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A study on Vesicular Arbuscular Mycorrhizal (VAM) fungi in the soils of Sacred Grove at kotarakara, kollam, kerala

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Received on 8-2-2017
Accepted on 5-4-2017

Abstract

The Sacred Groves play a significant role in the conservation of Biodiversity. The mycorrhiza have a vital role in maintaining the biodiversity of plants by giving them Phosphorus, Nitrogen, Magnesium and Carbon. A study was undertaken to understand the diversity of VAM fungi in the soils of Sacred Grove. For this the Sacred Grove of Vendar Bhagavathy Temple at Vendar Village belongs to Kottarakara Taluk, Kollam District, Kerala was selected. Soil samples were collected from five locations selected randomly in the Grove, and the samples were collected by making a pit of 20 cm depth. Soils are collected from the pit in depth wise 0-10 cm from the top and 10-20cm. Soil physical and chemical properties like soil moisture content, pH, Organic Carbon and available Phosphorus were recorded. VAM fungal spores were isolated using Wet- sieving and Decanting method with slight modification. The average VAM spore density per 10g of soil in the upper layer is 39.4 and the average density of VAM spore in Lower layer is 26.6. It was observed that the VAM fungal genera *Scutellospora* accounts maximum diversity in the upper soil layers in the study area followed by *Acaulospora*. From lower layers it was observed that maximum diversity observed by A*caulospora*, followed by *Gigaspora.* From the study retrieved 28 VAM fungal species belongs to five genera.

Key words: sacred groves, soil pH, VAM fungi, Mycorrhiza

Introduction

Culturally protected forest fragments, popularly known as Sacred Grove are often relics of original forests that covered the region before forest cutting and burning with the spread of civilization. These preserved forest patches, which originally covered larger areas, are usually close to human settlements. In ancient times, sacred groves were places of sanctuary and worship for the Dravids (Basu 2000, Jamir and Pandey 2003). They have became refuges for plants, birds, mammals and other forest dwelling animals (Dash 2005) and local community depends upon them for various products used in everyday life (Wadley and Colfer 2004).

Studies highlights that, groves support a good number of rare and endemic species, which are extra sensitive compared to common species and persist only in favorable niches, and the sacred groves are ideal places for them (Jamir and Pandey 2003, Jayarajan 2004, Sukumaran and Raj 2007). The sacred groves are found throughout the world in different temporal and spatial scales, and have contributed significantly for conservation of rare and endangered species (Magamia and Oba 2003). They have great significance in the biodiversity conservation because they contain some important species of flora and fauna that have been lost from the surrounding area. They also have considerable role in water and soil conservation. Hence the importance of sacred grove in nature conservation has been increased manifold in recent time especially after the declaration of Convention on Biological Diversity. More over the natural disturbance, unintentional introduction of new members are also responsible for increasing species diversity (Chandran and Hughes 1997).

Present day groves are under various threats which are mostly human induced. The threat to the groves include urbanization, over exploitation of resources, deforestation, land conversion, fragmentation, land quarrying invasive species, sanskritisation, developmental destruction etc. In our country most studies on sacred groves have harbored around floral and faunal diversity and maintenance of rare threatened and endemic species with sketchy description of the ecological profiles and disturbance regions. Most investigation over looked the importance of below ground diversity of sacred grove, which are responsible for the species richness of sacred grove. The studies of Vesicular Arbuscualr Mycorrhizal (VAM) fungi in relation to sacred grove is being rare. In this circumstances the present investigation was undertaken to fulfill the lacuna of Arbuscular Mycorrhizal Fungal spore diversity in soils of a sacred grove.

Materials and Methods

Study area

The present study was carried out in the Sacred Grove of Vendar Bhagavathy Temple at Vendar Village belongs to Kottarakara Taluk, Kollam District, Kerala. The Sacred Grove is located near to the Vendar Bhagavathi Temple. The Sacred Grove and the temple are extended about

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two acres. The grove is covered with massive trees and twisting serpentine vines. The main idol is Serpent King, *The Nagaraja*. The history of this Sacred Grove is mentioned in the light of old books available within the temple and in accordance with the members of the Kadalamadom family, who were installed the temple during 17th century, as well as the opinion from old devotees lives around the temple. The grove is now a sacred place where *Serpentine God* is worshipped.

The vegetation present in the Grove is undisturbed consists of luxuriant and multi layered trees mixed with shrubs, liners and herbs. The Sacred Grove is mostly dominated by *Vateria indica*, belonging to Dipterocarpaceac family. About 30 to 40 ft. tall trees and medium height trees are present. The *Vateria indica* present in the Grove have some peculiarities, it has 2-3 flowering time per year and also the seeds do not germinate outside. In addition to *Vateria indica*, many trees, shrubs and herbs grows inside the Grove. Many Pteridophytes, Lichens, Puff balls and many species variety of mushrooms present inside the grove. The fauna include different categories of Snakes, Ptyas, Frogs, Millipeds, Termites, Ants, Earthworm, Snails, Rats, Squirrels, Birds, etc. Bats, Insects, Warps, Honey bees, Butterflies and Beetles present here. Many migratory birds are visited in the Grove.

Soil Collection and Processing

For the present study soil samples were collected from different areas of the sacred grove. Five areas were located randomly in the grove. The soil samples were collected from the selected localities in such a way that a pit of 20 cm was made using a spade. Soil samples from the pits were collected from 0-10 cm from the top and 10-20 cm from the bottom. Care should be taken to avoid in the mixing of soils from the two layers. Soil from each layer was taken to a clean polythene bag. About 200 gm of soil from each layer was collected and labeled and then bring to the laboratory for further studies. In the laboratory the soil samples were processed in such a way that the soil from each layer was mixed well and stones, roots and other debris were removed. The processed sample was made into working samples of 10 g each and utilized for VAM isolation as well as for soil physical and chemical analyses.

For determining the Soil pH, 10g of the soil sample was weighed and mixed with 50 ml distilled water. Stirred it using a clean glass rod and kept it for some time to sediment the heavier soil particles. The pH was measured by using a portable digital pH meter. For determining the moisture content 10g of soil from each working sample was taken out and kept in a hot air oven at 80^0C for 48 hr. The dry weight of the sample was measured using a balance. The percentage moisture content of the sample was calculated.

The percentage of organic carbon in the soil samples was determined. For that 10 g of oven dried working soil sample was transferred to 500 ml conical flask and added 10 ml IN Potassium dichromate ($K_2 Cr_2 O_7$) solution and 20 ml con. Sulphuric acid ($H_2 SO_4$). It was then mixed by gentle stirring. The flask was kept for 30 minutes for the mixture to react. After the reaction is over the content was diluted with distilled water and added 10 ml of Phosphoric acid, followed by 1 ml of Diphenyl amine as indicator. The sample is then titrated against 0.4 N Ferrous Ammonium Sulphate. At the end point color changes to brilliant green. A blank with same quantity of the chemicals without soil was aso titrated as control and percentage of carbon was calculated.

Available Phosphate was estimated by taking 3g soil dissolved in 200ml of 0.002 N H_2SO_4 in a conical flask. The flask was kept for half an hour for dissolution of the soil. After half an hour, the sample was filtered and 10 ml of sample was taken and added 5 ml of Ammonium Molibdate solution followed by 2-3 drops of Stannous Chloride solution. A blue color appeared and spectral reading at 690 nm was taken after 5 min. with distilled water blank containing same amount of reagents. The Percentage of available Phosphorus was calculated.

Wet sieving and decanting (Gerdeman and Nicolson 1963) with modification was employed for isolation of VAM fungal spores from soil. For wet sieving and decanting method, 10 g of air dried soil from the working sample were taken in a 1000 ml beaker, stirred thoroughly with tap water and kept for some time to sediment heavier soil particles and other debris. The supernatant was decanted through a series of test sieves with 50ìm, 100ìm, 250ìm, 500ìm and 750ìm mesh. This process was repeated for five to eight times until the solution became clear. The sieving from the first three sieves were collected in 250 ml Erlenmeyer flask using a wash bottle, mixed thoroughly and kept for sometime. The supernatant was filtered through Watman No.1 filter paper (120 mm) and observed under Stereo Binocular Microscope. VAM fungal spores from the filter paper surface were selected and transferred to a drop of Polyvinyl-Lacto-Glycerol (PVLGA) or Polyvinyl Alcohol (PVA) mounted on a clear microscopic glass slide using a sharpened wooden dowel. VAM fungal spore preparations with and without Melzer's reagent were made to reveal details on spore inner wall layers and other spore characteristics. Identification of VAM fungal spores was made up to species level following taxonomic descriptions of Schenck and Perez (1990) and Morton (1993). Spore characteristics such as spore colour, shape, spore wall structure, subtending hyphae, presence or absence of special structures like germination shield, suspensor, spore ornamentation, etc. were used for identification.

Data generated from the field and laboratory experiments were processed. ANOVA (Analysis of Variance) was used to determine the VAM population in different soil layers. One-way analysis of variance (ANOVA) is used to determine whether there are any significant differences between the means of independent (unrelated) groups. The one-way ANOVA compares the means between the groups and determines whether any of those means are significantly different from each other. Specifically, it tests the null hypothesis. In addition to this correlation analysis was made to compare the population of VAM with the soil physical and chemical changes. Shannon- Weiner (H') indices was

used to detect the diversity of VAM fungal spores from the soil. Diversity indices provide more information about community composition than simply species richness, (i.e., the number of species present).

Results and Discussion

In the present study, the soil samples collected from five different regions of sacred grove are subjected to the determination of soil pH. Average pH of upper layer is 5.94 and that of lower layer is 5.88. The pH varied from 5.08 to 6.56 showing slight acidic to moderately alkaline in nature (Table 1).The present study shows that the percentage of moisture content in the upper layers varies from 7.7% to 8.9% while the lower layer ranges from 9.2to 10.4 % (Table 1). The soil organic carbon concentration in the five samples exhibited almost similar amount. It was found that the Organic Carbon content was very high. The average organic carbon content per gram of soil is found 5.624 mg (Table 1). On analyzing the available Phosphorus content in the soil samples, it was found that the average available Phosphors per gram of soil present in upper layer is 0.47 % and lower layer is 0.11 %. This shows that in upper layers the amount of phosphorus is higher than that of lower layers. The Phosphorus content decreased with increase in soil depth. (Table 1).

Table 1. Soil physical and chemical properties in the study area.

Sl No	Sample	Soil pH	Moisture content	% organic Carbon	% available Phosphorus	VAM fungi
			Upper layer (0-10 cm)			
1	I	5.86	8.9	5.9	0.85	26
2	II	6.31	7.7	5.42	0.32	59
3	III	5.23	8.73	5.8	0.5	37
4	IV	5.90	8.1	4.9	0.3	49
5	V	6.43	8.8	6.1	0.6	26
			Lower layer (10-15 cm)			
1	I	5.86	10.28	4.2	0.55	18
2	II	6.31	9.9	5.0	0.28	38
3	III	5.23	9.19	4.9	0.3	29
4	IV	5.90	10.4	4.5	0.31	32
5	V	6.43	9.5	5.2	0.51	16

While analyzing the soil samples for VAM density resulted a very high number of VAM spores present in each sample. The average VAM spore density per 10g of soil in the upper layer is 39.4 and the average density of VAM spore in Lower layer is 26.6. The VAM density decreased with increase in soil depth. It may be due to mostly the mycorrhizal colonization abundant in upper region than lower region hence the spore amount is relatively more (Table 1).

In the present investigation highest spore density was observed in soil with rich organic matter and alkaline soil. Soil pH and percentage of soil moisture are factors for determining spore density. In the present study resulted alkaline pH in the soils at different locations of the Scared Grove. It will helps for the richness in biodiversity of VAM. The alkaline pH is may be due to the high amount of litter content on the soil surface.

For the determination of the variation of spore distribution in the different soil samples of the study area, one way analysis of Variance was performed. The results depicted in Table 2. From the results it was found that the calculated value of F is almost equal to the table value. Hence it was clear that there was no significant difference in the means of the population of VAM spores in different soil layers.

Table 2. ANOVA of VAM fungi in the study area

Source of Variation	SS	df	MS	F	P-value
Between Samples	409.6	1	409.6	2.748071	0.135959
Within Samples	1192.4	8	149.05		

The VAM spore isolated and identified from the present study are listed in Table 3. The VAM Fungal Genera *Acaulospora, Sclerocytis, Glomus, Scutellospora* and *Gigaspora* were isolated and identified. The *Glomus* accounts nine species. The *Acaulospora* species include *A. appendicula, A. bireticulata, A. delicate, A foveata, A. scrobiculate* and some unidentified *Acaulospora* spores were obtained from the present study. The *Gigaspora* accounts with *G. gigantea, G. candida, G. margarita, G. decipiens* were identified. The *Glomus* species include *G. albidum, G. australe, G. botryoides, G. deserticola, G. fasiculatum, G. invermaium, G. maculosum, G. mossae, G. reticulatum* and several unidentified species were obtained. The *Scutellospora* species include *S. heterogamae* and *S. reticulata*. *Sclerocystis microcarpa* and some unidentified black spores were obtained from the soil samples. Similar to density, the VAM diversity is also higher in upper layers. It is found out that *Glomus* and *Acaulospora* were dominant in the samples (Table 3).

Table 3. VAM fungal species present in the study area

VAM Fungi	**I**		**II**		**III**		**IV**		**V**	
	U	**L**	**U**	**L**	**U**	**L**	**U**	**L**	**U**	**L**
*Acaulospora appendicula S*pain, Sieverding & Schenk	1	2	2	1	2	-	2	2	2	-
A. bireticulata Rothwell& Trappe	-	-	1	2	1	1	2	3	3	2
A.delicata walker, pfeffer& Bloss	1	-	1	-	-	-	4	-	-	-
A. foveata Trappe & Janos	3	1	1	-	-	2	-	-	1	1
A. Scrobiculata Trappe	3		1	1	1	-	-	-	-	-
Acaulospora sp.	-	2	3	1	-	-	1	-	1	-
Gigaspora gigantia (Nicol.& Gerd.) Gerdemann &Trappe	2	1	2	2	3	2	4	3	4	2
G. rosea Nicol&Shenk	-	-	-	-	-	-	-	-	1	
G. Margarita Becker & Hall	1	-	-	1	-	-	-	-	1	1
Gigaspora sps	-	-	3	-	-	-	-	1	1	
G*lomus albidium* Walker & Rhodes	2	-	1	-	-	-	-	-	-	-
G. austral (Berk.) Berch.	-	-	1	-	-	-	-	-	-	-
G. botryoides Rothwell&Victor	3	-	-	-	-	1	1	2		1
G. deserticola Trappe,Bloss&Menge	2	-	-	-	-	-	-	-	-	-
G. fasiculatum (Thaxter sensu Gerdemann) Gerdemann&Trappe	-	1	-	1	-	-	-	-	-	-
G. invermaium Hall	-	-	1	-	3	-	-	-	-	-
G. Maculosum Miller&Trappe	1	-	-	-	1	1	-	-	-	-
G. Mossaeae (Nicol.&Gerd.) Gerdmann&Trappe	-	-	-	-	-	-	-	-	-	1
G. constrictum Trappe	1	-	-	-	-	-	-	-	-	-
Glomus sps	-	1	-	1	1	1	1	1	1	-
G. Microcarpum Tul.&Tul.	-	-	1	-	1	-	-	-	-	-
Sclerocystis mircocarpus Iqbal&Bushra	-	-	1	-	-	-	-	1	-	2
Scutellospora heterogama (Nicol.&Gerd.)Walker	-	1	-	-	1	-	-	-	-	-
S. reticulate	1	-	-	-	-	-	-	-	-	-
Scutellospora sps.	1	-	-	1	-	1	2	-	1	-
Black Spore	4	1	-	4	5	3	5	3	2	3

The diversity of VAM species in the study area was calculated using Shannon- Weiner (H') diversity index. The results are as follows (Table 4.)

Table 4. Diversity of VAM fungi in the upper layers

Sl No	VAM Fungi	Shannon- Weiner (H')	
		Upper layer	Lower layer
1	*Acaulospora* sps.	1.0625	0.677419
2	*Gigaspora* sps.	0.6875	0.419355
3	*Glomus* sps.	0.71875	0.387097
4	*Sclerocystis* sps.	0.03125	0.096774
5	*Scutellosora* sps.	2.5	0.032258
6	Unidentified spores	0.5	0.451613

In the present study five VAM fungal genera were identified. It was observed that the number of *Scutellospora* accounts maximum diversity in the upper soil layers in the study area. Followed by *Acaulospora*. The least occurred taxa was *Sclerocysts* (Fig 1).

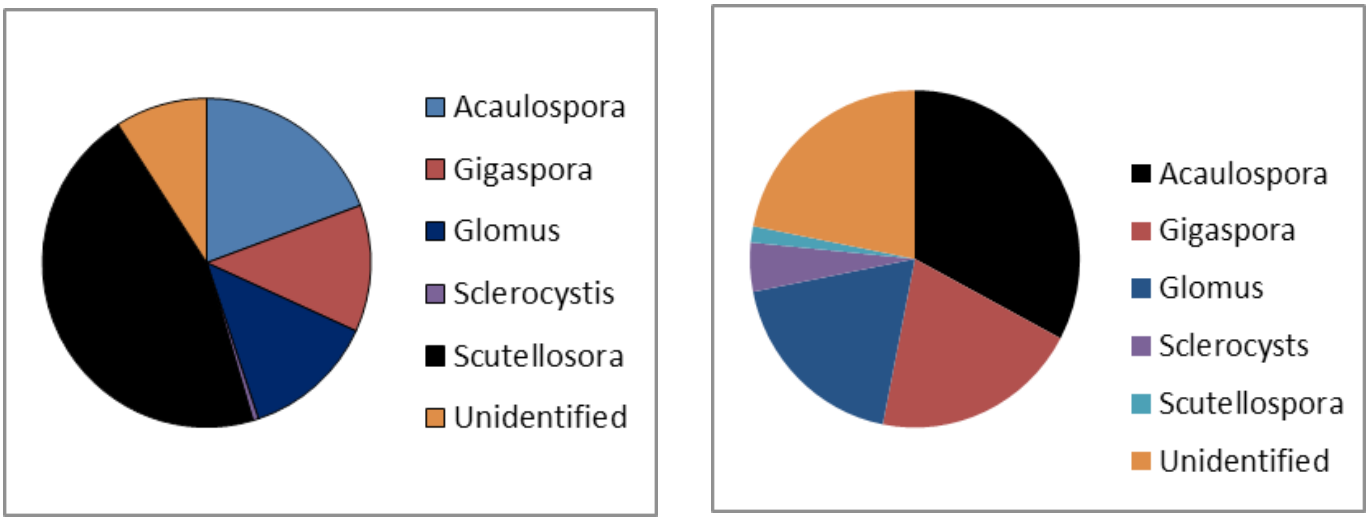

Fig.1. The diversity of VAM fungi in the upper and lower soil layers in the Sacred Grove

The diversity of VAM fungi in the lower layers also calculated. It was observed that maximum diversity exhibited by *Acaulospora*, followed by *Gigaspora* (Fig. 2.).

Mostly VAM diversity related to the biodiversity of ecosystem. We know that sacred groves exists a link between biodiversity, culture, religious and ethnic heritage. The VAM diversity associated with Sacred Grove studies are mostly compare their relationship. The present study mainly focus on the biodiversity of VAM in corresponds to Sacred Grove soil conditions. This study shows the abundance of *Acauloposra* and *Glomus* specie. It is in agreement with Jaykumari and Potty (2010). They have characterized and identified four AM Fungi belonging to three genera Viz, *Glomus, Acaulospora* and *Scutellsopra*. The VAM diversity depends on the type of vegetation. The study conducted by Meitini *et al* in 2013 identified five genera consisting of 13 species (*Acaulsopora, Gigaspora, Scutellopora, Entrophosphora* and *Glomus*) in cashew nut plantation .

The present study resulted there was no significant difference in spore density and number of species. Similar results obtained in several other studies. The VAM genera of *Acaulosopra*, *Glomus, Scutellospora* and *Gigarpora* diversity is almost similar in Degraded forest and Rubber plantation (Uma et al, 2014). In regions where, mycorrhizae are abundant, shows a decline in phosphorus content. This is because the mycorrhizae absorb phosphorus in that region.

The Diversity of AM fungi in different land system in Kerala part of Niligri Biosphere Reserve study Sankaran, et al. (2005) shows that *Acaulospore* have the highest spore density than other genera. The species of *Gigaspora* and *Sclerocystis* were rather restricted in distribution. Overall, it is evident from the above data that shows the number of AM fungal spores from each sample was not correlated with any of the soil parameters tested, total N, available P and organic Carbon, Ca and Mg, and soil pH.

The Mycorrhizas functions as a below ground sink for the Carbon and increased net photosynthesis rates of mycrrrhizal plants (Douds *et al*, 1988). A mycorrhiza dependant species shows clear disadvantages in low fertility

soil, which lacks mycorrhizal propaguls in quality and quantity. Investigators like Ameeta Sharma and Yadav *(2013)* have the resulted three main genera such as *Glomus, Gigaspora* and *Scutellospora* in barley field. But Aldo *et al.* (2006) identify 34.444/30 g of soil VAM spores and the dominant genera are *Glomus, Paraglomus* and *Acaulospora* in the study area Papaya plantation. In most study the VAM diversity increase or decrease depend upon the abiotic factors and the cultivation in that area.

The VAM study related to Sacred Grove are rare. Most studies focus on the biodiversity and its conservation significance of sacred grove. The role of sacred grove with Mycorrhizae independent species, which will dominant in the community (Janos, 1988). As we discussed the above studies, we understand that mycorrhiza make a significant but contrasting nutritional contributions to plants in different ecosystem. Diversity is the essence of integrations of nature. Mycorrhizas functions as below ground integrators of populations and communities of plants by influencing the dynamics, distribution and diversity..

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