MYCORRHIZA NEWS
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About TERI

TERI (The Energy and Resources Institute) is a not-for-profit research organization engaged in research on various aspects of energy, environment, and biotechnology, and on documentation and information dissemination. The genesis of these activities lie in TERI's firm belief that the efficient utilization of energy, sustainable use of natural resources, large-scale adoption of renewable energy technologies, and reduction of all forms of waste would move the process of development towards the goal of sustainability.

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Mycorrhiza Network was set up at TERI in 1988 with the objective to strengthen research, encourage cooperation, promote exchange of information and germplasm, and facilitate transfer of technology to the field through the establishment of a mycorrhiza research network. Within the Network, a Mycorrhiza Information Centre (MIC) has been set up with support from the Department of Biotechnology, Government of India, with an objective to function as a specialized centre for collection, compilation, and dissemination of information and resources on mycorrhiza; promote networking and resource sharing.

Mycorrhiza News

The *Mycorrhiza News*, a quarterly publication since 1988, provides a forum for dissemination, acquisition, interaction and communication of scientific information on mycorrhizal research activities. It publishes papers/research findings from eminent scientists covering biology, ecology, and other related aspects of mycorrhiza, including biodiversity and conservation of mycorrhizae. Among other components encompassing the newsletter include: notes on important breakthroughs; brief accounts of new approaches and techniques; research activities highlighting the Centre for Mycorrhiza Culture Collection, forthcoming events on Mycorrhiza and related events; important references of research papers published in different national and international journals.

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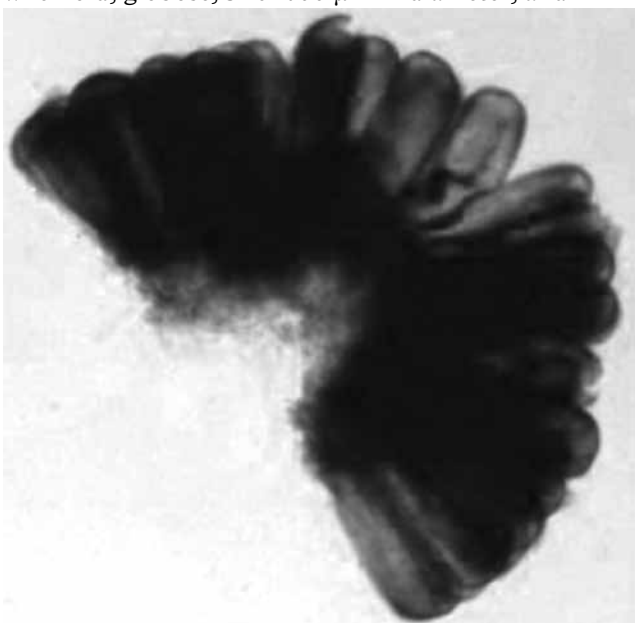
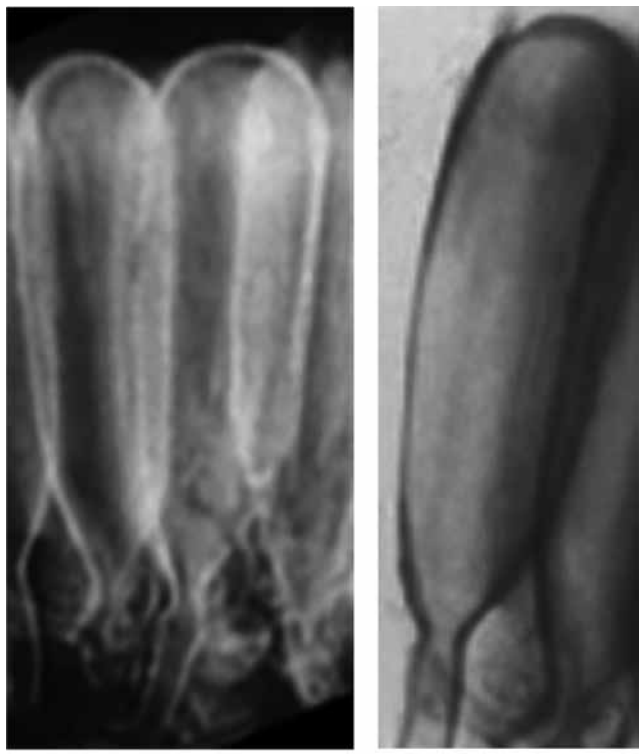
Mycologia **82**: 703–714, 1990
= *Sclerocystis pakistanica* Iqbal and Bushra *Transactions of Mycological Society Japan* **21**: 57–63, 1980 (Plate 19)

Sporocarps are yellow when young, and dark brown when old, globose, 520–600 µm in diameter, and consist of chlamydospores radially arranged on a central plexus of hyphae (Plate 19a). Peridium tightly



(a)

Plate 19(a) A hemispheric sporocarp of *Glomus pakistanica* with the central plexus of interwoven hyphae (× 150)



(b) **(c)**

Plate 19(b) Clyindro-clavate to clavate and elongated chlamydospores of *Glomus pakistanica* without thickened apex (× 400)

Plate 19(c) A single chlamydospore of *Glomus pakistanica* (× 1000)

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enclosing sporocarp and consists of thick-walled interwoven hyphae. Chlamydospores are brown, clyindro-clavate to clavate, elongated, 85–200 × 32–55 µm in diameter, with a short hyphal stalk (Plates 19b and 19c). The chlamydospore wall is pale brown to dark brown and 2 µm thick. The spore is without a thickened apex (Plates 19b and 19c) but generally thickest at the base.

Distinguishing feature: Chlamydospores are clyindro-clavate to clavate and elongated, without a thickened apex.

***Distribution*:** Recorded from Bicholim in July with 33.33% frequency of occurrence.

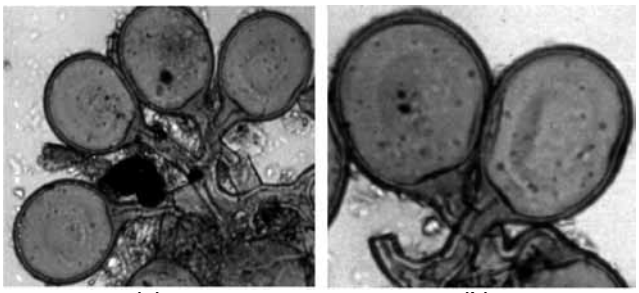
***Association*:** Found in association with *Carica papaya* L. plants from plateau region of Goa, India.

20. *Glomus rubiforme* (Gerdemann and Trappe), Almeida and Schenck,

Mycologia **82**: 703–714, 1990 (Plate 20)

Sporocarps brown to dark brown, subglobose to ellipsoid, 150–180 × 310–450 µm in diameter, and consist of a single layer of chlamydospore surrounding the central plexus of hyphae (Plate 20a). The peridium is nearly absent. The individual spores at times are partially enclosed in a thin network of tightly appressed hyphae. Chlamydospores are brown to dark brown, globose to obviod, 37–60 × 40–80 µm in diameter (Plate 20b), with small pores opening into thick-walled subtending hypha. The spore wall is laminated (3–8 µm, up to 15 µm thick) at the base of the spore (Plates 20a and 20b). The chlamydospore is often perforated on the inner surface. The attached hypha is 10–30 µm in diameter. The wall of attached hypha extending down is usually thicker than the spore wall (Plates 20a and 20b).

Distinguishing feature: Globose to obviod, non-septate chlamydospores with wall of attached hypha usually thicker than the spore wall.



(a) (b)

Plate 20(a) Crushed sporocarp of *Glomus rubiforme* (× 300)
Plate 20(b) Chlamydospores of *Glomus rubiforme* (× 400)

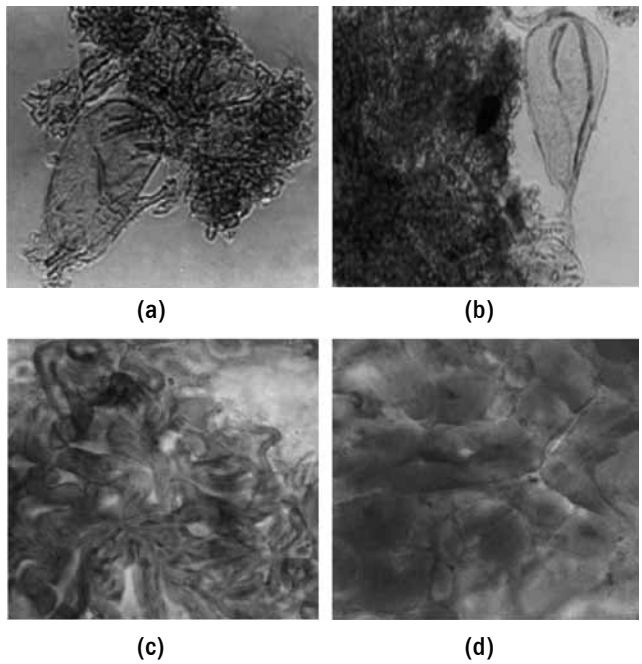
***Distribution*:** Recorded from Valpoi in December with 12.5% frequency of occurrence.

***Association*:** Found in association with *C. papaya* plants from Western Ghats of Goa, India.

21. *Glomus sinuosum* (Gerdemann and Bakshi) Almeida and Schenck

Mycologia **82**: 703–714, 1990 (Plate 21)

Sporocarps reddish-brown to brown, globose to subglobose, and 248–412 µm in diameter. The peridium encloses a sporocarp and is composed of thick-walled interwoven hyphae. Peridial hyphae sinuous (Plates 21a and 21c), which is evident in young and mature sporocarps. Sinuous features may be indistinct in older sporocarps (Plates 21b and 21d). The peridium is 8–20 µm thick. The chlamydospores are globose, subglobose, obovate to clavate, elliptical, fusiform-elliptical to clavate, and radiate from central plexus (45–150 × 30–75 µm in diameter), with 1–2 attached hyphae. The chlamydospore walls are brown, evenly or unevenly thickened (5–22 µm), usually thickened at the apex or lateral side, and generally thickest at the spore base.



(a) (b)

(c) (d)

Plate 21(a) Young chlamydospore of *Glomus sinuosum* with sinuous hyphal peridium (× 400)
Plate 21(b) Old chlamydospore of *Glomus sinuosum* with indistinct sinuous peridium (× 400)
Plate 21(c) Sinuous peridium in young sporocarps (× 1000)
Plate 21(d) Indistinct sinuous peridium in old sporocarps (× 1000)

Distinguishing feature: Peridium consists of the sinuous hyphae.

***Distribution*:** Recorded from (1) Valpoi in December with 50% frequency of occurrence, and in April and October with 37.50% frequency of occurrence; (2) Valpoi, Kodar, Old Goa, Collem, Quepem, Colva in December with 100% frequency of occurrence; (3) Kodar in April, July, October, and January with 75% frequency of occurrence; (4) Old Goa in July and January with 37.50% frequency of occurrence, and in June, July, August, and September with 80% frequency of occurrence.

***Association*:** Found in association with *C. papaya* plants from Western Ghats, plateaus and coastal areas of Goa, India.

22. *Glomus taiwanense* (Wu and Chen) Almeida and Schenck

Mycologia **82**: 703–714, 1990 (Plate 22)

Sporocarps reddish brown, brown or dark brown, globose to subglobose, 200–300 × 180–280 µm in diameter, with spores formed radially in a single, tightly packed layer around a central plexus of hyphae (Plates 22a and 22b). The peridium is lacking. Chlamydospores are clavate, cylindro-clavate, 40–85 × 22–30 µm in diameter, and with or without a septum at the spore base (Plates 22c and 22d). Chlamydospore wall laminate or single, with a hyaline separable outer layer (1 µm thick), yellowish-brown inner layer, 6–18 µm thick at the apex, 2–5 µm thick at sides, and generally thickest at the apex (Plates 22c and 22d).

Distinguishing feature: Distinguished from its morphologically allied species (*Glomus clavisporum*) by presence of a hyaline outer wall in the chlamydospores.

***Distribution*:** Recorded from (1) Collem in December with 50% frequency of occurrence; (2) Valpoi, Old Goa, and Collem in December with 50% frequency of occurrence; (3) Valpoi in April, October, and January with 50% frequency of occurrence; (4) Kodar in April with 12.5% frequency of occurrence; (5) Old Goa in July with 50% frequency of occurrence.

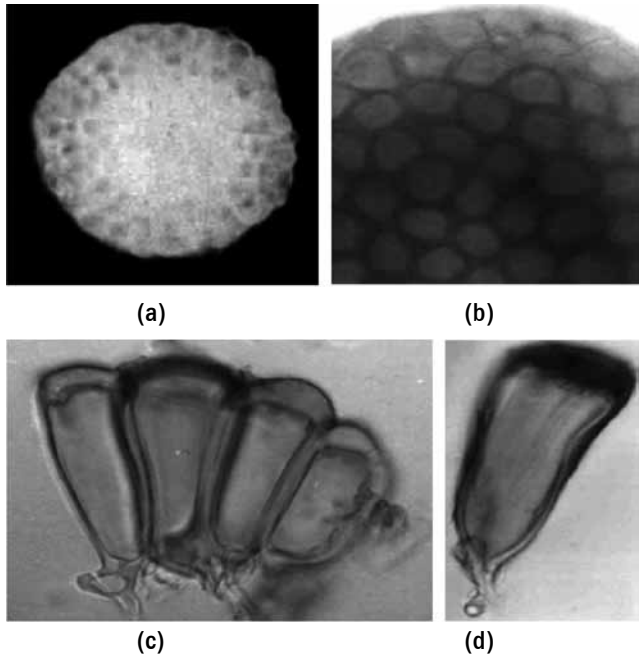


Plate 22(a) Young sporocarp of *Glomus taiwanense* (× 200)
Plate 22 (b) Surface view of sporocarp of *Glomus taiwanense* (× 400)
Plate 22(c) Chlamydospores of *Glomus taiwanense* with thickened apex (× 400)
Plate 22(d) Single chlamydospore of *Glomus taiwanense* with thickened apex (× 1000)

***Association*:** Found in association with *C. papaya* plants from Western Ghats, plateaus, and coastal areas of Goa, India.

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Impact of rhizospheric conditions on AM diversity, succession, and colonization in two plantations of *Acacia auriculiformis* and *Eucalyptus tereticornis*

Somdatta Ghosh[⋆] *and N K Verma*[#]

Introduction

Though AM are present in almost all soil types, their diversity and behaviour are influenced by soil conditions such as soil pH (Davies, Young, and Linderman 1983) , soil temperature (Schenck and Smith, 1974), moisture (Hetrick, Kitt, and Wilson 1987), nutrient availability (Menge, Stierle, Bagyaraj, *et al*. 1978), and so on. Hence, seasonal variation of these factors may influence AM status in a particular area. Plants of the same area vary in root colonization. AM fungi have no host specificity (Van der Heijden, Boller, Wiemken, *et al*. 1998).

Similar soil conditions at a locality may gradually change from time to time due to rhizospheric interaction of different plants and soil, which, in turn, influence AM status. Though ecological and rhizospheric specificity have not been studied adequately, there are several reports in its support (McGonigle and Fitter 1990; Verma 2000). In this study, we have investigated how rhizosphere of 2–3 species plantations influences AM colonization and sporulation during four prominent seasons (summer, monsoon, winter, and spring). Soil depth is a factor in AM sporulation. Seasonal impact on sporulation in different soil depths has also been studied.

Materials and methods

The study site is *Acacia auriculiformis* and *Eucalyptus tereticornis* plantations located at the Vidyasagar University campus in Midnapore district of West Bengal. The site is located at 22.30˚N latitude and 87.20˚E longitude, where during the dry summer (March–June) the average temperature is 30˚C, with a maximum of 42˚C. In winter, the average temperature is 18˚C (11˚C–26˚C). Most of the rainfall occurs between June and September. The soil is acid lateritic, with pH range 5.5–6.3. Up to a depth of 30 cm, the soil contains coarse sand (35%), sand (30%), silt (20%), and clay (15%), available nitrogen 0.0064%, available phosphorus 0.0021%, available potassium 0.0028%, and organic matter 0.4%.

For each season, soil and root samples were collected in three replicates. pH was measured by electric pH meter. Soil moisture content (%) was calculated by the following formula: [weight of moist soil (g) – weight of oven-dried soil (g)]/weight of oven-dried soil (g) X 100. Organic matter was assessed by Walkley and Black's rapid filtration method.

AM fungal spores were isolated by the method of Gerdemann and Nicholson (1963). The spores were mounted in PVLG and PVLG + Melzar reagent in intact and slightly broken state to study the cell wall structure. Spores were identified on the basis of shape, size, cell wall, attachments, contents, and so on following the manuals of Schenck and Perez (1990) and INVAM databases (http://invam.caf.wvu.edu).

Root colonization percentage was evaluated after treating a root sample with 10% KOH solution and staining with trypan blue (Phillips and Hayman 1970). Colonization percentage was obtained according to the formula: Colonization % = [number of infected root segments (1cm) × 100]/number of total root segments observed. Statistical analyses were done by 'Statistica' 5.0.

Results and discussion

Soil pH, moisture content, and organic carbon were slightly high in *A. auriculiformis* rhizosphere (see Table1). Soil pH showed positive but not significant correlation with colonization or spore density. Organic carbon is highly correlated with monsoon-enhanced colonization ($r=77$; $p<0.05$), and so is moisture content with colonization ($r=0.84$; $p<0.05$), but negatively with sporulation ($r=-72$; $P<0.05$). Colonization was maximum during monsoon, followed by winter, and minimum in summer (see Figure 3). Spore density and diversity were maximum in winter and minimum in monsoon (see Figures1 and 2, and Table 2). Colonization, spore density, and AM species diversity were higher in all seasons in *A. auriculiformis* than in *E. tereticornis*. Succession or sporulation of AM species varied with season and age of plant. Level of pH tolerance varied from species to species. Soil pH may affect spore germination and species distribution (Davies, Young, and Linderman 1983), which may explain the succession of AM in different seasons.

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Table 1 Seasonal variation of pH, moisture content, organic carbon, and soil temperature in rhizosphere of *Acacia auriculiformis* (A.a) and *Eucalyptus tereticornis* (E.t)

Season	Soil pH	Moisture content	Organic carbon	Soil
Temp (°C)				
Winter				
A.a	5.6 ± 0.05	2.2 ± 0.6	1.3	20 ±0.3
E.t	5.6 ± 0.4	1.8 ± 0.5	1.05	19.2 ± 0.4
Spring				
A.a	5.7 ± 0.05	2.8± 0.4	2.0	24 ±0.2
E.t	5.6 ± 0.08	2.2± 0.3	2.0	25.1± 0.4
Summer				
A.a	5.5 ± 0.06	1.2± 0.1	2.6	35 ± 0.3
E.t	5.5 ± 0.03	0.7± 0.08	2.2	36.2 ± 0..5
Monsoon				
A.a	5.8 ± 0.06	5.6± 0.3	3.9	22.2 ± 0.4
E.t	5.9 ± 0.04	5.4 ±0.2	3.2	23 ±0.3

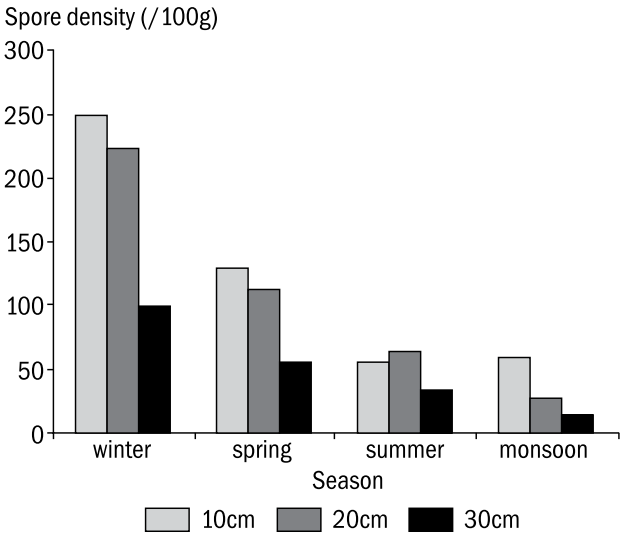

Figure 1 Seasonal variation of spore population at three different soil depths in *E. tereticornis*

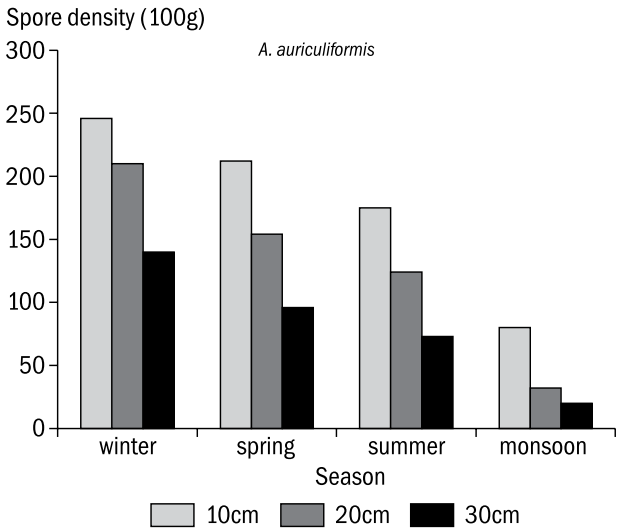

Figure 2 Seasonal variation of spore population at three different soil depths in *A. auriculiformis*

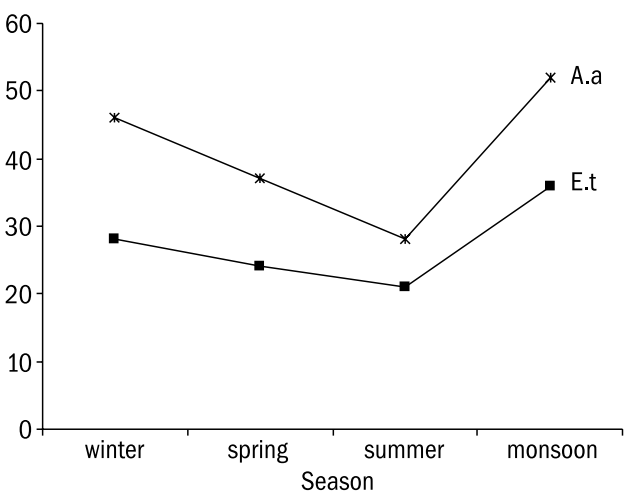

Figure 3 Seasonal change in AM colonization % in root of *Acacia auriculiformis* (A. a) and *Eucalyptus tereticornis* (E. t).

The observed AM species mainly belonged to *Acaulospora, Gigaspora*, and *Scutellospora*. *Glomus aggregatum* was present throughout the season. Members of *Acaulospora* such as *Gigaspora, G. heterogama, G. margarita,* and *G. gigantea* were found in a pH range 4.2–6.2 (Porter, Robson, and Abbott 1987). *G. aggregatum* was reported in acid soil (Aziz and Habte 1990; Habte 1999). Soil acidity factor also plays an important role in regulating spore germination (Porter, Robson, and Abbott 1987). Reduced soil pH increases the hydrogen and mineral ion concentration, depending on physical and chemical properties of soil (Abbott and Robson 1985), which possibly inhibits spore germination (Hepper 1979; Soedarjo and Habte 1995). This might be an explanation of poor colonization in summer.

Soil moisture levels have been shown to influence the distribution of AM fungi (Perry, Molina, and Amaranthas 1987). Spore production, germination, and root colonization were reported to be maximum at optimum soil moisture supporting plant growth (Redhead 1975).

The AM fungi vary in their spatial distribution in soil (see Figures1 and 2). There is also seasonal influence on spore population. Increased temperature (r=-78) and moisture(r-72) level had negative impact on spore populations. The spore density was gradually decreased up to 30 cm depth. At 20–30 cm, the density was very poor, at nearly half of that at 10–20 cm depth. In summer and monsoon, at 10 cm. depth, spore density gets reduced by almost half. The spore density was high at all depths overall in *A. auriculiformis.* In *E. tereticornis*, the upper two levels of soil depth showed no major difference, while in summer, 10–20cm depth showed maximum spore density.

Moisture and organic carbon may play a major role in AM colonization, but temperature and less moisture are the main criteria for sporulation for most AM species, especially *Gigaspora* and *Scutellospora.* Those genera are observed in winter and spring, except *S. nigra that* appears in monsoon only. Temperature also influenced AM species' succession in nature (Verma 2000).

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Inoculation studies in some shade-tree species with a Glomus sp. obtained from tea gardens

*S Routaray, U C Basak, and N Gupta**

Introduction

Tea is a shade-loving plant. Shade plants are mostly leguminous trees such as *Albizia lebbeck*, *(L.) Benth, A. moluccana, A. porcera (Roxb.) Benth*, *A. stipulata (Roxb.), Acacia nilotica (L.) Del.*, and *A. auriculiformis A. Cunn. ex. Benth.*. Several reports are available on the studies of mycorrhization of *Acacia* and *Albizia* (Mehrotra and Mehrotra 2000; Jammaluddin, Chandra, and Goswami 2001). All these species are used as shade trees in tea plantations at Bhuyanpirh in Orissa. The present study deals with the mycorrhizal association of these tree species with the AM fungi obtained from tea garden at Bhuyanpirh, Orissa.

Materials and methods

The AM fungi were isolated from the tea (*Camellia sinensis* L.) plantations at Bhuyanpirh tea estate in Tarmakanta, situated 48 kilometres from Keonjhar, Orissa. The plantation has been raised at a place—which was once covered by dry and mixed deciduous sal forests—at an elevation of more than 600 msl. This 15–17 year old plantation is interspread with *Acacia auriculiformis A. Cunn. ex. Benth, Albizia lebbeck (l.) Benth., A. odoratissima (Linn.f.) Benth.*, and *Leucaena leucocephala* for providing shade.

The soil was collected in polythene bags from 10 cm depth of the root zone of tea plants, and brought for analysis. Isolation of AM fungi was carried out by following the method of Gerdemann and Nicolson (1963). AM spores obtained from the tea plantation were purified following the funnel technique (Menge and Timmer 1982). The pure culture of AM fungi was identified according to Schenck and Perez (1987). The pure culture of *Glomus* sp. was maintained and multiplied on maize roots using ¼ strength Hoagland solution for daily irrigation (Menge and Timmer 1982).

Seven shade tree species, namely, *Acacia auriculiformis* A.Cunn.ex.Benth., *A. dealbata* Link., *A. decurrens* (Wend.)Willd, *Albizia molluccana* (Benth), *A. stipulata* Roxb., *A. odoratissima* (Linn.f.) Benth., and *A. lebbeck* (L.) Benth. were considered for the study. The seeds were surface sterilized with 0.01% $HgCl_2$, and sown in polybags (9 kg size) containing sterilized black cotton soil. After germination, the 21-day-old seedlings were transplanted for the study.

In the glasshouse, polybags of 2 kg capacity were filled with sterilized (autoclaved at 15 lb, 121° C, 3 h), black cotton soil supplemented with compost and sand at the ratio of 3:1:1. Inoculation of different AM isolates was done by spreading soil inoculum (100 g), containing about 200–250 spores in each pot separately for each treatment. Only sterilized soil was taken as control. Plants of individual tree species were transplanted in 10 replicates separately, and watering was done as per the requirement. Hoagland nutrient solution without phosphorus (1/4 strength) was added (25ml plant) fortnightly during the experiment.

Plants (150 days old) were harvested and their roots were treated for clearing and staining following the method of Phillips and Hayman (1970) to determine AM colonization. The analysis of % colonization in the roots of different plants was done according to the slide method (Kormainic and McGraw 1982). Data obtained in triplicate were subjected to statistical analysis (Sokal and Rolf 1973).

Results and discussion

The result of AM inoculation conducted under glasshouse conditions with AM fungi on seven tree species are presented in Table 1. The isolated and purified AM culture was identified as *Glomus* sp.. All species of forest trees were found to be mycorrhizal. The maximum root colonization was recorded in the roots of *Albizia stipulata* (19.40±1.10), followed by *Albizia odoratissima* (14.67±4.37), *Acacia decurrens* (14.00±1.67), and *Acacia auriculiformis* (14.00±1.23). The results exhibited very low range of colonization in *Albizia lebbeck* (8.33 ±4.21%). Rahangdale and Gupta (1999) reported very low level of % colonization in *Albizia lebbeck*. In this connection, number of vesicles/root also varied from 1.80±0.25 (in *Acacia moluccana*) to 5.417±0.37 (in *Albizia lebbeck*). Other tree species, namely, *Acacia auriculiformis*, *A. decurrens*, and *Albizia odoratissima* showed more or less similar response. *Acacia dealbata* and *Albizia stipulata* had almost the same number of vesicles (4.00 ±0.34 and 4.13 ±0.030, respectively). The results indicated a wide spectrum infection potential of this fungi. Since, very low percentage of colonization in forest tree species has been observed, it cannot be correlated as an efficient parameter for determination of AM infectivity and

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affectivity. Mycorrhizal association provides additional support to plants for nutrient uptake in deficient soil. Incidence of fairly low degree of % root colonization was apparent in all seven tree species.

The present study showed the relative effects of *Glomus* sp. on the growth of host plant. As far as symbiotic benefits were concerned, most of the host plants, except *A. moluccana*, achieved increment in shoot growth and dry biomass (see Table 1). Inoculation resulted in enhancement of plant height as compared to uninoculated control. Similar differential responses due to AM inoculations were observed in plant root length. Significant differences were observed for the number of leaves in the treated plants, as compared to the control. Dry biomass of shoot and root was enhanced to the maximum over the control. All inoculated plants showed increase in the fresh and dry weight of root over the control. Similar responses of AM were observed for biomass measurement of leaves and it was proved to be the best in enhancing dry weight of leaves over the control. The response of AM inoculation on nodulation was also studied during the experiment. The maximum number of nodules was recorded in AM-infected plants. Results also attributed towards the suitability of this *Glomus* species as the best inoculant for forest trees species being used as shade trees in tea gardens. This is because it increased the plant dry biomass, though % colonization was rather low.

Proportional distribution of dry biomass by tree species grown under mycorrhizal and non-mycorrhizal conditions was also determined. Three plant species, namely, *Acacia auriculiformis*, *Albizia stipulate*, and *A. odorata* showed higher % of shoot biomass, where as *Acacia moluccana* and *Acacia decurrens* exhibited higher leafy biomass with mycorrhizal and non-mycorrhizal treatments. Similarly *A. auriculiformis* showed higher leafy biomass under mycorrhizal conditions. *Acacia dealbata* exhibited more or less equal amounts of leaf, shoot, and root biomass under both mycorrhizal and non-mycorrhizal conditions. Generally, higher yield in shoot biomass, followed by leafy and root dry biomass, was recorded in all the tree species studied.

Almost all the shade trees showed more or less similar shoot-root ratio. *A. stipulata* and *A. odorata* were, however, showed higher values under mycorrhizal and non-mycorrhizal conditions, respectively (see Table 2). Similarly, in case of shoot-root ratio of dry biomass, uniform value was obtained under non-mycorrhizal conditions, except for *A. auriculiformis* where mycorrhizal plants showed higher shoot-root ratio of dry biomass. AM-inoculated seedlings of tree species had a significantly lower shoot-root ratio, than uninfected plants. But

Table 1 Effect of AM inoculation on growth and AM colonization of shade tree species

Parameters	Treatment	1	2	3	4	5	6	7
% Colonization		14.00 ± 1.23	10.00 ± 2.10	14.0 ± 1.67	11.75 ± 3.45	19.4 ± 1.10	14.67 ± 4.37	8.33 ± 4.21
No. of vesicles		2.56 ± 0.46	4.00 ± 0.34	2.52 ± 0.24	1.80 ± 0.25	4.13 ± 0.30	2.05 ± 0.40	5.41 ± 0.33
Shoot length (cm)	NM	25.40 ± 4.97	27.60 ± 1.99	23.00 ± 3.23	28.33 ± 1.67	45.97 ± 8.07	57.33 ± 20.80	47.67 ± 6.49
	M	26.67 ± 4.92	38.14 ± 6.54	28.38 ± 2.92	28.33 ± 3.28	62.00±12.34	70.00 ± 30.41	56.67 ± 13.23
Root length (cm)	NM	16.33 ± 1.29	19.71± 1.48	19.50 ± 1.54	35.00 ± 7.64	26.67 ± 1.67	27.00 ± 4.04	26.67 ± 6.36
	M	17.00 ± 1.53	19.8 ± 1.05	19.38 ± 2.31	38.33 ± 13.33	25.33 ± 2.60	40.00 ± 10.07	33.83 ± 0.60
Shoot dry wt (g/plant)	NM	0.57 ± 0.36	0.20 ± 0.05	0.16 ± 0.02	0.12 ± 0.05	1.24 ± 0.38	4.16 ± 1.96	4.35 ± 1.67
	M	0.70 ± 0.30	0.22 ± 0.14	0.12 ± 0.06	0.15 ± 0.01	3.10 ± 2.42	7.76 ± 3.30	4.52 ± 3.50
Root dry wt (g/plant)	NM	0.11 ± 0.03	0.16 ± 0.05	0.18 ± 0.06	0.08 ± 0.04	0.43 ± .24	0.28 ± 0.19	0.91 ± 0.11
	M	0.49 ± 0.07	0.28 ± 0.16	0.21 ± 0.03	0.14 ± 0.09	0.72 ± 0.61	0.38 ± 0.17	0.90 ± 0.20
Leaf dry wt (g/plant)	NM	0.08 ± 0.03	0.18 ± 0.05	0.45 ± 0.22	0.42 ± 0.26	0.77 ± 0.39	0.23 ± 0.17	0.58 ± 0.27
	M	1.79 ± 0.74	0.25 ± 0.17	0.86 ± 0.50	1.92 ± 0.50	2.26 ± 1.21	0.83 ± 0.65	2.64 ± 0.12
Leaf no. (per plant)	NM	14.22 ± 2.03	10.00± 1.17	9.13 ± 1.27	9.00 ± 0.58	7.33 ± 0.88	14.0 ± 0.58	14.67 ± 1.67
	M	16.67 ± 2.03	15.43± 3.15	10.50 ± 0.93	13.00 ± 0.45	12.33± 1.67	14.0 ± 3.61	15.67 ± 1.20
Nodule no. (per plant)	NM	4.00 ± 1.16	6.10 ± 1.04	5.67 ± 3.28	9.67 ± 0.33	14.67± 3.18	6.33 ± 0.67	20.67± 11.47
	M	7.00 ± 3.44	7.43 ± 3.00	11.0 ± 7 .00	17.67 ± 4.41	25.67± 8.76	10.67± 7.45	72.33± 12.88

1=*Acacia auriculiformis*; 2= *A. dealbata*; 3= *A. decurrens*; 4 = *Albizia molluccana*; 5 = *Albizzia stipulate*; 6= *A.odoratissima*; 7 = *A. lebbeck* ; ± = Standard error of 5 replicates; NM = non-mycorrhizal (uninoculated); M = mycorrhizal (inoculated)

Table 2 Proportional dry biomass of tree species under non-mycorrhizal and mycorrhizal conditions (%)

	Non-mycorrhizal			Mycorrhizal		
Tree species	Shoot	Root	Leaf	Shoot	Root	Leaf
Acacia auriculiformis	75.0	14.47	10.52	23.49	16.44	60.07
Acacia dealbata	37.03	29.62	33.33	29.33	37.33	33.33
Acacia decurrens	20.25	22.78	56.96	10.08	17.65	72.27
Acacia moluccana	19.35	12.9	67.74	6.79	6.33	86.88
Albizzia stipulata	50.81	17.62	31.55	50.99	11.84	37.17
Albizia odoratissima	89.07	5.99	4.92	86.51	4.24	9.25
Albizia lebbeck	74.48	15.58	9.93	56.08	11.17	32.75

the decreased root of the inoculated seedlings was probably functionally substituted by the external mycelium of AM fungi.

Though, AM inoculations enhanced growth of shade-tree species, the differences in plant height and biomass among the control and inoculated plants were not very significant in *Acacia dealbata*, *Albizia odorata*, and *Albizia lebbeck* (see Table 3). However, the overall performance of *Acacia auriculpformis*, *A. decurrence*, *A. moluccana*, and *Albizia stipulata* was encouraged through mycorrhization. Leaf number was found to be significantly higher in the inoculated plants, as compared to the control.

Data of the intergeneric and intrageneric level were also analysed statistically for the nodulation status (see Table 4). In non-mycorrhizal shade trees, significant variation was recorded at the intergeneric level between *Acacia* and *Albizia*; whereas difference in the nodulation status at the intrageneric level of

Table 3 Shoot-root ratio in different tree species under non-mycorrhizal and mycorrhizal conditions

	Non-mycorrhizal		Mycorrhizal	
Tree species	S/R length	S/R dry wt.	S/R length	S/R dry wt.
Acacia auriculiformis	1.55	5.18	1.56	1.42
Acacia dealbata	1.40	1.25	1.92	0.78
Acacia decurrens	1.79	0.88	1.46	0.57
Acacia moluccana	0.81	1.50	0.73	1.07
Albizzia stipulata	1.70	2.88	2.44	4.30
Albizia odoratissima	2.12	1.48	1.75	2.04
Albizia lebbeck	1.787	4.78	1.67	5.02

Acacia was not significant. But for *Albizia*, it showed a significant difference.

In all the shade-tree species studied, data recorded for % colonization and number of vesicles were treated for ANOVA (see Table 5). Though colonization percentage recorded for all shade-tree species differed, a significant variation among them could not be observed at the intrageneric and intergeneric levels. These plants varied in the occurrence and the number of vesicles in their roots, with a highly significant difference occurring among both the genera *Acacia*

Table 5 Two-way ANOVA for comparison among the tree species at generic and species level (F value)

	Intergeneric	Intrageneric	
Parameters		Albizia	Acacia
% colonization	4.072*	4.256	4.51
No. of vesicles	32.96**	15.22*	82.49**
No. of nodule (NM)	8.919**	5.487*	32.07**
No. of nodule (M)	65.91**	0.563	1.88.5**

*= significant at 5% level, ** = significant at 1 % level,

Table 4 Two-way ANOVA (F value) for different parameters of different tree species grown under non-mycorrhzial and mycorrhizal conditions

Tree species	Shoot length	Root length	Leaf no.	Shoot dry wt.	Root dry wt.	Leaf dry wt.
Acacia auriculiformis	0.198	0.151	6.52**	0.116	43.32*	17.40
Acacia dealbata	13.66	0.002	4.74	0.033	0.98	1.02
Acacia decurrens	2.29	0.004	48.71*	0.231	1.00	2.67
Acacia moluccana	0.00	0.098	311.2**	1.289	4.32	35.98
Albizzia stipulata	42.98*	0.053	120.2**	1.286	0.374	2.518
Albizia odoratissima	0.184	6.581	0.00	0.008	0.917	1.922
Albizia lebbeck	2.657	3.175	0.364	0.004	0.037	83.7*

* = significant at 5% level; ** = significant at 1% level

and *Albizia*. Not only at the generic level, but these groups also showed significant variations in the number of vesicles among the species.

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Incidence of arbuscular mycorrhizal fungi (AMF) in tree species in arid zones of Ajmer region of Rajasthan

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Introduction

The arid zone of India constitutes about 12% of the country's geographical area. The desert, or arid zone ecosystem is characterized by scanty and erratic rainfall. High temperatures, excessive evapo-transpiration, and strong winds on sandy landforms cause massive soil erosion and soil movement. Drought is a recurring feature of the region, while moisture stress is one of the major limiting factors for productivity and regeneration of woody species. Different tree species such as *Acacia, Prosopis, Albizia, Cassia, Tecomella, Eucalyptus, Azadirachta, Dendrocalamus, Dalbergia, Terminalia, Polyalthia*, and so on are commonly grown in the arid and semi-arid desert regions of India. These species are economically important as sources of tannin, timber, fodder, gum, shelter, building-making material, and fuel for livelihoods. They are also extremely suitable for afforestation and reclamation of wastelands in the arid zones. Presence and utilization of AMF have markedly increased the success of rehabilitation of these moisture-deficient regions. AMF can bind soil particles together into larger aggregates that are necessary for a stable and porous physical structure of the soil. AMF can play an important role in stabilizing survival and growth of desert and sand dunes, colonizing plant species, and creating shelterbelts. AMF enhance drought tolerance in plants (Sanchez-Diaz and Honrubia 1994; Quilambo 2003) by tapping a large volume of soil, mobilizing scarce nutrient sources, and modifying the root environment (Hayman 1983). AMF associations with plants are wide spread, both taxonomically and geographically. Most of the plants growing in Indian deserts carry AMF infection on their roots (Quilambo 2003). The colonization of AMF in desert plants species differs in ecological requirements and adaptability. Therefore, the present investigation was carried out to examine the AMF status in plants of different tree species in arid zones of Ajmer region.

Material and methods

A survey was conducted on the occurrence of AMF on trees growing at different attitudes on several fields. Thirty -six tree species belonging to 18 families of angiosperms from various forests, and the institute's plantation (approximately one year old plants) located at various places in the arid zones of Ajmer district in western Rajasthan were included. Roots and soils (200g soil in each sample) of three samples of each species were collected randomly, and brought to the laboratory for estimation of AMF association. Mycorrhizal infection in roots was determined (Biermann and Liadermann 1981). The root samples were washed thoroughly in tap water and fixed in formalin acetic acid alcohol for further studies. After washing, 1cm segments were cut from the fine roots. Thereafter, the samples were cleaned with 10% KOH, acidified with 1N HCL, and stained in 0.05% trypan blue following the method of Phillips and Hayman (1970). Mycorrhizal infection in the roots was expressed as the percentage of segments containing fungal structures like mycelium/fungal hyphae, arbuscules, and vesicles (100 root segments per sample were evaluated for mycorrhizal infection).

$$\% \text{ of mycorrhizal association} = \frac{\text{No. of mycorrhizal segments}}{\text{Total no. of segments screened}} \times 100$$

Individual AMF spores in soil showing hyphal connection were isolated using the modified wet sieving and decanting method of Daniels and Skipper (1982) and Gerdemann and Nicolson (1963) from the air-dried rhizosphere soil samples collected from different tree species. Characterization of individual AMF spores was carried out after being subjected to morphogenetic and micrometric analysis based on their colour, diameter, shape, wall layers, surface content, hyphal colour, hyphal width, and hyphal attachment with the wall. On this basis, dominant genera of AMF were categorized (see Table 1), and the identification was made at species level with the help of relevant literature (Trappe 1982; Morton and Benny 1990; Schenck and Perez 1990; and Wu, Hao, Lin, *et .al.* 2002). For pH estimation, soil suspension (1:4, W/V) was prepared as described by Jackson (1973).

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Results and discussion

The collected soil samples for estimation of AMF density were of different types and had varying pH values. In the samples, the pH range was from 7.5 to 8.5. The mycorrhizal spore population in soil was higher in this pH range, and dominant genera of AMF were categorized as *Glomus*.

The average of mycorrhizal spore density ranged from 8–15 spores per 10g of dry rhizosphere soil in the collected tree species. A variety of spores were recovered from soils and root washings, mainly belonging to the genus *Glomus*. However, azygospore of *Acaulospora* and *Gigaspora*, and sporocarps of *Sclerocystis* were also recovered, though these were rare.

The results of root samples (shown in Table 1) indicate the prevalence of AMF colonization in roots of different tree species occurring in various places in arid zones of Ajmer region of Rajasthan. The average of mycorrhizal root colonization ranged from 26–68% in different tree species. Most of the tree species had prominent AMF association in the form of vesicular, arbuscular, and mycelium structure, while one sample had all of these. The frequency of AMF infection and the percentage of root colonization varied within the family and among different host genera. AM fungal structures were found in 33 species of plants belonging to 16 families. These fungal structures were frequently seen in the inner cortex of younger fine roots. Arbuscules were commonly recorded in 30 species belonging to 15 families, while vesicles were noticed in 33 species belonging to 16 families and were usually found in the cortical tissue of roots. The AM fungal hyphae were broader and thicker in the roots, and varied from plant to plant. However, the mycorrhizal infection was found in 34 species of plants from 16 families of angiosperms.

Vesicles and arbuscules were present in the roots of trees species, and it was very difficult to identify the AM fungus species. To facilitate identification, the rhizosphere soil samples of all the tree species were processed for isolating different fungal propagules. The rhizosphere soil samples of the entire tree contained AM fungal spores. Based on the spore characters, the following fungi were identified: two species of *Acaulospora*, namely, *A. tuberculata* and *A. laevis*; six species of *Glomus*, namely, *Glomus aggregatum*, *Glomus constrictum*, *Glomus fasciculatum*, *Glomus intraradices*, *Glomus mosseae*, and *Glomus microcarpum*; two species of *Gigaspora*, namely *G. calaspora* and *G. gigantean*; one species of *Sclerocystis nigra*; and one unidentified spore each of *Glomus, Gigaspora*, and *Sclerocystis*.

G. fasiculatum seems to be the most predominant species, found in most of the rhizosphere soil samples of different tree species (31 species); followed by *G. mosseae (*19 tree species*), G. intraradices* (9 tree species*), G. aggregatum* (7 tree species), *G. constrictum* (6 tree species), and *G. microcarpum* (5 tree species).

The spore of *A. tuberculata* was found in three tree species, while that of *A. laevis* was found only in one tree species in the rhizosphere soil samples. The tree species were *Leucaena leucocephala*, *Prosopis juliflora*, *Terminalia arjuna*, and *Dendrocalamus strictus* respectively. The spore of *Gigaspora* (*G. calaspora*) was noticed in one species, namely *Eucalyptus camaldulensis*; while *G. gigantea* was found in three tree species of rhizosphere soil samples, namely *A. sinuate*, *Azadirachta indica*, and *D. latifolia*. The spore of *Sclerocystis* was found only in the rhizosphere soil samples of two tree species, namely, *Ailanthus excelsa* and *Cassia fistula*, and one unidentified species in *Sisymbrium irio* soil sample (see Table 1).

The AM fungal infection consisted of fungal hyphae, vesicles, and arbuscules. The percentage of infection varied among the plant species. No definite correlation could be established between spore and mycorrhizal root colonization. As multiplication of an endomycorrhiza depends on its association with plant roots, the number of its spores in soils is likely to differ, as shown in the present investigation. The prevalence of AM fungi suggests that the mycorrhizae may be of great importance for plant growth and development in tropical soils, especially in arid and semi-arid zones, which have extremely low soil moisture and are relatively poor in macro- and micro-nutrients. The activity of mycorrhizae fungal population, in terms of root infection and the number of spores, has been shown to be greatly affected by soil conditions (Mukerji, Sabharwal, Kochar, *et al.* 1984).

The present study reveals that AM fungi are quite common in all the tree species examined from various places in the arid zones of Ajmer region, with species of *Glomus* found to be widely spread among the trees of this region. The *Glomus* species of AM fungi help the various trees of the arid region in afforestation and reclamation of wasteland development in a sustainable manner.

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Table 1 AM fungi association in roots and spore propagules in soil for different tree species growing in their natural habitat in the arid zone of Ajmer, Rajasthan

Plant species/ family	"Soil pH"	Hyphal type		AMF structure		"Infection level (%)"	"AMF spores population per 10g soil"	"AM fungal species "
		Broad	Thin	Arbuscule	Vesicle			
Acacia catechu (Mimosaceae)	8.1	0	***	**	**	56a	12	Gf,Ga,Gi
A. sinuata(Mimosaceae)	8.2	*	0	*	**	45	11	Gf,Gm,Gg
A. leucophloea (Mimosaceae)	8.3	0	**	**	**	55	12	Gf,Gm
A. melanoxylon (Mimosaceae)	7.9	0	0	0	0	45	10	Ga,Gf,Gc
A. nilotica (Mimosaceae)	7.4	0	**	**	***	59	8	Gf,Gm
A. senegal (Mimosaceae)	8.1	**	0	0	**	65	14	Gm,Gi,Gm
A. tortilis (Mimosaceae)	8.0	0	***	**	***	56	12	Ga,Gf,Gm
Ailanthus excelsa (Simaroubaceae)	8.2	**	0	**	***	45	12	Gf,Gm,Sn
Albizia lebbeck (Mimosaceae)	8.5	0	***	**	**	63	13	Gf,Gi,Gc
Azadirachta indica (Meliaceae)	8.1	**	0	**	**	68	13	Gf,Gg,Gmi
Bambusa arundinacea (Poaceae)	8.0	0	**	**	**	66	15	Ga,Gf,Gg
Bauhinia variegata (Caesalpiniaceae)	7.8	0	*	*	**	62	11	Gf,Gm,Gc
Cinnamomum verum (Lauraceae)	7.7	0	*	*	**	50	12	Gf,Gm,Sc
Cassia fistula (Caesalpiniaceae)	8.1	0	*	0	**	64	12	Gf,Gi,Gm,Sn
Cassia siamea (Caesalpiniaceae)	8.2	*	0	0	**	55	15	Gf,Gm,Gs
Citrus aurantifolia (Rutaceae)	8.1	0	*	*	**	49	12	Gf,Gm,Gc
Cordia myxa (Ehretiaceae)	8.3	*	0	*	***	52	13	Gf,Gm
Dalbergia sissoo (Fabaceae)	8.2	0	***	**	**	68	15	Gf,Gi,Gs,Gmi
D. latifolia (Fabaceae)	7.8	0	**	**	***	55	11	Gf,Gi,Gg
Dendrocalamus strictus (Poaceae)	7.7	0	**	**	***	55	14	Gf,Gm,Gc.Al
Euphorbia tirucalli (Euphorbiaceae)	7.9	0	0	0	0	0	8	Gs,Gca
Eucalyptus camaldulensis (Myrtaceae)	8.1	0	*	*	**	45	13	Gf,Gm,Gci
E. tereticornis (Myrtaceae)	8.2	0	0	**	***	64	15	Gf,Gc,Gmi,Gs
Ficus religiosa (Moraceae)	8.0	0	0	0	0	0	8	Gf,Gc
Leucaena leucocephala (Mimosaceae)	8.1	**	0	**	***	67	16	Ga,Gf,At,Sc
Moringa oleifera (Miriagaceae)	8.1	0	0	*	**	59	14	Gf,Gi,Gs,Gsp
Polyalthia longifolia (Annonaceae)	7.6	*	0	**	***	45	13	Gf,Gm
Pongamia pinnata (Fabaceae)	7.9	0	*	**	**	44	12	Gf,Gm,Gmi
Prosopis juliflora (Mimosaceae)	7.7	0	***	**	**	56	14	Gi,At,Ga
P. cineraria (Mimosaceae)	7.9	0	**	**	**	45	10	Ga,Gf
Sisymbrium irio (Capparaceae)	7.9	0	*	*	**	46	11	Gf,Gm,Sp
Syzygium cumini (Myrtaceae)	8.0	0	*	*	**	39	12	Gi,Gm,Gs
Tectona grandis (Verbenaceae)	8.1	**	0	*	**	26	8	Gf,Gs,Sp
Terminalia arjuna (Combretaceae)	8.2	0	**	**	**	45	12	Ga,Gf,At
Tecomella undulata (Bignoniaceae)	8.0	0	*	**	**	45	11	Gf,Gi
Zizyphus mauritiana (Rhamnaceae)	8.2	0	*	*	**	55	13	Gf,Gm

* =1–30% infection level, Poor; ** = 31–70% infection level, Moderate; *** = above 70% infection level, Abundant; 0 = Absent

[a] Each figure represents the mean of three replicates

AL= Acaulospora laevis; At = Acaulospora tuberculata; Ga = Glomus aggregatum;Gc = Glomus constrictum; Gf = Glomus fasciculatum; Gi = Glomus intraradices;Gm = Glomus mosseae;Gmi = Glomus microcarpum; Gs = Glomus species; Gci = Gigaspora calaspora;Gg = Gigaspora gigantea; Gsp = Gigaspora spp.; Sn = Sclerocystis nigra; Sc = Sclerocystis spp.

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CENTRE FOR MYCORRHIZAL CULTURE COLLECTION

Phytoremediation of Cr-contaminated soil using Aloe vera and Chrysopogon zizanioides along with AM fungi and filamentous saprobe fungi: a research study towards possible practical application

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Introduction

Today, heavy metal pollution in soil as a result of various industrial activities is increasingly becoming a source of worry for farmers and plant producers around the world. The tannery industry, which regularly uses basic chromium (Cr) sulfate for tanning process is a major contributor to the high proportion of Cr in the biosphere, accounting for 40% of the total industrial use (Barnhart 1997). Cr is a known carcinogen and toxic heavy metal that, even in low concentrations, is reported to affect humans and aquatic life adversely. In India, around 0.9×10^9 kg of hide and skin are processed every year, releasing 30×10^9 l to 40×10^9 l of liquid effluent annually (Thanikaivelan, Rao, Nair, *et al.* 2005), which generally contains 1.09–8 g/l of Cr (Hafez, El-Manharawy, and Khedr 2002; Hintermeyer, Lacour, Padilla, *et al.* 2008). The discharged effluent eventually contaminates soil and water bodies and the whole environment. Today, the mushrooming tannery industry in North India has converted the holy Ganges into a dumping ground. Several analyses reveal high concentrations of Cr even in supposedly 'treated' effluents by common effluent treatment plants (CETPs) (Ramteke, Awasthi, Srinath, *et al.* 2010). The residues can be traced even in crops grown using water taken from the river. According to Bureau of Indian Standard (BIS) the industrial effluent permissible discharge level of total Cr and Cr(VI) into inland water is 2 and 0.1 mg/l respectively (Baral and Engelken 2002).

The main centres of tanning industry in India are located in the states of Tamil Nadu, Andhra Pradesh, Uttar Pradesh, Bihar, Gujarat, Maharashtra, Karnataka, Punjab, Rajasthan, and West Bengal. Since 1990, awareness about environmental damages has been growing on the issue of discharge of untreated effluents by tanneries into adjoining land areas, streams, and rivers. Recently, the Department of Environment has identified the tannery industry as the principal pollutant in the country, and has taken legal actions against 1,423 industries in between September 2009 and February 2010. Experts believe that untreated tannery wastes discharged in such a manner is causing a variety of health hazards. These wastes discharged in the adjoining land areas form large wastelands that contain significant amounts of a number of toxic elements, especially Cr. Phytoremediation is a promising low-cost biotechnological solution for such sites and in extracting Cr as a value addition. Metal-tolerant plant species, along with indigenous microbial community (including AM fungi), can be used to clean up metal-contaminated sites in a much more efficient manner.

Research strategies and approach followed

Most of the known and studied metal hyperaccumulator plants are either slow growing or negligible producers of biomass or fall under the food chain category. Hence, there is a need to select plants that exhibit rapid growth, high biomass producing ability, along with high metal accumulation ability. Generally, in metal-polluted soil, the normal flora and microbial diversity get highly distorted, which, in turn, does not support growth of plants (Terry 1981). The AM fungi are known to occur in plants growing in metal-polluted soil (Khade and Adholeya 2009). In exchange for carbon from plant hosts, the AM fungi can facilitate plant uptake and transport of lesser amount of mobile soil nutrients (such as phosphorus) (Bolan 1991; Jakobsen, Gazey, and Abbott 2001), enhance drought tolerance (Ruiz-Lozano, Collados, Barea, *et al.* 2001; Kaya, Higgs, Kirnak, *et al.* 2003)

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and reduce pathogenic infections (Newsham, Fitter, Watkinson, *et al.* 1995; Abdalla and Abdel-Fattah 2000). It has also been reported that mycorrhizal fungi can impact plant uptake or translocation of soil metals (such as Cd, Zn, Pb, Cu, and Al) (Leyval, Turnau, and Haselwandter 1997; Khan, Kuek, Chaudhry, *et al.* 2000). Besides AM fungi, other beneficial micro-organisms such as filamentous saprobe fungi are also known to enhance growth and heavy metal uptake in plants (Arriagada, Herrera, Garcia-Romera, *et al.* 2004; Arriagada, Herrera, Borie, *et al.* 2007). In the present study, taking into account the important roles of AM fungi and filamentous saprobe fungi in plant growth and heavy metal uptake from metal-contaminated soil, research strategies and approach were developed to select some of the best combinations of hyperaccumulator plants and AM fungi and other beneficial saprobe fungi for phytoremediation of Cr-contaminated soil.

Greenhouse evaluation studies

To select the best combinations of plants and micro-organisms, altogether 22 different plant species (see Table 1) were screened for their ability to grow and accumulate Cr in their biomass. To achieve the primary objective, greenhouse studies have been initiated at the Centre for Mycorrhizal Research (CMR) since 2003. Soon after, the Central Pollution Control Board (CPCB) identified the tannery industry as a major problem, and targeted chrome recovery and reusability along with waste management as a major challenge. All greenhouse experiments were conducted in absolute tannery sludge (see Table 2) at TERI, Gual Pahari, Haryana. The tannery sludge used was alkaline in nature and contained Cr in very high concentration (25,762.14 mg/g). Further, the effect of different AM fungi—separately and in different combinations—was studied on the enhanced plant growth and heavy metal uptake by plants from the absolute tannery sludge. Three different species of *in vitro* grown AM fungi (*Glomus intraradices, G. proliferum*, and *G. lamellosum*) separately and in different combinations, and five trap cultures (TC1, TC2, TC3, TC4, and TC5) separately and in different combinations with two different plant growth promoting filamentous fungi (having ability to solubilize phosphate) were studied for enhanced plant growth and heavy metal uptake ability. The trap cultures were developed from five different rhizospheric soils of plants growing in Cr-contaminated soil collected from sludge disposal site of CETP, Jajmau (Kanpur, Uttar Pradesh, India). After extensive greenhouse experiments, five plant species were selected (see Table 3). The final microcosm experiment was conducted for six months in greenhouse conditions using trap culture (TC2), *in vitro* grown *G. intraradices*, *in vitro* consortium (mixture of *G. intraradices*, *G. proliferum*, and *G. lamellosum*), and two filamentous fungi (*Aspergillus niger* and *Aspergillus flavus*) separately and in different combinations to select the best combinations of plants and AM fungi and other beneficial filamentous saprobe fungi. The filamentous saprobe fungi selected were isolated from the tannery sludge and were found to possess the capability of hyper accumulating chromium. They were also incorporated in the evaluation studies because of their phosphorus solubilization ability, which could be beneficial for plant growth.

Table 1 List of plants screened for growth and Cr metal uptake from absolute tannery sludge (Control treatments were set up in soil)

S. No	Plants	Substrate
	Economically important plants	
1	*Jatropha curcas* (Vijaywada)	Sludge and soil
2	*Jatropha curcas* (Barwaha)	Sludge and soil
3	*Aloe vera*	Sludge and soil
4	*Melia azadirachta*	Sludge and soil
5	*Dalbergia sissoo*	Sludge and soil
6	*Tabacum nicotiana*	Sludge and soil
	Vegetable crops	
7	*Lycopersicon esculentum*	Sludge and soil
8	*Brassica oleracea var. capitata*	Sludge and soil
8	*Brassica oleracea var. botrytis*	Sludge and soil
10	*Spinacia oleracea*	Sludge and soil
11	*Allium cepa*	Sludge and soil
	Agricultural crops	
12	*Zea mays*	Sludge and soil
13	*Brassica juncea* (*Pusa jai kishan*)	Sludge and soil
14	*Brassica juncea (Pusa agrani)*	Sludge and soil
15	*Brassica juncea (Pusa mahak)*	Sludge and soil
16	*Brassica juncea (Pusa bold)*	Sludge and soil
17	*Brassica juncea (Pusa varuna)*	Sludge and soil
18	*Helianthus annuus*	Sludge and soil
	Grasses	
19	*Chrysopogon zizanioides*	Sludge and soil
20	*Phragmites communis*	Sludge and soil
	Other plants	Sludge and soil
21	*Sesbania bispinosa*	Sludge and soil
22	*Amaranthus spinosus*	Sludge & soil

Table 2 Elemental profile of tannery sludge collected from the sludge disposal site of CETP, Jajmau (Kanpur, Uttar Pradesh, India)

pH	Cr (mg/l)	Al (mg/l)	Co (mg/l)	Ni (mg/l)	Mn (mg/l)	Zn (mg/l)	Pb (mg/l)	Cu (mg/l)	Cd (mg/l)	Fe (mg/l)
8.07	25,762.14 ±3402.88	1,663.34± 249.42	11.98± 2.41	52.75± 5.89	186.87± 10.62	90.66± 3.27	39.83± 1.80	45.41± 1.57	1.45± 0.43	14,168.26 ± 176.06

Table 3: Details of plants, AM fungi, and filamentous saprobe fungi used for the microcosm experiments under greenhouse conditions.

S. no.	Plant	AM fungi type	Filamentous saprobe fungi type
1	*Vetiver zizanioides*	Trap culture 2 (TC2)	*A. niger*
2	*Dalbergia sissoo*	*In vitro* grown *Glomus intraradices*	*A. flavus*
3	*Melia azadirachta*	*In vitro* consortium (mixture of *Glomus intraradices, G. proliferum*, and *G. lamellosum*)	—
4	Aloe vera	—	—
5	Jatropha curcas	—	—

Salient findings

- Almost all the plants that were screened, exhibited metal accumulation property. Plants like *Tabacum nicotiana*, *Brassica oleracea*, *Spinacia oleracea*, and *Brassica juncea* were able to grow aggressively in the tannery sludge (see Figure 1).
- In general, it was observed that the indigenous AM fungi (trap culture), separately and in combination with filamentous saprobe fungi, were far more effective than the *in vitro* grown AM fungi, in terms of enhanced plant growth and Cr uptake by plants.
- In general, all plants exhibited higher Cr metal uptake in roots than in the shoot.
- From the microcosm experiment (see Figures 2 and 3), it was observed that out of five plants, the *Aloe vera* with trap culture (TC2) and *C. zizanioides* with trap culture (TC2) + *A. niger* were found to be effective for enhanced plant growth and Cr metal uptake by the plants.
- The biomass obtained in case of *Aloe vera* was 44.5 gram (in presence of TC2), and in case of *C. zizanioides*, it was 62.7 gm in presence TC2 + *A. niger*, which were significantly higher than the control treatments (non-microbial treatment).
- The colonization percentage obtained in case of *Aloe vera* was 60.2 by TC2, and in case of *C. zizanioides* was 62.18 by TC2 (in presence of *A. niger*).
- *Aloe vera* with TC2 exhibited accumulation of 486.54 mg Cr/kg of shoot and 1254.35 mg Cr/kg of root from the absolute tannery sludge.
- The *C. zizanioides* with TC2 + *A. niger* have shown the accumulation of 445.36 mg Cr/kg of shoot and 838.09 mg Cr/kg of root from the absolute tannery sludge.

Figure 1 Comparative growth of *Brassica oleracea var. capitata* in soil and sludge

Figure 2 Microcosm experiment set up and comparative growth of plants in soil and sludge as a substrate after six months of plantation. Plants were comparatively healthier and with higher biomass in case of tannery sludge than in soil

Figure 3 *Aloe vera* and *C. zizanioides* growing in absolute tannery sludge after one month (left) and after six months (right) of plantation.

Discussion and conclusions

Most of the naturally occurring plants in metal-contaminated soil did not exhibit higher application potential mainly due to their slow growth and low biomass producing ability (Kubota and Takenaka 2003). Another category of plants mentioned in the existing literature belongs to the food chain category. However, for heavy metal uptake study such plants are not suitable. Neither are they feasible options for phytoremediation of metal-contaminated soil (Singh and Sinha 2005; Rajkumar and Freitas 2008; Ma, Rajkumar, and Freitas 2009). Besides, most of the results already obtained and mentioned in existing literature are derived from pot experiments, with metal salts used as the source of heavy metals. These pot experiments are not the ideal representative of natural field conditions, under which metals usually accumulate in a less-available chemical form (Ma, Rajkumar, and Freitas 2008; Rajkumar and Freitas 2008). The present study reveals the ability of two different plants (*Aloe vera* and *C. zizanioides*) to grow and accumulate significant amount of Cr from the absolute tannery sludge (containing 25,762.14 mg/kg of Cr). Both the plants are perennial and have an aggressive ability to grow in absolute tannery sludge, even in the absence of microbial inoculants. *Aloe vera* has shown the ability to accumulate 486.54 mg Cr/kg of shoot and 1,254.35 mg Cr/ kg of root in presence of TC2; while *C. zizanioides* has shown the ability to accumulate 445.36 mg Cr/kg of shoot and 738.09 mg Cr/kg of root in presence of TC2+ *A. niger*. Both the plants accumulate a major proportion of Cr in the root. However, the amount of Cr both the plants accumulate in their shoot is also quite significant. In fact, it is higher than what is commonly reported in the upper parts of Cr-accumulator plants (Singh and Sinha 2005; Chandra, Bhargava, Yadav, *et al.* 2009). In this study, after five years of extensive greenhouse experiments, *Aloe vera* with TC2 and *C. zizanioides* with TC2 + *A. niger* were selected as the best combinations of plants and micro-organisms for possible application in the phytoremediation of Cr-contaminated soil. The interesting point that *Aloe vera* and *C. zizanioides* carry from the phytoremediation point of view is that *Aloe vera* is a shallow-rooted plant that can be used for removal of Cr from the upper layer of soil. On the other hand, *C. zizanioides* is a deep-rooted plant that can be utilized for removal of Cr from lower soil levels. The cultivation of both these plants in combination would be ideal for removal of Cr from contaminated soil. The harvested plants can be used for the recovery of pure form of Cr, as a green method of disposal.

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