DIVERSITY OF ARBUSCULAR MYCORRHIZAL FUNGI IN SHOLA FORESTS OF KODAIKANAL

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INTRODUCTION

Mycorrhiza is the mutualistic symbiosis (non-pathogenic association) between certain soilborne fungi and plant roots (Sieverding, 1991). Mycorrhizal fungi often connect plant root systems over broad areas through their mycelial networks. These fungi frequently comprise the largest portion of soil microbial biomass (Högberg and Högberg, 2002). Arbuscular mycorrhiza (AM) is the most widely distributed association in plants. About 80% of all terrestrial plant species form this type of symbiosis (Smith and Read, 1997). Plant roots have evolved to accommodate, utilize and control mycorrhizal fungi. Both molecular and fossil evidence indicate that the earliest land plants were mycorrhizal (Redecker et al., 2000). Plants could not have colonized land without fungal partners capable of acquiring nutrients from the undeveloped soils that existed during the Silurian and Devonian (Pirozynski and Malloch, 1975). There are plants, however, that have been shown to be mycorrhiza free, such as Proteaceae (Nicholson, 1967; Brundrett et al., 1996), Cruciferae, Zygophyllaceae (Varma, 1998) Dipterocarpaceae, Betulaceae, Myrtaceae and Fagacaeae (Nicholson, 1967). The reason why some plants do not form mycorrhizas is not fully known, but it may be related to the presence of fungal toxic compounds in root cortical tissue or in root exudates. It may also be due to interactions between the fungus and the plant at the cell wall and (or) middle lamella level (Tester et al., 1987). High concentrations of salycilic acid have been found to reduce mycorrhization (Medina et al., 2003), meaning that plants with a genetic basis for high salycilic acid content have evolved to be non-mycorrhizal.

Arbuscular mycorrhizal morphology is distinguished into *Arum* – type and *Paris* – type. The *Arum*-type association is characterized by intercellular hyphal growth in the root cortex, with short lateral branches into cortical cells forming arbuscules (Smith and Smith, 1997). Intracellular–hyphal coils frequently having intercalary arbuscules spreading cell to cell in the cortex characterize the *Paris*– type association , has been found to be more frequent in natural ecosystems (Yamato and Iwasaki, 2002; Ahulu *et al* ., 2005; Tsuyuzaki *et al* ., 2005) . Three main components are involved in AM association: the soil, the fungus and the plant. The fungal component involves the fungal structure within the cell of the root and the

extraradical mycelium in the soil. The last may be quite extensive under some conditions, but does not form any vegetative structures (Smith and Read, 1997). Its primary function is the absorption of resources from the soil. The increased efficiency of mycorrhizal roots versus non-mycorrhizal roots is caused by the active uptake and transport of nutrients by mycorrhizae.

AM fungal diversity is the major factor in the maintenance of plant biodiversity and ecosystem stability and function. Several studies indicate that AM fungi alter plant community structure by affecting the relative abundance of plant species and plant-species diversity (Grimme *et al.*, 1987; Gange *et al.*, 1990; Sanders and Koide 1994). Interplant transport of assimilates from the dominant canopy species via a common mycorrhizal network to subordinate plant species, has been suggested as a mechanism by which AM fungi affect the floristic diversity of plant communities (Grimme *et al.*, 1987). Another mechanism by which AM fungi may affect plant community structure is the differential growth response of plant species to colonization by AM fungi, the so called "mycorrhizal dependence" (Gederman, 1975; Plenchete *et al.*, 1983; Habate and Manjunath, 1991).

The species composition and diversity of AM fungal communities has the potential to determine plant population and plant community structure. The fact that plant species vary in the degree of response to AM fungal species has important implications for growth of individual plant species. In turn, this will affect a plant's ability to coexist with other plant species in a community (Van der Heijden *et al.*, 1998). On the other hand, established mycorrhizal plants may serve as important sources of inoculum for initially non-mycorrhizal, conspecifics, which may affect regeneration and could contribute to patchy distributions of species within the community (Koide and Dickie, 2002).

Arbuscular mycorrhizal fungi occur in all kind of landforms including mountains (Shi et al., 2007), plateaus (Pan et al., 1997), hills, plains (Gai et al., 2006), islands (Liu et al., 2001) and basins (Wang et al., 2006). Generally, the AM fungal spores are isolated from field soil and identified based on their morphology and sub cellular characters. More than 200 AM fungal species are described based on spore morphology (Schüßler et al., 2001), but characterization of spore morphology requires considerable experience (Clapp et al., 2001). Spore counts may not reflect the true composition of the AM fungal or plant communities (Turnau et al., 2001).

This lack of reliability arises from the taxon-specific differences existing between sporulation and root colonization rates. The most common and widely distributed, AM fungal genus in the tropics is *Glomus*, followed by *Acaulospora* and *Scutellospora*. Therefore, information regarding the active AM fungi in roots is crucial for any ecological field studies. Understanding AM morphology with in roots at best allows discriminating AM fungi at the family or genus level (Merryweather and Fitter, 1998).

The unique combination of forests and grassland comprise the Shola forest. They are stunted evergreen forest found as patches in grasslands especially in Valleys. The Sholas are dark damp throughout the year, because Shola soil absorbs and retains water like a sponge. However, wide diversity and unique floral distribution, no systematic investigation has been carried out to explore the root fungal associations in plant species of shoal forests. When compare to other ecosystems, shoals are poorly explored for AM fungal distribution. In shoals of Western Ghats region, 29 plant species has been studied for AM fungal association (Bagyalakshmi et al., 2010). The root fungal associations of 107 medicinal and aromatic plant species have been assessed in Western Ghats region (Muthukumar et al., 2006). Six plant species in shoal forest of Velliangiri hills, western Ghats, Southern India, has been examined for AM fungal association and spore numbers (Muthukumar et al., 2018). Mycorrhizal status of sixteen epiphytic and terrestrial ferns has been explored from Kodaikanal Hills of Southern India (Raju et al., 1995). Arbuscular mycorrhizal association of 60 ferns and lycophytes were observed from Palni hills, Western Ghats region southern India (Muthukumar rt al., 2014). These studies insist that the importance of mycorrhizal research which deserves much attention is the investigation of more plant species for their mycorrhizal status. In addition, the results of this investigation are primarily used for revegetation programs in shoal forest. The seasonal dynamics of AM fungi is essential to quantify the functioning and ecological significance of AM in natural ecosystems. The increase in the AM fungal spore numbers suggests a period in which the fungi act as a carbon sink (Smith and Read, 2008). Therefore the present investigation was carried out to fulfill the following objectives, (i) To assess the incidence and the types of AM association in shola plant species, (ii) To evaluate the arbuscular mycorrhizal (AM) fungal diversity, (iii) To record the seasonality of AM fungi in shola forest, (iv) To observe if any relationship between plant diversity and AM fungal diversity, (v) To identify the nutrient uptake mechanisms of shola species.

Materials and Methods

Study site

The study site, Kodaikanal (longitude 77° 26' to 77° 33' E and latitude 10° 12' to 10° 15'N) is located within the Eastern offshoot of the Western Ghats and the spur aligned on a east west and north south axis. The shoal forest is occupied by upper elevations of the Palani hills. Sholas are patches of jungles isolated from forests varied with plant species composition and size. The streams running through the shoals and trees showed stunted growth. The research was conducted among shoals with altitudes ranging from 360m - 2550m. The annual rainfall is quite variable in the hills (1300 mm) with temperatures ranging from 13 to 24°C in summer and winter ranged from 7 to 16°C in the summer.

Sampling

Root and soil samples for each species were collected from five individuals at different stages of growth (vegetative and reproductive). Care was taken during collection that roots of shrubs and tree species could be positively identified. For this reason, samples of herbs were usually made by uprooting the plants. Roots were washed and stained within 24h or preserved in formalin acetic acid-alcohol before staining. Rhizosphere soil from roots and adjacent to plants was collected. Soil samples collected from different individuals of a species were mixed to form a composite sample. These composite soil samples were used for the isolation of AM fungal spores.

Preparation of roots and AM assessment

Fixed roots were washed free of formalin acetic acid alcohol (5:5:90; V/V) (FAA) and examined under a dissection microscope (X 20) for AM fungal spores attached to roots. After examination, the roots were cut into 1-cm fragments, cleared in 2.5% KOH (Koske and Gemma 1989), acidified with 5 N HCl and stained with trypan blue (0.5% in lactoglycerol) overnight. Roots that remained dark after clearing were bleached in alkaline H_2O_2 prior to the acidification. The stained roots were examined with a compound microscope (X 200–400) for AM fungal structures and the percentage of root length colonization was estimated according to the magnified intersection method (McGonigle et al. 1990). In addition, the number of hyphae, arbuscule and vesicle intersections were noted. It was thus possible to quantify both the root length colonized by AM structures and total root length colonization. Only species in which arbuscules found were considered to have arbuscular mycorrhizae.

The AM-morphology was classified as *Arum*- or *Paris*-type based on whether the fungal hyphae were present mainly as hyphae running through intercellular spaces or within cells as coils respectively following descriptions of Dickson (2004). Since we examined whole and squashed roots, we could not reliably distinguish among the intermediate sub-type morphologies as described and classified by Dickson (2004). However, wherever the parallel running hyphae were seen intracellular, the morphology was designated as the Intermediate-type.

Isolation, enumeration and identification of AM fungal spores

The soil samples from rhizosphere soil were used for enumerating AM fungal spores. One hundred grams of the soil samples were dispersed in 1L water and the suspension was decanted through 710 to 37µm sieves. The residues in the sieves were washed into beakers. The sievates were dispersed in water and filtered through gridded filter papers. Each filter paper was then placed spread on a Petri dish and scanned under a dissection microscope at X40 magnification and all intact spores (non-collapsed spores with cytoplasmic contents and free from parasitic attack) were counted. Sporocarps and spore clusters were considered as one unit. The soils of the pot culture were used for identification of AM fungi. After isolation of the spores as described above, the intact spores were transferred using a wet needle to polyvinyl alcohol-lacto glycerol with or without Melzers reagent on a glass slide for identification. Spores were identified from spore morphology and sub cellular characters and compared to original descriptions (Schenck and Perez 1990). Spore morphology was also compared to the culture database established by INVAM (<u>http://invam.cag.wvu.edu</u>).

Trap cultures

Rhizosphere soil samples were mixed thoroughly to form a composite soil sample. Two-liter capacity pots were filled with 1 L of thrice pasteurized (120°C for 60 min) sandy soil followed by 500ml of the mixed composite rhizosphere soil sample. The pots were seeded with *Eleusine coracana* (L.) Gaertn., and the seedlings were thinned to 5 seedlings per pot after germination. A total of 12 pots, were arranged in a randomized block design. At the end of the growth period the soil samples were taken from each pot and AM fungal spores were isolated by a modified wet-sieving and decanting method as detailed above.

Identification of AM fungal spores

Intact and crushed spores in polyvinylalcohol-lactophenol and in Melzer's reagent were examined and identified according to Schenck and Perez (1990). Spore colour was examined under a dissection microscope on fresh specimens immersed in water. Classification, spore wall characters and the spelling of scientific names are as suggested by Morton and Benny (1990), Walker (1983, 1986) and Walker and Trappe (1993).

Life-history attributes and plant nomenclature

Each plant species recorded during the survey was categorized for life-form and life-cycle attributes as determined from the literature (Parin 1981a,b; Toby and Hodd 1982; Nair and Henry 1983; Henry et al. 1987,1989) or field observations. Nomenclature and authorities are as used by Nair and Henry (1983) and Henry et al. (1987, 1989).

RESULTS

Occurrence of AM association

Of the 71 plant species (in 35 families) examined, all the families were colonized by AM fungi except two species in a genus *Psychotria*(Table 1). AM association was observed in members of supposedly non-mycorrhizal families Commelinaceae, Cleomaceae and Convolvulaceae. Only those species in which arbuscules or arbusculate coils were found were considered to have AM association. The fungal entry into roots was characterized by the presence of appresorium (Plate 1.). Further invasion of the roots varied depending upon the AM types.

AM morphology

Thirty four of the plant species had *Arum*-type morphology, 25 had Intermediate- type and 12 had typical *Paris*-type morphology. The *Arum*-type was characterized by the presence of intercellular hyphae, vesicles and intracellular arbuscules. Intracellular hyphal coils, arbusculate coils and intracellular vesicles characterized the *Paris*-type morphology. The Intermediate-type had intracellular hyphal coils, as well as intercellular hyphae, arbuscules / arbusculate coils and inter / intracellular vesicles (Figure 1).

AM morphology in life forms

In herbs 20 species had Arum type morphology, 10 had Paris type morphology and 14 had Intermediate type morphology. In Shrubs, 8 species had Arum, one species had Paris and 5 species had Intermediate type morphology. In Tree species 4, 1 and 6 plant species had Arum, Paris and Intermediate type morphology respectively (Table 1; Figure 2).

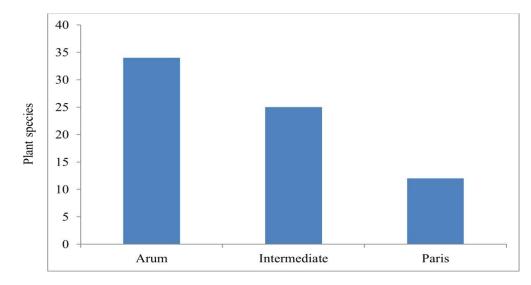
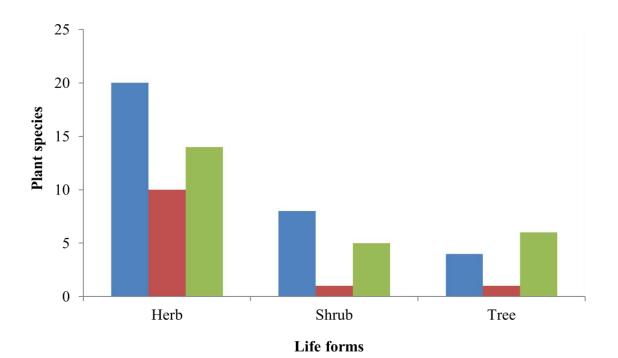


Figure 1. Arbuscular mycorrhizal fungal morphology in shoal plant species of Kodaikanal

Figure 2. Arbuscular mycorrhizal fungal morphology in various life forms of shoal species in Kodaikanal



	TT.1.4	Fungal	
Family/Plant species	Habit	association	AM type
Acanthaceae	Charach	4 N <i>I</i>	A
Justicia adhatoda F. Muell.	Shrub	AM	Arum
Justicia txanquebariensis Roxb.	Herb	AM	Arum
Rungia repens Nees.	Herb	AM	Arum
Thunbergia fragrans C. Presl,	Herb	AM	Paris
Acorus calamus L.	Herb	AM	Paris
Amaranthaceae			
Achyranthus aspera	Herb	AM	Intermediate
Aerva lanata	Herb	AM	Arum
Hypoxidaceae			
Curculigo orchioides Gaertn.,	Herb	AM	Arum
Annonaceae			
Annona squamosa L.,	Herb	AM	Arum
Apiaceae			
Centella asiatica (L.) Urb.	Herb	AM	Paris
Apocynaceae			
Cascable thevetia	Tree	AM	Intermediate
Holarrhena antidysenterica	Tree	AM	Intermediate
Aristolochiaceae			
Aristolochia bracteolata	Herb	AM	Arum
Aristolochia indica	Tree	AM	Intermediate
Asclepiadaceae			
Gymnema sylvestre	Herb	AM	Intermediate
Asteraceae			
Anaphalis lawii (Hook.f.) Gamble.	Herb	AM	Intermediate
Ageratum conyzoides L,	Herb	AM	Arum
Balsamiaceae			
Impatiens campanulata Wight,	Herb	AM	Intermediate
Begoniaceae			
Begonia malabarica Buch	Herb	AM	Paris
Caesalpinaceae	nero	7 1111	1 0/15
Cassia fistula L.,	Tree	AM	Intermediate
Delonix regia (Bojer) Raf.,	Tree	AM	Arum
Cleomaceae	1100	1 1111	<u> </u>
Cleome gynandra L.	Herb	AM	Intermediate
Combretaceae	11010	1 1111	memeut
<i>Terminalia arjuna</i> (Roxb. Ex DC.) Wight & Arn.	Tree	AM	Intermediate

Table 1. Arbuscular mycorrhizal (AM) fungal morphology and colonization in shola plant species of Kodaikanal

Commelinaceae			
Commelina benghalensis Wall.,	Herb	AM	Arum
Convolvulaceae			
Evolvulus alsinoides (L.) L	Herb	AM	Arum
Ipomoea batatas L.	Shrub	AM	Arum
Cucurbitaceae			
Mukia leiosperma	Shrub	AM	Intermediate
Euphorbiaceae			
Acalypha indica Vell.,	Herb	AM	Intermediate
Euphorbia hirta L.	Herb	AM	Intermediate
Jatropha gossypiifolia L.	Shrub	AM	Arum
Phyllanthus amarus Schumach. & Thonn.	Herb	AM	Intermediate
Phyllanthus maderaspatensis Thouars ex Baill.	Herb	AM	Intermediate
Labiatae			
Leonotis nepetifolia (L.) R. Br.,	Shrub	AM	Paris
Leucas aspera Link,	Herb	AM	Paris
Plectranthus caninus Roth	Herb	AM	Arum
Malvaceae			
Abutilon indicum (L.) Sweet	Shrub	AM	Arum
Sida acuta Burm. F.	Herb	AM	Intermediate
Sida cordifolia Forssk.,	Herb	AM	Arum
Mimosaceae			
Mimosa pudica L.	Herb	AM	Arum
Prosopis cineraria	Tree	AM	Arum
Acacia pinnata	Tree	AM	Arum
Myrtaceae			
Syzygium cumini (L.) Skeels,	Tree	AM	Arum
Nyctaginaceae			
Boerhavia diffusa	Herb	AM	Arum
Oxalidaceae			
Biophytum intermedium var. pulneyensis	Herb	AM	Intermediate
Oxalis ausensis R. Knuth	Herb	AM	Arum
Papilonaceae			
Desmodium triflorum (L.) DC.,	Herb	AM	Arum
Indigofera tinctoria Chapm.	Shrub	AM	Arum
Acacia melanoxylon R. Br.	Tree	AM	Paris
Passifloraceae			
Passiflora leschenaultia Dc.,	Shrub	AM	Arum
Poaceae	2111.00		
Bambusa bambos	Herb	AM	Paris
Cynodon dactylon (L.) Pers.	Herb	AM	Arum
Echinochloa colona (L.) Link	Herb	AM	Arum
Setaria verticillata (L.) P. Beauv.	Herb	AM	Intermediate

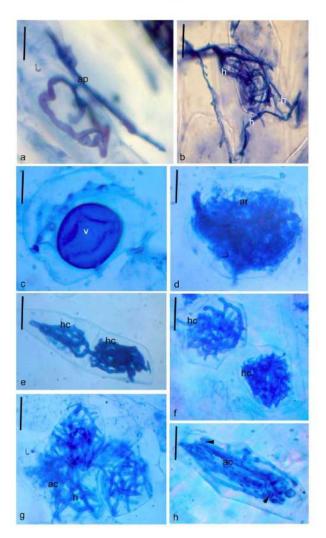
Eragrostis nigra Nees ex Steud	Herb	AM	Paris
Polygonaceae			
Polygonum glabrum Willd.	Herb	AM	Paris
Rubiaceae			
Hedyotis puberula	Herb	AM	Arum
Lacianthus acminatus	Shrub	AM	Intermediate
Psychotria octosulcata	Shrub	NAM	
Psychotria nilgriensis var. astephana	Tree	AM	Intermediate
Psychotria nilgiriensis Deb & M. Gangop.,	Tree	NAM	
Lasianthus attenuates Jack	Shrub	AM	Intermediate
Morinda pubescens Sm.	Shrub	AM	Arum
Rutaceae			
Toddalia asiatica (L.) Lam.,	Shrub	AM	Intermediate
Sapindaceae			
Cardiospermam helicacabum	Herb	AM	Paris
Solanaceae			
Solanum pubescens Roxb.	Shrub	AM	Intermediate
Solanum giganteum Jacq	Shrub	AM	Arum
Urticaceae			
Elatostema sessile	Herb	AM	Intermediate
Verbenaceae			
Clerodendrum phlomides	Herb	AM	Arum
Lantana camara L.	Herb	AM	Intermediate
Lippia javanica (Burm.f.) Spreng.	Herb	AM	Arum
Violaceae			
Hybanthus enneaspermus (L.) F. Muell	Herb	AM	Paris

Extent of AM association

There were large differences in the extent of AM colonization and root lengths with AM fungal structure between plant species. Total root length colonization (%RLTC) ranged from 25.84 % (*Commelina benghalensis*, Commelinaceae) to 95.14% (*Impatiens campanulata*, Balsamiaceae) and varied significantly among plant species ($F_{70,213}$ = 44.49; P<0.001)(Figure 3). The percentage root length with inter or intracellular hyphae (%RLH) ranged from 3.96% (*Oxalis ausensis*, Oxalidaceae) to 43.23% (*Halorrhena antidysenterica*, Apocynaceae) and varied significantly amoung plant species ($F_{410, 213}$ = 55.38; P<0.001). Similarly percentage root length with hyphal coils (%RLHC) ranged from 1.13 % (*Justicia tranquebariensis*, Acanthaceae) to 33.55% (*Eragrostis nigra*, Poaceae) and varied significantly amoung plant species ($F_{70,213}$ = 68.86; P<0.001). In colonized plants, percentage root length with arbuscules (%RLA) ranged from 1.67% (*Hybanthus enneaspermus*,

Violaceae) to 24.08% (*Curculigo orchioides*, Amaryllidaceae) and varied significantly among plant species ($F_{70, 213} = 35.55$; P<0.001). The percentage root length with vesicles (%RLV) ranged from 0.42% (*Anaphalis lawii*, Asteraceae) to 26.81% (*Impatiens campanulata*, Balsamiaceae) and varied significantly among plant species ($F_{70,213} = 54.15$; P<0.001). The percentage of root length with arbusculate Coils (%RLAC) ranged from 1.67% (*Echinocola colona*, Poaceae) to 22.67% (*Anaphalis lawii*, Acanthaceae) and varied significantly among plant species ($F_{70,213} = 17.13$; P<0.001) (Table 2).





	% Colonization							
Family/Plant species	RLH	RLV	RLA	RLAC	RLHC	RLTC	(100g Soil)	
Acanthaceae								
Justicia adhatoda	25.15 ± 1.66	20.13 ± 1.26	15.80 ± 0.59	6.92 ± 1.26	2.52 ± 0.63	50.60 ± 4.00	3.14 ± 0.60	
Justicia txanquebariensis	19.77 ± 1.13	14.12 ± 0.56	12.43 ± 1.49	1.69 ± 0.98	1.13 ± 1.13	35.40 ± 4.34		
Rungia repens	27.83 ± 0.16	19.26 ± 0.54	$12.47{\pm}~0.77$	3.89 ± 1.31	2.75 ± 0.97	47.26 ± 2.18	5.33±0.56	
Thunbergia fragrans	8.52 ± 1.19	2.92 ± 0.97	11.27 ± 0.64	6.76 ± 1.21	22.94 ± 1.12	52.41 ± 2.49	6.53±0.51	
Acorus calamus	7.64 ± 2.67	0.00 ± 0.00	2.31 ± 0.55	4.32 ± 0.62	25.34 ± 1.82	39.61 ± 2.84	5.88±3.13	
Amaranthaceae								
Achyranthus aspera	26.54 ± 0.70	20.77 ± 0.16	18.46 ± 0.68	11.53 ± 0.09	10.38 ± 0.08	66.94 ± 0.52	3.97±1.15	
Aerva lanata	26.94 ± 1.29	18.64 ± 1.40	12.30 ± 0.61	6.08 ± 1.30	3.84 ± 1.00	49.50 ± 3.51	5.31±0.46	
Amaryllidaceae								
Curculigo orchioides	12.45 ± 1.22	0.00 ± 0.00	24.08 ± 0.84	8.64 ± 0.97	2.46 ± 0.61	47.63 ± 2.00	7.71±0.69	
Annonaceae								
Annona squamosa	28.27 ± 1.84	20.25 ± 1.26	8.86 ± 0.73	7.60 ± 1.27	8.02 ± 1.12	53.12 ± 1.50	7.56±0.66	
Apiaceae								
Centella asiatica	3.48 ± 0.59	4.86 ± 1.48	15.68 ± 1.94	6.92 ± 2.18	21.31 ± 1.47	52.25 ± 4.59	6.02 ± 1.55	
Apocynaceae								
Cascable thevetia	36.21 ± 4.34	19.54 ± 1.15	10.92 ± 0.58	3.45 ± 1.99	1.72 ± 0.00	52.29 ± 4.49	3.49±1.13	
Holarrhena							3.42 ± 0.91	
antidysenterica	43.23 ± 1.38	10.42 ± 2.27	8.34 ± 2.60	6.25 ± 1.80	7.81 ± 3.25	66.71 ± 4.58		
Aristolochiaceae								
Aristolochia bracteolata	27.68 ± 0.23	19.16 ± 0.53	12.42 ± 0.90	5.02 ± 0.73	2.72 ± 0.94	48.15 ± 2.27	9.20±0.57	
Aristolochia indica	31.22 ± 0.25	22.77 ± 0.57	12.66 ± 0.79	9.69 ± 0.78	8.02 ± 0.48	61.76 ± 0.90	4.96±0.95	
Asclepiadaceae								
Gymnema sylvestre	31.75 ± 1.19	18.31 ± 0.85	12.18 ± 0.55	10.15 ± 0.72	6.52 ± 0.50	60.76 ± 1.01	4.24±0.65	
Asteraceae								
Anaphalis lawii	11.10 ± 0.74	0.42 ± 0.42	15.78 ± 1.69	22.76 ± 2.49	25.41 ± 2.14	75.47 ± 0.49	5.16 ± 0.58	

Table 2. Extent of arbuscular Mycorrhizal (AM) fungal colonization and spore numbers in shola plant species of Kodaikanal.

Ageratum conyzoides	8.64 ± 0.50	6.05 ± 0.95	21.29 ± 3.09	8.18 ± 0.31	4.83 ± 1.45	48.99 ± 2.07	7.60±0.99
Balsamiaceae							
Impatiens campanulata	38.23 ± 0.47	26.81 ± 0.93	9.27 ± 0.43	2.92 ± 0.21	17.91 ± 0.44	95.14 ± 0.39	4.35 ± 1.10
Begoniaceae							
Begonia malabarica	8.52 ± 0.59	5.29 ± 0.55	4.36 ± 0.59	5.76 ± 1.84	29.26 ± 1.25	53.18 ± 3.65	13.38±0.62
Caesalpinaceae							
Cassia fistula	30.80 ± 0.42	22.78 ± 0.73	13.08 ± 0.42	9.70 ± 0.84	8.01 ± 0.42	61.74 ± 1.43	$5.24{\pm}1.05$
Delonix regia	25.15 ± 1.66	20.13 ± 1.26	15.80 ± 0.59	6.92 ± 1.26	2.52 ± 0.63	50.60 ± 4.00	4.44 ± 0.72
Cleomaceae							
Cleomy gynandra	26.65 ± 1.63	21.42 ± 1.08	17.28 ± 0.15	10.84 ± 0.07	7.51 ± 1.10	62.65 ± 0.69	13.30±0.69
Combretaceae							
Terminalia arjuna	21.82 ± 0.34	9.86 ± 0.70	7.02 ± 0.57	3.50 ± 0.67	3.65 ± 0.76	36.25 ± 1.87	5.10±0.53
Commelinaceae							
Commelina benghalensis	15.34 ± 2.26	9.65 ± 0.52	6.31 ± 1.06	2.08 ± 0.10	2.08 ± 0.10	25.84 ± 2.00	6.31±0.52
Convolvulaceae							
Evolvulus alsinoides	27.95 ± 1.71	14.63 ± 1.01	10.35 ± 0.75	5.33 ± 1.09	2.75 ± 0.57	46.56 ± 1.70	3.60 ± 0.84
Ipomoea batatas	5.15 ± 1.19	0.00 ± 0.00	22.38 ± 0.45	8.18 ± 0.32	3.80 ± 1.37	39.51 ± 1.30	5.04 ± 0.91
Cucurbitaceae							
Mukia leiosperma	14.97 ± 1.58	2.89 ± 0.94	19.51 ± 0.53	6.31 ± 0.89	8.53 ± 0.55	52.21 ± 2.81	11.00±0.96
Euphorbiaceae							
Acalypha indica	26.38 ± 0.49	22.18 ± 0.04	17.62 ± 0.94	12.64 ± 0.52	10.21 ± 0.16	66.90 ± 0.40	4.53±0.35
Euphorbia hirta	29.88 ± 2.97	22.43 ±0.13	12.92 ± 0.95	10.22 ± 1.70	8.07 ± 0.63	61.30 ± 0.81	9.30±0.49
Jatropha gossypiifolia	25.18 ± 0.88	15.24 ± 0.78	11.89 ± 1.02	3.29 ± 0.63	3.32 ± 0.68	43.92 ± 0.93	3.55 ± 0.35
Phyllanthus amarus	27.41 ± 0.95	20.77 ± 0.16	18.46 ± 0.68	11.48 ± 0.08	10.38 ± 0.08	67.76 ± 0.38	2.65 ± 0.87
Phyllanthus							9.80±0.65
maderaspatensis	28.25 ± 1.61	23.26 ± 2.81	8.85 ± 0.67	7.16 ± 1.51	8.04 ± 1.21	52.71 ± 1.67	
Labiatae							
Leonotis nepetiifolia	29.33 ± 0.77	13.33 ± 0.77	12.00 ± 0.77	12.00 ± 0.77	11.11 ± 1.18	64.84 ± 1.63	5.11±0.88
Leucas aspera	29.21 ± 0.78	14.60 ± 0.71	11.94 ± 0.72	11.51 ± 0.91	10.61 ± 0.73	63.52 ± 0.74	5.31±0.46
Plectranthus caninus	4.11 ± 1.07	0.00 ± 0.00	30.89 ± 1.68	13.46 ± 0.59	4.81 ± 1.12	53.26 ± 1.85	4.23±0.60

Malvaceae							
Abutilon indicum	28.28 ± 0.51	18.18 ± 1.51	14.65 ± 0.50	5.56 ± 1.01	3.54 ± 1.33	52.47 ± 2.68	6.55 ± 0.55
Sida acuta	33.41 ± 2.26	19.21 ± 0.39	12.55 ± 0.39	10.20 ± 0.39	7.06 ± 0.68	63.44 ± 1.71	5.52 ± 0.55
Sida cordifolia	28.07 ± 1.68	12.17 ± 0.27	10.42 ± 0.38	3.43 ± 0.94	2.86 ± 0.46	44.93 ± 0.33	2.75 ± 0.35
Mimosaceae							
Mimosa pudica	28.87 ± 0.73	17.61 ± 1.35	14.45 ± 1.03	6.44 ± 1.01	4.29 ± 0.59	54.26 ± 2.38	5.41±0.69
Prosopis cineraria	25.45 ± 1.05	12.73 ± 1.82	6.66 ± 0.61	3.03 ± 0.61	1.21 ± 0.61	36.57 ± 1.07	11.97 ± 1.50
Acacia pinnata	27.10 ± 1.77	19.83 ± 1.50	12.40 ± 0.94	4.50 ± 0.50	4.52 ± 1.13	48.90 ± 3.99	5.45 ± 0.59
Myrtaceae							
Syzygium cumini	$25.44{\pm}0.69$	13.30 ± 1.43	7.61 ± 0.95	2.38 ± 0.40	1.92 ± 0.09	37.37 ± 0.99	7.22 ± 0.58
Nyctaginaceae							
Boerhavia diffusa	24.71 ± 0.15	12.26 ± 1.26	6.49 ± 0.63	2.91 ± 0.52	1.77 ± 0.07	35.90 ± 0.09	7.79 ± 0.70
Oxalidaceae							
Biophytum intermedium							5.35 ± 0.45
var. pulneyensis	43.72 ± 2.04	9.21 ± 0.62	0.64 ± 0.04	5.09 ± 0.17	8.43 ± 0.29	67.09 ± 2.48	
Oxalis ausensis	3.96 ± 0.94	5.86 ± 1.76	34.75 ± 2.76	5.51 ± 1.01	5.44 ± 0.63	55.51 ± 5.62	4.64 ± 0.60
Papilonaceae							
Desmodium triflorum	27.83 ± 0.16	19.20 ± 0.48	12.47 ± 0.77	3.89 ± 1.31	2.75 ± 0.97	47.26 ± 2.18	2.80 ± 0.32
Indigofera tinctoria	25.29 ± 1.36	13.87 ± 2.33	8.83 ± 1.59	3.16 ± 0.62	1.90 ± 0.02	39.18 ± 3.45	11.63±0.84
Acacia melanoxylon	10.50 ± 1.01	3.38 ± 0.55	1.94 ± 1.36	4.65 ± 1.19	38.49 ± 2.25	58.96 ± 3.94	4.14 ± 0.58
Passifloraceae							
Passiflora leschenaulti	22.63 ± 4.03	14.76 ± 1.39	17.60 ± 2.17	$16.87{\pm}\ 1.20$	8.81 ± 0.86	80.67 ± 3.78	7.66 ± 0.73
Poaceae							
Bambusa bambos	28.95 ± 0.76	13.16 ± 0.76	11.84 ± 0.76	11.74 ± 0.85	10.87 ± 1.18	63.79 ± 1.99	9.52±0.43
Cynodon dactylon	27.26 ± 0.63	16.17 ± 1.31	10.37 ± 0.37	3.90 ± 0.10	2.57 ± 0.57	44.29 ± 1.08	12.01±0.95
Echinochloa colona	19.44 ± 0.56	15.55 ± 1.47	14.44 ± 2.42	1.67 ± 0.96	1.67 ± 0.96	37.54 ± 2.95	5.71±0.58
Setaria verticillata	32.54 ± 0.40	21.72 ± 3.28	13.09 ± 0.69	9.52 ± 0.69	7.54 ± 0.79	62.94 ± 1.95	2.41 ± 0.42
Eragrostis nigra	2.50 ± 0.66	1.85 ± 0.33	2.63 ± 0.62	2.31 ± 0.32	33.55 ± 2.15	42.84 ± 3.04	7.75 ± 0.75
Polygonaceae							
Polygonum glabrum	7.49 ± 1.08	0.00 ± 0.00	1.78 ± 0.34	4.49 ± 1.07	31.13 ± 2.19	44.89 ± 3.22	5.95 ± 0.25

Rubiaceae							
Hedyotis puberula	27.41 ± 0.95	20.77 ± 0.16	18.46 ± 0.68	11.54 ± 0.09	10.38 ± 0.08	67.82 ± 0.35	12.57±0.91
Lacianthus acminatus	42.60 ± 1.10	3.12 ± 0.11	6.22 ± 0.25	10.32 ± 0.45	9.43 ± 0.52	71.69 ± 0.51	5.95±0.43
Psychotria octosulcata	13.33 ± 0.48	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	13.33 ± 0.48	8.88±0.93
Psychotria nilgriensis							7.32±0.64
var. astephana	23.21 ± 0.26	2.63 ± 0.20	1.92 ± 0.11	0.00 ± 0.00	4.44 ± 0.47	32.20 ± 0.69	
Psychotria nilgiriensis	8.67 ± 0.25	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	8.67 ± 0.25	5.25 ± 0.57
Lasianthus attenuatus	23.04 ± 1.10	2.64 ± 0.12	0.00 ± 0.00	0.65 ± 0.07	1.31 ± 0.09	27.64 ± 1.05	5.24 ± 0.51
Morinda tinctoria	29.66 ± 0.43	19.55 ± 0.45	14.88 ± 1.03	5.28 ± 1.33	4.17 ± 0.99	54.33 ± 2.54	3.37±0.53
Rutaceae							
Toddalia asiatica	10.27 ± 1.33	0.00 ± 0.00	22.53 ± 4.16	17.94 ± 2.48	22.76 ± 2.30	73.51 ± 6.99	5.42 ± 0.52
Sapindaceae							
Cardiospermam							3.12±0.39
helicacabum	7.27 ± 0.42	3.51 ± 0.72	2.16 ± 0.55	8.31 ± 1.29	26.60 ± 1.28	47.84 ± 1.21	
Solanaceae							
Solanum pubescens	30.87 ± 0.33	22.06 ± 0.73	16.20 ± 1.00	9.11 ± 0.99	6.36 ± 0.39	62.67 ± 1.48	8.67±1.44
Solanum giganteum	13.88 ± 1.66	8.87 ± 2.17	23.40 ± 1.52	6.42 ± 0.67	1.19 ± 0.14	53.75 ± 2.07	5.44 ± 0.46
Urticaceae							
Elatostema sessile	26.67 ± 0.54	0.67 ± 0.09	0.00 ± 0.00	0.00 ± 0.00	6.00 ± 0.79	33.34 ± 1.03	6.22 ± 0.94
Verbenaceae							
Clerodendrum phlomides	21.10 ± 0.59	15.16 ± 1.03	13.16 ± 0.82	2.97 ± 0.50	2.36 ± 0.46	39.75 ± 1.44	6.04 ± 0.90
Lantana camara	28.27 ± 1.84	20.25 ± 1.26	8.86 ± 0.73	7.60 ± 1.27	8.02 ± 1.12	53.12 ± 2.15	10.12 ± 1.20
Lippia javanica	10.71 ± 0.88	4.68 ± 1.39	13.61 ± 1.91	5.81 ± 1.90	1.76 ± 0.23	36.57 ± 1.54	8.31±0.57
Violaceae							
Hybanthus enneaspermus	6.20 ± 0.88	3.41 ± 0.60	1.67 ± 0.39	2.98 ± 0.99	29.10 ± 1.12	43.36 ± 0.71	5.21±0.54

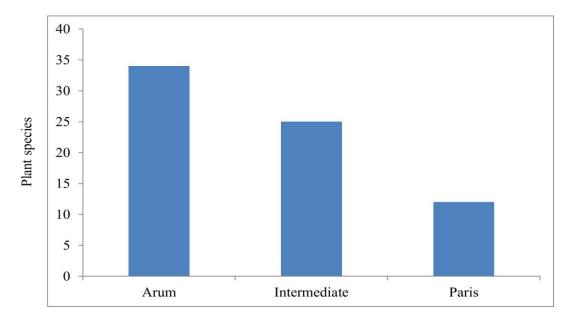
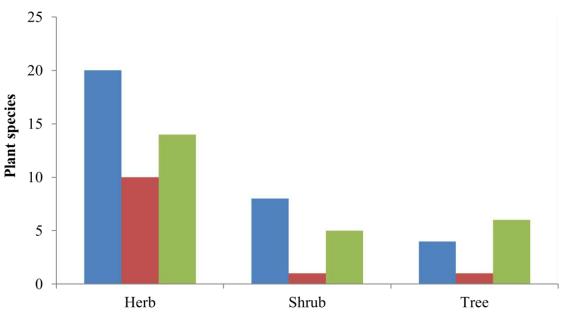


Figure 1. Arbuscular mycorrhizal fungal morphology in shoal plant species of Kodaikanal

Figure 2. Arbuscular mycorrhizal fungal morphology in various life forms of shoal species in Kodaikanal



Life forms

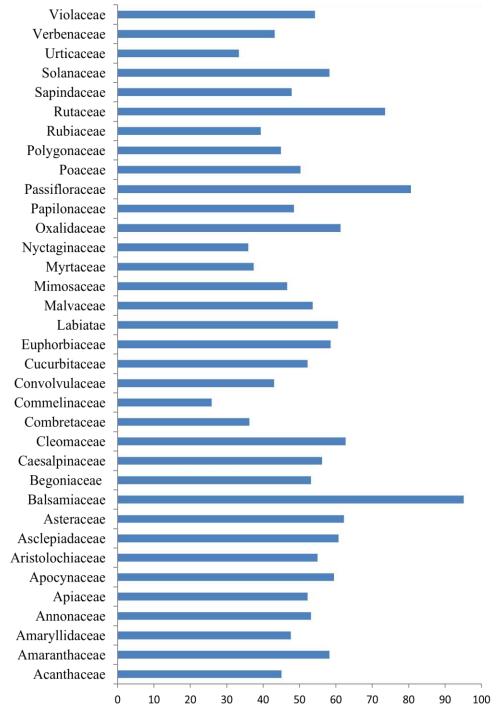


Figure 3. Average arbuscular mycorrhizal fungal colonization in plant species of shoal forests in Kodaikanal

Average percentage total colonization

AM fungal characteristics of the study sites

A total of Seven AM fungal morphotypes could be distinguished on the basis of spore morphology, to the species level (Plate 2; Table 3). These included one species in *Acaulospora. scrobiculata* Trappe), *Scutellospora calospora* Walker and Sanders, *Funneliformis geosporum* (Nicol. and Gerd.) Walker, *Glonus aggregatum* Schenck and Sm. emend. Koske, *Glomus sinuosum* (Gerd. and Bakshi) Almeida and Schenk, *Glomus viscosum* Nicolson, and *Funneliformis mosseae* (Nicolson&Gerd.) C. Walker &A.Schubler. Distribution of AM fungal spores (Table. 5) ranged from 2 spores 100 g⁻¹soil (*Setaria verticillata*, Poaceae) to 13 spores 100 g⁻¹ soil (*Cleome gynandra*, Cleomaceae)(Table 3). The spore numbers were not related to the extinct of AM colonization (r = -0.10; p >0.05; n =

213). The diversity indices like Shanon - Weaver ndex (H') ranged from 0.25(Site I) to 0.48 (Site III) and the Simpson index (D) ranged from 2.08 (Site III) to 4 (Site I) (Figure 4). *Glomus aggregatum* was the most frequent species in shoal forest. In Seasonal pattern of AM fungal spore numbers shows that, 24 spore in the month of September and six spores in the month of July (Figure 5).

Table 3. Arbuscular mycorrhizal fungal spore morphotypes isolated from different sites in sholas at
Kodaikanal. (X indicates the presence).

Fungal species	Site I	Site II	Site III	Site IV
Acaulospora scrobiculata Trappe	Х		Х	Х
Scutellospora calospora Scutellospora calospora	Х			
(T.H. Nicolson & Gerd) C. Walker & F.E.				
Sanders		Х		
Glomus aggregatum N.C. Schenck & G.S. Sm	Х			
Funneliformis mosseae (T.H. Nicolson & Gerd.)	Х			Х
C. Walker & A. Schüßler		Х		
Glomus sinuosum T.H. Nicolson	Х	Х		
Glomus viscosum	Х		Х	
Funneliformis geosporum (T.H. Nicolson &				Х
Gerd.) C. Walker & A. Schüßler		Х	Х	

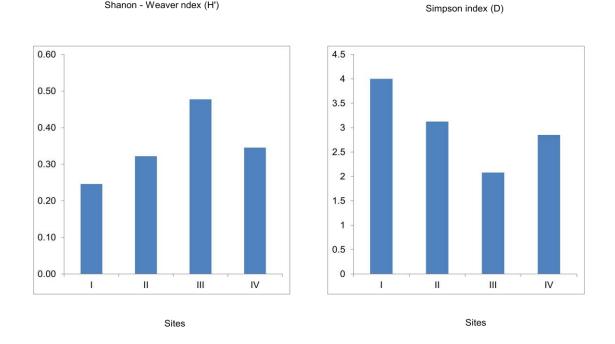
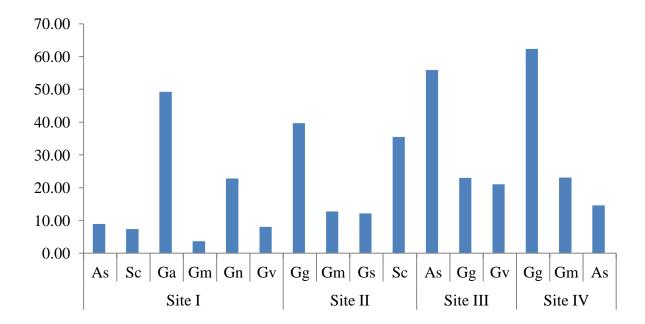


Figure 4: Arbuscular mycorrhizal diversity indices of shoal forests in Kodaikanal

Shanon - Weaver ndex (H')

Figure 5: Frequency of arbuscular mycorrhizal fungal species in the shoal forests of Kodaikanal



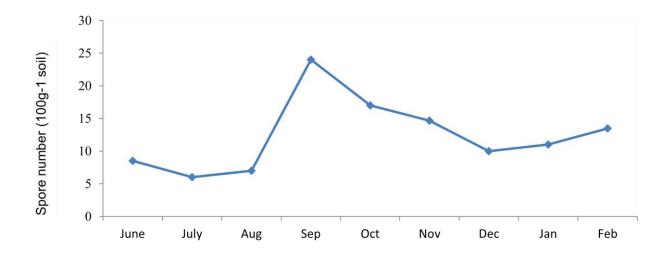


Figure 6: Arbyscular mycorrhizal fungal species dynamics in various seasons of Kodaikanal

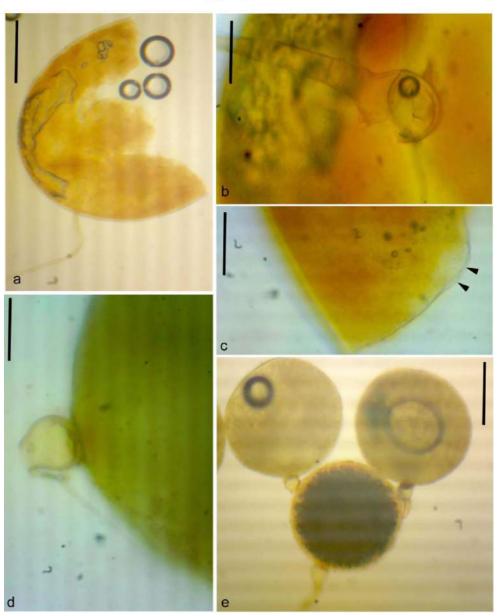


Plate - 2

Discussion

AM fungi colonize the roots of most land plants, where they facilitate mineral nutrient uptake from the soil in exchange for plant assimilated carbon. Though about 80% of the land plants are assumed to form AM association only slightly over 10,000 plant species i.e. around 3% of the known plant species have been examined for AM association (Wang and Qui, 2006). In recent years more information has been gathered regarding the mycorrhizal status of plants in natural ecosystems (Muthukumar *et al.*, 2006; Radhika and Rodrigues, 2007; Gai *et al.*, 2006; Grippa *et al.*, 2007; Tawaraya *et al.*, 2003; Tanumi Fuman and Monoranjan Ghose, 2008). In particular, there is evidence that AM fungi are common in plant groups once considered as non-mycorrhizal (Muthukmar *et al.*, 2004; Radhika and Rodrigues, 2007). This study was intended to generate more information on the occurrence of AM fungal association in Shola plant species.

The incidence of mycorrhiza (97%) in plant species of Shola forest was higher than those reported for angiosperms by Trappe (1987) and Wang and Qui (2006). Seventy percent of the 6,500 angiosperms indexed by Trappe (1987) and 80% of the 2,964 angiosperms listed by Wang and Qui (2006) were mycorrhizal. The higher mycorrhizal incidence of angiosperms in the present study could be attributed to the low nutrient status of the soils along with high plant competition. Non-mycotrophy was low (3%) in this study compared with reports from other vegetation types worldwide (Ragupathy and Mahadevan, 1993; Muthukumar and Udaiyan, 2000; Zhao *et al*, 2001; Muthukumar *et al.*, 2003). The phenomenon of nonmycotrophy is often associated with high levels of disturbance, or under extreme environmental conditions, the low incidence of non-mycotrophy in the present study is not surprising. However, plants that lacked mycorrhizal plant families (Wang and Qui, 2006).

Surveys of earlier literature showed that *Paris* type colonization occurs more predominantly in wild angiosperms (Smith and Smith, 1996; 1997; Menoyo et al., 2007). In the present study, 35% (25/71) of mycorrhizal plant species had Intermediate-type of AM morphology and typical Paris-type morphology occurred only in 17% (12/71) of the mycorrhizal species and 48% (34/71) of the shoal species had Arum- type AM morphology. It has been shown that host plants control the morphological types of AM. Gerdemann (1965) demonstrated that the same species of AM fungi formed the Paris-type in Liriodendron and Arum- type in maize, respectively. Likewise, Jacquelinet – Jeanmougin and Gianinazzi – Pearson (1983) showed that the *Paris*- type in *Gentiana* was formed by the same fungus which formed the Arum- type in Allium. Brundrett and Kendrick (1990) suggested that the types of AM are determined by the presence of continuous longitudinal air-spaces in the root cortex, i.e. the Arum- type is formed in their presence and the Paris-type is formed in their absent. However, even though the fungal identity could determine the morphological types of AM in some cases, it still seems likely that only a single type is found in a plant in most cases, which indicates the morphological types of AM depend on the characteristics of plants rather than those of Fungi (Yamato and Iwasaki, 2002).

The spore numbers of 2 to 13 spores per 100g soil is low compared to 14 to 93 per 100g soil reported by Muthukumar *et al* (2003) and 55 to 191 spores per 100g soil reported by Zhao *et al.* (2001) from Primary forest of Xishuangbanna, southwest China. The low density of AM fungal spores of the present study corroborates the reports from humid tropical forest where spore numbers tend to be low or infrequent (Janos, 1980; Fischer *et al.*, 1994). Generally, AM fungal spores in natural soils are dead or parasitized and are merely spore cases (Muthukumar and Udaiyan, 1999). The spore number reported in this study is intact healthy spores. In addition, a range of environmental, host and fungal factors influences AM fungal sporulation and spore numbers tend to decrease during root growth, but tend to increase during root inactivity or senescence (Brundrett, 1991). In undisturbed forest, spores may be relatively less important than other vegetative propagules, and primarily the soil hyphal networks initiate the colonization of new roots (Jasper *et al.*, 1989). As a result, forests with root growth throughout the year usually have small spore populations and high mycorrhizal colonization levels (Muthukumar *et al.*, 2003; Zandavalli *et al.*, 2008). In this study, AM fungal spores were present in the rhizosphere of two shoal plants species lacking

AM colonization. In natural soils, roots of adjacent plants often grow in close proximity and are interwoven, so spores in the rhizosphere of a host could be contributed by a companion plant species (Muthukumar and Udaiyan, 2000).

A total of seven AM fungal species were identified based on the morphological characters of the spores. This number is about half of those reported in semiarid Mediterranean ecosystems (Ferrol *et al.*, 2004) and semi- arid areas in Brazil (Silva *et al.*, 2005) where 23 and 21 AM fungal species were reported. However, a high AM fungal diversity has also been reported in other natural ecosystems. Muthukumar and Udaiyan (2000) reported 6–22 species per site from Western Ghats region of South India. Forty four AM fungal species were isolated from grasslands of Namibia (Uhlmann *et al.*, 2004), 43 from an arid steppe of inner Mongolia (Tian *et al.*, 2008) and 27 species from tropical rain forest of Xishuangnanna, southwest China (Zhao *et al.*, 2003). In this study, the rhizosphere soil samples of plant species contained spore morphotypes of 1 to 4 AM fungal species. This shows that several fungal species can colonize the roots of an individual plant in a natural ecosystem (Van Tuinen *et al.*, 1998; Helgason *et al.*, 1999), thus showing lack of host specificity (Smith and Read 1997). AM fungal spores belonging to *Glomus* predominated species diversity, which is in accordance with the observations that species of *Glomus* dominate tropical soils (Muthukumar and Udaiyan, 2000; Zaho *et al.*, 2001; 2003).

The lack of correlation between the AM fungal spore numbers and percent root length colonization is consistent with several previous reports in which the lack of demonstrable relationship was reported between these mycorrhizal variables (Brundrett, 1991; Zhaka *et al.*, 1995; Brundrett *et al.*, 1996). As a wide range of plant, fungal and environmental factors influence AM fungal colonization and sporulation the observed lack of relationship between these mycorrhizal variable is tenable (Helgason and Fitter, 2005).

In conclusion, AM fungal association was found to be wide spread in the plant species of shoal forests of Kodaikanal Hills, Western Ghats region. AM fungi can enhance root functions of native plants in natural ecosystems, where they are exposed to extreme competition. The future phase of this study is to entail experimental studies of these rare and economically important plant species to determine the effects of fungal inoculants on growth to restoration of shoal species in forestry.

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Summary

In natural ecosystems plant roots are colonized various microorganisms which affects plant distribution, survival and growth in different mechanisms. Most prevalent microorganism in most of the ecosystems is AM fungi. However, the prevalence of this association has been well reported from several natural ecosystems, information on AM fungal association and their abundance are unknown for shola ecosystems. In the present study, 71 plant species (in 35 families) examined, all the families were colonized by AM fungi except two species in a genus Psychotria. AM association was observed in members of supposedly non-mycorrhizal families Commelinaceae, Cleomaceae and Convolvulaceae. Only those species in which arbuscules or arbusculate coils were found were considered to have AM association. The fungal entry into roots was characterized by the presence of appresorium. Thirty four of the plant species had Arum-type morphology, 25 had Intermediate- type and 12 had typical Paristype morphology. In herbs 20 species had Arum type morphology, 10 had Paris type morphology and 14 had Intermediate type morphology. In Shrubs, 8 species had Arum, one species had Paris and 5 species had Intermediate type morphology. In Tree species 4, 1 and 6 plant species had Arum, Paris and Intermediate type morphology respectively. There were large differences in the extent of AM colonization and root lengths with AM fungal structure between plant species. Total root length colonization (%RLTC) ranged from 25.84 % (Commelina benghalensis, Commelinaceae) 95.14% to (Impatiens *campanulata*, Balsamiaceae). The percentage root length with inter or intracellular hyphae (%RLH) ranged from 3.96% (Oxalis ausensis, Oxalidaceae) to 43.23% (Halorrhena antidysenterica, Apocynaceae). Similarly percentage root length with hyphal coils (%RLHC) ranged from 1.13 % (Justicia tranquebariensis, Acanthaceae) to 33.55% (Eragrostis nigra, Poaceae). In colonized plants, percentage root length with arbuscules (%RLA) ranged from 1.67% (Hybanthus enneaspermus, Violaceae) to 24.08% (Curculigo orchioides, Amaryllidaceae. The percentage root length with vesicles (%RLV) ranged from 0.42% (Anaphalis lawii, Asteraceae) to 26.81% (Impatiens campanulata, Balsamiaceae). The percentage of root length with arbusculate Coils (%RLAC) ranged from 1.67% (Echinocola colona, Poaceae) to 22.67% (Anaphalis lawii, Acanthaceae). A total of Seven AM fungal morphotypes could be distinguished; these included species in Acaulospora. Scrobiculata, Scutellospora calospora Walker and Sanders, Funneliformis geosporum (Nicol. and Gerd.) Walker, Glonus *aggregatum* Schenck and Sm. emend. Koske, *Glomus sinuosum* (Gerd. and Bakshi) Almeida and Schenk, *Glomus viscosum* Nicolson, *and Funneliformis mosseae* (Nicolson&Gerd.) C. Walker &A.Schubler. Distribution of AM fungal spores ranged from 2 (*Setaria verticillata*, Poaceae) to 13 spores 100 g⁻¹ soil (*Cleome gynandra*, Cleomaceae). The spore numbers were not related to the extinct of AM colonization. *Glomus aggregatum* was the most frequent species in shoal forest.

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