STUDIES ON THE ARBUCULAR MYCORRHIZAL FUNGAL ASSOCIATION IN THE PLANT SPECIES OF PONNUTHU HILLS, WESTERN GHATS COIMBATORE DISTRICT, TAMILNADU

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ABSTRACT

The present study analyzed the arbuscular mycorrhizal fungal spores in root colonization and spore population in rhizosphere soils samples in various medicinal plant species at Ponnuthu hills, Western Ghats of Coimbatore district, Tamilnadu, 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 36 plant species belonging to 21 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 21 AM fungal spores were recovered from the rhizosphere soil samples in this study region. The Glomus was dominant and found in rhizosphere soil samples in all the medicinal plant species. The maximum spore population was found in the rhizosphere soil samples of Hemidesmus indicus (573/100g of soil) which belongs to the family Asclepiadaceae and the lowest spore population was observed in the Abutilon indicum (145/100g of soil) which belongs to Malvaceae family. The highest 81% AM fungal colonization was found in roots of Gymnema sylvestre which belongs to the family Apocynaceae. While the lowest 16 % AM fungal colonization was found in the root of Tridax procumbens which belongs to the family Asteraceae.

Keywords: Glomus aggregatum, Medicinal plants, Ponnuthu hills.

1. INTRODUCTION

India is recognized as one of the seventeen mega biodiversity zones of the world. The forest of Western Ghats, in view of their floristic diversity and numerous multipurpose species, are considered as a varietal storehouse of economically important plants and beneficial microbial communities. AM fungi are geographically ubiquitous in occurrence that have a broad range of dissimilar environments (1,2) from the arctic to the tropics and occupy a wide range of ecological niches (3). The fossil record suggests that AM were also present in the subterranian parts of the earliest land regions (4). Mycorrhizas are one such examples of a plant-fungal association that is found in plants under a range of abiotic conditions. Mycorrhizal symbiosis occurs in a vast majority of vascular plants except for members of a few families, including Cruciferae, Brassiaceae and Zygophyllaceae (5,6).

Arbuscular mycorrhizal symbiosis that appeared with the first land plants more than 400 million years ago, is still formed by the large majority of extent plant species with no host specificity (7). Occurrence of AM fungi has been reported from an exceptionally wide range of plant and different ecosystems and plays a major role in better nutrition, species diversity and survival. (8). Almost all higher plants in the terrestrial ecosystems are known to be associated with mycorrhizal fungi (9).

Associations between plants and arbuscular mycorrhizal fungi are common in natural and agricultural ecosystems. Soil microorganisms play important role in plant-soil interactions. Microbes alter nutrient availability, immobilize heavy metals in soils, and bind soil particles into stable aggregates (10). Of the several types of mycorrhizal fungi, the arbuscular mycorrhizal fungi (AMF) form important symbiosis with the flora prevalent in serpentine grasslands.

In developing countries and rural societies, the use of medicinal plants is both a valuable resource and necessity and furthermore it provides real alternative for primary health care systems (11). Globally about 85% of the traditional medicine used for primary healthcare are derived from plants. In many countries scientific investigations of medicinal plants have been initiated because of their contribution to healthcare. Herbal medicines have good values in treating many diseases including infectious diseases, etc.

2. MATERIALS AND METHODS

2.1. Study area Description

Kurudi Malai is the hill at the base of which Ponnuthu Anman temple is situated. It lies between 11.1186° N, 76.8923° E. Few even call it as Ponnuthu Malai. The summit is at a rough elevation of 1200m (3900ft). The terrain is rocky at the beginning and an
abrupt rise in elevation arises as tracks of salt could be clearly seen on those rocks indicating the past traces of waterfalls. 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 Ponnuthu hills.

2.2. Sample collection
In this present study, root and rhizosphere soil samples of 36 plant species were collected for the duration of August, 2018- March, 2019. The collected soil and root samples were placed in the polyethylene bags, labelled 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 Philips and Hayman's). The collected roots samples were cut into 1-2 cm 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 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:

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: Raman and Mohankumar (12) Schenk and Perez (13) and Schubler and Walker (14). The Photomicrographs were taken with the help of a Magnus Olympus Microscope.

2.5. Soil pH
The pH of the soil samples was determined (soil-water suspensions 1:5) with the help of pH meter (Elico) and values were recorded.

3. RESULTS AND DISCUSSION
AM fungal colonization and spore population of 36 plant species belongs to 21 families and also analysis of the pH ranges between 4.8 and 6.9 are represented in the (Tables-1 & 2). The maximum temperature was recorded 36.5°C while the maximum rain fall were noted in August, 390 mm.

The maximum spore population was observed in the plant species of *Hemidesmus indicus* (573/100g of soil) belonging to Asclepiadaceae and minimum was observed in *Abutilon indicum* (145/100g of soil) belonging to Malvaceae. In the present investigation the highest AM fungal infection was recorded in *Gymnema sylvestre* (81%) belonging to Apocynaceae and minimum was noticed in *Tridax procumbens* (16%) belonging to Asteraceae.

The plant species like *Acorus calamus* 28% (Acoraceae), *Barleria prionitis* 24% (Acanthaceae), *Catharanthus pusillus* 22% (Apocynaceae), *Commelina benghalensis* 28% (Commelinaceae), *Plectranthus barbatus* 38% (Lamiaceae), *Corchorus aesteruans* 33% (Tiliaceae), *Capparis zeylanica* 30% (Capparidaceae), showed 20 to 40 % of infection.

The other plant species like *Areva lanata* 59% (Amaranthaceae), *Azadiractha indica* 55 % (Meliaceae), *Anisomeles malabarica* 43% (Lamiaceae), *Achyranthes aspera* 44 % (Amaranthaceae), *Abutilon indicum* 52 % (Malvaceae), *Argemone mexicana* 58 % (Papaveraceae), *Blepharis maderapaptens* 48% (Acanthaceae), *Barleria cristata* 46% (Acanthaceae), *Catharanthus roseus* 41% (Apocynaceae), *Datura innoxia* 50% (Solanaceae), *Evolulus alsinoides* 53% (Convolvulaceae), *Hibiscus micranthus* 46 % (Malvaceae), *Hibiscus vitifolius* 42 % (Malvaceae), *Ipomoea obscura* 58% (Convolvulaceae), *Leucas aspera* 43% (Lamiaceae), *Mimosa pudica* 44% (Mimosaceae), *Oxalis corniculata* 52% (Oxalidaceae), *Ocimum sanctum* 49% (Lamiaceae) showed above 41 to 60% of infection.

The rest of the species like *Bacopa monnieri* 61% (Plantaginaceae), *Euphorbia hirta* 67% (Euphorbiaceae), *Ficus benghalensis* 64 % (Moraceae), *Oldenlandia umbellata* 69% (Rubiaceae),
Portulaca oleracea 65% (Portulacaceae), showed above 61 to 80% of infection. The plant family like Amaranthaceae and Juncaceae were thought to be mycorrhiza free, most of the species were found to be infected under natural stressed rangeland conditions (15). The plants that do not form mycorrhizas may be related to the presence of fungi toxic compounds in root cortical tissue or in root exudates. It may also be due to interactions between the fungus and the plant at the cell wall and (or) middle lamella level (16). High concentrations of salycilic acid have been found to reduce mycorrhization. But in the in the present study revealed that the plant family Amarathaceae showed the mycorrhizal infection. The research clearly showed that AMF enhanced nutrient uptake and growth of endangered plants (17,18).

Many studies conducted in different ethnic communities, have reported frequently the use of leaves was widely accepted for traditional therapies may be due to large quantity of biologically active components present inside them. Apart from leaves, almost all the other parts of medicinal plants such as flower, bark, stem, seed, fruit are also used. The utilization of leaves in traditional medication may also be due to their easy availability.

Table: 1. List of plants species collected from the Ponnuthu hills, and their medicinal uses.

| S. No | Plant species                          | Family           | Habit   |
|-------|---------------------------------------|------------------|---------|
| 1.    | Areva lanata (L.) Juss. Ex schult.    | Amaranthaceae    | Herb    |
| 2.    | Azadirachta indica A. Juss.           | Meliaceae        | Tree    |
| 3.    | Anisomeles malabarica (L.) R.Br.ex Sims | Lamiaceae        | Herb    |
| 4.    | Achyranthes aspera L.                 | Amaranthaceae    | Herb    |
| 5.    | Abutilon indicum L.                   | Malvaceae        | Shrub   |
| 6.    | Acalypha indica L.                    | Euphorbiaceae    | Herb    |
| 7.    | Acorus calamus L.                     | Acoraceae        | Herb    |
| 8.    | Argemone mexicana L.                  | Papaveraceae     | Herb    |
| 9.    | Blepharis maderaspatensis (L.) Heyne ex Roth. | Acanthaceae    | Herb    |
| 10.   | Bacopa monnieri (L.)                  | Plantaginaceae   | herb    |
| 11.   | Barleria cristata L.                  | Acanthaceae      | Shrub   |
| 12.   | Barleria prionitis L.                 | Acanthaceae      | Shrub   |
| 13.   | Crotalaria retusa L.                  | Fabaceae         | Herb    |
| 14.   | Corchorus aescuans L.                 | Tiliaceae        | Herb    |
| 15.   | Capparis zeylanica L.                 | Capparaceae      | Shrub   |
| 16.   | Catharanthus pusillus (Murray) G. Don  | Apocynaceae      | Herb    |
| 17.   | Catharanthus roseus (L.) G.Don         | Apocynaceae      | Shrub   |
| 18.   | Commelina benghalensis L.             | Commelinaceae    | Herb    |
| 19.   | Datura inoxia Mill.                   | Solanaceae       | Shrub   |
| 20.   | Evolulus alsinoides (Linn)             | Convolvulaceae   | Herb    |
| 21.   | Euphorbia hirta L.                    | Euphorbiaceae    | Herb    |
| 22.   | Ficus benghalensis L.                 | Moraceae         | Tree    |
| 23.   | Gymnema sylvestre R.Br.               | Apocynaceae      | Shrub   |
| 24.   | Hibiscus micranthus L.f.              | Malvaceae        | Shrub   |
| 25.   | Hibiscus vitifolius L.                | Malvaceae        | Herb    |
| 26.   | Hemidesmus indicus (L.)               | Asclepiadaceae   | Shrub   |
| 27.   | Ipomoea obscura (L.) Ker Gawl.        | Convolvulaceae   | Herb    |
| 28.   | Leucas aspera Linn.                   | Lamiaceae        | Herb    |
Table 2. Arbuscular Mycorrhizal fungal spore population and root colonization in the plant species of Ponnuthu hills, a part of Western Ghats, Coimbatore district, Tamilnadu, during 2018-2019.

| S. No | Plant Species                     | pH  | Types of infection | Spore Population (100g/soil) | (%) root colonization |
|-------|-----------------------------------|-----|--------------------|-------------------------------|-----------------------|
| 1.    | Areva lanata (L.) Juss. Ex schult.| 5.2 | + - +              | 320                           | 61                    |
| 2.    | Azadirachta indica A. Juss.       | 4.8 | + + -              | 280                           | 55                    |
| 3.    | Anisomeles malabarica (L.) R.Br.ex Sims | 6.2 | + + -              | 410                           | 43                    |
| 4.    | Acorus calamus L.                 | 5.5 | + - +              | 185                           | 44                    |
| 5.    | Abutilon indicum L.               | 5.7 | + + -              | 387                           | 52                    |
| 6.    | Acalypha indica L.                | 6.0 | + - +              | 156                           | 39                    |
| 7.    | Acorus calamus L.                 | 5.8 | + + -              | 270                           | 28                    |
| 8.    | Argemone mexicana L.              | 5.1 | + - +              | 355                           | 58                    |
| 9.    | Blepharis madraspatensis (L.)     | 4.9 | + + -              | 190                           | 67                    |
| 10.   | Bacopa monnieri L.                | 5.3 | + - +              | 177                           | 65                    |
| 11.   | Barleria cristata L.              | 6.7 | + + -              | 445                           | 74                    |
| 12.   | Barleria prionitis L.             | 6.6 | + - +              | 390                           | 24                    |
| 13.   | Crotalaria retusa L.              | 5.9 | + + -              | 365                           | 43                    |
| 14.   | Corchorus aescuans L.             | 5.4 | + - +              | 402                           | 33                    |
| 15.   | Capparis zeylanica L.             | 5.3 | + - +              | 339                           | 30                    |
| 16.   | Catharanthus pusillus (Murray) G. Don | 6.1 | + - +              | 130                           | 22                    |
| 17.   | Catharanthus roseus (L.) G.Don     | 6.4 | + - +              | 470                           | 60                    |
| 18.   | Commelina benghalensis L.         | 5.2 | + + -              | 293                           | 22                    |
| 19.   | Datura innoxia Mill.              | 6.3 | + - +              | 245                           | 51                    |
| 20.   | Evolvulus alsinoides              | 5.5 | + + -              | 310                           | 53                    |

Note: Fabaceae: shrub, Oxalidaceae: Herb, Lamiaceae: Herb, Rubiaceae: Herb, Asteraceae: Herb.
| No. | Species Name                  | Habit | Distribution | AM Fungi | AM Spore |
|-----|------------------------------|-------|--------------|----------|----------|
| 21. | Euphorbia hirta L.           | 6.8   | +            | -        | 360      | 67       |
| 22. | Ficus benghalensis L.        | 5.7   | +            | -        | 387      | 68       |
| 23. | Gymnema sylvestre R.Br.      | 6.9   | +            | -        | 422      | 81       |
| 24. | Hibiscus micranthus L.f.     | 5.6   | +            | +        | 126      | 46       |
| 25. | Hibiscus vitifolius L.       | 6.1   | +            | -        | 405      | 63       |
| 26. | Hemidesmus indicus (L.)      | 6.5   | +            | -        | 573      | 55       |
| 27. | Ipomoea obscura (L.) Ker Gawl.| 5.8   | +            | +        | 280      | 58       |
| 28. | Leucas aspera Linn.          | 5.0   | +            | +        | 410      | 43       |
| 29. | Mimosa pudica L.             | 5.1   | +            | -        | 185      | 44       |
| 30. | Oxalis corniculata L.        | 5.4   | +            | +        | 387      | 52       |
| 31. | Ocimum sanctum L.            | 5.2   | +            | -        | 156      | 49       |
| 32. | Oldenlandia umbellata L.     | 5.5   | +            | +        | 270      | 49       |
| 33. | Plectranthus barbatus Andrews| 6.2   | +            | -        | 355      | 38       |
| 34. | Phyllanthus amarus Schumach & Thonn.| 6.3 | +            | +        | 190      | 47       |
| 35. | Portulaca oleracea L.        | 5.7   | +            | -        | 420      | 65       |
| 36. | Tridax procumbens L.         | 5.3   | +            | +        | 445      | 16       |

Fig. 2. Habit wise distribution of plant species in Ponnuthu hills.
From the rhizosphere soils sample of Ponnuthu hills, totally 21 AM fungal species were isolated and identified. Of these 4 species of Acaulospora, Aca. delicata, Aca. denticulatum, Aca. gdanskensis Aca.levies, 1 species of Ambispora, Amb. appendicula, 1 species of Gigaspora, Gig.candida, 13 species of Glomus, Gl. aggregatum, Gl. albidum, Gl. ambisporum, Gl. arborescens, Gl. australe, Gl. canadense, Gl.citricola, Gl. delhiense, Gl. deserticola, Gl. dimorphicum, Gl. radiatum, Gl. segmentatum, Gl. versiforme, 1 species of Redeckera, Red. fulvum, 1 species of Rhizophagus, Rhi. fasciculatus were observed.

The names of the species are represented in Table 3. In addition with Santhoshkumar and Nagarajan (19) were studied on AM spore population in the plants species at Sirumalai hills, Eastern Ghats of Dindugul district. Totally 39 AM fungi species belongs to 6 genera were isolated and identified. To isolate and identification of the AM fungal spores in rhizosphere soils samples in different regions such as Yellanahalli hills reported by (20), Kondranghi hills (21), Bargur hills (22).
Fig. 3. AM fungal spore population of the plant species of Ponnuthu hills.

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

Fig. 5. Isolation and Identification of AM fungal spores in rhizosphere soils of Ponnuthu hills, Western Ghats of Coimbatore district.

Fig. 6. Dominant genus was recovered from the rhizosphere soils samples in Ponnuthu hills.

4. CONCLUSION

From above results we concluded that the present study the AM fungal root colonization and spore population in all the plant species in Ponnuthu hills. In this symbiotic association of AM fungi in the plant species to absorb the soil nutrients, zinc, copper especially phosphorous and also increased plant resistance to various stresses like drought, salt and heavy metal. In future, the AM fungal spores were cultured under in vitro condition for raise agricultural crops plant species growth and development.

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