MYCORRHIZA NEWS

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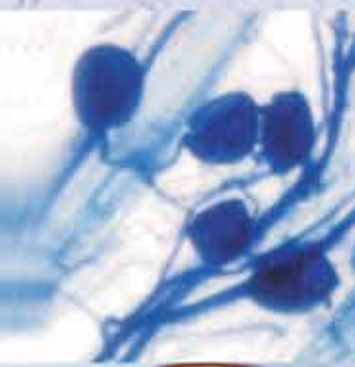

About TERI

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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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Arbuscular Mycorrhizal Status of Some Alien Forest Invasive Species Invading Hoollongapar Gibbon Sanctuary, Jorhat, Assam, India–1

Ankur Jyoti Saikia[1] and Vipin Parkash[2]

Introduction

Species which cross over their natural distribution and get introduced to new habitats are known as alien species (Saxena, 1991). Plant species that move from one geographical region to the other (either accidentally or intentionally), establish and proliferate there and threaten native ecosystems, habitats and species are known as invasive alien plants (Richardson *et al,* 2000a). Invasive alien plant species are characterized by rapid growth rates, widespread dispersal capabilities, large and rapid reproductive output along with a wide environmental tolerance. These alien plants while they adversely affect biodiversity, thus aggravating agricultural and forestry-related losses. In the Indian sub-continent, a total of 173 plant species belonging to 117 genera under 44 families were documented as invasive alien plant species, representing 1 per cent of the Indian flora (Reddy, 2008). The major invasive species in the National Parks and Wildlife Sanctuaries of Assam, India are *Mikania micrantha*, *Mimosa* sp., *Ipomea* sp. and *Chromolaena odorata* (Lahkar *et al*, 2011). Two non-grass species, namely *Leea asiatica*, an under-shrub naturalized in the grassland and *Bombax ceiba*, a tree species commonly occurring in the adjacent forest areas of the biosphere reserve have been found extensively growing in the Manas Biosphere Reserve, Assam (Baruah *et al,* 2003). However, no any such kind of report on the status of alien invasive plant species in Hoollongapar Gibbon Sanctuary in Jorhat district of Assam, India has been observed yet.

Biological invasions are among the anthropogenically-mediated perturbation threatening the native biodiversity, preventing natural ecological succession and changing the community structure and composition (Vitousek *et al*, 1996; Mack *et al*, 2000). The enormous ecological and socio-economic damage inflicted by plant invasions (Pimentel *et al,* 2005) has stimulated great interest in the possible contribution of mutualistic and antagonistic microorganisms in the success or failure of alien plants invading non-native ecosystems (Shah *et al*, 2009). For effective management of alien invasive species; knowledge about their ecology, morphology, phenology, reproductive biology, physiology and biochemistry is very essential (Raghubanshi *et al,* 2005). The factors influencing a plant's ability to invade the ecosystem are not clearly understood (Bhatt *et al*, 2012). Many mechanisms influence a plant's ability to invade. The importance of different mechanisms depends on the specific invasion. The growth and fitness of many alien plant species depends upon their mutualistic associations with soil microbes, such as arbuscular mycorrhizal (AM) fungi (Smith and Read, 1997; Richardson *et al,* 2000b).

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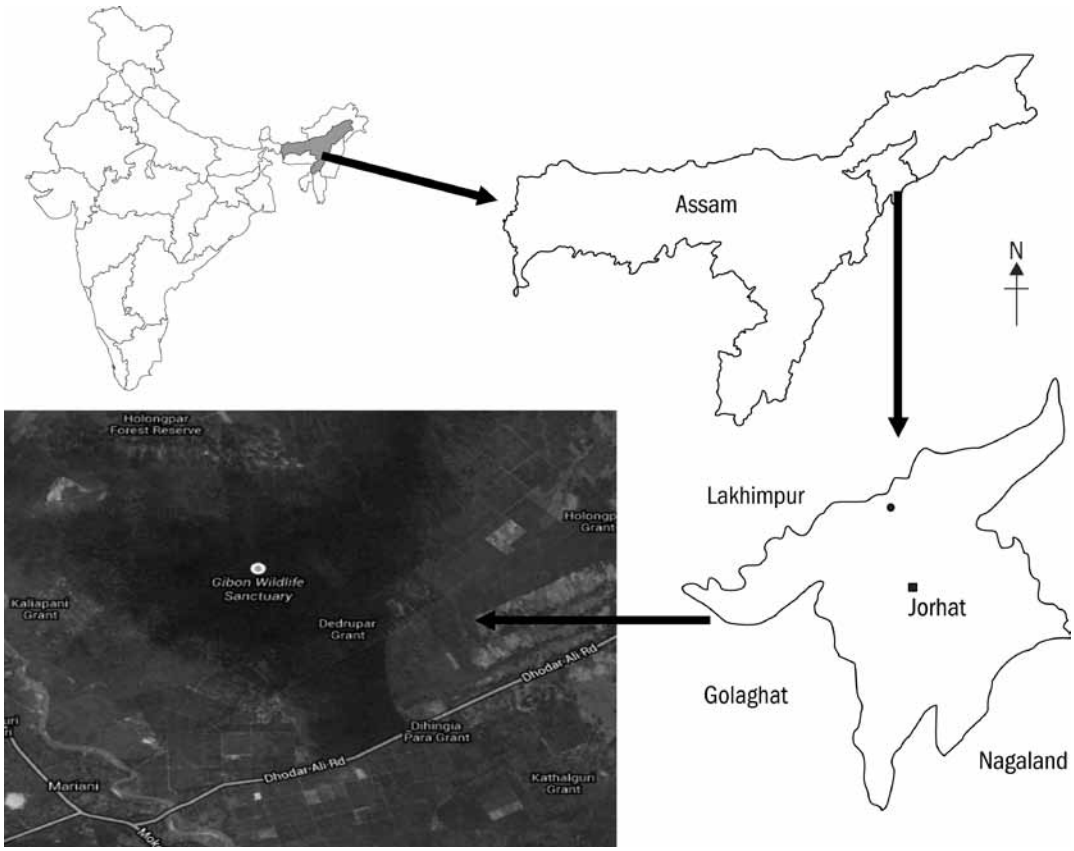
AM fungi are an important soil asexually reproducing microbial community that forms symbiotic relationship with nearly 90 percent of the flowering plants (Brundrett, 1991). It is the most ancestral and the commonest type of mycorrhizal symbiosis (Brundrett, 2002), in which the fungal hyphae penetrate the cortical cell wall of the host plant root. It is characterized by arbuscules and vesicles formed by the aseptate, obligately symbiotic fungi of the phylum Glomeromycota (Schüßler *et al,* 2001). AMF associations benefit the ecosystem by improving the soil stabilization, increasing the nutrient cycling (Bethlenfalvay and Linderman, 1992), increasing the plant diversity and productivity (Dhillion and Gardsjord, 2004), and facilitating the plant community succession (Janos, 1980; Greipsson and El-Mayas, 2000). It is estimated that more than 80 per cent of the terrestrial plant species, including invasive ones, have a symbiotic relation with the mycorrhizal fungi (Wang and Qiu, 2006).

In spite of the multifaceted role of AM fungi in determining structure and dynamics of the plant community, a few surveys exploring the mycorrhizal status of alien invasive plants have been carried out with respect to bio-geographic regions (Shah *et al,* 2009) and protected forest areas (Hazarika *et al*, 2009; Hemavani and Thippeswamy, 2013) in India. In this context, the extent and type of AM fungal occurrence in alien plant species in the Hoollongapar Gibbon Sanctuary, Jorhat district of Assam, India was evaluated.

Materials and Methods

Study area and field survey

Field surveys were conducted in the Hoollongapar Gibbon Sanctuary (26°45'00"N and 94°25'00"E) located in the Mariani Forest Range, Jorhat district of Assam, India (Figure 1). The region has an area of about 2,098.62 ha within an altitudinal range of 100-120 m above sea level. The annual rainfall is 1870.5 mm and the average maximum and minimum temperatures are 20.15 °C and 28.75 °C, respectively. The sanctuary is a tropical moist deciduous diterocarp-dominant forest and its floral diversity is represented by 489 plant species (Chetry *et al.* 2007). During 2013, specimens of three alien plant species *viz.* - *Ageratum conyzoides* L., *Chromolaena odorata* (L.) R.M. King & H. Rob. and *Mikania micrantha* Kunth. (Plate 1) belonging to Asteraceae family, were collected from different compartments. They were identified with the help of the herbaria of Rain Forest Research Institute and were examined for the extent and type of endomycorrhizal colonization. Among the



Figures 1: Map of study site (Hoollongapar Gibbon Sanctuary, Jorhat district of Assam, India)

three invasive plant species investigated in the present study, *Chromolaena odorata* and *Mikania micrantha* were the high risk species, while *Ageratum conyzoides* was a low risk species (Sanjeev *et al*, 2012).

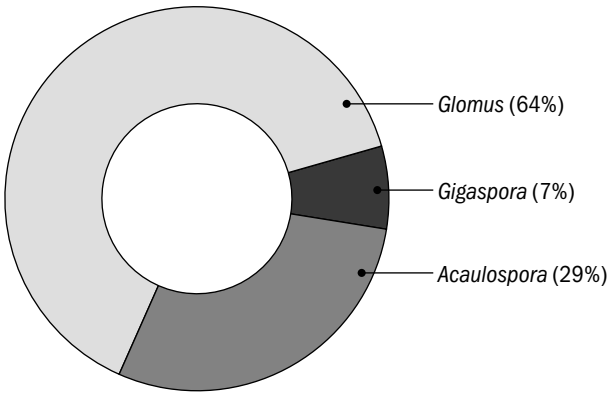
Mycorrhizal analysis

Isolation, quantification and root colonization of VAM spores was done by using wet sieving and decanting technique (Gerdemann and Nicolson, 1963). The quantitative estimation of AM spores was done by modified method of Adholeya and Gour (1994). To study the colonization of Arbuscular Mycorrhizae, the rapid clearing and staining method by Phillips and Hayman (1970) was employed. The AM fungi were identified using the keys of Trappe (1982), Walker (1983), Schenck and Perez (1990), Morton and Benny (1990), Mukerji (1996), Morton and Redecker (2001) and Sharma *et al.* (2008, 2009). They were also identified using websites (Morton, 2013; Blaszkowski, 2003; Parkash, 2013).

Results and Discussion

Qualitative analysis revealed that the arbuscular mycorrhizae found in the rhizospheric soil of *Mikania micrantha* Kunth. belongs to three genera viz. *Glomus*, *Acaulospora* and *Gigaspora*. Among these the maximum spores belonged to *Glomus*, while minimum spores belonged to the genus, *Gigaspora*. In the rhizospheric soil sample of *Chromolaena odorata*, spores of *Acaulospora* sp. showed the maximum presence, while *Glomus* spp. were in low numbers. In the rhizospheric soil sample of *Ageratum conyzoides*, *Acaulospora* sp. were in very high number than *Glomus* sp. (Figures 2, 3 and 4; Plate 1).

Maximum percentage of hyphal colonization (100 per cent) was observed in *Ageratum conyzoides* and *Chromolaena odorata*, while minimum was observed in *Mikania micrantha* (60 per cent). Highest percentage of arbuscular and vesicular infection was found in *A. conyzoides* (60 and 50 per cent, respectively) followed by *C. odorata* (50 per cent and 20 per cent). No arbuscular or vesicular infection was observed in *M. micrantha*. The quantification of AM spore numbers per 50 g of soil was highest in *A. conyzoides* (175±9.42), followed by *M. micrantha* (132±7.07). *C. odorata* represented the lowest spore quantity (73±8.48) (Figure 5; Plate 1).



Figures 2: Generic diversity of AM fungi associated with *Mikania micrantha* Kunth

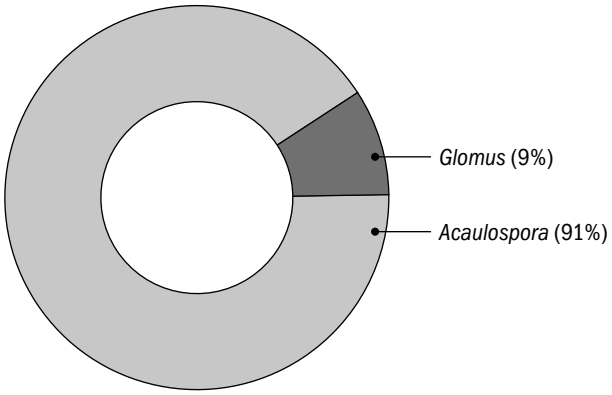


Figure 3: Generic diversity of AM fungi associated with *Chromolaena odorata* (L.) R.M. King & H. Rob

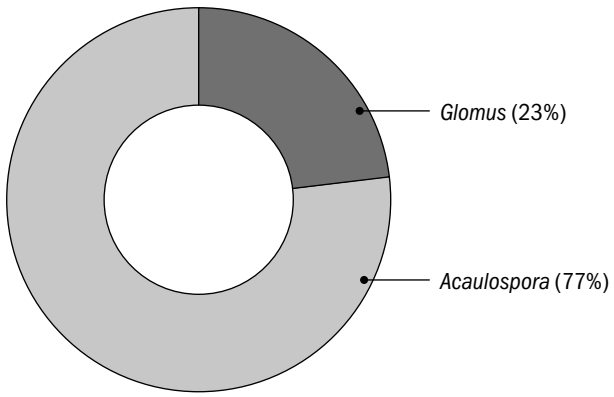


Figure 4: Generic diversity of AM fungi associated with *Ageratum conyzoides* L.

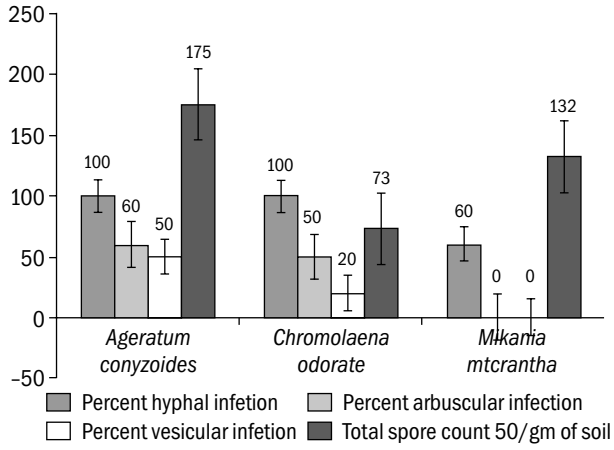


Figure 5: Percent root infection of *Ageratum conyzoides* L., *Chromolaena odorata* (L.) R.M. King & H. Rob. and *Mikania micrantha* Kunth.

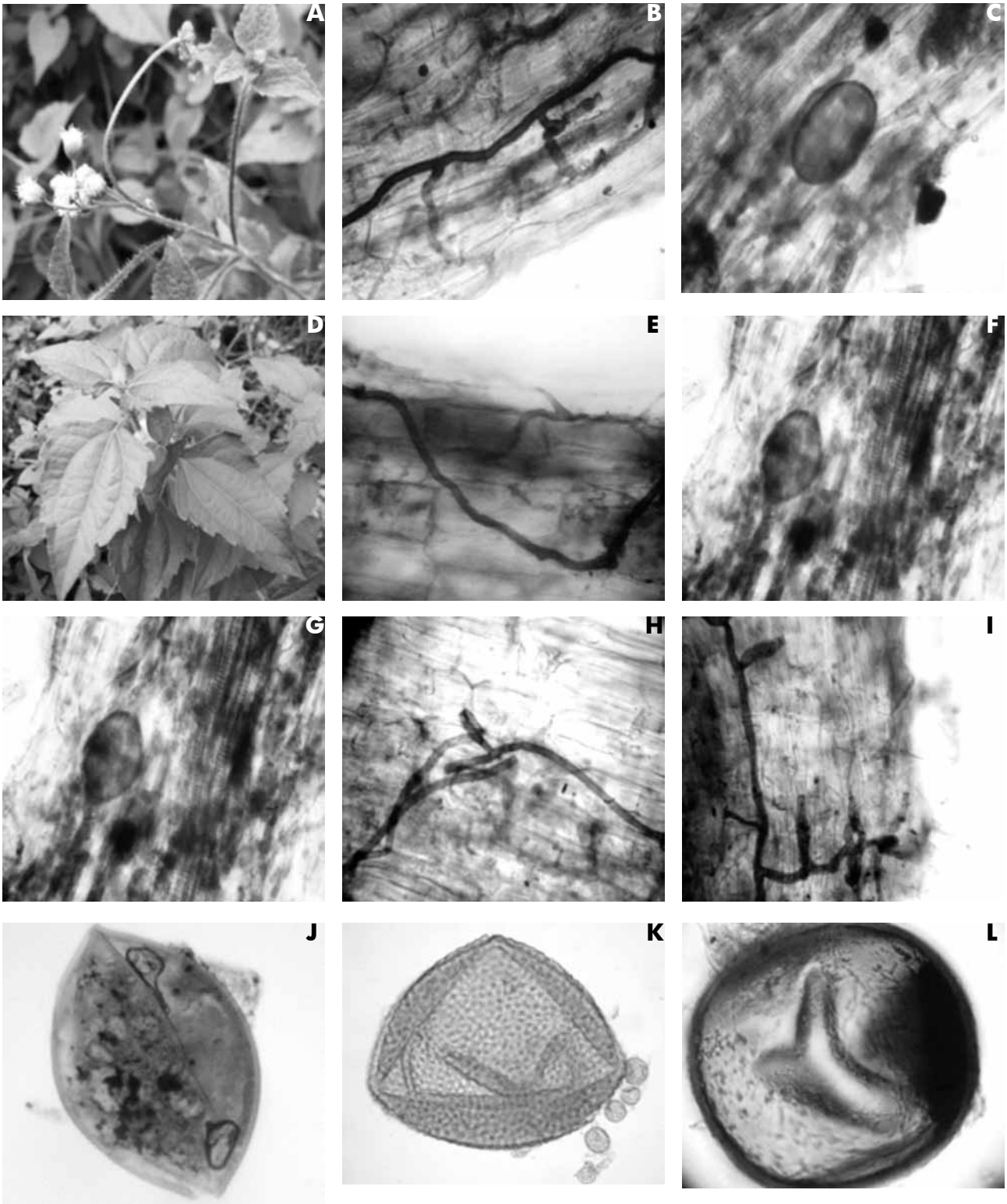


Plate 1

A- *Ageratum conyzoides*, B- Hyphal infection in *A. conyzoides*, C- Arbuscule in *A. conyzoides*, D- *Chromolaena odorata*, E- Hyphal infection in *C. odorata*, F- Arbuscule in *C. odorata*, G- *Mikania micrantha*, H- Hyphal infection in *M. micrantha*, I- J shaped Hyphal infection in *M. micrantha*, J- *Glomus* sp. Tulasne & Tulasne, K- *Acaulospora scrobiculata* Trappe, L- *Acaulospora* sp. Gerd. & Trappe emend. Berch.

The findings of this study also divulged the common occurrence of Arum-type AM association in the investigated invasive plant species which showed similar results as observed by earlier researchers (Yamoto, 2004; Shah *et al*, 2009). They also suggested that Arum-type AM colonization may be held responsible for the fast-growing nature of invasive plant species.

Alien species are more mycorrhizal than native species in United Kingdom (Fitter, 2005); although Page *et al.* (2006) and Pringle *et al.* (2009) have reported opposite results for invasive species in Canada and California, respectively. It was observed that in contrast to the observations of Vogelsang *et al.* (2004) that naturalized alien plants are poor hosts of AM fungi; *Ageratum conyzoides* and *Chromolaena odorata*, were found to be highly endomycorrhizal in this study. Some recent studies have shown an enhanced invasiveness of some alien plant species through association with AM fungi (Fumanal *et al*, 2006; Shah and Reshi, 2007; Shah *et al,* 2008). The presence of a fine root system in *A. conyzoides* and *C. odorata* facilitates higher AM fungal colonization (Zangaro *et al*, 2005). Enhanced colonization helps in the establishment and survival of host plants (Zangaro *et al,* 2003) in disturbed habitats. The absence of arbuscular and vesicular infection in *Mikania micrantha*, categorizes it as a facultative symbiont or mycoheterotroph. However, colonization by AMF depends on many other factors such as host plant species phenology (Sanders and Fitter 1992a, 1992b), AMF diversity and species composition within different habitats and communities (Gange *et al,* 1990), or/and seasonal and ontogenetic variations (Jakobsen *et al,* 2002). Therefore, the intensity of AMF colonization in roots can be influenced by specific habitat conditions (Štajerová *et al*, 2009). Root colonization by AM fungi, is generally very low or absent during the first few weeks of rapid root growth following emergence. The levels of root colonization reach a maximum when growth of root systems slows at the end of the growing season (Gemma, 1987; Sutton, 1973).

Pringle *et al.* (2009) have discussed that a nonmycorrhizal or facultatively mycorrhizal plant (regardless of whether the plant is specific or obligate, and the mycorrhizal association is transportable or not transportable) can successfully invade any geographical region. On the other hand, obligative and specific mycorrhizal plants failed to establish or spread within a novel habitat unless they are moved with intact symbioses. An obligate mycorrhizal plant may also successfully invade, if it is flexible and can associate with the local fungi. Finally, a plant that obligately requires the mycorrhizal association but is both flexible and also moved with intact symbioses is very likely to be a successful invasive species. In other words, invasion is facilitated when organisms are not associated in mutualism, or are facultative mutualists. Invasion may be hindered if the organism (here, invasive plant) is associated in an obligative mutualism. Zhang *et al.* (2010) demonstrated that soil occupancy by an invasive herb causes changes in the composition of AMF communities that favour growth of that invasive species over certain natives in both monoculture and in mixture. Invasive mycorrhizal plants do not have a competitive advantage over native species, unless they utilize the mycorrhizal symbiosis in an unusual way (Richardson *et al,* 2000a). Such a mechanism can determine their success and affects the resident plant community and ecosystem functioning (Callaway *et al*, 2004).

In conclusion, the present study highlights the inadequacy of data regarding mycorrhizal status of invasive alien species occurring in the undisturbed and reserved forest areas. It stressed the need for detailed investigation of the mycorrhizal status of *Mikania micrantha*, *Ageratum conyzoides*, *Chromolaena odorata* and other invasive plants in diverse bio-geographical regions and different protected forest areas of the Indian subcontinent for better understanding of the multi-faceted role or mechanism of AM fungi colonization in alien plant invasions**.** The understanding of these associated AM fungal species may be useful in developing effective eco-restoration management practices.

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Microbial Restoration of Degraded Lands through Plantation Forests

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Introduction

The mining lands are chemically, physically, and biologically unstable and deficient in nutrients. Plantation is the most effective technique to reclaim mined overburdens and to restore the land productivity through microbial population. Mycorrhizal fungi play an important role in facilitating the survival and growth of plant species. Vesicular Arbuscular Mycorrhiza helps in the eco-restoration by enabling a host plant to establish itself in degraded soil, thereby improving soil quality and health (phytoremediation). This eco-friendly biotechnology of using VAM fungi as biofertilizer has been considered worldwide for better management, survival, and sustainability of the forest tree seedlings in the nutrient deficient soil of the tropical and subtropical countries (Klironomos *et al,*2000; Bever *et al,* 2001; Burrows and Pfleger, 2002a, 2002b; O'Connor *et al,* 2002). Further, AM fungi are capable of increasing the soil nutrients and restoring native microflora (Brown, 2002; Chaubey *et al,* 2012, 2013). The present paper deals with the microbial restoration of degraded coal mine soil through establishment of multipurpose tree species, viz., *Acacia mangium, Pithecellobium dulce, Emblica officinalis, Eucalyptus camaldulensis, Acacia auriculiformis, Prosopis juliflora, Leucaena leucocephala* and *Dendrocalamus strictus* in different age group plantations in Northern Coalfield Limited, Singrauli.

Materials and Methods

Singrauli (24°46' 60"– 24°78' 33"N, 82° 49' 59"– 82° 83' 30"E, 275 –500m AMSL) was granted a district status on May 24, 2008, with its headquarters at Waidhan. It has a tropical climate with a mean maximum and minimum temperature of 48 °C and 21°C, respectively. The area receives an average rainfall of 1,000 mm. Large forest areas were cleared as a result of mining. The present study covers artificial plantations raised in the mined out Northern Coalfield Limited (NCL) area. Plantations of different age groups were raised in the eight project sites of NCL Singrauli, viz., Jayant, Dudhichua, Amlohri, Jhingurdah, Nigahi, Bina, Kakari, and Khadia opencast projects. The last three projects areas of Bina, Kakari, and Khadia are located in Uttar Pradesh, while the first five project areas are in Madhya Pradesh. A large number of over-burdened mixed and monoculture plantations are raised by MP State Forest Development Corporation and UP Forest Department in different areas of Singrauli coalfields. The species planted were mainly *Acacia catechu* (L. f.) Willd, *A. mangium* Willd, *A. nilotica* (L.) Del., *Aegle marmelos* (L.) Correa, *Albizia procera* (Roxb.) Benth., *Anthocephalus kadamba (*Roxb.) Miq., *Azadirachta indica* A. Juss., *Bauhinia variegata* Linn., *Bombax ceiba* (L.) Gaertn, *Cassia fistula* L., *C. siamea* Lamk., *Dalbergia sissoo* Roxb. ex DC., *Delonix regia* (Hook.)Raf., *Emblica officinalis* Gaertner., *Eucalyptus camaldulensis* Dehnh., *Gmelina arborea* Roxb., *Holoptelia integrifolia* (Roxb.), *Leucaena leucocephala* (Lam.) de Wit, *Madhuca indica* Roxb., *Mangifera indica* L., *Peltaphorum* sp *(Miquel)* Kurz, *Pongamia pinnata* (L.) Pierre, *Prosopis juliflora* (Sw.) DC., *Syzygium cumini* (L.) Skeels, *Tectona grandis* L. f., *Terminalia arjuna* (Roxb.) Wight & Arn., *T. belerica* (Gaertner) Roxb.,etc. The plants were placed at a distance of 2m in rows. A distance of 1.5m should be maintained between the rows. The plants were raised in pits of size 45cm^3. No amendment was given during plantations except farm yard manure (FYM), urea, and aldrin as soil insecticide. Six months old poly-potted seedlings were taken for plantations.

To analyse the influence of tree cover on biological properties, soil samples from the rhizosphere of different species were collected from the surface soil up to a depth of 30cm. Five surface soil samples of each species from age series of plantations were collected and mixed thoroughly to get a composite sample for each plantation. These samples were divided into five sub-samples/replicates for analysis of microbial properties, such as density of AM fungi, percent root colonization, and microbial biomass. Chloroform fumigation extraction method by Carter (1991) was used to estimate microbial biomass. The microbial biomass was expressed on oven-dried (105° C for 24 hours) soil samples. VAM spore count and VAM root infections were assessed for rhizospheric soil of different multipurpose tree species planted on different overburdened mine sites. Live spores were extracted from 100gm of rhizospheric soil using the method suggested by Gerdemann and Nicholson (1963). The root samples were properly washed under running tap water and cleared by boiling in 10 per cent aqueous KOH (Potassium Hydroxide) solution for 24 hours. The roots were again washed and stained by trypan blue, followed by washing in distilled water.

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They were then mounted on lactophenol mountant (Philip and Hayman, 1970). Usual microscopic techniques were followed to examine the root infection in different plant species; also the percentage root infection with AM fungi was calculated.

Results

The results of biological restoration in terms of AM fungi density, percent root colonization with AM fungi, and microbial biomass are given in Table1 for all the eight species, namely, *Acacia mangium, Pithecellobium dulce, Emblica officinalis, Eucalyptus camaldulensis, Acacia auriculiformis, Prosopis juliflora, Leucaena leucocephala,* and *Dendrocalamus strictus*.

Acacia mangium Willd, belonging to family Mimosaceae, is a multipurpose tree species. The status of AM fungi density, percent root colonization, and microbial biomass increased with the age group of plantations. The density of AM spores in the rhizosphere of different age group plantations was found to be 95 spores g^{-1} of soil in 1 to 5 years old plantation, 116 spores g^{-1} of soil in 6 to 10 years old plantation, 140 spores g^{-1} of soil in 11 to 15 years old plantation, and 176 spores g^{-1} of soil in 15 to 20 years old plantation. The percent root colonization was found to be 25 (1 to 5 years old plantation), 32 (6 to 10 years old plantation), 48 (11 to 15 years old plantation), and 52 percent (15 to 20 years old plantation), respectively. Similarly, there is a growth in the microbial biomass with the age group of plantations. It varied as 24.3mg kg^{-1} (1 to 5 years old plantation), 25.4 mg kg^{-1} (6 to 10 years old plantation), 29.6 mg kg^{-1} (11 to 15 years old plantation), and 33.7 mg kg^{-1} (15 to 20 years old plantation), respectively (Table 1).

The density of AM fungi, percent root colonization, and microbial biomass surrounding the roots of *Pithecellobium dulce* (Roxb.) Benth, member of family Mimosaceae, were also recorded (Table 1). The density of AM spores in the rhizosphere of different age group plantations varied as 105 spores g^{-1} of soil in 1 to 5 years old plantation, 117spores g^{-1} of soil in 6 to 10 years old plantation, 163 spores g^{-1} of soil in 11 to 15 years old plantation, and 230 spores g^{-1}in 15 to 20 years old plantation. The per cent root colonization was found to be 29 (1 to 5 years old plantation), 34 (6 to 10 years old plantation), 51 (11 to 15 years old plantation), and 57 percent (15 to 20 years old plantation), respectively. The microbial biomass showed an increase with the age group of plantations as 25.0 mg kg^{-1} (1 to 5 years old plantation), 29.4 mg kg^{-1} (6 to 10 years old plantation), 32.6 mg kg^{-1} (11 to 15 years old plantation), and 38.5 mg kg^{-1} (15 to 20 years old plantation), respectively.

Emblica officinalis L, belonging to family Euphorbiaceae, showed a successive growth in the AM fungi density, percent root colonization, and microbial biomass with the increasing age group of plantations. The density of AM spores in the rhizosphere of different age group plantations increased as 113 spores $g^{-1}$of soil in 1 to 5 years old plantation, 125 spores $g^{-1}$of soil in 6 to 10 years old plantation, 205spores $g^{-1}$of soil in 11 to 15 years old plantation, and 242 spores g^{-1} of soil in15 to 20 years old plantation. The percent root colonization was found to be 33 (1 to 5 years old plantation), 46 (6 to 10 years old plantation), 53 (11 to 15 years old plantation), and 61 percent (15 to 20 years old plantation), respectively. The microbial biomass varied as 26.7 mg kg^{-1} (1 to 5 years old plantation), 31.3 mg kg^{-1} (6 to 10 years old plantation), 35.6 mg kg^{-1} (11 to 15 years old plantation), and 40.3 mg kg^{-1} (15 to 20 years old plantation), respectively (Table 1).

Eucalyptus camaldulensis Dehnh is the member of the family Myrtaceae. It is also known as the River Red Gum. Though it is available in many parts of the world, it is native to Australia. The results (Table 1) indicated that the density of AM fungi, percent root colonization, and microbial biomass were increasing with the age group of plantations. The density of AM spores in the rhizosphere of different age group plantations was recorded as 120 spores $g^{-1}$of soil in1 to 5 years old plantation, 164 spores $g^{-1}$of soil in6 to 10 years old plantation, 217 spores $g^{-1}$of soil in11 to 15 years old plantation, and 250 spores g^{-1} of soil in 15 to 20 years old plantation. The percent root colonization was found to be 38 (1 to 5 years old plantation), 49 (6 to 10 years old plantation), 55 (11 to 15 years old plantation), and 63 percent (15 to 20 years old plantation), respectively. The microbial biomass also improved with the age group of plantations and was found as 28.6mg kg^{-1}(1 to 5 years old plantation), 32.4 mg kg^{-1}(6 to 10 years old plantation), 37.3 mg kg^{-1}(11 to 15 years old plantation), and 43.2 mg kg^{-1} (15 to 20 years old plantation), respectively.

Acacia auriculiformis A Cunn ex Benth, a member of family Mimosaceae, is also raised on plantations for fuel wood throughout Southeast Asia, Oceania, and Sudan. Its wood is good for making paper, furniture, and tools. It contains tannin, useful in animal hide tanning. In India, its wood and charcoal are widely used for fuel. Gum from the tree is commercially sold, but it is said not to be as useful as gum arabic. Extract of *Acacia auriculiformis* heartwood inhibits wood attacking fungi. The result (Table 1) indicated that the density of AM fungi, percent root colonization, and microbial biomass were increasing with the age group of plantations. The density of AM spores was recorded as 126 spores g^{-1} of soil in 1 to 5 years old plantation, 176 spores $g^{-1}$of soil in 6 to 10 years old plantation,

Table 1: Density of AM fungi, percent root colonization, and microbial biomass in rhizosphere of various species in different age group plantations at NCL, Singrauli

S. No.	Species	Parameters	Age group of plantations (Years)			
			1 to 5	6 to 10	11 to 15	16 to 20
1.	*Acacia mangium*	Density of AM fungi (No. of spores g^{-1} of soil)	95 (±22.56)	116 (±15.43)	140 (±36.92)	176 (±37.19)
		Percent root colonization with AM fungi	25 (±3.53)	32 (±4.74)	48 (±6.24)	52 (±5.52)
		Microbial biomass (mg kg^{-1})	24.3 (±2.01)	25.4 (±1.73)	29.6 (±3.01)	33.7 (±2.74)
2.	*Pithecellobium dulce*	Density of AM fungi (No. of spores g^{-1} of soil)	105 (±8.92)	117 (±18.14)	163 (±31.76)	230 (±28.26)
		Percent root colonization with AM fungi	29 (±2.12)	34 (±3.39)	51 (±3.80)	57 (±5.62)
		Microbial biomass (mg kg^{-1})	25.0 (±1.53)	29.4 (±2.39)	32.6 (±2.36)	38.5 (±1.60)
3.	*Emblica officinalis*	Density of AM fungi (No. of spores g^{-1} of soil)	113 (±31.40)	125 (±15.81)	205 (±31.45)	242 (±32.62)
		Percent root colonization with AM fungi	33 (±3.53)	46 (±5.74)	53 (±4.63)	61 (±4.64)
		Microbial biomass (mg kg^{-1})	26.7 (±2.59)	31.3 (±2.26)	35.6 (±3.65)	40.3 (±2.47)
4.	*Eucalyptus camaldulensis*	Density of AM fungi (No. of spores g^{-1} of soil)	120 (±38.47)	164 (±24.62)	217 (±45.83)	250 (±35.31)
		Percent root colonization with AM fungi	38 (±2.73)	49 (±6.89)	55 (±3.53)	63 (±4.41)
		Microbial biomass (mg kg^{-1})	28.6 (±2.18)	32.4 (±2.20)	37.3 (±2.19)	43.2 (±3.23)
5.	*Acacia auriculiformis*	Density of AM fungi (No. of spores g^{-1} of soil)	126 (±36.06)	176 (±26.11)	223 (±36.99)	252 (±47.27)
		Percent root colonization with AM fungi	42 (±3.39)	50 (±3.16)	58 (±6.63)	65 (±3.80)
		Microbial biomass (mg kg^{-1})	30.0 (±2.03)	34.4 (±3.01)	39.8 (±3.21)	43.8 (±3.22)
6.	*Prosopis juliflora*	Density of AM fungi (No. of spores g^{-1} of soil)	130 (±34.48)	185 (±26.79)	225 (±33.41)	270 (±37.50)
		Percent root colonization with AM fungi	47 (±2.44)	52 (±4.12)	59 (±4.94)	67 (±5.43)
		Microbial biomass (mg kg^{-1})	30.2 (±2.44)	35.4 (±2.76)	40.4 (±2.22)	44.6 (±3.53)
7.	*Leucaena leucocephala*	Density of AM fungi (No. of spores g^{-1} of soil)	170 (±38.98)	200 (±35.27)	229 (±34.33)	289 (±41.61)
		Percent root colonization with AM fungi	48 (±4.06)	53 (±3.39)	61 (±4.94)	70 (±4.95)
		Microbial biomass (mg kg^{-1})	31.6 (±2.77)	36.7 (±3.07)	41.3 (±1.95)	45.6 (±2.57)
8.	*Dendrocalamus strictus*	Density of AM fungi (No. of spores g^{-1} of soil)	181 (±31.32)	245 (±33.35)	280 (±44.54)	295 (±31.59)
		Percent root colonization with AM fungi	61 (±2.91)	63 (±6.24)	69 (±6.78)	76 (±5.52)
		Microbial biomass (mg kg^{-1})	36.0 (±2.48)	40.0 (±2.21)	42.6 (±2.19)	47.8 (±2.37)

223 spores $g^{-1}$of soil in 11 to 15 years old plantation, and 252 spores $g^{-1}$of soil in 15 to 20 years old plantation. The percent root colonization was found to be 42 (1 to 5 years old plantation), 50 (6 to 10 years old plantation), 58 (11 to 15 years old plantation), and 65 percent (15 to 20 years old plantation), respectively. The microbial biomass showed growth as 30.0 mg kg^{-1}(1 to 5 years old plantation), 34.4 mg kg^{-1}(6 to 10 years old plantation), 38.8 mg kg^{-1} (11 to 15 years old plantation), and 43.8 mg kg^{-1} (15 to 20 years old plantation), respectively.

Prosopis juliflora (Sw.) DC., of family Mimosaceae, is used in the forage, wood, and environmental management. The plant possesses an unusual amount of mesquitol, a form of flavan-3-ol in its heartwood. *Prosopis juliflora* is a fast growing, deciduous tree or shrub that is thorny and has a wide crown and deep roots. This specie grows in very hot and dry climates, with temperatures up to 48 °C and annual precipitation from 150 to 750 mm. It is found around 1500m above the sea level. The results (Table 1) depicted an increase in the AM fungi density, percent root colonization, and microbial biomass of different age group plantations as 130 spores $g^{-1}$of soil in 1 to 5 years old plantation), 185 spores $g^{-1}$of soil in 6 to 10 years old plantation), 225 spores $g^{-1}$of soil in 11 to 15 years old plantation), and 270 spores g^{-1} of soil in15 to 20 years old plantation. The per cent root colonization was found to increase as 47 (1 to 5 years old plantation), 52 (6 to 10 years old plantation), 59 (11 to 15 years old plantation), and 67 per cent (15 to 20 years old plantation), respectively. With the age group of plantations the microbial biomass increased as 30.2 mg kg^{-1}(1 to 5 years old plantation), 35.4 mg kg^{-1}(6 to 10 years old plantation), 40.4 mg kg^{-1}(11 to 15 years old plantation), and 44.6 mg kg^{-1} (15 to 20 years old plantation), respectively.

Leucaena leucocephala (Lam.) de Wit, belonging to family Mimosaceae, is a fast-growing tree native to southern Mexico and northern Central America (Belize and Guatemala), but is now naturalized throughout the tropics. It is known as Subabool in India. *L. leucocephala* is used for a variety of purposes, such as firewood, fiber, and livestock fodder. The results (Table 1) indicated that the AM fungi density, percent root colonization, and microbial biomass were increasing with the age group of plantations. The density of AM spores in the rhizosphere of different age group plantations varied as 170 spores $g^{-1}$of soil in 1 to 5 years old plantation, 200 spores $g^{-1}$of soil in 6 to 10 years old plantation, 229 spores $g^{-1}$of soil in 11 to 15 years old plantation, and 289 spores $g^{-1}$of soil in 15 to 20 years old plantation. The percent root colonization was found to be 48 (1 to 5 years old plantation), 43 (6 to 10 years old plantation), 61 (11 to 15 years old plantation), and 70 per cent (15 to 20 years old plantation), respectively. The microbial biomass increased as 31.6 mg kg^{-1}(1 to 5 years old plantation), 36.7 mg kg^{-1} (6 to 10 years old plantation), 41.3 mg kg^{-1} (11 to 15 years old plantation), and 45.6 mg kg^{-1} (15 to 20 years old plantation), respectively.

Dendrocalamus strictus (Roxb.) Nees, belonging to family Poaceae, is also known as green gold, poor man's timber, and friends of the poor. It occupies an unparalleled position in the plant kingdom in terms of its distribution, diversity, and usage in the tropics and subtropics. It is a fast growing woody plant found almost all over in India, except the Kashmir Valley. It has more than 1,500 documented uses. It is widely used to manufacture paper and rayon, and in construction, architecture, engineering, handicraft, food, and medicine. The results (Table 1) indicated an increase in the AM fungi density, percent root colonization, and microbial biomass with the age group of plantations. The density of AM spores in the rhizosphere of different age group plantations was found to be 181 spores $g^{-1}$of soil in 1 to 5 years old plantation, 245 spores $g^{-1}$of soil in 6 to 10 years old plantation, 280 spores $g^{-1}$of soil in 11 to 15 years old plantation, and 295 spores g^{-1} of soil in 15 to 20 years old plantation. The percent root colonization was recorded as 61 (1 to 5 years old plantation), 63 (6 to 10 years old plantation), 69 (11 to 15 years old plantation), and 76 percent (15 to 20 years old plantation), respectively. Similarly, microbial biomass varied with the age group of plantations as 36.0 (1 to 5 years old plantation), 40.0 (6 to 10 years old plantation), 42.6 (11 to 15 years old plantation), and 47.8 mg kg^{-1} (15 to 20 years old plantation), respectively.

Discussion

Soil microorganisms play a significant role in soil fertility and ecosystem functioning. Soil microbial biomass measurements were useful in determining the degree of disturbance as well as the subsequent recovery of degraded ecosystems. Rhizospheric microorganisms, particularly mycorrhizal fungi, promote establishment of plant species on mine spoil by immobilizing the heavy metal in the soil, there by restoring the fertility of mine spoil, leading to the development of aself-sustainable ecosystem. The AM fungi production *in vitro* has already been commercialized by TERI, New Delhi for reclamation of degraded lands by fly ash (Adholeya, 2003; Sharma and Adholeya, 2005). The density of AM fungi and percentage of AM root colonization are showing increasing trend from younger age group to older age group plantations in different multipurpose tree

species. Similarly, there was a gradual increase in microbial biomass from younger age group to older age group plantations. It was primarily due to increase in the microbial population in older plantations, as the availability of organic matter and humus had increased over a period of time. In younger age group plantations, the AM spores gradually decreased due to lesser availability of nutrients. Similarly, colonization of AM spores in the roots was achieved by all five genera of AM fungi including *Acaulospora* sp., *Glomus* sp., *Gigaspora* sp., *Scutellospora* sp., and *Sclerocystis* sp. Out of these, *Acaulospora* and *Glomus* showed a high frequency of colonization. The spore population was more in *Acaulospora* and *Glomus* compared to *Gigaspora* and *Scutellospora* species. *Sclerocystis* was recorded in a few samples, especially in older plantations. In terms of AM density, root colonization with AM fungi, and microbial biomass, *Dendrocalamus strictus* proved the most promising species, followed by *Leucaena leucocephala, Prosopis juliflora, Acacia auriculiformis, Eucalyptus camaldulensis, Emblica officinalis, Pithecellobium dulce,* and *Acacia mangium*. These species help in restoring the coal-mine spoil and enhancing the soil development. The findings were comparable with the observations recorded by Daft and Nicoloson (1974), Gupta and Shukla (1991), Jamaluddin and Chandra (2009), and Chaubey *et al* (2012, 2013) in different studies regarding VAM status and microbial biomass. A wide variation in the percent colonization, intensity of structural colonization, percent population, and the distribution of AM fungal spores may be the results of variable host susceptibility (Mehrotra, 1998), diverse type of AM fungi in the rhizosphere soils of individual plant species, host efficiency in soil resource capture and utilization (Koide, 1991; Clark and Zeto, 2000), soil types and quality (Raman and Gopinathan, 1992), root morphology (Hetrick, 1991) and mycorrhizal dependency of different host plants, and other edapho-climatic factors (Fontenla *et al.*, 1998; Abbott and Robson, 1991). The AM fungal colonization and subsequent spore production depend on the type of host as well as on the duration of infection of these symbiotic organisms. Generally, with increase in the growth period after infection, the host root colonization increases with little effect on spore production. It is the host type which is more important for spore production (Bever *et al.*, 1996; Eom *et al.*, 2000). The higher colonization and spore count in *D. strictus* may be due to the effectiveness of AM fungal consortium developed in the root morphology of this host. The improvement in AM fungal consortium would improve both above ground as well as below ground biomass (Gupta and Janardhanan, 1991; Silveira *et al.*, 2006). Similar effect has been observed in different bamboo species, including *D. strictus*, grown under tropical conditions (Ravikumar *et al.*, 1997; Muthukumar and Udaiyan, 2006).

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Morphotaxonomy of *Funneliformis mosseae* (accession CMCC/AM-1408)

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In continuation to our previous series of editorial regarding the morphotaxonomic studies of our successfully isolated and established monosporals, in this article we shall take up the study of **accession number CMCC/AM-1408.** This culture has been isolated and raised from the soils of Gual-Pahari, Haryana, India. The soil sample was made into a suspension and was passed through sieves of 60, 100, and 300 BSS mesh sizes (British Standard Size), respectively, in order to capture the mycorrhizal diversity of varying diameter. The sieving from each group was collected separately and critically observed with the help of a stereo-zoom microscope.

Morphologically similar looking and healthy spores were isolated. About 50 monosporals were initiated in order to obtain pure single species culture of AM fungi. The morphotaxonomic analysis of the spore and its wall layers, hyphal attachment, etc., was done on visible basis under compound microscope after preparing voucher specimens. Two kinds of mounting agents (Polyvinly Lacto glycerol and Polyvinly Lacto glycerol: Melzer's reagent at the ration of 1:1) were used for the studies.

Monosporals are raised using single spores along with solitary pre-germinated seed of a suitable host plant grown in micro tips. These cultures are then evaluated for their success rate after an interval of three months and beyond. The culture is considered to be pure when the spores isolated from them are morophotaxonomically are similar to the voucher specimen prepared from the mother cultures that were used during the initiation of the monosporals. As presented in Table 1, it took almost ten months for accession number CMCC/AM-1408 to set up with high spore count and good percentage colonization. Investigation shows that among all the generated monosporals, these cultures can be distinguished in the category of high sporulating cultures preserved in the CMCC bank.

Table 1: Spore count of accession CMCC/AM-1408 checked at different time intervals

Activity	Date	Spore count
Date of initiation	7 August, 2012	Single spore
Date of fourth checking	12 June, 2013	6 to 10 spore/gm
Date of fifth checking	5 November, 2013	20 to 55 spore/gm

Now for the reader we would enlist each of the characteristic parameters of this accession that were critically observed to reach the morphotaxonomic identification of this culture:

Colonization Assessment

Both intraradical and extraradical hyphae along with arbuscules and vesicles were distinctly visible in the colonized root. When stained with 3 % Ink vinegar, it produced a dark colour (Figure 1). Profuse coiling is observed near most of the entry point (Figure 2). On an average the hyphal width ranges between 4 µm to 6 µm.

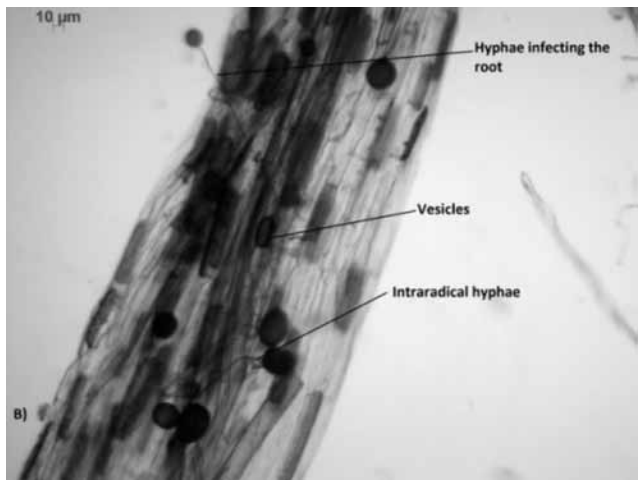

Figure 1: Stained images of root showing characteristic structures of AM Fungi

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\# Director, Biotechnology and Bioresources, Centre for Mycorrhizal Research, The Energy and Resources Institute, Darbari Seth Block, IHC Complex, Lodhi Road, New Delhi-110 003, India

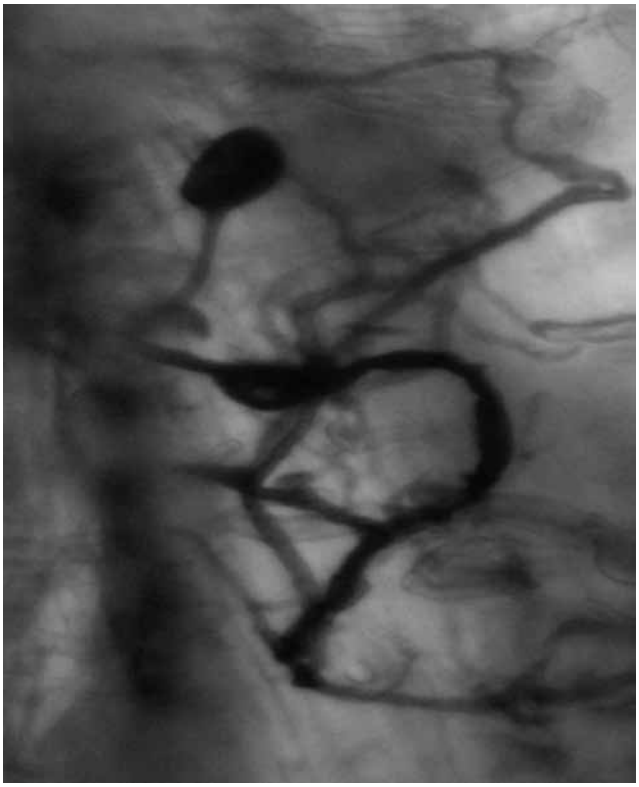

Figure 2: Profuse coiling near the entry point

Spore Morphology Assessment

Spores isolated from the culture were devoid of any sporocarp. They were single, large, dark yellowish to brown coloured structure (Figures 3a and 3b).

Spore Shape and Diameter Assessment

The spores are globose to subglobose in shape. In order to assess the diameter, averages of 11 spores were evaluated and the diameter range was found to be between 288.18 µm-389.41µm. (Figure 4). Mean diameter was found to be 348.4318 µm. The spores are found to be much bigger than the average size of most of the AMF species reported.

Spore Wall Layer Assessment

There are three distinct wall layers that are visible among most of the spores (Figures 5a and 5b).

Outer layer (L1)- It is a hyaline, mucilaginous, often seen as a crushed fragile layer in both PVLG and melzer's reagent (melzer's: PVLG- 1:1). Thickness of the outer layer varies from 4.22-4.83µm in mature spores.

Middle layer (L2)- It is a thick, continuous layer, which appears brownish in PVLG and reddish-brown in melzer's reagent. In mature spores the thickness varies from 3.36 µm to 5.63µm.

Inner layer (L3)- It appears as a continuous pale yellow coloured layer in both PVLG and melzer's reagent. This thin inner layer remains attached to the second layer. The thickness of the inner layer varies from 1.44-2.13µm.

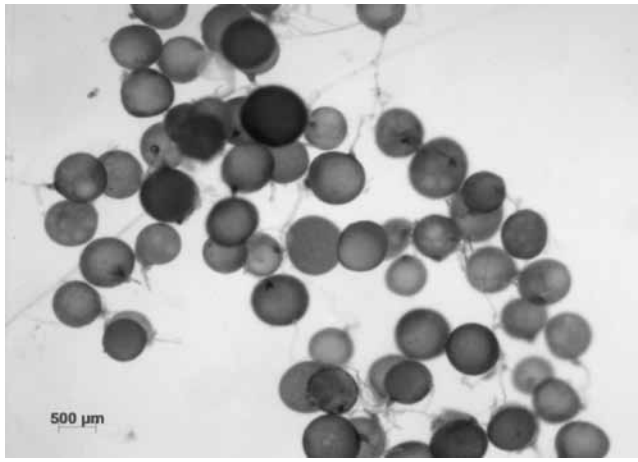

Figure 3a: Spores isolated from monosporal culture

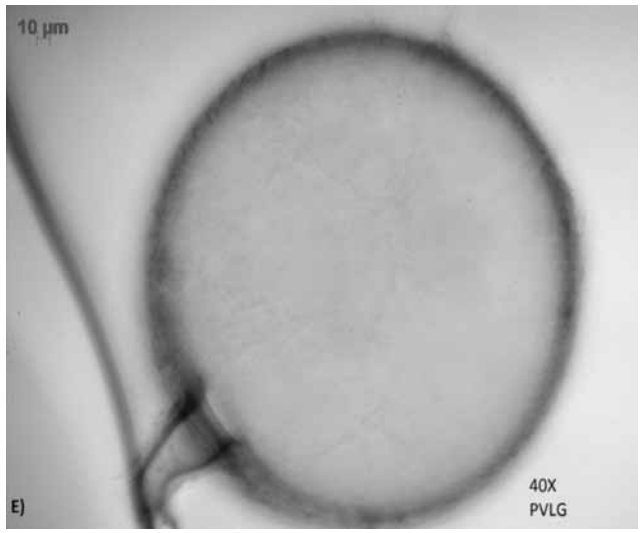

Figure 3b: Single yellow coloured spore

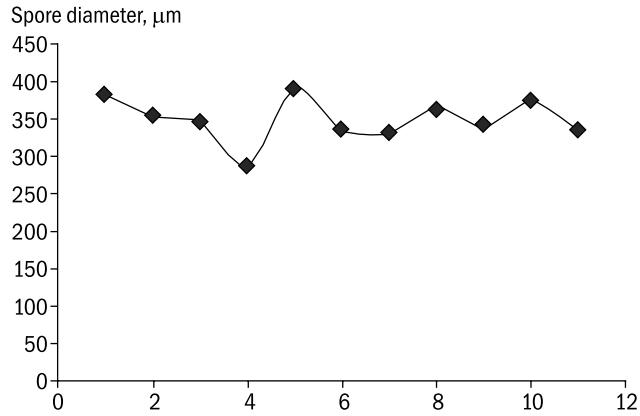

Figure 4: Spore diameter data of 11 healthy and intact spores were taken and plotted as a smooth line curve

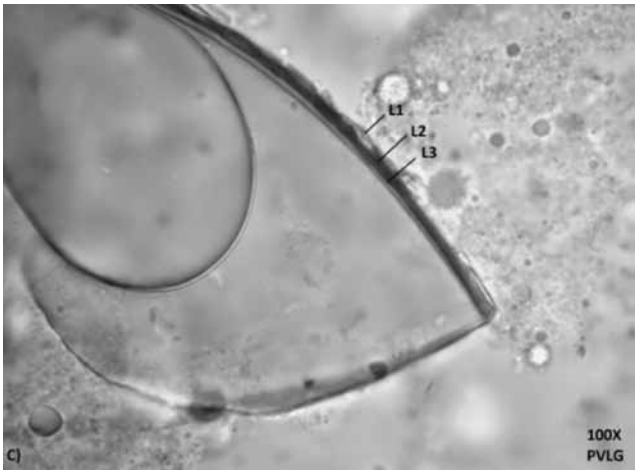

Figure 5a: Three different wall layers of spore

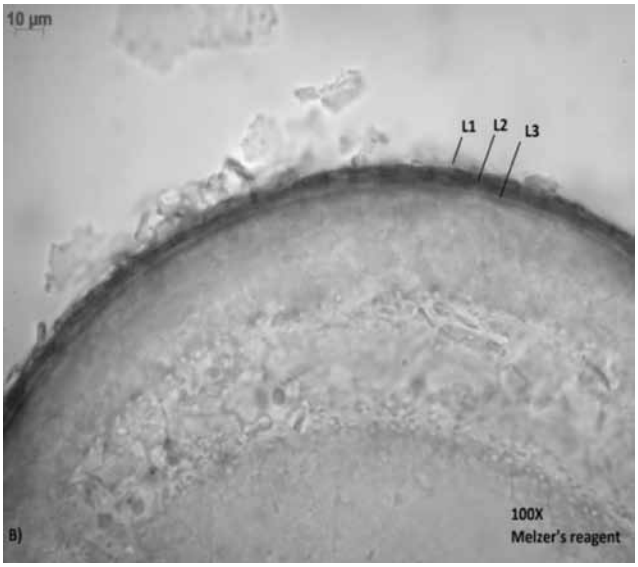

Figure 5b: Three different wall layers after Melzer's reaction

Subtending Hyphae Assessment

All the spores in this accession show an intact funnel or V-shaped subtending hyphae arising from the inner layer (L3). The thickness of the hyphal wall varies from 6.87-7.46µm (Figures 6a and 6b). This kind of subtending hype is mostly seen in *Funneliformis mosseae* and is also considered as one of its most characteristic features.

Occlusion Assessment

A recurved septum is seen at the point of attachment of the spore as shown in Fig 6a.

Most of the features that has been studied while doing the morphotaxonomic analysis as listed above shows that the culture may be similar to *Funneliformis mossae* or also known as *Glomus mossae*, a culture named in honour of Dr Barbara Mosse.

Classification Level

Glomeromycota

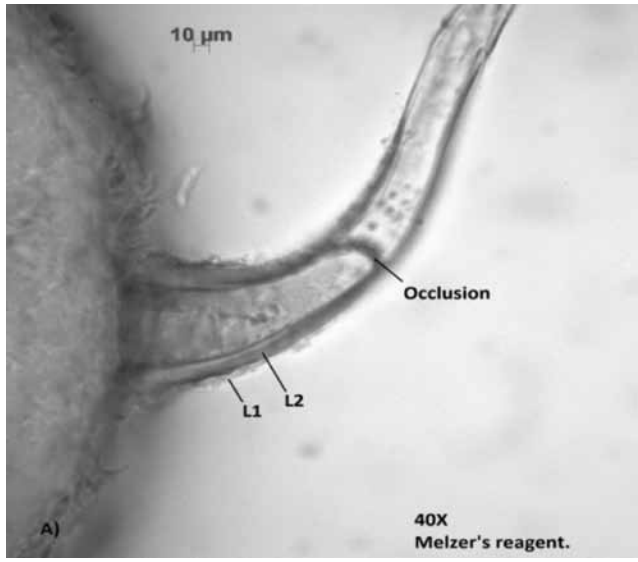

Figure 6a: Spore showing recurved septum at the point of attachment

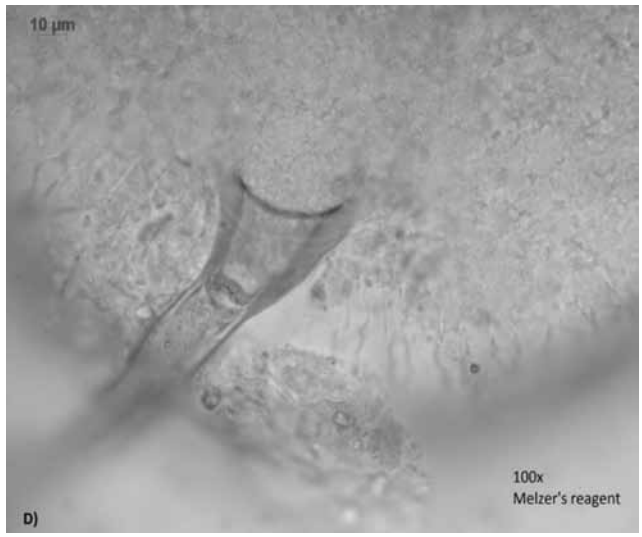

Figure 6b: Spore showing Funnel or V-shaped subtending hyphae

Glomeromycetes
Glomerales
Glomeraceae
Glomus mosseae

There are certain points of contention that needs to be counterchecked, such as absence of any peridium or sporocarp in the culture. An exact taxonomic conclusion can only be completed after doing the molecular characterization, which would be our next step.

Acknowledgements

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Recent references

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:

- *Agriculture, Ecosystems & Environment*
- *Applied Soil Ecology*
- *Biologia*
- *Canadian Journal of Microbiology*
- *Contemporary Problems of Ecology*
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- *Plant, Cell and Environment*
- *Restoration Ecology*
- *Russian Journal of Plant Physiology*
- *Science of The Total Environment*
- *Soil Biology and Biochemistry*

Name of the author(s) and year of publication	Title of the article, name of the journal, volume number, issue number, page numbers (address of the first author or of the corresponding author, marked with an asterisk)
Abdel-Fattah G M*, Asrar A-W A, Al-Amri S M, Abdel-Salam E M. 2014	**Influence of arbuscular mycorrhiza and phosphorus fertilization on the gas exchange status, growth and nutrients content of soybean (*Glycine max* L.) plants grown in a sandy loam soil** *Journal of Food, Agriculture and Environment* **12**(1): 150–156 [*Plant production Department, College of Food and Agriculture Sciences, King Saud University, Riyadh, P.O. Box 2460, Riyadh 11451, Saudi Arabia]
Ai-Dong Z*, Xiao-Qin W, Shen L, Xu X-L, Huang L, Ye J-R. 2014	**Profiling of differentially expressed genes in ectomycorrhizal fungus *Pisolithus tinctorius* responding to mycorrhiza helper *Brevibacillus reuszeri* MPt17** *Biologia* **69**(4): 435–442 [*College of Forest Resources and Environment, Nanjing Forestry University, Nanjing, Jiangsu, 210037, People's Republic of China]
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Brown R L*, Naeth M A. 2014	**Woody debris amendment enhances reclamation after oil sands mining in Alberta, Canada** *Restoration Ecology* **22**(1): 40-48 [Cardno ENTRIX, 801 Second Avenue, Suite 700, Seattle, WA, 98104, United States]

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Corrêa A*, Cruz C, Pérez-Tienda J, Ferrol N. 2014	**Shedding light onto nutrient responses of arbuscular mycorrhizal plants: Nutrient interactions may lead to unpredicted outcomes of the symbiosis** *Plant Science* **221–222**: 29–41 [*Depto. de Microbiología del Suelo y Sistemas Simbióticos, Estación Experimental del Zaidín, CSIC, Granada, Spain]
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Name of the author(s) and year of publication	Title of the article, name of the journal, volume number, issue number, page numbers (address of the first author or of the corresponding author, marked with an asterisk)
Pereira C M R*, Silva D K A da, Ferreira A C de Almeida, Goto B T, Maia L C. 2014	**Diversity of arbuscular mycorrhizal fungi in Atlantic forest areas under different land uses** *Agriculture, Ecosystems & Environment* **185**: 245–252 [*Programa de Pós-Graduação em Biologia de Fungos, Departamento de Micologia, Universidade Federal de Pernambuco, Avenida da Engenharia, s/n, Recife, PE 50740-600, Brazil]
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FORM IV

(as per Rule 8)

Statements regarding ownership and other particulars about Newsletter: Mycorrhiza News

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Signature of Publisher

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Date: 1 April 2014

Dr R K Pachauri

Call for papers for publication in the Mycorrhiza News

The Energy and Resources Institute (TERI) was formally established in 1974. With the headquarters in New Delhi, it has created an environment that is enabling, dynamic and inspiring for the development of solutions to global problems in fields of energy, environment and sustainable development. It has been ranked 20th in the list of top global think tanks on environment by Think Tank and Civil Societies Program, University of Pennsylvania.

The Centre for Mycorrhizal Research. In view of the stark reality of increasing demand for food production, food security, and nutrient deficiency, Mycorrhiza will in the future play a key role owing to its contribution to the plant apropos to phosphorus nutrition in agriculture. Mycorrhiza, with its mutualistic, symbiotic association, is of great relevance and significance to problems such as nutrient deficiency and marginal lands. With its main focus on basic and applied research using microbial resources for biotechnological intervention to address agriculture, energy, and environment-related issues, the Centre for Mycorrhizal Research at TERI, spearheaded by Dr Alok Adholeya, Director, Bioresources and Biotechnology has core competence in mycorrhizal technology and bioremediation.

The Centre has been publishing a quarterly newsletter called Mycorrhiza News, since 1988 to disseminate information on advancements in Mycorrhiza research globally. It presents original research finding papers from eminent scientists covering research into mycorrhizas including molecular biology, ecology, fungal systematics, development and structure and other related aspects of mycorrhiza including biodiversity and conservation of mycorrhizae.

While we appreciate your contribution in the field of Mycorrhiza, we are pleased to invite your research finding article/notes on important breakthroughs/ brief accounts of new approaches and techniques*/ Events**, for publication in the Newsletter. As for your paper, you may write about 3-4 manuscript pages plus 2 Figures 2 Tables and should be accompanied by a brief of their significance and submit the mss as email attachment to either of the following Email Id: tpsankar@teri.res.in or aloka@teri.res.in.

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* NEW APPROACHES
New approaches are invited for publication in the Mycorrhiza News. Under this column appear brief accounts of new techniques, modifications of available techniques, and new applications of other known techniques etc. in mycorrhiza research that have been published in reputed journals during the last two or three years.
** NEWS/EVENTS of common interest to members like seminars/workshops/ conferences attended, brief summaries of current research etc. may be communicated to us for future issues of the Mycorrhiza News.

The Mycorrhiza Information Centre (MIC) [visit our web site http://mycorrhizae.org.in/ at TERI [www.teriin.org], with support from the Department of Biotechnology, Government of India, serves primarily to meet information needs of researchers engaged in the field of Mycorrhiza by providing value-added information services. The center produces a range of on-line databases on mycorrhiza literature and other related information resources. It facilitates open access to current mycorrhiza research findings and development thereby catering to the needs of mycorrhiza researchers and promoting research and communication among mycorrhizologists, scientists, educators, agriculturists, and students, besides, exposing the researchers to the frontline research, innovation, new methodologies and bioprospecting.

The Centre is in the process of developing an international Directory of Mycorrhizologists. The objective is to create a network of scientists associated directly with research on mycorrhizae as also to identify global centers and institutions where this key research is being carried out. This will also help Mycorrhiza scientists to communicate among themselves and update their CVs.

We are pleased to include you as an expert in the Directory and this will certainly add great value. You may kindly provide details of your expertise by filling up the following and send the filled in form to the undersigned, so we can publish the updated directory in the Ministry web site as well.

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