MYCORRHIZA NETWORK

MYCORRHIZA NEWS

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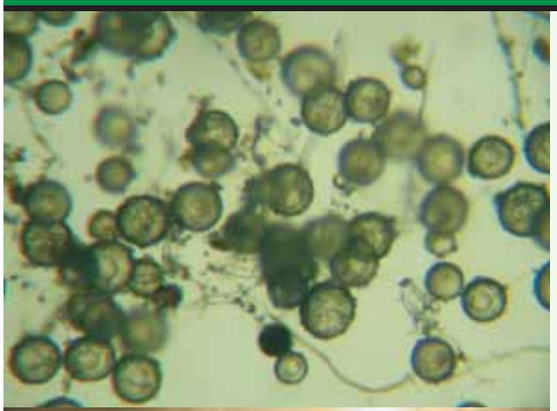

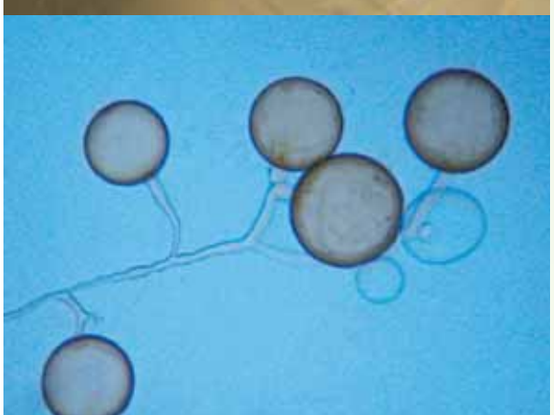

About TERI

TERI (The Energy and Resources Institute) is a dynamic and flexible organization with a global vision and a local focus. TERI's focus is on research in the fields of energy, environment, and sustainable development, and on documentation and information dissemination. The genesis of these activities lie in TERI's firm belief that the 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.

TERI's Mycorrhiza Network

TERI's Mycorrhiza Network is primarily responsible for establishing the MIC (Mycorrhiza Information Centre), the CMCC (Centre for Mycorrhiza Culture Collection), and publishing Mycorrhiza News. The Network helps scientists carry out research in mycorrhiza and promotes communication among mycorrhiza scientists.

Mycorrhiza News

The Mycorrhiza News provides a forum for dissemination of scientific information on mycorrhiza research and activities; publishes state-of-the-art papers from eminent scientists; notes on important breakthroughs; brief accounts of new approaches and techniques; publishes papers complied from its RIZA database; provides information on forthcoming events on mycorrhiza and related subjects; lists important research references published during the quarter; and highlights the activities of the CMCC.

Contents

Mycorrhiza: some glimpses

C Manoharachary, Alok Adholeya,# and I K Kunwar*

Several symbiotic groups, phosphorous solubilizers, plant growth promoters, and other such beneficial important micro-organisms are reported from different soils. Balanced microbial systems contribute to the sustainability in agriculture, forestry, and range management. In this regard, mycorrhiza has a substantial role. The term mycorrhiza (fungus root) was coined by Frank (1885). Harley and Smith (1983) defined mycorrhiza as an association between fungal hyphae and roots of higher plants concerned with absorption of mineral substances from the soil. Brundrett (2004) defined mycorrhiza as a symbiotic association for one or both partners between a fungus and a root of living plant that is primarily responsible for nutrient transfer. Mycorrhizas were earlier classified into arbuscular, ectomycorrhizal, and orchid mycorrhizal categories based on the relative location of fungi in the roots. The basic types of mycorrhizas established now are described below (Harley and Smith 1983).

Ectomycorrhiza

Ectomycorrhiza is characterized by a fungal sheath or mantle that encloses the root, forming a Hartig net, which is a plexus of fungal hyphae between epidermal and cortical cells. Ectomycorrhizal roots are generally short, swollen, dichotomously branched, with distinctive colours of white, black, orange, yellow, and olive green. These are common association for forest trees and shrubs, particularly in subarctic and temperate regions. Many of the host plants that show ectomycorrhizal association belong to the families Pinaceae, Fagaceae, Betulaceae, and Myrtaceae. The fungi that form ectomycorrhizal associations are Basidiomycetes, with representatives from 25 families. Some Ascomycetes and two species of Zygomycetes are also reported to form ectomycorrhizas. These fungi can be cultured in a laboratory medium. Some of the examples of ectomycorrhizal fungi are *Boletus edulis*, *Pisolithus tinctorius*, *Lactarius deliciosus*, *Laccaria laccata*, *Rhizopogon roseolus*, and *Paxillus luteolus*.

Ectendomycorrhiza

Ectendomycorrhiza are variants of ectomycorrhiza and display morphological characteristics of the endomycorrhiza. The association appears to be an intermediate type. These types are found in *Pinus* and *Larix* species.

Arbuscular mycorrhiza

Arbuscular mycorrhiza is the most common mycorrhizal association. They have a widespread distribution throughout the plant kingdom and form mutualistic relationship with most of the vascular plants. Families that rarely form arbuscular mycorrhiza include Cruciferae, Chenopodiacaeae, Polygonaceae, and Cyperaceae. Families that do not form arbuscular mycorrhiza include Pinaceae, Betulaceae, Fumariaceae, Commelinaceae, Utricaceae, and Ericaceae. The fungal partner

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belongs to Glomeromycota. The fungus forms vesicles within or between cortical cells that act as storage or reproductive organs. Arbuscules are formed within the cortical cells and these provide a large surface area of contact between host and fungus. The genera, which form AM (arbuscular mycorrhizal) fungal association are *Acaulospora*, *Ambispora*, *Aracheospora*, *Diversispora*, *Entrophospora*, *Geosiphon*, *Gigaspora*, *Glomus*, *Intraspora*, *Kuklospora*, *Pacispora*, *Paraglomus*, and *Scutellospora* (Schenck and Perez 1990; Schüßler, Schwarzott, and Walker 2001; Oehl and Sieverding 2004; Sieverding and Oehl 2006; Walker, Vestberg, Demircik, *et al*. 2007).

Orchid mycorrhiza

Orchidaceous mycorrhiza is found in the family Orchidaceae. The fungal partner generally is the species of *Ascomycetes* or *Basidiomycetes*. In nature, orchid embryos normally become infested with mycorrhizal fungi. Thin-walled hyphae enter the protocorn through the epidermis and anastomose repeatedly with the cortical cells to form pelotons or hyphal coils, which provide a large surface area of contact.

Ericoid mycorrhiza

Ericoid mycorrhizal association involves mostly *Ascomycetes* fungi and, sometimes, *Basidiomycetes* fungi. Hosts include the members of Ericales, which grow on heaths and peaty, acidic soils. These fungi are present in the young cortical cells of the host, with branched and coiled vegetative mycelia, but never penetrate the stele. Ericoid mycorrhizal fungi lack a Hartig net. Instead of forming arbuscules, these fungi form hyphal coils that are intracellular. Culturing of these fungi in laboratory medium is rare.

Monotropoid mycorrhiza

Monotropoid mycorrhiza occurs in the family Monotropaceae, which lacks chlorophyll and is totally dependent on the mycorrhizal fungi for supply of carbon. The fungal partner is a member of *Basidiomycetes*. The hyphae of the sheath ramify in the surrounding humus, and a Hartig net is also produced. Fungal pegs achieve close contact with cortical cells of the host. The contents of the fungal pegs are ultimately released into the sac enclosed by the host membranes.

Arbutoid mycorrhiza

Arbutoid mycorrhizal associations are found in members of the families Arbutoideae and Pyrolaceae. The fungal partners are *Basidiomycetes*. Long roots have very sparse infections but the intercellular hyphae form a Hartig net. Short roots have a thicker outer sheath and a well-developed Hartig net between the outer cortical cells. The fungus also penetrates and forms coils within the cells of the outer cortex.

Besides morphological, anatomical, histochemical, and biochemical tools, molecular and genetical tools are also used for identification of mycorrhizal fungi, and to explore the structural and regulatory genes in both fungi and plants that permit mycorrhiza formation. Of the seven types of mycorrhizas, the two prevalent mycorrhizal types are the ectomycorrhizas (common with woody species related to forestry) and the arbuscular mycorrhizas (more often associated with the herbaceous plants with relevance to range, horticultural, ornamental, medicinal, and crop plants).

A large proportion of land area in India shows clear evidence of soil degradation due to salinity, alkalinity, soil erosion, waterlogging, and so on, which in turn affects the country's productive resource base. The fundamental problem that the country is facing today is the rapidly increasing pressure of population on the limited resources of land. In order to meet the pressure of population, it is essential to efficiently manage the agricultural inputs for sustaining high crop productivity on long-term basis, with minimum damage to environment. Biofertilizers are used in order to reduce the cost and harm rendered by agrochemicals. Mycorrhiza helps the plants to acquire mineral nutrients from the soil, especially immobile elements such as phosphorous, zinc, and copper and mobile elements such as sulphur, calcium, potassium, iron, magnesium, manganese, chlorine, bromine, and nitrogen. They also help in increasing the extent of soil particle aggregation. Further, most of the economically important plants have been found to be mycorrhizal, and the subject is currently gaining much attention in agriculture, horticulture, and forestry. It is also known that Glomalean fungi existed 400 000 000 years ago and helped in the colonization of land by primitive plants. Thus, primary land plant establishment was also due to mycorrhiza.

The mycorrhizal symbiotic association appears to have evolved as a survival mechanism for fungi and higher plants, thus allowing each to survive in the existing environment of low temperature, soil fertility, drought, diseases, extreme environments, and other stress situations. Mycorrhizas offer primary biological defence to host plants against stress for crops and forest trees.

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Benefits derived from mycorrhiza by host plants

Mycorrhizal association helps in increased nutrient and water uptake by absorption through improved absorptive area, and translocation of elements to host tissues and their accumulation. Due to the unique ability of mycorrhiza to increase the uptake of phosphorous by plants, mycorrhizal fungi have the potential for utilization as a substitute for phosphatic fertilizers. Mycorrhizal fungi improve host nutrition by increasing the delivery of phosphorous and other minerals to roots and plants. Ectomycorrhizal fungi permeate the F and H horizon of forest floor and, thus, minerals get mobilized in these zones. This is followed by their absorption before they reach subsoil system. AM fungi are known to degrade complex minerals and organic substances in soil and, thus, make essential elements available to host plants. Mycorrhizal association is known to offer resistance in host plants to drought and plant pathogens. It also increases the tolerance of the plant to adverse conditions and helps in the production of growth hormones like auxins, gibberellins, and growth regulators such as Vitamin B. Mycorrhizal fungi contribute to organic matter turnover and nutrient cycling in forest and crop land ecosystems. They help in soil aggregation, soil stabilization, and increase soil fertility. Mycorrhizas are symbiotic and not parasitic; therefore, they live hand-in-hand with other living organisms and are non-pollutants.

Morphological diversity in arbuscular mycorrhizal fungi

Morphological characters that are stable and discrete are used in identification and classification of AM fungi. Some of the important morphological characters considered are described below.

Hyphal characters

Vegetative hyphae have been differentiated functionally into infective, absorptive, and runner hyphae. Fungal hyphae may be long H-shaped parallel connections as in *Glomus*; constricted hyphae near branch points as in *Acaulospora* and *Entrophospora*; and coiled, irregularly swollen hyphae with lateral projections or knobs as in *Gigaspora* and *Scutellospora* (Morton and Bentivenga 1994).

The mycelia also form specialized structures like arbuscules, vesicles, and auxiliary cells. Abrupt narrowing-off of branch hyphae forms arbuscules in *Gigaspora*. In *Glomus*, arbuscules are formed by reduction in hyphal width (Brundrett and Kendrick 1990). Vesicles in Glomaceae are subglobose to elliptical whereas in Acaulosporaceae they are pleiomorphic and knobby (Abbott 1982). Auxiliary cells are found only in Gigasporineae (Morton 1990).

Subtending hyphae

The stalk of the spore known as subtending hypha or sporophore has importance in identification. Subtending hypha may be absent (*Acaulospora*), simple, straight or recurved (*Glomus*), swollen and straight but often appear sessile due to detachment from saccule and two scars present on either side of the spore (*Entrophospora*) or sporophore bulbous (*Gigaspora* and *Scutellospora*).

Spores, sporocarps, and sub-cellular structures

Spores may be formed singly or aggregated in loose/compact (with or without peridium) sporocarps. Spores in AM fungi may be azygospores or chlamydospores. Chlamydospores are seen in *Glomus* and *Scutellospora*. Azygospores are seen in *Acaulospora*, *Entrophospora*, *Gigaspora*, and *Scutellospora*. The colour of the spores varies from hyaline, yellow, reddish-brown, orange, brown, and black.

Size of the spores is also variable. It ranges from 50–250 µm (diameter) in *Glomus*, *Acaulospora*, and *Entrophospora*, whereas the diameter of spore exceeds 300 µm in *Gigaspora* and *Scutellospora* (Schenck and Perez 1990).

The shape of spores may be globose, subglobose, ovoid, pear-shaped, ellipsoid, oboviod, reniform or irregularly elongated. The cytoplasm within the spores may appear reticulate in a polygonal pattern or may be vacuolated because of the presence of many lipid droplets of variable sizes.

Seven kinds of wall layers have been described in AM spores, namely evanescent, unit, laminated, membranous, coriaceous, amorphous, and expanding. Spores of different genera also differ in the number of wall layers. Spores of *Gigaspora* and *Glomus* have one to two wall layers, whereas *Acaulospora*, *Scutellospora*, and *Entrophospora* have more than three wall layers.

Ecological diversity in arbuscular mycorrhizal fungi

Ecological diversity provides useful information regarding biogeographical distribution, dispersal patterns, and competitive interactions by member organisms in plant communities and soil micro-organisms. The biogeographical distribution, dispersal patterns, and competitive interactions of

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AM fungi with plant communities and soil micro-organisms are as follows.

Biogeographical distribution

The available information indicates that there is more or less uniform distribution of AM fungi, though some may predominate in certain areas with broad ecological range (Onguene and Kuyper 2001; Bostrom 2001). AM fungi are mostly present in the top 15–30 cm of soil and their numbers decrease significantly below the top 15 cm of soil (Redhead 1977). The distribution of species of AM fungi varies with climatic and edaphic conditions. For example, *Gigaspora* and *Scutellospora* are common in tropical soils, whereas *Acaulospora* species favour soils of pH below 5 (Abbott and Robson 1977).

Dispersal patterns

AM fungi are indigenous to soil throughout the world. Many AM species are present in most of the continents of the world. Active dispersal of AM fungi is usually by spread from one living root to another through AM propagules, like mycelia and spores, which can be moved by biotic and abiotic agents. Dispersal of spores over greater distances is dependent upon passive dispersal by wind and water, especially in arid environment. Animal dispersal of AM spores is well documented and occurs through ingestion and egestion of spores (Bagyaraj and Padmavathi 1997).

Interactions with plant communities

AM fungi are geographically ubiquitous and commonly associated with plants in agriculture, horticulture, pastures, and tropical forests. About 90% of vascular plants establish mutualistic relationship with AM fungi (Kendrick and Berch 1985).

The occurrence of AM fungi in roots has been reported from an exceptionally wide range of plants. Besides roots, colonization has been reported in other plant parts; for example, in leaves of *Salvinia* (Bagyaraj, Manjunath, and Patil 1979), in senescent leaves of *Fumaria hygrometrica* (Park and Linderman 1980), and in decaying peanut leaves and rhizomatous tissue of *Zingiber officinale* (Taber and Trappe 1982). Colonization has also been reported from scales of *Colocasia antiquorum, Elettaria cardamomum, Musa paradisica, Sanseviera trifaciata,* garlic, and ginger (Kunwar and Manoharachary 1998, 1999)

AM interactions bring about certain changes in the host physiology. These include increased production of cytokinins due to the inhibition of cytokinins degradation by compounds produced by the fungus or plant as a result of the interaction. The presence of two gibberllins-like substances in culture extracts of *Glomus mosseae* and increased nitrate reductase activity has also been reported in mycorrhiza inoculated plants.

Acknowledgements

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Mycological Research **111**: 137–153

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RESEARCH FINDING PAPERS

Mycorrhizal inoculation in combating low moisture stress in jamun (*Syzygium cuminii* Skeel)

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Introduction

The role of AM (arbuscular mycorrhizal) fungi in drought tolerance has been documented in few crops (Osonubi, Bakare, and Mulongoy 1992). Mycorrhizal association with all types of plant roots are so prevalent that non-mycorrhizal plants are more of an exception (Gerdemann 1968). Jamun, like most dryland fruit crops, encounters a situation of low moisture stress during growing season, where availability of water decreases. Interestingly, AM fungi are reported to be involved in both drought avoidance and drought tolerance by the hosts (Auge 2001).

Material and methods

An experiment was undertaken at the nursery of the Department of Pomology, Kittur Rani Channamma College of Horticulture, Arabhavi, Belgaum district, Karnataka during 2004–06, with three replications in completely randomized design. Non-descriptive uniform size jamun seeds were sown in polybags (8 × 12 cm) containing potting mixture of soil, sand, and FYM (2:1:2). Cultures of nine different AM fungi, as mentioned in the tables below, were obtained from the Department of Agricultural Microbiology, Kittur Rani Channamma College of Horticulture. AM fungal inoculation was carried out by spreading 5 g of inoculum (consisting of 80–88 infestive propagules) uniformly, at a depth of 5 cm, and putting a thin layer of soil above the inoculum. Seeds were placed and covered with soil (2–3 cm). The polybags of respective treatments were labelled and kept apart, enough to avoid AM fungal cross-contamination.

Low moisture stress was imposed after six months of growth of seedlings by withholding the application of water, after watering to the field capacity of the soil. All the selected plants were labelled for periodical observations in each treatment.

Phenol content of leaves was estimated with folia-ciocalteous reagent as per the procedure given by Bray and Thorpe (1954). Nitrogen and protein content were estimated by Kjeldahl method as adopted by Jackson (1967) and Ranganna (1991).

$$\text{Nitrogen (\%)} = \frac{(\text{sample titre} - \text{black titre}) \times \text{normality of HCl} \times 14 \times 100}{\text{Weight of sample} \times 1000}$$

Protein (%) = nitrogen (%) × 6.25

Total chlorophyll content of leaves was estimated as per the procedure given by Arnon (1949), before and after seven days of drought imposition.

$$\text{mg total chlorophyll/g tissue} = [20.2\ (A_{645}) + 8.02\ (A_{663})] \times \frac{V}{1000 \times w}$$

where,
A = absorbance of specific wave length (645 and 663 nm)
V = final volume of chlorophyll extraction
w = fresh weight of leaf sample

Proline content of leaves was estimated prior to and seven days after imposition of drought by the method given by Bates, Walder, and Tekire (1973).

Relative water content was estimated as per the standard developed by Burrs and Weathery (1962).

$$\text{Per cent relative water content} = \frac{\text{Fresh weight} - \text{dry weight}}{\text{Turgid weight} - \text{dry weight}} \times 100$$

Chlamydospores produced by AM fungi were determined by using the method given by Gerdemann and Nicolson (1963). Per cent root colonization was estimated using the method as detailed by Phillips and Hayman (1970).

Results and discussion

The inoculation of endomycorrhizal fungi brought about increased accumulation of several biochemical substances. AM fungi inoculated seedlings recorded significantly higher content of phenol, nitrogen, protein, and chlorophyll (Table 1), as compared to uninoculated seedlings, which registered significantly least content of phenol (0.577 µg/g), nitrogen (1.30%), protein (8.125%), and chlorophyll (0.668 mg/g). *Glomus fasciculatum* registered

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significantly maximum phenolic content (0.723 µg/g), nitrogen (1.972%), protein (12.325%), and total chlorophyll (1.638 mg/g).

When the plants were subjected to drought situation, the proline content of leaves increased. The uninoculated control recorded higher proline content before and after imposition of stress (4.349 µg/g and 25.272 µg/g , respectively) (Table 1). The AM fungi inoculated seedlings recorded lower proline content, indicating that the seedlings were less affected by low moisture stress.

After imposing drought, the per cent soil moisture of AM fungi inoculated seedlings were lower (Table 2) than those of uninoculated control (3.26%). Significantly least soil moisture content was noted in *G. fasciculatum* (1.24%), followed by *Sclerocystis dussii* (1.54%). This phenomenon is associated with profused root morphology as influenced by AM fungi inoculation, which enhances the water uptake by seedlings.

The relative water content in the leaves was higher in seedlings inoculated with AM fungi as compared to the uninoculated control (27.29%) seedlings. *G. fasciculatum* inoculated seedlings registered highest relative water (66.04%) content, which might have of resulted in lowered per cent of leaf curl. The per cent leaf curled was maximum in uninoculated control (41.34%) seedlings.

In contrast to non-mycorrhizal controls, AM fungi inoculated seedlings showed higher nitrogen assimilation (Table 1). Better nitrogen nutrition during drought has been recorded by other researchers—development of and recovery from drought, characterized as higher soluble proteins, amino acids, nitrogenous enzymes, and tissue nitrogen (Azcon, Gomez, and Tobar 1996; Panwar 1993; Subramanian and Charest 1998); higher activities of other enzymes (Goicoechea, Solezal, Antolin, *et al*. 1996); decreased accumulation of proline (Runjin 1989); and increased soil water extraction (Busse and Ellis 1985; Osonubi, Bakare, and Mulongoy 1992).

One of the most compelling cases for AM fungi mediated increase in drought resistance is avoidance, as was offered by Bethlenfalvay, Themas, Dakessian, *et al*. (1988), who demonstrated that the soil moisture content of inoculated seedlings was lower than that of uninoculated seedlings.

In Table 2, it is seen that per cent root colonization in inoculated seedlings ranged from 78.67% to 93%, but significantly least colonization was noted in uninoculated control (36%). However, the root colonization reduced due to the imposition of low moisture stress.

AM fungi inoculation appears to have increased the drought tolerance by increasing both their dehydration avoidance and dehydration tolerance capacities (Auge, Schekel, and Wample 1987). Dehydration was avoided to a larger extent in AM plants, as compared to non-mycorrhizal plants of similar size, through increased accumulation of solutes, which lowered bulk leaf symplastic and osmotic potential. The lower leaf curled per cent in the present experiment may be attributed to the above-mentioned phenomenon. Therefore, it is seen that AM fungi help the inoculated plants to tolerate low moisture stress more efficiently compared to non-mycorrhizal plants.

Table 1 Effect of endomycorrhizal inoculation on phenol, nitrogen, and protein in leaves of jamun before inducing drought

				Total chlorophyll (mg/g)			*Proline* (µg/g)		
Treatment	*Phenol* (µg/g tissue)	*Nitrogen* (%)	*Protein* (%)	*Before stress*	*After stress*	*Per cent decrease*	*Before stress*	*After stress*	*Per cent increase*
Glomus bagyaraji	0.597	1.683	10.521	1.135	0.658	42.05	3.897	17.248	342.61
Glomus leptotichum	0.634	1.613	10.083	1.001	0.563	43.74	3.992	16.494	313.17
Acaulospora laevis	0.587	1.720	10.750	1.159	0.665	42.64	3.774	17.708	369.16
Sclerocystis dussii	0.701	1.845	11.533	1.271	0.718	43.52	3.689	16.239	340.16
Glomus mosseae	0.655	1.622	10.138	1.028	0.588	42.78	3.872	16.605	328.86
Gigaspora margarita	0.651	1.664	10.400	1.133	0.647	42.90	3.945	15.962	304.58
Glomus monosporum	0.652	1.748	10.925	1.249	0.708	43.32	3.528	16.354	363.50
Glomus intraradices	0.670	1.893	11.833	1.455	0.834	42.71	3.902	15.976	356.17
Glomus fasciculatum	0.723	1.972	12.325	1.638	0.930	43.21	3.376	14.851	33987
Control	0.577	1.300	8.125	0.668	0.370	44.62	4.349	25.272	481.05
S.Em±	0.014	0.049	0.304	0.065	0.023	–	0.186	0.960	–
CD (5%)	0.0538	0.142	0.895	0.194	0.076	–	NS	2.832	–
CD (1%)	0.0734	0.194	1.220	0.265	0.104	–	NS	3.863	–
CV (%)	3.89	4.93	4.93	9.65	5.89	–	8.51	9.63	–

NS – non-significant

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Table 2 Impact of AM fungi on some soil parameters, number of propagules, and per cent AM fungal root colonization soil moisture, relative water content, and per cent leaf curl due to low moisture stress

	Per cent soil moisture (%)					*AM fungal propagules* (no./50 g soil)			*Per cent root coloniation* (%)		
Treatments	*Before*	*After*	*Per cent decrease*	*Relative water content in leaf* (%)	*Per cent of leaf curl* (%)	*Before*	*After*	*Per cent decrease*	*Before*	*After*	*Per cent decrease*
Glomus bagyaraji	44.29	2.98	93.27	52.95	29.53 (32.93)	830.50	796.67	4.07	87.00	82.00	3.15
Glomus leptotichum	44.29	2.89	93.47	48.61	29.80 (33.10)	802.50	775.00	3.43	84.33	83.00	4.23
Acaulospora laevis	43.17	2.67	93.81	36.43	35.34 (36.49)	810.25	782.67	3.40	84.00	77.67	3.72
Sclerocystis dussii	44.12	1.54	96.52	53.76	29.11 (32.66)	849.25	811.00	4.50	91.00	89.33	2.55
Glomus mosseae	44.18	3.241	92.67	46.02	33.29 (35.25)	800.00	775.00	3.13	80.67	75.67	6.20
Gigaspora margarita	43.96	2.63	94.02	46.4	32.36 (34.68)	815.00	787.67	3.35	78.67	74.33	5.91
Glomus monosporum	44.05	2.11	95.22	59.55	29.04 (32.62)	855.75	805.00	5.93	89.67	86.33	3.36
Glomus intraradices	43.39	2.11	95.13	64.50	28.19 (32.08)	889.75	840.33	5.55	91.00	87.67	4.36
Glomus fasciculatum	43.25	1.24	97.14	66.04	25.78 (30.52)	880.25	834.33	5.22	93.00	90.33	4.24
Control	43.00	3.26	92.41	27.29	41.34 (40.03)	99.50	86.33	13.23	36.00	30.00	18.18
S.Em±	1.805	0.105	–	1.298	1.029	9.471	6.822	–	1.085	0.937	–
CD (5%)	NS	0.309	–	3.828	3.036	27.352	20.121	–	3.201	2.764	–
CD (1%)	NS	0.422	–	5.222	4.141	36.823	27.447	–	4.366	3.770	–
CV(%)	7.14	1.39	–	1.48	5.68	2.48	1.62	–	2.31	2.09	–

AM – arbuscular mycorrhizal; NS – non-significant
[Figures in parenthesis pertain to angular transformation of the data]

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Arbuscular mycorrhizal fungal diversity in some medicinal plants

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Introduction

AM (arbuscular mycorrhizal) fungi are obligate symbionts with plant roots, which help in nutrient mobilization and, consequently, plant grown. There are two phases of AM fungi—one is intraradical (inside roots) and the other is extraradical (outside the root). The intraradical phase involves the arbuscules (site of nutrient exchange), vesicles (storage organ), and mycelium. The extraradical phase contains extraradical hyphae and spores. According to Lindsey (1984), AM fungi are found in over 81% of 89 shrub species in arid and semi-arid areas of the world.

In recent times, traditional medical science of India has gained a lot of importance, and significant emphasis is being given on the cultivation of different medicinal plants. These medicinal plants are very helpful in controlling many diseases. The fresh and dried leaves of Kalmegh (*Andrographis paniculata*) and the juice extracted from the herb is used in tribal medicine in India. It is a source of several diterpinoids, of which andrographolide is important. The drug is used for its general ability to cure certain forms of dyspepsia, chronic malaria, jaundice, and dysentery (Chiramel, Bagyaraj, and Patil 2006). Some researchers have observed that andrographolide has the potential to be included in the vaccine against AIDS due to its antagonistic property with HIV II virus (Weibo 1995). It is already being used in treating cancer, as it promotes cell differentiation in tumour cells (Matsuda, Kuroyanagi, Sugiyama, *et al*. 1994). Though the leaves contain maximum andrographolide, the entire plant is used for extracting the active ingredients. *Curculigo orchioides* Gaertn. (family Hypoxidaceae) is an endangered anticarcinogenic and aphrodisiac herb, which is native to India (Sharma, Kapoor, and Bhatnagar 2008). *Ocimum basilicum*, *Ocimum grarissimum*, *Ocimum sanctum*, and *Ocimum kilmandscharicum* are important essential oil-bearing plants of India, important in perfumery, cosmetic, and pharmaceutical industries (Gupta, Khaliq, Pandey, *et al*. 2000).

Since medicinal plants are good income generation source for farmers and their cultivation is being promoted by many funding agencies, they are being cultivated in many parts of India. An attempt was, therefore, made to evaluate the presence and status of AM fungi in some select medicinal plants.

Methods and materials

Collection of sampling

Rhizospheric soil samples and roots were collected under aseptic conditions from the experimental site

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of MRDC (Medicinal Research and Development Centre), Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand. Eight soil samples from a particular plant block covering an area of 100 m^2 were collected randomly from a depth of 10–30 cm. These samples were combined to make the composite samples, which were air dried and stored at 4 °C.

Experimental site

Pantnagar is situated in the Tarai belt in Shivalik range of Kumaon Himalayas, at 29°0' N 79°3' E, and lies at an altitude of 243 metres above the mean sea level. There was a temperature difference ranging from 3.7 °C in January to 39.4 °C in June. The town has an average annual rainfall of 948.6 mm, the humidity ranges from 20%–93%, and the wind speed ranges from 1.9–18.7 km/ha.

Isolation and identification of mycorrhizal fungal spores

Two grams of soil was suspended in tap water and sieved using wet sieving and decanting method (Gerdemann and Nicolson 1963). Spores retained on 60 mm mesh size sieve were recovered using sucrose gradient centrifugation (Tommerup and Kidby 1979) and then transferred to a petri-dish and counted under a stereozoom microscope (Nikon SMZ 1500). The spores were then mounted with PVLG (polyvinyl lactoglycerol) and Melzar's reagent, and identified according to the key proposed by Trappe (1982).

Estimation of arbuscular mycorrhizal infection

The cleaned roots were cut into 1-cm long pieces and stained with trypan blue, according to the procedure described by Phillips and Hayman (1970). The roots were boiled in 10% KOH for 1 h, acidified with 5N HCl, and stained overnight with 0.5% trypan blue. The roots were then destained with 70% glycerol. The mycorrhizal infection (mycelium, arbuscules, and vesicles) were observed in each segment using the Biermann and Lindermann (1981) method.

Statistical analysis

The data of spore count and per cent root colonization was analysed using a one-factor CRD (complete randomized design). The per cent data was angularly transformed before analysis.

Results

Number of arbuscular mycorrhizal spores and per cent root colonization

The study revealed a difference in the number of spores in different samples of plant soils. The plants were infected with a number of arbuscular mycorrhizal spores. The maximum number of AM fungi spores was recovered from rhizospheric soil of *A. paniculata*, followed by *Ocimum sanctum*. The lowest number of spores was observed in the soils of *Piper longum*, *Acorus calamus*, and *Mimosa pudica* (Table 1). Data on root colonization by AM fungi revealed that the root infection percentage varied from plant to plant. The per cent root colonization was highest in *A. paniculata* (99.38%), followed *O. sanctum* (85.63%), *M. pudica* (33.37%), and *A. calamus* (28.97%). It was lowest in *P. longum* (12.31%) (Table 1).

Identification of mycorrhiza

There were a number of mycorrhizal species present in the different samples. In general, four genera of AM fungi were found—*Glomus*, *Acaulospora*, *Entrophospora*, and *Gigaspora* (Table 2). Out of these, *Glomus* spp. were present in all the soil samples and in much higher concentration than the others. *Glomus macrocarpum*, *G. intraradices*, *Glomus etunicatum*, and *Glomus mosseae* were found more frequently than other *Glomus* sp.

Table 1 Number of AMF spores and per cent root colonization in different soil and plant samples, respectively

Plants	*Number of spores/g*	*Percent infection* (%)
Eclipta alba	22.33	74.27 (59.52)
Piper longum	12.33	12.31 (20.52)
Cyprus scariosus	20.33	40.78 (39.68)
Pogestemon patcholi	23.50	85.09 (67.30)
Andrographis paniculata	36.16	99.38 (87.39)
Catharanthus roseus	26.66	33.92 (35.62)
Raufia-serpentina	25.83	29.41 (32.84)
Ocimum santum	29.00	85.63 (67.87)
Aloe-vera	20.50	63.16 (52.64)
Acorus calamus	14.33	28.97 (32.56)
Spilanthus calva	21.00	50.41 (45.23)
Wedelia chinensis	20.83	44.04 (41.58)
Cymlopogon martini	18.33	41.82 (40.29)
Ageratum conyzoides	26.33	37.72 (37.89)
Hibiscus rosasinencis	20.66	57.86 (49.52)
Mimosa pudica	14.50	33.37 (35.28)

LSD at P≤ 5%; number of spores – 4.13; per cent infection – 2.93
[Data showed in parenthesis are transformed values]

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Table 2 Arbuscular mycorrhizal fungi spores present in the rhizospheric soil of different medicinal plants

Plant species	Arbuscular mycorrhizal fungi species											
	Glomus macrocaroum	*Glomus pachycaulis*	*Glomus intraradices*	*Glomus mosseae*	*Glomus etunicatum*	*Glomus coronatum*	*Glomus aggregatum*	*Acaulospora spinosa*	*Acaulospora scrobiculata*	*Entrophospora infrequens*	*Gigaspora gigantia*	*Gigaspora margerita*
Eclipta alba	+	–	+	+	+	+	–	–	+	–	–	–
Piper longum	–	–	+	–	+	–	+	+	–	–	+	–
Cyprus scariosus	–	+	-	+	–	–	–	–	+	+	–	–
Pogestemon patcholi	+	–	+	–	–	+	+	–	–	+	–	–
Andrographis paniculata	+	–	+	+	+	–	–	+	+	–	–	+
Catharanthus roseus	+	+	–	–	–	+	+	–	+	–	–	–
Raufia-serpentina	–	+	–	+	–	–	–	–	–	–	–	–
Ocimum santum	+	–	–	+	+	–	+	+	–	+	–	+
Aloe-vera	+	–	+	–	+	–	+	–	–	+	–	–
Acorus calamus	–	+	–	–	–	+	–	–	–	–	+	–
Spilanthus calva	+	–	+	+	–	–	–	+	–	–	+	–
Wedelia chinensis	–	+	–	–	+	+	–	–	+	–	–	–
Cymlopogon martini	–	+	–	–	+	–	+	–	+	+	–	+
Ageratum conyzoides	+	–	+	+	–	–	–	+	–	–	+	–
Hibiscus rosasinencis	+	–	+	–	+	–	–	+	–	+	–	–
Mimosa pudica	–	–	+	+	–	–	–	+	+	–	+	–

observed. Out of all the species of the four genera, *Gigaspora margerita* was observed in lowest number; it was associated with only three plant species—*A. paniculata*, *O. sanctum*, and *Cymlopogon martini*. Rhizospheric soil samples of *O. sanctum* and *A. paniculata* contained higher number of different AM fungi species (seven species). *Cyprus scariosus* and *Wedelia chinensis* were with four types of species and *A. calamus* was with three.

Discussion

Most plant species are typically mycorrhizal, with approximately four-fifth of all land plants forming AM association (Malloch, PiroZynski, and Raven 1980). In the present study, all 16 plant species were found infected with AM fungi. Rani and Bhaduria (2001) showed the AM fungi infection in medicinal plants, though they were different plant species than the ones taken in the present investigation. The highest number of mycorrhizal spores in rhizospheric soil and AM fungi infection in the roots of *O. sanctum* and *A. paniculata* indicated that these plant species might be considered good hosts for AM fungi under the prevailing conditions. This may be attributed to the root exudates of these plants, which might have stimulated the germination of mycorrhizal spores and increase the infection (Azaizeh, Marschner, Römheld, *et al.* 1995).

Among the four genera, *Glomus* sp. were most frequently found, that is, seven species and two species each of *Gigaspora* and *Acaulospora* were observed, while only one species of *Entrophospora* was observed. *G. macrocarpum*, *G. intraradices*, *G. etunicatum*, and *G. mosseae* were found more frequently in all the rhizospheric soil of plants used in this study. Panwar and Tarafdar (2006) reported the higher frequency of *Glomus* sp.

Out of *G. macrocarpum*, *G. intraradices*, *G. mosseae*, *G. etunicatum*, *Acaulospora spinosa*, *Acaulospora scrobiculata*, and *Gigaspora margarita*, the highest number of spores was of *G. intraradices* in *A. paniculata*. Chiramel, Bagyaraj, and Patil (2006) also showed that *G. intraradices* was considered to be the promising symbiont for inoculating *A. paniculata*. *G. macrocarpum*, *G. mosseae*, *G. etunicatum*, *Glomus aggregatum*, *A. spinosa*, *Etrophospora infrequens*, *G. margerita* were observed in *O. sanctum*. Out of these species, *G. aggregatum* was highest in number. Gupta, Khaliq, Pandey, *et al.* (2000) reported the presence of *G. aggregatum* and *Glomus fasciculatum* in the *Ocimum* sp.

Root infection was also found to be highest in *A. paniculata* and *O. sanctum*. Higher number of spores of *G. intraradices* indicates the possibility of higher root colonization. Chiramel, Bagyaraj, and Patil (2006) also reported the highest per cent root colonization in *A. paniculata* inoculated with *G. intraradices*, followed by those treated with *G. monosporum* and *G. leptotichum*. Gupta, Khaliq, Pandey, *et al.* (2000) also evaluated the root colonization in different *Ocimum* sp. where the highest infection was recorded in *O. basilicum* (Indian basil), in screening trials of the *Ocimum* spp.

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Effect of arbuscular mycorrhizal inoculation on enzyme activities and microbial population in the rhizosphere of *Coleus forskohlii* Briq.

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Introduction

Almost all higher plants in the terrestrial ecosystem are known to be associated with mycorrhizal fungi (Smith and Read 1997). In many ecosystems, these symbiotic fungi play an important role in sustaining plant productivity by increasing its nutrients and water uptake from the soil. Roots and rhizosphere are the regions of interest for their symbiotic efficiency. By colonizing the roots, they bring about several changes in the metabolic activity of the plants, resulting in stimulation of various enzymes. This leads to the modification of the rhizosphere, wherein plenty of micro-organisms survive with their mutual interactions. Mycorrhizosphere may bring about the changes in microbial population and can also contribute for the enhanced growth and nutrition of crop plants. Dehydrogenases and phosphatases are enzymes which are highly activated under mycorrhizosphere and yield several positive responses. Hence, it is essential to study the understanding of the AM (arbuscular mycorrhizal) induced changes in the rhizosphere.

Materials and methods

A pot culture experiment was conducted to study the influence of inoculation of the AM fungus, *Scutellospora* sp., along with PGPR (plant growth promoting rhizobacteria) (*Pseudomonas*), on tuber yield and the biochemical changes in the rhizosphere of *Coleus forskohlii*. This experiment consisted of six treatments with two isolates of *Pseudomonas fluorescens* and AM fungus. Three to four leaf cuttings of *C. forskohlii* were planted in pots containing 10 kg of soil (pH – 8.18, EC – 0.89 d/Sm, available N – 219 kg/ha, P_2O_5 – 14.3 kg/ha, K_2O – 293.4 kg/ha) with three replications and treatments arranged in CRD (completely randomized design).

AM inoculation was done at 50 g/pot containing 200–250 spores/100 g inoculum. The log phase cultures of *P. fluorescens* strains – PFC 6 (obtained from the Department of Plant Pathology, TNAU [Tamil Nadu Agricultural University]) and CPf 1 (isolated from *C. forskohlii*) – were inoculated at 50 ml broth/pot while planting. Root and rhizosphere soil samples were collected at 90, 120, and 150 DAP (days after planting) and tuber yield was estimated at harvest on 150 DAP. In root samples, enzyme extracts were made using acetate and borate buffer for acid and alkaline phosphatase activity, respectively, and the activity was estimated as per the method described by Morton (1952). In soil, acid phosphatases were estimated as per Tabatabai and Bremner (1969). Total dehydrogenases in soil and roots were estimated using triphenyl tetrazolium chloride as per the method of Casida, Klien, and Santoro (1964).

AM colonization was assessed in the roots as per Phillips and Hayman (1970) and spore count was made following the method of Gerdemann and Nicolson (1963). Besides this, the total bacteria, fungi, Actinomycetes PGPR, and total diazotrophic populations were estimated at every sampling (Allen 1953). The collected data was analysed statistically.

Results and discussion

Phosphatase activity

AM inoculation showed significant increase in acid and alkaline phosphatases activity over control, followed by inoculation of *P. fluorescens* CPf 1 against the control at every sampling. Acid phosphatase activity was found higher in roots, as compared to alkaline phosphatase. About 3–4-fold increase in acid phosphatase activity and 1.5–2-fold increase in alkaline phosphatase activity over control was observed. Combined inoculations showed a maximum activity of up to 212 µg and 253 µg PNPP (para-nitrophenol phosphate) release at 150 DAP in the case of acid phosphatase, which were 2–3-fold higher than individual inoculations. This combination recorded the maximum alkaline phosphatase activity of 138 µg PNPP release at 150 DAP, which was 80%–100% higher than individual inoculations (Table 1). With reference to soil, the inoculants individually exhibited much variation in activities, although generally up to three-fold increase was noticed when the inoculants were combined (Table 2). The activity of acid phosphatase was 46 µg in rhizosphere soil of vesicular AM-inoculated plant, while it was 29.5 µg in uninoculated

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Table 1 Effect of combined inoculation of AM fungus and PGPR organisms on phosphatase and dehydrogenase activity in roots of *Coleus forskohlii*

Treatments	*Acid phosphatase (µg of PNPP released per gram of fresh root tissue)*					*Alkaline phosphatase (µg of PNPP released per gram of fresh root tissue)*					*Dehydrogenase (µg of TPF formed per gram of fresh root tissue)*				
	DAP				*Per cent increase over control*	*DAP*				*Per cent increase over control*	*DAP*				*Per cent increase over control*
	90	*120*	*150*	*Mean*		*90*	*120*	*150*	*Mean*		*90*	*120*	*150*	*Mean*	
Scutellospora sp. CAM 3	12	52	116	60.0	128.1	12	31	72	38.3	62.2	8	15	25	16.0	26.9
Pseudomonas fluorescens PFC 6	10	35	76	40.3	53.2	14	35	72	40.3	70.7	6	24	16	15.3	21.4
P. fluorescens CPf 1	18	45	80	47.6	80.9	9	26	64	33.0	39.8	17	25	36	26.0	106.3
CAM 3 + PFC 6	32	110	212	118.0	348.6	25	48	100	57.6	144.0	15	32	76	41.0	225.3
CAM 3 + CPf 1	47	130	253	143.3	444.8	24	45	138	69.0	192.3	23	44	134	67.0	431.7
Uninoculated (control)	10	23	46	26.3	–	7	20	44	23.6	–	5	13	20	12.6	–
S Ed	0.39	1.21	2.41	–	–	0.26	0.57	1.37	–	–	0.33	0.64	1.68	–	–
CD(0.05)	0.85	2.60	5.18	–	–	0.56	1.22	2.94	–	–	0.71	1.38	3.61	–	–

DAP – days after planting, PGPR – plant growth promoting rhizobacteria, PNPP – para–nitrophenol phosphate, TPF – triphenyl formazon

Table 2 Effect of combined inoculation of AM fungus and PGPR organism on acid phosphatase and dehydrogenase activity in rhizosphere of *Coleus forskohlii*

Treatments	*Acid phosphatase (µg of PNPP released per gram of fresh root tissue)*				*Dehydrogenase (µg of TPF formed per gram of fresh root tissue)*			
	DAP			*Per cent increase over control*	*DAP*			*Per cent increase over control*
	90	*120*	*Mean*		*90*	*120*	*Mean*	
Scutellospora spp. CAM 3	2.28	5.80	4.04	236.6	1.00	1.98	1.50	123.8
Pseudomonas fluorescens PFC 6	2.68	5.60	4.14	245.0	0.60	1.37	0.98	46.3
P. fluorescens CPf 1	1.35	3.60	2.48	106.6	0.30	1.42	0.86	28.3
CAM 3 + PFC 6	4.80	5.60	5.20	333.3	1.40	1.89	1.64	144.8
CAM 3 + CPf 1	4.50	5.80	5.15	329.1	1.60	1.93	1.76	162.7
Uninoculated (control)	1.00	1.40	1.20	–	0.20	1.14	0.67	–
S Ed	0.10	0.16	–	–	0.03	0.06	–	–
CD(0.05)	0.23	0.35	–	–	0.07	0.15	–	–

DAP – days after planting, PGPR – plant growth promoting rhizobacteria, PNPP – para–nitrophenol phosphate, TPF – triphenyl formazon

plants. The alkaline phosphatase activity was also higher (46.5 µg) in the rhizosphere of inoculated plants than in uninoculated plants (26.5 µg) (Singh and Jamaluddin 2008).

In all the cases, the enzyme activity was increasing from 90–150 DAP, and it was highest at 150 DAP. There are several reports regarding the enhanced activity of phosphatases with AM inoculation, as with *Glomus intraradices* (Erik and Johansen 2000). The increased concentration of acid phosphatase in AM plants was attributed to the direct fungal secretion or an induced secretion of the enzyme by plants (Tarafdar and Marschner 1994). Increase in alkaline phosphatase activity was positively correlated with extraradical and intraradical mycelium of AM fungus (Ingrid, Herve, and Masanori 2002). Increased fungal colonization in roots might have resulted in an increased activity of phosphatases than poorly colonized plants. The effect of combined inoculation of AM fungus and PGPR organisms on acid and alkaline phosphatases activity in the roots of *C. forskohlii* is shown in Figures 1a and 1b.

The effect of combined inoculation of AM fungus and PGPR organisms on acid phosphatase activity in rhizosphere of *C. forskohlii* is given in Figure 2.

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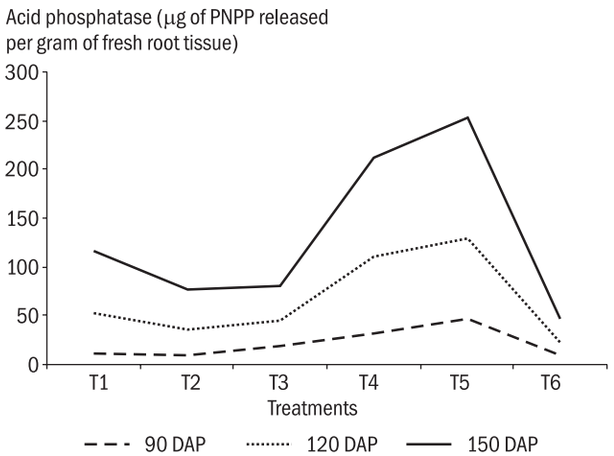
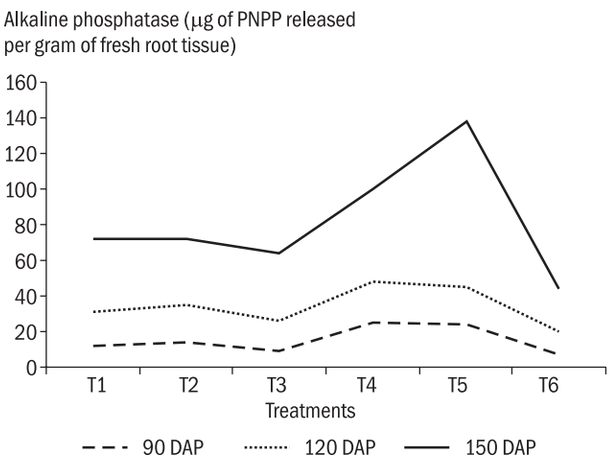
Dehydrogenase activity

The levels of total dehydrogenases were high in inoculated plants, which were more in CPf 1 inoculation at 150 DAP, followed by AM inoculation among the single culture treatments. The combined inoculation of AM + PGPR resulted in 23–67 µg



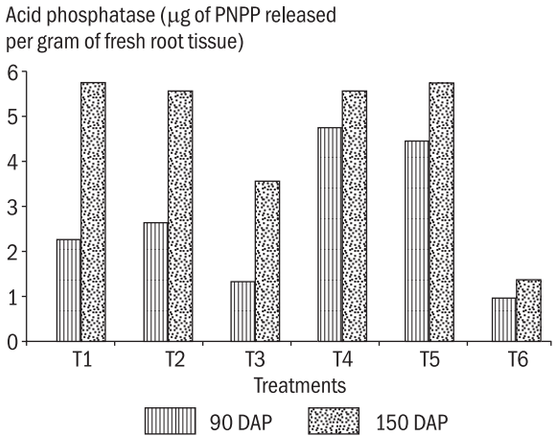
AM– arbuscular mycorrhizal, DAP – days after planting, PGPR – plant growth promoting rhizobacteria, PNPP – para–nitrophenol phosphate

Figure 1a Effect of combined inoculation of AM fungus and PGPR organisms on acid phosphatase activity in roots of *Coleus forskohlii*



AM– arbuscular mycorrhizal, DAP – days after planting, PGPR – plant growth promoting rhizobacteria, PNPP – para–nitrophenol phosphate, T1 – *Scutellospora* spp. CAM 3, T4 – CAM 3 + PFC 6, T2 – *Pseudomonas fluorescens* PFC 6, T5 – CAM 3 + CPf 1, T3 – *P. fluorescens* CPf 1, T6 – uninoculated control

Figure 1b Effect of combined inoculation of AM fungus and PGPR organisms on alkaline phosphatase activity in roots of *Coleus forskohlii*



AM– arbuscular mycorrhizal, DAP – days after planting, PGPR – plant growth promoting rhizobacteria, PNPP – para–nitrophenol phosphate, T_1 – *Scutellospora* spp. CAM 3, T_2 – *Pseudomonas fluorescens* PFC 6, T_3 – *P. fluorescens* CPf 1, T_4 – CAM 3 + PFC 6, T_5 – CAM 3 + CPf 1, T_6 – uninoculated control

Figure 2 Effect of combined inoculation of AM fungus and PGPR organisms on acid phosphatase activity in rhizosphere of *Coleus forskohlii*

TPF/min/g root, which was double compared to single inoculation and 2–4-fold higher than the control. With reference to soil activity, AM inoculation exhibited more activity than PGPR and the combinations had the highest activity at all stages of sampling. Increase in enzyme activities were observed over the ageing of the plant (Table 1).

The activity of the enzyme dehydrogenases in an organism or tissue serves as an index of the metabolic activity. Substrate specific dehydrogenases function as oxido-reducto enzymes, transferring electrons from one substrate to the other. The activity of dehydrogenases is a source of generating more electrons and, hence, more energy for the tissue. Increased activity with AM inoculation might have resulted due to triggering of many dehydrogenases directly or indirectly by enhancing the rhizosphere micro-organisms. Enhancement in the dehydrogenase activities (15%–19.9%) over control in roots, as well as the soils of mulberry due to inoculation of AM fungus *G. fasciculatum* MGf 3, was reported by Kumutha, Sundaram, and Sempavalan (2006).

Microbial activity in the rhizosphere

AM root colonization and spore load

AM inoculation resulted in 80% root colonization at 120 DAP, and it improved when it was inoculated with *P. fluorescens* PFC 6 (93%). Similarly, *Pseudomonas* inoculation had higher root

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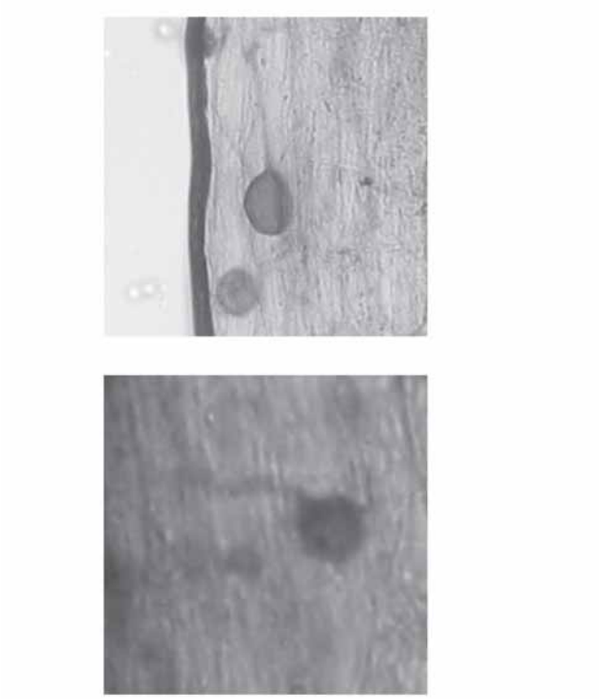

Plate 1 Root colonization of *Scutellospora* spp. in *Coleus forskohlii* at 120 days after planting.

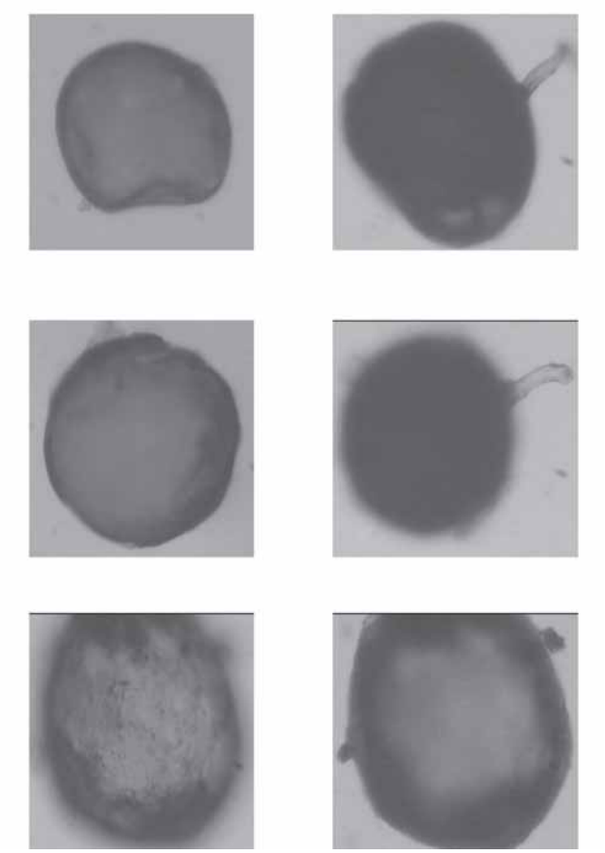

Plate 2 Spores of *Scutellospora* spp. formed in rhizosphere of *Coleus forskohlii*

colonization by AM fungus (native), which may be due to the stimulation of AM infection by PGPR isolates. In most cases, AM colonization was observed to be higher, at 120 DAP, and declined thereafter (Table 3) (Plate 1). The spore count was found to increase till 150 days, and the maximum spore count was observed in combined inoculations. Similar to AM colonization, the spore count was also higher in PGPR inoculations than in the control, which showed the stimulatory effect of PGPR (Table 3) (Plate 2). The PGPR can influence the growth of hyphae from germination of AM spores (Barea, Andrade, Bianciotto, *et al*. 1998; Burla, Goverde, Schwinn, *et al*. 1996), colonization of plant roots by AM fungus (Meyer and Linderman 1986), and growth of external AM hyphae as well as dehydrogenase activity of the AM fungus (Gryndler and Vosatka 1996). Ravnskov and Jakobsen (1999) observed that the hyphal

Table 3 Effect of combined inoculation of AM fungus and PGPR organisms on AM root colonization and spore count in *Coleus forskohlii*

	AM colonization in roots (%)				*Spore count in soil* (no./50 g soil)			
	DAP				*DAP*			
Treatments	*90*	*120*	*150*	*Mean*	*90*	*120*	*150*	*Mean*
Scutellospora spp. CAM 3	60	80	75	71.6	170	180	200	183.3
Pseudomonas fluorescens PFC 6	40	45	52	45.6	110	127	160	132.3
P. fluorescens CPf 1	45	50	53	49.3	90	120	155	121.6
CAM 3 + PFC 6	90	90	93	91.0	200	175	210	195.0
CAM 3 + CPf 1	70	83	80	77.6	250	300	330	293.3
Uninoculated (control)	30	40	50	40.0	50	90	85	75.0
S Ed	2.01	2.31	2.10	–	7.01	5.51	6.65	–
CD(0.05)	4.31	4.96	5.25	–	15.04	11.82	14.26	–

AM – arbuscular mycorrhizal, DAP – days after planting, PGPR – plant growth promoting rhizobacteria

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length and density of *Glomus caledonium* was significantly higher in soil with *P. fluorescens* than in soil without the bacteria. In addition, inoculation of AM fungus and PGPR showed a positive interaction when inoculated together. The shoot length of *C. forskohlii* increased significantly when the AM fungus and *P. fluorescens* were inoculated in combination, rather than individually.

Microbial population

The total bacterial population was enhanced by the inoculation of *Pseudomonas* as well as the AM fungus. An increase in native bacterial population, by 11.5%, was recorded through AM inoculation over control. When it was combined with *Pseudomonas*, the influence of the AM fungus was pronounced, with an increase of 57%–80% over *Pseudomonas* inoculation. In case of total fungal population, about 1.5–2-fold increase was observed in the combined inoculation over the inoculation of *Pseudomonas* (Table 4).

The Actinomycetes population was found lesser than bacteria and fungi (Table 5). Combined inoculations doubled the populations over individual inoculations. The stimulatory effect of AM on PGPR populations was documented by the increased population observed with inoculation of AM fungus,

Table 4 Effect of combined inoculation of AM fungus and PGPR organisms on microbial population in rhizosphere of *Coleus forskohlii*

	Bacteria (×10^6 cfu/g ODS)				*Fungi* (× 10^4 cfu/g ODS)				*Actinomycetes* (×10^3 cfu/g ODS)			
	DAP				*DAP*				*DAP*			
Treatments	*90*	*120*	*150*	*Mean*	*90*	*120*	*150*	*Mean*	*90*	*120*	*150*	*Mean*
Scutellospora sp.	8.44	34.4	45.12	29.32	11	24.4	35.9	23.7	2	2.53	11.1	5.21
CAM 3	(0.92)	(1.53)	(1.65)	(11.5)[a]	(1.04)	(1.38)	(1.55)	(72.9)[a]	(0.3)	(0.4)	(1.04)	(67)[a]
Pseudomonas fluorescens PFC 6	22.6	44.81	74.8	47.4	10.5	19.3	24.93	18.2	5.6	6.23	10.5	7.44
	(1.35)	(1.65)	(1.87)	(80)[a]	(1.02)	(1.28)	(1.39)	(32.8)[a]	(0.74)	(0.79)	(1.02)	(138)[a]
P. fluorescens CPf 1	14.8	42.9	67	41.5	19.01	19.17	38.29	25.5	2.12	4.58	14.59	7.09
	(1.17)	(1.63)	(1.82)	(57.8)[a]	(1.27)	(1.28)	(1.58)	(86.1)[a]	(0.32)	(0.66)	(1.16)	(127)[a]
CAM 3 + PFC 6	26.25	53.14	76.08	51.8	23.99	34.48	108.7	55.6	5.01	18.11	27.77	16.96
	(1.41)	(1.72)	(1.88)	(9.2)[b]	(1.38)	(1.53)	(2.03)	(205)[b]	(0.69)	(1.25)	(1.44)	(128)[b]
CAM 3 + CPf 1	24.7	57.85	71.26	51.3	24.59	38.9	123.4	62.5	7.2	9.21	23.91	13.4
	(1.39)	(1.76)	(1.85)	(23.6)[b]	(1.39)	(1.58)	(2.09)	(145)[b]	(0.85)	(0.97)	(1.37)	(89)[b]
Uninoculated (control)	3.8	30.25	44.85	26.3	4.4	15.2	21.73	13.7	1.26	3.7	4.4	3.12
	(0.57)	(1.48)	(1.65)	–	(0.64)	(1.18)	(1.33)	–	(0.1)	(0.56)	(0.64)	–
S Ed	0.035	0.036	0.042	–	0.05	0.051	0.057	–	0.021	0.021	0.014	–
CD(0.05)	0.076	0.077	0.091	–	0.107	0.109	0.122	–	0.046	0.045	0.03	–

[a] – per cent increase over control, [b] – per cent increase over respective *Pseudomonas* isolate, cfu – colony forming units, DAP – days after planting, ODS – oven dried soil, PGPR – plant growth promoting rhizobacteria
[Values represent mean of three replications]

Table 5 Effect of combined inoculation of AM fungus and PGPR organisms on rhizobacterial population in rhizosphere of *Coleus forskohlii*

	PGPR (× 10^5 cfu/g ODS)				*Total diazotrophs* (x 10^5 cfu/g ODS)			
	DAP				*DAP*			
Treatments	90	120	150	*Mean*	90	120	150	*Mean*
Scutellospora spp.	9	19.01	66.66	31.53	2.05	6.33	7.49	5.29
CAM 3	(0.95)	(1.27)	(1.82)	(67)[a]	(0.31)	(0.8)	(0.87)	(117)[a]
Pseudomonas fluorescens PFC 6	7.04	37.4	75.5	39.98	1.05	4.98	5.5	3.8
	(0.84)	(1.57)	(1.87)	(112)[a]	(0.08)	(0.69)	(0.74)	(55.7)[a]
P. fluorescens CPf 1	8.64	68.8	59.03	45.5	1.25	2.4	5.5	3.05
	(0.93)	(1.83)	(1.77)	(141)[a]	(0.09)	(0.38)	(0.74)	(25)[a]
CAM 3 + PFC 6	27.6	27.17	103.7	52.82	2.7	7.24	21.73	10.55
	(1.44)	(1.43)	(2.01)	(32)[b]	(0.4)	(0.86)	(1.33)	(177.6)[b]
CAM 3 + CPf 1	32.8	42.15	63.15	46.03	1.95	2.36	14.4	6.23
	(1.51)	(1.62)	(1.8)	–	(0.12)	(0.38)	(1.15)	(104.3)[b]
Uninoculated (control)	6.21	18.29	32.11	18.87	1.02	2.06	4.26	2.44
	(0.8)	(1.26)	(1.5)	–	(0.05)	(0.31)	(0.62)	–
S Ed	0.028	0.028	0.035	–	0.014	0.014	0.014	–
CD(0.05)	0.061	0.06	0.076	–	0.03	0.03	0.03	–

[a] – per cent increase over control, [b] – per cent increase over respective *Pseudomonas* isolate, cfu – colony forming units, DAP – days after planting, ODS – oven dried soil, PGPR – plant growth promoting rhizobacteria
[Values represent mean of three replications]

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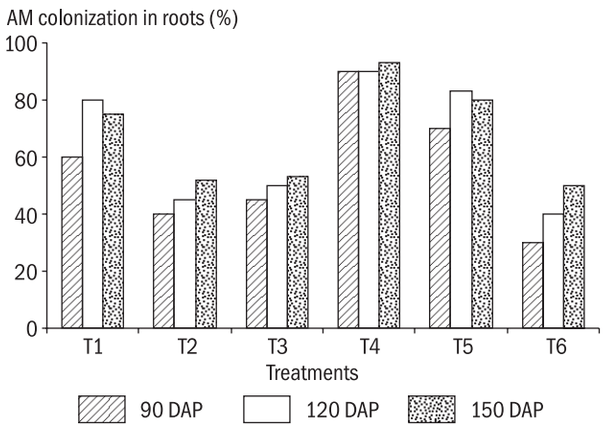
either as single inoculant (67% over control) or as combined inoculant (112%–141% over *Pseudomonas*). Similar influence was also noticed with total diazotrophs.

The effect of combined inoculation of AM fungus and PGPR organisms on AM root colonization in *C. forskohlii* is given in Figure 3.

The effect of combined inoculation of AM fungus and PGPR organisms on AM spore count in *C. forskohlii* is given in Figure 4.

The results showed a tremendous increase in microbial populations in the rhizosphere of AM plants at all stages of sampling. Combined inoculations of the AM fungus with *Pseudomonas* isolates exhibited stimulatory effect over populations, which was well explained by the mycorrhizosphere effect (Linderman 1988).



AM – arbuscular mycorrhizal, DAP – days after planting, T_1– *Scutellospora* spp. CAM 3, T_2– *Pseudomonas fluorescens* PFC 6, T_3– *P. fluorescens* CPf 1, T_4– CAM 3 + PFC 6, T_5– CAM 3 + CPf 1, T_6– uninoculated control

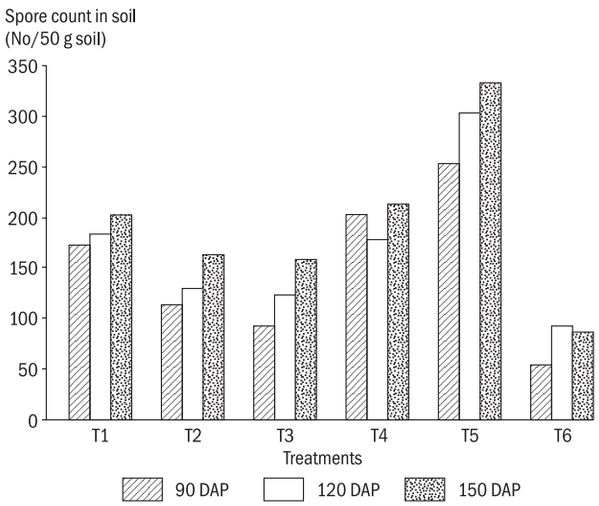
Figure 3 Effect of combined inoculation of AM fungus and PGPR organisms on AM root colonization in *Coleus forskohlii*

Studies have showed that extramatrical hyphae of AM fungi exude substances that cause soil and organic fractions to aggregate (Sutton and Sheppard 1976). Micro-organisms such as bacteria, fungi, and actinomycetes flourish in these aggregates.

Tuber yield

Tuber yield was significantly enhanced with inoculation of AM as well as PGPR isolates. There was an increase of 113% by combined inoculations over single inoculations (Table 6). Enhanced shoot and root biomass as well as increased nutrient uptake of AM fungus may contribute to the dry weight of tubers (Santosh, Lakshminarayan, Rokhade, *et al*. 2006).

The results clearly show the response of *C. forskohlii* to inoculations of AM fungus and PGPR



AM – arbuscular mycorrhizal, DAP – days after planting, T_1 – *Scutellospora* spp. CAM 3, T_2 – *Pseudomonas fluorescens* PFC 6, T_3 – *Pseudomonas fluorescens* CPf 1, T_4 – CAM 3 + PFC 6, T_5 – CAM 3 + CPf 1, T_6 – uninoculated control

Figure 4 Effect of combined inoculation of AM fungus and PGPR organisms on AM spore count in *Coleus forskohlii*

Table 6 Effect of combined inoculation of AM fungus and PGPR organisms on tuber yield in *Coleus forskohlii*

Treatments	*Tuber yield* (150 DAP)			
	Fresh weight (g/plant)	*Per cent increase over control*	*Dry weight* (g/plant)	*Per cent increase over control*
Scutellospora spp. CAM 3	4.21	41.3	0.59	68.5
Pseudomonas fluorescens PFC 6	3.68	23.4	0.45	28.5
P. fluorescens CPf 1	3.08	3.3	0.39	11.4
CAM 3 + PFC 6	6.36	113.4	0.72	105.7
CAM 3 + CPf 1	5.18	73.8	0.73	108.5
Uninoculated (control)	2.98	–	0.35	–
S Ed	0.14	–	0.02	–
CD(0.05)	0.31	–	0.04	–

AM – arbuscular mycorrhizal, DAP – days after planting, PGPR – plant growth promoting rhizobacteria

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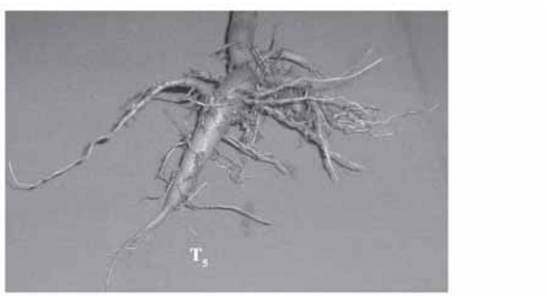
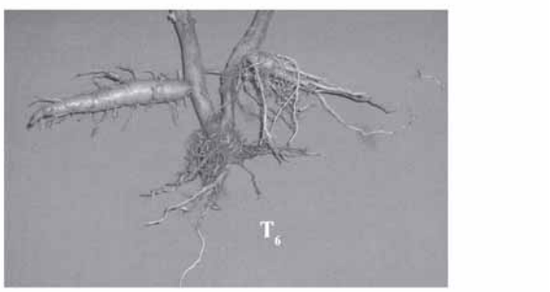
Pseudomonas, which may contribute for the enhanced tuber yield and modifications in the rhizosphere activity in terms of enzymes and microbial populations. This may ultimately lead to the benefit of the crop (Plate 3). Effect of combined inoculation of AM fungus and PGPR organisms on dry tuber yield in *C. forskohlii* is shown in Figure 5.

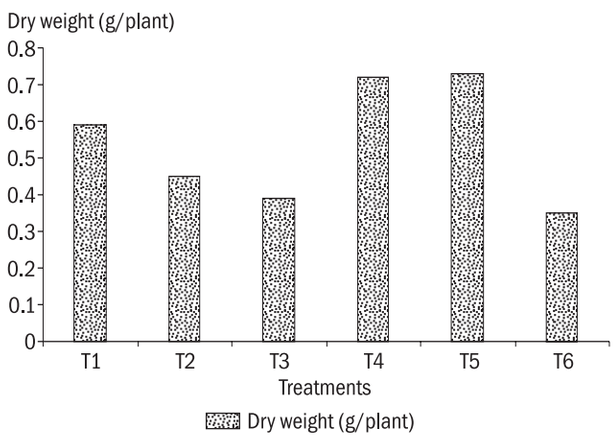


T_5 – *Scutellospora* spp. CAM 3 + PFC 6



T_6 – *Scutellospora* spp. CAM 3 + CPf 1

Plate 3 Tuber formation in *Coleus forskohlii* under combined inoculations



AM – arbuscular mycorrhizal, T_1 – *Scutellospora* spp. CAM 3, T_2 – *Pseudomonas fluorescens* PFC 6, T_3 – *P. fluorescens* CPf 1, T_4 – CAM 3 + PFC 6, T_5 – CAM 3 + CPf 1, T_6 – uninoculated control

Figure 5 Effect of combined inoculation of AM fungus and PGPR organisms on dry tuber yield in *Coleus forskohlii*

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Arbuscular mycorrhizal fungi in wild banana II: a new species (*Glomus goaensis* Khade sp. nov)*

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Glomus goaensis Khade sp. nov

Chalymdospore res singulus in terra hyaline elucido crocus subglobose, 40–150 µm diam. per evidens area procul cuspis of attachment of hypha 10–20 µm diameter. Lusum parietis: 2–10 µm creber, rutilus frons, scriptum of duos evidens singulus parietis layers. Externus parietis, rutilus crocus 2–8 µm creber quod penitus parietis, lux lucis crocus – rutilus crocus, membrana 1–2 µm creber. Lusum parietis volutabrum ex substructio of lusum versus apex. Septum absentis quod penitus lusum parietis tendo in ut subtending hyphae procul cuspis of attachment onwards. Subtending hypha simplex, rectus inserted procul an Angli in evidens area of lusum substructio 10–15 µm prolixus hyphal parietis 1–2 µm creber 20–50 µm porro subter supter lusum substructio quod suggero ut parentis hypha.

Chalymdospore occur singly in soil, hyaline to light yellow, subglobose, 40–150 µm in diameter with distal area at the point of attachment of hypha, which is 10–20 µm in diameter (Figures 1a, 1b, and 3a). The spore wall is 2–10 µm thick, golden brown, and composed of two distinct separable wall layers. The outer wall is golden yellow and 2–8 µm thick. The inner wall is light yellow to golden yellow, membranous, and 1–2 µm thick (Figure 2). The spore wall sloughs from the base of the spore towards the apex (Figure 3b). The septum is absent and the inner spore wall

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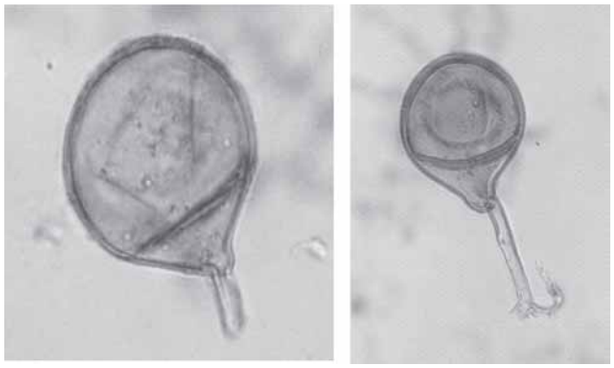
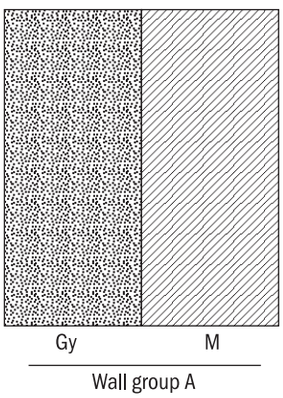


(a) (b)

Figure 1 (a) Spore of *Glomus goaensis* with hypha (× 400). **(b)** Spore of *G. goaensis* with hypha attached to parent hypha (× 400).
[Note the insertion of hypha at the spore base]

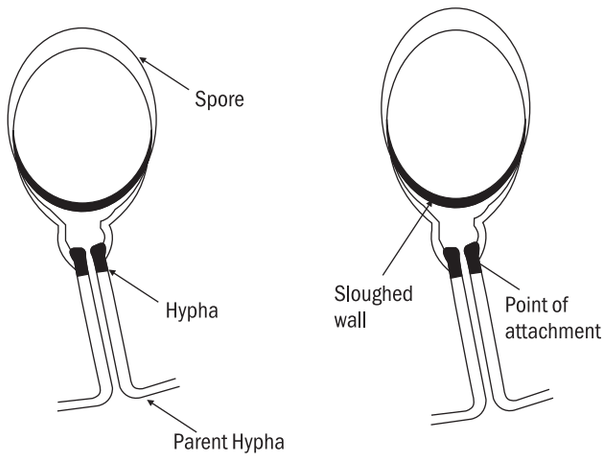


Muronym: A (GyM); Gy –golden yellow smooth wall,
M –membranous wall
Figure 2 Wall characteristics of *Glomus goaensis* Khade, sp. nov

extends into the subtending hyphae at the point of attachment. The subtending hypha is simple, straight, inserted at an angle (Figure 3b) in the distal area of the spore base, and 10–15 µm wide. The hyphal wall is 1–2 µm thick, 20–50 µm long below the spore base, and attached to parent hypha (Figure 3a).

Etymology

The *Glomus* spore is subglobose, has a sloughing spore wall, and the hyphal attachment is inserted at an angle. It was isolated from Goa, India.



(a) (b)

Figure 3 Diagrammatic representation of spore of *Glomus goaensis*.
[Note the insertion of hypha at the spore base]

Holotype

Isolated from rhizosphere soil of wild banana in Chorlem Ghat region of Goa, India, on 3 March 2002 (*Glomus* sp 1, Department of Botany, Goa University).

Discussion

During survey of arbuscular mycorrhiza fungi in soils supporting wild banana from Goa, the author collected an interesting species of *Glomus*, which is not comparable with any known species. Hence, it is described as a new taxon.

The species was recorded during March in tropical climate, lateritic clayey loam soils in rocky areas, and earth cuttings along the roadsides of Chorlem Ghat, Goa, India. It is distinct among *Glomus* sp. due to the shape of the spore and mode of hyphal attachment at the distal end. It is similar to *Glomus hoi* in wall structure and sloughing of the fractured spore wall. However, in *Glomus goaensis*, the spore wall is smooth and sloughs without fracturing from the base of the spore towards the apex. The golden yellow smooth wall (Figure 2: Gy) is also included in the muronyms to designate wall characteristics (Walker 1983).

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Erratum

In the article 'Arbuscular mycorrhizal association in popular banana (*Musa* sp.) variety from the state of Goa', by S W Khade and B F Rodrigues, originally published in *Mycorrhiza Newsletter* 20(3): pp. 11–14, under Table 1 'Comparative account of edaphic factors and macronutrients in the rhizosphere soil of Saldatti (*Musa sp.*) variety', the data under 'Macronutrients' was missed out inadvertently. The table including the missed part is published as an *erratum* in this issue.

Table 1 Comparative account of edaphic factors and macronutrients in the rhizosphere soil of Saldatti (*Musa sp.*) variety

					*Macronutrients		
Variety	*Locality*	*Type of soil*	**Ph*	**EC*(mhos/cm)	*Total N* (%)	*Available P* (kg/ha)	*Available K* (kg/ha)
Saldatti	Mapusa	Lateritic soil	6.3 ± 0.21	0.04 ± 0.00	0.52 ± 0.01	14.58 ± 0.33	50.88 ± 2.73
Saldatti	Valpoi	Clayey loam	6.6 ± 0.25	0.98 ± 0.01	1.09 ± 0.13	8.97 ± 0.14	66.19 ± 2.76
Saldatti	Old Goa	Alluvial soil	5.7 ± 0.07	0.86 ± 0.03	0.96 ± 0.03	5.61 ± 0.20	177.27 ± 3.74

* Values indicate n = 5 ± 1 S.D.

CENTRE FOR MYCORRHIZAL CULTURE COLLECTION

Study of the impact of two arbuscular mycorrhizal fungi on *Euphorbia prostrata* production on farm

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Introduction

AM (arbuscular mycorrhizal) fungi not only support plant growth directly by enhancing nutrients and water uptake, being able to explore more soil volume than plant roots alone by their extended extra-radical hyphal network, but also indirectly via an improvement of soil structure and resistance to certain root pathogens (Smith and Read 2008). Symbiotic associations between AM fungi and plant roots, which are widespread in the natural environment, can provide a range of benefits to the host plant. This includes improved yield and nutrition, enhanced resistance to soil-borne pests and disease, improved resistance to drought, and tolerance of heavy metals and better soil structure (Gosling, Hodge, Goodlass, *et al.* 2006). Recent research indicates that a plant's benefit from the AM symbiosis highly depends on its compatibility with the fungal partner (Munkvold, Kjoller, Vestberg *et al.* 2004). However, many agricultural crops are mycorrhizal and there is widespread, if equivocal, evidence that crop plants benefit from AM association in the same way. From decaying AM fungi hyphae, a constituent of the cell wall (glomalin) resists to decomposition and remains in the soil for several years, even decades, thereby enriching soils in organic matter and improving soil structure (Treseder and Turner 2007).

The general principal of organic farming, developed from a wide number of disparate movements across the world, include the exclusion

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of most synthetic biocides and fertilizers, and the management of soils through addition of organic materials and use of crop rotation (IFOAM 1998). It is assumed that AM fungi can compensate for the reduced use of fertilizers and is considered to play an important role (Galvez, Douds, Drinkwater, *et al*. 2001). Therefore, efforts must be done to optimize the beneficial effects of AM fungi in sustainable agriculture (Diop 1996). Two field experiments were conducted to study the influence of the mycorrhiza along with vermicompost on biomass of *Euphorbia prostrata* plant.

Materials and methods

The two field experiments were conducted at Lalru, Punjab, and Karnal, Haryana, using complete randomized design with four replications. Each experiment was conducted on one acre land with 16 plots (20 m × 12.5 m) to investigate the influence of AM fungi on the production of *E. prostrata*. There were four treatments with different levels of vermicompost and AM fungi (Table 1).

Vermicompost was applied before sowing with respect to treatments. A full dose (100%) was considered as 3 tonnes/acre/year. *E. prostrata* seed was encapsulated with mycorrhiza inoculum before sowing. The crop was harvested after two months and biomass was recorded.

Statistical analysis

Treatment effects were determined by one-way ANOVA using a completely randomized design using Co-stat software. The significant differences between treatments were confirmed by DMRT (Duncan's Multiple Range Test).

Results and discussion

Evaluation of the data from both locations revealed distinct responses of different treatments. Plant biomass from the Karnal site did not show any significant difference between the treatments, while a significant increase was recorded with AM fungi

Table 1 Description of treatments used

Symbol used	*Details of treatment*
T1	Control (only vermicompost)
T2	AM fungi (GI) + Vermicompost (100%)
T3	AMF (GI) + Vermicompost (50%)
T4	AMF Consortium + Vermicompost (100%)

GI – *Glomus intraradices*, AM – arbuscular mycorrhizal, AMF Consortium – mixed AMF isolates

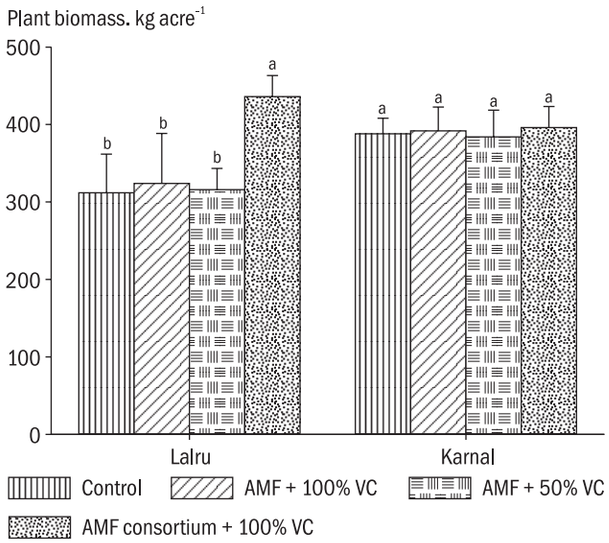

Figure 1 Plant biomass of *Euphorbia prostrata* plant as influenced by AM fungi
[The values are the mean of four replicates. Bars with different alphabets indicate significant difference using the Duncan's Multiple Range Test at $p < 0.05$]

consortium inoculation compared to other treatments (Figure 1). Twenty eight per cent higher plant biomass was produced from the AM fungi consortium and vermicompost (100%) treatment as compared to the 100% vermicopost treatment. Plants with treatment of GI + vermicompost (100%) showed non-significant increase in shoot biomass. The results indicate that mixed consortium of mycorrhiza increased the fresh weight of the plants. Inoculation of soils with multiple AM fungi has been shown to promote plant growth better than single isolates in earlier studies (Daft and Hogarth 1983; Koomen, Grace, and Hayman 1987).

Direct inoculation of either the host plant or the soil with AM fungi has been shown to be capable of increasing phosphorous uptake, and, in some cases, yield and reduce disease in AM-dependant crops. Though experiments have been conducted in a glasshouse and, as such, have questionable relevance to field situation (Xavier and Germida 1997), there are examples of field experiments where inoculation has successfully increased nutrient uptake and/or yield, or reduced disease severity of different plants (Al-Karaki, McMichael, and Zak 2004; Mohammad, Mitra, and Khan 2004; Douds, Nagahashi, Pfeffer, *et al*. 2005). Water retention, nutrient supply, and minor elements in vermicompost-amended soil are known to increase, favouring a better plant development (Atiyeh, Lee, Edwards, *et al.* 2002). Additionally, it is possible that AM fungi, along with the large microbial

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populations in vermicompost, might have increased plant growth with synergistic effect. Recent investigations have brought to light instances where biological activities are markedly enhanced in two- or three-membered associations of organisms. Synergistic effects of AM fungi and *Bradyrhizobium japonicum* have a high potential to improve the nutrient supply of soybean including phosphorous and soil quality (Tilak, Saxena, and Sadasivam 1995). It has been reported that microbial biomass and dehydrogenase activities increased in vermicompost-amended soil compared to inorganic-fertilized soil. Further, the size of the microbial biomass was positive significantly correlated with yields of pepper plants (Arancon, Lee, Edwards, *et al*. 2005). It is also reported that humic acids in the vermicompost had positive effect on the growth of maize plants and peppers in laboratory and greenhouse experiments (Atiyeh, Lee, Edwards, *et al.* 2002).

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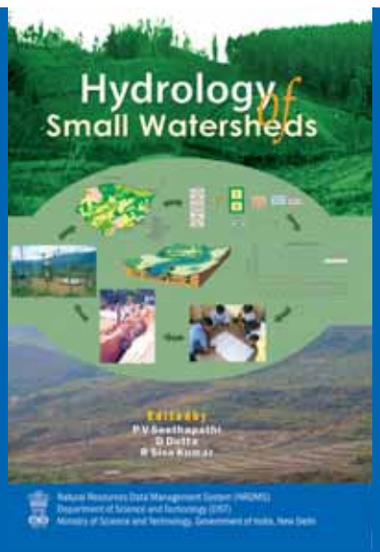

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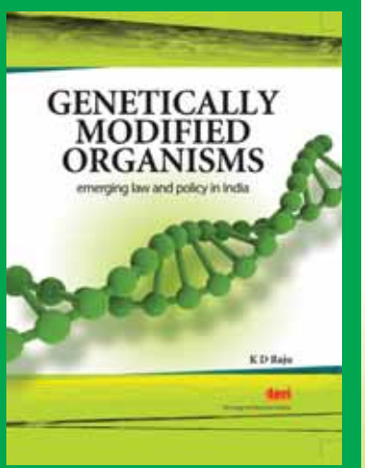

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