MYCORRHIZA NEWS

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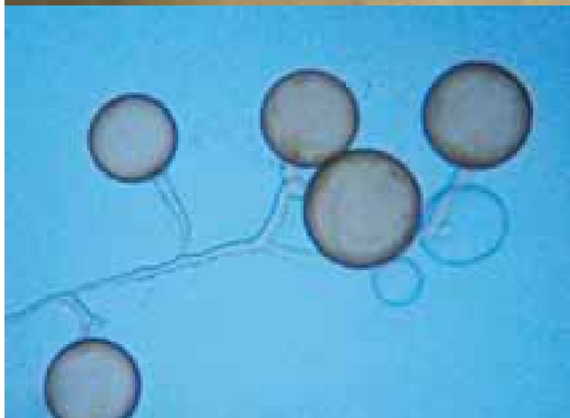

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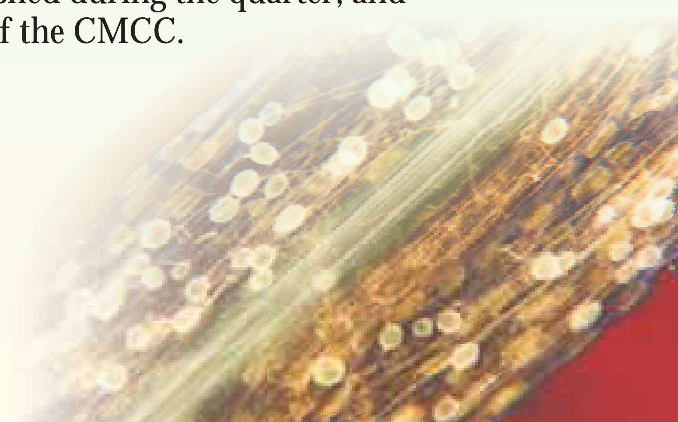
TERI's Mycorrhiza Network is primarily responsible for establishing the MIC (Mycorrhiza Information Centre), the CMCC (Centre for Mycorrhiza Culture Collection), and publishing Mycorrhiza News. The Network helps scientists carry out research in mycorrhiza and promotes communication among mycorrhiza scientists.

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.



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Research finding papers

Some aspects of Ectomycorrhiza with reference to Conifers

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Introduction

The mutually beneficial relationship between the feeder roots of plants and fungi is called 'mycorrhiza'. The term 'mycorrhiza' meaning 'fungus root' in Greek was coined by Frank in 1885 to describe the symbiotic association of plant roots and fungi. Recently, Brundrett (2004) defined mycorrhiza as 'a symbiotic association essential for one or both partners between a fungus (specialized for life in soil and plants) and a root (or other substrate-contacting organ) of a living plant, that is primarily responsible for nutrient transfer. Mycorrhizae occur in a specialized plant organ where intimate contact results from synchronized plant–fungus development.

Presently, the mycorrhizal association and its beneficial role towards plants are accepted as a universal phenomenon. Mycorrhizal associations are so prevalent that the non-mycorrhizal plant is more of an exception than the rule (Gerdemann 1968). Mycorrhizal associations are placed in seven categories (Harley and Smith 1983), that is, ectomycorrhizae, ectendomycorrhizae, AM (arbuscular mycorrhizae), orchidaceous, ericaceous, arbutoid, and monotropoid mycorrhizae.

Mycorrhizae are active living components of the soil and have some properties like those of roots and some like those of microorganisms. Mycorrhizal fungi extend the plant's possibilities for exploring the soil (Gerdemann 1968, Mosse 1973). The fungus supplies the basic enzymatic machinery for absorbing, translocating, and assimilating major mineral ions like phosphate and inorganic nitrogen, plus a number of genes required for symbiosis. The mycorrhizal fungi derive most, if not all, of their needed organic nutrition (carbohydrates, vitamins, and amino acids) from plants. The plant provides the peculiar ecological niche that is necessary for fungal growth and development including the completion of the sexual cycle, and it contributes the enzymes that assimilate the important nutrients (Martin and Hilbert 1991).

Busgen (1901) on the basis of the intensity with which the occupied soil volume is tapped, has distinguished 'intensive' and 'extensive' types of root systems. In the former many roots penetrate a smaller soil volume whereas in the latter fewer roots penetrate a greater soil volume. In general, the conifers have extensive and broad-leaved plants have an intensive root system. The intensive root system appears to possess both ecto- and endomycorrhizae, whereas the extensive root system seems to be restricted to endomycorrhizal condition. The long root of conifers show racemose branching and are capable of indefinite growth, unlike the dichotomously branched short root, which grow for only a restricted period. The growth rates of long roots are considerably greater and the duration of growth more prolonged than that observed in short roots.

Ectomycorrhizae

An ectomycorrhiza, a specialized root organ, is the result of a complex interaction leading to a finely tuned symbiosis between a plant and a compatible ectomycorrhizal fungus (Harley and Smith 1983). This type of association occurs on about 10% of the

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world flora. Trees belonging to the Pinaceae (pine, larch, spruce, hemlock), Fagaceae (oak, chestnut, beech), Betulaceae (alder, birch), Salicaceae (poplar, willow), Juglandaceae (hickory, pecan), Myrtaceae (Eucalyptus), Ericaceae (*Arbutus*), and a few others form ectomycorrhizae. Some tree genera, such as *Alnus*; *Eucalyptus*; *Casuarina*; *Cupressus*; *Juniperus*; *Tilia*; *Ulmus*; and *Arbutus* form both ectomycorrhizae and arbuscular mycorrhizae, depending on soil conditions and tree age (Harley and Smith 1983).

Numerous fungi have been identified as forming ectomycorrhizae. Worldwide, there are over 5000 species of fungi that can form ectomycorrhizae on some 2000 species of woody plants. Riviere, Diedhiou, Diabate, *et al.* (2007) studied genetic diversity of ectomycorrhizal basidiomycetes from African and Indian tropical rain forests. Among the basidiomycetous fungi, species of Hymenomycetes (mushrooms) in the genera *Boletus*; *Cortinarius*; *Suillus*; *Russula*; *Gomphidius*; *Hebeloma*; *Tricholoma*; *Laccaria*; *Lactarius* and species of the *Gasteromycetes* (puffballs) in the genera *Rhizopogon*, *Scleroderma*, and *Pisolithus* form ectomycorrhizae. Certain orders in the Ascomycetes, such as Eurotiales (*Cenococcum geophillum*); Tuberales (truffles); and Pezizales, have species that also form ectomycorrhizae. Many ectomycorrhizal fungi can be grown routinely in pure culture. They cannot exist saprophytically in nature without a plant-host association, spores or resistant hyphae may survive long periods in soil without a plant host but the fungi from these propagules cannot grow independent of their plant host as saprophytes (Kendrick and Berch 1985).

Ectomycorrhizal infection is initiated from spores or hyphae (propagules) or the fungal symbionts inhabiting the rhizosphere of the feeder roots. Propagules are stimulated by root exudates and grow vegetatively over the feeder (short) root surface, thus, forming a fungus mantle. Following fungal mantle development, hyphae develop intercellularly around root cortical cells and form the 'Hartig net', which may completely replace the middle lamella between the cortex cells. 'Hartig net' is the main distinguishing feature of ectomycorrhizae (Harley and Smith 1983). Ectomycorrhizal colonization normally changes the feeder root morphology and colour. They may be unforked, bifurcated, nodular, multiforked (coralloid) or in other shapes. Their colour, which is usually determined by the colour of the mycelium of the fungal symbiont, may be black, red, yellow, brown, white or blends of these colours. Ectomycorrhizal colonization is limited to the primary cortex (with intercellular hyphae surrounding cortical cells) and does not spread beyond the endodermis or into meristem tissues of the feeder root (Martin and Hilbert 1991).

Trees with abundant ectomycorrhizae have a much larger, physiologically active root fungus area for nutrient and water absorption than trees with less ectomycorrhizae. This increase in surface area comes both from the multi-branching habit of most ectomycorrhizae and from the extensive vegetative growth of hyphae of the fungal symbionts from the ectomycorrhizae into the soil. The extramatrical hyphae functions as additional nutrient and water absorbing entities and assure maximum nutrient capture from the soil by the host. Ectomycorrhizae are able to absorb and accumulate nitrogen, phosphorus, potassium, and calcium in the fungus mantle more rapidly and for longer periods of time than non-mycorrhizal feeder roots. Ectomycorrhizae remain active for periods ranging from several months to three years (Marx and Shafer 1989).

Ectomycorrhizae increase the tolerance of trees to drought, high soil temperatures, soil toxins (organic and inorganic), and extreme soil acidity caused by high levels of sulphur or aluminium. Ectomycorrhizae deter infection of feeder roots by root pathogens such as species of *Pythium* or *Phytophthora*. Hormone relationships, induced by fungal symbionts, cause ectomycorrhizal roots to have greater longevity (length of physiological activity) compared to non-mycorrhizal roots (Slankis 1973).

The fungal sheath or mantle

The fungal mantle consists of weft of interwoven hyphae enveloping short roots. Hyphae in the mantle may be loose or tightly interwoven. Different ectomycorhhizal fungi, with different hosts, form distinctive mantles in varying thickness, texture, and colour. The hyphal sheath is formed by plectenchymatous hyphae. Mantle may be smooth or with radiating hyphae, which enter into soil to increase the absorbing surface of short roots. Mantle surface can range from thin to profuse and texture can vary from smooth, cottony, velety, warty to granular. The hyphae, radiating from the mantle surface, may be simple or branched, bearing simple or clamped septa (Zak 1973). The fungal hyphae in the mantle are more or less coated with cement, which has been described as a coating layer, an interfacial matrix, and an apposition layer or the external layer or the hyphal wall. The cement appears to consist of polysaccharides associated with proteins (Dexheimer and Gerard 1988).

Hartig net

The region where host and fungus come into close contact and where nutrient exchange takes place between the two symbionts is known as Hartig net. Hyphae from the fungal mantle also penetrate

through the epidermis into the intercellular spaces of the cortical cells, apparently replacing the middle lamella and forming an interconnecting network, that is the Hartig net. In most cases, this net spreads slowly inward until it reaches the endodermis, which effectively bars any further penetration, though in some angiosperms the Hartig net may not penetrate beyond the first layer of cortical cells.

As the hyphae insinuate themselves between the cortical cells, the latter simply separate at the middle lamella, and an almost complete single layer of fungal hyphae eventually seperates and virtually encapsulates each cell, though plasmodesmatal connections may remain between cortical cells. The presence of the fungal net actually prolongs the life of the cortical cells and of the root as a whole (Kottke and Oberwinkler 1986 a). It has been shown that sugars are translocated from the root via the Hartig net to the fungal mantle where they tend to accumulate. As sugars pass from plant to fungus they are converted into trehalose, mannitol, and glycogen, all three being typical fungal carbohydrates (Nylund 1981).

Electron microscopic examination (Nylund 1981) of Hartig net has revealed that it consists of complicated fan-like systems of hyphae, which provide a very large surface of contact between cells of the two symbionts. Kottke and Oberwinkler (1986b) have reviewed the process of Hartig net development. It starts when hyphae come into contact with unsuberized living cortical or epidermal cells and is characterized by changes of hyphal growth and morphology. Hyphae are oriented transversely to the root axis and begin to branch out irregularly, septation is rare, the hyphae penetrate in the direction of the endodermis and growth in longitudinal direction through the intercellular spaces is rather restricted.

Characterization and identification of ectomycorrhizae

Ceruti and Bussetti (1962) have identified fungal symbionts of five ectomycorrhizae of Tilia species. Fontana and Centrella (1967) have named symbiotic fungi of eight mycorrhizae of *Castanea*, *Carpinus, Fagus*, *Pinus*, and *Quercus* species. Trappe (1967) has described a black mycorrhiza and a tuberculate mycorrhiza of 'Douglas fir'. He has also described four Douglas fir mycorrhizae synthesized in pure culture of *Hebeloma crustuliniforme*, *Suillus subolivaceous*, *Rhizopogon colossus*, and *Astraeus pteridis*.

Zak (1969) described two new ectotrophic mycorrhizae of Douglas fir, each of which is formed by a distinct strain of Poria terrestris. He has named each mycorrhiza according to tree and fungal species including strain of the latter as denoted by characteristic staining of respective sporocarps.

Zak (1973) laid down certain criteria for characterization and identification of mycorrhizae. He has characterized and classified ectomycorrhizae formed by *Rhizopogon vinicolor* with *Pseudotsuga menziesii* roots. He has re-examined the tuberculate mycorrhiza of Douglas fir and opined that the fungal symbiont described earlier as a phycomycete and basidiomycete by Trappe (1965), was actually *R. vinicolor*.

Ectomycorrhizae formed by *Lactarius rufus* and *Picea sitchensis*, in the field and under aseptic conditions, are described and compared (Alexander 1981). Acsai and Largent (1983) have described fifteen ectomycorrhizae for Abies concolor of which the mycobiont is known for six. They have also described thirteen ectomycorrhizae for *Pseudotsuga menziesii*, of which three mycobionts were identified. They have described, comprehensively, the methods applied for characterization and identification of ectomycorrhizae and have also summarized the available literature. They have identified the mycorrhizae of spruce with *Lactarius deterrimus*, *L. picinus*, *Russula ochroleuca,* and *R. xerampelina* by tracing hyphal connections between the fruit bodies and the mycorrhizae.

Mycorrhizosphere

Mycorrhizosphere has been described (Davey 1971) as a complex habitat characterized by the presence of many exudates of the roots and mycorrhizal fungi, sloughed root cells and lysed hyphal cells, various nutrients existing in a range of forms, valencies, and oxidation states, and chelated compounds adsorbed on cation exchange sites. Influence of ectomycorrhiza on root exudates is very important for the mineralization process in the soil. The nutrient sorption by the plants is by release of exudates in the form of low molecular weight carboxylates, which in turn mobilize the required elements and form organometallic complexes that are in a form suitable to be taken up by plants for metabolic purposes (Kavety 2007). Atmosphere in the mycorrhizosphere ranges from highly aerobic to anaerobic and contains varying but relatively high contents of CO_2 (carbon dioxide) and occasionally CO (carbon monoxide), NH_3 (ammonia), CH_4 (methane), H_2S (hydrogen sulphide), HCN (hydrogen cynide) and a range of other volatile, low molecular weight metabolites. The acidity (pH) of the mycorrhizophere varies in intensity, capacity, and physiological effects depending on its origin from H or Al ions.

Garrett (1960) has described rhizosphere as the outermost defence of the plant against attack by root pathogens, and it has been demonstrated to support a much greater population of microorganisms than

is found in non-rhizospheric soils. Tribunskaya (1955), comparing the rhizosphere of mycorrhizal and non-mycorrhizal pine seedlings, has identified a considerable increase and a change in composition of the rhizospheric microflora around the mycorrhizal rootlets. He has discovered 9 to 10 times as many fungi in the rhizosphere of the latter and a large number of bacterial saprophytes and bacteria capable of degrading organic substances. Foster and Marks (1967) in an electron microscopic examination of P. *radiata* have observed about 16 times more bacterial population in the outer layers of the mantle than that found in the outermost region of the rhizosphere.

Processes occurring in the rhizosphere undoubtedly influence rooting characteristics, mycorrhizae formation, and probably many other factors bearing directly or indirectly on tree seeding vigour. In the healthy rhizosphere, a dynamic balance exists between plant, mycorrhizal fungi, and other organisms. There interactions are, within limits, stable against external perturbation. However, disrupting the balance can change the community of rhizosphere organisms from one that is mutualistic with tree to one that is pathogenic (Finlay 1985). Rambelli, Freccero, and Fanelli (1972) in an investigation of the mycorrhizosphere of *Pinus radiata*, have studied the variation of the rhizosphereic microbial population for the whole range of seasons. They have noted an extremely irregular distribution of the microflora throughout the four seasons.

Systematics of fungal symbionts

Many species of fungi are normally involved in the ectomycorrhizal associations of a forest stand, on a single tree species, on an individual tree or even on a small segment of lateral root. Three species of fungi have been isolated from an individual ectomycorrhiza (Zak 1973). A single fungal species can enter into ectomycorrhizal association with numerous tree species on the same site. A fungus can also develop numerous biotypes or clones in a very limited area of a pure stand. Some fungi are apparently host specific; others have broad host ranges and form ectomycorrhizae with members of numerous tree genera in diverse families (Marx and Cordall 1988). All ectomycorrhizal fungi, with only a few exceptions, belong to Basidiomycetes and only some to Ascomycetes. Miller (1982) has recorded mycorrhiza-forming activity in representatives of 73 Basidiomycete genera distributed among 27 families in nine orders. Most ectomycorrhizal fungi can be accurately identified only from their macroscopic fruit bodies, which are produced during a relatively short season each year. General technical works for the identification of ectomycorrhizal fungi are those of Singer (1975).

Many species of Boletaceae have been shown to be ectomycorrhizal and most are believed to be so. Sharma and Lakhanpal (1981) have recorded 20 species of boletes, which form mycorrhizal association with forest trees like *P. wallichiana*, *Quercus* sp., *Rhododendron* sp., and *Betula* species.

Suillus is the most promising ectomycorrhizal genus in the family Boletaceae, usually associated with members of the Pinaceae. Asian *Suillus* species and North American species are confirmed to be ectomycorrhizal by synthesis experiments and European species (Treu 1987) are confirmed by direct observations of hyphal connections between basidiocarps and ectomycorrhizae.

Cotter (1987), while working on the systematics and ecology of boletes with special references to the genus *Suillus* and its ectomycorrhizal relationships, has recognized and described nine species of *Suillus* from Nepal. He has provided synoptic keys to the basidiocarps, and to the cultures of different species worked out by him. Mycorrhizal synthesis confirmed that the six *Suillus-Pinus* relationships were ectomycorrhizal.

Physiological studies on mycobiont

Peng and Chien (1988) have investigated the effects of temperature, pH, salt tolerance, and water stress on growth of *Boletus griseus* and *Suillus grevillei* by growing them in Potato Dextrose Agar and modified MNM (Melin Norkran's Medium). They have selected suitable strains and optimum growth conditions (for reproduction of ectomycorrhizal inoculums) on the basis of these studies. Chang and Chien (1988) have cultured 38 species of ectomycorrhizal fungi on MMN in a temperature range of 5 °C to 40 °C. They have concluded that optimum temperature for most of the strains ranged from 22 °C to 27 °C with the optimum at 25 °C.

In vitro synthesis of ectomycorrhiza

In vitro mycorrhizal synthesis studies help in determining the ability of a fungal isolate to form mycorrhizae and provides much needed anatomical and morphological data on selected plant fungus combinations produced under controlled conditions. Morphology and anatomy, combined with other distinctive characters, provide essential data for identifying mycobionts from field connections.

Melin (1921) has successfully demonstrated, for the first time, that ectotrophic mycorrhizae could be produced in synthetic cultures by inoculating seedlings of *Picea abies, Pinus sylvestris*, and *Larix europaea* with appropriate fungi. The most used and most successful substrate for synthesis cultures has been sand moistened with a nutrient solution.

Marx and Bryan (1970) have further improved the substrate by stabilizing the acidity with an addition of finely ground sphagnum peat moss. They have synthesized ectomycorrhizae by *Thelephora terrestris* and *P. tinctorius* on different conifer hosts. They have observed that mycorrhizae formed by *T. terrestris* were macroscopically and microscopically different from those of *P. tinctorius*, but mycorrhizae formed by different isolates of *T. terrestris* were indistinguishable from each other, regardless of host. Results suggested that the fungal symbiont determines colour and morphology of ectomycorrhizae.

Marx and Ross (1970) have synthesized ectomycorrhizae on Pinus taeda by basidiospores of *T. terrestris*. Cultures isolated from the mycorrhizae were identical with the culture used to form the basidiocarp. Molina (1979) has tested cultures of 208 ectomycorrhizal fungi in pure culture synthesis for mycorrhiza formation with red alder and found only four formed characteristic ectomycorrhizae. Fortin, Pieche, Lalonde (1980) have described synthesis of ectomycorrhiza on *P. strobes* seedlings within five days after inoculation with *P. tinctorius*. Alexander (1981) has described and compared ectomycorrhizae formed by *Lactarius rufus* and *Picea sitchensis* in the field and under aseptic conditions. Nylund and Unestam (1982) have studied the process of *in vitro* mycorrhiza formation in Norway spruce using the fungus *Piloderma croceum*.

Palm and Stewart (1984) have reported mycorrhizal synthesis between *Pinus resinosa* and *Suillus americanus*, *S. brevipes*, *S. luteus*, and *S. neoalbidipes* and between *P. strobes* and *S. americanus*, *S. brevipes*, *S. qranulatus*, *S. pictus*, and *S. punctipes*. Their study indicated that mycorrhizae formed by has all combinations were similar in that all had multiple dichotomous branches and in mantle organization. Mycorrhizae differed in the macroscopic colour of the mantle and surrounding hyphae and hyphal strands.

Warmbrodt and Eschrich (1985) have studied ectomycorrhizae of *Pinus sylvestris*, synthesized in vitro with *Suillus variegatus*. They have compared the structure of mycorrhizas produced *in vitro* with that to naturally occurring mycorrhizas on the same host species. Ceruti, Tozzi, and Reitano (1986) have worked out the mycorrhizal synthesis between *Boletus aereus* and *Castanea sativa* by inoculation of mycelium from culture, *in vitro* on seedlings in sterile vermiculite. Miller, Jenkins and Dery (1986) have achieved mycorrhizal synthesis under laboratory conditions between *Amanita muscaria*, *Pinus taeda*, and *P. virginiana*. They reisolated the fungus from the mantles and verified its identity by cultural characteristics.

Duddridge (1986a, b) has synthesized ectomycorrhiza on *Picea sitchensis*. *P. abies*, and *Pinus sylvestris* using mycelium of the fungus *Suillus grevillei*. Kannan and Natarajan (1987) have reported pure culture synthesis of *Pinus patula* ectomycorrhizae with *Scleroderma citrinum*. Theodorou and Reddell (1991) have selected 11 species of ectomycorrhizal fungi from stands of *P. radiata* and *Eucalyptus* spp. and have confirmed the ectomycorrhizal relationship by *in vitro* synthesis. Kasuya, Muchovej, Bellei, *et al.* (1992) have determined mycorrhizal formation in six species and varieties of pine with six species of ectomycorrhizal fungi, using an in vitro synthesis technique, which allowed for observation of mycorrhizal formation without disturbing the host plants.

Inoculums and inoculation techniques

Ectomycorrhizae may be initiated by several different kinds of inoculums, which can be categorized as natural inoculum in the form of airborne spores; soil already colonized by an ectomycorrhizal fungus or fungi; seedlings already colonized by an ectomycorrhizal fungus or fungi that is bearing mycorrhizal roots; fungus sporophore, spores or sclerotia specially collected for the purpose; and fungal mycelium produced in axenic cultures. Each has advantages and disadvantages in relation to the objectives and economics of the inoculation programme. If the procedures are followed properly, these methods usually produce abundant ectomycorrhizae on seedlings. Soil inocula, taken from beneath ectomycorrhizal host trees, have been used extensively, expecially in the developing countries (Mikola 1970). In bare root nurseries up to 10% by volume of soil inoculum is incorporated into the soil (top 10 cm of beds) before sowing. Parke, Lindermann, and Black (1983) have reported enhanced growth of Douglas fir container seedlings inoculated with litter and humus taken from beneath Douglas fir trees. One of the most serious disadvantages of soil inoculums is that weed seeds, rhizomes, and potential pathogens may also be transported into the nursery with the soil.

Spores or macerated fruiting bodies of some ectomycorrhizal mushrooms, puffballs, and truffles provide good inoculum. Spore inoculum is prepared by blending freshly collected fruit bodies with tap water at high speed for two to three minutes. Li and Castellano (1987) have found beneficial microorganisms within and on the surface of mature fruiting bodies of various ectomycorrhizal fungi; these organisms should be encouraged, not excluded. Spores are applied 6 to 12 weeks after sowing, either with a standard watering can or through the existing irrigation system. Spores of different fungi have been successfully used to inoculate and stimulate growth of pines in Australia and South Africa. Marx (1980)

had similar success with inoculating *P. tinctorius* onto assorted pine species in the US. Pure mycelial or vegetative inoculums of ectomycorrhizal fungi have been recommended as the most biologically sound material for inoculation. A pure culture of a particular fungus is obtained by inoculating fungal material (vegetative tissue explant) onto special media and grown under aseptic conditions to produce inoculums.

Industrial fermentation and entrapment in calcium alginate beads have been successfully employed to produce pure culture inoculums in France (Le Tacon, Garbaye, Bouchard, *et al.* 1988). In Canada, vegetative inoculum of several ectomycorrhizal fungus species have been successfully produced in industrial fermentors for operational applications in container and bare root nurseries. Mycorrhizal tablets are now commercially available in the Philippines from the National Institute of Biotechnology and Applied Microbiology (Marx, Reuhle, and Cordell 1991).

Moser (1958) in Austria was one of the first to make a serious attempt to produce vegetative inoculums of ectomycorrhizal fungi. For production of inoculum, mycelium of *Suillus plorans* was first grown in liquid culture then in sterile peat moss. Hacskaylo and Vazzo (1967) have grown *Cenococcum geophilum*, *Suillus cothuranatus*, *Corticium bicolour*, and *Rhizopogon roseolus* in polypropylene cups containing a 2:1 ratio of sterile peat moss and vermiculite moistened with nutrient solution.

Vermiculite-based inoculums have been used successfully to form *P. tinctorius* ectomycorrhizae in fumigated soil on pine, oak, and pecan seedlings in experimental microplots. In nursery tests, seedlings with abundant *P. tinctorius* ectomycorrhizae grew larger than control seedlings with naturally occurring mycorrhizae (Marx 1980). Vermiculite-based inoculum also has been used successfully in forming *P. tinctorius* ectomycorrhizae on container-grown tree seedlings in various media and container types. It was found that fertility, type of container, growing medium, fungicides, inoculum storage, and frequency of watering all influence effectiveness of the inoculums. Dramatic improvements in survival and growth of pine seedlings with abundant *P. tinctorius* ectomycorrhizae over control seedlings produced in containers or base root nurseries have been reported from studies on acid coal spoils in Appalachia (Walker, West, and Melaughlin 1980). Similar improvements in pine seedlings performance is reported on routine reforestation site (Ruehle, Marx, Barnet, *et al.* 1981). Staudenrausch, Kaldorf, Renker, *et al.* (2005) have studied diversity of ectomycorrhiza community at a uranium-mining heap. Karkouri, Martin, and Mousain (2004) have reported *Suillus collinatus* to be the most abundant ectomycorrhizal fungus in *Pinus halepensis* plantations in fire-disturbed site of Rieucoulon (France). Ugawa and Fukuda (2005) have suggested that the density of inoculum was more important than the amount of inoculum.

Selection of the mycobiont

The most important first step in any nursery inoculation programme is the selection of the mycobiont. One criterion is host specificity. Certain fungi are consistently associated with a few specific tree hosts (Trappe 1962). Many other fungi are associated with a variety of different tree hosts (Marx 1977). It is imperative, therefore, that the candidate fungi exhibits the physiological capacity to form mycorrhizae on the desired hosts. Another criterion is the ability of the selected fungi to grow rapidly in pure culture.

The candidate fungi must be an early stage fungus in normal succession if it is to be effective on seedlings in the nursery and in the early successional stage of stand establishment (Trappe 1962). There appears to be a distinct early stage and late stage fungi in ectomycorrhizal fungus succession in forests (Marx, Ruehle, and Cordell 1991). Only the early stage fungi are able to rapidly colonize seedlings in natural, non-sterile soil that harbours competitors and other environmental stresses. Upper and lower temperature limits of the candidate fungi should be determined. Moser (1958) has studied the ability of fungi to survive long periods of freezing at –12 °C, and to grow at 0 °C to 5 °C. He has found that high elevation ecotypes of Suillus variegatus survived freezing for two months. *P. tinctorius* can grow at temperatures as high as 40 °C to 42 °C and has a hyphal thermal death point of 45 °C.

Reaction of candidate fungi to soil moisture, organic matter, and pH are also important traits to consider. Bowen (1973) has shown that nutrient uptake is greater in fungi that produce hyphal strands. The production of sclerotia and hyphal strands by *P. tinctorius* and *C. geophilum* in soil enhances the ability of these fungi to survive harsh soil conditions. Therefore, hyphal strands and sclerotia production are also a favourable trend in candidate fungi. All the criteria mentioned are meaningless unless the candidate fungus is aggressive and can form abundant ectomycorrhizas on seedlings as soon as short roots are produced.

Artificial inoculation, growth, and development of seedlings

The need of many species of forest trees for ectomycorrhizal association was initially observed when attempts to establish plantations of exotic

pines routinely failed until the essential fungi were introduced. Field performance of tree seedlings is improved by forming ectomycorrhizae on them in nurseries with specific fungi ecologically adapted to the planting site. The need of pine and oak seedlings for ectomycorrhizae has also been convincingly demonstrated in the afforestation of former treeless areas such as the grasslands of Russia and Great Plains of the US (Briscoe 1959).

Large-scale inoculation experiments have been done in the US with *P. tinctorius* on different pine species including *P. taeda*, *P. virginiana*, *P. ponderosa*, *P. strobes*, and *P. resinosa*. Marx and Schenck (1983) have reviewed the potential of mycorrhizal symbiosis in agricultural and forest productivity. Inoculation of containerized seedlings with pure culture is being practised.

In a review by Cordell, Omdal, and Marx (1991), 66 species of ectomycorrhizal fungi have been used experimentally to form ectomycorrhizae on 49 tree species. Over 40% of the publications dealt with *P. tinctorius* forming ectomycorrhiza on 29 different tree species. *Cenococcum geophilum*, *Hebeloma crustuliniforme*, *Laccaria bicolor*, *L. laccata*, *Suillus granulatus*, *S. luteus*, and *T. terrestris* have been evaluated to a lesser extent on six or more tree species.

Effect of artificial inoculation on nutrient uptake of seedlings

Ectomycorrhizal fungi's beneficial effects on plant nutrition have been known. Hatch (1937) has reported that mycorrhizal eastern white pine (*P. strobus*) weighed significantly more and contained more N (nitrogen), P (phosphorus), and K (potassium) than did non-mycorrhizal plants. Ectomycorrhizae are capable of increasing P uptake, especially in low fertility soils. Recently Paul, Chapman, and Chanway (2007) have demonstrated that tuberculate ectomycorrhizae of *S. tomentosus* are sites of significant nitrogenase activity in *Pinus contorta*. It was shown in New Zealand and Australia that the stimulating effect on conifer growth depends largely on the uptake of phosphorus liberated by the mycorrhizal fungi in the soil. The role of the mycorrhizal fungi in the liberation of nutrients, from complex compounds in the forest soil, has been discussed. The inverse relationship between soil fertility and ectomycorrhizal infection has long been recognized and ectomycorrhizal infection is often reduced by application of fertilizer (Richards and Wilson 1963).

In one of the few studies on minor element uptake, excised mycorrhizal roots of *P. radiata* absorbed more zinc than non-mycorrhizal roots. Naturally occurring ectomycorrhizal fungal symbionts, in association with *P. taeda* and *P. echninata* roots, were as efficient as *P. tinctorius* in the uptake of B (boron), Cu (copper), Fe (iron), Mo (molybdenum), Mn (manganese), and Zn (zinc) from sewage sludge applied to nursery beds (Bowen, Skinner, and Bevege 1974). Mycorrhizal fungi, in association with plant roots, seem likely to increase P uptake more thorough exploration of soil volume, thereby making positionally unavailable nutrients 'available'. This is achieved by decreasing the distance for diffusion of phosphate ions and by increasing the surface area for absorption (Bowen, Skinner and Bevege 1974).

Harley (1989) has suggested that production of phosphatases by ectomycorrhizal fungi is important in the solubilization of organic phytates, which constitute a large fraction of total phosphate in humic soils. These enzymes are many times more active than those on non-mycorrhizal roots. The evidence for the induction of phophatase activity in response to the lack of inorganic phosphate by ectomycorrhizal fungi has been given (Mousin, Bousquet, and Polard 1988). Ectomycorrhizae have been shown to produce large amounts of calcium oxalate, which may be involved in the chelation of Fe and Al and, thereby release P for plant uptake (Treeby, Marschner and Romheld 1989).

In experiments using 32P it was found that the mantle of ectotrophic mycorrhizae accumulates and stores P. This accumulation remains consistent unless there is a dramatic change in the plant's P status. A phosphorus deficiency in the plant may stimulate the release by the mantle to the plant. However, whether or not an ectotrophic mycorrhiza can store other nutrients is questionable (Harley 1989).

Mejstrik (1975) has investigated the effect of mycorrhizal infection of *Pinus abies* by two *Boletus* species on the accumulation of phosphorus. Following mycorrhizal development, the increase of the dry weight of roots and shoots over controls was highly significant in all combinations. The phosphorus content in mycorrhizal seedlings was higher than in non-mycorrhizal control. Bolan (1991) has reviewed the role of mycorrhizal fungi in the uptake of phosphorus by plants. Koide (1991) has given a comprehensive review of nutrient supply, nutrient demand, and plant response to mycorrhizal infection. Rousseau, Reid, and English (1992) have studied the relationship between biomass of the mycorrhizal fungus, *P. tinctorius* and phosphorus uptake in Loblolly pine seedlings. Ineichen, Wiemken, Wiemken (1995) have reported considerable higher root biomass (+57%) at elevated CO_2 level in *P. sylvestris* colonized by *P. tinctorius*. Ray, Reddy, Lapeyrie, *et al.* (2005) have studied the effect of coal ash on the growth and metal uptake by six Ectomycorrhizal fungi and found *P. tinctorius* to be the most tolerant. The study may be helpful for the reclamation of coal ash over burdened sites.

Ectomycorrhizal research in India

Ectomycorrhizal research in India did not get under way until the early fifties. Chaudhuri, (1945) was the first to report the mycorrhizal association in *Abies spectabilis*, *Cedrus deodara*, *Picea morinda*, *Pinus roxburghii*, and *Taxus baccata*. However, the credit for firmly establishing mycorrhizal research goes to Dr Bakshi at the FRI (Forest Research Institute), who studied ectomycorrhizal association in *Abies pindrow*, *C. deodara*, *P. morinda*, and *P. roxburghii* (Bakshi 1957). The work on ectomycorrhizae in India has been reviewed by Lakhanpal (1991).

Characterization and identification

Characterization and identification of mycorrhiza has been done in a number of plants. Bakshi (1957) has studied the morphology of mycorrhiza in spruce, silver fir, sal and deodar. Subsequently Bakshi and Thapar (1960, 1966) and Mukerjee and Rehill (1962) studied the morphology and structure of ectomycorrhizae of pines. Sharma and Mishra (1982) studied ectomycorrhizal association in gymnosperms of Meghalaya. Thapar and Paliwal have (1982) investigated the status of mycorrhiza in pine nursery seedlings.

Kumar and Lakhanpal (1983) have reported monopodial type of mycorrhizal roots in spruce. Lakhanpal and Kumar (1984) have reported brown type (Type-I) and yellowish white type (Type-II) mycorrhizae in spruce; and creamish white, yellowish, white and black types of mycorrhizae in *Pinus gerardiana*. Sharma and Singh (1985) have conducted studies on the mycorrhizal association of *P. roxburghii*. Sharma and Lakhanpal (1988) have characterized and identified the mycorrhizal types in A. *pindrow*. Verma and Shukla (1989) have determined the ectomycorrhizal status of *Pinus kesiya* in the soils of Cherapunji. Thakur (1990) has conducted studies on mycorrhizae of some conifers of Himachal Pradesh. Mehrotra and Thapar (1990) have described the morphological and anatomical details of mycorrhiza in *P. kesiya*. Singh (1992) has studied the morphological and anatomical characteristics of ectomycorrhizal roots of *Cedrus deodara*. Pande, Palni, and Singh (2004) have recorded 55 species of ectomycorrhizal fungi from conifer forests of the Western Himalayas.

Studies on mycorrhizosphere

Eleven fungi and myceliua of some unidentified Basidiomycetes were isolated from mycorrhizosphere of *Picea smithiana* (Lakhanpal and Kumar 1984), frequency distribution of *Fusarium*, *Penicillium*, *Rhizopus*, and *Alternaria* was found to be higher than others. Wadhwani and Srivastava (1985) have reported rhizosphere and rhizoplane fungi of coralloid roots of *Cycas revoluta*. Sharma and Lakhanpal (1988) have isolated 21 species of fungi from the mycorrhizosphere of *A. pindrow*. Chaudhary and Lakhanpal (1988) have isolated 13 fungal species and one unidentified Basidiomycetous mycelium from the mycorrhizosphere of *Pinus gerardiana*. Thakur (1990) has isolated 22 fungi from *Abies spectabilis*, 14 fungi from *P. roxburghii*, and 27 fungi from the mycorrhizosphere of *Taxus baccata*. Natarajan, Senthilrasu, Kumaresan, *et al.* (2005) have studied diversity in Ectomycorrhizal fungi of a dipterocarp forest in the Western Ghats.

Systematics and cultural characteristics of mycobionts

Bakshi (1974) has reported the association of *Amanita verna*, *Astraeus hygrometricus*, *Cantharellus cibarius*, *Cenococcum graniforme*, *Lactarius scrobiculatus*, and *Scleroderma* spp with different species of *Pinus* and other trees. Sharma and Lakhanpal (1981) have reported 22 species of Boletaceae, found to be mycorrhizal with different trees. Natarajan and Raman (1983) have described the association of *Pinus patula* with *Scleroderma citrinum*, *Russula parazurea*, *Suillus brevipes*, *S. pallidiceps*, *S. punctatipes*, and *S. subluteus*.

Lakhanpal (1987) has recorded 72 species of fungi belonging to families Amanitaceae, Agaricaceae, Hygrophoraceae, Tricholomataceae, Russulaceae, Strophariaceae, and Paxillaceae to be mycorrhizal with different trees in north-western Himalayas. Raman and Mahadevan (1987) and Natarajan and Purushothoma (1987) have reported *Lycoperdon perlatum* as a fungal symbiont of *P. patula*. Singh and Thapar (1988) have identified 29 fungal symbionts, which were in intimate association with different plant species and presented a diagonostic key for their identification. Raman and Mahadevan (1988) have reported results on the selection of fungi for ectomycorrhizal inoculation in *P. patula*, *A. muscaria*, *L. laccata*, *L. perlatum*, and *S. citrinum* were described to be mycorrhizal with *P. patula*. Sharma and Singh (1990) have observed that *L. sanguifluus*, *S. sibricus*, *B. edulis*, and *T. terrestris* formed symbiosis with *P. roxburghii*.

Jha, Sharma and Mishra (1990) have recorded maximum colony growth in *L. laccata* at pH 5 on MNM. Collybia *radiata* grew at pH 6 and *P. tinctorius* at pH 7. Modified MNM, NM (Norkran's medium), and HM (Hagem's medium) were tested to find a suitable medium for mass multiplication of *P. tinctorius*. MNM was the best medium that promoted maximum colony diameter and dry weight of fungi (Rangarajan, Narayana, Kandaswamy, *et al.*, 1990). *L. laccata* and *A. muscaria* were grown at 20 °C, 24 °C, 28 °C, 32 °C, 36 °C, both in the light and in the dark. *Laccaria laccta* and *A. muscaria* grew well at 24 ˚C and

28 ˚C, respectively, and in total darkness (Raman and Thiagarajan 1988). Wild strains of *L. laccata* grew well at 24 °C and 30 ˚C and mutant strains grew well at 24 °C, 30 °C and 36 ˚C (Raman 1990). Reddy, Singhla, Natrajan, *et al.* (2005) have described *P. indicus*, a new species of ectomycorrhizal fungus from the Western Ghats.

In vitro synthesis and inoculation with ectomycorrhizal fungi

Not much has been done in India in this regard but whatever has been done holds a good promise for the future. Bakshi (1974) has synthesized mycorrhiza of *P. patula* with *Scleroderma geaster*. Kanan and Natarajan (1987, 1988) have synthesized ectomycorriza of *S. citrinum* and *A. muscaria* with *P. patula*. Raman (1988) has reported mass production of ectomycorrhizal spawn of *L. laccata* and *A. muscaria* in sorghum grains. Kumar (1989) and Kumar and Lakhanpal (1991) have reported results of artificial inoculation of *P. gerardiana* and *P. smithiana* seedlings with mycorrhizal associates isolated from mycorrhizoplane of natural roots. Singh (1992) has reported *in vitro* synthesis of ectomycorrhizae between *C. deodara* and *Trappinda himalayansis*. Ray and Adholeya (2008) have developed molecular markers for four heavy-metal tolerant isolates of *Laccaria fraterna* and *P. tinctorius*.

Studies on physical and chemical status

Analysis of physical and chemical status of mycorrhizal and non-mycorrhizal plants of *Picea smithiana* have been reported by Kumar and Lakhanpal (1983). The mycorrhizal plants were reported to attain better shoot-root ratio (Fresh weight and dry weight), and to exhibit higher shoot-root ratio compared to non-mycorrhizal plants. Marked differences in needle nutrient content have been observed with higher concentration of P, Ca, and Mg. In a similar study on *C. deodara*, considerable improvement in growth and development and nutrient uptake of the seedlings was observed.

Mycorrhizas as symbionts are known to play an important role in nutrient mobilization and plant growth along with coexistence of microorganisms. The ectomycorrhizal fungi supply the necessary biochemical and physiological machinery for absorption, translocation, and assimilation of major mineral ions. Approximately 5000 ectomycorrhizal fungi are reported. Present review has elaborated the diversity; structural; and functional components of ectomycorrhiza, its characterization and identification; nature of fungal symbiosis; physiological studies; in vitro studies; inoculum and inoculation techniques; selection of ectomycorrhizal symbionts; artificial inoculation into the conifer; mycorrhizosphere, and related aspects from all around the world and also added a note on ectomycorrhizal research in India.

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Arbuscular mycorrhizal fungi associated with *Carica papaya* L. during reproductive stages in agro-based ecosystem

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Introduction

Carica papaya L. is valued for its rich nutrient content. The ripe fruits of papaya are used for table purpose and raw fruits are cooked as vegetable. Immature fruits of papaya are used for the extraction of proteolytic enzyme papin, which is mainly used as a drug in digestive disorders and in treating ulcers and diphtheria. Papaya fruit is known for its medicinal use. It improves digestion and is said to cure chronic constipation, enlarged liver, and spleen. It also has varied industrial uses viz., tanning industry, degumming of silk, and so on (Reddy 2000). It is cultivated on a commercial scale in Karnataka, Bihar, Gujarat, Kerla, Tamil Nadu, Andhra Pradesh, Maharashtra, and Madhya Pradesh. Thus, papaya which remained as a backyard crop, hitherto, has become an important commercial crop over the years. This is precisely because of the increased demand from papin industry for tutti frutti making and for small sized fruits in big cities (Reddy 2000).

In State of Goa, large-scale cultivation of papaya is seen only in government agricultural farms and/with few progressive farmers. In Goa, papaya cultivation is mostly confined to kitchen garden and along the bunds due to constraints in productivity resulting from fungal and viral diseases. Therefore, at present, most of the demand for papaya is met by importing the fruit from the neighbouring states.

Arbuscular mycorrhizal (AM) fungi play an important role in this context since, the plants colonized by AM fungi are better able to obtain their nourishment in the soil and resist environmental stresses which gives fungal symbionts a bio-fertilizing and crop protection role. In agriculture, the increased uptake of soil minerals by colonized plants means that it is possible to consider substantially, reducing applications of fertilizers and pesticides and at the same time obtain equivalent or even higher crop yields (Abbott and Robson 1991 a, b). Through appropriate management of arbuscular mycorrhizae in agriculture it is also possible to maintain soil quality and sustainability while protecting the environment over the long term and reducing the costs of production.

Mostly, diversity studies of AM fungi in field soils have been studied based on spore populations. However, observation of spore communities alone may not provide adequate information about AM fungal community structure because of the difference in growth and sporulation time among the species. Studies on AM status during reproductive stages of *Carica papaya* L. have been studied in Goa, India by Khade (2003). In the present paper, AM fungi commonly associated with the two reproductive stages viz., flowering stage and fruiting stage of *Carica papaya* L. are studied.

Materials and methods

Study site

Papaya plantations in agricultural farm located at Old Goa (North Goa) were selected for the study. The soil at this site was well drained, dark grey brown to dark brown gravelly sandy loam to sandy clay loam with medium water-holding capacity. The study was conducted from July 2001 to September 2001.

Four dioecious varieties viz., CO-1, CO-2, Honey Dew, and Washington were planted in monoculture. It was observed that *Carica papaya* L. produces numerous feeder roots on the surface in response to soil moisture. So, the collections were carried out at the depth of 0–25 cm throughout the study period.

Collection of samples

Generally, papaya seedlings are raised in the nursery for two months and then they are transplanted in the field. Two stages of development viz., flowering-I (first week of July), flowering-II (last week of July), fruiting–I (mid August), and fruiting –II (mid-September) were considered for the study with the time of sampling given in parenthesis.

During each stage two healthy plants per variety were randomly selected for the collection of rhizosphere soil and root samples. For each plant three random soil cores were collected from within 60 cm of each plant, each at the depth of 0–25cm. Sub-samples collected from both plants were then combined to make composite sample after thorough mixing. From each composite sample, five sub-samples were made for quantification of spore density.

Quantification of spore density of AM fungi

Spores and sporocarps of AM fungi were isolated by wet sieving and decanting method (Gerdemann and Nicolson 1963) and quantification of spore density of

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AM fungi was carried out using the method described by using Gaur and Adholeya (1994).

Identification of AM fungi

Diagnostic slides containing intact and crushed spores and sporocarps of AM fungi were prepared in PVLG (polyvinyl alcohol lactoglycerol). Spore morphology and wall characteristics were considered for the identification of AM fungi, and these characteristics were ascertained using compound microscope, Leica WILD MP3, and Nikon E 800.

Arbuscular mycorrhizal fungi were identified to species level using bibliographies provided by Almeida and Schenck (1990), Morton and Benny (1990), Schenck and Perez, (1990, Bentivenga and Morton (1995), Walker and Vestberg (1998), Redecker *et al.*, (2000). Taxonomic identification of spores was also carried out by matching the descriptions provided by international collection of vesicular arbuscular mycorrhizal fungi (http:// invam.caf.wvu.edu).

Frequency of occurrence-

Frequency of occurrence of AM fungi was calculated using the following formula (Beena *et al.* 2000).

$$\text{Frequency (\%)} = \frac{\text{Number of soil samples that possess spores of particular species}}{\text{Total number of soil samples analyzed}} \times 100$$

Relative abundance

Relative abundance of AM fungi was calculated using the following formula (Beena *et al.* 2000).

$$\text{Relative abundance (\%)} = \frac{\text{Number of AM fungal spores of particular species}}{\text{Total number of spores of all species}} \times 100$$

Results

In the present study, flowering stage-1 exhibits onset of flowering (Plate 1A), flowering stage-2 exhibits late flowering stage (Plate 1B), while fruiting stage-1 represents onset of fruiting (Plate 1C), and fruiting stage-2 represents late fruit bearing stage (Plate 1D) in female *Carica papaya* L plants. In the present study, six species of AM fungi (Plate 2), viz., *Acaulospora spinosa* Walker and Trappe (Plat 2 a,b), *Acaulospora myriocarpa* Spain, Sieverding & Schenck, *Gigaspora margarita* Becker and Hall, *Glomus claroideum* Schenck and Smith emend. Walker and Vestberg, *Glomus coremioides* (Berk. and Broome) Redecker, Morton and Bruns (Plate 2 c,d) and *Dentiscutata reticulata* (Koske, Miller and Walker) Sieverding, De Souza and Oehl were commonly occurring during two reproductive stages of *Carica papaya* L. The frequency of occurrence of these AM fungi is depicted in Figures 1 and 2, and their relative abundance is depicted in Figures 3 and 4.

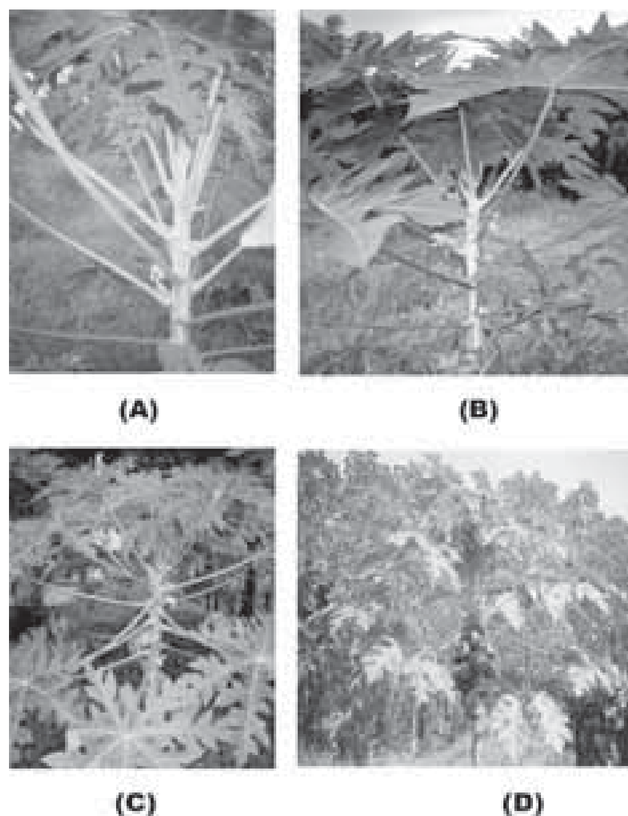

Plate 1 A) On set of flowering stage (Flowering stage-1) in female
B) Late flowering stage (Flowering stage-2) in males
C) Onset of fruiting stage (Fruiting stage-1) in female
D) Late fruiting stage (Fruiting stage-2) in female

In general, during flowering stage-1, the frequency of occurrence of AM fungi was ranging from 16. 5% (*Gi. margarita*) to 87.5 % (*A. myriocarpa* and *D. reticulata*) [Figure 1 A] with species names given in parenthesis. In flowering stage-2, the frequency was ranging from 12.5% (*G. coremioides* and *Gi. margarita*) to 87.5% (*D. reticulata*) [Figure 1B] with species names given in parenthesis. In fruiting stage-1, the frequency was ranging from 12.5% (*G. coremioides* and *Gi. margarita*) to 87.5% (*D. reticulata*) [Figure 2 A] with species names given in parenthesis. In fruiting stage-2, the frequency was ranging from 12.5% (*G. coremioides*) to 100 % (*D. reticulata*) [Figure 2 B] with species names given in parenthesis.

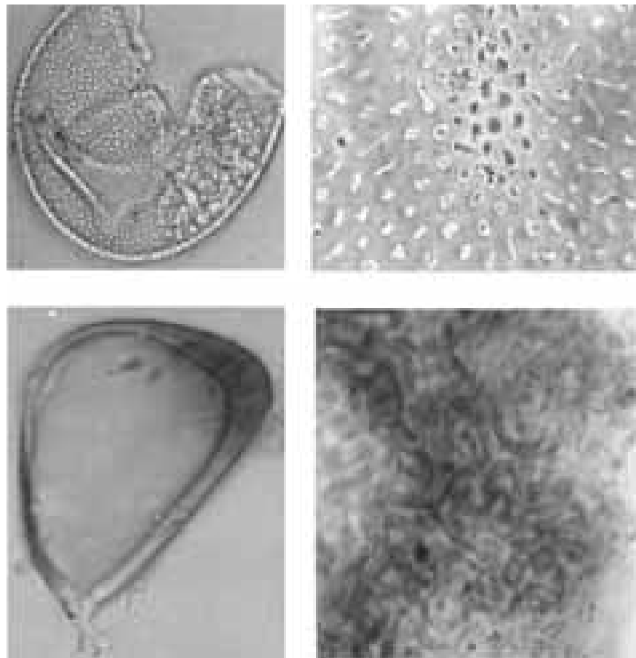

Plate 2 A) Crushed spore of *Acaulospora spinosa* (×400)
B) A surface view of spore wall of *Acaulospora spinosa* showing the spines (×1000)
C) A single spore of *Glomus sinuosum* (×400)
D) A surface view of sinuous hyphae comprising the peridium (×1000)

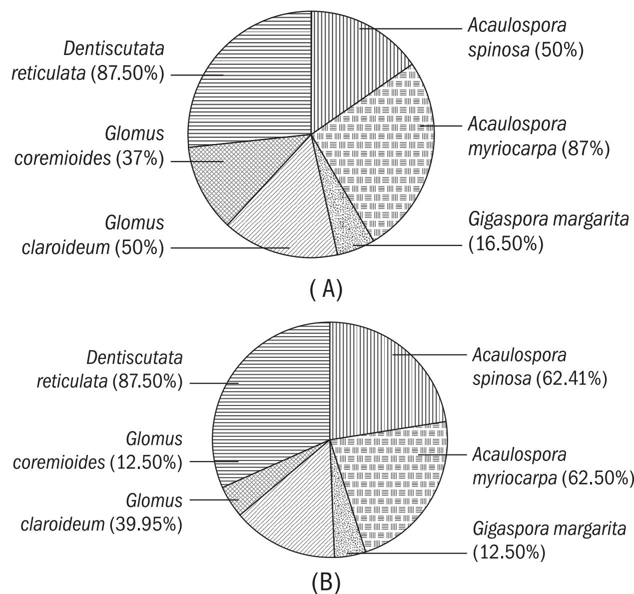

Figure 1 Frequency of occurrence of AM Fungi during (A) flowering stage 1 (B) flowering stage 2

Further, during flowering stage-1, the relative abundance of AM fungi was ranging from 3.59% (*G. coremioides*) to 30.93 % (*A. myriocarpa*) [Figure 3A] with species names given in parenthesis. In flowering stage-2, the relative abundance of AM fungi was ranging from 0.4% (*G. coremioides*) to 23.77% (*A. myriocarpa*) [Figure 3B] with species names given in parenthesis. In fruiting stage-1, the relative abundance of AM fungi was ranging from 0.6% (*G. coremioides*) to 33.53% (*A. spinosa*) [Figure 4 A] with species names given in parenthesis. In fruiting stage-2, the relative abundance of AM fungi was ranging from

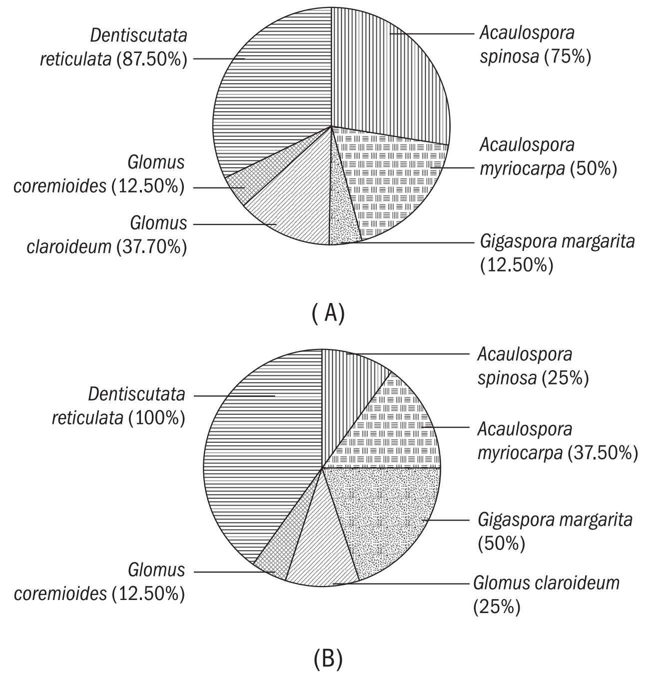

Figure 2 Frequency of occurrence of AM Fungi during (A) Fruiting stage 1 and (B) Fruiting stage 2

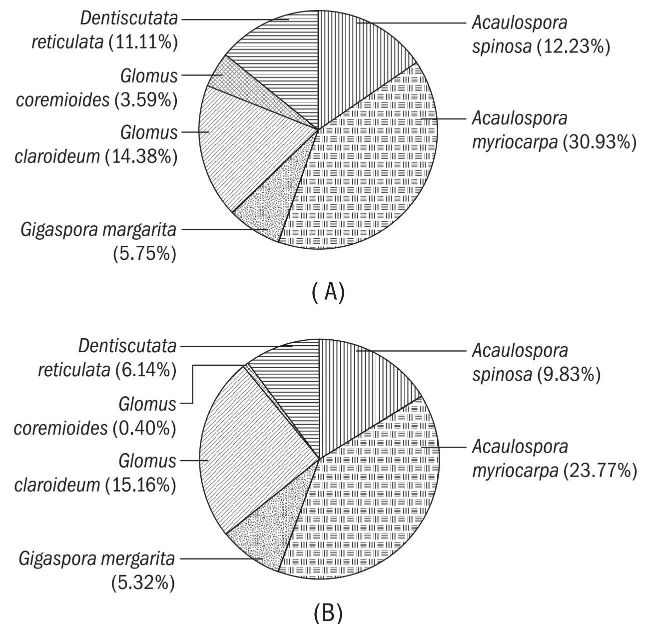

Figure 3 Relative abundance of AM fungi during (A) flowering stage 1 (B) flowering stage 2

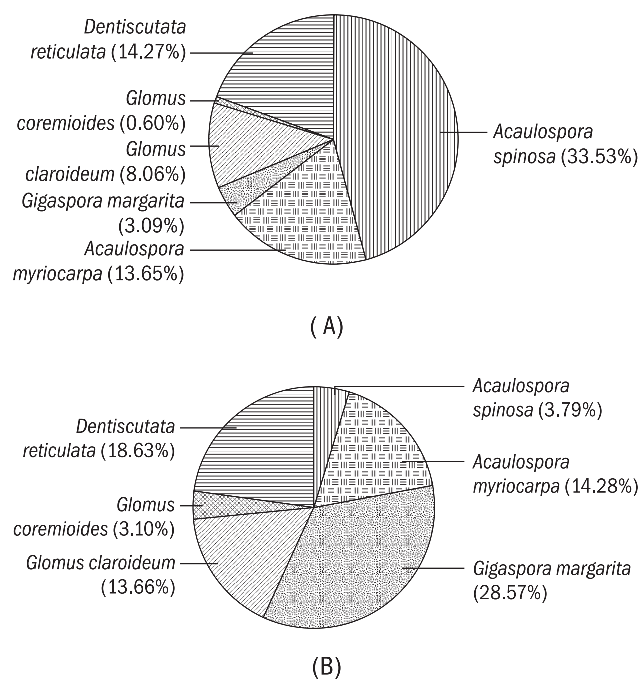

Figure 4 Relative abundance of AM fungi during (A) fruiting stage 1 and (B) fruiting satge 2

3.7% (*A. spinosa*) to 28.57 % (*Gi. margarita*) [Figure 4 B] with species names given in parenthesis.

Furthermore, the highest -frequency of occurrence for *A. myriocarpa* and *A. spinosa*, were 87% and 75%, respectively. The highest frequency for occurrence for *Gi. margarita* during the period of study was 50%. The highest frequency of occurrence for *G. claroideum* and *G. coremioides*, were 50% and 37%, respectively. While the highest frequency for occurrence for *D. reticulata* during the period of study was 100% [Figure 5]. Similarly, maximum relative abundance for *A. myriocarpa* and *A. spinosa* were 30.93% and 33.5% respectively. The maximum relative abundance for *Gi. margarita* during the period of study was 28.57%. The maximum relative abundance for *G. claroideum* and *G. coremioides*, were

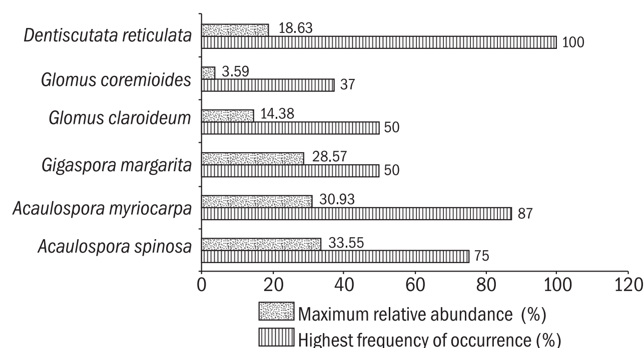

Figure 5 Highest frequency of occurrence and maximum relative abundance of AM during reproductive stages of *Carica papaya* L.

14.38% and 3.59%, respectively. While the maximum relative abundance for *D. reticulata* during the study period was 18.63% [Figure 5].

Discussion and conclusion

Two of the six species of AM fungi recorded the highest frequency of occurrence and relative abundance for the same stage of growth viz. , *A. myriocarpa* in flowering stage-1 and *D. reticulata* in fruiting stage-2. The frequency of occurrence and relative abundance were not correlated in the present study. Similarly, Stürmer and Bellei (1993) have reported an absence of correlation between the frequency of occurrence and relative abundance of AM fungi in dune soils on the island of Santa Catarina, Brazil. They attributed it to the non-uniform spatial distribution of spores in soil. However, the sample collection procedures in the study were modified to avoid non-normal distribution of spores. Therefore, the non-uniform distribution of spores in the present study may be due to various factors, the most obvious being the threshold of mycorrhizal biomass needed to induce spore production (Stürmer and Bellei 1993).

In the present study, *D. reticulata* (87%–100%) was frequently occurring during the flowering and fruiting stages while *A. myriocarpa* was most abundant during flowering stages and *A. spinosa* was abundant during fruiting stage-1 and during fruiting stage -2, *Gi. margarita* was most abundant. No trend was evident in the frequency of occurrence and relative abundance of AMF between flowering and fruiting stages. Further, the time and interval of sampling did not influence the distribution of AM fungi in the present study. In conclusion, the AM fungal species were randomly distributed during the reproductive stages of *Carica papaya* L.

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Influence of *Glomus fasciculatum* and bioformulations on shelf life, quality, and yield of *papaya cv. Red Lady*

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Introduction

Papaya (*Carica papaya* Linn.) is an important fruit crop of the tropics, and since a long time it is considered to be a wonder fruit. It gives higher production of fruits per hectare and generates income next only to the income generated by banana production. Papaya, being a climacteric and highly perishable fruit, the main problem lies in its post-harvest handling and marketing, which needs to be standardized in order to generate useful information on extending the shelf life of the fruit. Over ripen fruits become unfit for consumption and are also not be fit for transportation to distant markets. It seems necessary to retard the ripening processes of fruits that can help in sending the fruits to distant markets. Hence, the present study was planned to find out the effect of AM (arbuscular mycorrhizal) fungi and bioformulations on shelf life, fruit quality, and yield.

Material and methods

The present investigation was carried out at the Department of Pomology, Kittur Rani Channamma College of Horticulture, Arabhavi, during 2004/05. The experiment was laid out in split plot with AMF and without AMF as the main treatments and bioformulations and their combinations as seven sub-treatments (panchagavya, amrit pani, microbial consortia, panchagavya + amrit pani, panchagavya +

microbial consortia, amrit pani + microbial consortia, and RDF (recommended dose of fertilizer) in each replication was allotted randomly. The experiment was replicated thrice. The mycorrhizal inoculation was done to papaya seeds by placing AM fungi inoculum uniformly— 5 grams per bag at two centimetres depth comprising of 19 chlamydospores per gram of the soil.

The bioformulations, that is, panchagavya, amrit pani, and microbial consortium were used at the rate of 3% concentration in the form of soil application except for panchagavya, which was applied both in the form of soil application and as a spray. The bioformulations and combination of these bioformulations were applied at monthly intervals to the respective plots as per treatments. Chemical fertilizer was added to the RDF treatment at the rate of 250: 250: 500 g NPK per plant per year at split doses (Anonymous 2004).

The quality parameters, such as TSS (total soluble solids), reducing sugars, non-reducing sugars, total sugars, physiological loss in weight, shelf life, and yield characters were recorded from each plot and mean values were used for statistical analysis. The data was subjected to Fisher's method of analysis of variance and interpretation of data was done as given by Panse and Sukhatme (1967).

Results and discussion

The inoculation of AM fungi had significantly increased the quality parameters, viz., TSS, reducing sugars, total sugars, shelf life, and yield as compared to uninoculated plants (Table 1). Nine days after harvest, maximum PLW (physiological loss in weight) was recorded in uninoculated plants, wherein minimum PLW was recorded in *Glomus fasciculatum* inoculated plants (Table 2). Plants supplied with panchagavya and amrit pani recorded higher TSS, maximum reducing sugars, higher total sugars and yield, and maximum shelf life followed by the treatment panchagavya + microbial

Table 1 Influence of *Glomus fasciculatum* and bioformulations on quality parameters of papaya

Treatment	*Total soluble solids* (%)		*Reducing sugars* (%)		*Non-reducing sugars* (%)		*Total sugars* (%)	
M_1S_1	11.67		10.76		1.10		11.86	
M_1S_2	11.47		11.07		0.82		11.89	
M_1S_3	11.60		10.94		0.99		11.92	
M_1S_4	12.67		11.20		1.50		12.70	
M_1S_5	12.13		11.02		1.44		12.46	
M_1S_6	11.07		11.00		1.02		12.02	
M_1S_7	11.00		10.40		1.17		11.57	
Mean (M_1)	11.66		10.91		1.15		12.06	
M_2S_1	11.00		10.54		0.97		11.51	
M_2S_2	10.46		10.13		1.24		11.37	
M_2S_3	10.47		10.35		1.13		11.48	
M_2S_4	11.67		10.91		0.87		11.78	
M_2S_5	11.20		10.30		1.02		11.32	
M_2S_6	11.07		10.35		0.93		11.28	
M_2S_7	10.53		9.40		1.62		11.02	
Mean (M_2)	10.91		10.28		1.11		11.39	
S_1	11.33		10.65		1.04		11.69	
S_2	10.98		10.60		1.03		11.63	
S_3	11.03		10.65		1.06		11.70	
S_4	12.17		11.06		1.19		12.24	
S_5	11.67		10.66		1.23		11.89	
S_6	11.07		10.68		0.98		11.65	
S_7	10.77		9.90		1.40		11.30	
For comparing the means of	S.Em±	C.D.at 5%	S.Em±	C.D. at 5%	S.Em±	C.D. at 5%	S.Em±	C.D.at 5%
Main (M)	0.023	0.142	0.044	0.269	0.018	NS	0.100	0.606
Sub (S)	0.108	0.317	0.056	0.163	0.064	0.186	0.091	0.265
S at same M	0.153	0.448	0.079	0.231	0.090	0.263	0.128	NS
M at same S	0.144	0.420	0.085	0.249	0.085	0.249	0.155	NS

M_1 – With *Glomus fasciculatum*; M_2 – Without *Glomus fasciculatum*; S_1 – Panchagavya; S_2 – Amrit Pani; S_3 – Microbial consortia; S_4 – Panchagavya + Amrit Pani; S_5 – Panchagavya + microbial consortia; S_6 – Amrit Pani + microbial consortia; S_7 – RDF; DAT – Days after transplanting; NS – Non-significant

consortia as compared to uninoculated plants. In the interaction effects, *Glomus fasciculatum* inoculated plants registered significantly higher TSS, among sub-treatments, panchagavya + amrit pani followed by panchagavya + microbial consortia registered maximum reducing sugars, minimum PLW, and higher yield as compared to other treatments (Table 3).

The beneficial effects of AM fungi on papaya were evident from the present investigation, where all the quality parameters and yield exhibited significant improvement in the AM fungi inoculated plants (Tables 1 and 2). Similar increase in yield of papaya with inoculation of AM fungi had been reported by Pandey and Mishra (1975), Shrinivas (1988) and Manjunath, Patil, Swamy, *et al.* (2002). However, there are no reports available on the additive effects of bioformulations on the VA mycorrhizal papaya that could influence qualitative and reproductive parameters. Increased yield, as observed in the present investigation with the inoculation of *Glomus fasciculatum* and use of bioformulations, might be attributed to the formation of higher sink capacity by retention of more carbohydrates and also the translocation of carbohydrates from other parts to reproductive parts during development. The increase in quality parameters might be attributed to the increased biochemical parameters over uninoculated control plants.

In the present investigation it was observed that the fruits obtained due to inoculation of *Glomus fasciculatum*, along with application of bioformulations panchagavya + amrit pani had least PLW, followed by the treatment amrit pani. Shelf life extension was highest in the treatment panchagavya + amrit pani followed by panchagavya alone, indicating their effectiveness in controlling weight loss, which might be attributed to the reduced rate of respiration and transpiration from fruit surfaces as indicated by Rao and Chundawat (1991). The decrease in the respiration could be further attributed to lowering of succinate and malate dehydrogenase activities associated with the TCA (tricarboxylic acid) cycle (Mehta, Raj, and Raju *et al.* 1986). Thus, in the present investigation, the presence of some unidentified metabolic components like GA3 in panchagavya, amrit pani, and microbial consortia might had acted as ripening retardants leading to the reduced respiration, transpiration, and weight loss with extended shelf life. Rao and Chundawat (1991), who have worked on banana, have also confirmed that GA3 treatment extended the shelf life with delayed ripening, transpiration, and respiration.

Table 2 Influence of *Glomus fasciculatum* and bioformulations on yield and yield parameters of papaya

Treatment	*Yield* (kg/plant)		*Yield* (t/ha)	
M_1S_1	43.43		134.70	
M_1S_2	42.51		131.11	
M_1S_3	36.04		111.04	
M_1S_4	64.47		198.95	
M_1S_5	51.16		155.29	
M_1S_6	47.63		144.92	
M_1S_7	34.02		104.99	
Mean (M_1)	45.61		140.14	
M_2S_1	23.25		71.75	
M_2S_2	22.72		69.47	
M_2S_3	20.97		64.71	
M_2S_4	31.12		96.31	
M_2S_5	27.74		85.61	
M_2S_6	23.24		71.72	
M_2S_7	18.71		57.74	
Mean (M_2)	23.97		73.90	
S_1	33.34		103.23	
S_2	32.62		100.29	
S_3	28.51		87.88	
S_4	47.80		147.63	
S_5	39.45		120.45	
S_6	35.44		108.32	
S_7	26.36		81.36	
For comparing the means of	S.Em±	C.D.at 5%	S.Em±	C.D. at 5%
Main (M)	0.191	1.163	0.179	1.088
Sub (S)	0.336	0.979	0.266	0.776
S at same M	0.475	1.385	0.376	1.098
M at same S	0.479	1.398	0.392	1.143

M_1 – With *Glomus fasciculatum*; M_2 – Without *Glomus fasciculatum*; S_1 – Panchagavya; S_2 – Amrit Pani; S_3 – Microbial consortia; S4 – Panchagavya + Amrit Pani; S_5 – Panchagavya + microbial consortia; S_6 – Amrit Pani + microbial consortia; S_7 – RDF; DAT – Days after transplanting

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Table 3 Influence of inoculation of and application of bioformulations on keeping quality of papaya fruits

Treatment	*Physiological loss in weight* (%)								*Shelf life* (days)	
	3 days after harvest		*6 days after harvest*		*9 days after harvest*		*12 days after harvest*			
M_1S_1	10.10		16.72		22.25		28.62		8.33	
M_1S_2	8.62		14.80		18.00		21.92		8.00	
M_1S_3	12.20		16.66		21.07		27.08		7.00	
M_1S_4	7.34		11.33		15.00		20.16		8.67	
M_1S_5	9.22		13.25		17.62		23.33		8.00	
M_1S_6	7.94		14.10		18.72		24.66		7.67	
M_1S_7	8.90		17.27		22.30		27.72		7.00	
Mean (M_1)	9.19		14.88		19.28		24.78		7.81	
M_2S_1	7.20		15.27		21.66		26.14		6.33	
M_2S_2	7.80		14.34		18.28		22.02		6.33	
M_2S_3	10.92		16.10		23.80		28.18		6.00	
M_2S_4	10.40		16.53		22.65		26.33		6.67	
M_2S_5	12.72		16.03		20.00		23.76		6.33	
M_2S_6	9.62		15.90		21.50		26.17		6.00	
M_2S_7	9.33		16.25		24.33		28.82		6.00	
Mean (M_2)	9.71		15.78		21.75		25.92		6.24	
S_1	8.65		16.00		21.96		27.38		7.33	
S_2	8.21		14.57		18.14		21.97		7.17	
S_3	11.56		16.38		22.44		27.63		6.50	
S_4	8.87		13.93		18.83		23.25		7.67	
S_5	10.97		14.64		18.81		23.55		7.17	
S_6	8.78		15.00		20.11		25.42		6.83	
S	9.12		16.76		23.32		28.27		6.50	
For comparing the means of	S.Em±	C.D. at 5%	S.Em±	C.D. at 5%	S.Em±	C.D. at 5%	S.Em±	C.D. at 5%	S.Em±	C.D. at 5%
Main (M)	0.348	NS	0.186	NS	0.263	1.598	0.268	NS	0.233	1.419
Sub (S)	0.656	1.915	0.343	1.000	0.371	1.083	0.433	1.264	0.281	0.819
S at same M	0.928	2.709	0.485	1.415	0.525	1.532	0.612	1.787	0.397	NS
M at same S	0.927	2.706	0.486	1.418	0.952	1.612	0.627	1.830	0.435	NS

M_1 – With *Glomus fasciculatum*; M_2 – Without *Glomus fasciculatum*; S_1 – Panchagavya; S_2 – Amrit Pani; S_3 – Microbial consortia; S_4 – Panchagavya + Amrit Pani; S_5 – Panchagavya + microbial consortia; S_6 – Amrit Pani + microbial consortia; S_7 – RDF; NS – Non-significant

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Growth and alkaloid yield of *Coleus forskohlii* with the inoculation of arbuscular mycorrhiza fungi and plant growth promoting rhizobacteria

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Introduction

AM (arbuscular mycorrhizae) are symbiotic associations, formed between plants and soil fungi that play an essential role in plant growth, plant protection, and soil quality. There are reports providing evidence that infection with mycorrhizal fungi facilitates better nutrient uptake (Adivappar 2001) and the PGPR (plant growth promoting rhizobacteria) like *Pseudomonas* sp. appear to be better options enhancing the plant growth. Hence, to exploit these biological tools, a pot experiment was carried out and the response on growth and alkaloid yield of *Coleus forskohlii* was studied.

Materials and methods

A pot experiment was carried out in the Department of Agricultural Microbiology, TNAU (Tamil Nadu Agricultural University) during 2006/07 to study the effect of AM inoculation (*Scutellospora*) with two PGPR isolates (*Pseudomonas* sp.) on *Coleus forskohlii*. Pots of 30 x 28 cm size were filled with 10 kg soil (pH-8.2, EC-0.89 dSm-1, available N-219 Kg/ha, P_2O_5-14.3 Kg/ha, K_2O-293 Kg/ha) and the AM (CAM 3 isolate, isolated and purified from soils of *Coleus forskohlii*, Salem District, Tamil Nadu) inoculation was done at 50 g per pot (containing 250–300 spores/100g inoculum) at 2.5–5 cm depth and added with 50 ml (liquid culture [containing 10^8 cfu/ml]) of *Pseudomonas* isolates per pot as per the treatment in the planting hole. Local variety of *Coleus forskohlii* was planted at the rate of two cuttings per pot. Following treatments were replicated thrice in a completely randomized design.

Treatments

1 *Scutellospora* sp. CAM 3
2 *Pseudomonas fluorescens* (PFC 6)
3 *Pseudomonas fluorescens* (CPf 1)
4 CAM 3 + PFC 6
5 CAM 3 + CPf 1
6 Uninoculated control

Results and discussion

Plant samples were taken at 90, 120, and 150 DAP (days after planting) for estimating growth and yield parameters as well as AM root colonization. On perusal of the results, it was evident that AM and PGPR inoculation exhibited significant increase in growth of *Coleus forskohlii*. Among the treatments, CAM 3 + PFC 6 combination was superior, which influenced the plant height, number of leaves, and dry matter production. It recorded the maximum shoot length of 35.8 cm, the root length of 56.0 cm per plant with 50, and a 286.2% increase over control respectively at 150 DAP (Table 1). Similarly, this combination registered 49.5% and 17.6% increase in the number of leaves produced and girth of the stem (Table 2) (Plate 1). The results were supported by the work of Boby and Bagyaraj (2003), who have found the enhanced growth of *Coleus forskohlii* with the dual inoculation of AM(arbuscular mycorrhiza)

Table 1 Effect of combined inoculation of AM fungus and PGPR organisms on growth of *Coleus forskohlii*

No.	Treatments	*Shoot length* (cm plant^{-1}) DAP 90	120	150	Mean	*Per cent increase over control*	*Root length* (cm plant^{-1}) DAP 90	120	150	Mean	*Per cent increase over control*
1.	*Scutelliospora* spp. CAM 3	25.50	31.20	34.20	30.3	18.8	8.00	15.00	28.50	17.1	80.0
2.	*Pseudomonas fluorescens* PFC 6	29.70	30.00	35.40	31.7	24.3	6.50	14.50	36.10	19.0	111.1
3.	*Pseudomonas fluorescens* CPf 1	29.00	29.40	35.00	31.1	21.9	8.00	15.20	30.00	17.7	86.3
4.	CAM 3 + PFC 6	29.80	32.50	45.30	35.8	40.4	12.40	18.00	56.00	28.8	203.1
5.	CAM 3 + CPf 1	30.50	31.50	43.70	35.2	38.0	8.50	13.80	46.80	23.0	142.1
6.	Uninoculated Control	21.40	25.00	30.20	25.5	–	5.05	9.00	14.50	9.5	–
	S Ed	0.92	0.99	1.24	–	–	0.27	0.46	1.32	–	–
	CD (0.05)	1.97	2.12	2.67	–	–	0.59	1.00	2.82	–	–

DAP- Days after planting

Table 2 Effect of combined inoculation of AM fungus and PGPR organisms on number of leaves and stem girth in *Coleus forskohlii*

		No. of leaves (per plant)					*Stem girth* (cm)				
		DAP				*Per cent increase*	*DAP*				*Per cent increase*
No.	*Treatments*	*90*	*120*	*150*	*Mean*	*over control*	*90*	*120*	*150*	*Mean*	*over control*
1.	*Scutelliospora* spp.CAM 3	45	98	155	99.3	49.0	3.0	3.4	3.5	3.3	13.8
2.	*Pseudomonas fluorescens* PFC 6	74	95	120	96.3	44.6	2.9	3.0	3.8	3.2	10.3
3.	*Pseudomonas fluorescens* CPf 1	52	100	148	100.0	50.1	2.8	3.1	4.2	3.3	13.8
4.	CAM 3 + PFC 6	78	108	142	109.3	64.1	3.2	3.3	4.0	3.5	20.6
5.	CAM 3 + CPf 1	65	102	152	106.3	59.6	3.4	3.4	5.1	3.9	34.5
6.	Uninoculated Control	40	65	95	66.6	–	2.5	3.0	3.4	2.9	–
	S Ed	1.94	3.25	4.76	–	–	0.09	0.05	0.13	–	–
	CD (0.05)	4.15	6.97	10.21	–	–	0.21	0.11	0.28	–	–

DAP – Days after planting

T_1 – *Scutelliospora* spp. CAM3; T_2 – *P. 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
Plate 1 Effect of AM fungi and PCPR organisms on growth of *Coleus forskohlii*

fungi and PGPR organisms. Enhanced dry matter production was observed with individual as well as combined inoculation treatments and the combination of AM + PFC 6 registered 27.5%–50.3% increase over individual inoculation of AM and PFC 6 over the observations made from 90–150 DAP (Table 3). Increased dry matter production with AM inoculation was observed in grapevine (Bavaresco, Cantu, and Trevisan 2000) and cassava (Howler, Cadavid, and Burckhard 1982), which was 15–30 fold higher than control.

AM root colonization was found to be 60%–80% in *Scutellospora* sp. CAM 3 inoculated plants and the spore load was 17–200 spores/50 g soil. In *Pseudomonas* inoculated plants AM colonization, as well as spore load, was observed to be high, which showed the stimulatory effect of *Pseudomonas* isolates. In combined inoculation, much increase in spore numbers (210–330 spores) as well as root colonization was noticed (80%–93%) than single inoculations of AM/PGPR isolates (Table 4). PGPR can influence the growth of hyphae from germinating arbuscular mycorrhizal spores (Barea, Andrade, Bianciotto, *et al.* 1998), colonization of plant roots by AM fungi, and growth of external hyphae. It was also hypothesized that *Pseudomonas* (MHB [mycorrrhiza helper bacterium]) could soften the cell wall and the middle lamella between the cells of the root cortex by producing enzymes, and thus making fungal penetration easier (Duponnois 1992). These results tend to indicate some sort of tropic stimulation of the fungal growth by the bacteria in the main mechanism involved. Maximum spore count of 330 spores 50 g^{-1} soil, observed with dual inoculation, highlighted the existence of synergistic interaction in the rhizosphere. It was explained that the fungus could be suffering

Table 3 Effect of combined inoculation of AM fungus and PGPR organisms on dry matter production in *Coleus forskohlii*

No.	Treatments	*Dry matter production* (g plant $^{-1}$) *DAP* 90	120	150	*Mean*	*Per cent increase over control*
1.	*Scutelliospora* spp. CAM 3	8.28	9.48	27.64	15.1	38.8
2.	*Pseudomonas fluorescens* PFC 6	9.27	10.73	32.85	17.8	63.3
3.	*Pseudomonas fluorescens* CPf 1	8.68	9.40	19.83	12.6	15.5
4.	CAM 3 + PFC 6	10.33	11.61	46.28	22.7	104.5
5.	CAM 3 + CPf 1	10.07	11.14	42.25	21.2	94.5
6.	Uninoculated Control	6.62	8.26	18.05	10.9	–
	S Ed	0.30	0.36	1.07	–	–
	CD (0.05)	0.65	0.77	2.03	–	–

DAP– Days after planting

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

No.	Treatments	*AM colonization in roots* (%) *DAP* 90	120	150	*Mean*	*Spore count in soil* (No / 50 g soil) *DAP* 90	120	150	*Mean*
1.	*Scutelliospora* spp. CAM 3	60	80	75	71.6	170	180	200	183.3
2.	*Pseudomonas fluorescens* PFC 6	40	45	52	45.6	110	127	160	132.3
3.	*Pseudomonas fluorescens* CPf 1	45	50	53	49.3	90	120	155	121.6
4.	CAM 3 + PFC 6	90	90	93	91.0	200	175	210	195.0
5.	CAM 3 + CPf 1	70	83	80	77.6	250	300	330	293.3
6.	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	–

DAP– Days after planting

from auto inhibition by producing some fungistatic compounds, which could be metabolized by the accompanying microbes and this allows the growth of mycelium and spore production (Azcon-Aguilar and Bago, 1994).

Nutrient uptake

With reference to the uptake of nutrients, AM inoculation had exhibited significant influence on uptake of N (nitrogen); P (phosphorous); and K (potassium), and it was further improved by the coinoculation of *Pseudomonas*. Combinations of *Scutellospora* sp. CAM 3+ *Pseudomonas* PFC 6 registered 7–8 fold increase in N uptake, 1.5 fold increase in P uptake, and 2–6 fold increase in K uptake of Coleus forskohlii over a period of 90–150 DAP (Table 5). Mycorrhizal fungi may affect mineral nutrition of the host plant either directly or indirectly. In many cases AM association usually enhanced the growth of plants solely by enhancing the uptake of nutrients. The increase may be attributed to the contribution of hyphal transport of N in the form of NO_3 or NH_4 (Taylor, Remy, Hass, *et al.*, 1995), P_2O_5 (Bolan, 1991), and K_2O. These results suggests two scenarios; one may be due to the increased absorption through extramatical hyphae of AM fungi and the second may be the enhanced growth of AM roots facilitated in the absorption of more nutrients from soil than non-AM roots.

Tuber and alkaloid yield

Not only the growth but also a significant enhancement in tuber yield was resulted with the inoculation of AM fungi (by 68.5 % over control) and the tuber yield was doubled by the combined inoculations (Table 6) (Plate 2). Improvement in the alkaloid forskohlin content (50%) was observed by AM inoculation over control and further a two-fold increase was observed when AM was inoculated with *Pseudomonas* (CPf 1) (Figure 1). This may be explained by the stimulation of specific metabolic pathways as reported in wheat (Walter, Fester, and Sracl 2000) by the combined inoculations.

Table 5 Effect of combined inoculation of AM fungus and PGPR organisms on uptake of nutrients in *Coleus forskohlii*

No.	*Treatments*	*Nutrient uptake* (mg plant -1) *N*	*Per cent increase over control*	P_2O_5	*Per cent increase over control*	K_2O	*Per cent increase over control*
1.	*Scutelliospora* spp. CAM 3	737.0	497	116	81.2	610.0	496.8
2.	*Pseudomonas fluorescens* PFC 6	481.0	289	98	53.0	399.0	290.4
3.	*Pseudomonas fluorescens* CPf 1	529.0	329	70.0	9.4	438.0	328.6
5.	CAM 3 + PFC 6	891.0	622	164.0	156.2	738.0	622.1
6.	CAM 3 + CPf 1	876.0	610	118.0	84.4	725.0	609.4
7.	Uninoculated Control	123.4	–	64.0	–	102.2	–
	S Ed	14.3	–	1.90	–	11.8	–
	CD (0.05)	30.7	–	4.08	–	25.5	–

DAP – Days after planting

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

No.	*Treatments*	*Tuber yield (150 DAP)* *Fresh weight* (g plant -1)	*Per cent increase over control*	*Dry weight* (g plant -1)	*Per cent increase over control*
1.	*Scutelliospora* spp. CAM 3	4.21	41.3	0.59	68.5
2.	*Pseudomonas fluorescens* PFC 6	3.68	23.4	0.45	28.5
3.	*Pseudomonas fluorescens* CPf 1	3.08	3.3	0.39	11.4
4.	CAM 3 + PFC 6	6.36	113.4	0.72	105.7
5.	CAM 3 + CPf 1	5.18	73.8	0.73	108.5
6.	Uninoculated Control	2.98	–	0.35	–
	S Ed	0.14	–	0.02	–
	CD (0.05)	0.31	–	0.04	–

DAP – Days after planting

T_5-*Scutelliospora* spp. CAM 3+ PFC 6

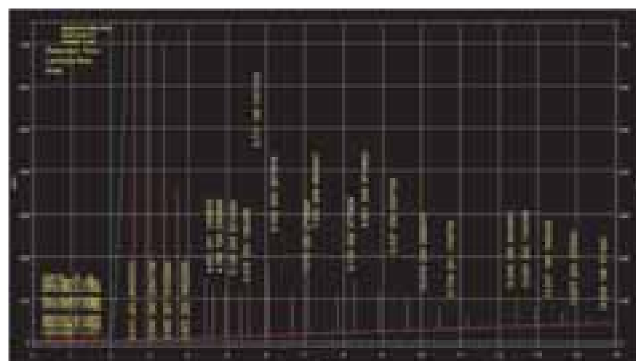
T_6-*Scutelliospora* spp. CAM3 + CPf 1

Plate 2 Tuber formation in *Coleus forskohlii* under combined inoculations

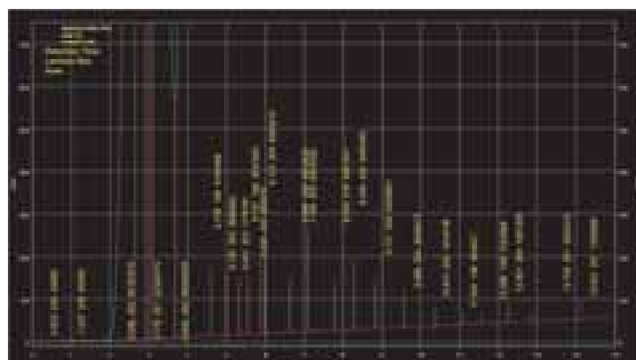
Conclusions

Inoculation of native strain of AM fungus (*Scutellospora* sp. CAM 3) with *Pseudomonas* sp. had tremendous response in the medicinal herb *Coleus forskohlii* by the way of augmenting plant growth, nutrient status, as well as by enhancing the tuber yields and the yield of the medicinal principle, the alkaloid forskohlin. *Scutellospora*, being compatible

HPLC GRAPH - FORSKOHLIN – STANDARD



HPLC GRAPH content of forskohlin due to combined inoculation of CAM 3 + PFC 6 in *Coleus forskohlii*



HPLC GRAPH content of forskohlin due to combined inoculation of CAM 3 + CPf 1 in *Coleus forskohlii*

Figure1 Effect of combined inoculation of AM fungus and PGPR organisms on forskohlin content of in *Coleus forskohlii*

with *Pseudomonas* isolates, combined inoculations with both the isolates performed better than individual inoculations and these combinations may be the right choice for the improvement of *Coleus forskohlii*.

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Exploring possibilities of partial drought mitigation in upland rice (*Oryza sativa* L.) through enhancing native arbuscular mycorrhizal association

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Introduction

Drought tolerance in mycorrhizal plants, through inducing favorable alterations in water relations and associated parameters, have been reported (Smith 1988). The present investigation explored the possibility of partial drought mitigation through enhancing AM (arbuscular mycorrhizal) association in upland rice, a direct sown crop grown under rainfed ecosystem and aerobic soil conditions favourable for AM activities.

Materials and methods

Native AMF inoculum preparation

Two types of AM (arbuscular mycorrhizal) fungi inoculum, that is, PI (pot inoculum) and MI (mass inoculum) were prepared by multiplying the NI (nucleus inoculum) under sterilized, controlled conditions, and partially sterilized, natural conditions, respectively. Two types of inoculum were applied in the field at different doses to simulate differential inoculum load (AM fungi) under field conditions.

(a) NI: Chlamydospores of native AMF consortium, predominated by *Glomus* and *Acaulospora* (Maiti, Variar, and Saha 1995) were isolated following wet sieving-decanting method (Gerdmann 1955). NI was prepared by multiplying the spores on roots of Sorghum plant grown from surface sterilized seeds in sterilized 'soil : sand : FYM' mixture (1 : 1 : 1; v/v/v; SSF substrate) in plastic pots under glass house conditions (day temperature maintained up-to 35 +/–50 °C) for 30 days with regular watering.

(b) PI: PI was prepared by multiplying NI for 30 days in bigger pots under similar conditions like that of NI.

(c) MI: MI was prepared by multiplying NI on Sorghum roots, grown in partially sterilized soils of micro-plots (4×4 m^2) in field for 30 days (Maiti and Barnwal 2003). The soils of micro-plots were partially sterilized by soil solarization (Katan, Greenberger, Alon, *et al.* 1976) using transparent, thin (1–2 mm), LDPE (low density poly-ethylene) film as mulching over ploughed, levelled, and moist soil for 30 days during peak summer months (April-May) of this region.

Field experiment

Three doses (1.25, 2.5 and 5.0 t/ha) of PI and two doses (2.5 and 5.0 t/ha) of MI were compared with untreated control in three replications under field condition during the wet season of 2000 using rice variety 'Vandana' (an improved upland rice variety of 90–95 days duration). Normal agronomic practices were followed for growing the crop. The inoculum was applied to soil just prior to seed sowing (direct sowing in lines). The higher doses (2.5 and 5.0 t/ha) were broadcasted followed by mixing in soil and the lower doses were band placed just beneath the seeds for application convenience. The crop was fertilized with 40:20:20 kg $N:P_2O_5:K_2O$ in the forms of urea, SSP (single super phosphate), and MOP (muriate of potash), respectively. One fourth dose of N and full doses of P_2O_5 and K_2O were applied as basal and rest of the N was top dressed in two equal splits at 'maximum tillering' and 'panicle initiation' stages, respectively.

Based on the results of WS, 2000, the experiment was repeated during WS, 2001, with only MI of narrower dose ranges (0.75, 1.00, 1.25 and 1.50 t/ha) for identifying optimum dose more precisely.

The physiological parameters of leaf, that is (1) relative water content (RWC %), (2) transpiration rate (ug/cm/sec.), (3) diffusive resistance (s/cm), and (4) temperature (°C) were monitored during natural drought spells resulting into apparent drought symptoms (leaf rolling). RWC (relative water content) was recorded following the standard procedures (Kramer 1969) and for other parameters a 'Steady State Porometer' (Make- LI-COR, USA; model-LI 1600) was used. The extent of drought effects (wilting score) was recorded using modified 'Standard Evaluation System' (IRRI 1996) on a 0–7 scale. Based on this, the symptomatic leaf rolling severity (0= no leaf rolling, 1= <2% leaves rolled, 2= 2-5% leaves rolled, 3= 6-25% leaves rolled, 4= 26-50% leaves rolled, 5= 51-75% leaves rolled, 6= 100% leaves rolled, 7= 100% leaves rolled tightly) was recorded.

Rice roots were sampled at 60 DAE (days after emergence) from up to 20 cm depth following the

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standard procedure. Sampled roots were cut into pieces of about 1 cm length and fixed in Formalin 5 ml: acetic acid 5 ml: 70% alcohol 90 ml (formalin: acetic acid: alcohol) solution for 48 hours. The fixed roots were cleared using 10% KOH solution and stained by Trypan blue following the method of Kormanik, Bryan, Schultz, *et al*. (1980). The stained roots were observed under stereo-zoom microscope (STEMI 2000C; Carl Zeiss) and root length colonization (% RLC) by native AM fungi were assessed using systematic gridline-intersect method (Giovennetti and Mosse, 1980).

Results and discussion

In the wet season of 2000, one major drought spell of nine non-rainy days coinciding with late PI (panicle initiation) stage (56 to 65 days after emergence; DAE) of the crop occurred during the cropping period. In WS, 2001, however, the crop was exposed to two consecutive drought spells (spaced by only one rainy day) of five and seven non-rainy days coinciding respectively with milk (72 to 76 DAE) and dough (77 to 83 DAE) stages.

All the treatments with inoculum resulted in higher AMF colonization in rice roots in both the years. The lowest inoculum dose (1.25 t/ha) applied as band placement beneath the seeds, however, led to highest colonization over that of higher doses (2.5 and 5.0 t/ha) applied as soil mixing during 2000 (WS). This was because band placement of lowest dose provided comparatively higher concentration/load of IP of AM fungi in the rice rhizosphere than that of higher doses applied as broadcasting followed by soil mixing. Higher AM fungi colonization in rice roots maintained elevated leaf RWC (%) over that of treatments with lower colonization in both the years during peak drought spells as function of non-rainy days. This also resulted in concomitant higher levels of leaf transpiration rate and lower levels diffusive resistance of indicating lesser drought effects. As a result, associated leaf temperature was also reduced with lower wilting scores. The observations evidenced that an higher AM fungi colonization (above 30% RLC) induced by application of soil-root based native AM fungi (consortium) inoculum could help rice plants to withstand drought spells up to 7–9 non-rainy days (leading to soil moisture depletion up to as low as 10.9 to 11.5% and 14.2 to 15.4% respectively at 5–10 and 10–20 cm soil depth) during reproductive phases. Such effects could be mediated through greater exploration of soil moisture from outside moisture depletion zone around the root surface through extramatrical mycelial network of the associated AM fungi, extended beyond root network, leading to more efficient extraction of water from soil profile (Smith 1988). The exploratory observations, thus, confirmed the possibilities of partial drought mitigation through inducing higher native arbuscular mycorrhizal association in upland rice.

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Effect of bioinoculants and stone mulch on performance of biodiesel and medicinal plant species on limestone mined spoil

Anuj Kumar Singh and Jamaluddin#*

Introduction

The limestone mined spoils also reduce the level of infectivity of AM fungi due to soil disturbance while AMF have been advocated for successful rehabilitation of different mine spoils. Mulching has also been reported to be helpful for establishment of plants on mined overburdens. Mulches are known for reducing water loss from the soil and may be helpful in maintaining moisture regime in the rhizosphere of the plant (Sarles and Emanuel 1977, Morse 1979).

The present investigation was carried out to study the role of mixed culture of AMF and PF (*Pseudomonas fluorescens*) bacteria in combination with stone mulch on performance of plant species – including *Ailanthus excelsa*, *Jatropha curcas*, and *Withania somnifera* – which were planted in limestone mined spoil. The fragments of waste limestone rocks were used as stone mulch.

Materials and methods

Six month old seedlings of each plant species were planted after onset of monsoon in July. All the microbial cultures used in this study were isolated from limestone mined spoils. Microbes including different species of arbuscular mycorrhizal fungi and a bacterial species that is, PF (*pseudomonas fluorescens*) were multiplied in bulk using suitable host and growth medium respectively. The broth culture of PF was diluted four times and inoculated in the seedlings @ 30ml per seedlings. At the same time AMF mixed culture containing *Glomus mosseae*, *Glomus intraradices*, *Glomus deserticola*, *Gigaspora rosea*, *Gigaspora margarita*, *Acaulospora scrobiculata*, and *Acaulspora denticulata* was placed in the rhizosphere at 50 g soil inocula per seedling. The planting pits were immediately refilled with excavated soil. After inoculation of plants with above bio-inoculants, stone mulch treatment was given. The un-inoculated plants without mulch were maintained as control. Growth parameters – like plant height and collar diameter – were recorded after one year of inoculation, while development of AM (arbuscular mycorrhiza) in planted species were observed at intervals of six months to one and a half year. The periodical status of development of AM was studied by observing the root colonization and AM spore population. Isolation of AM spores was made by following wet sieving and decanting technique of Gerdemann and Nicolson (1963) and extraction of viable spores were worked out by sucrose centrifugation method of Daniel and Skipper (1982). Method provided by Phillips and Hayman (1970) was followed for the study of root colonization.

Results and discussion

The data generated by this study reveals that the *Withania somnifera* attained the maximum height (53.30) out of three study plants. The height of W. somnifera increased significantly at 5% probability as compared to control (35.16 cm). *Jatropha curcas* showed 41.46 cm height with mixed AMF + PF + stone mulch and 31.53 cm in control plants. *Jatropha curcas* also increased significantly over control. *Ailanthus excelsa* also followed the same trend and attained the plant height of 29.93 cm with AM fungi + PF + stone mulch, while it remained only 21.55 cm in control plants. *Ailanthus excelsa* also showed significant increase in height over control. Collar diameter (Table1b) also shows a clear difference between treated and control plants. All the plant species gave higher diameter in comparison to control.

Withania somnifera exhibited 4.80 cm collar diameter with mixed AMF + FP + stone mulch and 2.93 cm in control plants. Increase in collar diameter was significant over controls. *Jatropha curcas* gained collar diameter of 2.42 cm in treated plants while it remained only 1.70 cm in controls. The increase in collar diameter was highly significant at 5% probability in comparison with controls.

Ailanthus excelsa also showed a significant increase in collar diameter in treated plants. It gained collar diameter of 2.13 cm in treated plants whereas limited collar diameter (1.27 cm) was recorded in control (untreated) plants. The increase in collar diameter was highly significant at 5% level of probability. Similar results were noticed by Jasper and Abbott (1989) that AMF inoculation enhanced the growth of *Acacia* spp. in mined over burden dumps. Kamal Prasad (2006) has reported the positive synergistic effect of

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Table 1a Effect of mixed AMF and PF in combination with stone mulch at a height of different tree species after one year of plantation

S. No.	Plant species	Plant height (in cm)		CD	SE (±)
		Control	AMF+ PF + stone mulch		
1.	*Ailanthus excelsa*	21.55	29.93	6.406	2.8417
2.	*Jatropha curcas*	31.53	41.46	6.9390	3.3153
3.	*Withania somnifera*	35.16	53.30	7.2884	3.3982

Table 1b Effect of mixed AMF and PF in combination with stone mulch on collar diameter of different tree species after one year of plantation

S. No.	Plant species	Collar diameter (cm)		CD (0.05)	SE (±)
		Control	AMF + PF + stone mulch		
1.	*Ailanthus excelsa*	1.27	2.13	1.6928	0.7483
2.	*Jatropha curcas*	1.70	2.42	0.32887	0.15713
3.	*Withania somnifera*	2.93	4.80	0.9561	0.44579

VAM (vesicular abscrular mycorrhiza) and phosphate solubilizing bacterium on growth of *Azadirachta indica* in nursery soil. Chandra and Jamaluddin (2004) have found enhanced growth of *Albizia procera* in coal mine spoil when inoculated with AMF and amended with husk mulch. Panwar and Bhardwaj (2000) have reported increased height of *Leucaena leucocephala* and *Acacia* spp. planted in sandstone mine spoil when amended with FYM (farm yard manure) as mulch.

Effect of mulching, in enhancing the plant growth, may be attributed to better moisture status and reduction of thermal stress there by increasing the availability of nutrients and water in root zone (Singh, Manjhe, Behari 1994) and development of AMF in roots. Similar results were noticed by Abbott and Robson (1982) and Menge (1984) that inoculation of AMF with mulches increased root length of plants. This might be due to better moisture status of soil under mulches.

Development of arbuscular mycorrhiza in host plants

The planted species, viz. *Ailanthus excelsa*, *Jatropha curcas* and *Withania somnifera*, were inoculated with AMF consortium containing different species of *Glomus, Acaulospora*, *Gigaspora*, and *Scutellospora*. The number of spores and percentage of infection was considerably increased in inoculated plants. There was accelerated multiplication of AM spores and increment in root colonization in plants inoculated with AMF and PF bacteria and amended with stone mulch. There was an increase in AM spores population and percentage of root colonization with the increase in age of plantation of each species under study. There was a significant increase in spore density in the rhizosphere of *Ailanthus excelsa* and *Jatropha curcas*. However, *Withania somnifera* did not show significant increase in spore density.

Increase in per cent root colonization in *A. excelsa* was highly significant while in the case of *J. curcas* and *W. somnifera* it was comparatively less. Spore population exhibited significant difference in sporulation rate, which was successively enhanced. However, it varied species to species. This variation in AM development might be due to primary succession of species and due to other ecological and edaphic factors, which govern the distribution of mycorrhizal fungi and their association with plants. Similar results were also noticed by Howler,

Table 2 Development of arbuscular mycorrhiza in different plant species

S.No.	Plant species	Development of AMF			CD (0.05)	SE(±)
		After ½ year	After 1 year	After 1½ years		
1.	*Ailanthus excesla*					
	AMF spores/100g soil	65.33a	140.00b	170.7c	25.775	9.2836
	Root colonization (%)	31.67a	42.00 b	48.33b	7.9613	2.8674
2.	*Jatropha curcas*					
	AMF spores /100g soil	97.00a	151.3b	189.00c	32.095	11.560
	Root colonization (%)	44.33a	53.33 a	53.33a	14.218	5.1208
3.	*Withania somnifera*					
	AMF spores/100g soil	96.00ab	143.00ba	171.3cb	57.638	20.760
	Root colonization (%)	43.33a	51.67a	54.00a	12.278	4.4222

Same alphabet followed by same row is statistically not different from each other at 5% rejection level

Sieverding and Saif (1987), Katiyar, Das, Choudhury (1995), and Chandra (1996) in different plant species. The survival of inoculated AMF species (Dodd and Thompson 1994) and time of sporulation (Sieverding 1991) might have influenced the spore density in the rhizosphere. The successive increase in root colonization and AM spore density exhibited the survival and establishment of inoculated AM species with the host plant species under study. The increased sporulation and root colonization was observed with the age of plantation in the rhizosphere of each planted species. The rate of sporulation and root colonization were at a very slow rate in the un-inoculated plants.

This study concludes that the consortium of different species of AM fungi and PF bacteria when added with stone mulch, proved effective for the growth and establishment of plants on calcareous mined spoil. The plant species like *Jatropha curcas* and *Ailanthus excelsa*, thus may be utilized for restoration of limestone mined overburden spoils. *Withania somnifera*, which is a very important medicinal herb may also be successfully grown in mined out areas.

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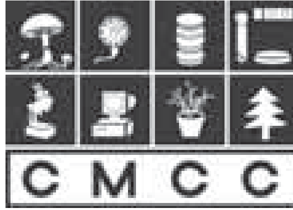

Centre for Mycorrhizal Culture Collection

Ectomycorrhizas: extending the capabilities of Chromium-nanoparticles biosynthesis

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Introduction

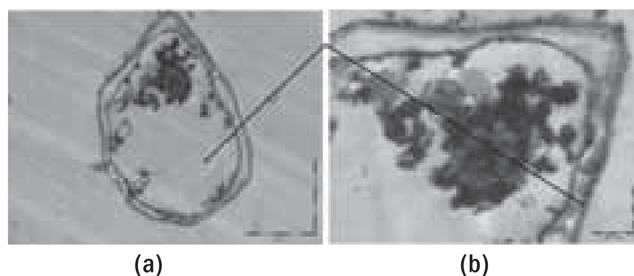
The development of reliable, eco-friendly process for the synthesis of nanoparticles is an important aspect of nanotechnology today. The biological synthesis of nanomaterials, using microorganism, is a very interesting and exigent area of nanotechnology. It is widely accepted that specific strains of microorganisms that can tolerate heavy metal stress may be potential biofactories for the synthesis of metal nanoparticles. Microorganisms – such as bacteria, yeast, and fungi – play an important role in remediation of toxic metals through reduction of metal ions (Fortin and Beveridge,2000; Mukherjee et al 2001). It is well known that some microbes – such as bacteria (Beveridge and Murray 1980, Golab 1981, Brierley 1990), yeast (Huang *et al.* 1990), fungi (Frilis and Myers Keithi 1986, Volesky 1990, Niu *et al.* 1993), and algae (Darnall *et al.* 1986) – are able to adsorb and accumulate metal and can be used in the reduction of environmental pollution and also for the recovery of metals from waste. One approach that shows immense potential is based on the biosynthesis of nanoparticles using microorganisms such as ECM (Ectomycorrhiza) fungi. Our work concentrated on the use of ECM fungi in the biosynthesis of metal nanoparticles. The ECM isolates were taken from CMCC (Centre for Mycorrhizal Culture Collection, TERI, New Delhi).

Material and methods

Twenty ECM fungi were selected for synthesis of chromium nanoparticles and maintained on MMN (Modified Melin Norkrans) medium slants at 25 °C. Stock cultures were maintained by sub culturing at monthly intervals. Twenty ECM were grown in 200 ml MMN broth containing (g/l) malt extract 3.0, D-glucose 10, KH2PO4 0.5, (NH4)2HPO4 0.25, MgSO4.7H20 0.15, CaCl2.2H2O 0.05, FeCl3 0.025, thiamine 100μg and pH-5.5-6.0 in 500 ml conical flask was adjusted and kept on shaker (150 rpm)at 25 oC for 15–18 days. After full growth the biomass was harvested by filtering through Whatman filter paper no. 1 and then extensively washed with distilled water to remove any medium component. Harvested ECM biomass was transferred to 250 ml conical flask containing 100 ml 1 mm Cr2SO4 salt solution and flask kept on shaker (150 rpm) at 25 oC and biomass was allowed to interact for 72 hours for synthesis of chromium nanoparticle. Biomass was analysed for nanoparticles using TEM (Transmission Electron Microscopy) and EDX (Energy Dispersive X-ray) study.

Result and discussion

Twenty ECM isolates were analysed for the Cr nanoparticle using the TEM and EDX. Among 20 isolates one isolate, E1 (*Paxillus invlutus*), showed positive result for nanoparticle synthesis. Metal accumulation as nanoparticle was confirmed through TEM and EDX. This confirmed the metal presence in the cell. Fungi synthesized the nanoparticle intracellularly in cytoplasm and cell wall, possibly due to reduction of the metal ions by enzymes present in the cell wall and on cytoplasmic membrane. TEM picture was recorded from the thin section of *Paxillus invlutus* mycelium with Cu-grid for the chromium nanoparticles. At higher magnification the morphology and size of the particles can be clearly visualized (Figure a and b). It can be seen from TEM micrograph that the distribution of chromium nanoparticles are polydispersed, and their size and morphology is highly variable. Optical microscopy analysis of many such TEM images gave an average size of dimension of 5–50 mm. Further evidence for the intracellular formation of Cr-nanoparticles is provided by EDX analysis (Fig C) of the *Paxillus invlutus* biomass. The two prominent peak are Cu, which is used for the preparation of grid to support the specimen. In this, chromium presence was conformed with less intensity peak. Few successful studies (Fu *et al.* 1999, 2000; Li *et al.* 1990; and Ahmad *et al.* 2003) demonstrate that some microbes are potentially useful in the preparation of metal nanoparticles under normal air pressure and at room temperature.



(a) (b)

Figure a and b TEM micrographs recorded from thin section of *Paxillus invlutus* fungus cells after reaction with Cr ions for 72 hours at different magnification

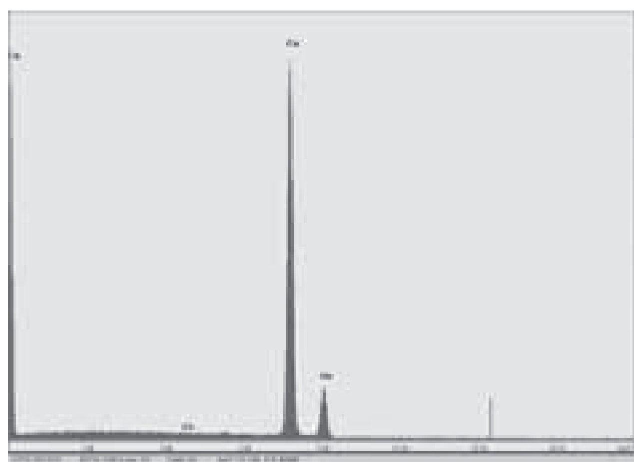


Figure C Energy Dispersive X- Ray Analysis spectrum of *Paxillus invlutus* fungi for chromium nanoparticle

Conclusion

The intracellular biosynthesis of Cr-nanoparticles of various morphology and sizes in ECM culture like *Paxillus invlutus* has showed positive results. A biological process with the ability to strictly control the shape and quantity of the particles produced would, therefore, be an exciting prospect. However, the cellular mechanism leading to the biosynthesis of chromium nanaoparticles is not yet fully understood. Further research will, therefore, focus on the development of fundamental mechanism at a cellular and molecular level, including compounds and enzyme responsible for the reduction of Cr-ions.

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The latest additions to the network's database on mycorrhiza are published here for the members' information. The list consists of papers from the following journals.

- *Acta Edulis Fungi*
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- *Botany-Botanique*
- *Guizhou Agricultural Sciences*
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- *Mycorrhiza*
- *Mycoscience*
- *Mycotaxon*
- *Pedobiologia*
- *Photosynthetica*
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- *Scientia Horticulturae*
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