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Status of Arbuscular Mycorrhizal Fungi in Tree Species from Eastern Ghats of Tamil Nadu, India

S. Vinoth Ponpandian[1], A. Egbert Selwin Rose[2]

[1,2]Department of Botany, St. Joseph's College, Tiruchirappalli, Tamil Nadu, India

Abstract: ***The Arbuscular Mycorrhizal (AM) status of 10 tree species in Pachaimalai Hill, Eastern Ghats of Tamil Nadu was surveyed. All the tree species were observed as colonizer of AM fungi. The root segments were found to contain any of the AM fungal structures i.e. coenocytic hyphae, hyphal coils, vesicles and arbuscules. The extent of AM colonization varied among the tree species. The average percentage of AM fungal colonization ranged from 12 (Cleistanthus collinus) to 89 (Bauhinia purpurea). Roots of all tree species formed hyphae, vesicles and hyphal coils however all tree species lacked arbuscules except Melia azedarach. A total of 25 morphotypes of AM fungi corresponding to 7 genera and 3 families were recorded. Of the 25 morphotyes, 11 belonged to the genus Acaulospora, 6 to the genus Glomus, 2 each to the genera Gigaspora, Scutellospora, Sclerocystis, one each to Entrophosphora and Rhizophagus. Species richness was highest in B. purpurea (20 species) and lowest in M. azedarach (4 species).***

Keywords: Arbuscular mycorrhizal fungi (AM fungi), spore density, tree species, Pachaimalai hill

1. Introduction

Arbuscular Mycorrhizal (AM) fungi are symbionts abundant in soil of most ecosystems [1] throughout the World ranging from the Artic to the tropical rain forests [2, 3]. These fungi form obligate symbiotic associations with the roots of over 80% of terrestrial plant species [4, 5]. They facilitate plants to uptake water, phosphorus and other immobile nutrients [6, 7], thereby increase plant growth rates protect the plant against pathogens [8, 9] and drought. As phosphorus is a major limiting nutrient in many of the ecosystems AM fungi play a key role in ecosystem functioning. Therefore the presence of these fungi has been shown to influence on plant community structure, productivity, and the course of succession [10, 11].

AM fungal research in tropical forest has long history. All over the World numerous studies have investigated the association of AM fungi with tree species. In India, many authors have documented the occurrence of AM fungi in natural forest, covering various areas including subtropical evergreen forest and arid zone [12, 13, 14, 15, 16, 17, 18, 19, 20]. The evaluation of AM fungal association in the forests of Western Ghats has also been recorded in Nilgiri districts [21, 22], Kalakad forest reserve [23, 24], Kodayar forest [25], Maruthamalai Hills [26, 27] and Mollem forest [28].

The Eastern Ghats constitutes an important biogeographic region in Indian and is a major centre of plant diversity with a high endemism. Ranging from Orissa, Andhra Pradesh to Karnataka and Tamil Nadu, the Eastern Ghats spread over an area of about 75,000 sq km through a chain of fragmented and disjunct hill ranges. AM fungal association in Eastern Ghats of Tamil Nadu has been documented only in Kolli Hills [29] and Servarayan Hills [30, 31]. However no work on AM fungal association from Pachaimalai hill has been reported so far. Hence we made an attempt to study the occurrence and distribution of AM fungi in tree species from Pachaimalai hill located in Eastern Ghats of Tamil Nadu.

2. Materials and Methods

Study site

Pachaimalai hill, part of Eastern Ghats in Tamil Nadu was selected for the present study. This hill 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. It spread over an area of 527.61sq km and altitudes range of 160m a.s.l.to 1072m a.s.l., enjoy a sub-tropical climate with temperatures varying from 25°C to 31°C and annual rainfall ranging from 800mm to 900mm. The hill harbors eight vegetation types, of which tropical dry deciduous forests are widespread with rich diversity.

Collection of soil and root samples

Root and rhizosphere soil samples of 10 tree species (Table 1), belonging to 7 families were randomly collected in the months of September and November 2015. Three rooting zone soil samples with fine roots were collected in three different directions from each plant. Three individuals of each plant species were randomly selected for sampling. During sampling, care was taken to trace back the feeder roots of the selected tree species. Samples collected from individual plant species were packed in sterile polythene bags and transported to the laboratory. Root samples were freshly analyzed whereas the soil samples were air dried and stored at 4^{o}C until processing. A portion of soil samples was used for assessing various soil parameters and the remaining for enumeration and extraction of AM fungal spores. The host tree species were identified at Botanical Survey of India, Coimbatore.

Analysis of soil samples

Soil samples were mixed thoroughly and analyzed for pH (1:1 soil to water ratio) using a digital pH meter (L1 model, Elico, India). The total nitrogen and total

Table 1: Edaphic characteristics of the samples collected from study sites

Tree species	Family	pH	Ec (mS dsm-1)	N(kg/ha)	P (kg/ha)	K (kg/ha)
Majidea zanguebarica J. Kirk *ex* Oliv.	Sapindaceae	6.3	0.19	64.4	3.5	113
Sapindus emarginatus Vahl.	Sapindaceae	6.4	0.16	78.4	0.5	172
Melia azedarach L.	Meliaceae	4.9	0.43	134.4	6	113
Pongamia pinnata (L.) Pierre.	Fabaceae	6.2	0.19	100.8	0.5	316
Bauhinia purpurea L.	Caesalpiniaceae	6.2	0.17	126	0.5	93
Anogeisses latifolia (Roxb. *ex* DC.) Wall *ex* Guill. & Perr.	Combretaceae	5.8	0.1	149.8	2.5	194
Terminalia chebula Retz.	Combretaceae	6.6	0.22	135.8	2	237
Terminalia paniculata Roth.	Combretaceae	6.3	0.19	93.8	0.5	90
Wrightia tinctoria R.Br.	Apocynaceae	6.4	0.19	141.4	3.5	188
Cleistanthus collinus (Roxb.) Benth. ex Hook. f.	Euphorbiaceae	6.8	0.23	65.8	0.5	46

phosphorus were determined according to Jackson [32] and available potassium was determined by following the ammonium acetate method as described by Merwin and Peach [33].

3. Assessment of AMF colonization and spore density

The root samples were washed thoroughly with tap water cut into 1cm segments, cleared in 10% (w/v) KOH by heating to approximately 90^{o}C in a water bath for 2-3 h, acidified with 1N HCl, treated with trypan blue (0.05% in lactophenol) and left over night for staining[34]. The stained roots were examined under an Olympus microscope (Model CX-21i) for AM fungal structures and percentage root colonization was estimated using slide method [35].

4. Isolation and identification of AMF spores

For isolation of AM fungal spores, wet sieving and decanting method proposed by Gerdemann and Nicolson[36] was followed. 100gm of soil sample was dispersed in one liter of water and the suspension was decanted through two mesh sieves, 700µm and 37µm. The residues in the sieves were washed into the beakers and passed through filter papers. Each filter paper was then spread on a Petri dish and scanned under a dissection microscope. Intact fungal spores were counted and transferred using a wet needle to polyvinyl alcohol - lactoglycerol on a glass slide for identification. Spores were identified based on spore size, colour and wall layers and hyphal attachments using INVAM website by Joe Morton: http://invam.caf.wvu.edu. and other suitable references [37, 38, 39, 40, 41].

5. Results and Discussion

The soil type of study sites were red loamy and had a pH of acidic to near neutral (Table 1). The highest soil pH (6.8) was observed in the soil collected from rhizosphere of *Cleistanthus collinus* where as the lowest pH (4.9) was observed in *Melia azedarach*, which is considered to be highly acidic. Electrical conductivity ranged from 0.10 mS dsm-1 to 0.43 mS dsm-1. The total soil N, P and available K were 64.4 - 149.8 kg/ha., 0.5 - 6.0 kg/ha. and 90 - 316 kg/ha., respectively.

The results showed that all tree species found to exhibit AM fungal colonization (Table 2). It is well known that mycorrhizal fungi preferentially colonize young roots [42]. These roots are the sites where most exudate release [43] and which may attract AM fungi [4]. In the present study the samples were collected during the wet season when the tree species develop more young roots, hence all the selected trees species from the tropical forest were said to be mycorrhizal. This is in agreement with the observations made on other tropical forest tree species [2, 28, 44, 45, 46, 47, 48, 49, 50]. The root segments of the tree species were found to contain any of the AM fungal structures i.e. coenocytic hyphae, intercellular hyphae or intracellular hyphal coils, vesicles and arbuscule. Hyphae and hyphal coils found in all tree species, however *M. azedarach* lacked hyphal coils. Vesicles were found to be present in all tree species except *Wrightia tinctoria*. *M. azedarach* was the only tree species formed arbuscules. The frequency of occurrence of arbuscules was lower than vesicles and hyphal coils. One possible explanation is that 90% of tree species screened may form typical *Paris*-type or intermediate-type mycorrhizae, which is in agreement with the findings of Kubota et al.[51] and D'Souza & Rodrigues [52] who reported dominance of *Paris*-type morphology in natural ecosystems. The absence of arbuscules in most of the species also suggests that the hyphal coils may serve the functions of arbuscules [53]. The extent of AM colonization varied significantly among the tree species examined. The average percentage of total AM fungal colonization ranged between 12 (*C. collinus*) and 89 (*Bauhinia purpurea*). This is in accordance with the earlier report on AM fungal association of plants of the Western Ghats (12-90%) by Muthukumar et al. [27]. Songachan & Kayang [54] reported AM fungal colonization from forest of Megalaya (66-71%), which is slightly lesser than our finding, suggesting that the intensity of AM fungal colonization could be influenced by specific habitats conditions. The highest root colonization was reported from *B. purpurea*.and *Pongamia pinnata*. Both members belong to Legumes, which are generally known to be highly dependent on AM fungal association that is mainly implicated to the higher phosphorus demand for nodulation and nitrogen fixation [55, 56, 57]. Correlation analysis revealed that AM fungal colonization and P concentration are positively correlated for *P. pinnata and B. purpurea*, however negatively correlated for rest of the tree species assessed. The average percentage root colonization for all tree species sampled was 54.5%, which is in agreement with the findings of Mohankumar & Mahadevan [23] and Khade & Rodrigues[28].

A total of 25 morphotypes of AM fungi corresponding to 7 genera and 3 families were recorded from the rhizosphere soils of different tree species (Table 3).

Nandakwamg et al. [58] described 24 morphotypes from indigeneous forest trees of Thailand. However Singh et al. [59] detected a total of 51 morphotypes associated with tea growing in natural and cultivated ecosites. The difference may be due to the nutrient composition of soil. In general high level of P content has negative effect on AM fungal distribution [60, 61, 62, 63]. Several authors have indicated that increasing P content significantly reduced the species diversity of AM fungi [64, 65, 66]. This is in agreement with our findings that most soil samples have low to medium P content. The low P contents may have contributed to the high species richness and vice versa.

All the morphotypes were identified to species level except two. From the 25 AM fungal morphotypes a total of 11 species belong to the genus *Acaulospora*, 6 to the genus *Glomus*, 2 each to the genera *Sclerocystis*, *Gigaspora*, *Scutellospora* and one each to the genera *Rhizophagus* and *Entrophospora*. The above results showed that *Acaulospora* was the predominant genus followed by *Glomus*. These findings are in accordance with results of Mangan et al. [67], Zhao & Zhao [68], Nandakwang et al. [58], Wongmo [69] and Emmanuel et al. [70]. Such wider occurrence of *Acaulospora* is due to their facultative symbiotic nature, adapted to a wide array of soil and host species [45, 71, 72]. Moreover, *Acaulospora* are frequently associated with soil with low pH [73, 74].

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

Tree species	AM colonization					Spore number per 100 g soil
	Hypha	Hyphalcoil	Arbuscule	Vesicle	Mean root colonization (%)	
M. zanguebarica	+	+	-	+	35 ± 3.22	325 ± 37.15
S. emarginatus	+	+	-	+	41 ± 4.26	350±70.13
M. azedarach	+	-	+	+	49 ± 4.91	273±58.20
P. pinnata	+	+	-	+	88 ±5.21	340 ±23.11
B. purpurea	+	+	-	+	89±4.12	240 ± 70.23
A. latifolia	+	+	-	+	81 ± 3.97	350 ± 51.25
T. chebula	+	+	-	+	52 ± 1.26	460 ± 26.90
T. paniculata	+	+	-	+	56 ± 2.38	525 ± 45.05
W. tinctoria	+	+	-	-	42±3.26	425 ± 6.97
C. collinus	+	+	-	+	12 ± 1.58	78 ± 8.02

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

Hose Plant Species	AM fungal species	Species richness
M. zanguebarica	*A. denticulata, A. laevis, A. spinosa, A.* sp.1, *G. aggregatum, G. viscosum, G. geosporum*	7
S. emarginatus	*A. bireticulata, A. cavernata, A. delicata, A. laevis, A. mellea, A. spinosa, A. scrobiculata, A. spinosa, A.* sp.1, *Entrophospora schenckii,*	17
	Gi. gigantea, G. aggregatum, G. geosporum, G. macrocarpum, G. viscosum, Sclerocystis rubiformis, R. fasciculatum	
M. azedarach	*A. denticulata, A. decipiens, A. laevis, G. aggregatum*	4
P. pinnata	*A. bireticulata, A. cavernata, A. delicata, A. denticulata, A. laevis, A. scrobiculata, A. spinosa, A.* sp.1, *A.* sp.2, *Entrophospora schenckii,*	19
	Gi. decipiens, Gi. gigantea, G. aggregatum, G. macrocarpum, G. mosseae, G. viscosum, Scutellospora calospora, Scutellospora cerradensis,	
	R. fasciculatum	
B. purpurea	*A. bireticulata, A. capsicula, A. cavernata, A. delicata, A. denticulata, A. mellea, A. scrobiculata, A. spinosa, A.* sp.1, *A.* sp.2, *Entrophospora schenckii, Gi. decipiens, Gi. gigantea, G. aggregatum, G. macrocarpum, G. mosseae, G. viscosum, Sclerocystis rubiformis, Scutellospora cerradensis, Rhizophagus fasciculatum*	20
A. latifolia	*A. denticulata, A. laevis,A. spinosa,Gi. gigantea, G. aggregatum, G.geosporum, G. macrocarpum, G. viscosum, Sclerocystis sinuosa*	9
T. chebula	*A. delicata, A. denticulata, A. mellea, A. spinosa, A.*sp.1, *Gigaspora decipiens , Gi. gigantea, G. aggregatum, G. viscosum.*	9
T. paniculata	*A. bireticulata, A. capsicula, A. cavernata, A. denticulata, A. laevis, A. mellea, A. scrobiculata, A.spinosa, Gi. gigantea, G. aggregatum, G. macrocarpum, G. mosseae, G. viscosum, G. geosporum, Scutellospora cerradensis*	15
W. tinctoria	*A. bireticulata, A. laevis, A.* sp.1, *A.* sp.2, *Gi. gigantea, G. aggregatum, G. macrocarpum, G. viscosum,*	8
C. collinus	*A. bireticulata, A. delicata, A. denticulata, A. mellea, A.* sp.2, *Gi. gigantea, G. aggregatum, G. geosporum, G. mosseae, G. rubiformis, G. viscosum, Rhizophagus fasciculatum, Sclerocystis rubiformis, Sclerocystis sinuosa*	14

In the present study the genus *Glomus* was the second most representative type after *Acaulospora*. The possible reasons for the predominance of *Glomus* sp. are that spores of *Glomus* sp. have different temperature and pH preferences for germination [75]. Hayman and Stovold [76] also proved that AM fungi, especially *Glomus* are able to live in broad range of pH and can reduce acidic stress in plants growth regions. Dominance of genus *Glomus* has been reported earlier by

Selvaraj et al. [77], Rajkumar et al. [78].

Shi et al. [79] reported that *Acaulospora* and *Glomus* were the dominant genera in the rhizosphere of 14 genera of Meliaceae in a tropical forest in China (including *M. azedarach*). However, in our study we found *Acaulospora* dominant over *Glomus* in *M. azedarach.* This difference in AM fungal community composition could be linked to geographic environmental variations or the plant genotypes involved [80]. However, *Gigaspora, Sclerocystis, Entrophosphora, Scutellospora* and *Rhizophagus* represented only less number of species in our studies. These results are consistent with other investigation conducted in tropical forests [28, 48, 49, 81, 82].

There were significant differences in the species richness AM fungi in the rhizosphere soil of the tested tree species of Pachaimalai hill. The tree species from tropical forests exhibit differential responses and compatibility in growth in relation to AM fungal species [83]. Our results confirm this, a high diversity of AM fungi (20 species) associated with *B. purpurea* followed by *P. pinnata* (19 species), *Sapindus emarginatus* (17 species), *Terminalia paniculata* (15 species), *C. collinus* (14 species), *Anogeissus latifolia, T. chebula* (9 species each), *Wrightia tinctoria* (8 species), *Majidea zanguebarica* (7 species) and *M. azedarach* (4 species). The pattern of species distribution may be due to ecological factors like seasonality, host dependence, age of the host plants, sporulation capability of the AM fungi, and the dormancy of AM fungal spores in soils [84, 85, 86, 87, 88, 89, 90, 91]. The species richness was relatively high and varied with host plant species and somewhat relation to soil properties, especially P content [92]. In the present study there was correlation found between the percentages of root colonization and AM fungal species richness for certain tree species. For instance, the percentage of root colonization was nearly 90 in *B. purpurea* and *P. pinnata*, and both tree showed highest AM fungal species richness. These findings are not in accord with the results of Brundrett [93], Zahka et al. [94] and Brundrett et al. [95]. However, it was not the case for *M. azedarch* that comprised of least number of AM fungal species whereas the root length colonization was not least.

Enormous variation in spore abundance that ranges from 78 to 525 spores per 100gm soil sample was documented in the present investigation (Table 2). The results are in agreement with Rajkumar et al. [78] who have reported 15 to 520 spores. The variation in AM fungal spore abundance between samples could be due to the factors such as climatic, edaphic properties, spatial and temporal variation, vegetation, host specificity, age of the host plants, disturbance and differential sporulation ability [96, 97]. In this study the highest number of spores was recorded in the rhizosphere soil of *T. paniculata* (525/100gm of soil) and the lowest in *C. collinus* (78/100 of soil). The number of AM fungal spore and percentage of root colonization observed in the present study correlated certain tested tree species but not all. The relationship between AM fungal spore density and percentage of root colonization are influenced by many biotic and abiotic environmental factors such as AM fungal species, plant host and soil nutrients [98]. The present study showed a significant negative correlation between spore density and P content (Tables 1 and 2), which is in agreement with Udaiyan et al [99], Kahiluoto et al. [65], Belay et al. [100]. The decrease in spore density with an increase in P content observed in this study can be attributed to the fact that P content of soil suppresses AM fungal spore density.

6. Conclusion

The present study revealed the distribution of AM fungi in 10 tested tree species of Pachaimalai hill, Eastern Ghats of Tamil Nadu. The tree species are good colonizers of AM fungi and support variety of AM fungi. Our small-scale field survey confirms that attention should be given to all plant species of Pachaimalai hill, including herbs and shrubs to understand the overall status of AM fungi in this forest.

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