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# Occurrence and Distribution of AM Fungi in Cultivable Land and Forest Ecosystem

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**ABSTRACT: Arbuscular mycorrhizal fungal (AMF) density and diversity of rhizosphere soil samples of cultivable land (maize, onion and paddy) and forest ecosystem (teak, rose wood, porasu, flame of the forest and Indian kino) has been the focus of this study. One of the major components in influencing the AM fungal spore diversity is the unequal distribution of arbuscular mycorrhizal fungal spores and the complexity of the below-ground root system. To determine the natural existence of AM fungi in a certain area, it is crucial to study the diversity and distribution of AM fungus in different plant species. Although AM fungal spore density being more in cultivable land, there is no difference in terms of AMF diversity among the two ecosystems. The spore density was in the range of 68±1.67 (Indian Kino, Siruvani) to 102±4.41 (Onion, Narasipuram) spores 100 $g^{-1}$ soil. From 4 rhizosphere of cultivable and 5 rhizosphere of forest soils, 12 AM fungal species with 7 genera were identified. They include *Acaulospora* sp, *Ambispora leptotica*, *Glomus hoi, Glomus microcarpum, Glomus ambisporum, Glomus aggregatum, Septoglomus deserticola, Septoglomus constrictum, Paraglomus* sp, *Funneliformis geosporum, Gigaspora margarita* and *Gigaspora gigantea.* Percent occurrence of these spores range from 11.11% (*Acaulospora* sp, *Ambispora leptotica* and *Paraglomus* sp) to 66.66% (*Glomus hoi* and *Glomus microcarpum*). This study also revealed a positive correlation between total glomalin and spore density and a negative correlation between available phosphorus level and spore density.**

**Keywords:** Cultivable land, forest ecosystem, AMF diversity.

## INTRODUCTION

Arbuscular mycorrhizal fungi, one of the significant soil microorganisms, create symbiotic associations with most of the terrestrial plants (Smith and Read 2008). The extra radical mycelium develops spores, extends away from root zone to colonize, and absorbs nutrients (Ahmed *et al.,* 2019), whilst the intraradical mycelium penetrates root epidermis and cortex zone and forms structures notably arbuscules and vesicles. The host plant provides carbohydrates and fatty acids to the AMF, and in return, the AMF supports the plants by enhancing inorganic nutrient uptake, increasing tolerance to stressors caused by salt (Dehn and Schuepp 1990), AMF also improves soil structure via aggregation by synthesizing a glycoprotein known as glomalin (Gao *et al.,* 2019). The abundance and diversity of these fungi may be influenced by the plant species in the environment. The prevalence of AM fungus in both natural and disturbed grassland habitats is well documented. Numerous researches conducted over the last few years have revealed the variety of AM fungi (Wang *et al.,* 2020). In order to preserve and enhance the ecosystem's stability, it is crucial to comprehend the diversity and distribution of AMF. Here, we assessed the AMF diversity in cultivable land and forest ecosystem of Coimbatore district of Tamil Nadu.

## MATERIALS AND METHODS

*A. Sampling location*

Rhizosphere soil samples were collected from cultivable (TNAU and Narasipuram) and forest ecosystem (Velingiri and Siruvani Hills) of Coimbatore Tamil Nadu. Rhizosphere soil samples of onion (TNAU Orchard and Narasipuram), maize (TNAU), and rice (Wetland, TNAU) were collected from different locations. Similarly, rhizosphere soil samples of rosewood, teak, flame of forest and Indian Kino of Siruvani hills and Porasu tree of Velingiri hills were collected and stored at refrigerated condition for further processing.

*B. Physico-chemical and fertility status of soil samples*

The dried soil samples were crushed, sieved through 2 mm sieve, physico-chemical characteristics (pH and

EC) and fertility status (organic carbon, available nitrogen, phosphorus, and potassium) of collected soil samples were determined. Standard protocols were followed to measure pH, electrical conductivity, available nitrogen, phosphorus, and potassium (Subbiah and Asija 1956; Olsen *et al.,* 1954; Toth and Prince 1949). The volumetric approach was used to calibrate organic carbon content of the soil (Walkley and Black 1934).

*C. Collection and identification of AMF spores*

**(i) AM spore extraction and morphological characterization of AMF spores.** By wet sieving and decantation technique (Gerdemann and Nicolson, 1963), AM fungal spores were extracted from different soil samples. Total number of spores in a gram of soil sample was calculated. Morphological identification of spores was done using physical characteristics such as colour, size, wall thickness, and hyphal attachment (Schenck and Perez, 1990). Spores were stained with a solution mixture consisted of 1:1 ratio of Melzer's reagent (Morton, 1991) with PVLG (Koske and Testier, 1983) and observed under microscope.

The macro-characteristics such as sporocarp, spore, and subtending hypha were employed to distinguish between different species (Walker, 1992). The micro-characteristics of spore wall layer, texture, and colour were explored to discriminate between specific AM fungi. The visual differences in morphology of fungal hyphae and vesicles inside the roots were observed for more precise identification of AM fungi (Mehrotra and Mehrotra 1999).

**(ii) Glomalin content of soil samples.** The amount of glomalin in the soil samples was measured by following the methodology of Wright and Upadhyaya (1998). One gram soil sample was mixed with 8 ml of 50 mM sodium citrate buffer (pH 8.0) and autoclaved for 60 minutes at 121°C. Reddish brown supernatant was collected by centrifuging the contents at 7000 rpm for 15 minutes. To the pellet, 50mM sodium citrate buffer was added, autoclaved and centrifuged. This procedure was repeated until the reddish-brown colour disappeared. The collected supernatant from each replicate was then pooled. The protein content of the extract was estimated by Bradford's procedure using Bovine Serum Albumin as standard (Bradford 1976). Readings were taken at 595 nm with three replications in a microplate reader (Spectramax i3x) and expressed in mg $g^{-1}$ soil.

**(iii) Studies on AMF diversity.** Species richness, relative abundance and isolation frequency were calculated to quantify AM fungal diversity. The number of AM fungal species reported at each research site was used to calculate species richness. The relative abundance (RA) was calculated according to the equation: RA = A / $\Sigma a$ × 100, where A = abundance of individual "i th" species, $\Sigma a$ = sum of abundances of all species. The isolation frequency was defined as the percentage of samples from which particular species were isolated. Pearson correlation study was carried out to understand the influence of available phosphorous on AMF diversity indices.

**(iv) Statistical analysis.** All the data were statistically analysed using SPSS software (version 16.0). The mean values were compared through Duncan's multiple range test (DMRT) carried out at $P \leq 0.05$ (Duncan, 1955).

## RESULT AND DISCUSSION

*A. Physico-chemical properties of soil*

Results of physico-chemical properties and fertility status of rhizosphere soil samples are given in Table 1. The soils were mildly acidic to alkaline, with pH values ranging from 6.4 to 8.0. The soil reaction of rhizosphere samples of cultivable lands was slightly alkaline to alkaline. While the soil reaction of forest soil samples was neutral to acidic. The soluble salts concentration (EC) of soil samples of both cultivable and forest ecosystem ranged from 0.18 to 0.42 $dsm^{-1}$.

**Table 1: Physico-chemical properties of the collected soil samples.**

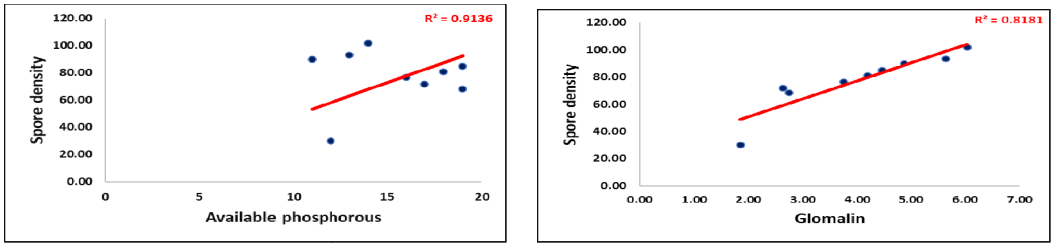
| Samples | Crop type | Soil type | Location | pH | EC ($dSm^{-1}$) | Avail. N (Kg $ha^{-1}$) | Avail. P (Kg $ha^{-1}$) | Avail. K (Kg $ha^{-1}$) | Organic carbon (%) |
|---|---|---|---|---|---|---|---|---|---|
| **Cultivable land** | | | | | | | | | |
| S1 | Onion | Clay loam | Orchard, TNAU | 7.6±0.06$^{b}$ | 0.34±0.02$^{b}$ | 276±1.20$^{ef}$ | 11±1.17$^{d}$ | 216±0.88$^{c}$ | 0.37±0.03$^{bc}$ |
| S2 | Maize | Sandy clay loam | TNAU | 7.7±0.09$^{b}$ | 0.36±0.02$^{ab}$ | 274±1.76$^{f}$ | 13±0.81$^{cd}$ | 234±1.76$^{b}$ | 0.32±0.02$^{c}$ |
| S3 | Onion | Silty clay | Narasipuram | 7.8±0.15$^{ab}$ | 0.42±0.01$^{a}$ | 263±0.88$^{g}$ | 14±0.68$^{cd}$ | 219±1.53$^{c}$ | 0.39±0.02$^{bc}$ |
| S4 | Paddy | Clay loam | Wetland, TNAU | 8.0±0.12$^{a}$ | 0.34±0.03$^{b}$ | 280±1.86$^{e}$ | 12±1.07$^{d}$ | 242±1.20$^{a}$ | 0.42±0.03$^{b}$ |
| **Forest ecosystem** | | | | | | | | | |
| S5 | Porasu tree | Sandy clay loam | Velingiri hills | 6.4±0.06$^{d}$ | 0.23±0.01$^{c}$ | 379±0.88$^{ab}$ | 19±1.09$^{a}$ | 167±1.20$^{d}$ | 0.89±0.03$^{a}$ |
| S6 | Rosewood | Silty clay loam | Siruvani forest | 6.8±0.03$^{c}$ | 0.21±0.01$^{c}$ | 346±1.76$^{c}$ | 18±0.47$^{ab}$ | 156±1.76$^{e}$ | 0.79±0.04$^{a}$ |
| S7 | Teak | Silty clay loam | Siruvani forest | 6.7±0.12$^{c}$ | 0.18±0.02$^{c}$ | 341±1.53$^{d}$ | 16±0.31$^{bc}$ | 148±1.15$^{f}$ | 0.81±0.05$^{a}$ |
| S8 | Flame of forest | Silty clay loam | Siruvani forest | 6.9±0.09$^{c}$ | 0.21±0.03$^{c}$ | 382±0.58$^{a}$ | 17±1.73$^{ab}$ | 152±1.73$^{ef}$ | 0.83±0.02$^{a}$ |
| S9 | Indian kino | Silty clay loam | Siruvani forest | 6.8±0.01$^{c}$ | 0.19±0.03$^{c}$ | 376±1.45$^{b}$ | 19±0.88$^{a}$ | 163±2.03$^{d}$ | 0.85±0.04$^{a}$ |

Rhizosphere soils of cultivable lands registered greater EC values. Maximum EC value of 0.42 $dsm^{-1}$ was observed in onion rhizosphere soil sample of Narasipuram. The lowest value of 0.18 $dsm^{-1}$ was noticed with teak rhizosphere soil. Available nitrogen varied from 263 to 382 kg $ha^{-1}$, phosphorus varied from 11 to 19 kg $ha^{-1}$, and potassium ranged between 148 and 242 kg $ha^{-1}$ respectively. The organic carbon content of the samples ranged from 0.32 to 0.89 %. Maximum organic carbon content of 0.89 % was observed in Porasu samples collected form Velingiri Hills.

The lowest organic carbon content of 0.32 % was found in maize rhizosphere of TNAU. However, the organic carbon content of rhizosphere soil samples of cultivable ecosystem was almost similar and did not vary with location and the type of rhizosphere. Similarly organic carbon content of soils of forest ecosystem did not vary with the kind of rhizosphere. It is quite obvious that organic carbon content of forest soils is greater than the soils of cultivable land. Due to continuous addition of litter and steady and slower rate of decomposition, there is build-up of organic carbon in the forest ecosystem. While in cultivable lands, first of all there is no addition of plant litter due to removal of all plant residues during weeding. Even if there is addition of litter, the rate of decomposition is faster in managed ecosystem like agricultural lands than forest ecosystem. These factors could have contributed for lesser organic carbon content of cultivable lands.

*B. Total glomalin content*

Total glomalin content of rhizosphere of various crops (S1, S2, S3, S4, S5, S6, S7, S8, S9) ranged from 1.86±0.18 to 6.04±0.07 mg $g^{-1}$ (Table 2). The highest glomalin content was observed in onion rhizosphere sample collected from Narasipuram (6.04±0.07 mg $g^{-1}$) and the lowest was in the paddy rhizosphere soil of Wetlands of TNAU (1.86±0.18 mg $g^{-1}$). The glomalin content of forest rhizosphere soil was in the range of 2.63±0.03 mg $g^{-1}$ (flame of the forest) to 4.46±0.15 mg $g^{-1}$ (porasu). Results of the study revealed a direct correlation between glomalin and AMF spore density and a less positive relationship between available phosphorus and AMF spore density (Fig. 1).




**A.** **B.**

**Fig. 1.** Relationship of Spore density with available phosphorous and glomalin.

*C. AM fungal spore density*

The AMF spore density differed greatly with the type of rhizosphere. It ranged from 68±1.67 to 102±4.41 per 100-gram soil (Table 2). Compared to forest ecosystem, a greater number of spores was found in the rhizosphere soils of cultivable ecosystem. However, paddy rhizosphere harbored lesser number of AMF spores (30±2.89 spores 100 $g^{-1}$ soil). Among nine soil samples, onion (Narasipuram) rhizosphere scored the highest value of 102±4.41 spores 100 $g^{-1}$ soil. It was followed by maize rhizosphere sample of TNAU which recorded 93±4.41 spores 100 $g^{-1}$ soil). Among rhizosphere samples obtained from forest ecosystem, Porasu rhizosphere had more spores (85±2.89 spores 100 $g^{-1}$ soil) followed by rose wood (81±2.08 spores 100 $g^{-1}$ soil). Pearson correlation studies revealed negative correlation between spore density and available phosphorus ($R^2$ = 0.91) (Fig. 1a) and positive correlation with glomalin content in the soil ($R^2$ = 0.81) (Figure 1b). Soil AM fungal diversity is indirectly correlated with available phosphorus content. The results of current study confirm the earlier reports that AMF spore population reduces with increase in available phosphorus content. Cell wall protein of glomalin in hyphal thread and spore is responsible for soil aggregation and carbon sequestration. This also governs the total glomalin content of the soil. In this study also, the total glomalin content of various rhizosphere soil samples were found to be positively correlated with AMF spore density.

**Table 2: Estimation of AM fungal spore density and total glomalin content in the collected soil samples.**

| Samples | Soil Type | Crop Type | Location | Total glomalin content (mg $g^{-1}$) | Spore density (100/g soil) |
|---|---|---|---|---|---|
| **Cultivable land** | | | | | |
| S1 | Clay loam | Onion | Orchard, TNAU | 4.87±0.18$^{c}$ | 90±2.89$^{bc}$ |
| S2 | Sandy clay loam | Maize | TNAU | 5.64±0.06$^{b}$ | 93±4.41$^{ab}$ |
| S3 | Silty clay | Onion | Narasipuram | 6.04±0.07$^{a}$ | 102±4.41$^{a}$ |
| S4 | Clay loam | Paddy | Wetland, TNAU | 1.86±0.18$^{g}$ | 30±2.89$^{f}$ |
| **Forest ecosystem** | | | | | |
| S5 | Sandy clay loam | Porasu Tree | Velingiri Hills | 4.46±0.15$^{d}$ | 85±2.89$^{bcd}$ |
| S6 | Silty clay loam | Rosewood | Siruvani Forest | 4.20±0.02$^{d}$ | 81±2.08$^{cd}$ |
| S7 | Silty clay loam | Teak | Siruvani Forest | 3.75±0.05$^{e}$ | 77±1.67$^{de}$ |
| S8 | Silty clay loam | Flame of forest | Siruvani Forest | 2.63±0.03$^{f}$ | 72±1.67$^{e}$ |
| S9 | Silty clay loam | Indian Kino | Siruvani Forest | 2.74±0.05$^{f}$ | 68±1.67$^{e}$ |

*D. AM fungal diversity*

Totally 698 AM fungal spores were collected from the nine distinct soil samples using the wet sieving and decantation method. This AMF spore population was found to have 12 different AM fungal species with 7 genera. These AMF species include *Acaulospora* sp*, Ambispora leptotica*, *Glomus hoi, Glomus microcarpum, Glomus ambisporum, Glomus aggregatum, Septoglomus deserticola, Septoglomus constrictum, Paraglomus* sp*, Funneliformis geosporum, Gigaspora margarita* and *Gigaspora gigantea.* The most abundant genus was *Glomus* (4 species). It was followed by *Gigaspora* (2 species), and *Septoglomus* (2 species). While other genera such as *Acaulospora, Ambispora, Funneliformis*, and *Paraglomus* had only one species each. The qualitative analysis of distribution of AMF spores indicated uneven distribution of AM fungal species in the natural habitat. Similar to the current study, *Glomus, Gigaspora,* and *Acaulospora* were reported to be most common AMF genera (Ambili *et al.,* 2012) in any rhizosphere sample. This may be due to wide range of hosts preferred by *Glomus* sp (Ming-Yuan *et al.,* 2007). Further, it was also reported that the tiny spores of *Glomus and Acaulospora* makes them the easiest to reproduce and generate a great quantity of spores at faster rate (Hepper, 2018). Among the rhizosphere, maximum species richness of 7 species was observed in maize collected from TNAU, Orchard samples (S2). Minimum number of species (2 species) was observed in paddy soils collected from TNAU (S3). Maximum occurrence (66.66%) was noticed with *Glomus hoi* and *Glomus microcarpum*. They were identified in six samples. The percent occurrence of *Gigaspora margarita* and *Septoglomus constrictum* was 55.55%. Among these two, the former was observed only in forest soil samples, while the latter was noticed in both cultivable and forest soils. Other AMF found in forest soils alone is *Funneliformis geosporum*. AMF of cultivable land alone include *Acaulospora* sp, *Ambispora leptotica* and *Paraglomus* sp which accounted for 11.11% (Table 3). Other AMF with 33.33% occurrence noticed in both cultivable and forest ecosystem were *Glomus ambisporum, Glomus aggregatum* and *Gigaspora gigantea.* The results of current study confirm the observations of Helgason *et al.,* (2002); Bhattacharjee and Sharma (2011) who reported *Glomus microcarpum* as dominant AMF spore in the paddy rhizosphere. *Acaulospora foveata* and *Septoglomus constrictum* were the prevalent species amongst forest ecosystem and were tightly tied to edaphic variables, and AMF diversity was shown to be highly related to soil carbon and pH (Wang *et al.,* 2019).

**Table 3: Distribution pattern of AM fungi in the collected soil samples.**

| Sr. No. | AM species identified | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | Occurrence (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | *Glomus hoi* | + | + | + | - | + | + | + | - | - | 66.66 |
| 2. | *Septoglomus deserticola* | + | + | + | + | - | - | - | - | - | 44.44 |
| 3. | *Glomus microcarpum* | + | + | + | - | + | + | | + | - | 66.66 |
| 4. | *Gigaspora margarita* | - | - | - | - | + | + | + | + | + | 55.55 |
| 5. | *Septoglomus constrictum* | + | + | + | - | + | + | - | - | - | 55.55 |
| 6. | *Funneliformis geosporum* | - | - | - | - | + | - | + | - | + | 33.33 |
| 7. | *Acaulospora sp.* | - | - | - | + | - | - | - | - | - | 11.11 |
| 8. | *Ambispora leptotica* | - | + | - | - | - | - | - | - | - | 11.11 |
| 9. | *Glomus ambisporum* | - | - | + | - | - | - | + | + | - | 33.33 |
| 10. | *Paraglomus sp.* | - | + | - | - | - | - | - | - | - | 11.11 |
| 11. | *Gigaspora gigantea* | - | + | + | - | - | - | - | + | - | 33.33 |
| 12. | *Glomus aggregatum* | + | - | - | - | - | + | - | - | + | 33.33 |
| | **Species richness** | **5** | **7** | **6** | **2** | **5** | **5** | **4** | **4** | **3** | |

## CONCLUSION

This study revealed that the AMF fungal density was greater in cultivable land. However, the diversity was found to be almost equal in both the ecosystem. But some of AMF genera were found to be unique to the ecosystem. For example, *Gigaspora margarita* was found in all rhizosphere soil samples of forest ecosystem while it was absent in the soil samples of cultivable land. On the contrary, *Septoglomus deserticola* was evidenced in all the rhizosphere soil samples of cultivable ecosystem and absent in forest soil. Another interesting observation is presence of *Acaulospora* sp in wet land rhizosphere alone and absent in all other rhizosphere of both cultivable and forest ecosystem. The dominant genera found in both the ecosystem are *Glomus hoi, Glomus microcarpum* and *Septoglomus constrictum.* Further studies are needed to ascertain the use of AMF genera of forest origin to boost the growth of agricultural crops.

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**Conflict of interest.** None.

## FUTURE SCOPE

For the purpose of predicting plant growth, it is crucial to comprehend the ecological dynamics of arbuscular mycorrhiza (AM) fungi and their response to soil characteristics.

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