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 phyllanthus 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. Ethnobotanical 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, Karulai hills.

1. INTRODUCTION

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...
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 ethnobotanical 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 Cholanaikkalan 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 hectares 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/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 into 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 H₂O₂ for 30 minutes at 25°C until bleached. They were rinsed thoroughly with water to remove the H₂O₂ acidified in dilute HCl and stained as described earlier. In some cases the modified method of Merryweather...
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 sieves 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 Cholanaikkkan 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 Cholanaikkkan call themselves as ‘Malanaikan’ or ‘Sholanaikan’. They are called Cholanaikkan because they inhabit the interior forests. ‘Chola’ or ‘sholas’ means deep ever green forests. And ‘naikan’ means king. The Cholanaikkkan numbered 360 individuals 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 2017

| 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           |

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 |
|-------|-----------------------------|--------------|-----------|---------|----------------------|---------------------|-------------------------------|
| 1     | Abrus precatorius L.        | Leguminosae  | Climber   | 5       | + - + +              | 58                  | 693                           |
| 2     | Andrographis paniculata     | Acanthaceae  | Herb      | 5.8     | + - -                | 27                  | 372                           |
|       | (Burm.f) Nees               |              |           |         |                      |                     |                               |
| 3     | Asparagus racemosus         | Asparagaceae | Armed vine| 5.1     | + - -                | 22                  | 329                           |
|       | Wild                        | Oxalidaceae  | Herb      | -       | + + -                | 27                  | 268                           |
| 4     | Calotropis gigantea (L.) DC.| Asclepiadaceae| Shrub    | 4.8     | + - -                | 18                  | 325                           |
| 5     | Canavalia gladiate (Jacq.) | Leguminosae  | Twinig herb| 4.6     | + + +                | 75                  | 693                           |
| 6     | Cassia auriculata (L.)      | Caesalpiniae  | Shrub     | 5.6     | + + -                | 58                  | 683                           |
| 7     | Catharanthus roseus         | Aponynaeae   | Shrub     | 5.9     | + - -                | 19                  | 427                           |
| 8     | Centella asiatica (L.) Urb.| Apiaceae     | Herb      | 4.2     | + - -                | 27                  | 276                           |
| 9     | Cheilocostus speciosus      | Costaceae    | Herb      | 5.5     | - - -                |                     | 197                           |
|       | (J.Koenig C.D.Specht)       |              |           |         |                      |                     |                               |
| 10    | Clitoria ternatea (L.)      | Leguminosae  | Climber   | 6       | + - +                | 57                  | 572                           |
| 11    | Costus pictus D.Don.        | Costaceae    | Herb      | 5.1     | - - -                | 213                | 231                           |
| 12    | Crotalaria pallida          | Leguminosae  | Shrub     | 5.2     | + + +                | 73                  | 842                           |
| 13    | Aiton.                      |              |           |         |                      |                     |                               |
| 14    | Curculigo orchoides         | Hypoxidaceae | Herb      | 5.3     | - - -                |                     | 174                           |
| 15    | Curcuma aromatic Salisb     | Zingiberaceae| Herb      | 4.8     | - - -                |                     | 266                           |
| 16    | Cymbopogon flexuosus        | Poaceae      | Herb      | 5.4     | + + -                | 38                  | 372                           |
|       | (Nees ex steud) W.Watson    |              |           |         |                      |                     |                               |
| 17    | Desmodium L.                | Leguminosae  | Climber   | 5.6     | + + +                | 64                  | 874                           |
| 18    | Datura metal L.             | Solanaceae   | Subshrub  | 5.9     | + + +                | 72                  | 624                           |
| 19    | Derris pilulifera           | Leguminosae  | Herb      | 5.6     | + + +                |                     | 874                           |
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 |

H- Hyphae, A- Arbuscules, V- Vescicle, +- Present, - - Absent
| S. No. | Plant name                                      | Family           | Spores name                                                                 |
|-------|------------------------------------------------|------------------|----------------------------------------------------------------------------|
| 1     | Abrus precatorius L.                           | Leguminosae      | Acaulospora alpine, Gigaspora albida, Glomus arboresce, 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     | Biophytmum 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 albium, Glomus multicaule, Scutellospora striata |
| 7     | Cassia auriculata L.                           | Caesalpinaceae   | Claraideoglomus clarioideum, Glomus ambisporum, Rhizophagus fasciculatus   |
| 8     | Catharanthus roseus (L.) G.Don.                | Apocynaceae      | Acaulospora foveat, Diversispora celata, Glomus arboresce                   |
| 9     | Centella asiatica (L.) Urb.                    | Apiaceae         | Acaulospora undulate, Gigaspora albida, Pacispora scintillans              |
| 10    | Cheilocostus speciosus (J.Koenig) C.D.Specht  | Costaceae        | Acaulospora undulate, Glomus albium, Glomus multicaule                     |
| 11    | Clitoria ternatae L.                           | Leguminosae      | Acaulospora undulate, Glomus albium, Glomus multicaule, Rhizophagus fasciculatus |
| 12    | Costus pictus D.Don                            | Costaceae        | Acaulospora alpine, Diversispora arenaria, Glomus arboresce, Rhizophagus fasciculatus |
| 13    | Crotalaria pallida Aiton.                      | Leguminosae      | Claraideoglomus clarioideum, Glomus ambisporum, Rhizophagus fasciculatus   |
| 14    | Curculigo orchideoides Gaertn                  | Hypoxidaceae     | Acaulospora tuberculata, Funneliformis coronatum, Rhizophagus fasciculatus |
| 15    | Curcuma aromatic Salisb.                       | Zingiberaceae    | Claraideoglomus clarioideum, Gigaspora                                    |
| 16    | Cyclea peltata (Lam.) Hook.f.&Thomson          | Menispermacae    | ramisporophora                                                             |
| 17    | Cymbopogon flexuosus (Nees ex steud) W.Watson  | Poaceae          | Dentiscutata erythropus, Glomus albium, Glomus arboresce                    |
| 18    | Datura mental                                  | Solanaceae       | Acaulospora foveat, Entrophospora infrequens, Glomus globiferum, Rhizophagus fasciculatus |
| 19    | Desmodium gangeticum (L.) DC.                  | Leguminosae      | Acaulospora undulate, Glomus albium                                         |
| 20    | Desmodium triflorum (L.) DC.                   | Leguminosae      | Claraideoglomus clarioideum, Entrophospora infrequens, Rhizophagus fasciculatus |
| 21    | Elephantopus scaber L.                         | Compositae       | Diversispora arenaria, Funneliformis coronatum, Rhizophagus fasciculatus   |
| 22    | Emilia sonchifolia (L.) DC.ex                  | Compositae       | Archaeospora trappe, Gigaspora albida, Glomus canadense                    |
| S. No. | Botanical Name | Family | Local Name | Habit  | Part used         |
|-------|----------------|--------|------------|--------|-------------------|
| 23    | Eute superbum (Roxb.) Cheesm. | Musaceae |  |  |  |
| 24    | Euphorbia hirta L. | Euphorbiaceae |  |  |  |
| 25    | Gliricidia sepium (Jacq.) Walp. | Leguminosae |  |  |  |
| 26    | Gloriosa superb L. | Liliaceae |  |  |  |
| 27    | Helicertes isora L. | Malvaceae |  |  |  |
| 28    | Hemidesmus indicus (L.) R.Br.ex Schult | Apocynaceae |  |  |  |
| 29    | Hydnocarpus pentandra (Buch-Ham) | Flacouriaceae |  |  |  |
| 30    | Justicia adhatoda L | Acanthaceae |  |  |  |
| 31    | Justicia gendarussa Burm.f | Acanthaceae |  |  |  |
| 32    | Leucas aspera (Willd.) Link | Lamiaceae |  |  |  |
| 33    | Maranda arundinacea L. | Marandaceae |  |  |  |
| 34    | Microsorum diversifolium G.Forst | Polypodiaceae |  |  |  |
| 35    | Mimosa pudica L. | Mimosaceae |  |  |  |
| 36    | Oscimum sanctum L. | Lamiaceae |  |  |  |
| 37    | Pandanus odoratissimus L.F | Areaceae |  |  |  |
| 38    | Phyllanthus amarus Schumach & Thonn. | Euphorbiaceae |  |  |  |
| 39    | Phyllanthus emblica L. | Euphorbiaceae |  |  |  |
| 40    | Piper longum L. | Piperaceae |  |  |  |
| 41    | Plumbago zeylanica L. | Plumbaginaceae |  |  |  |
| 42    | Pseudarthria viscida (L.) Wight & Arn. | Leguminosae |  |  |  |
| 43    | Psidium guajava L. | Myrtaceae |  |  |  |
| 44    | Rotala aquatica Lour | Euphorbiaceae |  |  |  |
| 45    | Scoparia dulcis L. | Scrophulariaceae |  |  |  |

Table 5. Details of enumerated plants used by the Cholanaikkan tribes from Karulai.
| No. | Scientific Name                          | Family            | Common Names                  | Description               |
|-----|----------------------------------------|-------------------|-------------------------------|---------------------------|
| 3.  | *Asparagus racemosus* Willd.           | Asparagaceae      | Sathavari.                    | Armed vine Herb Tuberous root. |
| 4.  | *Biophytum sensitivum* (L.) DC.         | Oxalidaceae       | Mukkutthi                      | Herb Aerial part          |
| 5.  | *Calotropis gigantea* (L.) R.Br        | Asclepiadaceae    | Erikkku                        | 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.) U.            | Costaceae         | 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.            | Leguminosae       | 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 | Menispermacae     | Padathali,Pattiechevian       | Climber Leaves,Roots      |
| 17. | *Cymbopogon flexuosus* (Nees ex steud) W.Watson | Poaceae           | Inchipullu, Thilappulu         | 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 Hook.f.&Thomson | Compositae       | Mualchevian                    | Diffuse herb Whole plant. |
| 23. | *Ensete superba* (Roxb.) Cheesman      | Musaceae          | Kalluvazha, Malavazha          | Erect 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         | Menthonnii                     | Climber Leaves            |
| 27. | *Heliceres isora* L.                   | Malvaceae         | Edampiri-valampiri             | Large shrub Fruit         |
| 28. | *Hemidesmus indicus* (L.) R.Br.ex Schult | Asclepiadaceae    | Nannari                         | Climber Root, Leaves      |
| 29. | *Hydrangea arborescens* (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          | Lamiaeceae        | Thumba                         | Herb whole plant          |
| 33. | *Maranda arundinacea* L.               | Marandaceae       | Kuvva                          | Shrub Rhizome             |
| 34. | *Microsorum diversifolium* G.Forst     | Polypodiaece      | Panal chedi                    | Shrub Tuber               |
| 35. | *Mimosa pudica* L.                     | Mimosaceae        | Thottavadi                     | Sub shrub Whole plant     |
| 36. | *Ocimum sanctum* L.                    | Lamiaeceae        | 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             |
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.    | Costus pallida Aiton.        | Swelling                  | The poultice made of the root applied in painful swelling of joint                                                                                     |
| 14.    | Curculigo orchioidees 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.    | Desmodium gangeticum (L.) DC. | Headache, Stomachache     | The oil extracted from the leaves is used directly to the skin for headache, stomachache, muscle pain                                                   |
| 18.    | Desmodium triflorum (L.) DC.  | Snake poison              | Fruit paste applied for snake poison                                                                                                                    |
| 19.    | Desmodium triflorum (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                                           |

Table 6. Mode of administration for the ailments of the medicinal plants used by the Cholanaikkan tribes from Karulai.
| No. | Plant Name | Part Used | Uses |
|-----|------------|-----------|------|
| 21. | *Elephantopus scaber* L. | Fresh roots | Remedy for kidney stone. |
| 22. | *Emilia sonchifolia* (L.) DC.ex DC. | Juice of the root | Used against head lice. |
| 23. | *Ensete superba* (Roxb.) Cheesman | Flower heads | Chewed and kept in the mouth for about 10 minutes to protect teeth from decay. |
| 24. | *Euphorbia hirta* L. | Fresh roots | Used to prepare a blend which is best for combating vomiting. |
| 25. | *Gliricidia sepium* (Jacq.) Walp. | Insect repellent | The leaves paste is used as a sedative and insecticides. |
| 26. | *Gloriosa superba* L. | Root paste | Used in the treatment of chronic rheumatism and used for bathing during child birth. |
| 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 | Skin diseases | Crushed inflorescence is mixed with water and sprayed over mosquito affected areas. |
| 29. | *Hemidesmus pentandra* (Buch-Ham) | Cough & cold | Oral administration of leaf juice is used for cough and cold. |
| 30. | *Hemidesmus pentandra* (Buch-Ham) | Headache | Chewing of crushed fruits can reduce tooth ache. |
| 31. | *Hemidesmus pentandra* (Buch-Ham) | Skin diseases | Leaves paste is used for curing stomachache, headache, skin diseases, insect bites and itching. |
| 32. | *Justicia gendarussa* Burm.f | Cuts and wounds | Crushed leaf juice is applied over cuts and wounds. |
| 33. | *Maranda arundinacea* L. | Mosquito repellent | Crushed inflorescence is mixed with water and sprayed over mosquito affected areas. |
| 34. | *Microsorum diversifolium* G.Forst | Mosquito repellent | The root juice along with milk consumed in the morning is good to cure jaundice. |
| 35. | *Mimosa pudica* L. | Eye diseases & Diabetes | Amla juice used to treat eye disease, diabetes, common cold and cough. |
| 36. | *Ocimum sanctum* L. | 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. | Cuts and wounds | Crushed leaf juice is applied over cuts and wounds. |
| 40. | *Piper longum* L. | Headache | Leaves paste is used for curing stomachache, headache, skin diseases, insect bites and itching. |
| 41. | *Plumbago zeylanica* L. | Internal bleeding | Oral administration of leaf paste is used for internal bleeding. |
| 42. | *Pseudarthria viscosa* (L.) Wight & Arn. | Stomach problems | Leaves paste is used to the treatment of diarrhea and stomachache. |
| 43. | *Psidium guajava* L. | Stomach ache | Consumption of root decoction is used for stomach ulcer. |
| 44. | *Rotala aquatica* Lour | 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.
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%), Asparagusaceae, Biophyrum sensitivum (27%), Oxlalidaceae, Calotropis gigantea (18%), Asclepiadaceae, Catharanthus roseus (19%), Apocyanaeaceae, Centella asiatica (27%), Apiceae, Cycla peltata (26%), Menispermacese, Gloriosa superba (21%), Liliaceae, Justicia adhatoda (22%), J. gendarussa (28%) both species belong to Acaentaceae member, Maranda arundinacea (27%), Phyllanthus amarus (17%), Euphorbiaceae, Scoparia dulcis (19%), Scrophulariaceae, Hydrocarpus pentandra (25%), Flacourtiaaceae, Piper longum (18%), Costaceae, 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%), Apcieae, Leucas aspera (4%), Lamiaceae, Mimosa pudica (38%), Leguminosae, Osmium sanctum (47%), Lamiaceae, Microsorum diversifolium (31%), Polypodiaceae, showed 30 to less than 60% 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 members of Elephantobus scaber, Emilia sonchifolia infected 63 and 69% respectively, and one species Pseuderthria 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 Areaceae 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 (Table 5). The study as carried out related to Cholnayyan 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 followed by shrub (24.4%), climber (8.8%), sub shrub (8.8%), erect shrub (4.4%), large shrub (4.4%), tree (4.4%) and scendent 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 (Mosse14). Seasonal fluctuations in moisture, temperature, pH 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%. Mycorrhizal association occurred naturally with many important forest trees. Ectomycorrhizae mostly occur in temperate forest whereas in tropics endomycorrhizae 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 Papilomaceae 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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