

Diversity Status of Arbuscular Mycorrhizal (AM) Fungi in Association with Selected Mangrove Plants in Tamil Nadu

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ABSTRACT

An investigation was carried out for three mangrove plant species from two coastal regions - Parangipettai and Pazhayar - of Cuddalore and Mailaduthurai districts, respectively, Tamil Nadu, India to determine their symbiotic association potential with arbuscular mycorrhizal (AM) fungi. All mangrove plants developed AM fungal colonization in their root tissues with a mean range of 85% - 95%. All the soil sediment samples had AM fungal spores with a density range from 196 - 1403 spores/100g air-dried soil sediment. Variations in AM fungal root colonization and soil spore densities were found statistically significant. Maximum percent root colonization and soil spore population of AM fungi were recorded in samples of *Rhizophora mucronata* in both the study locations. Frequency distribution of AM fungi was also determined, and it was found that the rhizosphere samples of three mangrove species had maximum fungal population of *Rhizophora mucronata*, which was followed by that of *R. apiculata* in both the study locations. Significance of the findings is discussed in detail.

Keywords: AM fungi, *Acaulospora*, *Glomus*, *Gigaspora*, Mangroves, *Avicennia*, *Rhizophora*

INTRODUCTION

Mangrove ecosystems are one of the most productive and biologically diverse of its kind on earth. They are influenced wet land ecosystem within intertidal zones of tropical and subtropical regions located between latitude 25° N to 25° S. They have unique biological adaption characteristics and can grow in extreme conditions such as high temperature, fluctuating salinity and muddy, and aerobic soil conditions. Mangrove biomes are associated with salt tolerant plants, animals, and microbes, most of which are very admirably adapted to live in these extreme conditions and are very important for the sustenance of these biomes. Also, mangroves are potential bio-resource of important beneficial microorganisms, which can be utilized for valuable products and services.

Mangroves ecosystems provides essential services of fuel wood and serve as the nursery and feeding ground for marine as well as estuarine organisms, particularly important fishery resources, maximum depository area for carbon in the largely anaerobic sediments, which is otherwise called as blue carbon. The total amount of carbon deposited in soil is reported to vary from < 0.1% to > 40% of soil dry weight by a grand median of 2.2%. Carbon accumulation in mangrove soils attracts greater importance in terms of excess carbon dioxide sequestration from the atmosphere leading to mitigation of climatic change effects and related other disturbances (Alongi, 2002, 2009).

Mangrove forests exhibit high range of ecological stability and accomplished greater significance in both ecological and economical aspects. A study of

earth satellite data revealed that total mangrove area in 2000 was 1,37,760 sq km across the world in 112 countries and their territories. The estimated mangrove cover of the total tropical forests in the world is 0.7%. The total extent of mangroves in different continents is reported in the order of 42% in Asia, 20% in Africa, 15% each in North and Central America, 12% in Oceania and 11% in South America. Around three-fourth of the mangrove forests are concentrated in 15 countries only (Giri *et al.*, 2008, 2010). As per the India State of the Forest Report 2017, the total extent of mangroves in India is 4,921 sq km and it showed increase in the area under mangroves in three states *viz.*, Andhra Pradesh, Maharashtra, and Gujarat (ISFR, 2017).

In mangrove ecosystems, the flora is classified into (i) true or exclusive mangroves and (ii) mangrove associates. Tamil Nadu has an estimated 950 km of coastal line areas with major mangrove areas such as Pitchavaram and Muthupet. Apart from this, five districts of Tamil Nadu such as Chidambaram, Cuddalore, Nagapattinam, Ramanathapuram and Thanjavur have mangrove vegetation cover.

Soil is essential for all life forms on earth, and it supports growth of vegetation in multitude of ways including nutrition, aeration, microbial interactions, etc., and above all, it is the basis for agricultural productivity that offer food for human beings in the world. Rhizosphere region is a part of the soil where most intense interactions between microbes and plants take place. Beneficial rhizosphere microbes occur in either symbiotic or free-living relationship with most of the terrestrial plants. They play a vital role in the growth and survival of the host plants.

Arbuscular Mycorrhizae are one of the most common root colonizing fungal symbionts that form beneficial associations with the roots of more than 80% plant species. They act as extensions of plant root systems and help the plant to mobilize the important soil mineral nutrients, to provide protection/tolerance against environmental stressors and soil and root borne pathogens (Berruti *et al.*, 2016; Schouteden *et al.*, 2015).

Mangroves harbour multitude of organisms, many of which are commercial in nature. Mangrove wetlands are known to possess novel microbial components, which are yet to be explored for many of their properties. Microbial-nutrient-plant relationship in the terrestrial environment especially that of rhizosphere beneficial microbes is fully established and voluminous literature is available on the topic. However, not many studies have been carried out on salt-tolerant beneficial microbes especially the AM fungi of mangrove wetland ecosystems (Reef *et al.* 2010). The salt tolerant rhizosphere microorganisms of halophytes can be utilized as bio-inoculants for improving salinity tolerance to plants and for promoting crop production in saline soils (Etesami and Beattie, 2018). Also, relatively a huge research gap exists in the habitat and host preference of these microbes in mangroves. The salt-tolerant beneficial micro flora that can be explored for plant growth and biomass development under salt stress soil environment is relatively less explored. Hence, the present study was taken up to explore the status of AM fungi in association with selected mangrove plants in Tamil Nadu.

MATERIALS AND METHODS

Study sites

Three mangrove species viz., *Avicennia marina*, *Rhizophora apiculata* and *R. mucronata* were selected from two coastal regions at Parangipettai under Cuddalore district and Pazhayar under Mailaduthurai district of Tamil Nadu, India.

Sample collection

Soil sediment samples together with roots of selected mangrove plants of *Avicennia marina*, *Rhizophora apiculata* and *R. mucronata* were collected in neat and clean self-sealing covers by adopting standard methods. All the samples were labelled properly and brought to Forest Protection Division laboratory, IFGTB, Coimbatore for further analysis. All the samples were preserved under refrigerated condition in the laboratory before processing for studying the status of AM fungal association.

Assessment of AM fungal spore population

Air dried soil sediment samples were taken in four replicates for the determination of AM fungal spore population. The AM fungal spores were extracted

using the modified wet-sieving and decanting method of Daniels and Skipper (1982) and Gerdemann and Nicolson (1963) from soil sediment samples collected from the rhizosphere of different mangrove plant species. The contents from all the sieves were taken on filter paper and AM fungal spore density was counted under stereo binocular microscope.

Assessment of AM fungal colonization in roots

Approximately 5.0 g (fresh weight) of fine roots were used for staining and the assessment of AM fungal colonization in all the mangrove plants. Freshly collected roots were thoroughly washed with water several times and cut into uniform pieces approximately 1 cm long. Then the roots were cleared with 10% KOH solution, acidified with 1 N HCl, and stained in 0.05% Trypan blue stain method by Philips and Hayman (1970). Mycorrhizal colonization in roots was expressed as percentage of segments containing fungal structures like mycelia, vesicles and arbuscules.

Identification of AM fungal species

AM fungal spores were separated under stereo binocular microscope and mounted in PVLG and PVLG Melzer's reagent. Gentle pressure was applied on coverslip to rupture the spores for details of wall layers. Characterization of individual AM fungal spores was carried out after being subject to morphogenetic and micrometric analysis based on their colour, diameter, shape, wall layers, surface content, hyphal colour, hyphal width, and hyphal attachment with the wall. The AM fungi were identified at species level with the help of relevant literature (Trappe, 1982; Morton and Benny, 1990; Schenck and Perez, 1990; Hao *et al.*, 2002). The identity of the AM fungi was further confirmed with the species description of INAM (<http://inAM.wvu.edu/>).

Statistical Analysis

Observations of AM fungal root colonization and soil spore density were analyzed using SPSS (SPSS Inc. version 17.0). Results were subjected to one-way analysis of variance and the significant difference was determined according to Duncan's Multiple Range Test at significant level of $P < 0.05$.

RESULTS AND DISCUSSION

AM fungal root colonization

Root samples of all the mangrove plant species collected from two different coastal localities in Tamil Nadu were analysed for AM fungal root colonization and estimated the percent root colonization. It was observed from the data that the root samples of *Rhizophora mucronata* in both Parangipettai and Pazhayar had highest mean value

of AM fungal root colonization *i.e.*, $92 \pm 7.58\%$ and $95 \pm 3.54\%$, respectively. High AM fungi colonization was recorded in *Rhizophora mucronata*

species effectively and this is followed by *Avicennia marina* and *R. apiculata* (Table 1 and Figure 1).

Table 1: AM fungal root colonization percentage of the three mangrove species collected from two coastal localities in Tamil Nadu.

Location	Mangrove species	N	AM fungal root colonization (%)		
			Minimum	Maximum	Mean \pm SD
Parangipettai	<i>Avicennia marina</i>	5	80.00	100.00	88.00 ± 7.58
	<i>Rhizophora apiculata</i>	5	85.00	95.00	89.00 ± 4.18
	<i>Rhizophora mucronata</i>	5	80.00	100.00	92.00 ± 7.58
Pazhayar	<i>Avicennia marina</i>	5	80.00	95.00	89.00 ± 5.48
	<i>Rhizophora apiculata</i>	5	80.00	95.00	85.00 ± 6.12
	<i>Rhizophora mucronata</i>	5	90.00	100.00	95.00 ± 3.54
Total		30	80.00	100.00	89.67 ± 6.29

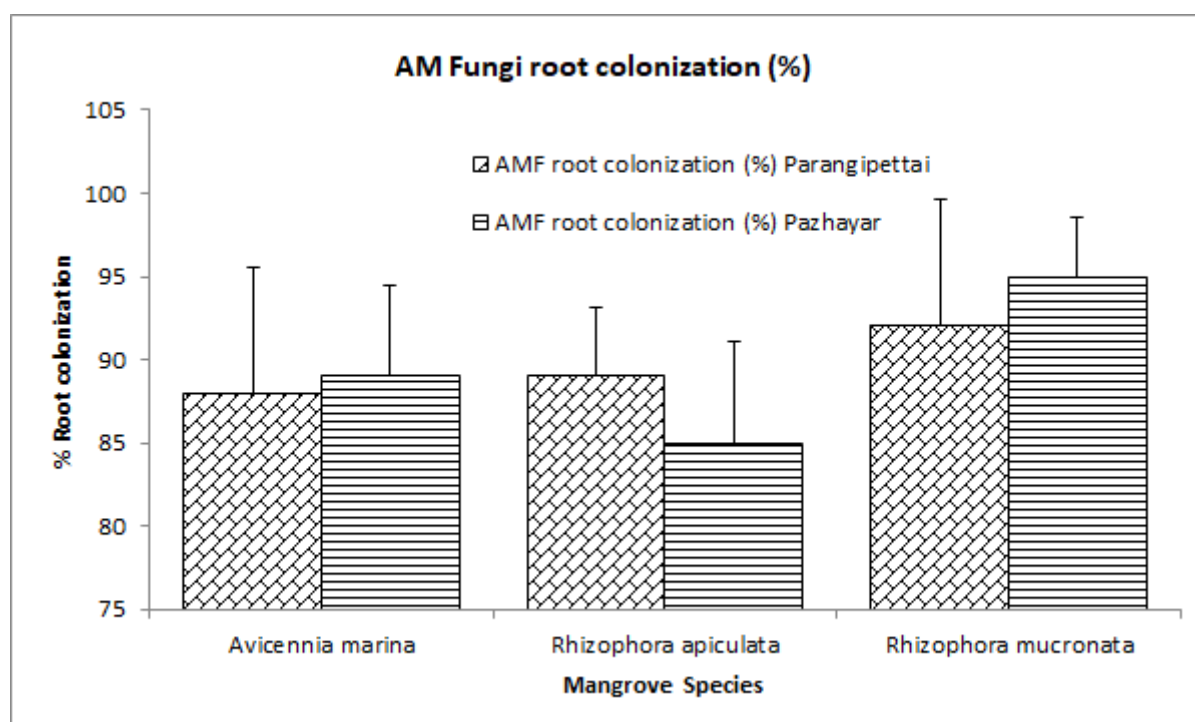


Figure 1: Arbuscular mycorrhizal fungal root colonization percentage of the three mangrove species (*Avicennia marina*, *Rhizophora apiculata*, and *Rhizophora mucronata*) collected from two coastal localities in Tamil Nadu.

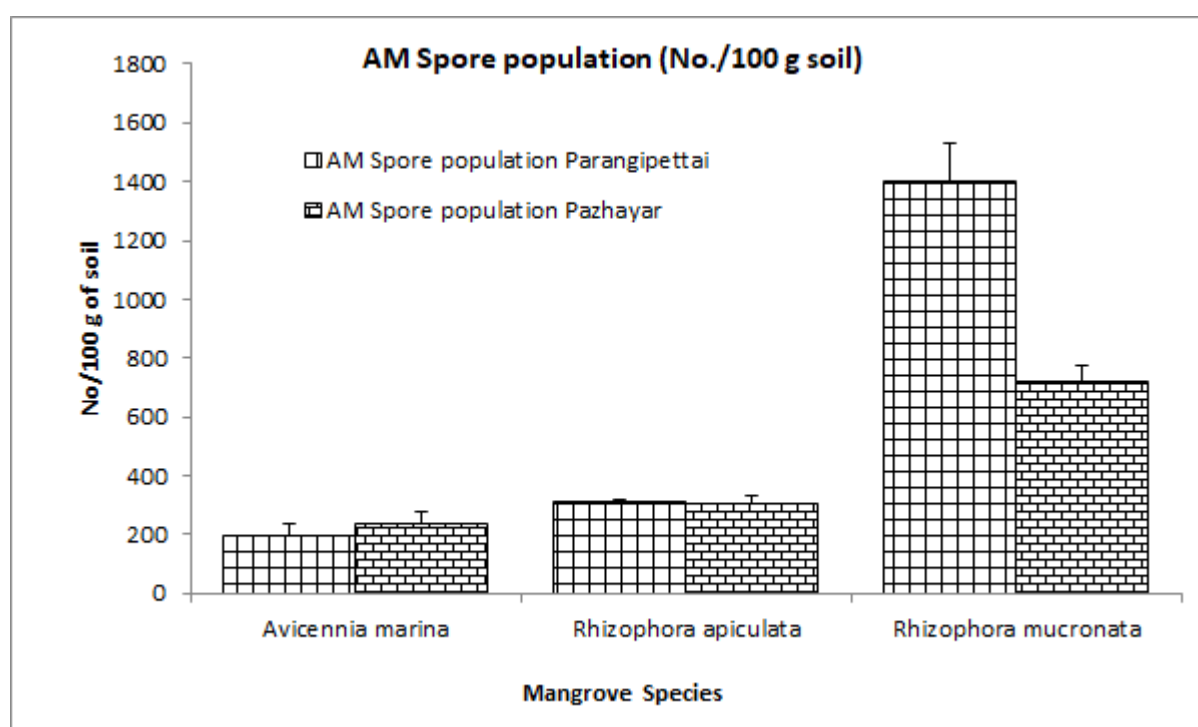
AM fungal spore population

Soil sediment samples collected from the rhizosphere of mangrove plant species from two different coastal areas were processed and enumerated for the population density of AM fungal spores by wet sieving and decanting method. The results of the AM enumeration in different samples revealed that all rhizosphere soil sediment samples had AM fungal spores but varied in population density (Table 2 and Figure 2). It was observed that *Rhizophora mucronata*

had highest mean abundance of AM spores' population in both the samples from Parangipettai (1403.33 ± 128.97 /100 g soil) as well as Pazhayar (720 ± 51.96 /100 g soil) followed by *R. apiculata*, while that of *Avicennia marina* had lowest. It was concluded from the data that rhizosphere soil associated with *Rhizophora mucronata* species had high AM spore presence irrespective of coastal locality.

Table 2: AM fungal spore population in the rhizosphere soil sediment samples of three selected mangrove plant species from Parangipettai and Pazhayar, Tamil Nadu.

Location	Mangrove Species	N	AM fungal spore population (No./100 g of soil)		
			Minimum	Maximum	Mean \pm SD
Parangipettai	<i>Avicennia marina</i>	4	168.00	246.00	196.00 \pm 43.41
	<i>Rhizophora apiculata</i>	4	304.00	320.00	311.33 \pm 8.08
	<i>Rhizophora mucronata</i>	4	1260.00	1510.00	1403.33 \pm 128.97
Pazhayar	<i>Avicennia marina</i>	4	197.00	278.00	237.00 \pm 40.51
	<i>Rhizophora apiculata</i>	4	278.00	322.00	306.67 \pm 24.85
	<i>Rhizophora mucronata</i>	4	690.00	780.00	720.00 \pm 51.96
Total		24	168.00	1510.00	529.06 \pm 442.51

**Figure 2:** Arbuscular mycorrhizal fungi spore population in the rhizosphere soil sediment samples of three selected mangrove plant species from Parangipettai and Pazhayar, Tamil Nadu.

Frequency distribution of AM fungi recorded in rhizosphere soil samples collected from three different mangrove plants in Parangipettai, Cuddalore district, Tamil Nadu

Data on the frequency distribution of AM fungi recorded in the rhizosphere soils of 3 different mangrove plants in Parangipettai, Cuddalore district is presented in **Table 3**. A total of 12 different species of AM fungi belonging to three genera such as *Acaulospora*, *Glomus* and *Scutellospora* were isolated and identified from the rhizosphere samples of three different mangrove plants screened during

period of investigation. Among them, the genus *Glomus* was found to be the most predominant one. It was also recorded that the AM fungal genera *Acaulospora* had 2 species and *Scutellospora* had one species during the period of investigation. The genus *Glomus* had 9 different species. Among the different AM fungi isolated, *Glomus geosporum* and *Acaulospora scrobiculata* were the most frequent AM fungi (100% each) recorded from the rhizosphere of all the three mangrove plants rhizosphere samples. The AM fungal species such as *Acaulospora laevis*, *Glomus albidum* and *Glomus macrocarpum* were recorded as low frequency distribution (33.3% each).

Table 3: Frequency distribution of AM fungi recorded in rhizosphere soil samples collected from three different mangrove plants in Parangipettai, Cuddalore district, Tamil Nadu.

S. No.	AM Fungi	Mangrove Plants			Frequency (%)
		<i>Avicennia marina</i>	<i>Rhizophora apiculata</i>	<i>Rhizophora mucronata</i>	
1	<i>Acaulospora laevis</i>	-	-	+	33.3
2	<i>Acaulospora scrobiculata</i>	+	+	+	100.0
3	<i>Glomus albidum</i>	-	-	+	33.3
4	<i>Glomus claroideum</i>	-	+	+	66.7
5	<i>Glomus fulvus</i>	+	-	+	66.7
6	<i>Glomus geosporum</i>	+	+	+	100.0
7	<i>Glomus intraradices</i>	+	-	+	66.7
8	<i>Glomus macrocarpum</i>	-	+	-	33.3
9	<i>Glomus microcarpum</i>	-	+	+	66.7
10	<i>Glomus occultum</i>	+	-	+	66.7
11	<i>Glomus pubescens</i>	-	+	+	66.7
12	<i>Scutellospora calospora</i>	-	+	+	66.7

Frequency distribution of AM fungi recorded in rhizosphere soil samples collected from three different mangrove plants in Pazhayar, Mailaduthurai district, Tamil Nadu

Data on the frequency distribution of AM fungi recorded in the rhizosphere soils of 3 different

mangrove plants in Pazhayar, Mailaduthurai district is presented in **Table 4**. A total of 15 different species of AM fungi belonging to three genera such as *Acaulospora*, *Gigaspora* and *Glomus* were isolated and identified from the rhizosphere samples screened during period of investigation.

Table 4: Frequency distribution of AM fungi recorded in rhizosphere soil samples collected from three different mangrove plants in Pazhayar, Mailaduthurai district, Tamil Nadu.

S. No.	AM Fungi	Mangrove Plants			Frequency (%)
		<i>Avicennia marina</i>	<i>Rhizophora apiculata</i>	<i>Rhizophora mucronata</i>	
1	<i>Acaulospora</i> sp.	-	+	+	66.7
2	<i>Acaulospora scrobiculata</i>	+	-	+	66.7
3	<i>Glomus albidum</i>	-	+	+	66.7
4	<i>Glomus claroideum</i>	-	+	+	66.7
5	<i>Glomus clarum</i>	-	+	+	66.7
6	<i>Glomus fulvus</i>	+	-	+	66.7
7	<i>Glomus fasciculatum</i>	+	+	+	100.0
8	<i>Glomus geosporum</i>	+	+	+	100.0
9	<i>Glomus intraradices</i>	+	-	+	66.7
10	<i>Glomus macrocarpum</i>	-	+	-	33.3
11	<i>Glomus microcarpum</i>	-	+	+	66.7
12	<i>Glomus multicaulae</i>	+	+	+	100.0
13	<i>Glomus occultum</i>	+	-	+	66.7
14	<i>Glomus pubescens</i>	-	-	+	33.3
15	<i>Gigaspora gigantea</i>	-	+	+	66.7

Among them, the genus *Glomus* was found to be the most predominant one. It was also recorded that the AM fungal genera *Acaulospora* had two species and *Gigaspora* had one species during the period of investigation. The genus *Glomus* had 12 species. Among the different species of the genus *Glomus* isolated, *Glomus fasciculatum*, *Glomus geosporum* and *Glomus multicaulae* were the most frequent AM fungi (100% each) recorded from the rhizosphere of different mangrove plant samples. The AM fungal species such as *Glomus macrocarpum* and *Glomus pubescens* had less frequency distribution (33.3% each) during the period of investigation.

On a global scale, AM fungi are virtually ubiquitous being present in tropical, temperate, and arctic regions. Within the different global regions AM fungi have a broad ecological range (Harley and Smith, 1983; Gianinazzi and Schuepp, 1994; Mohan and Menon, 2015). They are found in most ecosystems including dense rain forests, open woodlands, scrub, savanna, grasslands, heaths, sand-dunes, and arid and semiarid deserts. Their occurrence within these systems varies according to localized environmental conditions and plant cover. AM are the most widespread in their distribution. Almost all land plants including cereals, legumes, millets, plantations and horticultural crops, ornamental and medicinal plants, and forest trees are reported to be the hosts of AM fungi (Barea *et al.* 1983; Chandra and Kheri, 2006).

The findings of the present study are in confirmation with the findings of many earlier workers (Sengupta and Chaudhuri, 1989, 1990, 2002; Selvaraj and Subramanian, 1991; Kothamasi *et al.*, 2006; Kumar and Ghose, 2008). The earlier studies have shown that occurrence of AM fungi is either absent (Mohankumar and Mahadevan, 1986), rare (Kothamasi *et al.*, 2006) or ubiquitous (Sengupta and Chaudhuri 1989, 1990, 2002; Kumar and Ghose, 2008) in Mangrove ecosystems. Selvaraj and Subramanian (1991) studied AM association in different mangroves. Kolthamasi *et al.* (2006) investigated the presence of AM fungi and phosphate-solubilizing bacteria (PSB) from the rhizosphere of the mangrove plants of Great Nicobar Islands and five species of AM fungi belonging to the genus *Glomus* and percent AM fungal colonization in the mangrove plants was found between 0 and 17%.

The present study is in accordance with the findings made by Kumar and Ghose (2008) and they have assessed the status of AM fungi of 15 species of Mangroves and one mangrove associate in 27 sites from Sunderbans and forty-four AM fungal species belonging to six genera, namely *Acaulospora*, *Entrophospora*, *Gigaspora*, *Glomus*, *Sclerocystis* and *Scutellospora* were recorded. Among them, *Glomus mosseae* exhibited highest frequency at all the inundation types; *Glomus fistulosum*,

Sclerocystis coremioides and *Glomus mosseae* showed highest relative abundance at sites inundated by usual springtides, summer springtides and diurnal tides, respectively. Similarly, Wang *et al.* (2010; 2014) studied AM fungal association with two mangrove swamps in south China and found AM fungi in the form of hyphae which were commonly associated with all the mangrove species and six AM fungal species belonging to two genera viz., *Glomus* and *Acaulospora* were identified by them.

DSouza and Rodrigues (2013a) have studied the diversity status of AM fungi in association with seventeen mangrove species belonging to eight families at seven riverine and fringe habitats in Goa, West India. Among them, sixteen species were found to be mycorrhizal, and one species showed no AM fungal colonization. AM root colonization was recorded at all seven sites and ranged from 6% to 77%. Maximum percent root colonization was recorded in *Excoecaria agallocha* (77%) and minimum percent root colonization was recorded in *Avicennia marina* (6%). AM fungal root colonization and spore density varied by mangrove plant species and the study sites. In total, 28 AM fungal species of five genera, viz., *Glomus*, *Acaulospora*, *Scutellospora*, *Gigaspora* and *Entrophospora* were recorded by them. Among these, *Glomus* was found to be the dominant genus. In another study, DSouza and Rodrigues (2013b) determined seasonal dynamics of AM fungal community composition in three common mangrove plant species, namely, *Acanthus ilicifolius*, *Excoecaria agallocha*, and *Rhizophora mucronata*, from two study sites in Goa, India and observed variation in AM fungal spore density and a total of 11 AM fungal species representing five genera were recorded. Neelakandan and Mahesh (2015) studied the diversity status of AM fungi in salt marsh plants of Southeast Coastal area of Muthupet, Tamil Nadu and determined the impact of physico-chemical factors in relation to the quantitative and qualitative assessment of AM fungi in saline soils. They have reported ten species of AM fungi belonging to five genera viz., *Glomus*, *Sclerocystis*, *Acaulospora*, *Gigaspora* and *Scutellospora* and among them, *Glomus aggregatum* and *G. mosseae* were found as most dominant saline tolerant strains of AM fungi. Gupta *et al.* (2016) have assessed the species diversity of AM fungi in different salinity zones of Bhitarkanika mangroves of Orissa, India, and recorded total of 45 AM species belonging to five genera namely, *Glomus*, *Acaulospora*, *Gigaspora*, *Scutellospora* and *Enterophospora* from three salinity zones of Bhitarkanika mangrove ecosystem. Gopinathan *et al.* (2017) studied the rhizosphere soils of *A. marina* in different seasons for the occurrence and distribution of AM fungi and a total of eighteen AM fungal spores belong to five genera viz., *Glomus*, *Acaulospora*, *Gigaspora*, *Sclerocystis* and *Scutellospora* were observed in Muthupet, Tamil Nadu.

The present study indicates that the genus *Glomus* was found predominant as compared to other AM

fungus genera in both the study locations. Similar observations have already been reported by other researchers on other plant species (Muthukumar and Udaiyan, 2000; Mohan *et al.*, 2007). The possible reason for the predominance of *Glomus* spp. is known to be more common in natural and slightly alkaline soil condition (Mukerji *et al.*, 2002). The variation in spore population density and root colonization of AM fungi in different mangrove plants may be generated by a variety of mechanisms, including variation in host plant species and their phenology, mycorrhizal dependency, host mediated alterations of the soil microenvironment or other host plant species traits. Results from the present study revealed the status of AM fungi in symbiotic association with different mangrove plants. The potential for employing AM fungi as a bio-inoculant on a wide scale in afforestation programmes of degraded mangrove areas is dependent on mass multiplication of superior, dominating and growth-promoting AM fungal species isolated from mangrove forest. Therefore, selection of more adapted AM fungal species for introduction into mangrove environments is needed to maintain and restore the plant-soil equilibrium in natural ecosystem.

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