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EXPLORING THE SYMBIOTIC RELATIONSHIP BETWEEN ARBUSCULAR MYCORRHIZAL FUNGI AND MEDICINAL PLANTS IN MIZORAM, NORTH EAST INDIA

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Abstract– Arbuscular mycorrhizal fungi are the most common symbiotic microbes that associate with more than 70% of vascular plants. In this work, the arbuscular mycorrhizal fungal characteristics of 12 medicinal plant species in Mizoram, northeast India were studied. *Kalanchoe pinnata* (Lam.) Pers. showed the highest colonization (87%) and it is lowest in *Hedyotis scandens* (Roxb.) R.J.Wang (1.72%). The spore density of arbuscular mycorrhizal fungi ranges from 477 to 1479 per 50 g of soil. The most dominant genus of arbuscular mycorrhizal fungi was found to be *Glomus*. Isolation frequency of the arbuscular mycorrhizal fungi is considerably high for all species. The Shanon-Wiener diversity index range from 1.14 to 2.24. In conclusion, medicinal plants and arbuscular mycorrhizal fungi show various extent in terms of colonization and species diversity and the dynamic significance of AM fungus in the cultivation of medicinal plants deserves more investigation.

INTRODUCTION

Arbuscular mycorrhiza (AM) is the most common terrestrial symbiosis with 70–90% of land plant species with fungi belonging to phylum Glomeromycota (Parniske, 2008). The function of mycorrhizal symbiosis may differ greatly between fungal species (Smith and Read, 2008). The diversity of these symbiotic associations has been directly linked with plant diversity, primary productivity and herbivory by animals (van der Heijden, Boller and Wiemken, 1998; Vannette and Rasmann, 2012).

The presence of Arbuscular mycorrhizal fungal (AMF) in medicinal plants was first documented by Taber and Trappe (1982) and from then onwards, it has been found that most of the medicinal plants are able to form symbiotic association with mycorrhizal fungus (Chen *et al*., 2014). AMF could qualitatively and quantitatively affect the production of secondary metabolites in their host plant (Porcel *et al*., 2012; Ahanger *et al*., 2014; Cicatelli *et al*., 2014; Salam *et al*., 2017; Kaur and Suseela, 2020). The dynamics of synthesis of plant secondary metabolites are influenced by AMF, which in turn increases the tolerance of medicinal plants to abiotic and biotic stress and renders them beneficial to human health, mainly through their antioxidant activity (Seeram, 2008).

Diversity of AMF associated medicinal plants has been exploited in several states of India like Karnataka, Himachal Pradesh, Haryana, Madhya Pradesh, Goa, etc. However, this association is not much exploited in the state of Mizoram that possesses about 400 medicinal plants currently (Soren, 2021). The current study examined the diversity, spore density, and level of Arbuscular mycorrhizal fungal colonization in a few medicinal plants from Mizoram, Northeast India, taking into account the aforementioned circumstances.

MATERIALS AND METHODS

Plant Material

Ten medicinal plants that grew under natural environmental conditions have been selected. The selected plant species with their respective family to which they belong are listed in Table 1.

Sample Collection

The root samples and rhizospheric soil samples were collected from ten randomly selected healthy individual per plant species of medicinal plants. At a depth of 0–30 cm, three random soil cores from ten duplicate plants were collected and mixed. In total, root samples of 12 plant species and 120 soil samples were collected. Only healthy, undamaged individual plants were chosen for the study. The collected roots and soil samples of each selected plant kept in Ziplock bags were brought back to the laboratory for further processing.

Estimation of AMF colonization

The collected root samples were carefully washed under tap water in order to remove soil particles. Then the roots were heated at 90° C with 10% KOH after cutting into 1-2 cm segments. The roots segments after rinsing undergo acidification with 2% HCl for 15-20 mins. After this, the roots were stained with 0.05% trypan blue in lactophenol for 5 minsand excess stain were removed by clear lactophenol (Phillips and Hayman, 1970). Forty root segments (1-2 cm) for each plant sample were then mounted on a glass slide with lactophenol and observed under light microscope (Olympus BX 53) in 100X magnification. The degree of AM colonization was estimated using the magnified intersection method (Mc Gonigle, 1990). The plane of focus was moved through the entire root to evaluate each intersection, and any arbuscules, vesicles, or hyphae intersected by the vertical crosshair were noted. The numbers of arbuscules and vesicles were divided by the total number of intersections analyzed to estimate arbuscular colonization (AC) and vesicular colonization (VC). Hyphal colonization (HC) was calculated as the proportion of intersections where all the fungal structures (arbuscles, vesicles and hyphae) were observed. The percentage of arbuscular colonization (AC), vesicular colonization (VC) and hyphal colonization (HC) was calculated by using the following formula:

$$AC = \frac{\text{Count of Arbuscules}}{\text{Total number of intersections examined}} \times 100$$

$$VC = \frac{\text{Counts of vesicles}}{\text{Total number of intersections examined}} \times 100$$

$$HC = \frac{\text{Total number of intersections examined-Negative intersection}}{\text{Total number of intersections examined}} \times 100$$

Table 1. Selected medicinal plants with their respective plant families

Plant Species	Family
Centella asiatica (L.) Urb.	Apiaceae
Acmella paniculata (Wall. ex DC.) R.K. Jansen	Asteraceae
Acmella ciliata (Kunth) Cass	Asteraceae
Eupatorium odoratum L.	Asteraceae
Synedrella nodiflora (L.) Gaertn.	Asteraceae
Cheilocostus speciosus (J. Koenig) C.D.Specht	Costaceae
Kalanchoe pinnata (Lam.) Pers.	Crassulaceae
Mimosa pudica L.	Fabaceae
Sida acuta Burm.fil.	Malvaceae
Phyllanthus urinaria L.	Phyllanthaceae
Hedyotis scandens (Roxb.) R.J.Wang	Rubiaceae
Lantana camara L.	Verbanaceae

Identification and quantification of AM fungal spore

AM fungal spores were extracted by wet sieving and decanting method (Gerdemann and Nicolson, 1963). Isolated spores were mounted in polyvinyl lactoglycerol (PVLG) and a dissecting microscope was used to count the spores, and the number of spores per 100 g of soil was reported as the spore density (SD). The morphological identification of the AMF was conducted based on spore size, spore color, thickness of wall layer,spore surface ornamentation and subtending hyphae, using the identification manual available at the International Collection of Vesicular and AM Fungi website (http://invam.wvu.edu/) and the amf-phylogeny.com.

Diversity and statistical analysis

The structure of AM fungi communities was described using spore density (SD), species richness (SR), isolation frequency (IF), Evenness(J) and the Shannon-Wiener index (H) (Simpson, 1969; Franke-Snyder *et al*., 2001).

Data generated were statistically analyzed using the SPSS Statistics Version 26 (SPSS Inc., USA). Difference in mycorrhizal colonization and diversity parameters were tested using one-way ANOVA and means were compared by least significant difference at $P<0.05$.

RESULTS

AMF Colonization

The root colonization rate of AM fungi is shown in

Table 2 and the various structures of arbuscular mycorrhizal fungi are shown in Figure 1. The arbuscular colonization percentage range from 0.50% to 25.64% and the average AC is 5.18%. The arbuscular colonization was found to be highest in *Sida acuta* Burm.fil. and lowest in *Cheilocostus speciosus* (J.Koenig) C.D.Specht. There is no arbuscular colonization observed in *Hedyotis scandens* (Roxb.) R.J. Wang. The average vesicular colonization percentage is 5.87% and ranges from 0.44% to 20.8%. *Kalanchoe pinnata* (Lam.) Pers. has the highest vesicular colonization and it is lowest in *Acmella paniculata* (Wall. exDC.) R.K. Jansen. There is no vesicular colonization in *Hedyotis scandens* (Roxb.) R.J.Wang. The average hyphal colonization is 60.68% and ranges from 87.83% to 1.72% with the highest colonization observed in *Kalanchoe pinnata* (Lam.) Pers. and lowest in *Hedyotis scandens* (Roxb.) R.J. Wang.

Spore density and diversity index in the rhizosohere soil of 12 medicinal plants are shown in Table 3. The average spore density per 50 g of soil is 839.61 and ranges from 1479.67 to 477.67. The highest was observed in *Phyllanthus urinaria* L. and lowest in *Sida acuta* Burm.fil. Species richness is highest (11) in *Centella asiatica* (L.) Urb. and lowest

Table 2. AM Colonization percentage of 12 medicinal plant species

Plant Species	Colonization %		
	AC	VC	HC
Centella asiatica (L.) Urb.	2.79 0.81b	4.85 1.37bc	65.29 9.99bc
Acmella paniculata (Wall. ex DC.) R.K.Jansen	18.77 5.43a	0.44 0.29bc	52.53 5.69cd
Acmella ciliata (Kunth) Cass	0.54 0.34b	6.96 1.55bc	66.25 2.73bc
Eupatorium odoratum L.	1.71 1.07b	5.37 2.47bc	71.33 8.46ab
Synedrella nodiflora (L.) Gaertn.	1.76 1.19b	5.00 2.07bc	79.26 3.76a
Cheilocostus speciosus (J. Koenig) C.D.Specht	0.50 0.05b	3.50 1.71bc	30.75 2.53d
Kalanchoe pinnata (Lam.) Pers.	1.93 0.67b	20.80 5.99a	87.83 2.28a
Mimosa pudica L.	2.00 0.71b	7.00 2.48bc	79.00 5.10a
Sida acuta Burm.fil.	25.64 3.69a	1.70 0.43bc	47.66 2.41cd
Phyllanthus urinaria L.	5.50 1.50b	11.50 0.86ab	77.18 3.38a
Hedyotis scandens (Roxb.) R.J.Wang	0.00 0.00b	0.00 0.00c	1.72 0.74e
Lantana camara L.	1.05 0.36b	3.33 1.28bc	69.33 2.64bc

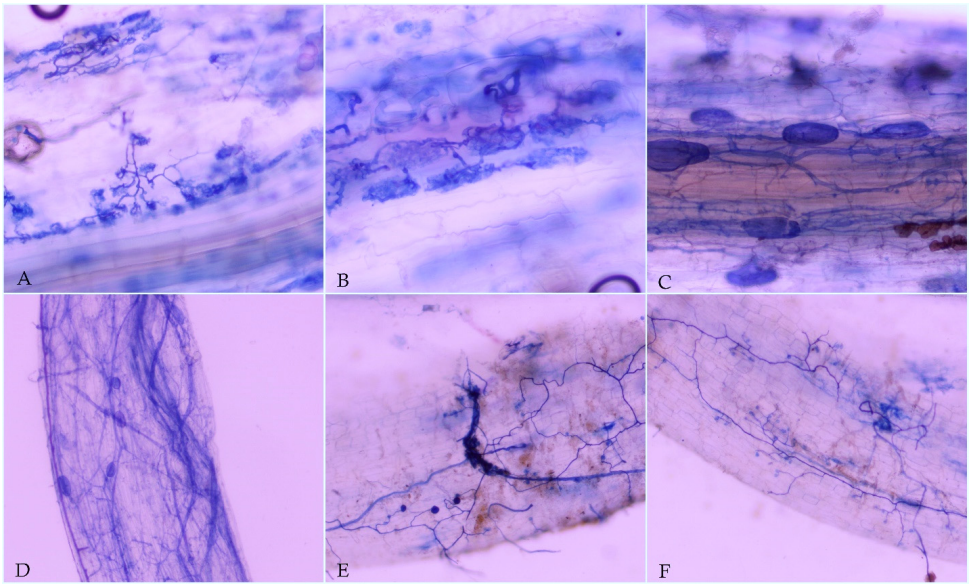

Fig. 1. Microscopic images of different structures of AM fungi in the roots.A: Arbuscles in the roots of *Centella asiatica* (L.) Urb. (100X); B: Arbuscles in the roots of *Acmella ciliata* (Kunth) Cass (200X); C: Vesicles in the roots of *Eupatorium odoratum* L. (200X); D: Intraradical hyphae and vesicles in the roots of *Synedrella nodiflora* (L.) Gaertn.(100X); E-F: Extraradical hyphae with terminal spores in the roots of *Sida acuta* Burm.fil. (100X).

(4) in *Hedyotis scandens* (Roxb.) R.J.Wang with an average of 9.42. *Eupatorium odoratum* L. (2.24) shows the highest H and lowest in *Hedyotis scandens* (Roxb.) R.J.Wang (1.14). The evenness (J) of AM fungi is between 0.63 to 0.98 with an average of 0.79.

Eight species of *Glomus*, five species of *Acaulospora*, two species of *Funneliformis* and *Rhizophagus*, one species each of *Claroideoglomus*, *Gigaspora*, *Scutellospora*, and *Septoglomus* were isolated and identified. In total, 21 AMF species from 8 genera were identified.

DISCUSSION

Although AM fungus infected all 12 medicinal plants, the extent of colonization and spore density differed amongst plant species. This might be caused by variations in AM species' sporulation capacities (Turnau *et al*., 2001). Members of Asteraceae usually showed high colonization rate between 65-100% (Rodrigues and Furrazola, 2013; Vanlalmuana *et al*., 2021). There is a very low colonization percentage in *Hedyotis scandens* (1.72%). This may be because some plants typically remain fungusfree because of their root's great resistance to mycorrhizal fungal hyphae (Tester *et al*., 1987; Brundrett, 1991; Giovannetti and Sbrana, 1998). It's possible that many AM species that infect plant roots but don't sporulate in the soil went unnoticed in our investigation (Tian *et al*., 2009). This problem

Table 3. Spore density, Species Richness, Shannon-Wiener index and Evenness of medicinal plants

Plant Species	SD/50g	SR	H	J
Centella asiatica (L.) Urb.	627.33 14.84	11 0.89	1.78 0.13	0.98 0.05
Acmella paniculata (Wall. ExDC.) R.K.Jansen	656.67 9.71	5 0.23	1.33 0.07	0.76 0.06
Acmella ciliata (Kunth) Cass	503.67 4.4	8 0.45	1.42 0.12	0.96
Eupatorium odoratum L.	920.33 21.67	9 0.84	2.24 0.09	0.63 0.07
Synedrella nodiflora (L.) Gaertn.	1161.00 121.50	10 1.06	1.66 0.14	0.75 0.05
Cheilocostus speciosus (J.Koenig) C.D.Specht	621.67 52.44	8 1.11	1.85 0.18	0.67 0.05
Kalanchoe pinnata (Lam.) Pers.	775.33 64.35	10 1.01	2.02 0.16	0.86 0.06
Mimosa pudica L.	1047.00 34.10	9 0.98	1.78 0.11	0.77 0.03
Sida acuta Burm.fil.	477.67 67.09	9 0.47	1.82 0.17	0.76 0.08
Phyllanthus urinaria L.	1479.67 53.05	8 0.72	1.54 0.08	0.80 0.06
Hedyotis scandens (Roxb.) R.J.Wang	588.00 33.07	0.89	1.14 0.13	0.89 0.07
Lantana camara L.	1217.00 39.40	9 0.23	1.36 0.07	0.68 0.04

Table 4. Isolation frequency of different AM fungal species

AM fungi species	Isolation Frequency %
Acaulospora bireticulata Rothwell & Trappe	33.33 5.11
Acaulospora denticulata Sieverding & Toro	41.67 4.12
Acaulospora foveata Trappe & Janos	33.33 3.01
Acaulospora scrobiculata Trappe	41.67 2.91
Acaulospora sporocarpia Berch	50.00 4.22
Claroideoglomus claroideum (N.C. Schenck & G.S. Smith) C. Walker & A. Schüßler	41.67 3.66
Funneliformis geosporum (Nicolson & Gerdemann) Walker	41.67 3.24
Funneliformis mosseae (Nicolson & Gerdemann) Gerdemann & Trappe	66.67 5.32
Gigaspora margarita Becker & Hall	8.33 1.01
Glomus albidum Walker & Rhode	50.00 2.22
Glomus aggregatum Schenck & Smith.	41.67 5.01
Glomus claroideum Trappe & Gerdemann	41.67 3.25
Glomus clarum Nicolson & Gerdemann	33.33 4.78
Glomus convolutum Gerd. & Trappe	58.33 5.76
Glomus etunicatum Becker & Gerdemann	16.67 4.02
Glomus melanosporum Gerdemann & Trappe	75.00 7.98
Glomus multicaule Gerdemann & Bakshi.	33.33 4.66
Rhizophagus clarus (T.H. Nicolson & N.C. Schenck) C. Walker & A. Schüßler	50.00 5.43
Rhizophagus irregularis (Blaszk., Wubet, Renker & Buscot) C. Walker & A. Schüßler,	50.00 3.98
Scutellospora calospora Nicolson & Gerd	33.33 4.07
Septoglomus constrictum Trappe	50.00 4.43

might be resolved by more research employing molecular techniques to identify AM fungi that invade the roots but do not sporulate.

The findings demonstrated a robust symbiotic association between 12 medicinal plants and AM fungus; nevertheless, notable variations were noted across the plant species. It is also possible that the preferences of various AM fungi for various host plants in our study may be reflected at the species or family level, given that studies have revealed non-random differences in distribution among various AM fungi species and genera in the field (Husband *et al*., 2002; Helgason *et al*., 2002).

Based on morphological studies, the present investigation described the diversity and composition of AM fungus. The findings showed that *Glomus* was the most common genus, with *Acaulospora* coming in second. Within the phylum Glomeromycota, which forms symbiotic interactions with plant roots, *Glomus* is the most frequent and largest genus (Rodrigues and Rodrigues, 2020). The study of spore density and diversity of arbuscular mycorrhizal fungi in medicinal and seasoning plants also shows that *Glomus* was the most dominant genus, followed by *Acaulospora* among the identified AMF (Urcoviche *et al*., 2014; Wang 2015).

CONCLUSION

In the current investigation, the diversity and colonization degree of AM fungus of medicinal plants in Mizoram were investigated. From the research, we can conclude that there is an abundant biodiversity of AM fungi associated with medicinal plants. Among them, *Glomus* was the most common genus. Spore density and the degree of colonization varied significantly among the plant species. Medical plants may benefit from arbuscular mycorrhizal fungus in a number of ways, including increased stress tolerance and growth stimulation. AMF may also promote the buildup of active compounds in such plants. In conclusion, considering the potential of mycorrhizal fungi in growth improvement and production enhancement of secondary metabolites in medicinal plants, more attention should be focused on the dynamic function of AM fungi in the cultivation of medicinal plants.

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Conflict of interest

There is no conflict of interest concerning the publishing of this paper.

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