limited application in controlling algal blooms (Owens and Novotny, 1958) in comparison to other algicides. Comparison of sensitivities of the cyanobacterium *Nostoc* sp. and VAM to dichlone revealed that VAM is found to be more resistant than to *Nostoc*. Concentrations above 2.5 ppm were effective against VAM (50% survival at 3.0 ppm) whereas concentrations as low as 0.5 ppm were effective against *Nostoc* (50% survival at 0.25 ppm). In a previous study it was observed that among cyanobacteria, filamentous forms were more resistant in comparison to unicelluar forms and cyanobacterium *Anabaena cylindrica* was more resistant (Kashyap and Gupta, 1981) among filamentous forms. It was observed that dichlone alterd phycocyanin concentration earlier than other pigments leading to assumption that nitrogen fixation process might be impaired in the presence of dichlone, resulting in the increased sensitivity of *Nostoc* in comparison to VAM as the latter do not contain phycocyanin. Further, the differential sensitivities due to different cellwall structure cannot be ruled out also.

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Pesticides-mycorrhiza interactions on the growth and development of pigeonpea (*Cajanus cajan*)

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Though diverse groups of pesticides are widely used for the control of weeds, nematodes, insects and diseases in plants, many a times the application of these pesticides result in indiscriminate killing of non-pathogenic/beneficial microorganisms (Domsch, 1964). It has now been well demonstrated that vesicular-arbuscular (V-A) mycorrhizal associations can greatly increase the growth of host plants, particularly when the available soil phosphorus is a limiting factor (Mosse, 1973). However, pesticides commonly used in farming systems may exert adverse influence on the symbiotic relationship between plant roots and the fungal endophyte. The inhibitory effects of fungitoxicants applied as seed as well as soil treatments on the development of V-A mycorrhiza and phosphate transport in wheat have been demonstrated (Jalali and Domsch, 1975; Jalali, 1979). The present study was undertaken with a view to quantify the impact of some commonly used pesticides on the colonization of V-A mycorrhiza, host growth as well as N, P and K uptake in pigeon pea (*Cajanus cajan*).

The pigeonpea seeds (cv. Manak) were sown in pots (24 cm) containing nutrient-deficient sterilized soil (15 lb. psi for 2 h.). Bavistin, thiram and aldrin were used as soil as well as seed dressing; vitavax and captafol as seed treatment; and BHC, heptachlor, furadan and phorate as soil applications. The observations were recorded for dry matter production, plant height, N, P and K content of plants and mycorrhizal colonization of plant roots at different growth stages of the host plant. Mycorrhizal colonization was recorded by grading (grade 0-4) mycorrhizal infection along each root segment (Jalali and Domsch, 1975). N, P and K content of the plants were determined by the standard methods followed by Jackson (1958).

Among the five pesticides used as seed treatment, thiram had most inhibitory effect on mycorrhizal colonization while captafol reduced total dry matter production drastically. Nutrient uptake was significantly reduced by the application of these pesticides as compared to untreated control. P-uptake was appreciably suppressed as compared to N and K.

Of the soil pesticides, basalin had the most inhibitory effect on mycorrhizal colonization, plant height, total dry matter production and nutrient uptake followed by thiram and bavistin. Heptachlor had the least effect on various growth parameters followed by furadan and phorate.

These observations clearly indicate that VA-mycorrhizal infection is considerably inhibited by the application of different pesticides. Of the test pesticides evaluated, the most drastic effects were observed with basalin, bavistin, captafol and thiram applications, which interfered, in varying degress with the growth and nutrient uptake of the plants. These results have some practical significance, since any interference with mycorrhizal development may ultimately have a depressive effect on plant growth and development. Therefore, the repeated use of pesticides in normal farming practices need careful consideration in our future plant protection strategies.

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Effect of fungicides on mycorrhizal and rhizobial development in soybean

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Soybean (*Glycine max* L.) requires adequate phosphorus supply for nodule production and nitrogen fixation (Van Schreven, 1958). Vesicular-arbuscular (VAM) fungi are known to improve plant phosphorus nutrition particularly in low P soils and other immobile elements. Inoculation of soybean with VAM and rhizobium in some soils was found to have synergistic beneficial effect on nodulation, nitrogen fixation and soybean growth (Carling *et al.*, 1978). Soybean suffers from several seedborne pathogens which reduce plant stand in the field and thus result in reduction of grain yields. In order to control seedborne pathogens seed treatment with fungicides has been invariably carried out with fungicides like thiram, captan and carbendazim (Vyas, 1984). There is considerable evidence that the fungicide affects development of VAM fungi (Vyas, 1988). Hence, the present investigation was carried out to investigate the interactions of mycorrhizae, rhizobia and fungicides on the development of mycorrizal infection and colonization, nodulation and plant growth which is vital for rational use of fungicides in plant disease control without adversely affecting growth promoting mycorrhizal and rhizobial associations.

The result of the present investigation indicate that the soil had a low level of indigenous VAM. Inoculation with *Glomus* spp. increased the percentage of mycorrhizal infection and colonization of roots of soybean plants and number of chlamydospores in the soil. The results further indicate that the inoculation of soybean with *Rhizobium japonicum* significantly increased number of nodule per plant and their weight and also plant dry weight. Similarly *Glomus* spp. also increased plant dry weight. The results also indicate that dual inoculation with *Rhizobium* and VAM increased mycorrhizal colonization and chlamydospores number,nodule number and their weight and plant dry matter weight. This response in likely due to improvement in nutrient balance of the host plant, especially nitrogen, phosphorus, zinc and copper supply by VAM inoculation which results in enhanced nitrogen fixation (Carling *et al.*, 1978). Seed treatment with thiram (0.3%) or carbendazim (0.15%) had no adverse effects on the dual inoculation with mycorrhizal and rhizobial as well as their various benefial effects to the plants. Similar effects were also reported by Groth and Martinson (1983). Kumar and Jayaraman (1987) observed abverse effects of carbendazim, thiram and captafol seed treatment with mycorrhizal inoculation but this effect was nullified in the presence of fertilizers and farm yard manure. This and other interactions with agronomic practices need experimentations to elucidate the complexcity.

It is concluded from this study that seed treatment with carbendazim (0.15%) and thiram had no adverse effects on the nodulation and mycorrhizal colonization in soybean cultivar, JS-72-44.

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Effect of insecticides on wheat crop inoculated with phosphate solubilizing bacteria (PSB) and VAM fungi

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The effect of pesticides such as phorate and carbofuran applied to soil at recommended field dosage was studied on wheat crop. The seeds were inoculated with PSB (*Pseudomonas striata*) and soil was inoculated with *Glomus fasciculatum*. Simple application of these biocides slightly improved the growth, grain and straw yields. Seed treatment with PSB improved the growth and yield of the crop appreciably. Phorate slightly reduced the growth and yields but carbofuran application was compatible with PSB treatment. VAM application improved the yield of the crop over the control but was not better than PSB. The use of the insecticides did not inhibit the growth of the plant over the VAM inoculated treatment. The dual inoculation of PSB and VAM augmented the growth and yields of the crop and the biocides were compatible with the dual inoculation system.

The same treatments were repeated in soil amended with superphosphate at 60 kg ha^{-1}. The yields were improved due to use of phosphates and the best effect was obtained with dual inoculation and the pesticides only slightly affected the growth and yield of the crop.

Recent advances and trends in ectomycorrhizal research

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Inspite of the fact that mycorrhiza research has been pursued for several decades, it has not taken off of in a big way to attract the attention of nursery men and nursery managers for exploiting full potential of mycorrhiza in the production of superier planting stock. The thrust areas which should attract serious attentions are screening and selection of efficient fungi and strains, inoculum production, initiation of mycorrhiza in a soil system in containerized programmes, production of mycorrhizal plants with spores inocula (encapsulation of seeds, hydrogel bead inocula) and testing of field performance of inoculated nursery seedlings. While the efficacy of pure culture inoculation has been established, inoculation of nursery seedlings with a mixture of mycorrhizal fungi needs to be given a fair trial.

The work on fungicide/mycorrhizae interaction is confined to solitary reports. Few studies are documented which throw light on the toxic effect of fungicides on mycorrhizal development in seedlings and tolerance of mycorrhizal fungi to fungicides *in vitro*. The nitrogen fixing capability of mycorrhizal fungi is viewed with caution and not many reports have appeared to confirm the claim. This aspect of work has received scant attention and studies are warranted specially when sophisticated instrumentation facilities are available in all biochemical and physiological laboratories. Considerable amount of work has appeared in the past few years on biological suppression of root disea es in agricultural crops by VAM fungi in forest tree species, some attempts were made to initiate parallel studies with the ECM fungi but the work could not advance further. The role of ECM fungi in control of root diseases in genera of *Eucalypts* and *Albizia* need to be defined. Studies on ultrastructure of mycorrhiza to resolve differences between the various classes of mycorrhizal fungi are important and deserve more attention. Fungus gardens which serve as a source of mycorrhizal inoculum on the nursery sites need to be established in the areas where the density of the native mycorrhizal fungi is low.

Inspite of considerable upsurge in mycorrhiza research in advanced countries and third world nations more is known about the basic metabolic functions of mycorrhizae than is known to correct plant maladies and effectively supply knowledge gained as far on a broad practical scale even without knowledge of basic answers. Most researchers agree that in order to justify expenditure on mycorrhiza research it is time to apply existing basic information on developmental efforts to solve global problems of energy resources deforestations and timber shortage. The management system that produces high yield in agriculture and forest tree in developed nations are not condusive to depleted economics of growing nations as some of these systems are already under censure, especially in United States. The mycorrhizal technology developed in U.S.A. has found practical application in reclamation on waste lands, acid coal soils and forestation of mined areas, borrow pits with astounding success. The American experience convincingly establishes that some species of ectomycorrhizal fungi under certain environmental conditions are more beneficial to trees than other fungal species which occur naturally. Future research therefore, should be directed at country wide scaning, selecting, propagating, manipulating and managing more desirable fungal symbionts to improve tree survival and growth. Zobel (1979) pleaded that the major challenge to forest scientists is to generate the biological technologies needed to grow productive forests and remaining land now considered to be submarginal for economic production of either food or timber crops. Acording to Tinker (1982) unless the practical benefits of mycorrhizal inoculation are demonstrated convincingly in field trials and such hopes are too long deferred there could be a reaction amongst research managers against mycorrhiza research. However, when one considers the millions of hectares of potential exotic forests that might be established in the third world nations as well as millions of hectares of forests land awaiting artificial regeneration in the develop world the importance of such inexpensive treatments as for instance mycorrhizal inoculations becomes apparent and perhaps the only sound choice.

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Desert plantation and mycorrhizae-current state of art

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The importance of VA mycorrhiza to agriculture and forest crops has been well documented but associations in arid and semi arid plants and wild vegetation have received little attention. VA mycorrhizae may be advantageous to mycorrhizal desert plants where phosphorus exists as practically insoluble calcium phosphate and the diffusion of ions in the soils is decreased by low moisture. Mycorrhizae are an important consideration in maximising rangeland and arid land productivity. The understanding of mycorrhizal associations of semi arid desert, rangeland vegetations, the distribution of mycorrhizal fungi and soils is necessary for wise management of these fragile habitats.

Recent scientific results provide data which support the hypothesis that mycorrhizal plants are effective colonizers of disturbed habitats and that the lack of of mycorrhizal fungi exert profound influences on species composition (Tommereup and Abbott, 1981). Ninty nine percent of the plant cover was mycorrhizal in arid and semiarid habitats. Mycorrhizae are an important consideration in maximising rangeland and arid land productivity. The understanding of mycorrhizal associations of semiarid, desert and rangeland vegetation and the distribution of mycorrhizal fungi and soil is necessary for wise management of these sunbaked sand dunes (Trappe, 1981).

Mycorrhizal fungi directly mediate interaction between plant in atleast four ways (a) They allow trees to compete successfully with grasses and herbs for resources and they detoxify allelochemicals produced by these plants as well. (b) Mycorrhizae may decrease competetive interactions between the plants and increase the productivity of species mixture, particularly in soils where phosphorus is limiting. (c) Mycorrhizal hyphae link the same and different species act as a route of material transfer among plants. In drought stress environments legume seedlings often associate in disproportionate number with perennial grasses, where their survival is probably enhanced because of the rich concentration of mycorrhizal inocula. (d) Mycorrhizae and other microbes affect soil formation and structural characteristics by producing humic compounds, accelerating decomposition of primary minerals and producing organic 'glues' that bind soil particles into water stable aggregates. Aggregation in turn influences soil properties providing the diversity of pores necessary to permit both water drainage, therefore aeration (Rose, 1988; Maxel and Reid, 1973). Through close mutual interactions between plants and soil organisms, these ecosystems create the conditions that allow the systems to persist. Severing the close links between plants and soil has contributed to degradation of many ecosystems and restoring these links in an important step towards rehabilitation (Trappe and Awameh, 1981).

Vesicular-arbuscular mycorrhizae are cosmopolitan and almost universal in host range. The most dominant mycorrhizal spores in arid and semi-arid regions were *Glomus macrocarpum*, *G. fasciculatum*, *G. mosseae*, *Gigaspora albida*, *Sclerocystis sinuosa* and *Scutellospora calospora*. Some spores possess very characteristic features not commonly encountered elsewhere. The rhizosphere of the edible cacti were heavily infested with spores of *Glomus macrocarpum* and *Sclerocystis sinuosa*. They formed ramifying hyphae and the vesicles in the root system.

Out of the 6507 species of angiosperms that have been studied 70% are consistently found to be mycorrhizal and 12% are apparently facultatively mycorrhizal (Trappe, 1987), sometimes forming mycorrhizae or sometimes not. By and large the plants of arid and semi-arid rangeland are mycorrhizal. It is perhaps a testament to the strength of a healthy link between plants and rhizosphere organisms that despite stressful environments, their ecosystems are not necessarily unproductive.

Much still needs to be learnt, but one conclusion already seems warranted. Diversity in the plant community, the microbial community and the ecosystem as a whole plays a seminal role in buffering against disturbance and maintaining healthy links between plant and soils. Management system in these difficult terrain aimed at protecting diversity are an important step towards sustainable resource utilization. Studies are urgently needed for many habitats in question.

Indigenous mycorrhizal fungi are not necessarily the best for optimum growth of desired forage species in a given soil. The introduction of more efficient fungi to a site to selectively promote desired forage species and land deserves research attention.

Mycorrhizal and bacterial inocula need to be developed in two 'generations'—the first consisting of naturally occurring bacteria, fungi and the second consisting of genetically engineered microbes. The mass inoculum production must also be utilized and the quality of their output controlled with respect to inoculum density and biological activity.

We need to understand the interaction among mycorrhizal fungus species, host species and environment. Future research must therefore strengthen scope to include the mycorrhizosphere and ecosystem and as a whole.

The actual compounds transferred from the fungus to the host are essentially unknown. It is proposed that glutamine, as the most abundant and first formed amino compound is a probable candidate (Varma, 1989). Characterization and regulatory role of amino compounds need elucidation.

The ecological importance of the enzymatic reactions at the mycorrhizal or hyphal surfaces has not been fully considered but undoubtedly would seem to provide sources of nutrient in addition to the standing concentration of the soil solution in the immediate locality of a hyphae or rootlet.

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Vesicular-arbuscular mycorrhizal root colonization and spore production in maize inoculated with *Glomus fasciculatum*

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Surveys on the numbers and types of spores present in soil have shown that among the various vesicular-arbuscular mycorrhizal (VAM) fungi, *Glomus fasciculatum* (Thaxter sensu Gerd.) Gerd. & Trappe has a worldwide distribution both in agricultural and natural sites (Gerdemann and Trappe, 1974). Its occurrence is also widespread in Malaysia (Nadarajah and Nawawi, 1989). As an obligate symbiont, *G. fasciculatum* is a beneficial soil fungus forming associations with roots of a variety of agricultural crops as well as uncultivated plants. This fungus occurs in soil as (i) spores (singly or in loose aggregates), (ii) colonized roots in the form of hyphae, vesicles, coils and arbuscules or (iii) hyphe. Each of these components may have the ability to initiate an infection (Daft *et al.*, 1987).

A time course study was thus conducted to determine the infectivity of the components of a local isolate of *G. fasciculatum* and the development pattern of root colonization and spore production with maize (*Zea mays* L.) since this plant is used widely for routine production of spores.

Spores of *G. fasciculatum* were isolated from rhizospheres of cocoa plants (*Theobroma cacao* L.) using the wet-sieving and decanting technique (Gerdemann and Nicolson, 1963). The pH and available Bray No. 2 P of these soils ranged from 5.0 to 5.8 and 11 to 45 ppm, respectively (Nadarajah and Nawawi, 1989). *G. fasciculatum* was multiplied in pot cultures with maize. The growth medium consisted of methyl bromide fumigated garden soil : sand mixture (pH 5.3 and 11 ppm available P). All pot cultures were grown for 4 months in a greenhouse with normal sunlight and temperatures ranging from 24 to 39 ± 2°C. Pots were fertilized weekly with quarter-strength Hoagland's solution without phosphorus and were watered as needed with distilled water.

The fungal treatments were inoculation with (i) soil, (ii) spores, (iii) mycorrhizal root pieces, (iv) external hyphae, (v) vesicles or (vi) filtered washings. Soil inoculum (1 g) consisted of soil, spores, hyphae and mycorrhizal root fragments from plants grown in pots. Spore inoculum (100 spores) consisted of spores isolated from soil by wet sieving and decanting. External hyphae (0.5 g) were also obtained by wet-sieving and decanting. Root inoculum (0.5 g of chopped mycorrhizal roots) was obtained by picking the root pieces from sievings, then washed free of adhering spores and cut into 1 to 5 mm pieces. Vesicles (100 vesicles) were removed gently from mycorrhizal root pieces by maceration. Noninoculated controls received leachings of the fungus passed through a 45 um sieve three times.

Surface sterilized seeds of maize were germinated in moist, sterile sand. After 10 days, seedlings were transplanted singly into polyvinylchloride bags containing 3 kg fumigated garden soil. For each treatment, the inoculum was placed onto a filter paper and wrapped around the roots of the seedlings or placed 3-5 cm below the seedlings. Growth conditions were the same as those for pot cultures. There were four replicates for each form of inoculum.

Plants were harvested at 1, 2, 3, and 4 months after inoculation. Percentage mycorrhizal colonization in roots was determined by staining the roots with trypan blue (Phillips and Hayman, 1970) and spore numbers in soil by wet-sieving and decanting.

All plants, except controls, became mycorrhizal and produced spores at varying levels. Although infection was observed one month after inoculation, root colonization by spores, external hyphae and vesicles were much lower than that by soil or root inocula. However, by 4 months colonization levels by soil, root and spore components were over 80% while the external hyphae and vesicle components had 45 and 65%, respectively.

Sporulation pattern followed similar trends 4 months after inoculation. Of the various components tested, soil and root inocula started producing spores at 2 months with higher spore production occurring at 4 months with soil inoculum.

The soil component was more effective as inoculum for root colonization and spore production than the other components. This is because soil inoculum contains various forms of propagules (spores, mycorrhizal root fragments and external hyphae) which are capable of initiating an infection. Root colonization levels developed more quickly from colonized root pieces than from spores. The initial delay in spore infection may be related to factors such as age and viability of spores or because some spores produce a pre-infection phase in soil (Powell, 1976). Internal vesicles are often present in roots colonized by *G. fasciculatum*. These are considered to be storage organs and may become thick-walled and function as spores (Gerdemann and Trappe, 1974). In this study, vesicles were infective and colonized roots. This confirms observations by Biermann and Linderman (1983) who suggested that vesicles contribute to inoculum potential of mycorrhizal roots. External hyphae produced the lowest levels of colonization. This concurs with the findings of Daft *et al.* (1987), where the hyphal fragments were least effective in establishing VAM with *Medicago sativa*.

Powell (1976) suggested that the different infective patterns of VAM fungi were probably related to the amount of nutrient reserves in the specific inoculum component. The varying levels of development of VAM root colonization could also be attributed to the inoculum concentration of each component being not optimized. As there are conflicting reports on the infectivity and effectiveness of components of VAM fungi with other hosts (Biermann and Linderman, 1983; Daft *et al.*, 1987; Powell, 1976), further research is necessary to determine the inoculum potential of components of other VAM fungi.

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Development of ectomycorrhizae on pine and its effect on the growth of *Pinus kesiya* under different moisture regimes

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It is well known that water is a universal solvent. All physiological and biochemical activities are governed by the availability of water in living system. Microbial activities in soil are also influenced by the availability of moisture in soil. Water in soil may influence the mycorrhizal formation (Bakshi, 1974). Mycorrhizal plants can also tolerate more water stress conditions than non-mycorrhizal ones (Parke *et al*, 1983). An attempt was, therefore, made to study the influence of soil moisture on the colonization and development of mycorrhiza with pine seedlings.

The experiment was conducted in glass house conditions. Plastic pots of 15cm diameter were filled with sterilized sandy loam soil (pH 5.2, Organic matter 3.5%, N 0.094%, P 0.021% and K 0.183%). Pine (*Pinus kesiya* Royle Ex Gorden) seedlings of 4 cm length raised aseptically in laboratory, were transferred to the pots. Thereafter 10 days, the spore suspension of *Boletus edulis* and *Scleroderma aurantium* (1.3×10^4 spores/ml) was inoculated. Uninoculated seedlings of pine were maintained as control. Three levels of moisture content i. e. 10, 30 and 55% were maintained. Seedlings were harvested after 6 months with soil attached to root system. Acid phosphatase activity in soil and root surface was estimated by the method of Dodd *et al.* (1987). Colonization of mycorrhizal fungi was studied under stereobinocular microscope. Growth of mycorrhizal and nonmycorrhizal seedlings was measured.

Colonization of both mycorrhizal fungi on pine seedlings differed with different moisture levels. *S. aurantium* showed significantly higher colonization at 30% moisture level than other levels of moisture. However, *B. edulis* exhibited better symbiosis at 55% moisture content. Lowest moisture level (10%) inhibited colonization of both the mycorrhizal fungi.

Shoot growth of seedlings at 10 and 30% moisture content was insignificantly more in seedlings inoculated with *S. aurantium* than control ones. At 55% moisture level, *B. edulis* inoculated seedlings had significantly enhanced growth than control. Shoot and root dry weights of seedlings were observed better at medium moisture level with *S. aurantium.*

Maximum acid phosphatase activity in mycorrhizal roots and soil was observed in pine seedlings inoculated with *S. aurantium* at medium moisture level, whereas *B. edulis* inoculated roots and soil showed better activity at 55% moisture level. Mycorrhizal fungi enhanced the growth of pine seedlings more at low as well as high levels of moisture content differently than uninoculated seedlings.

The colonization of *S. aurantium* was reduced at highest moisture level which may be due to low hyphal entry points in root epidermis, resulting into the reduced infection (Reid and Mekal, 1977). Mycorrhizal plants have been reported to grow better in low moist condition than uninoculated ones due to their capacity to explore the new or larger soil zone through their extended hyphal root system (Ponder, 1983). *B. edulis* was better adapted to high moisture level than *S. aurantium* which suggested that the later one is highly aerobic fungus than the earlier one. Another reason for less efficiency at high moisture level may be attributed to the higher dilution rate of nutrients resulting in increase in acidity of soil which might have inhibited the growth of *S. aurantium* and stimulated *B. edulis.*

Acid phosphatase activity of the surface of mycorrhizal roots and in rhizospheric soil was correlated with colonization of mycorrhizal fungi and has been affected by the aeration at high moisture level and temperature of soil.

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Identification of endogonaceous fungi from Delhi

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Ten soil samples were collected from three different soil types from Indian Agricultural Research Institute fields, New Delhi. These soils were analysed for their pH, moisture contents and carbon and nitrogen ratio.

Twenty different species belonging to six genera of Endogonaceae viz., *Glomus, Gigaspora, Endogone, Entrophospora, Acaulospora* and *Complexipes* were collected. *Acaulospora trapei, A. scrobionta, A. spinosa, A. leavis, Endogone flammicorona, E. tactifolia, Entrophospora enfrequence, complexipes moniliformis, Gigaspora decipens, G. nigra, Glomus fragilis* and *G. wum*, were added for the first time from India. Several *Glomus* species were also recorded which are quite dominant in the area.

Interaction between *Rhizobium* (cowpea miscellany) and mycorrhizal fungi and their stimulatory effects on *Acacia nilotica* (L.) Del.

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VAM occur widely under various environmental conditions and are found in association with a number of leguminous plants. VAM fungi which constitute a group of important soil micro-ogranisms are ubiquitous throughout the world and are known to improve the plant growth through better uptake of nutrients. They also improve the activity of N_2 fixing organisms in the root zone (Mosse *et al.*, 1976).

It is found that simultaneous inoculation of legumes with *Rhizobium* and VAM causes synergistic beneficial effects (Bagyaraj *et al.*, 1979). The present report deals with the results of pot experiments to assess the effect of inoculating seeds of *Acacia nilotica* with *Rhizobium* sp. (cowpea miscellany) and soil with VAM fungi (*Gigaspora margarita* and *Glomus fasciculatum*) singly or in combination on dry matter production, nodulation, N_2-fixation (N_2-ase activity) and nutrient uptake by plants under sterile conditions.

Plants were inoculated with 5 ml/seedling of rhizobial inoculum containing 3×10^9 cells/ml (AN-R). Mycorrhizal spores were selected through the wet sieving and decanting technique (Gerdemann and Nicolson, 1963). The mycorrhizal inoculum consisted of a mixture of spores of endophyte belonging to *G. margarita* and *G. fasciculatum* on an average 50 VA spores were included in each pot. Inoculum contained about 250 spores per 50 g soil. A thin layer of inoculum (50 g) was placed below the surface before sowing seeds. Soil without any microbial additions served as control. Experiment was conducted during May to September months. Eight different treatment combinations were used. The total plant and nodular proteins were estimated according to the method of Lowry *et al.* (1951).

Quantitative estimation of nodular leghaemoglobins was done as per haemochromogen method of Hartee (1957). Nitrogen content was estimated following Kjeldahl digestion method (Bremner, 1960) and phosphorus was determined according to a modified molybdate method (Golterman, 1970). The total soluble sugars were measured by phenyl-sulphuric acid method (Dubois *et al.*, 1951) and the total chlorophyll content was estimated following the method of Arnon (1949).

The height of plants was significantly more in combinations of *Rhizobium* and VAM as compared to *Rhizobium* or VAM treatments alone and also with that of control treatment. Dry weights of plants also showed the same response to the treatments.

Similarly, dual infection with *Rhizobium* and VAM resulted in best nodulation. Number and weight of nodules in this treatment were significantly more than that of *Rhizobium* alone. Amount of leghaemoglobin in root nodules, treated with *Rhizobium* alone or in combination with VAM was almost the same.

The nitrogenase activity (N_2–fixation) by acetylene reduction assay in the root nodules obtained from the plants inoculated with *Rhizobium* plus VAM was higher than *Rhizobium* inoculated alone.

All plants were tested for sugar, chlorophyll and protein contents and it was seen that combined inoculum gave the best results. Similarly, the values for N and P were the highest in plants which had received the combined inoculum of *Rhizobium* and VAM.

The parameters selected to examine the effect of *Rhizobium* and VAM either individually or in combination were plant height, weight, nodule number, nodule weight, nodule protein, Lb content in nodules, plant protein, sugar and chlorophyll contents and uptake of nutrients (N and P). Data clearly indicate that dual infection resulted in better growth and nodulation in *Acacia nilotica*. This observation is in confirmity with earlier work of Brgyaraj *et al.* (1979) on soyabean. In view of these observations, it may be logical to conclude that *Glomus* and *Gigaspora* spores used in the present investigation are very effective with *Acacia nilotica*.

In the present study conducted in sterilized soil *Rhizobium* with VAM stimulated nodule number, nodule weights, total leghaemoglobin content and N_2–fixation (N_2–ase activity) in *Acacia nilotica*. This is in confirmity with the observations made in legumes by Varma (1979).

Uptake of N and P by inoculated plants (singly and dually inoculated) was significantly more than that of uninoculated controls. The dually infected plants probably derive considerable benefits from the physiological activities of the endophytes and the major elements N and P are mitigated by the endophytes.

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Studies on the effect of *Rhizobium* (cowpea miscellany) and endomycorrhizal interaction in *Dalbergia sissoo* (Roxb.)

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The leguminous plants can form two types of symbiotic associations with microorganisms. One with *Rhizobium* sp. involved in N_2-fixation, the other with VAM fungi, concerned with the uptake of P and other nutrients (Crush, 1974). It is now established that the enhanced growth of plant is due to absoption of ions especially P by the fungus from the soil and subsequent transfer in plant (Hayman and mosse, 1972). Inoculation of legumes with VA mycorrhizal fungi can stimulate nodulation and N_2-fixation (Mosse, 1981).

In the present study, response of *Dalbergia sissoo* to inoculation with *Rhizobium* sp. (cowpea miscellany) and VA mycorrhiza (*Glomus fasciculatum*) is presented.

The surface sterilized seeds of *Dalbergia sissoo*, inoculated with corresponding *Rhizobium* sp. (cowpea miscellany) containing 4×10^8 cells, were sown in pots filled with sterilized sandy loam soil (2 kg each). The endomycorrhizal spores as mycorrhizal inoculum, to provide 250 per 50 g soil was also added in each pot. Soils with no microbial additions served as control. Three seedlings were maintained in each of the five replicates of the treatment. The pot trial was carried out from April to August months, using diffrent treatment combinations.

The total plant and nodular proteins were estimated according to the method of Lowry *et al.* (1951). Quantitative estimation of nodular leghaemoglobin was done as per haemochromogen method of Hartee (1957), nitrogen content of plant was estimated following Kjeldahl digestion method (Bremner, 1967). P was determined acording to a modified molybdate method (Golterman, 1970). The total soluble sugars were measured by phenol sulphuric acid method (Dubois *et al.*, 1951). Total chlorophyll content was estimated following the method of Arnon (1949) and N_2-ase activity of nodules were measured by Acetylenereduction technique with the help of Gas chromatograph.

Analysis of growth in terms of shoot and root length, their dry weight, total chlorophyll content and total plant protein content indicates that, mycorrhizal and rhizobial plants grew much better than the untreated ones. The differences were very significant for control and rhizobial plants and also between mycorrhizal and *Rhizobium* plus mycorrhiza inoculated plants. The maximum growth was recorded in the double inoculated plants.

The dual inoculation considerably stimulated root nodulation (nodule number, fresh and dry wt., nodule protein and nodule leghaemoglobin) than plants inoculated with *Rhizobium* alone. The nitrogenase activity of nodules by Acetylene Reduction Assay (ARA) was also enhanced in double inoculated plants than with single inoculation (*Rhizobium* alone). Further, the specific activities of the ammonia assimilation enzymes viz., giutamine synthetase (Mn^{+2} dependent transferase assay), alanine aminotransferase and aspartate aminotransferase, except glutamine dehydrogenase were higher in nodules of double inoculated plants than of *Rhizobium* inoculation alone.

The uptake of elements N and P was also affected in plants inoculated by *Rhizobium* and VA mycorrhiza. Higher values were obtained in plants with double inoculations in comparison to single inoculation and uninoculated control.

The results clearly indicate that the dual inoculation of *Rhizobium* and VA mycorrhiza promoted more nutrient uptake (N and P) and consequently the growth in *Dalbergia sissoo.* This is in confirmity with earlier work (Abbott and Robson, 1982). Even response of plants to single inoculation with mycorrhiza or *Rhizobium* was better over inoculated control.

In the present investigation the nodulation in terms of nodule number, dry weight of nodules, total leghaemoglobin content and nitrogenase activity (N_2-fixation) in nodules was also enhanced in *Dalbergia sissoo* inoculated with *Rhizobium* and VA mycorrhiza. This is in keeping with the earlier observation made in legumes (Varma, 1979). Further, the specific activity of ammonia assimilation, viz., glutamine synthetase, aspartate amino transferase and alanine amino transferase was considerably stimulated as a result of dual interaction between *Rhizobium* and VA mycorrhiza.

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Nitrogen and carbon nutrition studies of endophytes of ophioglossales

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Having established the identity of the endophytes *Ophioglossum reticulatum* L., *O. pedunculosum* and *Botrychium virginianum* (Nair, 1988) it was necessary to ascertain the role they play in the nutritional cycle of the plant. Hence some aspects of their nutrition were studied using different nitrogen and carbon sources.

Most of the ammonium salts did not support the growth of *Fusarium oxysporum*-A. There was no sporulation and the hyphae were abnormal and appeared beaded. The terminal cells of these hyphae were elongated. Ammonium nitrate was slightly better and the growth with ammonium tartrate and ammonium citrate was better compared to other salts of ammonium, especially in solid cultures. With potassium nitrate there was normal and good growth and sporulation.

The nitrogen sources selected for both cultures were, ammonium oxalate, casein, urea, asparagine, sodium nitrate and ammonium nitrate.

In submerged cultures ammonium nitrate proved to be the best source of nitrogen. In these cultures almost all sources of nitrogen produced normal hyphae, though sporulation was absent.

In stationary cultures ammonium nitrate gave the best growth. Sources of other ammonium salts and casein unlike that in solid media, were good sources of nitrogen. The hyphae also were normal and sporulated, though in ammonium oxalate and ammonium nitrate few abnormal hyphae with swollen cells having dense cytoplasm were observed. The pH of the medium was 3 after 144 hours of growth.

In *Fusarium oxysporum*-B also ammonium chloride, ammonium oxalate and ammonium sulphate produced beaded haphae which did not sporulate. However, ammonium citrate, ammonium tartrate and ammonium nitrate supported the growth of this species with sporulation. Casein, like control, had very feeble growth and lacked sporulation. Asparagine, glycine, urea, peptone, sodium nitrate and potasslum nitrate proved to be very good sources both for growth and sporulation. Trpptophane did not show very good growth.

In submerged cultures ammonium nitrate was the best source of nitrogen though sodium nitrate and urea also supported comparatively better growth. The pH went down to 3 from 5.5.

In broth cultures, asparagine was the best source of nitrogen. Next to it were ammonium oxalate, sodium nitrate, ammonium nitrate, urea and casein in prefering their sequence.

Fusarium solani colonies appeared reddish yellow in colour and had stunted growth in ammonium chloride, ammonium oxalate, ammonium sulphate, ammonium tartrate and ammonium citrate. Growth actually started after 96 hours and the hyphae were abnormal, beaded terminating in long cells. There was no sporulation. However, ammonium nitrate did not produce abnormal hyphae though there was no sporulation. Casein had slightly better growth, though the colonies did not show its normal cotony growth, tryptophane, glycine, asparagine, urea, peptone, sodium nitrate and potassium nitrate, supported very good growth and sporulation though potassium nitrate and urea were the best sources.

In submerged cultures sodium nitrate gave the best results with asparagine and potassium nitrate the fungus did grow but the dry weight of the mycelium was less. Urea was a good source for the growth of this fungus in stationary broth cultures.

In case of *Fusarium oxysporum*-A all the thriteen sources of carbon tried proved to be good sources for the growth. The growth of hyphae started and was visible even after 48 hours of incubation. Out of the various sources, maltose proved to be the best source of carbon. For broth cultures sucrose, dextrose and starch were selected. For submerged and stationary broth cultures starch gave better growth though in solid cultures there was comparatively less growth. This indicates that in presence of aeration and agitation, polysaccharides are better utilized.

Fusarium oxysporum-B also showed good growth in all the carbon sources. However, sucrose and maltose were the best amongst the lot. In both submerged and stationary cultures starch showed better growth.

Fusarium solani showed good growth in all the thirteen carbon sources tried. Amongst the thirteen carbon sources, maltose and sorbitol were better sources. Sucrose and dextrose gave good growth in broth cultures.

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Distribution of VAM in Tamil Nadu

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A survey on the distribution of VAM in coastal and plain of Tamil Nadu was made. Rhizosphere soils and root samples from different areas were screened for VAM spores and VAM infection, respectively. Of the 203 samples screened, 121 displayed different degrees of infection ranging from 10 to 100%. This includes 35 plants with 0–20% infection, 33 with 21–40%, 20 with 41–60%, 12 with 61–80% and 21 with 81–100%. The identified VAM species were *Gigaspora albida, G. margarita, Glomus aggregatum, G. ambisporum, G. citricolum, G. claroideum, G. fasciculatum, G. intraradices, G. occultum, G. pustulatum* and *G. tenerum* which occurred at 3–17% frequency. *Gigaspora candida, Glomus australe, G. botryoides, G. clarum, G. deserticola, G. dimorphicum, G. mosseae* and *G. multisubstensum* had a frequency of 2%. However, spores of *Acaulospora longula, A. myriocarpa, A. icolsonii, Glomus albidum, G. hetersporum, G. pansihalos* and *sclerocystis clavispora* were less frequent (1%).

Root samples which showed heavy infection (90–100%) were used to inoculate onion *(Allium cepa)* in pots containing sterile soil. Twenty five days after inoculation, the onion roots were screened for VAM infection and rhizosphere soils for spores. Infected roots of *Catunaregam spinosa* devloped 100% infection in onion. *Scilla indica* developed 80% infection, *Bauhinia racemosa* 75%, *Lepidogathis* sp. and *Bulbostylis barbata* 65%, *Caralluma adscendens* 60%, *Dentella repens* and *Spermococe articularis* 55%, *Sesamum laciniatum* 50%, *Asystasia gangitica* 35% and *Justicia tranguebariensis* 30%. The onion roots did not develop VAM infection when inoculated with infected roots of *Cleome viscosa, Asperagus recemosus, Borreria hispida* and *Sida cordata.* The VAM spores isolated from rhizosphere soils of the test plants are listed below. In parantheses are the host plants whose roots served as inoculum. *Gigaspora albida (Asystasia gangetica, Catunaregam spinosa), Glomus aggregatum (Cleome viscosa), G. ambisporum (Asparagus racemosus, Bulbostylis barbata, Cleome viscosa, Detella repens, Scilla indica), G. claroideum (Dentella repens, Sesamum laciniatum), G. constrictum (Sida cordata, Spermococe articularis), G. fasciculatum (Catunaregam spinosa), G. leptotichum (Caralluma adscendens), G. pustulatum (Justicia tranguebariensis), G. reticulatum (Bauhinia recimosa), Sclerocystis microcarpos (Borreria hispida).*

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