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ARBUSCULAR MYCORRHIZAL FUNGI ASSOCIATED WITH SOME AGROFORESTRY TREE SPECIES FROM EASTERN GHATS OF TAMIL NADU

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ABSTRACT

Six commonly occurring indigenous tree species of agroforestry importance from Pachaimalai hill located in Eastern Ghats of Tamil Nadu was selected for the present study. All the six species examined were said to be mycorrhizal. *Pongamia pinnata,* a leguminous plant showed the highest percentage of root colonization (88%) and spore density (340 spores 100 g^{-1} rhizosphere soil). A total of 39AM fungal species representing eight genera namely *Glomus, Acaulospora, Gigaspora, Rhizaphagus, Entrophosphora, Scutellospora, Sclerocystis* and *Pacispora* were recorded.

KEYWORDS: AM Fungi, Agroforestry, Mycorrhization, Spore Density, Pachaimalai Hills

Agroforestry is a term used to describe the inclusion of trees with in farming systems. This has been a traditional land use developed by subsistence farmers throughout most of the world (Zomer et al 2009). Farmers have been growing trees on their farms for generations to maintain healthy soil, secure food supplies, and for timber and fuel (Chavan et al 2015). Further, the agroforestry systems render benefits to ecosystem by way of microclimate moderation, biodiversity conservation, carbon sequestration, protecting water sources, soil erosion and pollution control (Stamps and Linit 1998; Price and Gordon 1999; Thevathasan and Gordon 2004; Chavan et al 2014). Thus agroforestry systems have the potential for being an effective tool in climate change mitigation and adaptation steps (Chavan et al 2014).

India has become the first nation in the world to adopt an agroforestry policy (Dhyani et al 2013). Studies reveal that these agroforestry systems have the potential to generate employment opportunities of 450 man-days per hectare per year (Dhyani et al 2013). But the practice of agroforestry in India has been declining sharply in the past few decades (Dhyani et al 2013). According to Forest Survey of India (FSI: 2013) the current area under agroforestry in India is estimated as 11.54 m ha, which is 3.39% of the geographical area. Maharashtra (1.18%), Gujarat (1.16%) and Rajasthan (0.84%) rank high in state-wise area under agroforestry, however Tamil Nadu with only 0.46%.

In Tamil Nadu, the indigenous agroforestry system was more prevalent in Pachaimalai hills. They were found on bench terraces, moderately sloping lands and steeply sloping lands. The agroforestry system in the hills has been disappearing considerable over the decades due to primarily to changing socio-cultural values and population. These agroforestry have become center of conservation and environmental issues because of loss of beneficial effects on the local people and the environment.

One of the key players that can help in the conservation of agroforestry is the Arbuscular Mycorrhizal (AM) fungus (Janos 1980; van der Heijden et al 1998). They form symbiotic associations with the majority of agroforestry tree species. This mutualistic association can provide a number of benefits to the host plant including increased nutrient uptake, especially phosphorus, improved water relations, and protection from pathogens. Moreover, success of any reforestation intervention is depend on the co-establishment of diverse AM fungi together with seedlings in the nursery (Sieverding, 1991; Francis and Read, 1994). In India not many studies are available on AM fungal association in tree species of agroforestry system. Pande and Tarafdar (2004) surveyed AM fungi in neem (*Azadirachta indica* L.) based agroforestry systems in Rajasthan. Prasad and Mertia (2005) made a similar survey in three agroforestry tree species of arid zone. Most of the studies of this kind were carried out in arid zones while studies on the tropical hills are scarce. Therefore the present study was initiated to quantify AM fungal diversity occurring in some of the tree species of agroforestry in Pachaimalai hills and to identify the dominant AM fungi found there in.

MATERIALS AND METHODS

Study Sites and Sample Collection

Pachaimalai hills are part of Eastern Ghats in India that is situated at the mid regions of Tamil Nadu with latitudes 11°09'00" to 11°27'00" N and longitudes 78°28'00" to 78°49'00" E. They occupy an area of about 527.61 sq. km and altitudes range of 160 to 1072 m a.s.l. The mean annual temperature is 28°C and the average annual precipitation is 850 mm. The study was carried out

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with six tree species of agroforestry importance namely, *Dodonaea viscosa, Hardwickia binata, Melia azedarach, Peltophorum pterocarpum, Pongamia pinnata* and *Senna siamea*. Rhizosphere soil and roots were collected between September and November 2016. Three individuals of each plant species were randomly selected for sampling. Three rooting zone soil samples with fine roots were collected in three different directions from each plant. The root samples were obtained from the selected tree species by tracing thick roots from the base of the trunk to their ultimate branching. Each sample consisted of usually 1kg of soil sample and approximately 5g of fine root materials. The samples were placed in thin polyethylene bags and brought to the laboratory. The roots were washed in water and fixed in FAA (Formalin acetic acid alcohol) for further analysis. Rhizosphere soil of individual plant was shade dried, sieved to remove larger soil particles and was mixed thoroughly to obtain a composite samples.

Soil Analysis

Soil samples were analysed for pH and electrical conductivity in the laboratory using Elico model pH meter. Analyses of total nitrogen, total phosphorus and available potassium were carried out at the Government Soil Testing Laboratory, Tiruchirappalli, Tamil Nadu, India.

Estimation of AM Fungal Colonization

Roots were rinsed in distilled water to remove any trace of soil, cleared in 2% KOH, heated at 90°C, acidified with 1% HCl and stained with trypan blue (Koske and Gemma 1989). The stained roots were examined on an Olympus microscope (Model CX-2li) for AM fungal structures and percent root colonization was estimated using the slide method (Giovannetti and Mosse 1980).

Isolation and Taxonomic Identification of AM Fungal Spores

AM fungal spores were isolated by wet sieving and decanting method (Gerdemann and Nicholson 1963). One hundred grams of soil was suspended in one litre of water, stirred for few minutes and sieved. The residues on the sieves were spread on Whatman filter paper No.1 and examined under dissection microscope to count the viable spore population. They were identified according to their spore morphology and wall characteristics using the culture database established by INVAM (International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi) (http://invam.caf.wvu.edu) and Schüßler and Walker system of classification (2010) given in the website www.amf-phylogeny.com.

RESULTS AND DISCUSSION

Soil Characteristics of Study Site

The results of the soil physico-chemical characteristics are given in Table 1. Soils of study sites were acidic that the pH ranged between 4.9 and 6.4. The low pH strongly suggested that nutrient cations were low (Padamsee et al. 2016). Electrical conductivity ranged from 0.16 to 0.43 mS dsm^{-1}. Nitrogen content was significantly higher in the soil around *M. azedarach* and *S. siamea* (134.4 k/ha each). The P content in most of the examined rhizosphere soil samples seemed to be poor. It was just 0.5 kg/ha in the rhizosphere soil of *D. viscosa* and *P. pinnata.* In the present study the maximum P content was recorded in the rhizosphere soil sample of *S. siamea* (25 kg/ha). The K content of soil ranged from 113 k/ha (*M. azedarach*) to 367 k/ha (*H. binata*).

Table 1: Physico-chemical characteristics of the soil samples of the study sites

Tree species	pH	Ec (mSdsm^{-1})	N(kg/ha)	P (kg/ha)	K (kg/ha)
Dodonaea viscosa Jacq.	5.4	0.21	71.4	0.5	118
Hardwickia binata Roxb.	6.0	0.21	103.6	3.0	367
Melia azedarach L.	4.9	0.43	134.4	6.0	113
Peltophorum pterocarpum (DC.) K.Heyne	6.3	0.31	103.6	3.0	315
Pongamia pinnata (L.) Pierre	6.2	0.19	100.8	0.5	316
Senna siamea (Lam.) Irwin et Barneby	6.4	0.16	134.4	25	324

AM Fungal Colonization

AM fungi colonized roots of all six agroforestry taxa examined (Table 2). However, there were substantial differences among different host plants, i.e. levels of colonization ranged from 43% in *D. viscosa* to 88% in *P. pinnata.* In the present study the percentage of colonization seemed to be higher in leguminous tree species, because they need higher level of phosphorus for nodulation and nitrogen fixation (Smith & Daft 1977;

Carling et al. 1978; Plenchette et al. 1983). AM fungi take up immobile phosphate from soil, solubilize and transfer them to the host plant (Koide et al 1999). Hyphae, hyphal coils and vesicles were predominant AM fungal structures found in most of the taxa examined whereas the arbuscules were found only in *H. binata* and *M. azedarach.* This is because that the taxa form typical *Paris*-type or intermediate-type mycorrhizae. This is in accordance with the observations made by Kubota et al (2005) and D'Souza & Rodrigues (2013) who reported dominance of *Paris*-type morphology in natural ecosystems. The absence of arbuscules in most of the plant species suggests that the hyphal coils may serve the functions of arbuscules (Mago et al, 1992; Bukhari and Rodrigues, 2005). The average root colonization 63.66% recorded in the present investigation is in agreement with many of the previous studies.

AM Fungal Spore Density

The AM fungal spore density in the rhizosphere of six agroforestry taxa is shown in Table 2. It ranged from 230 to 340 per 100 g soil, with an average of 294. Spore density recorded in the present investigation is much higher than that of Mohankumar and Mahadevan (1988) observations, however consistent with the observations of Khade and Rodrigues (2003). In the present investigation the AM fungal spore density in the rhizosphere of *P. pinnata* was the highest, and the lowest in *D. viscosa.* The variable spore levels are likely due to their differential capacity of each AM fungal taxa to sporulate (Bever et al 1996). The spore density values were varied with the soil P content but not in relation to percentage of root colonization.

Table 2: Mean percent root colonization and soil spore density of AM fungi in the agroforestry tree species of Pachaimali hills, Eastern Ghats, Tamil Nadu

Tree species	Family	AM colonization					Spore density 100 g^{-1} soil
		Hypha	Hyphalcoil	Arbuscule	Vesicle	Root colonization (%)	
D. viscosa	Sapindaceae	+	+	-	+	43±3.22	230± 37.15
H. binata	Caesalpiniaceae	+	+	+	+	71± 4.26	325± 70.13
M. azedarach	Meliaceae	+	-	+	+	49 ± 4.91	273 ± 58.20
P. pterocarpum	Caesalpiniaceae	+	+	-	-	63± 2.21	310 ±23.11
P. pinnata	Fabaceae	+	+	-	+	88 ± 5.21	340 ± 23.11
S. siamea	Caesalpiniaceae	+	+	-	+	68± 3.34	290 ± 51.25

AM Fungal Diversity and Distribution

From the rhizosphere soil samples spore and sporocarp were obtained using wet-sieving method. We tried our level best to identify the spores and sporocarp to their species level by their morphological characters, but two specimens were identified only to genus level as they lacked distinguishable, fine taxonomic characters. The species composition of AM fungi in rhizosphere soil samples revealed the presence of 39 taxa representing eight genera (Table 3). Of the 39 taxa, 13 belonged to the genus *Glomus,* 12 to *Acaulospora,* 5 to *Gigaspora,* 3 to *Rhizophagus,* 2 each to *Entrophospora* and *Scutellospora,* one each to *Sclerocystis* and *Pacispora.* AM fungal diversity depends on season, climatic conditions, host, age of host, soil type and the dormancy and the distribution pattern (Koske and Halvorson 1981; Walker et al 1982; Gemma and Koske 1988; Greipsson and El-Mayas 2000; Zhao et al 2001; Yang et al 2010). Francis and Read (1994) reported that high species diversity characteristic of phosphorus deficient grassland ecosystem dominated by plant species with AM fungi may be attributed to a low level of host specificity. *Glomus* and *Acaulospora* were the dominant genera in all the samples analyzed. These results are consistent with other investigations conducted (Zhao et al 2001; Muthukumar et al 2003; Tawaraya et al 2003). The occurrence of dominant AM taxa with more than one tree species attributes a non-host specific relationship (Kumar et al 2013). Also *Glomus* and *Acaulospora* were the most representative types in the present study. The predominance of these genous in tropical soils have been reported by other workers (Thapar and Khan 1985; Raghupathy and Mahadevan 1993; Khade and Rodrigues 2003). The occurrence of other six genera was very low. In the present study we were able to establish a positive correlation between percentage of root colonization and species richness. These findings are in

agreement with Muthukumar et al (2001) but contrast with the findings of Brundrett (1991), Zahka et al (1995), Brundrett et al (1996) and Shi et al (2006).

Table 3: Distribution of AM fungi in the rhizosphere soil samples in the agroforestry tree species of Pachaimali, Eastern Ghats, Tamil Nadu

Host Plant Species	AM fungal species	Species richness
D. viscosa	*A. cavernata, A. denticulata, A. rehemii, Gi.decipiens, Gi. Gigantea, G. aggregatum, G. claroidium, G. macrocarpum, G. microaggregatum, G. rubiformis, G. viscosum, Pasispora boliviana, Sclerocystis sinuosa*	13
H. binata	*A. cavernata, A. solaidea, A. spinosa*, *Entrophospora kinentensis,E. schenkii, Gi. margaratia, G. aggregatum, G. macrocarpum, G.microaggregatum, G. microcarpum, G.mosseae, G. rubiformis, G. sinuosa, G. thaivansis, P. boliviana, R. intraradices,*	16
M. azedarach	*A. denticulata, A. decipiens, A. laevis, G. aggregatum*	4
P. pterocarpum	*A. cavernata,Gi. decipiens, Gi. roseae, G. aggregatum, G. micoaggregatum, G. microcarpum, G. minutum, G.rubiformis, G. thaivansis, G.viscosum, R. Intraradices*	11
P. pinnata	*A. bireticulata, A. cavernata, A. delicata, A. denticulata, A. laevis, A. scrobiculata, A. spinosa, A.* sp.1, *A.* sp.2, *Entrophospora schenckii, Gi. decipiens, Gi. gigantea, G. aggregatum, G. macrocarpum, G. mosseae, G. viscosum, Scutellospora calospora, S.cerradensis, R. fasciculatum*	19
S. siamea	*A. denticulata,A. solaideae, Gi. albida, Gi. margarita, G. aggregatum, G.geosporum, G. halonatum, G. macrocarpum, G. microaggregatum, G. microcarpum, G. mossae, G. sinuosa,P. boliviana, R. Diaphanous*	14

AM fungi may be important drivers of plant community composition in agroforestry ecosystems. The fungal species that occurred at all study sites showed different pattern of sporulation and distribution suggesting differences in functional diversity.

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