DOI:10.26524/krj.2020.19

Kong. Res. J. 7(2): 30-38, 2020
Publisher: Kongunadu Arts and Science College, Coimbatore.

https://www.krjournal.com

RESEARCH ARTICLE

STUDIES ON THE ARBUSCULAR MYCORRHIZAL FUNGAL ASSOCIATION IN SOME MEDICINAL PLANT SPECIES OF PAITHAL HILLS, WESTERN GHATS KANNUR DISTRICT, KERALA

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ABSTRACT

In the present study to analyzed that the arbuscular mycorrhizal fungal spores in root colonization and spore population in rhizosphere soils samples in various medicinal at Paithal hills, Western Ghats of Kannur district, Kerala, India. Root and rhizosphere soil samples were collected during the month of August, 2018-March, 2019 from the surface to 30 cm depth as well as pH were also recorded. Totally 30 plant species belonging to 19 families were collected and identified. The present result showed arbuscular mycorrhizal spore population in the rhizosphere soil and root colonization of all the plant species. A total of 19 AM fungal spores were recovered from the rhizosphere soil samples in this study region. The *Glomus* was dominant had seen in rhizosphere soil samples in all the medicinal plant species. The maximum spore population was found in the rhizosphere soil samples of *Mimosa pudica* (590/100g of soil) which belongs to the family Mimosaceae and the lowest spore population was observed in the *Terminalia bellirica* 135/100g of soil) belongs to Combretaceae family. The highest 78 % AM fungal colonization was found in roots of *Euphorbia hirta* belongs to the family Euphorbiaceae. While the lowest 11 % AM fungal colonization was found in the root of *Sida acuta* belongs to the family Malvaceae.

KEYWORDS: *Glomus aggregatum***,** Medicinal plants, Paithal hills.

1. INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are ubiquitous and abundant soil mycoflora and constitute an important functional component of the rhizosphere of 80-90% land plants in natural, agricultural and forest ecosystem (1). Mycorrhizae can develop when a hypha from a spore or an already infected root (2). In the tropics many crops are grown in acid infertile soils, where their establishment is frequently limited by the low levels of available phosphorus. In such soils an efficient mycorrhizal association can increase phopshorus uptake and crop yields (3). Stimulation of AM mycorrhizal fungi may secure the early establishment of symbiosis and benefit the host plant at an earlier stage of development.

The importance of mycorrhiza has been acknowledged in the fields of agriculture, forestry and other land use.AM fungal hyphae enter in to the plant roots, producing ballon like structures which increase the surface area of contact between hypha and cell cytoplasm to facilitate the transfer of nutrients. The AM fungi are widely distributed in most ecosystems and associated with many plant species. The beneficial effect of AM fungi on plant growth has been highlighted by (4) and by several researchers. It has been found that AM fungi contributed to increased rate of nutrient absorption especially phosphorus from soil, longevity of feeder roots, increased tolerance to drought, heavy metals, soil toxins extremes of soil pH and high temperature. Many commercially important tree species like *Acacias, Eucalyptus*, Teak etc., are naturally colonized by AM fungi.

AM fungi play very vital role in uptake nutrients particularly phosphorus from infertile and degraded soils. They infect large number of plants occurring in diverse environments. It is estimated that AM fungi occur over 90% of the earth plants species and in most soils (5). AM fungi are colonizing the majority of herbaceous plant roots and in natural ecosystems all over the world (6). AM colonization is common in infertile habitats (7) and typical grassland soils with low phosphorous level (8).

Members of the Glomaceae are believed to have been present as early as the Cambrian period (9). AM fungi are found in most Angiosperms, some Gymnosperms, Pteridophytes and Bryophytes (10) in which some listed plant

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families are believed to have little or no mycorrhizal association including Cruciferae, Chenopodiaceae, Caryophyllaceae, Polygonaceae, Juncaceae and Cyperaceae.

The situation in developing countries like India, fertilizer could be applied only for a few cash crops and stable food crops such as rice and wheat and not for afforestation of waste lands. Hence, microbial technologies hold great promise in the operation of scientific forest nursery managements by inoculating containers with biofertilizers viz, di-nitrogen fixing organisms, phosphate solubilising organisms and mycorrhizae.

2. MATERIALS AND METHODS

2.1. Study area

Paithalmala is a hill station in the Kannur district of Kerala in India. Located near Pottenplave village, at a height of 1372 m above sea level, this is the highest geographic peak in kannur. It is located at 40 km from Taliparamba and 65 km from Kannur. Nestled in the Kerala Karnataka border near to Kodagu forests, it lies in the Western Ghats (Figure 1). It has become a favorite spot for the nature enthusiasts, photographers, trekkers and leisurely visitors. The terrain is Rocky at the beginning and an abrupt rise in elevation arises from where a waterfall in the past must be flowing as tracks of salt could be clearly seen on those rocks. After which thick forest cover exists. Thorny shrubs can be found in abundance. Other smaller amphibians like newt were also spotted.

Fig. 1. Study area of Paithal Hills.

2.2. Sample collection

In this present study, root and rhizosphere soil samples of 30 plant species were collected for the duration of August, 2018- March, 2019. The collected soil and root samples were placed in the polyethylene bags, labeled and then transported to the laboratory. The root samples were freshly processed, whereas rhizosphere soil samples were analyzed for mycorrhizal spore population and AM fungal root colonization in study species.

2.3. Estimation of AM fungal root colonization

The root samples were cleared and stained in tryphan blue with a modified version of following method by (11). The collected roots samples were cut into 1-2 cm pieces, heated at 90ºC in 10% KOH for about 1 hour. For thicker and older roots, the duration was increased. The root segments were rinsed in water and acidified with dilute HCl. The root pieces were stained with 0.05% tryphan blue in lacto phenol for 5 minutes and the excess stain was removed with clear lacto phenol.

The percentage of AM fungal infection was calculated using the formula:

$$\text{Percentage of infection} = \frac{\text{No. of root segments infected}}{\text{Total no of root segments observed}} \times 100$$

2.4. Identification of AM fungi

The present study isolation and identification of AM fungal spores based upon their morphological characters such as spore size, color, hyphal attachment, cell wall layer characters, were identified in addition with nomenclature, keys of the following manual authors were used: (11 - 14). The Photomicrographs were taken with the help of a Magnus Olympus Microscope.

3. RESULTS AND DISCUSSION

In the present study the survey of medicinal plants were collected in Paithal hills Western Ghats of kannur district, Kerala. Totally 30 plant species belongs to 19 families were collected and identified. The collected plants species with their habit and their medicinal uses are presented in (Table -1 and Figure -1). Along with the Arbuscular Mycorrhizal fungal colonization and spore population of 30 plant species belongs to 19 families and pH (4.6 to 6.9) of

the rhizosphere soil sample present in the (Tables - 2).

The total number of 30 plant species belongs to 19 families were examined for AM fungal population and colonization. The maximum spore population was observed in the plant species of *Mimosa pudica* (590/100g of soil) belongs to Mimosaceae and minimum was observed in *Terminalia bellirica* (135/100g of soil) belongs to Combretaceae (Figure 2 and 3).

In the present investigation the highest AM fungal infection was recorded *Euphorbia hirta*78% belongs to Euphorbiaceaeand minimum was noticed in *Sida acuta*11 % belongs to Malvaceae. The plant species *Azadirachtaindica*25% (Meliaceae), *Acalypha indica* 38% Euphorbiaceae), *Alternanthera sessilis* 33% (Amaranthaceae), *Bauhinia purpurea*25% (Caesalpiniaceae), *Eclipta prostrate* 26% (Asteraceae), *Ficus bengnalensis*22% (Moraceae), *Impatiens balsamina* 35% (Balsaminaceae), *Ipomoea obscura*27% (Convolvulaceae), *Mimosa pudica*34% (Mimoceaese), *Mangifera indica*37% (Anacardiaceae), *Phyllanthus amarus* 32% (Phyllanthceae), *Tridax procumbens*38% showed 20 to 40 % infection.

The other plant species like *Abutilon indicum* 55% (Malvaceae), *Anacardium occidentale*57% (Anacardiaceae), *Areva lanata*54% (Amaranthaceae), *Amaranthus viridis* 45% (Amaranthaceae), *Chloris virgata*55% (Poaceae), *Hibiscus micranthus*51% (Malvaceae), *Leucas aspera*42% (Lamiaceae), *Pennisetum typhoideum* 48% (Poaceae), *Ocimum sanctum* 51% (Lamiaceae), *Terminalia bellirica*54% (Combretaceae), *Vernonia cinerea* 58% (Asteraceae).

The rest of plant species *Catharanthus roseus*63% (Apocynaceae),*Clitoria ternatea* 61% (Fabaceae), *Ixora coccinea* 64% (Rubiaceae), *Rauvolfia serpentine* 66% (*Rauvolfia serpentina*), *Tinospora cordifolia*63% (Menispermaceae) showed 61 to 80% infection.

From the rhizosphere soils sample of Paithal hills, totally 19 AM fungal species were isolated and identified. Of these 1 species of *Ambispora*, *A. appendiculatum*, 1 species of *Gigaspora,Gis. candida* 14 species of *Glomus*, *G. heterosporum, G. hoi, G. invermeyanum, G. macroporum, G. maculosum, G. microsporum, G. magnicule, G. monosporum, G. multicaulis,G. multisubstensum, G. segmantatum, G. versifome*, 1 species of *Sclerocytes, S. pachycaulis,* 1 species of *Paraglomus*, *P.occultum,* 2 species of *Rhizophagus*, *R. intraradix, R.manihotis,* and, 1 species of *Pasiphora, P. dominika* observed. The names of the species were present in (Table-3 and Figure-4).

Mycorrhizal plants produced significantly more leaves, and dry matter than the non-mycorrhizal plants. Santhaguru *et al*. (15) reported there was no infection in five plant species viz., *Albiziz lebbeck*, *Bauhinia tomentosa*, *Cassia soamia*, *Prosopis* species *spicifera* and *Tamarindus indica*. In contrast the present investigation the other plant species *Bauhinia purpurea* belongs to Caesalpiniaceae showed AM fungal infection.

The distribution of AM fungi also varies significantly. The occurrence of these fungi varies from species to species (Table- 4). In the present study observed that, arbuscular mycorrhizal fungi colonized all the medicinal plant species and the three stages of root colonization viz., hyphal, arbuscular and vesicular colonization were recorded. AM spore populations also showed variation in the rhizozphere soil of the shrubs and tree species. In the present study no definite correlation could be established between AM fungal root colonization and spore numbers. In the present investigation also revealed that the AM fungi belonging to genus Glomus were the most representative type in the rhizosphere soil of the plant species. The results of the present study hold the previous report that the *Glomus* is the dominant genus in Cholistan desert. Spore abundance had also been shown to be related with variations in moisture (16) pH (17) and temperature (18). However, such relationship did not always remain the same (19), did not find any correlation between AMF spore densities in a small spatial study. The same results also find out in the present study. Santhoshkumar and Nagarajan (20) reported that arbucular mycorrhizal fungal association in the rhizosphere soil and root colonization of some medicinal plant Species in Sirumalai Hills Eastern Ghats of Dindugul District, Tamilnadu and they were reported totally 39 AM fungal species belonging to six genera were isolated and identified. The genus *Glomus* were found dominate followed by *Acaulospora*, *Sclerocystis*, *Entrophospora* and *Gigaspora*. Priyadarshini *et al.* (21) also reported that occurrence of VAM fungi in Kalasalingam University campus. They were isolated totally 26 species of vesicular arbuscular mycorrhizal fungal spores from the rhizosphere soil samples of the

plant species belonging to 14 families was reported.

Table 1. List of plants species collected from the Paithal hills and their medicinal uses.

S. No	Plant species	Family	Habit	Part used	Medicinal uses
1.	*Abutilon indicum* L.	Malvaceae	Shrub	Roots and leaves	Curing fever
2.	*Azadirachtaindica* L.	Meliaceae	Tree	leaves	Antifungal, antibacterial, contraceptive and sedative.
3.	*Acalypha indica* L.	Euphorbiaceae	Herb	Leaves	Jaundice remedy
4.	*Anacardium occidentale* L.	Anacardiaceae	Tree	Leaves	Malaria
5.	*Areva lanata* (L.) Jus. Ex schult	Amaranthaceae	Herb	Leaves	Kidney stone
6.	*Alternanthera sessilis*L.	Amaranthaceae	Herb	Whole plant	Diuretic, cooling purposes
7.	*Amaranthus viridis* L.	Amaranthaceae	Herb	Leaves	Eye infection
8.	*Bauhinia purpurea* L.	Caesalpiniaceae	Tree	Leaves	Healing cuts and wounds
9.	*Catharanthus roseus* L.	Apocynaceae	Shrub	Whole plant	Stomach ache
10.	*Clitoria ternatea* L.	Fabaceae	Herb	Whole plant	Snakebite
11.	*Chloris virgata* Sw.	Poaceae	Herb	Leaves	Applied on wounds to prevent infection
12.	*Eclipta prostrata* L.	Asteraceae	Herb	Leaves	Used to wash open wounds
13.	*Euphorbia hirta* L.	Euphorbiaceae	Herb	Leaves	Diarrhea
14.	*Ficus bengnalensis* L.	Moraceae	Tree	Whole plant	Ulcer, Vomiting, Inflammation
15.	*Hibiscus micranthus* L.f.	Malvaceae	Shrub	Leaves	Antidote for snakebite
16.	*Impatiens balsamina* L.	Balsaminaceae	Herb	Leaves	Snakebite
17.	*Ixora coccinea* L.	Rubiaceae	Shrub	Leaves and Bark	Diarrhea
18.	*Ipomoea obscura* L.	Convolvulaceae	Herb	Leaves	Dysentery
19.	*Leucas aspera* Linn.	Lamiaceae	Herb	Whole plant	Fever, cold, and skin diseases
20.	*Mimosa pudica*L.	Mimosaceae	Shrub	Leaves	Antibacterial
21.	*Mangifera indica* L.	Anacardiaceae	Tree	Whole plant	Asthma and diabetics
22.	*Phyllanthus amarus* Schumach & Thonn	Phyllanthceae	Herb	Whole plant	Jaundice
23.	*Pennisetum typhoideum* Rich.	Poaceae	Herb	Whole plant	Anti-oxidant and anti inflammatory
24.	*Rauvolfia serpentina* (L.) Benth. Ex Kurz	Apocynaceae	Shrub	Root and leaves	Dysentery
25.	*Ocimum sanctum* L.	Lamiaceae	Herb	Whole plant	Skin diseases
26.	Sida acuta L.	Malvaceae	Shrub	Whole plant	Digestion
27.	*Terminalia bellirica* (Gaertn)Roxb.	Combretaceae	Tree	Leaves	Blocked nose

28.	*Tinospora cordifolia* (thumb.) miers	Menispermaceae	Shrub	Whole plant	Diabetes
29.	*Tridax procumbens* L.	Asteraceae	Herb	Whole plant	Healing of wounds
30.	*Vernonia cinerea* (L.) Less.	Asteraceae	Herb	Whole plant	Urinary incontinence

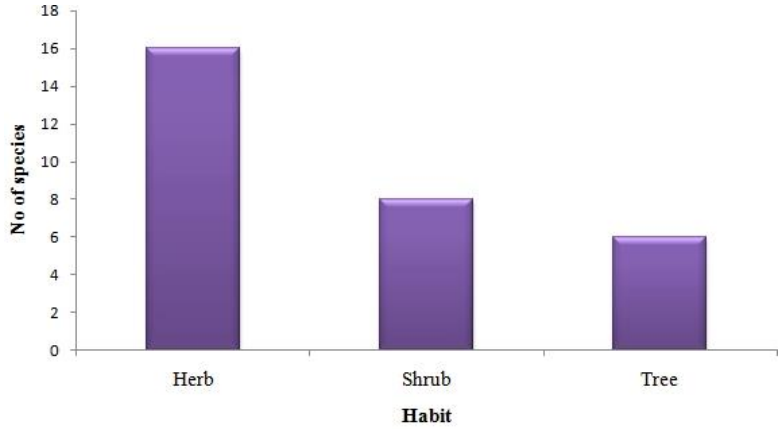

Fig. 2. Habit wise distribution of plant species in Paithal hills.

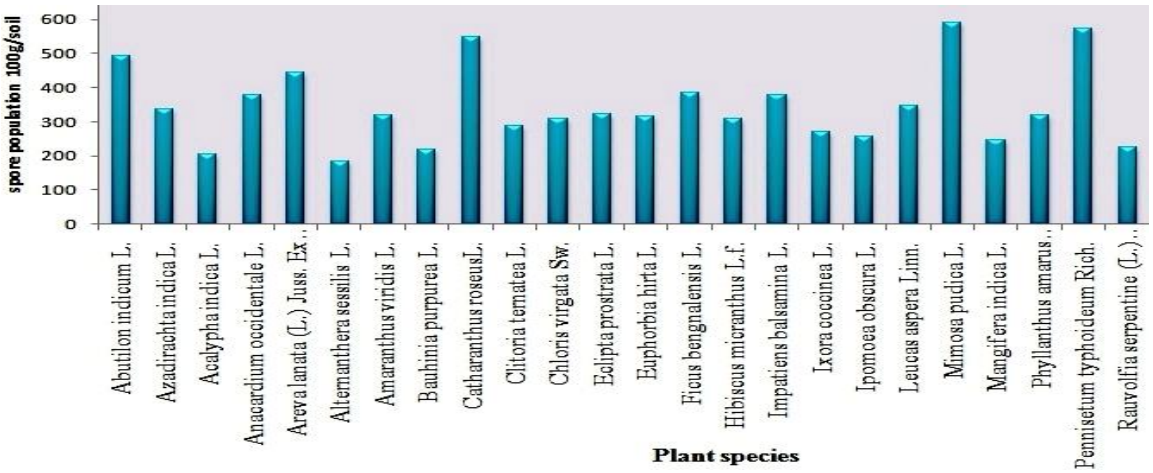

Fig. 3. AM fungal spore population of the plant species of Paithal hills.

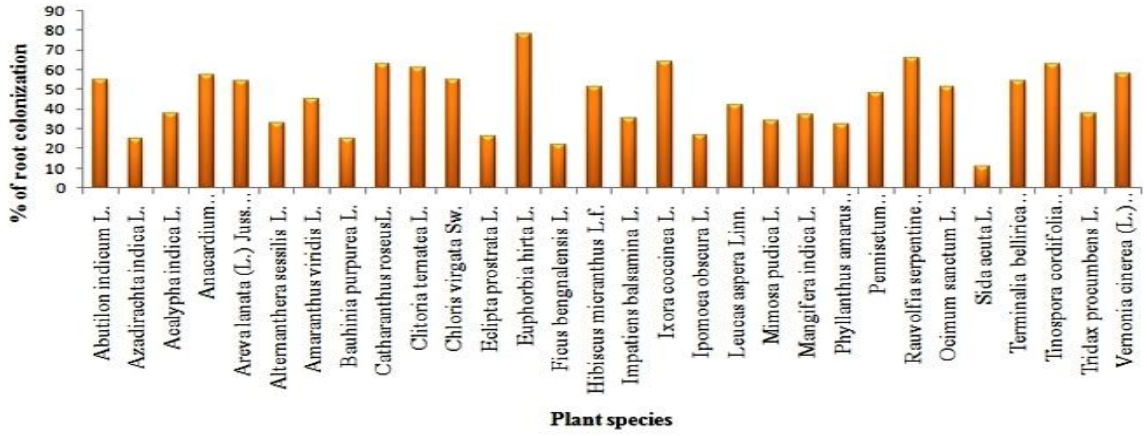

Fig. 4. AM fungal root colonization in collected plant species from Paithal hills.

Table 2. Arbuscular Mycorrhizal fungal spore population and root colonization in the plant species of Paithal hills, Western Ghats, Kannur district, Kerala, during 2018-2019.

S. No.	Plant Species	pH	Types of infection			Spore Population (100g/soil)	(%) root colonization
			Hyphae	Arbuscule	Vesicles		
1.	*Abutilon indicum* L.	5.6	+	-	+	492	55
2.	*Azadirachta indica* L.	4.9	+	+	+	337	25
3.	*Acalypha indica* L.	6.4	+	+	+	205	38
4.	*Anacardium occidentale* L.	5.3	+	-	+	380	57
5.	*Areva lanata* (L.) Juss. Ex schult	6.4	+	+	+	443	54
6.	*Alternanthera sessilis* L.	5.1	+	-	+	184	33
7.	*Amaranthus viridis* L.	4.6	+	+	+	320	45
8.	*Bauhinia purpurea* L.	5.2	+	-	+	219	25
9.	*Catharanthus roseus*L.	5.9	+	+	+	548	63
10.	*Clitoria ternatea* L.	5.7	+	-	+	287	61
11.	*Chloris virgata* Sw.	5.3	+	+	+	308	55
12.	*Eclipta prostrata* L.	5.6	+	-	+	324	26
13.	*Euphorbia hirta* L.	5.9	+	+	+	316	78
14.	*Ficus bengnalensis* L.	6.1	+	-	+	386	22
15.	*Hibiscus micranthus* L.f.	6.3	+	-	+	310	51
16.	*Impatiens balsamina* L.	6.5	-	-	-	377	35
17.	*Ixora coccinea* L.	5.6	+	-	+	270	64
18.	*Ipomoea obscura* L.	5.8	+	+	+	255	27
19.	*Leucas aspera* Linn.	5.4	+	-	+	346	42
20.	*Mimosa pudica* L.	5.7	+	+	+	590	34
21.	*Mangifera indica* L.	5.9	+	-	+	245	37
22.	*Phyllanthus amarus* Schumach & Thonn	5.1	+	-	+	319	32
23.	*Pennisetum typhoideum* Rich.	5.0	+	-	+	575	48
24.	*Rauvolfia serpentine* (L.) Benth. ex Kurz	6.4	+	+	+	226	66
25.	*Ocimum sanctum* L.	6.2	+	-	+	570	51
26	*Sida acuta* L.	5.1	+	-	+	326	11
27	*Terminalia bellirica (Gaertn)Roxb.*	4.8	+	+	+	135	54
28	*Tinospora cordifolia* (thumb.)miers	6.9	+	+	+	234	63
29	*Tridax procumbens* L.	5.3	+	-	+	455	38
30	*Vernonia cinerea* (L.) Less	6.4	+	+	+	314	58

Table 3. AM fungal spore recovered from the rhizosphere soils samples in Paithal hills during August, 2018-March, 2019

S.NO	AM Fungal genera	Species
1	*Ambispora*	*Amb. appendiculatum*
2	*Gigaspora*	*Gi.candida*
3	*Paraglomus*	*P.occultum*
4	*Pasipora*	*P. dominikii*
5	*Glomus*	*Gl. heterosporum, Gl. hoi, Gl. invermeyanum, Gl. macroporum,Gl. maculosum, Gl. microsporum, Gl. magnicule, Gl. monosporum, Gl. multicaulis, Gl. multisubstensum*
6	*Rhizophagus*	*R. intraradix P.occultum*
7	*Sclerocystis*	*Scl.pachycaulus*

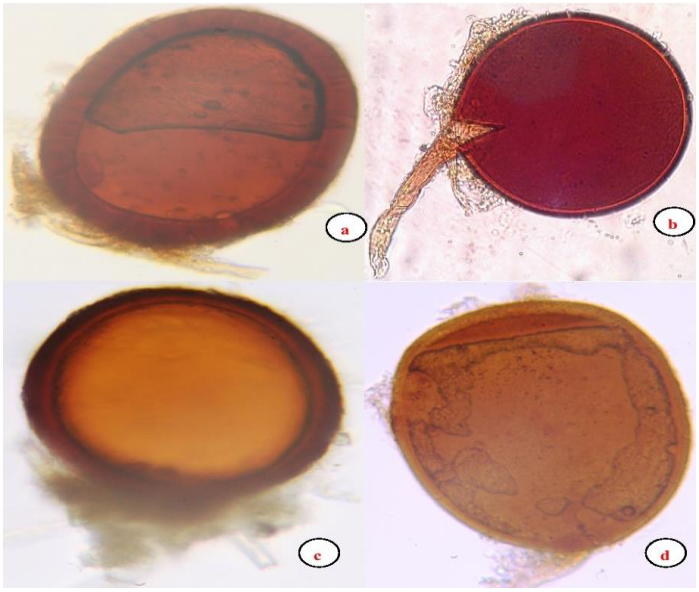

Fig. 5. a) *Glomus heterosporum*, b) *Glomus monosporum* c) *Glomus multicaulis* d) *Paraglomus occultum*.

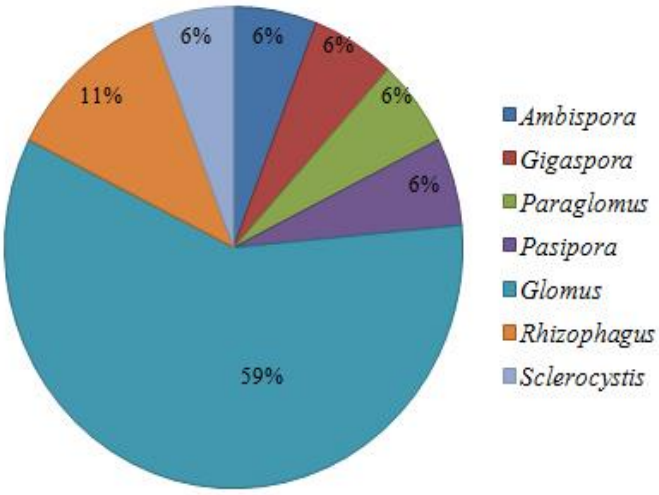

Fig. 6. Dominant genus was recovered from the rhizosphere soils samples in the study region.

Table 4. Distribution of AM fungal spores in the Plant species at the Paithal hills, Kannur Dist. Kerala.

S. No.	Plant species	AM fungal species
1.	*Abutilon indicum* L.	*candida ,Amb. Appendiculatum, R. intraradix*
2.	*Azadirachta indica* L.	*P. occultum, Gl. hoi*
3.	*Acalypha indica* L.	*Gl. multisubstensum,*
4.	*Anacardium occidentale* L.	*Gl. heterosporum, Gl. multicaulis, Gl. multisubstensum*
5.	*Areva lanata* (L.) Juss. Ex schult	*P. dominikii, Gl. invermeyanum*
6.	*Alternanthera sessilis* L.	*Gl. magnicule*
7.	*Amaranthus viridis* L.	*Amb. Appendiculatum, Gl. multisubstensum, Gl. maculosum, P. dominikii*
8.	*Bauhinia purpurea* L.	*Gi. candida, R. intraradix, Amb. appendiculatum ,Gl. multicaulis*
9.	*Catharanthus roseus* L.	*Scl.pachycaulus, P. dominikii, Gl. invermeyanum*
10.	*Clitoria ternatea* L.	*P. occultum, R. intraradix*
11.	*Chloris virgata* Sw.	*Gl. heterosporum, Gl. hoi, Gl. invermeyanum, Gl. macroporum, Gl. maculosum, Scl.pachycaulus*
12.	*Eclipta prostrata* L.	*R. intraradix, Amb. Appendiculatum, Gl. multisubstensum*
13.	*Euphorbia hirta* L.	*Amb. appendiculatum ,Gl. multicaulis*
14.	*Ficus bengnalensis* L.	*Gl. maculosum, P. dominikii*
15.	*Hibiscus micranthus* L.f.	*P. occultum*
16.	*Impatiens balsamina* L.	*Gl. multicaulis,Gl. maculosum, R. intraradix*
17.	*Ixora coccinea* L.	*Gl.microsporum, Gl. magnicule*
18.	*Ipomoea obscura* L.	*Scl.pachycaulus*
19.	*Leucas aspera* Linn.	*candida, Gl. hoi, R. intraradix, Gl. invermeyanum*
20.	*Mimosa pudica* L.	*Gl. heterosporum, Gl. magnicule*
21.	*Mangifera indica* L.	*Scl.pachycaulus, Gl. hoi*
22.	*Phyllanthus amarus* Schumach & Thonn	*Gl. heterosporum, Gl. hoi, Gl. invermeyanum, Gl. macroporum, Gl. maculosum*
23.	*Pennisetum typhoideum* Rich	*Amb. appendiculatum*

24.	*Rauvolfia serpentine(L.)* Benth.	*Scl.pachycaulus, Gl. monosporum*
25.	*Ocimum sanctum* L.	*P. dominikii*
26	*Sida acuta* L.	*Gi. candida, Gl. magnicule*
27	*Terminalia bellirica* . (Gaertn.) Robx.	*P. dominikii,Amb, Appendiculatum*
28	*Tinospora cordifolia* (thumb.) *miers*	*Amb. Appendiculatum, Gl. hoi, R. intraradix,P. dominikii*
29	*Tridax procumbens L.*	*Gi.candida*
30	*Vernonia cinerea* (L.) Less	*Gl. maculosum, P. dominikii, Gl. monosporum Gl. multisubstensum*

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