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SURVEY OF MYCORRHIZAL ASSOCIATION IN SOME TREES OF PACHAMALAI HILLS, THE EASTERN GHATS OF TAMIL NADU

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Abstract: **Arbuscular Mycorrhizal (AM) fungal status of eight tree species from Pachaimalai Hill, Eastern Ghats of Tamil Nadu was surveyed. AM fungal colonization was observed in the roots of all the tree species examined. The AM fungal colonization was characterized by the presence of any of the fungal structures such as 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 26 (*Strychnos nux-vomica*) to 86 (*Millingtonia hortensis*). Roots of all tree species formed hyphae, however the formation of vesicles, arbuscules and hyphal coil varied among the tree species. A total of 37 morphotypes of AM fungi corresponding to 13 genera and 8 families were recorded. Of the 37 morphotyes, 10 belonged to the genus Acaulospora, 6 to the genus *Glomus*, 4 each to the genera *Rhizoglomus, Gigaspora,* 3 to *Funneliformis*, 2 each to genera *Septoglomus, Paraglomus*, and one each to *Entrophosphora, Dominikia, Claroideoglomus, Scutellospora* and *Archaeospora*. Species richness was highest in *Tarenna asiatica*(23 species) and lowest in *Strychnos nus-vomica* (13 species).**

Keywords: **Arbuscular Mycorrhizal fungi (AM fungi), spore density, tree species, Pachaimalai hills.**

I. 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]. The evaluation of AM fungal association in the forests of Western Ghats has also been recorded in Nilgiri districts[16], [17], Kalakad forest reserve[18], [19], Kodayar forest[20], Maruthamalai Hills[21], [22] and Mollem forest[23].

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[24] and Servarayan Hills[25], [26]. 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.

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II. METHODS

Study site: Pachaimalai hills, part of Eastern Ghats in Tamil Nadu was selected for the present study. These hills 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, 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. These hills harbor 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 eight tree species (Table 1), belonging to 8families 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 phosphorus were determined according to Jackson[27]and available potassium was determined by following the ammonium acetate method as described by Merwin and Peach [28].

Assessment of AMF colonization: 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[29]. 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[30].

Isolation and identification of AMF spores: For isolation of AM fungal spores, wet sieving and decanting method proposed by Gerdemann and Nicolson[31] 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 at ×5 magnifications. 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[32], [33].

III. 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 (6.6) was observed in the soil collected from rhizosphere of *M. philippensis*whereasthe lowest pH (5.9) was observed in*S. nux-vomica*, which is considered to be acidic. Electrical conductivity ranged from 0.12 dSm-1 to 0.22 dSm-1. The total soil N, P and available K were 82.6 - 168.0 kg/ha., 0.5 - 2.0 kg/ha. and 42 - 209 kg/ha., respectively.

TABLE 1: Means and standard errors for rhizosphere soil characteristics of tree species in Pachamalai Hills, Eastern Ghats of Tamil Nadu.

Tree species	pH	EC dSm^{-1}	Nitrogen	Phosphorus	Potassium
Muntingia calabura	6.5 ± 0.02	0.18 ± 0.02	102.6 ± 1.02	0.5 ± 0.01	68 ± 1.85
Pterospermum acerifolium	6.5 ± 0.03	0.18 ± 0.01	84.0 ± 0.83	1.0 ± 0.02	113 ± 3.54
Ziziphus jujuba	6.4 ± 0.01	0.22 ± 0.03	91.0 ± 1.07	0.5 ± 0.04	140 ± 1.78
Leucaena leucocephala	6.1 ± 0.03	0.17 ± 0.01	89.6 ± 0.29	0.5 ± 0.02	42 ± 3.12
Tarenna asiatica	6.1 ± 0.02	0.12 ± 0.01	168.0 ± 1.23	2.0 ± 0.02	209 ± 1.57
Strychnos nux-vomica	5.9 ± 0.03	0.17 ± 0.03	100.8 ± 0.98	0.5 ± 0.03	41.5 ± 3.23
Millingtonia hortensis	6.3 ± 0.02	0.18 ± 0.03	82.6 ± 0.89	0.5 ± 0.02	132 ± 2.54
Mallotus philippensis	6.6 ± 0.02	0.22 ± 0.03	107.8 ± 0.99	0.5 ± 0.02	172 ± 1.12

All rhizosphere soil samples were red loamy
EC 0-0.12 very low level, 0.12-0.35 suitable range, 0.35-0.65 desirable range
Nitrogen, Phosphorus and Potassium kg acre^{-1}

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TABLE 2: Distribution of AM fungal structures, structural classes and mean percentage of root colonization

Tree species	Hyphae	Hyphal coils	Arbuscules	Vesicles	Structural class	% Root colonization
M. calabura	+	+	+	+	Intermediate	83±1.32
P. acerifolium	+	+	-	-	*Paris*	50±4.42
Z. jujuba	+	+	+	+	Intermediate	79±3.29
L. leucocephala	+	+	-	+	*Paris*	74±4.43
T. asiatica	+	+	-	+	*Paris*	46±4.54
S. nux-vomica	+	-	-	+	*Paris*	26±4.34
M. hortensis	+	+	+	+	Intermediate	86±3.33
M. philippensis	+	-	-	+	Intermediate	77±3.41

± indicates standard deviation

The table 2 shows that all tree species found to exhibit AM fungal colonization. It is well known that mycorrhizal fungi preferentially colonize young roots[34]. These roots are the sites where most exudate release[35] 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], [23], [36], [37], [38].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 vesicles found in all tree species, however *P. acerifolium* lacked vesicles. Hyphal coils were found to be present in most of the tree species except *S. nux-vomica* and *M. philippensis*. Arbuscules were found in three tree species only. The frequency of occurrence of arbuscules was lower than vesicles and hyphal coils. One possible explanation is that all the tree species screened formed typical *Paris*-type or intermediate-type mycorrhizae, which is in agreement with the findings of Kubota et al. [39] and D'Souza & Rodrigues[40] 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 [41]. The extent of AM colonization varied significantly among the tree species examined. The average percentage of total AM fungal colonization ranged between 26 (*S. nux-vomica*) and86(*M. hortensis*). This is in accordance with the earlier report on AM fungal association of plants of the Western Ghats (12-90%) by Muthukumar et al.[22]. Songachan & Kayang[42]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.

A total of 37 morphotypes of AM fungi corresponding to13 genera and 8 familieswere recorded from the rhizosphere soils of different tree species (Table 3). Nandakwamg et al.[43]described 24 morphotypes from indigeneous forest trees of Thailand. However Singh et al.[44] 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 [45]. Several authors have indicated that increasing P content significantly reduced the species diversity of AM fungi [46]. 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.

TABLE 3: Distribution of AM fungal species in tree species of Pachamalai Hills, Eastern Ghats of Tamil Nadu

Plant species	AM fungal species	Total
M. calabura	*Gl. microcarpum, Gl. rubiforme, Gl. sinuosum, F. geosporum, F. mosseae, S. viscosum, R. intraradices, R. microaggregatum, Ac. cavernata, Ac. denticulata, Ac. laevis, Ac.* spp. 1, *G. decipiens, Sc. calospora*	14
P. acerifolium	*Gl. botryoides, Gl. macrocarpum, Gl. sinuosum, S. constrictum, R. aggregatum, R. intraradices, R. microaggregatum, Cla. etunicatum, Ac. cavernata, Ac. denticulata, Ac. rehmii, Ac.* spp. 2, *G. decipiens, G. margarita, G. rosea, P. laccatum*	16
Z. jujuba	*Gl. macrocarpum, Gl. microcarpum, Gl. rubiforme, Gl. sinuosum, Gl. taiwanense, F. badium, F. geosporum, Septoglomus constrictum, R. aggregatum, R. intraradices, R. microaggregatum, Ac. bireticulata, Ac. cavernata, Ac. denticulata, Ac.* spp. 1, *G. decipiens, G. margarita, G. rosea, P. albidum, P. laccatum.*	20

L. leucocephala	*Gl. botryoides, macrocarpum, Gl. microcarpum, Gl. sinuosum, F. mosseae, R. aggregatum, R. intraradices, R. irregulare, R. microaggregatum, C. etunicatum, Ac. bireticulata, Ac. scrobiculata, Ac.* spp.1, *A. schenckii.*	14
T. asiatica	*Gl. botryoides, Gl. macrocarpum, Gl. microcarpum, Gl. rubiforme, Gl. sinuosum, F. geosporum, F. mosseae, S. constrictum, S. viscosum, R. aggregatum, R. intraradices, R. microaggregatum, Ac. cavernata, Ac. denticulata, Ac. laevis, Ac. rehmii, Ac. scrobiculata, Ac. spinosa, Ac.* spp. 2, *Ac.* spp. 3, *G. albida, G. decipiens, P. albidum.*	23
S. nux-vomica	*Gl. macrocarpum, Gl. microcarpum, Gl. sinuosum, F. geosporum, F. mosseae, R. aggregatum, R. intraradices, E. infrequens Ac. cavernata, Ac. denticulata, Ac. spinosa, A.* spp. 3.	12
M. hortensis	*Gl. macrocarpum, Gl. microcarpum, Gl. sinuosum, F. badium, F. geosporum, R. microaggregatum, Ac. bireticulata, Ac. denticulata, Ac. spinosa, G. albida, G. decipiens, G. margarita, G. rosea, A. schenckii.*	14
M. philippensis	*Gl. macrocarpum, Gl. microcarpum, Gl. rubiforme, Gl. sinuosum, Gl. taiwanense, S. viscosum, R. aggregatum, R. intraradices, R. microaggregatum, D. minuta, Ac. bireticulata, Ac. cavernata, Ac. denticulata, Ac. rehmii, Ac.* spp. 3,*Gi.decipiens, Gi. margarita A. schenckii*	18

All the morphotypes were identified to species level except three. From the 37 AM fungal morphotypes a total of 10 species belong to the genus *Acaulospora*, 6 to the genus *Glomus*, 4 each to the genera *Rhizoglomus*, *Gigaspora*, 3 to *Funneliformis*, 2 each to genera *Septoglomus*, *Paraglomus*, and one each to *Entrophosphora*, *Dominikia*, *Claroideoglomus*, *Scutellospora* and *Archaeospora*.The above results showed that *Acaulospora*was the predominant genus followed by *Glomus.*These findings are in accordance with results of Mangan et al.[47], Zhao & Zhao[48], Nandakwang et al.[43], Wongmo[49] and Emmanuel et al.[50]. Such wider occurrence of *Acaulospora* is due to their facultative symbiotic nature, adapted to a wide array of soil and host species [51]. Moreover, *Acaulospora* are frequently associated with soil with low pH [52], [53].

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 [54]. Hayman and Stovold [55] 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. [56], Rajkumar et al. [57].

There were significant differences in the species richness AM fungi in the rhizosphere soil of the tested tree species of Pachaimalai hills. The tree species from tropical forests exhibit differential responses and compatibility in growth in relation to AM fungal species[58]. Our results confirm this, a high diversity of AM fungi (23 species) associated with*T. asiatica*followed by *Z. jujuba*(20 species), *M. philippensis*(18 species), *P. acerifolium*(16 species), *M. calabura*, *L. leucocephala*, *M. hortensis*(14 species each) and *S. nux-vomica*(12 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[59],[60]. The species richness was relatively high and varied with host plant species and somewhat relation to soil properties, especially P content [61].

IV. CONCLUSION

The present study revealed the distribution of AM fungi in eight tested tree species of Pachaimalai hills, 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 speciesof Pachaimalai hills, including herbs and shrubs to understand the overall status of AM fungi in this forest.

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