RESEARCH ARTICLE

AM fungal association in the Rhizosphere soil of some Pteridophytic plant species in Valparai Hills, Western Ghats of Tamilnadu, India

Santhoshkumar S* and Nagarajan N

Post Graduate and Research Department of Botany, Kongunadu Arts and Science College (Autonomous), Coimbatore-641 029, Tamilnadu, India

**Corresponding author: santhosh.biology@gmail.com*

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ABSTRACT

In the present study to investigate the arbuscular mycorrhizal fungal colonization in the rhizosphere soil of some pteridophyte plant species. Soil samples were collected during the month of September 2013 from the surface to 30 cm depth and pH was analyzed. Rhizosphere soil root of 12 pteridophytic plant species belonging to 7 families were investigated. The result showed that the mycorrhizal populations, such as Glomus species were found dominate followed by Acaulospora, Sclerocystis, Entrophospora and Gigaspora. The maximum spore population was found in the rhizosphere of Adiantum capillus-veneris (640.20 /100 g soil) belongs to the family Adiantaceae and the least spore population was observed in the Adiantum radianum (130.90 /100g of soil). The highest (81.36 %) AM fungal infection found in roots of Angiopteris evecta belongs to the family Angiopteridaceae. The lowest (21.11 %) AM fungal association was found in the root of Pteris biaurita belongs to the family Pteridaceae. In conclusion the AM fungi are associated with pteridophyte plants in naturally for beneficially both of them. AM fungal may be increase the yield of agricultural crops and soil fertility.

Keywords: Pteridophytes, Arbuscular mycorrhizal fungi, Adiantum capillus-veneris, Glomus species.

INTRODUCTION

Soil, an underground terrestrial ecosystem shows the great diversity of Organism. Especially rhizosphere region is the most dynamic environment that harbors Arbuscular Mycorrhizae the most dominant fungal associations. Arbuscular mycorrhizal fungi formed by a very wide variety of host plant, including Angiosperms, Gymnosperms, Pteridophytes, mosses, Lycopods and psilotales and a comparatively small group of aseptate filamentous fungi, the Glomales. More than 90% of all plant families studied, agricultural and natural environments form mycorrhizal associations and they can be essential for plant nutrition (Maggirwar *et al.,* 2013). The ancestors of ferns may have been significant in the evolution of vascular plants. Ferns are still an important component of present day ecosystems, particularly in

tropical and subtropical regions (Ching, 1959). Pteridophytes constitute a significant and important group in the plant kingdom as early land plants. They show various adaptations as they have evolved to fill different habitats, thereby providing an ecological niche for many microorganisms like AM fungi that around the soil (Khade and Rodrigues, 2002). Arbuscular mycorrhizas (AM) have been found in pteridophytes from about 400 million years ago in the Devonian to Carboniferous periods of the Palaeozoic era (Hass *et al.,* 1994; Remy *et al.,* 1994).

India is being one of the twelve mega biodiversity regions of the World supports an enormous biodiversity of ancient lineage. It is estimated that over 45,000 plant species are accounted from the Indian subcontinent, which represents roughly 11% of the known plant species of the World. The forest of the Western Ghats, in view of their forest's diversity and numerous multipurpose species, is considered a varietal storehouse of economically important plants and beneficial microbial communities. The most prevalent beneficial microorganisms are associated with plants; however the soil inhabiting fungi that form a mutualistic root fungal association referred to as mycorrhiza (Rajkumar *et al.,* 2012). Arbuscular mycorrhizal fungi (AMF) are below the ground level of the symbiotic associations between plant roots and fungi which adds dimension to the plant-soil-microbe interaction (Sukla *et al.,* 2012).

In India, have documented studies on the arbuscular mycorrhizal colonization of the rhizosphere soil root of pteridophytic plant species (Mishra *et al*., 1980; Ragupathy and Mahadevan,1993). Colonization and diversity of AM fungi associated with common pteridophyte plant species have been investigated in Southwest of China. Modern, intensive agricultural practices, such as chemical fertilization and pest control, continuous monoculture, and tillage impact of AM fungal and plant interactions. These practices affect mycorrhizae both quantitative and qualitative. Thus, describing the diversity of the community of AM fungi at a site becomes, therefore, an important step in determining the effects of agricultural treatments upon AM fungi and the eventual development of management regimes for these fungi (Singh and Adholeya, 2013).

MATERIALS AND METHODS

Study Area: The present study area is confined to the major range in the Valparai hills of the Western Ghats that is rich biodiversity and indigenous population. It is located in the Western boundary range in Coimbatore district in the Southwest of Tamilnadu and lies between at 10.37 N 76.97 longitudes and E10.37:76.97 latitude form a portion of Tamilnadu. It has an average elevation of the hills ranges between 1193 meters (3914 feet) above (MSL) and about annual rainfall between 3523.3mm to 2882.7 mm, and temperature various between 23.6 °C to 19.9 °C. The vegetation type of forest is moist deciduous forest, and evergreen forest present (Fig- 1).

Sample collection: Root and rhizosphere soil samples were collected around the 12 pteridophytic plant species in the month of July 2011- June 2012. Samples were placed in the polyethylene bags, labeled and then transported to the laboratory. The root samples were freshly processed, whereas rhizosphere soil samples were analyzed for mycorrhizal spore population and AM fungal root colonization.

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.

Fig 1: Showing the study area map of Valparai hills, Western Ghats of Tamilnadu, India

Estimation of AM fungal root colonization **:** The root samples were stained by using modified method (Kousk and Gemma, 1989). Root samples of each pteridophytic plant species were washed gently under tap water and cleared in 2.5% KOH, acidified in 5 N HCL and stained in lacto glycerol with 0.05% Trypan blue. The stained roots were examined under a compound microscope (x40 – x100). Hundred root segments for each sample were randomly selected for microscopic observation and the degree of colonization was estimated using the slide method (Giovanneti and Mosse, 1980).

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

$$\% \text{ of colonization} = \frac{\text{No. of root segments colonized}}{\text{Total no of root segments observed}} \times 100$$

AMF spore identification**:** AM fungal spores were extracted from 25 g rhizosphere soil by wet-sieving and decanting method (Gerdemann and Nicolson, 1963), through a series of 710 to 37µm size sieve filter. For the identification and nomenclature of these AM fungal spore synoptic keys developed by (Raman and Mohankumar, 1988) and (Schenck and Perez, 1990) were used. The classification was based upon the color, shape, hyphae, structure, size, and cell wall thickness and spore diameter.

Identification of pteridophytes **:** Identification of pteridophytic plant species was carried out using 'The Manual of Pteridophyte Flora of Western Ghats, South India (Manickam and Irudhayaraj, 1992).

RESULTS AND DISCUSSION

Arbuscular Mycorrhizal fungal infection and spore population of 12 pteridophytic plant species belongs to 7 families and their pH of the rhizosphere soil samples was presented in (Table No.1) and (Fig 2). Totally 12 plant species belonging to 7 families were examined for AM fungal association and root colonization were recorded. Of these maximum spore populations was recorded in the plant species *Adiantum capillus-veneris* (640.20 /100g soil) belongs to the family Adiantaceae and the minimum spore population was recorded in the plant species of *Adiantum radianum* (130.90 /100g soil) belongs to the family Adiantaceae. The highest AM fungal infection found in roots of *Angiopteris evecta* (81.36 ± 1.02 %) belongs to the family Angiopteridaceae. The lowest fungal infection was found in *Pteris biaurita* (21.11 ± 0.56%) belongs to the family Pteridaceae.

Totally 34 AM fungal spore species was identified six genera from 12 plant species belongs to 7 families, Of these AM fungal spores of the genus *Glomus* was recorded as the most population, followed by *Aculospora*, *Gigaspora*, *Scutellospora*, *Sclerocystis and Entrophospora* are recorded in (Table 2; Fig 3, 4 & 5).

Table 1: List of Invertebrate Animal Species Use for Therapeutic Purpose by Mawasi Tribes of Chhindwara District of Madhya Pradesh, India

Sr. No	Plants name	Family	pH	Mean spore density 100^{-1}g of rhizosphere soil	% of root colonization	Types of infection		
						Hyphae	Arbuscles	Vesicles
1	*Adiantum caudatum* L.	Adiantaceae	4.8	270.40 ± 25.01	36.9 ±0.78	+	+	-
2	*Adiantum capillus-veneris* L.	Adiantaceae	5.1	640.20 ± 8.23	41.12 ± 0.77	+	_	+
3	*Adiantum radianum* (rq) Flickr	Adiantaceae	5.6	130.90 ±8.09	55.10 ± 0.74	+	+	-
4	*Adiantum zollingeri* Mett ex Kuhn	Adiantaceae	4.5	290.23 ± 11.23	31.133 ± 0.69	+	+	-
5	*Angiopteris evecta* (Forst.) Hoff	Angiopteridaceae	6.1	180.35 ± 6.93	81.36 ± 1.02	+	-	+
6	*Actinopteris radiata* (Link)	Actinopteridaceae	5.3	226.32 ± 4.86	60.08 ± 0.77	+	+	-
7	*Hemionities arifolia* Burm.f.) T. Moor)	Heminioteridaceae	4.7	342.26 ± 9.54	72.89 ± 0.41	+	_	+
8	*Pteris biaurita* L.	Pteridaceae	6.8	428.95 ± 7.0	21.11 ± 0.56	+	+	-
9	*Cheilanthus mysorensis* Wall	Pteridaceae	5.5	402.29 ± 8.32	57.01 ± 0.69	+	+	+
10	*Cheilanthus bullosa* kunze	Pteridaceae	4.6	298.0 ± 37.78	70.15 ±0.79	+	_	+
11	*Humata repens* (L.f.) J. Small ex Diels	Davalliaceae	6.9	508.93 ± 52.57	47.21 ± 0.28	+	-	+
12	*Botricum daucifolium* Wall. Hook & Grev.	Ophioglossaceae	5.3	316.26 ± 32.46	42.46 ± 0.43	+	+	-

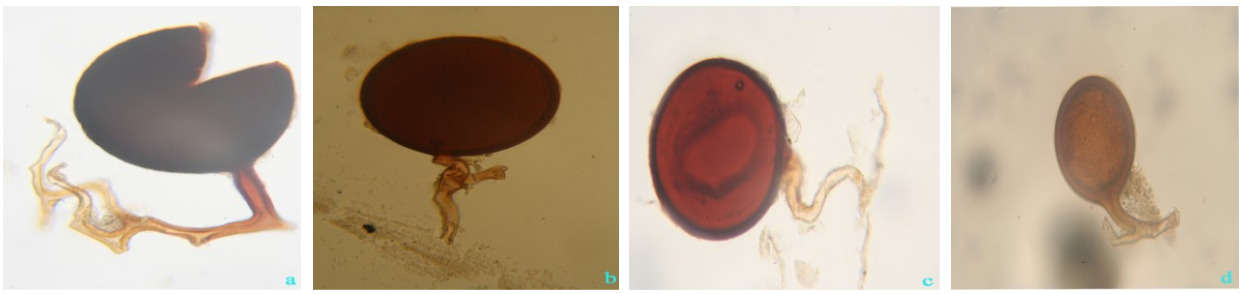
Each value represents the mean ± SD,

Table 2: AM fungal spore population was isolated from rhizosphere soil from Valparai Hills, Southern Western Ghats of Tamilnadu.

Sr.No.	AM Fumngal genera	AM Fungi Spore Species
1	***Glomus***	*Gl. albidum, Gl. arborensis, Gl. austral, Gl. canadense, Gl. citricola, Gl. claroids, Gl. clarum, Gl. constrictum, Gl. delhiense, Gl. dimorphicum, Gl. dominiki, Gl. etunicatum, Gl. fasciculatum, Gl. fistulosum, Gl. flavisporum, Gl. fragilistratum, Gl. fulvum, Gl. geosporum, Gl. glomerulatum, Gl. heterosporum, Gl. hoi, Gl. intraradix, Gl. invermeyanum, Gl. macrocarpum,*
2	***Gigaspora***	*Gi. candida*, *Gi. Decipiens*
3	***Acaulospora***	*A. gdanskensis*, *A. rehemii, A. thomii*,
4	***Sclerocystis***	*S. pachycaulis*, *S. rubiformis*
5	***Entrophospora***	*E. infrequens*
6	***Scutellospora***	*S. heterogama*, *S. Scutata*

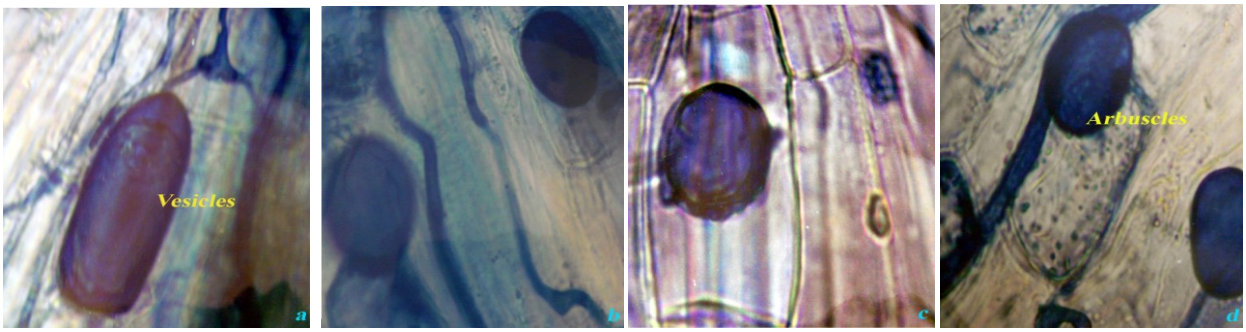
A. *Angiopteris evecta* **B.** *Pteris biaurita* **C.** *Cheilanthus mysorensis* **D.** *Hemionities arifolia*

Fig 2: Showing the pteridophytic plant species in Valparai hills, Western Ghats of Tamilnadu, India.



A. *Acaulospora thomii* **B.** *Gigaspora candida* **C.** *Scutellospora Scutata* **D.** *Glomus fasciculatum*

Fig.3: AM fungal species isolated from the rhizosphere soil samples of pteridophytic plant species of Valparai hills.



A. *Botricum daucifolium* ***B.*** *Cheilanthus mysorensis* **C.** *Actinopteris radiata* **D.** *Adiantum capillus- veneris*

Fig 4: Showing the vesicles, arbuscles type of hyphal infection in pteridophytic plant species, of Western Ghats.

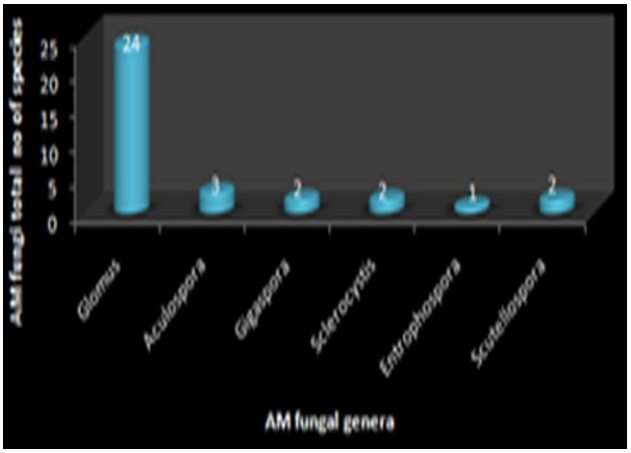


Fig 5: Showing AM fungal spore Species from the rhizosphere soil samples pteridophytes plant species in valaprai hills, Western Ghats of Tamilnadu.

Arbuscular mycorrhizal fungi have been described as 'keystone mutualists' in ecosystems due to their unique position at the root-soil interface (Kumar, *et al*., 2010). In this present study effort was made to the distribution, diversity, root colonization and richness of AM fungi in the pteridophytic plants species from the Western Ghats of Valparai hills. The spore population recorded in our study is much higher in ranges from 130 to 640 spores 100 g of rhizosphere soil. Similarly, Muthukumar and Udaiyan (2000) reported that the spore population ranges from 2.35-39.52 spore 100 g-1 rhizosphere soil of pteridophytes plant species in Western Ghats, Southern India. Zhao *et al*. (2000) investigated that the AM fungal structures were observed in 91% of the rhizosphere soil sample in 256 fern species and reported very low occurrence (17%) of AM fungi in a tropical region of Yunnan, southwest China.

Jun-Ki *et al.* (2002) observed that the AM fungi colonize in the roots of *Botrychium ternatum* collected two types of mycorrhizal, orchid (OM) and arbuscular mycorrhiza (AM) were observed in cortical cells of *B. ternatum* roots. In the present study revealed that the one species of *Botrychium daucalifolium* infected by AM fungal colonization, two types of mycorrhizal hyphae and arbuscular in the root cell of *Botrychium daucalifolium.* The AM fungi infected in the plant species in the range of 42% and spore population were recorded in 178/100 g of rhizosphere soil. The population of AM fungal diversity decrease or increase in the soil is also dependant on the factors like climate, soil type and the host plant root hairs (Hayman, 1982; Brundett, 1991; Dehne *et al*. (1987).

The arbuscular mycorrhizae suggest that these fungi were influential in root colonize in the terrestrial plants compared to aquatic plants (Smith and Read, 1997). Similarly, our study the aquatic plant species of *Marsilea Sp* were infected 32% root colonization were observed. Khade and Rodrigues (2002) observed that the AM fungal such as hyphae and vesicles colonization from the Pteridophytic plant species of an unidentified *Selaginella sp*, and *Adiantum lunulatum* of Selaginellaceae and Adiantaceae family respectively. In recent, Muthukumar and Prabha (2012) was recorded fungal association in gametophytes and young saprophytic roots of *Nephrolepis exaltata*.

Koul *et al*. (2012) recently reported that the total 42 AMF species associated with the medicinal plants and are predominantly distributed amongst 4 genera. The genus *Glomus* is represented by 21 species which is around 50 percent of the total AMF species, followed by genus Scutellospora (10), Aculospora (07) and Gigaspora (04) respectively. Similarly, present study 33 AM fungal species were associated in the pteridophytic plants species and 6 genera. The *Glomus* was maximum population 24 species followed by *Aculospora* (03), *Gigaspora* (02), *Scutellospora* (02), *Sclerocystis* (02) and *Entrophospora* (01) were recorded. Raei and Weisany, (2013) recently reported that the Arbuscular Mycorrhizal Fungi Associated with Some Aromatic and Medicinal Plant species. Our present result showed that the arbuscular mycorrhizal fungal association in pteridophytic plant species for Valparai hills, Western Ghats, Tamilnadu. Deotare *et al*., (2014) in recent times reported that the as the most dominant genera of *Glomus* (15 species) was recordedas followed by genes *Acaulospora* (03) in rhizosphere soil of *Opuntia humifusa*. Our present result showed that the genus *Glomus* (21 species) followed by Scutellospora (10 species), Aculospora (07 species), Gigaspora (04 species), *Scutellospora* (02), *Sclerocystis* (02) and *Entrophospora* (01) were recorded in the rhizosphere soil samples of Valparai hills Western Ghats of Tamilnadu.

CONCLUSION

In conclusion the present study revealed that the pteridophytic plant species had AM fugal spore density and root colonization.SS In this symbiotic association of AM fungi is not only angiosperms including pteridophytic 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 plant growth and development.

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