ijlpr 2020; doi 10.22376/ijpbs/lpr.2020.10.4.L109-116

International Journal of Life science and Pharma Research

Research Article

Botany for Medicinal science

Diversity of Arbuscular Mycorrhizal Fungi in Shola Forests of Kodaikanal, Southern India

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Abstract: Arbuscular mycorrhizal fungi establish symbiotic association with more than 80% of land plants and influence plant community composition and distribution. The mycorrhizal status of plant species in various ecosystems have been reported but failed to assess community composition of AM fungi in shola forests, therefore present study carried out to fulfill this research gap. A total of Seven AM fungal species could be isolated from Tiger and Kookal shoal and identified based on spore morphology. There is no significant difference among distribution of AM fungal spores in various seasons among sites. In both the sholas AM fungal spore numbers were high during the wet (September) season and the variation among spore numbers in different seasons among sites were insignificant. Arbuscular mycorrhizal spore of *Funneliformis geosporum* was the most frequent species and *Funneliformis mosseae* was the less frequent species. The frequency among various sites in both the Sholas was insignificant. The AM fungal species richness was high in Tiger Shola (six spores), whereas in Kookal Shola showed less species richness. The diversity indices like Shanon - Weaver index (H') and Simpson index (D) were calculated in all the sites and showed variation among shoals in four sites. Arbuscular mycorrhizal fungal community composition in this ecosystem have been elucidated to certain extent and used for conservation of shola species.

Keywords: AM fungi, Diversity, Seasonality, Shola forest, species richness

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Recieved On 23 April 2020
Revised On 01 June 2020
Accepted On 01 July 2020
Published On 10 October 2020

Funding This Research work is supported by University Grant Commission, UGC New Delhi Grant no. UGC MRP (F.MRP-6384/16).1.

Citation Kullaiyan Sathiyadash*, Jaffer Ali Jabeen, Karthigai Selvi Ezhilarasi and Eswaran Pillai Uma , Diversity of Arbuscular Mycorrhizal Fungi in Shola Forests of Kodaikanal, Southern India.(2020).Int. J. Life Sci. Pharma Res.10(4), L109-116 http://dx.doi.org/10.22376/ijpbs/lpr.2020.10.4.L109-116

1. INTRODUCTION

Arbuscular mycorrhizal fungi belong to the phylum, Glomeromycota, associated with more than 80% of the plant species[1] and form mutualistic symbiosis between certain soil-borne fungi and plant roots[2]. Arbuscular mycorrhizal fungi increase soil health, improves the uptake of nutrients particularly phosphorus, high water uptake under stress conditions[1,3], enhance seedling survival and plant growth[4], protect the plants from pathogens and uptake other nutrients like N, K, Ca, S, Cu, Zn, Fe and Mn[5]. Arbuscular mycorrhizal fungal diversity is the major fact the maintenance of plant biodiversity, ecosystem stability and function. Several studies indicate that AM fungi alter plant community structure by affecting the relative abundance of plant species and plant-species diversity[6]. The species composition and diversity of AM fungal communities has the potential to determine plant population and plant community structure. The fact that plant species vary in the degree of response to AM fungal species that has important implications for growth of individual plant species. In turn, this will affect a plant's ability to coexist with other plant species in a community[7]. On the other hand, established mycorrhizal plants may serve as important sources of inoculum for initially non-mycorrhizal, conspecifics, which may affect regeneration and could contribute to patchy distribution of species within the community[8]. The establishment, survival and maintenance of plant community diversity are influenced by mycorrhizal diversity[9]. However, AM fungal diversity plays a prime role in natural plant communities. The tremendous role played by AM fungi in natural ecosystems creates interest in researchers to reveal the distribution and diversity of AM fungi[10]. However, AM fungi influences both function and biomass of terrestrial ecosystems, but their spatio-temporal dynamics are largely unknown[11]. When compared to other microorganisms in global level the biogeography of AM fungi were relatively unknown[12]. The unique combination of forests and grassland comprise of the Shola forest. They are stunted evergreen forests found as patches in grasslands especially in Valleys. The Sholas are dark damp throughout the year, because Shola soil absorbs and retains water like a sponge. However, wide diversity and unique floral distribution, no systematic investigation has been carried out to elucidate the AM fungal community composition in shoal forests. When compared to other ecosystems, shoals are poorly assessed for AM fungal distribution. Few studies have reported for root fungal associations of medicinal and aromatic plant species in Western Ghats region[13], AM fungal association and spore numbers in Velliangiri hills (including shoal forests)[14] and South Indian Grasses[15]. In Sholas of Western Ghats region have been studied for AM fungal association[16] and not considered AM fungal species. Mycorrhizal status of sixteen epiphytic and terrestrial ferns has been explored from Kodaikanal Hills of Southern India[17] and AM fungal association of ferns and lycophytes was observed from Palni hills, Western Ghats region in southern India [18]. These studies insist that the importance of mycorrhizal research which deserves much attention is the investigation of more plant species for their mycorrhizal status and failed to assess community composition of AM fungi in this forest ecosystem. Hence, the aim of this study is to elucidate the seasonal dynamics of AM fungi, which is essential to quantify the functioning and ecological significance of AM in natural ecosystems. Therefore the present investigation was carried out to fulfill the following objectives, i. To evaluate the arbuscular mycorrhizal (AM) fungal diversity, (ii) To record the seasonality of AM fungi in shola forest of Kodaikanal

2. MATERIALS AND METHODS

2.1 Study site

The study site, Kodaikanal (longitude 77° 26' to 77° 33' E and latitude 10° 12' to 10° 15'N) is located within the Eastern offshoot of the Western Ghats and the spur aligned on a direction of East West and North South axis. The Shola forest is occupied by upper elevations of the Palani hills. Sholas are patches of jungles isolated from forests varied with plant species composition and size. The streams running through the Sholas and trees showed stunted growth. The research was conducted among Sholas with altitudes ranging from 360 m – 2550 m. The annual rainfall is quite variable in the hills (1300 mm) with temperatures ranging from 13 to 24°C in summer and winter ranging from 7 to 16°C.

2.2 Sampling

Rhizosphere soil was collected from two forests (Tiger Shola: 10° 14' 57"N 77° 31' 29.3"E, Elevation: 1886 m; Kookal Shola: 10°17′9″N 77°21′48″E, Elevation is 1,890 m). In both Sholas soil samples were collected from each two sites (Site I, II, III and IV). In each site, three replicate soil samples were collected from 1m distance. In a total of 15 samplings were done in two Shola forests comprising four sites from March 2018 to February 2019. Site wise sampling such as, Site I five samplings, Site II three, Site III three and Site IV four, a total of 45 soil samples were collected. The soil samples were brought to the laboratory, shade dried and stored in plastic bags. The stored soil samples were used to study the seasonality of AM fungal spores and diversity indices.

2.3 Isolation, enumeration and identification of AM fungal spores

The soil samples collected from Sholas were used for enumeration of AM fungal spores. One hundred grams of Shola soil were dispersed in 1L water and the suspension was poured through 710 to 37µm sieves. The sievates was dispersed in water and filtered through gridded filter papers. Each filter paper was then in a petri dish and scanned under a dissection microscope at X40 magnification and all intact spores (non-collapsed spores with cytoplasmic contents and free from parasitic attack) were counted. Sporocarps and spore clusters were considered as one unit. The soils of the pot culture were used for identification of AM fungi. After isolation of the spores as described above, the intact spores were transferred using a wet needle to polyvinyl alcohol-lacto glycerol with or without Melzer's reagent on a glass slide for identification. Spores were identified from spore morphology and subcellular characters and compared to original descriptions[19]. Spore morphology was also compared to the culture database established by INVAM *(http://invam.cag.wvu.edu)*. Spore colour was examined under a dissection microscope on fresh specimens immersed in water. Classification, spore wall characters and the spelling of scientific names are as suggested by Morton and Benny [20], Walker [21,22] and Walker and Trappe [23].

2.4 Frequency, Species richness and diversity indices

Frequency will be calculated as the ratio of the number of sites in which a particular spore morphotype presents to the total number of sites examined × 100. Species richness (SR) is the number of species present in a particular site. The Shannon Weaver index (H′) would be calculated from the equation H′= ∑Pi In Pi, where Pi is the relative abundance of the species compared to all the species in a sample. Simpson's index (D) are calculated from the formula D = $\sum Pi^2$

2.5 *Trap cultures*

Rhizosphere soil samples were mixed to form a composite soil sample. Two-litre capacity pots were filled with 1 L of pasteurized (120°C for 60 min) sandy soil followed by 500 g of composite rhizosphere soil sample. The pots were seeded with *Eleusine coracana* (L.) Gaertn., and the seedlings were thinned to 5-7 seedlings per pot after germination. A total of 48 pots, were arranged in a randomized block design. At the end of the growth period the soil samples were taken from each pot and AM fungal spores were isolated by a modified wet-sieving and decanting method as detailed above.

3. STATISTICAL ANALYSIS

Data on AM fungal spore numbers in various seasons and frequencies were subjected to Analysis of Variance (ANOVA; SPSS Version 16).

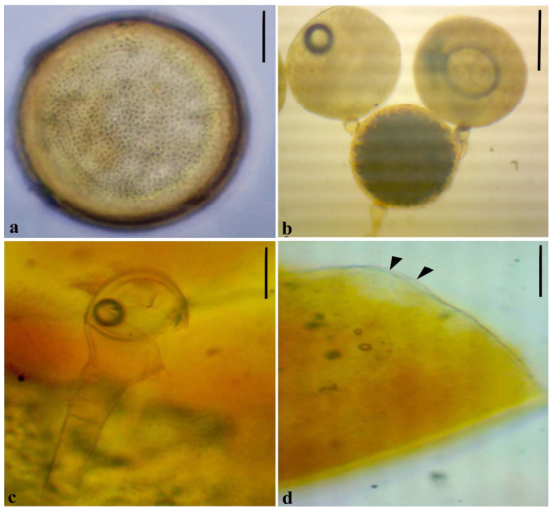
4. RESULTS

4.1 *Arbuscular mycorrhizal fungal characteristics of the study sites*

A total of seven AM fungal morphotypes could be distinguished on the basis of spore morphology, to the species level (Table 1; Fig. 1). These include one species in *Acaulospora. scrobiculata* Trappe), *Scutellospora calospora* Walker and Sanders, *Funneliformis geosporum* (Nicol. and Gerd.) Walker, *Glomus aggregatum* Schenck and Sm. emend. Koske, *Glomus sinuosum* (Gerd. and Bakshi) Almeida and Schenk, *Glomus viscosum* Nicolson, and *Funneliformis mosseae (*Nicolson Gerd.) C. Walker & A.Schubler.

Table 1. Arbuscular mycorrhizal fungal spore morphotypes isolated from different sites in sholas at Kodaikanal. (X indicates the presence).

Fungal species	Site I	Site II	Site III	Site IV
Acaulospora scrobiculata Trappe	X		X	X
Scutellospora calospora (T.H. Nicolson & Gerd) C. Walker & F.E. Sanders	X	X		
Glomus aggregatum N.C. Schenck & G.S. Sm	X			
Funneliformis mosseae (T.H. Nicolson & Gerd.) C. Walker & A. Schüβler	X	X		X
Glomus sinuosum T.H. Nicolson	X	X		
Glomus viscosum	X		X	
Funneliformis geosporum (T.H. Nicolson & Gerd.) C. Walker & A. Schüβler		X	X	X



a. Acaulospora scrobiculatum, b. Scutellospora calospora c. Subtening hyphae of Scutellospora calospora, d. Wall layer of Scutellospora calospora, Arrowheads indicate wall layers. Scale bars: 50µm

Fig 1. Arbuscular mycorrhizal fungal species in shola forests of Kodaikanal.

4.2 *Frequency, species richness and diversity indices of AM fungal species*

The frequency among various sites in both the Sholas were insignificant ($F_{3,15}$= 0.79;P>0.05). The AM fungal species richness was high in Tiger Shola (6 species), whereas in Kookal Shola showed only three species in both the sites (Site III and IV). The diversity indices like Shannon - Weaver index (H') ranged from 0.25(Site I) to 0.48 (Site III) and the Simpson index (D) ranged from 2.08 (Site III) to 4 (Site I) (Figure 2).

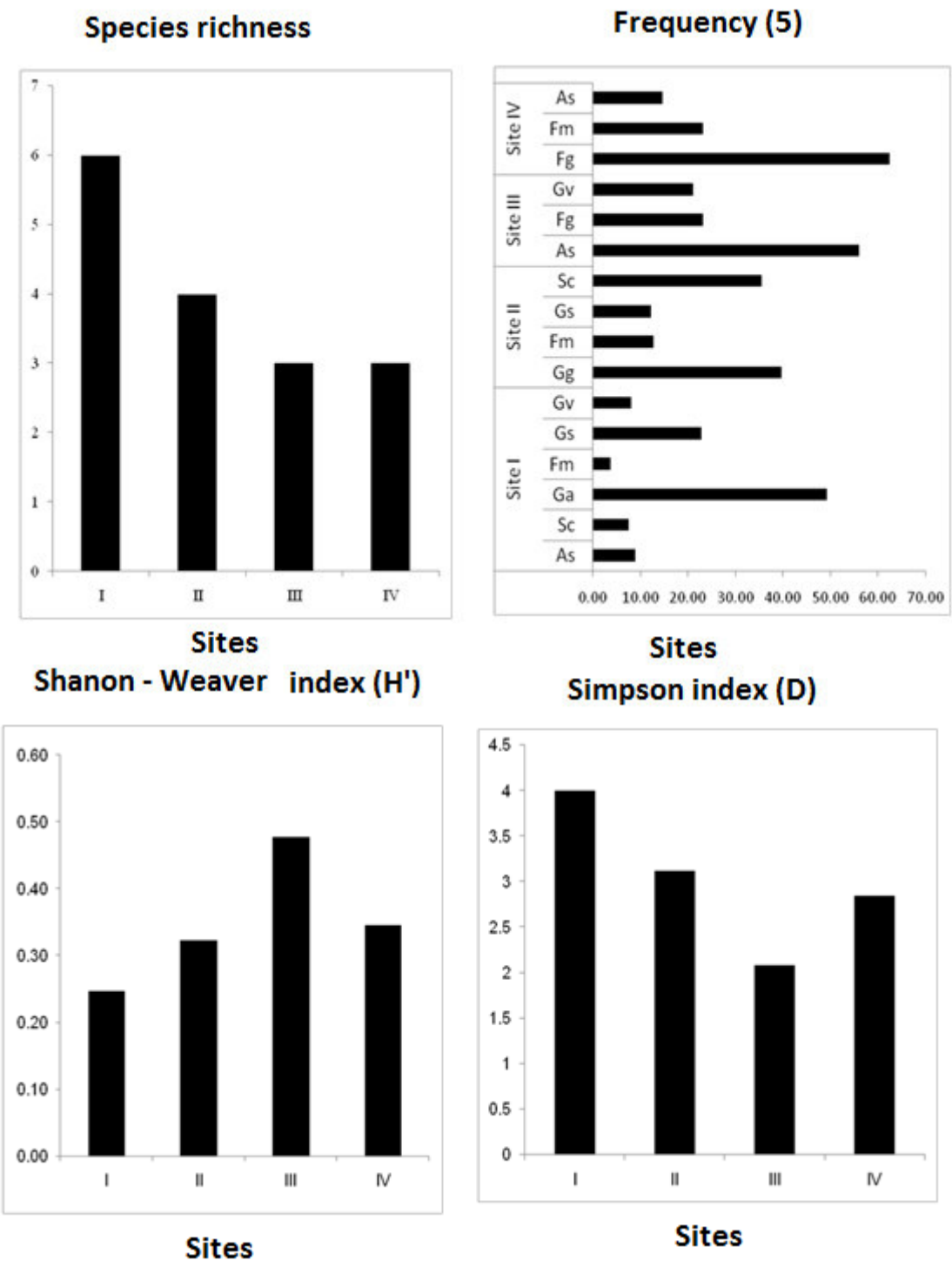


Fig 2. Arbuscular mycorrhizal species richness, frequency and diversity indices of shoal forests in Kodaikanal

4.3 Seasonal variation of AM fungi

There is no significant difference ($F_{3,47}$=0.13;P>0.05) among distribution of AM fungal spores in various seasons from four sites (Both the Sholas) . In Tiger Shola both the sites maximum (17 spores) number of AM fungal spores isolated in September and minimum (9 spores) in June. Similarly, in Kookal Shola maximum (17 spores) in September and December and minimum in January (10 spores) and May (9 spores) in both the sites (Site III and IV) respectively (Figure 3). Arbuscular mycorrhizal spores of *Funneliformis geosporum* were the most frequent (62%) species and *Funneliformis mosseae* was the least frequent (4%) species.

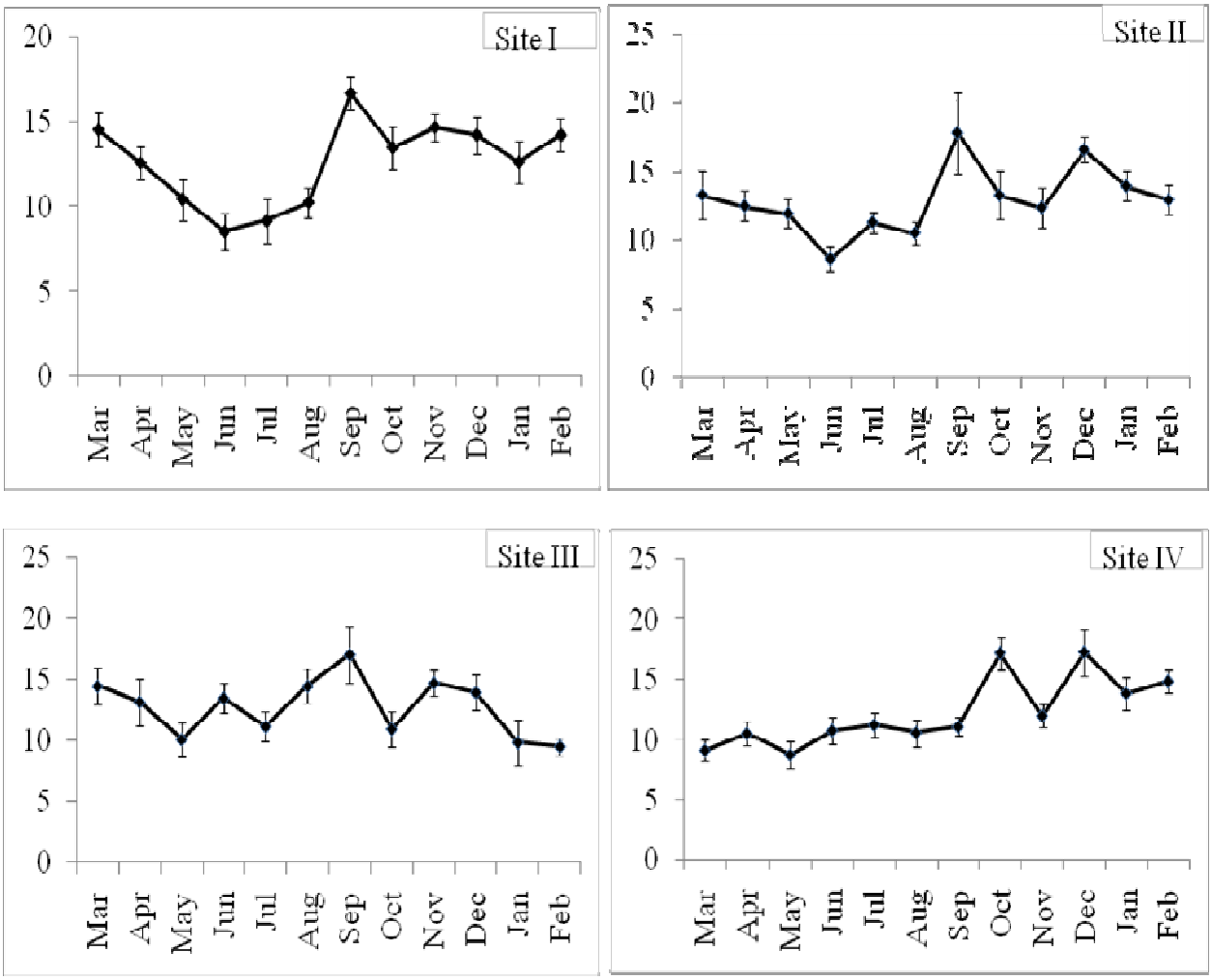


Fig 3. Arbuscular mycorrhizal fungal spore numbers in Tiger (Site I, II) and Kookal (Site III & IV) Sholas in various seasons of Kodaikanal

5. DISCUSSION

Analysis of soil for AM fungal populations should be a useful indicator of community composition and diversity[24]. In this study, a total of seven AM fungal species were identified based on the morphological characters of the spores. Similarly, various authors reported eleven AM fungal species from South Indian grasses[15] and AM fungal association of various vegetation types in Velliangiri hills, Western Ghats[14]. This number is about less in those reported from semiarid Mediterranean ecosystems[25] and semi- arid areas in Brazil[26] where 23 and 21 AM fungal species were reported. However, a high AM fungal diversity has also been reported in other natural ecosystems. Forty four AM fungal species were isolated from grasslands of Namibia [27], 43 from an arid steppe of inner Mongolia[28] and 27 species from tropical rainforest of Xishuangnanna, southwest China[29]. Availability of nutrients and moisture content can influence the AM fungal spore production[30]. In the present study, there is a difference in diversity of AM fungal communities in Tiger and Kookal Shola. The spore production may depend on spatiotemporal dynamics as well as the dispersal was also influenced by wind current [31]. These factors can reduce the AM fungal dispersal from one ecosystem to another may be the reason for site specific differences of diversity in this study[32]. The diversity analysis in present study indicated site specific variation in AM fungal community structure in both the Sholas. Arbuscular mycorrhizal fungal spores belonging to *Glomus* predominated species diversity and most frequent species, which is in accordance with the observations that species of *Glomus* dominate tropical soils[33-35]. The sporulation rates of *Glomus* are high[35] and they have rapid colonization potential, colonized using small fragments of mycelium or colonized roots[36]. The spore numbers of 8 to 18 spores per 100g soil is low compared to 14 to 93 per 100g soil reported by Muthukumar *et al* [29] and 55 to 191 spores per 100g soil reported by Zhao *et al.*[34] from Primary forest of Xishuangbanna, southwest China. The low density of AM fungal spores of the present study corroborates the reports from humid tropical forest where spore numbers tend to be low or infrequent [37,38]. Generally, AM fungal spores in natural soils are dead or parasitized and are merely spore cases[39]. The spore number reported in this study is intact healthy spores. In the present study, the seasonality of AM fungi showed maximum number of spores in the wet season (both the shoals). Similarly, studies in Yungas forest, high AM fungal spore numbers were observed during autumn (Sep. to Dec.) season[40]. In accordance with this study maximum spore numbers should be observed during high root growth in forest ecosystems[41,42]. Similar results were observed in Singapore, where peak sporulation was observed in locations receiving full rainfall[43]. This is due to the large spore of AM fungal species that were abundant in the wet season which was supported by Picone[44] in Nicaragua and Costa Rica. In addition, in this study the most frequent species is *Glomus* further supports the high sporulation during wet season in present study. In general ecological factors like prevailing season, edaphic factors, host dependence, host plant age, sporulation potential of AM fungi in specific edaphic conditions could influence the development and distribution

of AM fungal species[45]. In undisturbed forest, spores may be relatively less important than other vegetative propagules, and primarily the soil hyphal networks initiate the colonization of new roots[46]. As a result, forests with root growth throughout the year usually have small spore populations and high mycorrhizal colonization levels [47,48].

6. CONCLUSION

Seven AM fungal species could be isolated from shola forets and elucidated the frequent occurrence of *Funneliformis mosseae*. Arbuscular mycorrhizal fungal community composition in Shola forest of Kodaikanal has been elucidated to certain extent. In future, the studies are needed to assess a relationship among plant and AM fungal community structure, which is further useful for conservation of shola species.

7. FUNDING ACKNOWLEDGEMENT

Authors highly acknowledge UGC New Delhi for providing financial assistance in the form of UGC MRP (F.MRP-6384/16).

8. AUTHORS CONTRIBUTION STATEMENT

Dr. K. Sathiyadash, JA Jabeen and KS Ezhilarasi formulated the study objectives and carried out the work. Dr. E. Uma critically corrected the manuscript and added valuable points. All the authors contributed their role in finalizing the entire section of final manuscript.

9. CONFLICT OF INTEREST

Conflict of interest declared none.

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