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About TERI

A dynamic and flexible organization with a global vision and a local focus, TERI was established in 1974. While in the initial period the focus was mainly on documentation and information dissemination activities, research activities in the fields of energy, environment, and sustainable development were initiated towards the end of 1982. The genesis of these activities lay in TERI's firm belief that efficient utilization of energy, sustainable use of natural resources, large-scale adoption of renewable energy technologies, and reduction of all forms of waste would move the process of development towards the goal of sustainability.

The Bioresources and Biotechnology Division

Focusing on ecological, environmental, and food security issues, the Division's activities include working with a wide variety of living organisms, sophisticated genetic engineering techniques, and, at the grassroots level, with village communities. The Division functions through five areas—Centre for Mycorrhizal Research, Microbial Biotechnology, Plant Molecular Biology, Plant Tissue Culture, and Forestry/Biodiversity. The Division is actively engaged in mycorrhizal research. The Mycorrhiza Network has specifically been created to help scientists across the globe in carrying out research on mycorrhiza.

The Mycorrhiza Network and the Centre for Mycorrhizal Culture Collection

Established in April 1988 at TERI, New Delhi, the Mycorrhiza Network first set up the MIC (Mycorrhiza Information Centre) in the same year, and the CMCC (Centre for Mycorrhizal Culture Collection) – a national germplasm bank of mycorrhizal fungi – in 1993. The general objectives of the Mycorrhiza Network are to strengthen research, encourage participation, promote information exchange, and publish the quarterly newsletter, *Mycorrhiza News*.

The MIC has been primarily responsible for establishing an information network, which facilitates information sharing among the network members and makes the growing literature on mycorrhiza available to researchers. Comprehensive databases on Asian mycorrhizologists and mycorrhizal literature (RIZA) allow information retrieval and supply documents on request.

The main objectives of the CMCC are to procure strains of both ecto and VA mycorrhizal fungi from India and abroad; multiply and maintain these fungi in pure culture; screen, isolate, identify, multiply, and maintain native mycorrhizal fungi; develop a database on cultures maintained, and provide starter cultures on request. Cultures are available on an exchange basis or on specific requests at nominal costs for spore extraction/handling.



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Mycorrhiza and soil organic amendments

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Organic amendments to the soil may be in the form of material like chicken litter, oil cakes, rice husk, organic mulches, and sewage sludge or in the form of compost, farmyard manure, stable manure, and green manure. To understand the effect of organic amendments to the soil, it may be fruitful to understand the interaction between organic matter present in the soil (on forest floors or agricultural lands) and mycorrhiza.

Interaction between organic matter in soil and mycorrhiza

There is a lot of accumulation of litter on the surface of soil in forest ecosystems, especially in tropical and subtropical regions. Soil micro-organisms degrade this litter into humus and mineralize it for re-ploughing the nutrients from the organic source back into the soil. Mycorrhizal fungi extract the nutrients from organic matter in various stages of its degradation and thus make available to plants those nutrients which are generally in an unavailable form for the roots of non-mycorrhizal plants. Mycorrhizal fungi survive and, in most cases, grow saprophytically in accumulated organic matter on forest floors. They are also known to help in the mineralization of organic matter (Lakshman and Raghavendra 1995).

Decomposition of organic matter by mycorrhizal fungi

Symbiotic organisms are expected to cause little damage to root cells except modification of some tissues. Studies conducted at the Horticultural Research Institute, Wellesbourne, Warwick, England, on organic soil horizons of heath land and temperate forest ecosystem rich in phenolics showed that most ectomycorrhizal fungi have only low ability to degrade lignin and soluble phenolics and mycorrhizal fungi produce more phenol-oxidizing enzymes as compared to wood-decomposing fungi (Bending and Read 1997).

Some ectomycorrhizal fungi are, however, known to assist in decomposition of organic matter especially when organic matter is in advanced stages of decomposition. Decomposition of organic matter was examined in a series of experiments utilizing axenic association of Douglas fir with five ectomycorrhizal fungi (*Cenococcum geophilum*, *Rhizopogon vinicolor*, *Laccaria laccata*, *Amanita pantherina*, and *Suillus lakei*), in studies conducted at the Department of Botany and Plant Pathology, Oregon State University, Corvallis, USA. The decomposition was assessed by measuring $^{14}\text{CO}_2$ respired by mycorrhizal associations growing in peat-vermiculite amended with various ^{14}C -labelled test substrates (cellulose, hemicellulose, litter, and a model humic polymer). Uninoculated seedlings were the controls. All fungi were capable of decomposing each substrate to varying degrees. Hemicellulose was degraded more rapidly than cellulose and cellulose more rapidly than litter. Humic polymers were the most resistant to decomposition (Todd 1979).

In experiments conducted at the Laboratory of Developmental Biology, Institute of Botany, Katholieke Universiteit Leuven, Leuven, Belgium, Scots pine (*Pinus sylvestris*) seedlings were cultivated at a low rate of nutrient addition in plant containers where fresh leaf litter of beech (*Fagus sylvatica*) had been buried for up to six months. The saprophytic abilities of three ectomycorrhizal fungi (*Thelephora terrestris*, *Suillus bovinus*, and *Paxillus involutus*) were compared with degradation by the litter-decomposing basidiomycete, *Lepista nuda*, at two different pH levels; uninoculated leaves were the controls. The rate of litter decomposition was low with the mycorrhizal fungi as compared to that with *L. nuda*. Nitrogen was released only by *L. nuda* whereas leaves colonized by the mycorrhizal fungi showed no net release of nitrogen but a small accumulation of nitrogen in the

* Compiled from TERI database – Riza

litter. The ectomycorrhizal fungi did not efficiently decompose the ligno-cellulose matrix of the relatively recalcitrant beech litter. The degradation of this matrix was essential for the fungi to gain access to the leaf nitrogen pool of the fresh beech litter. A direct release of nitrogen from organic compounds by ectomycorrhizal fungi was confined to the older litter layers. The beech leaf litter contained an important fraction of easily mineralizable phosphorus which was not a growth limiting factor in the cultivation system and could, therefore, accumulate in the leaf litter colonized by the ectomycorrhizal mycelium (Colpaert and Tichelen 1996).

Nutrient mobilizing activities of the mycelia of the ectomycorrhizal fungi, *Suillus bovinus* and *Thelephora terrestris*, were investigated in studies conducted at the Department of Animal and Plant Sciences, University of Sheffield, United Kingdom. Discrete weighed samples of organic matter collected from the fermentation horizon of a pine forest soil were studied in transparent root observation chambers. The mycelia of mycorrhizal fungi grew from mycorrhizal plants of *Pinus sylvestris* over a homogeneous humified peat and colonized the introduced FHOM (fermentation horizon organic matter). The time from initial colonization to early senescence of mycelial patches was approximately 40 days. Colonization by *S. bovinus* reduced the concentration of nitrogen, phosphorus, and potassium in FHOM by 23%, 22%, and 30%, respectively; whereas colonization by *T. terrestris* led to decreases in nitrogen and potassium by 13% and 21%, respectively; increases in calcium and magnesium concentrations; but did not change phosphorus concentration. Analysis of the inorganic nitrogen pool of uncolonized FHOM incubated separately suggested that the mineralization rates were inadequate to explain the loss of nitrogen from the colonized material. The carbon-nitrogen ratio of the material colonized by both the fungi increased greatly relative to that of uncolonized material (Bending and Read 1995a).

In further studies conducted at the above university, FHOM was collected from a birch forest and placed in transparent observation chambers with (M) or without (NM) *Betula pendula* plants, the roots of which were infected with *Paxillus involutus*. The fungus colonized the FHOM of the M chambers. Activities of protease and polyphenol oxidase increased in the organic matter that had been occupied for 28–50 days and remained elevated in FHOM that had been occupied for 50–98 days. However, although phosphomonoesterase activity increased in FHOM that had been colonized for 28–50 days, amounts in FHOM colonized for 50–98 days decreased to below those found in uncolonized material. No differences in peroxidase activity were detected between colonized and uncolonized organic matter (Bending and Read 1995b).

Effect of organic matter application on mycorrhiza

In studies conducted at the Plant Biology Section, Environmental Science and Technology Department, Riso National Laboratory, Roskilde, Denmark, cucumber (*Cucumis sativus*) plants, uninoculated or inoculated with arbuscular mycorrhizal fungi, were grown in cross-shaped PVC (polyvinyl chloride) tubes where one horizontal compartment contained 100 g soil (quartz sand-loam ratio of 1:1) amended with 0.5 g ground clover leaves labelled with ^{32}P . The labelled soil was re-inoculated with a soil suspension without arbuscular mycorrhizal fungi to facilitate mineralization of organic matter. Labelling compartments were separated from a central root compartment by either 700 μm or 37 μm nylon mesh giving access to labelled soil to both hyphae and roots or only hyphae, respectively. The plants harvested after 30 days contained 15%–25% of added ^{32}P when roots had access to labelled soil. No differences between mycorrhizal and non-mycorrhizal roots were observed probably due to high root length densities (20–30 cm/cm^3) in the ^{32}P -labelled soil. The recovery of ^{32}P by hyphae alone was 5.5% and 8.6% for plants colonized with *Glomus* sp. and *G. caledonium*, respectively, but was only 0.6% in the non-mycorrhizal controls. Differences in the ^{32}P recovery between fungi were not related to root colonization rates or hyphal length densities (maximum for *G. caledonium*). Both fungi depleted the labelled soil of NaHCO_3 extractable P and ^{32}P compared to controls. Mycorrhizal fungi can thus compete successfully for P in the presence of other micro-organisms and P-sorbing clay minerals during mineralization of organic matter in soils (Joner and Jakobsen 1993 and 1994).

Erythrina americana seedlings were grown in a greenhouse and treated with three levels of organic matter (black soil) (25, 50, and 100 g), three rates of rock phosphate (20, 40, and 60 ppm), and mycorrhizal inoculation (*Glomus* sp. Zac-19), in studies conducted at the Instituto de Fitosanidad, Colegio de Postgraduados, Montecillo, Mexico. All the treatments increased almost all growth parameters recorded. In general, growth increased with increasing organic matter and rock phosphate concentration. Colonization with mycorrhiza was maximum (60.9%) with 25 g organic matter and 20 ppm rock phosphate (Gardezi, Garcia, Ferrera, et al. 1995).

In studies conducted at the Forest Pathology Division, Tropical Forest Research Institute, Jabalpur, India, bamboo (*Dendrocalamus asper*) plants were raised on two types of soil (sand-soil and sand-soil-organic manure ratios of 1:1 and 1:1:0.5 v/v, respectively) through tissue culture and were inoculated with three different arbuscular mycorrhizae (two collected from bamboo

rhizosphere and one from teak rhizosphere). After 12 months, significant positive effects of inoculum on phosphorus concentration in the shoot, root colonization, and spore production were observed in the sand + soil medium. In the organic manure amended medium, these parameters further improved. Amendment with organic manure significantly increased spore production (by 5.3- to 17.8-fold) and enhanced height and dry biomass (Verma and Arya 1998).

Studies were conducted at the Department of Botany and Plant Pathology, Oregon State University, Corvallis, USA, on Douglas fir and western red cedar (*Thuja plicata*). The seedlings were grown in untreated or pasteurized soils from undisturbed or cleared and burnt forest sites, with or without the addition of untreated or pasteurized litter. Mycorrhizae were abundant on Douglas fir seedlings grown in undisturbed forest soil but developed similarly on red cedar seedlings in either type of soil. Litter and humus included inocula of both vesicular-arbuscular and ectomycorrhizal fungi. Soil amended with litter usually enhanced the growth of host seedlings but this could not be fully attributed to the addition of mycorrhizal inoculum or nutrients provided by the litter (Parke, Linderman, and Trappe 1983).

Studies conducted at the Shenyang Agricultural University, Shenyang, Liaoming, China, showed that mycorrhizal infection of larch (*Larix* sp.) seedlings could reach 100% when humus (10 kg/m²) was applied to the soil. Untreated seedlings, seedlings treated with chemical pesticides, and those treated with humus showed damping off incidences of 95.6%, 8.5%, and 10%–16.5%, respectively. Plants treated with humus showed increases in height, diameter of the base, and the root system of the seedlings as compared to others (Wei, Liu, Yang, et al. 1987).

Sandy loam soil was kept for 14 months under two regimes (outdoor where surplus precipitation leached through the soil or indoor at constant moisture), without or with 9% (w/w) chopped wheat straw plus mineral nitrogen, in studies conducted at the Plant Biology Section, Riso National Laboratory, Roskilde, Denmark. The soils were then partially sterilized and placed in two-compartment pots separated with a 37 µm mesh. Non-mycorrhizal or mycorrhizal (*Glomus caledonium*) cucumber plants were placed in a root compartment and soils with different organic matter were placed in six parallel hyphal compartments. After 31 days, added straw increased hyphal length densities for outdoor and indoor soils by 34% and 62%, respectively. In another similar experiment only with outdoor soil and with *G. invermanium* as the VAM (vesicular-arbuscular mycorrhizal) fungus, three treatments were included: soil with no straw and with or without 0.5% (w/w) of ground *Trifolium subterraneum* leaves and soil with 9% (w/w)

straw plus mineral nitrogen. After 41 days, hyphal length density was twice as much in soil with added straw as with two other treatments (Joner and Jakobsen 1995).

Effect of litter manipulation on mycorrhiza in field experiments

In studies conducted at the Biological Station, Agricultural University, Kampsweeg, The Netherlands, five-month old, sterile grown Scots pine seedlings, uninoculated or inoculated with *Laccaria bicolor*, *Paxillus involutus*, or *Rhizopogon luteolus*, were used to see the effects of manipulation of litter and humus layer (removal, doubling, and control treatments) on the colonization potential of ectomycorrhizal fungi in two secondary stands of *Pinus sylvestris* (5 and 18 years old). In both stands, removal of litter and humus layers increased and addition of organic matter decreased the number of ectomycorrhizal types of seedlings after one growing season (Baar and Vries 1995).

In further studies conducted at the above university, ingrowth cores to a depth of 60 cm were installed in nitrogen-rich planted stands of Scots pine (*Pinus sylvestris*) in podzolic sandy soil, half a year after manipulation of litter and humus layers (removal, doubling, and control treatments) and in an untreated naturally established Scots pine stand on nutrient-poor, non-podzolic sandy soil for comparison. Ectomycorrhizal roots were found in all sampled soil depths up to 60 cm in all plots. Root growth and ectomycorrhizal development were more in the naturally established stand than in all plots in the planted stand. In general, there were more ectomycorrhizal root tips in the litter and humus removal plots than in the control plots in the planted stand. Doubling litter and humus did not significantly affect root length or number of ectomycorrhizal root tips. The nitrogen-dissolved NH₄⁺ and NO₃⁻ concentrations and the organic matter content in the upper 5 cm of the mineral soil in the planted stand on podzolic sandy soil were generally higher and the pH significantly lower than in the naturally established stand on non-podzolic sandy soil. Root growth and ectomycorrhizal development may have been negatively affected by the chemical composition of podzolic sandy soil (Baar 1997).

In studies conducted at the Department of Plant and Microbial Biology, University of California, Berkeley, USA, on three *Pinus sylvestris* stands of different ages (planted in 1987, 1963, and 1924), litter and humus layers and herbaceous vegetation were removed (sod cutting) in 1990 to create nutrient-poor sandy soils without overlying litter and humus layers. Untreated plots served as controls. Surveys conducted in 1991, 1992, and 1993 indicated that sod cutting enhanced the diversity of the species and sporocarp density of ectomycorrhizal fungi (Baar and Kuyper 1998).

Effect of litter extracts / leachates on mycorrhiza

Studies conducted at the CPG em Fitopatologia Piraciacaba, Brazil, showed that increasing the concentration of aqueous litter extracts in the culture medium reduced colony diameter and dry weight of two isolates of *Pisolithus tinctorius* from commercial *Eucalyptus grandis* stands (Auer and Bettiol 1986). Litter leachate greatly suppressed the number of mycorrhizal spores in soil and the inhibition increased with incubation time in studies conducted at the Centre of Advanced Study in Botany, University of Madras, Chennai, India. Extracts of leaves from an evergreen forest were toxic to *Glomus fasciculatum* spores. Catechin and tannic acid inhibited mycorrhizal activity (Mohankumar and Mahadevan 1987).

The growth rates of *Laccaria proxima* and *Rhizopogon luteolus* were negatively affected by the aqueous extracts of the Scots pine needles, in studies conducted at the Agricultural University, Biological Station, Kamsweg, The Netherlands. Only the high concentrations of the needle extracts had significant inhibitory effects on the growth rates of *Paxillus involutus* and *Xerocomus badius*. The needle extract significantly enhanced the growth rate of *Laccaria bicolor*. Shoot extract of the grass, *Deschampsia flexuosa*, inhibited the growth rates of *L. proxima*, *P. involutus*, and *R. luteolus* and significantly enhanced the growth rate of *L. bicolor*. The shoot extracts contained 3–5 times more high-molecular weight components, aliphatic acids, and phenolics than the root extracts (Baar, Ozinga, Sweers, et al. 1994).

Studies conducted at the Department of Horticulture, Pennsylvania University, USA, showed that the growth of ectomycorrhizal fungi, which are able to colonize red pine (*Pinus resinosa*), are differentially affected by red pine needles and its chemical components. Water extracts of pine needles stimulated the growth of *Suillus intermedium* but inhibited the growth of *Amanita rubescens*. Catechin and epicatechin gallate, components of water extracts, had a similar effect on the extract. The volatile compounds, alpha- and beta-pinene, also had differential effects on the growth of various species of ectomycorrhizal fungi (Koide, Suomi, Stevens, et al. 1998).

Effect of organic manures on mycorrhiza

Organic manures through their effects on the physical, chemical, and biological properties of soil improve the productive potential of soils and are invaluable for integrated nutrient and soil health management (Panja and Chaudhuri 1999).

Effect of farmyard manure

In studies conducted at the Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK Campus, Bangalore, India, the

influence of cropping sequence, with or without fertilizer and farmyard manure application, on VAM was studied over three consecutive seasons (maize in the first season; cowpea, groundnut, and finger millet in the second season; and sunflower in the third season). The application of farmyard manure stimulated VAM while fertilizers at the recommended level decreased the mycorrhizal propagules (Harinikumar and Bagyaraj 1989).

Studies conducted at the Department of Forest Pathology and Microbiology, Royal Forest Department, Bangkok, Thailand, showed an increased growth of *Eucalyptus camaldulensis* (in terms of increase in plant height, diameter at root collar, and total biomass) due to manure fertilization followed by ectomycorrhizal inoculation with *Pisolithus tinctorius* plus manure on old tin-mined soils. Growth of *Pinus caribaea* var. *hondurensis* was increased most by ectomycorrhizal inoculation plus manure followed by ectomycorrhizal inoculation alone. Too much manure increased soil pH and reduced the growth of *P. tinctorius* (Chalermpongse and Boonyuen 1990).

In studies conducted at the Department of Agronomy and Horticulture, Brigham Young University, Provo, USA, samples from manured and unmanured soils from a long-term field experiment were assessed. Treatment with manure did not have a significant effect on the number of VAM spores. In greenhouse experiments on dry bean (*Phaseolus vulgaris*), low levels of colonization (5%) were observed 21 days after planting, which increased to 58% 56 days after planting. Roots grown on subsoil treated with manure or compost manure showed higher colonization than roots from untreated subsoil but roots grown in topsoil had highest colonization. Topsoil promoted greatest percent colonization in early bean growth and this was reflected in greater zinc uptake during early growth stages. By day 56, plants grown in manured subsoil absorbed zinc equal to that in topsoil and more than that in the subsoil control. However, increased zinc uptake was not seen in plants grown in compost manured subsoil where a decrease in root and shoot height was observed and that seemed to decrease mycorrhizal efficiency (Tarkalson, Jolley, Robbins, et al. 1998a).

Further studies conducted at the above university showed that dry bean (*Phaseolus vulgaris*) yields were raised to similar levels as in the topsoil by manure application to the eroded or levelled silt loam soil. Manure application increased mycorrhizal colonization and zinc uptake in pot experiments with dry bean, which could explain the increased yields in the field. Similar field studies on wheat (*Triticum aestivum*) and sweet corn (*Zea mays*) showed that mycorrhizal root colonization was higher in untreated (treated with conventional fertilizers) than with the dairy manure treated wheat and sweet corn. Root colonization was also higher in subsoil than in topsoil in wheat but no

such difference was observed in case of sweet corn. In general, zinc and manganese concentrations in the shoot increased with increased root colonization for both species. However, wheat yield was highest for manure treated subsoil and topsoil compared to untreated soil. Thus mycorrhizal colonization was different between conventional and manure treated soils and also between topsoil and subsoil and these differences increased zinc and manganese uptake but this did not explain the improvement in wheat yields obtained with manure application (Tarkalson, Jolley, Robbins, et al. 1998b).

Studies conducted at the Department of Agricultural Microbiology, University of Agricultural Science, Dharwad, India, on three genotypes of wheat (DWR-39, DWR-163, and DWR-187), showed that organic amendments of soil had a positive influence on the proliferation of *Glomus fasciculatum*. Farmyard manure stimulated the greatest root colonization in artificially inoculated DWR-39 and DWR-187. Biogas spent slurry caused greatest root colonization is DWR-163. A corresponding trend was observed in plant height, shoot biomass, seed yield, shoot phosphorus, and seed protein concentration in these treatments. Organic amendments with a narrow C-N ratio had a greater influence on VAM proliferation compared with a wider C-N ratio (Gaonker and Sreenivasa 1994).

Effect of manure slurry

Studies were conducted at the Food and Agricultural Department, The Queen's University of Belfast, Northern Ireland, on long-term field experiment in which pig and cow dung slurries had been applied for 19 years (at 50, 100, and 150 m³ha⁻¹year⁻¹ for each slurry) to a cut grassland to examine VAM. Increasing the application of both pig and cow dung slurries produced a marked decrease in overall VAM infection of plant roots of *Lolium perenne*, *Agrostis stolonifera*, and *Poa* spp. in the sward with a corresponding increase in infection by the fine endophyte, *Glomus tenue*. Mycorrhizal infection was related to the botanical composition of sward, especially the proportion of *Lolium perenne*. Step-wise multiple regression analysis identified soil chemical properties, especially total zinc and pH, as more important explanatory variables in preference to botanical composition (Christie and Kilpatrick 1992).

Effect of compost

In studies conducted at the Department of Plant Science, University of Connecticut, Storrs, USA, compost of corn (*Zea mays*), high in available nutrients, and that of eastern hemlock (*Tsuga canadensis*) and sugar maple (*Acer saccharum*), both relatively low in nutrients, were each used as the

medium for container-grown hemlock, sugar maple, and rhododendron (*Rhododendron catawbiense*) over a three-year period. No VAM development was observed in a corn plant bioassay in corn compost while a moderate development in hemlock compost and high development in maple compost was found. Sugar maple was showed the greatest plant growth and incidence of VAM in maple compost while corn compost was lethal to 50% of the maples and produced a retarded growth and low incidence of VAM in the survivors. Hemlocks showed the greatest plant growth and incidence of ectomycorrhiza in unfertilized hemlock and maple composts. Rhododendrons showed the greatest growth in corn compost. Maples and rhododendrons showed a positive response to fertilized composts but hemlocks showed a significant retarded growth response to fertilizers (Guttay 1982).

Further studies were conducted at the same university, in which corn plants were grown in pot cultures on each of the composted plant residues of corn, eastern hemlock, and sugar maple with fertilizers as an added variable. Vesicular-arbuscular mycorrhiza on corn plants was quantitatively low in the corn and hemlock composts and high in the maple compost. Dry matter production was related to the fertility of the medium and was independent of the quantity of VAM. The incidence of VAM in maple compost was negatively affected by added superphosphate and positively affected by added ammonium nitrate, muriate of potash, and limestone. Also, the incidence of VAM was negatively correlated with extractable phosphorus and positively correlated with extractable potassium in the maple compost (Guttay 1983).

Four-week-old commercial lettuce (*Lactuca sativa*) and non-mycotrophic cabbage (*Brassica oleracea*) seedlings were studied at the New Year Research Centre, Agriculture Research Organization, Yishay, Israel. In both species, height, weight, and chlorophyll concentration of the seedlings were greater in media containing compost as compared to commercial peat-vermiculite medium. Lettuce seedlings from uninoculated media had greater height, weight, and chlorophyll concentration than those from mycorrhiza-inoculated media. Cabbage seedlings from media inoculated with mycorrhiza and *Trichoderma* had greater height, weight, and chlorophyll concentration than those from uninoculated media (Raviv, Zaidman, and Kapulnik 1998).

Studies conducted at the Division of Environmental Sciences, Indian Agricultural Research Institute, New Delhi, India, showed that crop yield of pearl millet can be significantly increased and nutritive value improved if the spent spawn of mushroom is used along with VAM (Kumar, Rai, and Jayaraman 1995).

In studies conducted at the Departamento de Microbiologia, Estacion de Experimental del

Zaidin, Consejo Superior de Investigaciones Científicas, Granada, Spain, maximum growth and nutrient uptake of alfalfa during three crop cycles were recorded in soil amended with sugar beet waste, treated by *Aspergillus niger* under the conditions of 10-, 20-, and 30-day solid state fermentation supplemented with rock phosphate. This effect was more pronounced in treatments with arbuscular mycorrhizal fungus grown in soil enriched with 10- and 20-day microbially treated sugar beet waste + rock phosphate when the average total plant growth increased by 233% and 343%, respectively, over non-mycorrhizal controls containing untreated sugar beet waste. Compared to other treatments, plant mycorrhization was ineffective when 30-day treated agrowaste was used (Rodríguez, Vassilev, and Azcon 1999).

Effect of compost and stable manure

In studies conducted at the Institut für Pflanzenbau und Tierhygiene in den Tropen und Subtropen, Göttingen, Germany, three organic soil amendments (fresh stable manure, composted stable manure, and composted plant remains [each sterilized or unsterilized]) were applied at 50, 100, or 300 dt/ha to pot-grown greenhouse *Capsicum annuum* inoculated with *Acaulospora longula*. The effect of inoculation on growth of *C. annuum* was better with unsterilized than with sterilized amendments but in general, decreased with increasing rates of application of organic manures due to better growth of non-mycorrhizal plants (Brechtel 1989 and 1990).

Effect of dairy manure, chicken litter, and leaf compost

Studies were conducted at the United States Department of Agriculture, Agricultural Research Service, Wyndmoor, USA, to examine the populations of VAM fungi and mycorrhiza formation in a field experiment with application of composted animal manures in rotation crops of *Zea mays* / vegetable crops (*Spinacea oleraceae* and/or *Capsicum annuum*) / small grain crops (*Avena sativa* or *Triticum aestivum*). Samples taken after three years showed that chicken litter / leaf compost and dairy cow manure / leaf compost enhanced spore populations of two type groups of VAM fungal species (*Glomus etunicatum* type and the general *Glomus* spp. group including *G. mosseae*) relative to those found in plots treated with raw dairy cow manure and conventional fertilizer. Populations of other groups were not affected by amendment probably due to the large amount of phosphorus added in composts and manure relative to the conventional fertilizer applied. Crop rotation point had consistent, significant effects with both lower population of spores and less mycorrhizal infectivity of soil in plots after the vegetable crop relative to maize and small grain crops. This was due to

non-mycorrhizal status and very low mycorrhization (approximately 1% root length colonization) of *S. oleraceae* and *C. annuum* (Douds Jr, Galvez, Frankesnyder, et al. 1997).

Effect of compost, stable manure, and organic mulches

In studies conducted at the Institut für Tropischen und Subtropischen Pflanzenbau der Universität Göttingen, Germany, organic manures consisting of grass mulch, stable manure, and three different kinds of compost were added in five concentrations (50, 100, 150, 100, and 300 dt/ha) in both unsterilized and sterilized and pulverized form to observe the efficiency of VAM fungus, *Glomus macrocarpum*, cultivated with *Capsicum annuum*. Mycorrhizal fungal inoculation resulted in maximum yield and phosphorus uptake increase at the lowest concentration of organic manure. Higher concentrations of all manures led to decreasing mycorrhizal efficiencies. This tendency was least pronounced with the grass mulch variant. Important factors of influence were the kind of starting material used for compost preparation and also whether the manures were mixed up rotten or in not rotten conditions (Brechtel 1987).

Effect of other miscellaneous organic amendments on mycorrhiza

Studies conducted at the Faculty of Agriculture, Department of Plant Pathology, Bidhan Chandra Krishi Vishwavidyalaya, Kalyani, West Bengal, India, on the effects of four different organic manures (rice husk, green leaf manure, farmyard manure, and oil cake) on mycorrhizal relations of *Cajanus* in a low-nutrient alluvial soil showed that root colonization intensity was not changed by the manures except by oil cake. At high concentrations, oil cake significantly reduced root colonization intensity. Promotion of root development by these manures resulted in a significant increase in mycorrhizal root biomass yield and spore production, except by oil cake which even at moderate concentration suppressed spore production significantly. Rice husk promoted root development and stimulated spore production. Farmyard manure and green leaf manure caused moderate stimulation of mycorrhizal root biomass and spore production in root association (Panja and Chaudhuri 1999).

In studies conducted at the Department of Microbiology, Annamalai University, Annamalai Nagar, Tamil Nadu, India, *Glomus fasciculatum* inoculated MCU-7 cotton plants applied with farmyard manure, biogas slurry, urban compost, and pressmud recorded 84.547%, 72.82%, 68.55%, and 62.6% root colonization, respectively, as compared to 56.04%, 52.06%, 45.05%, and 40.27% root colonization, respectively, in uninoculated cotton plants. The increase in growth and yield parameters observed with

farmyard manure was highest, followed by treatments with biogas slurry, urban compost, and pressmud. Mycorrhizal plants recorded higher values of growth and yield parameters than uninoculated plants (Srilakshmi, Sundaram, and Tholkappian 1999).

Effect of application of organic mulches on mycorrhiza

Organic mulches not only help mycorrhiza proliferation in soil but also help in suppressing weed plants. Studies conducted at the Ministère de l'Énergie et des Ressources, Direction Recherche et Développement, Service de l'Amélioration des Arbres Sainte-Foy, Quebec, Canada, showed that under laboratory conditions, cold water extracts of barley, oat, and wheat straws strongly inhibited the propagule growth of red raspberry (*Rubus idaeus*), a common forest weed. Shoot dry weights were 10%, 44%, and 68% of the control for oat, wheat, and barley straw extracts, respectively. Barley, oat, and wheat straws used as cover mulches inhibited red-raspberry establishment and nitrogen nutrition after clear cutting and site preparation (Jobidon, Thibault, and Fortin 1989a and 1989b).

Studies conducted at the Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK Campus, Bangalore, India, showed that soil amendments with rice straw, maize straw, and pongamia leaf increased the proliferation of VAM fungi. Addition of pongamia leaf showed the highest increase of native mycorrhizal fungi and this was followed by maize straw. Intercropping of soybean and maize stimulated proliferation of VAM fungi compared to monocropping with soybean or maize. In the soil cultivated with soybean, there were more mycorrhizal propagules as compared to maize (Harinikumar, Bagyaraj, and Mallesha 1990).

Studies conducted at the Centro de Edafología, Colegio de Postgraduados, Montecillo, Mexico, on the role of VAM fungi in zero tillage, legume-corn-squash system of sustained corn production, showed that intense microbiological activity resulted when the legume, *Stizolobium*, was mulched in the intercrop system. This enabled VAM hyphae already present in the soil to colonize corn rapidly since corn is planted only a few days after the legume is mulched. The absence of tillage favoured nutrient cycling since VAM mycelium in the soil was not disrupted and the nutrient transfer from the soil to the plant and from the plant to the soil could proceed effectively (Gonzalez-Chavez, Ferrera-Cerrato, and Garcia 1990).

In studies conducted at the Illinois Natural History Survey and University of Illinois, Urbana, USA, 38 mature (18–30 inches diameter at breast height) white oak (*Quercus alba*) trees with moderate to severe chlorosis were treated with approximately three inches of fresh, woodchip mulch, and ammonium nitrate (141 g/m²) was applied annually

for four years. After the treatment, soil bulk density and pH were lower in mulched (0.73 g/ml and 5.8, respectively) than in control samples (1.14 g/ml and 6.7, respectively). The moisture content, fine root development, and number of mycorrhizal roots increased under mulch. Also, foliar manganese increased and phosphorus decreased significantly. These changes were associated with a reduction of interveinous chlorosis (Himelick and Watson 1990).

Effect of green manuring on mycorrhiza

In studies conducted at the Ruhr-Stickstoff AG, Landwirtschaftliche Forschung, Hanninghof, Dulmen, Germany, inoculum potential of VAM fungi and the influence of green manure and nitrogen (100 and 200 kg/ha) on it were assessed in soils with winter wheat in a continuous monoculture and a four-year cereal crop rotation (winter wheat, winter barley, winter rye, and oats). Vesicular-arbuscular mycorrhiza was frequent in intensively cultivated soils. Compared with crop rotation, VAM inoculum potential was significantly reduced in continuous monoculture. A cumulation of this effect, with a particularly strong decrease of VAM, occurred when other factors unfavourable for the symbiosis coincided with continuous monoculture. Among these factors were the use of non-mycorrhizal *Raphanus sativus* and *Brassica napus* for green manure together with 200 kg/ha of nitrogen. Green manure and higher levels of nitrogen resulted in less disturbance of the VAM inoculum potential in crop rotation. No correlation between VAM inoculum potential in the soil and wheat yields was observed in crop rotation or continuous monoculture (Baltruschat and Dehne 1988).

Studies with similar four-year crop rotation (but with winter barley in a continuous monoculture) showed that VAM inoculum potential was high at both levels of nitrogen (100 and 200 kg/ha) although higher nitrogen level resulted in a reduction of inoculum potential in winter barley in both rotation and monoculture. No influence of nitrogen on inoculum potential was observed with oats and winter rye, which were cultivated in rotation. Green manure had a negative effect on inoculum potential in barley in monoculture but not in rotation. This may be due to annual application of green manure in monoculture and application of green manure only prior to the cultivation of oats and winter wheat in rotation. Except for the effect of green manure, large differences in inoculum potential were not found in rotation and monoculture of winter barley (Baltruschat and Dehne 1989).

Experiments conducted at the Central Soil Salinity Research Institute, Karnal, Haryana, on reclaimed sodic soils showed that after wheat harvest, there was a significant decrease in total VAM spore count with increasing soil phosphorus levels while green manuring resulted in an increase in

total spore count. Four VAM species observed in the experiment showed a differential response to phosphorus and green manuring. *Gigaspora* sp. and one *Glomus* sp. were significantly inhibited when soil phosphorus level crossed 10 kg/ha and green manure further reduced the spore count possibly due to increased soil phosphorus resulting from green manuring. The other two *Glomus* spp. were unaffected by the increasing soil phosphorus levels and the green manuring significantly increased their spore count in soil (Sharma 1995).

Studies were conducted at Ufrrj, Itaguaí, Brazil, to evaluate the effect of different green manures and fallow on potential of indigenous VAM fungal inoculum and yield of sweet potato (*Ipomoea batatas*). The treatments were: no vegetation, fallow, sunhemp (*Crotalaria juncea*), jack bean (*Canavalia ensiformis*), pigeon pea (*Cajanus cajan*), and velvet bean (*Mucuna aterrima*). Jack bean and velvet bean had a greater nitrogen, phosphorus, and potassium accumulation in the shoot than other treatments. Previous cultivation with leguminous crops increased sweet potato yield over fallow. Incorporation of leguminous crops into the soil reduced spore population in no vegetation, jack bean, and pigeon pea when compared with fallow. However, sunhemp, velvet bean, and jack bean had more infective propagules than no vegetation. Root colonization of sweet potato was greater in sunhemp, velvet bean, and fallow when compared with no vegetation. Yield of sweet potato had a direct correlation with the amounts of nitrogen, phosphorus, and potassium in the green manures (Espindola, DeAlmeida, Guerra, et al. 1998).

Studies conducted at the Centre for Advanced Studies in Botany, University of Madras, Chennai, India, on the effect of organic soil amendments on arbuscular mycorrhizal fungal activities of rice plants showed that arbuscular mycorrhizal spore density, per cent infection, and intensity of infection were increased while sheath blight disease caused by *Rhizoctonia solani* decreased. Certain amendments, especially green leaf manure, stimulated arbuscule development in rice plants (Baby and Manibhushanrao 1996).

Effect of sewage sludge on mycorrhiza

The effect of sewage effluents on mycorrhiza has been discussed in an earlier issue of Mycorrhiza News (Sujan Singh 1996).

Studies conducted at the AFRC Institute of Arable Crop Research, Rothamsted Experimental Station, Harpenden, United Kingdom, showed that treatment of soil with sewage sludge prior to 1961 had a strong negative effect of the heavy metals on the development of both native and introduced (*Glomus mosseae*) mycorrhizal fungi (Koomen and McGrath 1990).

Studies were conducted at the Department of Plant Biology and Pathology, University of Maine, Orono, USA, on soybean (*Glycine max*) grown for

7 weeks in fine loamy soil treated factorially with: (1) 0, 60, 150, or 270 mg/kg of phosphorus as monocalcium phosphate; (2) four sources of sewage sludge providing 100 mg/kg of phosphorus or a non-treated control; and (3) *Glomus fasciculatum* as mycorrhizal inoculum or no inoculum. Sludge reduced phosphorus uptake at 150-mg/kg phosphorus concentration or higher in non-mycorrhizal plants with little differences in plant growths among sludges. In treatments with mycorrhizal inoculum, growth and phosphorus uptake responses to sludge ranged from very beneficial with two sludges to a complete inhibition with another sludge. This inhibition was persistent and apparently due to suppression of the mycorrhizal fungi by toxic levels of NH_4^+ (Lambert and Weidensaul 1991).

Studies conducted at the University of Giessen, Institute of Zernernahrung, Sudanlage, Germany, showed that sewage sludge fertilization decreased mycorrhiza population in some localities possibly due to higher sensitivity of mycorrhizal fungi to heavy metals, especially zinc. In some localities, population of endophytes in the soil was rather high with spore densities of 31–97 per gram of dry soil (Loth 1996).

A pot experiment was conducted at the Soil, Water and Environment Research Institute, Agricultural Research Centre, Egypt, to study the effect of mycorrhizal inoculation and application of three types of organic manures (biogas residues, town refuse, and sewage sludge) on wheat (Giza 157) plants. Incorporation of organic manures into the soil enhanced plant growth as evidenced by increases in shoot dry weight and nitrogen and phosphorus uptake. Sewage sludge was the most effective manure, followed by biogas residues while town refuse compost exhibited the least influence. The applied organic manures caused a slight reduction in VAM infection. However, organic fertilizers reduced differences between the inoculated and uninoculated plants due to vigorous growth of uninoculated plants (Mikhaeel, Estefanous, and Antoun 1997).

Studies conducted at the Soil Microbiology Centre Via del Borghetto, Pisa, Italy, on application of compost from a mixture of the organic biodegradable fraction of municipal solid waste and sewage sludge in pot experiments with a low fertility soil supporting clover and sorghum, showed that addition of up to 10% compost on a dry weight basis was not inhibitory to *Glomus mosseae* and *Rhizobium trifolii* (Leporini, Pera, Vallini, et al. 1992).

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Research findings

Mycotrophy of sweet potato (*Ipomoea batatas*) in coastal soils of Tamil Nadu

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Introduction

Vesicular–arbuscular mycorrhizae, or VAM, have a mutualistic symbiotic relationship with most agricultural crops and enhance plant growth. Tuber crops are now being widely recognized as one of the major sources of food in tropical countries. Inoculation with VAM increases the growth of sweet potato (*Ipomoea batatas*) in low-phosphate fields. The occurrence of VAM species varies with climatic and edaphic factors as well as with land-use. In this study, a survey was carried out to observe the VAM status in sweet potato grown in coastal soils of Tamil Nadu.

Materials and methods

The plants of sweet potato and rhizosphere soil samples were collected from eleven locations of coastal Tamil Nadu. Wet sieving and decanting technique were used to assess VAM spores (Gerdemann and Nicolson 1963). The spores collected were identified following the synoptic key to the genera and species of *Endogonaceae* (Gerdemann and Trappe 1974). Per cent colonization of mycorrhizal roots was assessed by the method of Phillips and Hayman (1970).

Results and discussion

All the eleven locations in coastal Tamil Nadu showed VAM association in the roots of sweet potato, though there was a considerable variation in per cent

root colonization (Table 1). Samples from Tiruchendur showed maximum root colonization (32%) and spore density (44.3 per 10 g of soil). Samples from Marakanam showed least per cent root colonization (15.4%) and spore density (24.2 per 10 g of soil). In general, the locations had sandy loam soil and a neutral pH (6.8 to 7.5) except Marakanam (8.1) and Pudukottai (8.0) where higher pH was one of the reasons for poor root colonization.

Vesicular–arbuscular mycorrhizae have been reported from different geographical locations with a wide range of cultivated plant species (Tinker 1982). This study thus, besides confirming the ubiquitous nature of VAM fungi (Hayman 1982, Mosse 1981), brought out their prevalence in the roots and rhizosphere soils of sweet potato in coastal Tamil Nadu. Maximum response of sweet potato to VAM fungi in sandy loam soils of Tiruchendur and Puduchatram made it evident that the type of soil and its physico-chemical properties determined the mycotrophy of sweet potato.

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Table 1 Mycorrhizal status of sweet potato in coastal soils of Tamil Nadu

Location	Soil type	pH	Electrical conductivity (dsm ⁻¹)	Available P ₂ O ₅ (kg/ha)	Available N (kg/ha)	Available K ₂ O (kg/ha)	Organic carbon (%)	Root infection (%)	Spore density (number per 10 g of soil)
Orthur	Sandy loam	7.5	0.53	22.91	87.50	600.31	0.57	22.6	32.3
Palur	Sandy loam	7.2	0.49	24.24	112.68	470.28	0.55	21.8	31.4
Tindivanam	Sandy loam	7.3	0.34	22.33	127.72	590.64	0.49	24.4	34.7
Pudukottai	Sandy loam	8.0	0.29	27.64	138.17	475.75	0.85	17.6	27.7
Marakanam	Sandy loam	8.1	0.32	29.80	96.24	486.23	0.60	15.4	24.2
Ramnad	Sandy loam	7.3	0.40	20.73	150.13	537.18	0.50	25.4	37.1
Puduchatram	Sandy loam	7.0	0.35	13.44	103.42	606.27	0.53	30.6	41.6
Sethiathope	Sandy loam	7.1	0.51	15.17	95.29	564.62	0.47	29.3	39.9
Kanyakumari	Sandy	7.5	0.42	25.66	115.36	618.68	0.15	19.8	29.8
Tiruchendur	Sandy loam	6.8	0.33	11.28	127.64	540.73	0.72	32.0	44.3
Tuticorin	Sandy	7.0	0.51	18.34	134.34	426.66	0.31	27.4	39.3

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Growth stimulation of *Santalum album* seedlings by vesicular–arbuscular mycorrhizal fungi

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Introduction

Vesicular–arbuscular mycorrhiza, or VAM, is being widely used during the raising of nursery seedlings as it enhances nutrient availability (Peterson, Piche, and Plenchette 1984). *Santalum album* L., due to its economic value, is one of the important tree taxa being used by the state forest departments in afforestation programmes. In the present study, attempts were made to accelerate the growth of seedlings of *S. album* L., a semi root parasite, by applying two strains of VAM inoculum, namely *Glomus fasciculatum* and *G. aggregatum*.

Materials and methods

Sterilized soil mixture (red soil–sand ratio v/v of 1:3) was taken in pots (3 kg/pot) which were divided into four lots. One lot was inoculated with 200 g of soil containing *G. fasciculatum* spores and the other with that containing *G. aggregatum* spores. Surface sterilized (using 0.1% mercuric chloride) viable seeds of *S. album* L., soaked overnight in 0.05% GA₃, were sown (20 seeds/pot) and the pots were watered with half-strength Hogland's nutrient solution. In the third lot, 20 seeds soaked overnight in 0.05% GA₃, were sown without VAM inoculum. In the fourth lot, seeds without GA₃ treatment and with or without VAM inoculum were sown. Seeds germinated in all except the fourth lot. The seedlings were raised for 60 days and two seedlings from the pots with VAM inoculum were collected and the presence of VAM in their roots

established (Phillips and Hayman 1970). Growth parameters, namely stem thickness, leaf area, number of leaves, shoot and root length, and shoot and root biomass, of both VAM inoculated and control plants were recorded after separating plant parts from the pot soil mass. Leaf area was measured using a portable leaf area meter, LI–COR–3000 (USA).

Results and discussion

VAM inoculation improved the growth of the seedlings as indicated by increased shoot and root length, stem thickness, and surface area of the leaves (Table 1). Maximum growth promotion was recorded in *G. fasciculatum* treated plants in which the shoot length increased by 66.2%, fresh weight by 96.4%, and plant biomass by 94.7% over the control plant. Such an increase has been reported in citrus (Antones and Cardou 1991), tamarind (Reena and Bagyaraj 1990), and teak (Durga and Gupta 1995), and other forest tree taxa. Therefore, VAM, particularly *G. fasciculatum*, is significant in obtaining greater number of healthier planting stock of *S. album* L. seedlings.

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Table 1 Comparison of growth parameters of control and VAM (vesicular-arbuscula mycorrhiza) inoculated seedlings of *Santalum album* L.

Treatment	Stem thickness (cm)	No. of leaves/plant	Total leaf area (cm ²)	Shoot			Root		
				Length (cm)	Fresh weight (g)	Dry weight (g)	Length (cm)	Fresh weight (g)	Dry weight (g)
Control	0.9 ± 0.2	13.0 ± 2.1	3.25 ± 0.45	15.4 ± 2.4	0.56 ± 0.08	0.12 ± 0.03	7.7 ± 0.9	0.17 ± 0.04	0.07 ± 0.02
<i>G. fasciculatum</i>	1.4 ± 0.3	20.8 ± 1.5	5.57 ± 0.58	25.6 ± 3.2	1.1 ± 0.05	0.27 ± 0.04	12.2 ± 1.8	0.28 ± 0.05	0.10 ± 0.03
	(55.5)	(57.8)	(71.4)	(66.2)	(96.4)	(125.0)	(58.4)	(64.7)	(42.8)
<i>G. aggregatum</i>	1.1 ± 0.1	15.0 ± 2.0	4.10 ± 0.64	17.2 ± 2.5	0.7 ± 0.06	0.17 ± 0.03	11.3 ± 1.5	0.22 ± 0.03	0.08 ± 0.02
	(22.2)	(15.4)	(26.1)	(11.7)	(25.0)	(41.6)	(46.7)	(29.4)	(14.3)

Note Values expressed are mean ± standard deviation; Values in parentheses show percentage increase over control

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Arbuscular mycorrhizal association and incidence of root-knot nematode on some cucurbits in eastern Uttar Pradesh: a preliminary survey report

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Introduction

Cucurbits are cultivated extensively in India and are used as salad, vegetable, desert fruit, pickle, candy, etc. These summer crops are grown generally by the farmers under suboptimal dose of inorganic fertilizers and moisture tension and thus they might be depending on arbuscular mycorrhizae for their nutrient and moisture requirements.

Mycorrhizal association in plants is rule rather than exception (Gerdemann 1968) with more than 80% of plant species being mycorrhizal in nature (Gianinazzi, Gianinazzi-Pearson, and Trouvelot 1982). Some of the cucurbits are heavily infested by root-knot nematodes *Meloidogyne* Goeldi (Khan,

Saxena, and Siddiqi 1971; Seshadri 1993). Little information is available on the degree of mycorrhization, a measure of plant performance in adverse conditions, and the incidence of root-knot nematode in cucurbits in eastern Uttar Pradesh. This paper reports preliminary data on arbuscular mycorrhization and incidence of root-knot nematode in cucurbits commonly grown in this area.

Materials and methods

A survey was carried out in six districts of eastern Uttar Pradesh and root and soil samples were collected from around ash-gourd (*Benincasa hispida* (Thunb.) Cong.), bitter-gourd (*Momordica carantia*

L.), bottle-gourd (*Lagenaria ciceraria* (Mol.) Standley), cucumber (*Cucumis sativus* L.), muskmelon (*Cucumis melo* L.), pointed-gourd (*Trichosanthes dioica* Roxb.), pumpkin (*Cucurbita moschata* (Duch.) Poir.), round-gourd (*Citrullus vulgaris* var. *fistulosus* Duth. & Full.), sponge-gourd (*Luffa cylindrica* (L.) M.J.Roem.), and watermelon (*Citrullus lanatus* (Thunb.) Matsum & Nakai).

Roots were washed under running tap water. Incidence of root-knot nematode was recorded as gall index on 0–4 scale (0: no gall formation, 1: 25%, 2: 50%, 3: 75%, and 4: 100% of the roots galled). To quantify mycorrhizal association, properly washed roots were cleared in near-boiling 10% KOH aqueous solution for 48 hours and stained in trypan blue following several washings in distilled water to drain out KOH (Phillips and Hayman 1970). Stained roots were cut into 1 cm segments and 100 such segments were randomly picked up and examined under microscope. Arbuscular

mycorrhizal colonization was determined by Nicolson's formula (1955).

$$\text{Per cent colonization} = \frac{\text{Number of root segments colonized}}{\text{Total number of segments examined}} \times 100$$

Results and discussion

The degree of mycorrhization ranged from 0% to 95%. All the ten cucurbits were found mycorrhizal in nature though some samples were devoid of such an association. The average degree of mycorrhization in various crops and districts ranged from 16% to 44%. There was no distinct relationship between the crop and the location with the degree of mycorrhization (Table 1).

The incidence of root-knot nematode measured in terms of gall index in bottle-gourd, pointed-gourd,

Table 1 Arbuscular mycorrhizal colonization of some cucurbits in six districts of eastern Uttar Pradesh

Crop	Mycorrhizal colonization (%)	Districts					
		Bahraich	Faizabad	Jaunpur	Mirzapur	Sultanpur	Varanasi
Ash-gourd	Range	0-70	8-60	0-55	11-80	0-50	0-54
	Mean (SD) ^a	25 (28)	31 (21)	31 (18)	44 (23)	24 (18)	21 (17)
	n ^b	5	6	8	20	10	8
Bitter-gourd	Range	0-75	0-78	8-65	5-70	0-60	0-75
	Mean (SD)	25 (24)	28 (22)	32 (18)	30 (20)	27 (16)	30 (20)
	n	10	25	15	15	15	20
Bottle-gourd	Range	0-70	0-80	0-66	10-77	0-77	0-78
	Mean (SD)	28 (21)	33 (23)	28 (18)	30 (20)	25 (19)	30 (22)
	n	20	35	20	20	20	30
Cucumber	Range	0-38	0-70	0-60	5-50	0-50	0-70
	Mean (SD)	17 (11)	25 (19)	30 (17)	27 (14)	23 (14)	31 (19)
	n	15	20	15	15	16	15
Muskmelon	Range	8-70	8-60	0-80	5-78	10-65	0-50
	Mean (SD)	34 (22)	35 (16)	35 (23)	33 (22)	35 (19)	27 (17)
	n	10	10	35	25	10	10
Pointed-gourd	Range	8-95	0-70	0-70	8-80	11-68	8-78
	Mean (SD)	43 (32)	30 (21)	31 (18)	38 (27)	28 (21)	39 (21)
	n	15	20	20	10	10	15
Pumpkin	Range	0-80	0-70	0-75	5-60	0-55	0-80
	Mean (SD)	30 (19)	29 (23)	33 (19)	28 (18)	31 (19)	39 (21)
	n	20	15	15	15	8	15
Round-gourd	Range	0-35	4-55	0-65	5-65	0-50	0-44
	Mean (SD)	16 (11)	29 (18)	35 (20)	30 (23)	24 (17)	23 (16)
	n	10	10	15	10	8	15
Sponge-gourd	Range	0-85	0-80	5-90	5-85	0-50	0-75
	Mean (SD)	29 (23)	33 (22)	39 (23)	38 (22)	28 (14)	35 (21)
	n	25	35	30	20	20	25
Watermelon	Range	10-30	5-80	0-60	5-80	10-35	5-60
	Mean (SD)	19 (8)	26 (26)	36 (18)	31 (21)	20 (12)	29 (17)
	n	5	8	10	35	5	10

^astandard deviation; ^bnumber of samples

and sponge-gourd ranged from 2 to 4 in various districts and the average varied from 3.2 to 3.9 exhibiting a high degree of susceptibility of these crops to this nematode (Table 2). Ash-gourd, bitter-gourd, cucumber, muskmelon, pumpkin, round-gourd, and watermelon on the other hand exhibited poor host status as the average gall index in various districts ranged from 0.6 to 1.6. Saka and Carter (1987) have listed them under the category of non-host to *Meloidogyne incognita*. These crops have also shown, however, casually, the gall index rating of 3. In such cases, an association with *M. javanica* was found rather than with *M. incognita*. It was casually observed that highly susceptible crops like bottle-gourd, pointed-gourd, or sponge-gourd which exhibited low incidence of root-knot nematode were highly mycorrhized (80%–90%) pointing towards possible interaction between nematode and fungus symbiont. Such interactions have already been reported in various crops and the literature has been reviewed (Hussey and Roncadori 1982, Singh 1997).

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Table 2 Incidence of root-knot nematode on some cucurbits in six districts of eastern Uttar Pradesh

Crop	Gall index (0–4 scale)	Districts					
		Bahraich	Faizabad	Jaunpur	Mirzapur	Sultanpur	Varanasi
Ash-gourd	Range	0–2	0–2	0–3	0–3	0–3	0–3
	Mean (SD) ^a	0.8 (0.8)	1.0 (0.8)	1.3 (1.0)	1.3 (0.9)	1.3 (0.9)	1.3 (1.0)
	n ^b	5	6	8	20	10	8
Bitter-gourd	Range	0–3	0–3	0–3	0–3	0–3	0–3
	Mean (SD)	1.5 (0.9)	1.6 (0.9)	1.4 (1.0)	1.5 (0.9)	1.3 (0.8)	1.1 (0.9)
	n	10	25	15	15	15	20
Bottle-gourd	Range	2–4	2–4	3–4	3–4	3–4	3–4
	Mean (SD)	3.3 (0.7)	3.2 (0.7)	3.6 (0.5)	3.6 (0.5)	3.5 (0.5)	3.6 (0.5)
	n	20	35	20	20	20	30
Cucumber	Range	0–3	0–3	0–3	0–3	0–3	0–3
	Mean (SD)	1.1 (0.9)	1.0 (0.9)	1.0 (0.9)	1.0 (0.9)	1.0 (0.9)	0.9 (0.9)
	n	15	20	15	15	16	15
Muskmelon	Range	0–3	0–3	0–3	0–3	0–3	0–3
	Mean (SD)	1.6 (0.8)	1.5 (1.1)	1.3 (1.1)	1.0 (0.9)	1.5 (1.1)	1.5 (1.1)
	n	10	10	35	25	10	10
Pointed-gourd	Range	3–4	3–4	3–4	3–4	3–4	3–4
	Mean (SD)	3.8 (0.4)	3.9 (0.4)	3.9 (0.3)	3.8 (0.4)	3.9 (0.3)	3.9 (0.3)
	n	15	20	20	10	10	15
Pumpkin	Range	0–3	0–3	0–3	0–3	0–3	0–3
	Mean (SD)	1.3 (0.8)	1.3 (0.9)	1.3 (1.9)	1.2 (1.0)	1.4 (0.9)	1.0 (0.8)
	n	20	15	15	15	8	30
Round-gourd	Range	0–3	0–3	0–3	0–2	0–2	0–3
	Mean (SD)	1.0 (1.1)	1.3 (0.9)	1.1 (1.0)	0.9 (0.8)	0.9 (0.6)	0.9 (0.9)
	n	10	10	15	10	8	15
Sponge-gourd	Range	3–4	3–4	3–4	3–4	3–4	3–4
	Mean (SD)	3.8 (0.4)	3.7 (0.4)	3.8 (0.4)	3.6 (0.4)	3.8 (0.4)	3.7 (0.5)
	n	25	35	30	20	20	25
Watermelon	Range	0–3	0–2	0–2	0–3	0–1	0–2
	Mean (SD)	1.2 (1.3)	1.1 (0.8)	1.0 (0.8)	1.2 (0.9)	0.6 (0.5)	1.1 (0.9)
	n	5	8	10	35	5	10

^astandard deviation; ^bnumber of samples

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Influence of tillage practices and rice (*Oryza sativa* L.) based cropping systems on native VAM fungal population in rainfed upland ecosystem

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Introduction

Tillage and farming systems affect the spore population and colonization of VAM (vesicular–arbuscular mycorrhizal) fungi obligate symbionts potentially useful in sustainable agriculture (Douds Jr, Galvez, Janke, et al. 1995; Douds Jr Janke, and Peters 1993; Maiti, Variar, and Saha 1995). The affects of tillage practices and rice-based intercropping systems on native VAM fungi population under aerobic soil conditions of rainfed upland ecosystem were studied.

Materials and methods

Tillage practices

A long-term (1991–95) experiment was conducted at the research farm of CRURRS (Central Rainfed Upland Rice Research Station) with three different tillage practices involving wet-season (June to September) upland rice (variety: Vandana; fertilization: 40:30:20; N:P₂O₅:K₂O kg/ha). The tillage practices were

- 1 CT (conventional tillage): ploughing soil twice with bullock-drawn country plough (10–15 cm depth) followed by laddering;
- 2 DT (deep tillage): ploughing twice with tractor-drawn mould board plough (20–25 cm depth) followed by harrowing; and

- 3 NT (no tillage): weed control by spraying gramoxon (paraquat dichloride) 15 days prior to sowing.

In CT and DT, ploughing was done one day prior to direct line sowing at the end of June every year. In NT, however, seeds were dribbled after chemical weed control.

Three soil samples, each consisting of five pooled subsamples from each treatment, up to 15 cm deep, were collected from two replications of each treatment after harvest of rice in September 1995. Infective propagules of native VAM fungi in the soil samples were quantified following the ‘most probable number’ method (Porter 1979). Five root samples, each consisting of 250–300 feeder root pieces of 1–2 cm length, from each plot of rice at maturity stage (September 1995), were fixed and stained by the method described by Phillips and Hayman (1970). Per cent root colonization was assessed by non-systematic slide method using compound microscope. Root dry weight at maturity (September), up to 15 cm soil depth, was worked out by root sampling with the help of core sampler (15 cm diameter) from rice rows during 1993, 1994, and 1995.

Cropping systems

An experiment with rice-based cropping systems with upland rice (variety: Vandana) intercropped

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with maize (*Zea mays*), okra (*Abelmoschus esculentus* L.), tomato (*Lycopersicon esculentum*), and red gram (*Cajanus cajan* L.) in rice–intercrop row ratio of 4:1 was conducted in the research farm of CRURRS during the wet seasons of 1995 and 1996. Respective controls of sole crops of rice and intercrops were also maintained. All the treatments were sown (direct line sowing at the end of June) with four replications and fertilized 60:40:30; N:P₂O₅:K₂O kg/ha.

Soil samples from each plot were collected in the manner mentioned earlier after harvest during October 1995 and 1996. Total native VAM fungi spores from the soil samples were isolated following wet sieving and decanting (Gerdemann and Nicolson 1963) and quantified using stereo-zoom microscope.

Results and discussion

Tillage practices

Conventional tillage resulted in highest population of infective propagules and per cent root colonization in rice (at maturity) at the end of five years of practice (Figure 1). The population of infective propagules was substantially reduced in NT followed by DT. Deep tillage disturbed soil up to a depth of 20–25 cm leading to a drastic reduction in the population and efficacy of infective propagules through disruption of mycelial network in the soil

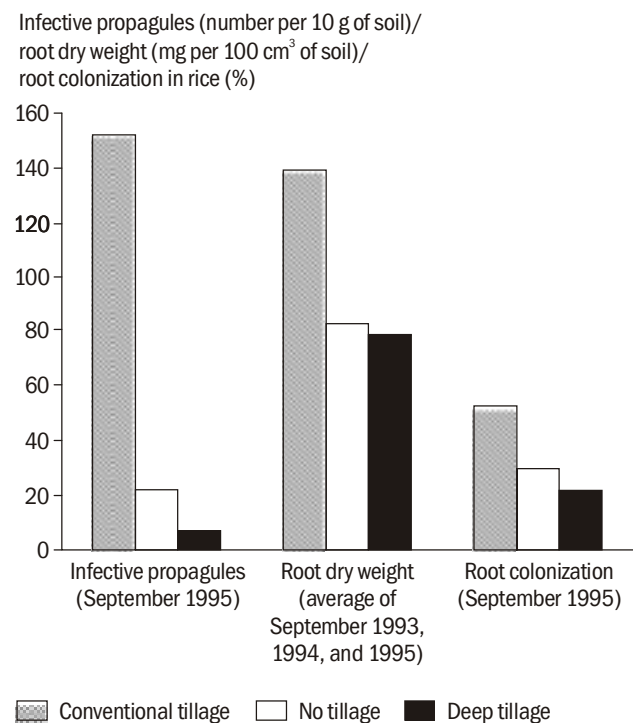


Figure 1 Effects of long-term (1991–95) tillage practices on native vesicular-arbuscular mycorrhizal fungi population, root (rice) dry matter yield, and root colonization in rice

(Jasper, Abbott, and Robson 1991; McGonigle and Millar 1993) as evidenced by reduced per cent root (rice) colonization (Figure 1). On the other hand, in NT, although there was no soil disturbance, mycelial network established with the roots of pre-dominant weed flora (Maiti, Variar, and Singh 1996) was disrupted due to destruction of weed and their roots by the weedicide. The process of population build-up was also interrupted by low root biomass production per unit volume of soil (evidenced by lower root dry matter yield) for colonization and multiplication of VAM fungi. One time (twice consecutively, prior to sowing) lower depth ploughing in CT, however, led to minimum disruption of established mycelial network as a consequence of minimum soil disturbance and resulted in a comparatively more steady build up of native VAM fungi infective propagule population in soil over a period of time (five years in this study) which in turn increased per cent root colonization, corroborating earlier findings of Maiti, Variar, Singh, et al. (1997).

Cropping systems

All the intercrops as sole resulted in significantly higher VAM fungi spore population in the soil at the end of both the growing seasons (Table 1) as compared to the rice sole. Only figurative differences were noticed among the intercrops. Only rice + tomato intercropping system led to significantly higher spore population over rice sole despite figurative increments in other intercropping systems. Such increment was attributed by Harinikumar and Bagyaraj (1988) to increased root density per unit volume of soil in intercropping systems which favours the rate of spread of the symbiotic fungi. The results, however, indicate that affinity of crop to

Table 1 Effects of rice-based intercropping systems on native vesicular-arbuscular mycorrhizal fungi spore population in the soil (October 1995 and 1996)

Cropping systems	Number of vesicular-arbuscular mycorrhizal fungi spores in 10 g of soil ^a	
	1995	1996
Rice sole	198.8 ^{cd}	194.8 ^{de}
Maize sole	259.5 ^{ab}	276.3 ^{abc}
Okra sole	283.3 ^a	304.5 ^a
Tomato sole	287.0 ^a	289.5 ^{ab}
Red gram sole	260.5 ^{ab}	256.8 ^{a-d}
Rice + maize	222.3 ^{bcd}	222.8 ^{de}
Rice + okra	191.5 ^d	183.5 ^e
Rice + tomato	260.8 ^{ab}	287.5 ^{abc}
Rice + red gram	253.3 ^{abc}	233.5 ^{b-e}

^amean of four replications

Note Means followed by a common letter are not significantly different at the 5% level by DMRT

VAM colonization plays a more important role for multiplication and spread of the fungi. Otherwise, rice + maize intercropping system, which has more root density as compared to other intercrops tested, would have the highest VAM fungi population. In intercropping systems, however, the spore populations were reduced over respective sole intercrops with significant reduction in rice + okra. This was probably because of lesser affinity of rice to VAM fungi colonization than the intercrops tested.

Inference

The observations indicate that

- Minimum soil disturbance and no chemical weed control intervention leads to uninterrupted build up of native VAM fungi population and higher efficacy of infective propagules in soil.
- In rainfed upland ecosystems, intercropping rice with suitable vegetables like tomato, besides legumes (Maiti, Variar, and Saha 1995), encourages the multiplication of native VAM fungi in soil.

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New approaches

First direct visualization of amino acids in ectomycorrhizal fungi by immunocytochemistry

Localization of amino acids was investigated by A Brun, M Chalot, J S Mathisen, P O Ottersen, and B Söderström (1998) in the ectomycorrhizal fungus, *Paxillus involutus*, by immunocytochemistry (<http://plantbio.berkeley.edu/~bruns/abstracts/brun.html> ICOM I: Proceedings of the second international conference on mycorrhiza, Uppsala, Sweden, 5–10 July 1998). Antibodies raised against glutamate and glutamine were produced which allow the utilization of immunogold labelling techniques to explore the cellular and tissular distribution of amino acids. Classical fixation procedures with glutaraldehyde and paraformaldehyde resulted in a loss of amino acids during the incubation step as demonstrated with [¹⁴C]-labelled amino acids. Therefore, a freeze-substitution technique was applied to fungal cells, which allows better conservation of vacuolar content. Immunogold labelling of freeze-substituted cells clearly showed a cytosolic localization of glutamate and glutamine. However, there was a great heterogeneity of labelling distribution between different cells, with some intensely

labelled while others poorly labelled. When using a double labelling protocol with two particle sizes of 10 and 30 nm for glutamate and glutamine, respectively, it appeared that glutamine amount was three- to fivefold higher than glutamate amount which is in good agreement with quantification of amino acids by HPLC. This study represents a first step towards the use of electron microscopic immunocytochemistry as a tool to assess the distribution and absolute concentration of amino acids in fungal cells and symbiotic tissues.

A simple staining technique for VAM fungi

A simple, reliable, and inexpensive staining technique was developed by H Vierheilig, A P Coughlan, Wyssll, Y Piche (1998) for staining vesicular mycorrhizal fungi (*Applied and Environmental Microbiology* 64(12): 5004–5007). After adequate clearing with Kott, an ink–vinegar solution successfully stained all fungal structures, rendering them clearly visible. Apart from application in research, this non-toxic, high-quality staining method could also be of great utility in leaching exercises.



List of cultures available with the Centre for Mycorrhizal Culture Collection as on 5 July 2000

S No.	Bank code	Name of the fungus	Host
1	EM-1078	<i>Thelephora terrestris</i>	Betula
2	EM-1276	<i>Tricholoma ustale</i>	Hetre
3	EM-1144	<i>Wilcoxinia mikolae</i>	—
4	EM-1283	<i>Scleroderma verucosum</i>	Sal
5	EM-1284	<i>Scleroderma cepa</i>	<i>Pinus wallichiana</i>
6	EM-1285	<i>Lycoperdon</i> sp.	<i>Pinus wallichiana</i>
7	EM-1286	<i>Russula</i> sp.	<i>Pinus smithiana</i>
8	EM-1287	<i>Suillus</i> sp.	<i>Pinus caribaea</i>
9	EM-1288	<i>Geastrum</i> sp.	<i>Eucalyptus tereticornis</i>
10	EM-1289	<i>Pisolithus tinctorius</i>	—

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Forthcoming events

Conferences, congresses, seminars, symposiums, and workshops

- Germany**
13-19 August 2000
3rd International Congress on Symbiosis 2000
Dr Hans Christian Weber, Spezielle Botanik und Mykologie, Fachbereich Biologie, Philipps-Universität, 35032 Marburg, Germany
Web site http://staff-www.uni-marburg.de/~b_morpho/symbio.html
- Kuhmo, Finland**
21-25 August 2000
3rd Workshop on Disturbance Dynamics in Boreal Forests
Workshop on Disturbance Dynamics, Department of Forest Ecology, PO Box 24, FIN-00014, University of Helsinki, Finland
Fax +358 9 191 7605
E-mail DIST2000@Helsinki.fi • *Web site* honeybee.helsinki.fi/dist2000/
- Lisbon, Portugal**
2-4 September 2000
7th Annual Conference of the European Forest Institute
Instituto Superior de Agronomia, Tapada da Ajuda, 1399 Lisboa, Lisbon, Portugal
or
Brita Pajari, European Forest Institute, Seminar Coordinator, Torikatu 34, FIN-80100
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E-mail brita.pajari@efi.fi
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World Potato Congress 2000
RAI Congress Centre, 8 Europaplein, 1078 GZ, Amsterdam, The Netherlands
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Web site www.potatocongress.org/congress2000.htm.
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11-15 September 2000
Chemical and Non-Chemical Soil and Substrate
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