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RESEARCH ARTICLE

STUDIES ON THE AM FUNGAL DIVERSITY OF SOME SIGNIFICANT ETHANO-MEDICINAL PLANTS OF KARULAI HILLS, MALAPPURAM DISTRICT, KERALA

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ABSTRACT

The study was carried out to observe the AM fungal diversity in some important medicinal plant species of Karulai hills, Malappuram district, Kerala. The root samples of all the collected plant species showed mycorrhizal infection. The percentage of AM fungal colonization ranged from 17 to 87. The highest AM fungal infection was exhibited in *Desmodium triflorum (*87%) and lowest in *phyllanthes amarus* (17%). The maximum spore population was observed in *Desmodium gangeticum* (874/100g of soil) and minimum in *Piper longum* (171/100g of soil). Totally 13 genera of AM fungi were found to be associated with the rhizosphere soil samples. Among them AM fungal species isolated, the dominant species is *Rhizophagus fasciculatus*. Ethanobotanical study reveals that the Cholanaykans tribes of Karullai hills posses great knowledge about the use of various herbal medicines to cure different ailments and are also conscious about the loss of their traditional medicinal practices. They know about number of medicinal plants and their applications.

Keywords: AM fungal, diversity, Ethanobotany, Cholanaykans, Karullai hills.

1. INRODUCTION

Limited availability of soil nitrogen and phosphorus is frequently a major factor limiting sustainable productivity of tropical tree plantations. 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.*, nitrogen fixing organisms, phosphate solubilising organisms and mycorrhizae. Of these, inoculation of forest trees with mycorrhizal fungi could help the plants to scavenge sparingly available nutrients in soil including phosphorus and also provide protection against plant pathogens and drought Baltruschat and Schonbeck (1).

Arbuscular mycorrhizal (AM) fungal symbiosis facilitates the survival, growth and establishment of plants in extreme habitats Asmelash (2). Many factors stimulate differential spore production by AM fungi in the rhizosphere, leads to seasonal fluctuation in AM fungal colonization and spore densities Koske (3), Gemma and Koske, (4). The most wide spread symbiosis amongst plants is mycorrhizal association which involves various root inhabiting fungi and feeder roots. Among the different type of mycorrhizal fungi, 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 Rafiq (5) 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 *Acacia*, *Eucalyptus*, Teak etc. are naturally colonized by AM fungi. It is well known that AM fungi protect plants against soil and root-borne pathogens Bagyaraj (6) thereby improving plant growth and vigor.

Microorganisms are present in great number on and near the feeder roots and they play vital roles in numerous physiological processes. These dynamic processes are medicated by association of microorganisms participating in saprophytic, pathogenic and symbiotic root activities. The major symbiotic associations on tree species are mycorrhizal fungi. AM fungi, play an important role in plant survival and in the community stability of vegetation in natural ecosystems. Mycorrhizal symbiosis plays a critical role in mineral nutrition of terrestrial plants. The mycorrhizal fungi are an important part of the soil microbial system because the prevalence of these associations on plants is so common under natural soil conditions.

Plants also find innumerable uses in the human civilization since its conception. The plants

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also find their use as medicine in human healthcare. Several traditional systems have evolved in the world, which use plants to cater to needs of healthcare and are still in practice around the world. The use of plants and natural products received a fillip when World Health Organization recognized plant and natural products based medicinal systems as alternative and complimentary. The use of medicinal plants for human healthcare is well documented in India, China, Egypt and Arab world Lalrinzuali (7).

The traditional systems of medicine prescribe drug as single plant products or a mixture of several plants depending on the disease, which are mainly administrated orally. The ethanobotanical and ethanomedicinal studies have great significance in the collection traditional knowledge, preparation of recorded data and in conservation of endangered medicinal plant species Prakash (8). The present work aims, to documentation of ethanobotanical importance of medicinal plants practiced by Cholanaikkan tribes and Enumeration of the arbuscular mycorrhizal fungal species in the rhizosphere soil samples of these plant species in Karulai, Malapuram district, Kerala.

2. MATERIALS AND METHODS

2.1. Study area

Karulai village is located in Nilambur, Malappuram district, Kerala, India. It is situated 10 km away from the sub-district headquarters Nilambur and 48 km away from district headquarters Malappuram. Total extent of Karulai range is 26560.76 hecters which is notified under two reserve notifications *viz*., Amarambalam Reserve and Karimpuzha reserve. Karulai is the "Gods' own village" in Kerala state with green forest (Fig. 1). The average annual temperature in Karulai is 27.7°C in a year and the average rainfall is 2500 mm (Table- 1). Karimpuzha is the largest tributary of Chaliyar River, Kerala, India. It is very near to Nilambur. Karimpuzha originates from Western slopes between Mukuthi peak and Avanlanche Dam in Nilgiri district of Tamil Nadu.

2.2. Sample collection

Totally 45 plant species belonging to the 31 families were collected from Karulai during the September, 2016 – March 2017. Root samples and rhizosphere soil samples of plant species growing in and around areas of Karulai were collected. The root and soil samples were transported to the laboratory immediately after collection.

2.3. Root samples

Root samples, 5-15 cm long, were collected from the plant species during all three seasons of 2016 to 2017. During collection, care was taken to ascertain individual plants for which roots could positively identified as belonging to a particular plant species. For identification and nomenclature of the plant species the following manual was used Gamble(9) Nair and Henry (10).

2.4. Soil samples

The rhizosphere soils, dug up to a depth of 10 cm, were collected from each plant species after removing the surface of the soil and litter covering. These samples were kept in sterilized bags and were transported to the laboratory immediately after collection for the examination of arbuscular mycorrhizal fungal spore isolation.

2.5. Soil pH

The pH of soil samples was determined (soil-water suspensions 1:5) with the help of pH meter (Elico).

2.6. Sample preservation

In the laboratory, the roots were separated from the soil by wet sieving. The roots were washed with water and processed a fresh whenever possible. Otherwise the washed roots were fixed in formaldehyde-acetic acid-ethanol (FAA) solution (90:5:5 V/N) modified method of Phillips and Hayman (11). The soil sample was air dried and stored at 4°C until processed. Each soil samples was used for chemical analysis, spore counts and classification in to various types and multiplication, concentration and separation of AM fungal spore for identification.

2.7. Evaluation of AM infection

The root samples were cleared and stained in tryphan blue with a modified version of the Phillips and Hayman's (12) method. Roots were cut in to 1-2 pieces, heated at 90°C 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 0.05% tryphan blue in lacto phenol for 5 minutes and the excess stain was removed with clear lacto phenol.

The pigmented roots were heated at 90°C in 10% KOH for 2 hours, washed with fresh 10% KOH and immersed in an alkaline solution of H_2O_2 for 30 minutes at 25°C until bleached. They were rinsed thoroughly with water to remove the H_2O_2, acidified in dilute HCl and stained as described earlier. In some cases the modified method of Merryweather

and Fitter (13) was followed where autoclaving and bleaching with H_2O_2, were omitted. In a few cases, direct observation of unstained, fresh and intact roots Arias(11) was made.

Arbuscular mycorrhizal infection in the roots was assessed following the grid line-intersect method of Giovannetti and Mosse (14). The stained root pieces were spread out evenly on a square plastic Petridish (10.2 x 10 cm). A grid of lines was marked on the bottom of the dish to form 1 cm inch squares. Vertical and horizontal gridlines were scanned under a dissecting microscope and the presence of infection was recorded at each point where the roots intersected a line. Four sets of observation were made, recording 100, 200, 300 and all the root gridline intersects. Each of the three replicates records was made on a fresh rearrangement of the same root sample.

The percentage of AM infection was calculated using the formula:

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

When sufficient root pieces are not available, the slide method Giovannetti and Mosse was followed. Root pieces, 1 cm long, were selected at random from a stained sample and mounted on microscope slide groups of 10. Presence of infection was recorded in each of the 10 pieces and present infection was calculated. To observe hyphae, vesicles and arbuscles under light microscope, the root pieces were mounted on glass slides either temporarily in lacto phenol. The cover slip was pressed gently to make the roots flattened and sealed with DPX medium.

2.8. Isolation of arbuscular mycorrhizal spores from the soil samples

Spores were recovered from the soil samples by the wet-sieving and decanting method Gerdemann and Nicolson (15). From each soil sample, 100 g of soil was taken and mixed with 1:1 of warm water in a large beaker until all the aggregates dispersed to leave a uniform suspension. Heavier particles were allowed to settle down. To remove organic matter and roots, the suspension was decanted through a 710 µm sieve. The suspension that passed through 710 µm was decanted 425 µm, 250 µm, 150 µm, 75 µm and 45 µm sieves consecutively. The residues in the respective sieve were collected in petridishes with about 10-20 mL water observed under a dissecting microscope for AM fungal spores.

The total spore count was calculated by counting the spores. Then the spores were separated using a glass pipette and segregated. The spore were mounted on clear glass slides using lacto phenol or polyvinyl alcohol lacto phenol (PVL), covered with cover slips and sealed with DPX medium.

2.9. Identification of AM fungi

Based upon microscopic characters, the AM fungal spores were identified. For identification and nomenclature, keys of the following manual authors were used: Raman and Mohankumar (16) and Redecker (17). Classification on based on color, size, shape, surface, structure, general nature of the spore contents and hyphal attachment. Photomicrographs were taken with the help of a Magnus Olympus Microscope.

2.10. Ethnobotanical study

Frequent field trips were conducted in the tribal villages located at Karulai hills, during the study period (2016-2017). Initial field trips were utilized to know about the land and people. As the tribal's are mostly illiterate, no structural questionnaire approach was used. Ethno medicinal data were collected through conversation with beneficiaries, traditional healers and elder people. During the interviews, local names, useful plant parts, method of preparation and dosage were recorded. Subsequent field trips were conducted in different season in the same localities for confirming the data collected and also for gathering, additional medicinal information. The medicinal plant species were collected from wild and also from the tribal peoples homestead gardens for herbarium preparation. The method of gathering information was the same as suggested by Jain (18).

2.10.1. Cholanaikkan tribes

The Cholanaikkans are an ethnic group and primarily inhabit the southern Kerala state, especially silent valley national park. The Cholanaikkan traditionally reside Karulai and Chunkathara forest ranges near Nilambur, Malappuram district. Until the 1960s, they were leading a secluded life with very limited contact with mainstream urban society. Since then, the Cholanaikkans traditional lifestyle has been altered. They currently have a 16% literacy rate. The Cholanaikkan call themselves as 'Malanaikan' or 'Sholanaikan'. They are called Cholanaikan because they inhabit the interior forests. 'Chola' or 'sholas' means deep ever green forests. And 'naikan' means king. The Cholanaikkan numbered 360 indivduals in the 1991 but only 191 members today. They are found widely scattered in the forest ranges. They subsist on food-gathering, hunting and minor forest produce collection. Their language is a mixture of Kannada, Tamil and Malayalam. They use rice as

their staple food, also use wild tubers, roots, seeds, fruits, and meat.

Table 1. Temperature and rain fall data of Malappuram, District, during the September 2016 to March

Year	Month	Temperature(0°C)		Rainfall (mm)	Humidity (%)
		Maximum	Minimum		
2016	September	29.5	24.0	253.2	84
	October	30.6	24.0	280.8	81
	November	31.3	23.6	68.6	77
	December	31.6	22.7	82.7	74
2017	January	31.9	22.9	19.4	67
	February	32.2	23.3	7.8	71
	March	33.1	24.9	1.5	74

2017

Table 2. AM Fungal spore population and root colonization of plants species in Karulai, Malappuram district, Kerala.

S. No	Plant name	Family	Habit	Soil pH	Type of colonization			% of Root Infection	Spore Population /100g of soil
					H	V	A		
1	*Abrus precatorius* L.	Leguminosae	Climber	5	+	+	+	58	693
2	*Andrographis paniculata* (Burm.f.) Nees	Acanthaceae	Herb	5.8	+	-	-	27	372
3	*Asparagus racemosus* Willd	Asparagaceae	Armed vine	5.1	+	-	-	22	329
4	*Biophytum sensitivum* (L.) DC.	Oxalidaceae	Herb	5	+	+	-	27	268
5	*Calotropis gigantea* (L.) R.Br	Asclepiadaceae	Shrub	4.8	+	-	-	18	325
6	*Canavalia gladiate* (Jacq.) DC.	Leguminosae	Twinig herb	4.6	+	+	+	75	693
7	*Cassia auriculata* L.	Caesalpiniaceae	Shrub	5.6	+	+	-	58	683
8	*Catharanthus roseus* (L.) G.Don.	Apocynaceae	Shrub	5.9	+	-	-	19	427
9	*Centella asiatica* (L.) Urb.	Apiaceae	Herb	4.2	+	-	-	27	276
10	*Cheilocostus speciosus* (J.Koenig) C.D.Specht	Costaceae	Herb	5.5	-	-	-	-	197
11	*Clitoria ternatea* L.	Leguminosae	Climber	6	+	+	-	57	572
12	*Costus pictus* D.Don	Costaceae	Herb	5.1	-	-	-	-	213
13	*Crotalaria pallida* Aiton.	Leguminosaea	Shrub	5.2	+	+	+	73	842
14	*Curculigo orchioides* Gaertn	Hypoxidaceae	Herb	5.3	-	-	-	-	174
15	*Curcuma aromatic* Salisb	Zingiberaceae	Herb	4.8	-	-	-	-	266
16	*Cyclea peltata* (Lam.) Hook.f.&Thomson	Menispermaceae	Climber	5.7	+	+	-	26	271
17	*Cymbopogon flexuosus* (Nees ex steud) W.Watson	Poaceae	Herb	5.4	+	+	-	38	372
18	*Datura metal* L.	Solanaceae	Subshrub	5.9	+	+	+	72	624
19	*Desmodium*	Leguminosae	Herb	5.6	+	+	+	64	874

20	*Desmodium gangeticum* (L.) DC. *triflorum* (L.) DC	Leguminosae	Herb	5.4	+	+	+	87	862
21	*Elephantopus scaber* L.	Compositae	Herb	5.1	+	+	-	63	758
22	*Emilia sonchifolia* (L.) DC.ex DC.	Compositae	Herb	5.6	+	+	-	69	649
23	*Ensete superba* (Roxb.) Cheesman	Musaceae	Shrub	5.5	+	+	-	47	483
24	*Euphorbia hirta* L.	Euphorbiaceae	Herb	5.4	-	-	-	-	287
25	*Gliricidia sepium* (Jacq.) Walp.	Leguminosae	Shrub	5.1	+	+	-	48	472
26	*Gloriosa superb* L.	Liliaceae	Climber	4.7	+	-	-	21	273
27	*Helicteres isora* L.	Malvaceae	Shrub	5	+	+	-	58	792
28	*Hemidesmus indicus* (L.) R.Br.ex Schult	Apocynaceae	Climber	5.6	+	-	-	32	341
29	*Hydnocarpus pentandra* (Buch-Ham)	Flacourtiaceae	Tree	5.1	+	-	-	25	432
30	*Justicia adhatoda* L	Acanthaceae	Shrub	5.7	+	-	-	22	276
31	*Justicia gendarussa* Burm.f	Acanthaceae	Shrub	5.8	+	-	-	28	372
32	*Leucas aspera* (Willd.) Link	Lamiaceae	Herb	5.8	+	+	-	44	537
33	*Maranta arundinaceae* L.	Marandaceae	Shrub	5.6	+	-	-	27	281
34	*Microsorum diversifolium* G.Forst	Polypodiaceae	Shrub	5.3	+	-	-	31	562
35	*Mimosa pudica* L.	Mimosaseae	Sub shrub	4.7	+	-	+	38	541
36	*Oscimum sanctum* L.	Lamiaceae	Herb	5.3	+	+	-	47	483
37	*Pandanus odoratissimus* L.F	Pandanaceae	Shrub	5.3	-	-	-	-	168
38	*Phyllanthus amarus* Schumach & Thonn.	Euphorbiaceae	Erect herb	5.3	+	-	-	17	228
39	*Phyllanthus emblica* L.	Euphorbiaceae	Tree	5.4	-	-	-	-	256
40	*Piper longum* L.	Piperaceae	Scadent shrub	5.3	+	-	-	18	171
41	*Plumbago zeylanica* L.	Plumbaginaceae	Subshrub	5.6	-	-	-	-	224
42	*Pseudarthria viscida* (L.) Wight & Arn.	Fabaceae	Subshrub	5.3	+	+	+	58	642
43	*Psidium guajava* L.	Myrtaceae	Shrub	5.8	+	-	-	26	331
44	*Rotala aquatica* Lour	Lythraceae	Shrub	5.3	+	-	-	27	462
45	*Scoparia dulcis* L.	Scrophulariaceae	Herb	5.6	+	-	-	19	254

H- Hyphae, A- Arbuscules, V- Vescicle, + - Present, - - Absent

Table 3. Identified AM fungal spore species list from Karulai, Malappuram district, Kerala.

S. No.	Genera	Species
1	*Acaulospora*	*Aca. alpine*, Aca. *foveat, Aca. tuberculata, Aca. undulate*
2	*Ambispora*	*Ambispora callosa*
3	*Archaeospora*	*Archaeospora trappei*
4	*Claroideoglomus*	*Claroideoglomus claroideum*

5	*Dentiscutata*	*Dentiscutata erythropus*
6	*Diversispora*	*Diversispora arenaria, Div. celata*
7	*Entrophospora*	*Entrophospora infrequens*
8	*Funneliformis*	*Funneliformis coronatum*
9	*Gigaspora*	*Gigaspora albida, Gi. decipiens, Gi. ramisporophora*
10	*Glomus*	*Gl. albidum, Gl. ambisporum, Gl. arborense, Gl. canadense, Gl. globiferum, Gl. multicaule*
11	*Pacispora*	*Pacispora scintillans*
12	*Rhizophagus*	*Rhizophagus fasciculatus*
13	*Scutellispora*	*Scutellispora spp, Scu. savannicola, Scu. striata*

Table 4. Distribution of AM fungal spores different plant species from Karulai, Malappuram district, kerala.

S. No.	Plant name	Family	Spores name
1	*Abrus precatorius* L.	Leguminosae	*Acaulospora alpine, Gigaspora albida, Glomus arborense, Rhizophagus fasciculatus*
2	*Andrographis paniculata* (Burm.f.) Nees	Acanthaceae	*Ambispora callosa, Diversispora arenaria, Pacispora scintillans*
3	*Asparagus racemosus* Willd	Asparagaceae	*Acaulospora foveat, Gigaspora ramisporophora, Glomus multicaule, Rhizophagus fasciculatus*
4	*Biophytum sensitivum* (L.) DC.	Oxalidaceae	*Acaulospora undulate, Dentiscutata erythropus, Pacispora scintillans, Rhizophagus fasciculatus*
5	*Calotropis gigantean* (L.) R.Br	Asclepiadaceae	*Ambispora callosa, Funneliformis coronatum, Rhizophagus fasciculatus*
6	*Canavalia gladiate* (Jacq.) DC.	Leguminosae	*Acaulospora alpine, Glomus albidum, Glomus multicaule, Scutellospora striata*
7	*Cassia auriculata* L.	Caesalpiniaceae	*Claroideoglomus claroideum, Glomus ambisporum, Rhizophagus fasciculatus*
8	*Catharanthus roseus* (L.) G.Don.	Apocynaceae	*Acaulospora foveat, Diversispora celata, Glomus arborense*
9	*Centella asiatica* (L.) Urb.	Apiaceae	*Acaulospora undulate, Gigaspora albida, Pacispora scintillans*
10	*Cheilocostus speciosus* (J.Koenig) C.D.Specht	Costaceae	*Acaulospora undulate, Glomus albidum, Glomus multicaule*
11	*Clitoria ternatea* L.	Leguminosae	*Acaulospora undulate, Glomus albidum, Glomus multicaule, Rhizophagus fasciculatus*
12	*Costus pictus* D.Don	Costaceae	*Acaulospora alpine, Diversispora celata, Glomus arborense, Rhizophagus fasciculatus*
13	*Crotalaria pallida* Aiton.	Leguminosaea	*Claroideoglomus claroideum, Glomus ambisporum, Rhizophagus fasciculatus*
14	*Curculigo orchioides* Gaertn	Hypoxidaceae	*Acaulospora tuberculata, Funneliformis coronatum, Rhizophagus fasciculatus*
15	*Curcuma aromatic* Salisb.	Zingiberaceae	*Claroideoglomus claroideum, Gigaspora ramisporophora*
16	*Cyclea peltata* (Lam.) Hook.f.&Thomson	Menispermaceae	*Dentiscutata erythropus, Glomus albidum, Glomus arborense*
17	*Cymbopogon flexuosus* (Nees ex steud) W.Watson	Poaceae	*Acaulospora foveat, Entrophospora infrequens, Glomus globiferum, Rhizophagus fasciculatus*
18	*Datura mental*	solanaceae	*Acaulospora undulate, Glomus albidum*
19	*Desmodium gangeticum* (L.) DC.	Leguminosae	*Claroideoglomus claroideum, Entrophospora infrequens, Rhizophagus fasciculatus*
20	*Desmodium triflorum* (L.) DC	Leguminosae	*Diversispora arenaria, Funneliformis coronatum, Rhizophagus fasciculatus*
21	*Elephantopus scaber* L.	Compositae	*Archaeospora trappei, Gigaspora albida, Glomus canadense*
22	*Emilia sonchifolia* (L.) DC.ex	Compositae	*Acaulospora foveat, Gigaspora ramisporophora,*

	DC.		*Glomus globiferum, Rhizophagus fasciculatus*
23	*Ensete superbum* (Roxb.) Cheesman	Musaceae	*Diversispora arenaria, Glomus albidum, Glomus arborense*
24	*Euphorbia hirta* L.	Euphorbiaceae	*Claroideoglomus claroideum, Funneliformis coronatum, Rhizophagus fasciculatus*
25	*Gliricidia sepium* (Jacq.) Walp.	Leguminosae	*Acaulospora foveat, Gigaspora albida, Glomus canadense, Scutellospora striata*
26	*Gloriosa superb* L.	Liliaceae	*Claroideoglomus claroideum, Glomus ambisporum, Glomus globiferum*
27	*Helicteres isora* L.	Malvaceae	*Entrophospora infrequens, Funneliformis coronatum, Glomus canadense, Rhizophagus fasciculatus*
28	*Hemidesmus indicus* (L.) R.Br.ex Schult	Apocynaceae	*Acaulospora alpine, Gigaspora ramisporophora, Glomus canadense, Rhizophagus fasciculatus*
29	*Hydnocarpus pentandra* (Buch-Ham)	Flacourtiaceae	*Archaeospora trappei, Gigaspora decipiens, Rhizophagus fasciculatus*
30	*Justicia adhatoda* L	Acanthaceae	*Archaeospora trappei, Glomus ambisporum, Glomus arborense*
31	*Justicia gendarussa* Burm.f	Acanthaceae	*Claroideoglomus claroideum, Gigaspora albida, Gigaspora decipiens, Rhizophagus fasciculatus*
32	*Leucas aspera* (Willd.) Link	Lamiaceae	*Archaeospora trappei, Glomus ambisporum, Glomus globiferum*
33	*Maranda arundinaceae* L.	Marandaceae	*Ambispora callosa, Gigaspora decipiens, Glomus canadense*
34	*Microsorum diversifolium* G.Forst	Polypodiaceae	*Acaulospora alpine, Glomus ambisporum, Scutellospora savannicola*
35	*Mimosa pudica* L.	Mimosaceae	*Dentiscutata erythropus, Entrophospora infrequens, Rhizophagus fasciculatus*
36	*Oscimum sanctum* L.	Lamiaceae	*Ambispora callosa, Glomus ambisporum, Glomus arborense*
37	*Pandanus odoratissimus* L.F	Arecaceae	*Acaulospora tuberculata, Gigaspora ramisporophora, Rhizophagus fasciculatus*
38	*Phyllanthus amarus* Schumach & Thonn.	Euphorbiaceae	*Dentiscutata erythropus, Gigaspora decipiens, Glomus canadense*
39	*Phyllanthus emblica* L.	Euphorbiaceae	*Diversispora celata, Entrophospora infrequens, Glomus arborense, Scutellispora* spp
40	*Piper longum* L.	Piperaceae	*Dentiscutata erythropus, Gigaspora decipiens, Rhizophagus fasciculatus*
41	*Plumbago zeylanica* L.	Plumbaginaceae	*Acaulospora tuberculata, Glomus arborense, Glomus globiferum, Rhizophagus fasciculatus*
42	*Pseudarthria viscida* (L.) Wight & Arn.	Leguminosae	*Claroideoglomus claroideum, Glomus ambisporum, Scutellospora savannicola*
43	*Psidium guajava* L	Myrtaceae	*Claroideoglomus claroideum, Gigaspora decipiens, Glomus canadense, Glomus globiferum, Scutellispora* spp
44	*Rotala aquatica* Lour	Euphorbiaceae	*Acaulospora foveat, Gigaspora decipiens, Glomus globiferum*
45	Scoparia dulcis L.	Scrophulariaceae	Acaulospora alpine, Gigaspora ramisporophora, Rhizophagus fasciculatus

Table 5. Details of enumerated plants used by the Cholanaikkan tribes from Karulai.

S. No.	Botanical Name	Family	Local Name	Habit	Part used
1.	*Abrus precatorius* L.	Leguminosae	Kunnikkuru	Climber	Leaves, Seed
2.	*Andrographis paniculata* (Burm.f.) Nees	Acanthaceae	Kiriyattu,Kiriyathu	Herb	Leaves, stem.

3.	*Asparagus racemosus* Willd.	Asparagaceae	Sathavari.	Armed vine	Tuberous root.
4.	*Biophytum sensitivum* (L.) DC.	Oxalidaceae	Mukkuttihi	Herb	Aerial part
5.	*Calotropis gigantea* (L.) R.Br	Asclepiadaceae	Erikku	Shrub	Leaves
6.	*Canavalia gladiata* (Jacq.) DC.	Leguminosae	Valpayar	Twinig herb	Seed
7.	*Cassia auriculata* L.	Caesalpiniaceae	Avara	Shrub	Whole plant
8.	*Catharanthus roseus* (L.) G.Don	Apocynaceae	Shavam Naari	Shrub	Whole plant
9.	*Centella asiatica* (L.) Urb.	Apiaceae	Kudangal,Mutthil.	Herb	Whole plant
10.	*Cheilocostus speciosus* (J.Koenig) C.D.Specht	Costaceae	Anakuva	Herb	Rhizome
11.	*Clitoria ternatea* L.	Leguminosae	Sangu pushpam	Climber	Leaves.
12.	*Costus pictus* D.Don.	Costaceae	Insulin chedi	Herb	Leaves
13.	*Crotalaria pallida* Aiton.	Leguminosaea	Kilukkachedi	Shrub	Roots
14.	*Curculigo orchioides* Gaertn	Hypoxidaceae	Nelappana	Herb	Rhizome
15.	*Curcuma aromatica* Salisb.	Zingiberaceae	Kasthurimanjal	Herb	Rhizome, oil
16.	*Cyclea peltata* (Lam.) Hook.f.&Thomson	Menispermaceae	Padathali,Pattichevian	Climber	Leaves,Roots
17.	*Cymbopogon flexuosus* (Nees ex steud) W.Watson	Poaceae	Inchipullu, Thilappullu	Herb	Leaves
18.	*Datura metal L.*	Solanaceae	Ummathu	Subshrub	Fruit
19.	*Desmodium gangeticum* (L.) DC.	Fabaceae	Orila	Herb	Leaves
20.	*Desmodium triflorum* (L.) DC	Leguminosae	Nilamparanda	Herb	Leaves
21.	*Elephantopus scaber* L.	Compositae	Anachuvadi	Herb	Leaves, Root
22.	*Emilia sonchifolia* (L.) DC.ex DC.	Compositae	Muyalchevian	Diffuse herb	Whole plant.
23.	*Ensete superba* (Roxb.) Cheesman	Musaceae	Kalluvazha, Malavazha	Erect shrub	Rhizome
24.	*Euphorbia hirta* L.	Euphorbiaceae	Nilappala	Herb	Root, Leaf
25.	*Gliricidia sepium* (Jacq.) Walp.	Leguminosae	Simakkonna	Short tree	Leaves
26.	*Gloriosa superb* L	Liliaceae	Menthonni	Climber	Leaves
27.	*Helicteres isora* L.	Malvaceae	Edampiri-valampiri	Large shrub	Fruit
28.	*Hemidesmus indicus* (L.) R.Br.ex Schult	Asclepiadiaceae	Nannari	Climber	Root, Leaves
29.	*Hydnocarpus pentandra* (Buch-Ham)	Flacourtiaceae	Marrotti	Tree	Seed
30.	*Justicia adhatoda* L	Acanthaceae	Aadalodakam	Shrub	Leaves
31.	*Justicia gendarussa* Burm.f	Acanthaceae	Vathakkodi	Shrub	Leaves
32.	*Leucas aspera* (Willd.) Link	Lamiaceae	Thumba	Herb	whole plant
33.	*Maranda arundinaceae* L	Marandaceae	Kuvva	Shrub	Rhizome
34.	*Microsorum diversifolium* G.Forst	Polypodiaceae	Panal chedi	Shrub	Tuber
35.	*Mimosa pudica* L.	Mimosaceae	Thottavadi	Sub shrub	Whole plant
36.	*Ocimum sanctum* L.	Lamiaceae	Tulsi	Herb	Leaves
37.	*Pandanus odoratissimus* L.F	Pandanaceae	Kaitha	shrub	Inflorescence
38.	*Phyllanthus amarus* Schumach & Thonn.	Euphorbiaceae	Keezharnelli	Erect herb	Whole plant
39.	*Phyllanthus emblica* L.	Euphorbiaceae	Nelli	Tree	Fruit
40.	*Piper longum* L.	Piperaceae	Thippali	Scadent	Fruit

				shrub	
41.	*Plumbago zeylanica* L.	Plumbaginaceae	Vellakoduveli	Subshrub	Root
42.	*Pseudarthria viscida* (L.) Wight & Arn.	Fabaceae	Moovila	Sub shrub	Leaves
43.	*Psidium guajava* L.	Myrtaceae	Perakka	Shrub	Leaves
44.	*Rotala aquatica* Lour	Lythraceae	Kallurvanchi	Shrub	Root
45.	*Scoparia dulcis* L.	Scrophulariaceae	Kallurukki	Herb	Whole plant

Table 6. Mode of administration for the ailments of the medicinal plants used by the Cholanaikkan tribes from Karulai.

S. No.	Botanical Name	Ailments	Mode of administration
1.	*Abrus precatorius* L.	Swelling	The leaves and seed powder is made paste with water, applied externally to relieve, Joint pains, swelling.
2.	*Andrographis paniculata* (Burm.f.) Nees	Diarrhea, Bronchitis, Chicken Pox and Coughs	Leaves and root decoction used for diarrhea, bronchitis, chicken pox, coughs, headaches and ear infection
3.	*Asparagus racemosus* Willd.	Stomach pain	Cooked tubers are eaten for stomach pain.
4.	*Biophytum sensitivum* (L.) DC.	Eye diseases	Juice taken from crushed plant parts is applied for eye itching and other eye problems
5.	*Calotropis gigantea* (L.) R.Br	Earache	The juice from the heated leaves of the plant is applied in to ear for earache.
6.	*Canavalia gladiata* (Jacq.) DC.	As a vegetable	The ripe seed can be eaten after cooking
7.	*Cassia auriculata* L.	Diabetes	Grind the dried bark, flowers, leaves and fruits in qualities boil with water. It is used to treat diabetes.
8.	*Catharanthus roseus* (L.) G.Don	Eye diseases	The extract of the plant is useful for eye infection and irritation.
9.	*Centella asiatica* (L.) Urb.	Memory power	Consumption of whole plant juice can improves memory power
10.	*Cheilocostus speciosus* (J.Koenig) C.D.Specht	Intestinal worms	Rhizome has been used to treat fever, rash, and intestinal worms.
11.	*Clitoria ternatea* L.	Head ache, Inflammation	Leaf juice is used as a nasal drops in headache. The leaf can be grind in to fine paste and applied any kind of inflammation.
12.	*Costus pictus* D.Don.	Diabetes	Juice prepared from the leaves is used to treat diabetes.
13.	*Crotalaria pallida* Aiton.	Swelling	The poultice made of the root applied in painful swelling of joint
14.	*Curculigo orchioides* Gaertn	Blood purifier	Crushed tubers are mixed with milk is used as blood purifier.
15.	*Curcuma aromatica* Salisb.	Skin diseases	The oil is used to reduce pain and inflammation associated with snake bite.
16.	*Cyclea peltata* (Lam.) Hook.f.&Thomson	Hair cleaner, Stomach pain	Leaves crushed with water and it is applied over the hair as hair cleaner. Powder obtained from dried tubers are mixed with hot water used for stomach pain
17.	*Cymbopogon flexuosus* (Nees ex steud) W.Watson	Headache, Stomachache	The oil extracted from the leaves is used directly to the skin for headache, stomachache, muscle pain
18.	*Datura metal L.*	Snake poison	Fruit paste applied for snake poison
19.	*Desmodium gangeticum* (L.) DC.	Kidney stones, Fever	A decoction of the leaves is used against kidney stones. The decoction of the root is employed to treat fever.
20.	*Desmodium triflorum* (L.) DC	Skin problems, Digestion.	The crushed leaves are applied externally on wounds and skin problems. The whole plant is used to promoting

			digestion
21.	*Elephantopus scaber* L.	Vomiting, Kill round worms	Fresh roots are used to prepare a blend which is best for combating vomiting. The decoction prepared from its roots or leaves is used to kill roundworms.
22.	*Emilia sonchifolia* (L.) DC.ex DC.	Diarrhoea, Protect teeth	The juice of the root is used to treat diarrhoea. The flower heads are chewed and kept in the mouth for about 10 minutes to protectteeth from decay.
23.	*Ensete superba* (Roxb.) Cheesman	Kidney stones	Raw fruits are eaten for kidney stone, diabetes and stomach ache. Flowers and pseudostem used as a vegetable.
24.	*Euphorbia hirta* L.	Impprove lactation, Remove warts on the face.	A decoction made from the root increases lactation in women. Leaf paste is used to treat swelling. Latex is used to treat warts on face.
25.	*Gliricidia sepium* (Jacq.) Walp.	Insect repellent	The leaves paste is used as a sedative and insecticides. It is used for the treatment of fracturesand wounds.
26.	*Gloriosa superba* L	Insect bites , Scorpion bites, Hair cleaner.	The crushed leaves are applied on treatment of snake bites, scorpion stings and sexually transmitted diseases. The leaf juice used against head lice.
27.	*Helicteres isora* L.	Ear drops	Crushed pods heated with castor oil used as an ear drop.
28.	*Hemidesmus indicus* (L.) R.Br.ex Schult	Snake bites& Scorpion bites	It is used for the treatment of snake bite, scorpion bite and other poisonous insect bite cases.
29.	*Hydnocarpus pentandra* (Buch-Ham)	Body pain	Oil extracted from the seeds are externally used for body pain
30.	*Justicia adhatoda* L	Cough & cold	Oral administration of leaf juice is used for cough and cold.
31.	*Justicia gendarussa* Burm.f	Chronic rheumatism	The decoction of leaves and tender young shoots are used in the treatment of chronic rheumatism and used for bathing during child birth.
32.	*Leucas aspera* (Willd.) Link	Cold and cough.	Crushed leaves juice is directly applied to the nose to get relief from cold and cough.
33.	*Maranda arundinaceae* L	Intestinal disorders.	Boiled starch is used to treat several stomach disorders like digestion and ulcers.
34.	*Microsorum diversifolium* G.Forst	Stomachache	Tuber used in the treatment of stomach ache.
35.	*Mimosa pudica* L.	Cuts and wounds	Crushed leaf Juice is applied over cuts and wounds.
36.	*Ocimum sanctum* L.	Headache& Skin diseases	Leaves paste is used for curing stomachache, headache, skin diseases, insect bites and itching
37.	*Pandanus odoratissimus* L.F	Mosquito repellent	Crushed inflorescence is mixed with water and sprayed over mosquito affected areas.
38.	*Phyllanthus amarus* Schumach & Thonn.	Jaundice	The root juice along with milk consumed in the morning is good to cure jaundice.
39.	*Phyllanthus emblica* L.	Eye diseases & Diabetes	Amla juice used to treat eye disease, diabetes, common cold and cough
40.	*Piper longum* L.	Tooth ache	Chewing of crushed fruits can reduce tooth ache.
41.	*Plumbago zeylanica* L.	Swelling.	Root paste applied externally for inflammatory swellings
42.	*Pseudarthria viscida* (L.) Wight & Arn.	Internal bleeding	Oral administration of leaf paste is used for internal bleeding
43.	*Psidium guajava* L.	Stomach problems	Leaves paste is used to the treatment of diarrhea and stomachache.
44.	*Rotala aquatica* Lour	Stomach ache	Consumption of root decoction is used for stomach ulcer.
45.	*Scoparia dulcis* L.	Kidney stones	Consumption of whole plant juice along with milk is remedy for kidney stone.

Their livelihood is totally dependent on the forest. The collection and selling of minor forest produce is the major source of income. The tribes, unlike any other tribes, under the leadership of the Mooppan (Elder) are willing to come out of the deep forest (Fig. 2).

3. RESULTS

AM fungal infection and spore population 45 plant species belongs to the 31 families and pH of the rhizosphere soil samples present in the Table 2 to 4. Totally 13 genera of AM fungi belonging to *Acaulospora, Ambispora, Archaeospora, Claroideoglomus, Dentiscutata, Diversispora, Entrophospora, Funneliformis, Gigaspora, Glomus, Pacispora, Rhizophagus* and *Scutellispora* were found to be associated with the rhizosphere soil samples (Fig. 3). Among them AM fungal species isolated, the *Glomus* is dominant genera and *Rhizophagus fasciculatus* is dominant species.

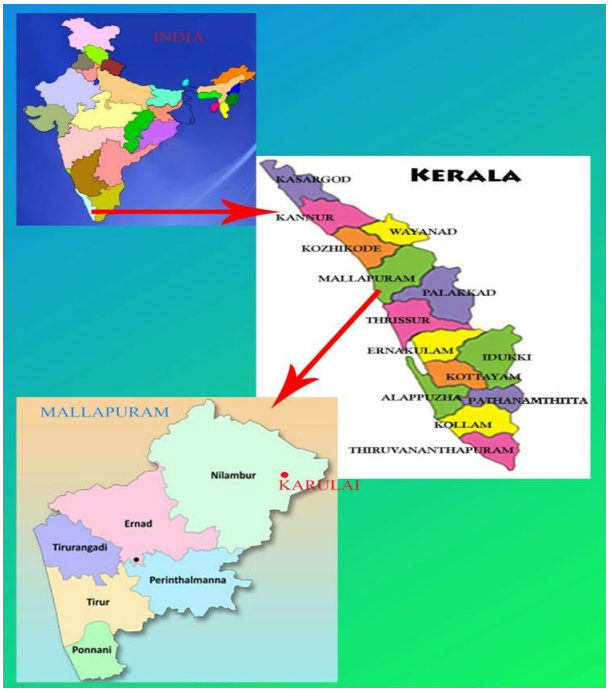

Fig. 1. The map showing the study area Karulai, Mallapuram, Kerala

Fig. 2. The Cholanaikkan tribes Karulai and Chunkathara forest ranges near Nilambur, Malappuram.

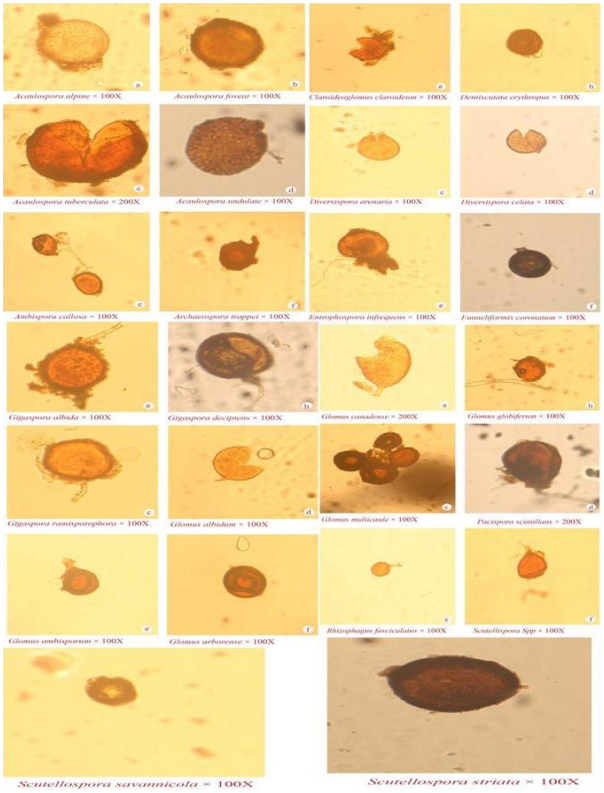

Fig. 3. Isolated AM fungal spore species from Karulai , Mallapuram, Kerala.

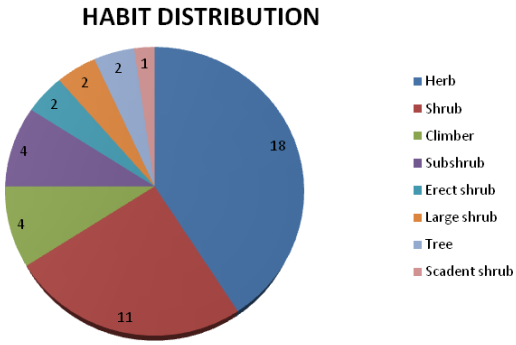

Fig. 4. Percentage of medicinal plants in different life-forms used by local healers of Karulai.

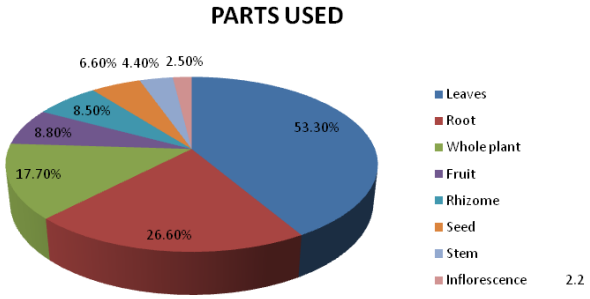

Fig. 5. Percentage of parts prescribed for the treatment of diseased by local healers of Karulai.

Totally 45 plant species belongs to 31 families were analyzed for AM fungal infection and spore population. Of these, the maximum spore population was observed in Leguminosae member of *Desmodium gangeticum* (874/100 g of soil) and minimum spore population was noticed in *Piper longum* (171/100 g of soil) belongs to the family Piperaceae.

The highest AM fungal infection was found in *Desmodium triflorum* (87%) belongs to Leguminosae and the least infection was recorded in Euphorbiaceae member of *Phyllanthus amarus* (17%). The plant species like *Andrographis paniculata* (27%), Acanthaceae, *Asparagus racemosus* (22%), Asparagaceae, *Biophytum sensitivum* (27%), Oxalidaceae, *Calotrapis gigantea* (18%), Asclepiadaceae, *Catharanthus roseus* (19%), Apocyanaceae, *Centella asiatica* (27%), Apiceae, *Cyclea peltata* (26%), Menispermaceae, *Gloriosa superba* (21%), Lilliaceae, *Justicia adhatoda* (22%), *J. gendarussa* (28%) both species belogs to Acanthaceae member, *Maranda arundinaceae* (27%), *Phyllanthus amarus* (17%), Euphorbiaceae, *Scoparia dulcis* (19%), Scrophulariaceae,, *Hydnocarpus pentandra* (25%), Flacourtiaceae, *Piper longum* (18%), Piperaceae, *Rotula aquatic* (27%), Boraginaceae*, Psidium guajava* (26%), Myrtaceae showed 10 to less than 30% of AM fungal infection.

The other species like *Abrus precatorius* (58%), Leguminosae*, Cassia auriculata* (58%), Caesalpiniaceae, *Clitoria ternatea* (57%), Leguminosae, *Cymbopogon flexuosus* (38%), Poaceae, *Ensete superbum* (47%), Musaceae, *Gliriciadia sepium* (48%), Leguminosae, *Helicteruse isora* (58%), Malvaceae*, Hemidesmus indicus* (32%), Apocynaceae, *Leucas aspera* (4%), Laminaceae, *Mimosa pudica (*38%), Leguminosae, *Oscimum sanctum* (47%), Lamiaceae, *Microsorum diversifolium* (31%), Polypodiaceae, showed 30 to less than60% of AM fungal infection.

The rest of the species like *Canavalia gladiate* (75%), *Crotalaria pellida* (73%), *Desmodium triflorum* (87%), *Desmodium gangeticum* (64%) all the four species belongs to Leguminosae, the Compositae membersof *Elephantobus scaber*, *Emilia sonchifolia* infected 63 and 69% respectively, and one species *Pseudarthria viscida* (58%) the member of Fabaceae showed 60 to less than 90% of AM fungal infection was found in the Costaceae members of *Costus pictus* and *Cheilocostus speciosus*. The species *Curculigo orchioides* belongs to Hypoxidaceae, *Cureuma aromatic* belongs to Zingiberaceae, the Arecaceae member *Pandanus odoratissimus*, the Euphorbiaceae member *Phyllanthus emblica* and *Plumbago zeylanica* the member of Plumbaginaceae, also there is no hyphae, vesicles and arbuscular infection surprisingly these all the plant species rhizosphere soil simply showed the spore population.

In ethanobotanical study, 45 medicinal plant species belonging to 31 families used traditionally as herbal medicines for curing various diseases (Table5). The study as carried out related to Cholanaykan tribes. Medicinal plants used in folk herbal remedies are prepared and administered in various forms in the Karulai hills.

Among these medicinal plants, herbs (40%) were found to be most used plants folloed by shrub (24.4%), climber (8.8%), sub shrub (8.8%), erect shrub (4.4%), large shrub (4.4%), tree (4.4%) and scadent shrub (2.2%) (Fig. 4). Similar pattern of life form was reported by Giday *et al.,* (2014). The most frequently utilized medicinal plant parts were leaves (53.3%) used for the preparation of medicines solely or mixed with other plant parts. It was followed by roots (26.6%), whole plant (17.7%), fruit (8.8%), rhizome (8.8%), seed (6.6%), stem (4.4%), and inflorescence (2.2%) (Fig. 5).

Medicinal plants used in folk herbal remedies are prepared and administered in various forms in the Karulai hills. Majority of the plant remedies were prepared by decoction and juice. The paste was prepared by grinding the fresh or dried plant parts with oil or water. Powder was prepared by the grinding of shade dried parts. The most frequently used mode of remedy administration is oral ingestion, followed by tropical uses, nasal drops, face crams, hair cleaners, and bath. The most treated illness of the Karulai hills using a number of medicinal plants are grouped in to several disorders. We found the highest number of plant species are used against cold, followed by cough, diabetes, kidney stones, stomachache, swelling, headache, eye diseases, ageist intestinal worms, toothache, snake and scorpion bites, mosquito repellent, vomiting, jaundice and rheumatism (Table 6). The present study noticed that, single disease can be cured with infusions of more than one plant. Similarly, the single plant can be utilized to cure more than one disease.

4. DISCUSSION

The arbuscular mycorrhizae are reported to be ubiquitous both geographically and ecologically Mosse(14). Seasonal fluctuations in moisture, temperature, p^H and soil nutrient status show high and dramatic effects on arbuscular mycorrhizal spore population and percentage of root colonization. Soil physiological characters played an important role in distribution and density of mycorrhizal fungi. All the plant species 45 belongs to 31 families of rhizosphere soil samples observed the AM fungal spores. Among the AM fungal species

Glomus is most common. All the plant species colonized by AM fungi. The plant species infected by hyphae, vesicles and arbuscules. Grasses they have evolved the fibrous root system or an alternative phosphate acquisition strategy which enables them to do without mycorrhiza.

In the present finding the Poaceae member *Cymbopogon flexuosus* infected by arbuscular mycorrhizae. The infection in the plant species has 38%. Mycorrizal association occurred naturally with many important forest trees. Ectomycorrhizae mostly occur in temperate forest whereas in tropics endomycorrizae are more common. The present finding is in agreement with the results obtained by seasonal workers.

Brundrett and Abbott (20) analyzed the most of the plant species in tropical rain forests and the members of Leguminosae and the subfamilies of Papilonaceae and Mimosaceae. The same results was obtained in the present investigation that the Leguminosae members of Abrus precatorius, clitoria pictus, crotalaria pallida infected by AM fungal infection. Arbuscular micorrhiza is most common in Angiosperms, Gymnosperms, Pteridophytes and Bryophytes. The association of AM fungi with all the plants studies confirms the ubiquitous nature of AM Hayman(11) although the extent of root infection and number of AM spores found in the rhizosphere were different.

In this investigation, the mycorrhizal colonization was vary this may be the host specificity. The major ecosystem function of mycorrhizae is to assist host plants in the acquisition of resources from soil. This study displays the different degrees of AM fungi in plant host specificity. Such as mycorrhizal symbioses play fundamental roles in shaping plant communities, terrestrial ecosystems and high value for sustainability of this ecosystem.

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