ORIGINAL RESEARCH PAPER VOLUME-6 | ISSUE-8 | AUGUST - 2017 | ISSN No 2277 - 8179 | IF : 4.176 | IC Value : 78.46

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

SPORE POPULATION, OCCURRENCE AND ROOT COLONIZATION OF AM FUNGI IN FIVE ETHNOMEDICINAL PLANTS OF MANIPUR, NORTH EASTERN INDIA.

Microbiology

Heigrujam Boboy Singh Laboratory of Microbiology and Biotechnology, Department of Forestry, North Eastern Regional Institute of Science & Technology, P.O. Nirjuli-791109, Arunachal Pradesh

Sorokhaibam Sureshkumar Singh Laboratory of Microbiology and Biotechnology, Department of Forestry, North Eastern Regional Institute of Science & Technology, P.O. Nirjuli-791109, Arunachal Pradesh - Corresponding Author

Sagolsem Mukta Singh Department of Botany, D.M. College of Science, Imphal-795001, Manipur

ABSTRACT

Spore population density, species occurrence and root colonization percentages of arbuscular mycorrhizal (AM) fungi were investigated for a period of four seasons spreading over two consecutive years on five wild ethnomedicinal plants (*Adhatoda vasica, Zanthoxylum acanthopodium, Solanum nigrum, Blumea balsamifera* and *Alpinia zerumbet*). found in three district of Manipur, north eastern India. The spore population of AM fungi varied from a minimum of 27 spores 10 g^{-1} soil in the rhizospehere of *A. vasica* (27) during season 3 to a maximum of 196 spores 10 g^{-1} soil in *Z. acanthopodium* during season 1. The AM fungal colonisation was observed highest in the roots of *B. balsamifera* (61.88%) during season 1 and the lowest was observed in the roots of *A. zerumbet* (22.67%) during season 2. A total of 20 species of AM fungi have been isolated from the rhizosphere soil of the five medicinal plants studied. *Glomus* was largest genus with 14 species followed by *Acaulospora* with 3 species, *Sclerosystis, Scutellospora* and *Gigaspora* with 1 species each. The number of AM fungi species was recorded highest in the rhizosphere soil of *B.balsamifera* (20), followed by *S.nigrum* (19) and *A.zerumbet* (18) while lowest of 17 AM fungi species was recorded from the rhizosphere of *A. vasica* and *Z. acanthopodium* respectively. *Acaulospora delicata* was most commonly occurring AM fungus with highest (100%) occurrence in the rhizosphere of all plant species and in all seasons. *Glomus macrocarpum* and *G. microaggregatum* were other AM fungi with 95% occurrences. There was significant variation in spore density and root colonization of AM fungi between and among five plant species. The influences of season on these two parameters of AM fungi were not stable but dynamic and therefore no clear pattern was observed. The present study recommends that cultivation practices for conservation and commercial exploitation of ethnomedicinal plants, especially the five species reported in this study may include three species of AM fungi (*A. delicata, G.macrocarpum* and *G.microaggregatum*) in the soil management activities for improving biomass production and growth performance.

KEYWORDS:

Acaulospora delicata, Arbuscular mycorrhizal (AM) fungi, spore density, *Glomus*, ethnomedicinal plants, north eastern India

Introduction

Endomycorrhizal symbiosis is highly interdependent mutualistic relationship between the root tissues of higher plants and a group of fungi where the host plant receives mineral nutrients and water through the network of fungal hyphae in the soil while the fungus obtains plant derived carbon compounds. Endomycorrhiza is the dominant type of mycorrhiza, colonizing approximately 85% of all land plants. Arbuscular mycorrhizal (AM) fungi are group of endomycorrhizal fungi which grows within the tissues of the plant roots by producing a specialized organ, finger like projection called arbuscles which are considered to be involved in exchange of nutrients inside the host cells. These fungi are known to occur in vast majority of land plants and reported to enhance biomass and growth rates, improve stress tolerance and defence mechanisms, stimulate mineral nutrients uptake and provides plants (Chandel, 2016, Smith and Read, 2010, Willmann *et al*, 2013, Zeng *et al*, 2013).

Ethnomedicinal plants play key role in traditional healthcare systems and socioeconomic conditions of the ethnic communities in all the north eastern states of India. These plants are continuously harvested and overexploited from wild habitats for use in traditional healthcare systems as well as source of daily income in most of the rural markets in the region. Additionally, large volumes of many high valued medicinal plants are harvested and used in pharmaceutical industries. These causes decline in natural population of important medicinal plants in their wild habitats and ultimately leads to status of threatened or endangered conditions. Therefore, there is a need for conserving the wild medicinal plants by human intervention through cultivation practices in a sustainable manner. One of the important aspects in the cultivation of wild medicinal is successful establishment in the cultivated fields which largely depends on the application and availability of endomycorhizal fungi as biofertilizers in the soil (Chanda and Sharma, 2012, Chanda *et al*., 2014, Chen *et al*, 2014). In this regard, understanding the diversity and occurrence of mucorrhizal fungi, particularly AM fungi in the rhizosphere of medicinal plants in wild habitats become prerequisite. A number of reports are available on diversity of AM fungi in the rhizosphere soils of medicinal plants from different parts of world and many states of the country (Koul *et al*, 2012, Radhika and Rodrigues, 2010, Rajkumar *et al*, 2012, Sinegani and Yeganeh, 2017, Wang and Jian, 2015). However, there has been scarce reports on AM fungi associated with ethnomedicinal plants of Manipur, north eastern India where a considerable amount of wild medicinal plants consumed in various traditional healthcare systems practiced by local healers known as "*Maibas*" and "*Maibees*" (male and female practitioners). Therefore, the present study investigates the diversity and occurrence pattern of AM fungi distribution in the rhizosphere of five ethnomedicinal plants commonly used in traditional healthcare practices of Manipur.

Materials and methods

Medicinal plants and soil sampling:

The present study was done on five ethnomedicinal plants occurring in two valley districts (Imphal East, Imphal West) and one hill district (Senapati) of Manipur (Table 1). The selected plant species are *Adhatoda vasica* (AV), *Zanthoxylum acanthopodium* (ZA), *Solanum nigrum* (SN), *Blumea balsamifera* (BB) and *Alpinia zerumbet* (AZ). Soil samples (0-30 cm) were collected seasonally from the rhizosphere of selected medicinal plants for a period of two years (2012 to 2014), twice a year based on seasonal period, wet summer seasons (Season 1 and Season 3) and again in the dry winter seasons (Season 2 and Season 4) respectively. Fresh soil samples were processed and used for further analysis of AM fungal population and identification. Plant roots were also collected from the replicate plants and analysed immediately to calculate percentage colonization.

Isolation of AM fungal spores:

Isolation of AM fungal spores was done by modified wet sieving and decanting method adopted by Gerdemann and Nicholson, (1963) and Singh and Tiwari (2001). All the AM fungal spores were counted under

a stereozoom microscope (Carl Zeis) and identified on the basis of morphological characteristic following related standard methods and literature up to species level. The number of AM fungal spores were expressed in triplicate means of soil samples analysed (i.e. number of spores 10 g^{-1} soil).

Analysis of AM fungal colonization and infection

Trypan Blue staining method was followed for analysis of infection and colonization of AM fungi in freshly collected root pieces (Kormaik and McGrew, 1982). The AM fungal infected roots were recorded and the percentage of root colonization was calculated as follows.

$$\text{Root colonization (\%)} = \frac{\text{No. of AM infected root sections x 100}}{\text{Total no. of root sections analysed}}$$

Identification of VAM fungi:

Isolated AM fungi were identified based on the methods adopted by Schenck and Perez (1987) and web resources INVAM (http://invam.wvu.edu) and by observing the characters such as spore morphology (colour, shape and size), wall ornamentation and architecture, mode of hyphal attachment, wall layers, etc.

Statistical Analysis:

The relationship between spore population and colonization of AM fungi was determined by calculating Pearson's correlation coefficient (*r*). The variation in distribution of AM fungal spore population between and among medicinal plant species was calculated using one way analysis of variance (ANOVA).

Results:

Spore population of AM fungi in the rhizosphere soils of five medicinal plants

The spore population of AM fungi varied from a minimum of 27 spores 10 g^{-1} soil in the rhizospehere of *A. vasica* (27) during season 3 to a maximum of 196 spores 10 g^{-1} soil in *Z. acanthopodium* during season 1 (Figure 1). The spore population of AM fungi in the rhizosphere of other medicinal plants have shown intermediate values between *A. vasica* and *Z. acanthopodium* (Figure 1). There was no clear pattern of seasonal variation in distribution of AM fungal spores in the rhizosphere soils of the five medicinal plants athough significant variations were observed among the plant species.

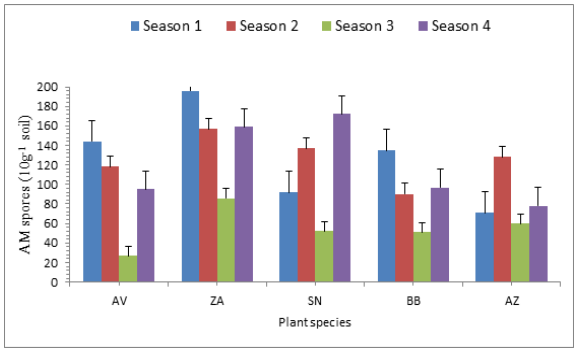

Figure 1. Distribution of AM fungal spore population in the rhizosphere soil of five medicinal plants (T-bars on the histogram represents standard deviation (±SD) of the triplicate mean values).

Percentage colonisation of AM fungal in the roots of five medicinal plants

The AM fungal colonisation was observed highest in the roots of *B. balsamifera* (61.88%) during season 1 and the lowest was observed in the roots of *A. zerumbet* (22.67%) during season 2 (Figure 2). The AM fungal colonization was observed comparatively higher in *S. nigrum* and *B.balsamifera* than other plant species in all seasons. The lowest colonization was recorded from *A. zerumbet* in all the seasons.

There was significant positive correlation between spore population and colonization percentage of AM fungi in the rhizosphere soils of *A. vasica* (r=0.74; $p \leq 0.05$) and *B. balsamifera* (r=0.6; $p \leq 0.05$) while no significant correlation was observed for *Z. acanthopodium, S. nigrum* and *A. zerumbet* in all seasons.

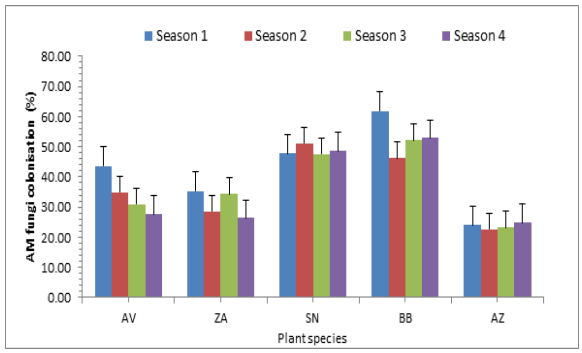

Figure 2. Percentage root colonisation in the roots of the five medicinal plants. (T-bars on the histogram represents standard deviation (±SD) of the triplicate mean values).

Distribution and occurrences of AM fungi in the rhizosphere of medicinal plants:

A total of 20 AM fungal species have been recorded from the rhizosphere soil of the five medicinal plants studied (Table 2). *Glomus* was largest genus with 14 species followed by *Acaulospora* with 3 species. Three other genera, *Gigaspora, Sclerosystis* and *Scutellospora* were recorded with only 1 species each in the present study. The highest number AM fungal species (17) was observed in the rhizosphere soil of *S. nigrum* which was followed by 16 species each in the rhizosphere of *Z. acanthopodium* and *A. vasica* during season 4 (winter). The lowest number of AM fungi species (8) was recorded from the rhizosphere of *A. vasica* during season 3 (second summer). There was a marked variation in number of AM species among the rhizosphere soils of the medicinal plants and between seasons.

The total percentage occurrences of 20 AM fungi in the rhisosphere of five medicinal plants for all seasons are shown in figure 3. *Acaulospora delicata* was most commonly occurring AM fungus with highest (100%) occurrence in the rhizosphere of all plant species and in all seasons. The other AM fungi with higher occurrences were *Glomus macrocarpum* and *G. microaggregatum* (95%) followed by *G.fasciculatum* and *G. mossae* (90%), *G.aggregatum* and *G.claroideum* (85%), etc. The lowest occurrence (10%) was recorded for *Acaulospora capsicula* and *Sclerocystis dussii.*

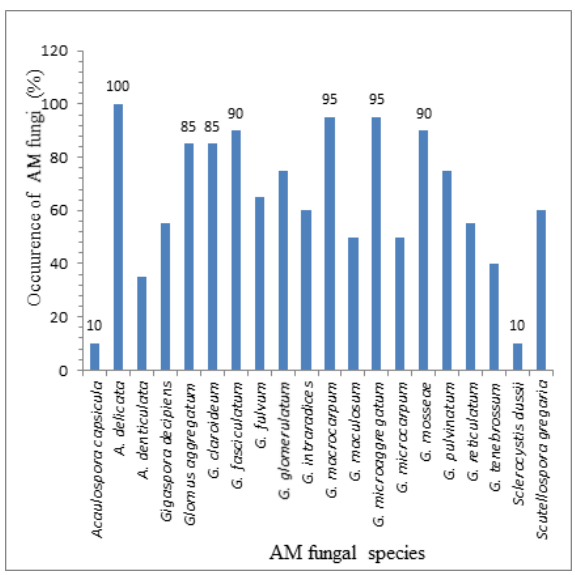

Figure 3. Total percentage occurrences of AM fungi in the rhizosphere of medicinal plants species for all seasons.

The number of AM fungi species was recorded highest in the rhizosphere soil of *B.balsamifera* (20), followed by *S.nigrum* (19) and *A.zerumbet* (18) while lowest of 17 AM fungi species was recorded from the rhizosphere of *A. vasica* and *Z. acanthopodium* respectively.

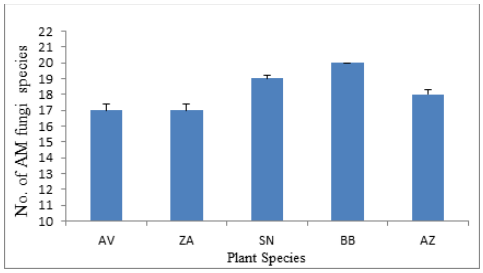

Figure 4. Total number of AM fungi species in the rhizosphere of each medicinal plants species for all seasons. (T-bars on the histogram represents standard deviation (±SD) of the mean values).

Table 3. ANOVA of spore population of AM fungi between and among medicinal plant species for all seasons (N=20).

Parameters	Source of Variations	F-values	p-values	Remarks
All seasons	AV x AZ	0.35	0.56	NS
	AV x BB	0.02	0.90	NS
	AV x SN	0.57	0.46	NS
	AV x ZA	5.31	0.03	S
	AZ x BB	0.34	0.57	NS
	AZ x SN	2.56	0.12	NS
	AZ x ZA	12.20	0.001	S
	BB x SN	1.09	0.31	NS
	BB x ZA	8.15	0.01	S
	SN x ZA	2.74	0.11	NS
	All plants	3.32	0.02	S

One way analysis of variance of the spore populations between and among all plant species for all seasons revealed that there were significant variation between *A. vasica* and *Z. acanthopodium* (F=3.31; $p \leq 0.05$), *A. zerumbet* and *Z. acanthopodium* (F=12.20; $p \leq 0.001$) and between *B.balsamifera* and *Z. acanthopodium* (F=8.15; $p \leq 0.01$) respectively (Table 3). There was significant variation in distribution of spore population among all plants (F=3.32; $p \leq 0.02$).

Discussion

The present study reveals that AM fungi occurs in the root systems as well as in the rhizosphere soils five ethnomedicinal plants. A total of 20 AM fungi were found to be associated these different species medicinal plants despite variations in seasonal distribution of spore population and root colonization pattern among the plant species. Mondal and Dutta (2017) have also reported presence of AM fungi in the rhizosphere soils and roots of medicinal herbs of acanthaceae family in Darjeeling, West Bengal. The spore density reported in this study are higher as compared to other studies (Debnath *et al*., 2015, Mondal and Dutta, 2017, Moreira *et al*, 2006, Sundar *et al*, 2011). The observation of highest spore population in the rhizosphere soils of *Z. acanthopodium*, *A. vasica* and *B.balsamifera* during first summer season suggests possible beneficial effects of increased moisture (water) and high temperature in hyphal growth and sporulation of AM fungi. Similar report of maximum number of AM fungal spores reported in the rhizosphere of many plant species during summer seasons than winter seasons (Setua *et al,* 2001, Chatterjee *et al* , 2010, Gaur and Kaushik, 2012). However, no specific pattern of seasonal variation was observed in this study since spore population of AM fungi was found comparatively lower during second summer season in the rhizosphere of all plants as compared to corresponding winter seasons. These findings are in agreement to the report that water logging in summer season for long time might cause low sporulation since AM fungi are obligately aerobic and therefore flooding will reduces sporulation process (Aziz and Sylvia, 1995). Analysis of variance also revealed significant variation in spore population of AM fungi which suggests distribution and abundance of AM fungi are significantly influenced by plant species irrespective of the seasons. Therefore, variation in the degree of spore density and number of species of AM fungi among plant species might be due to the prevailing micro-environmental conditions in the rhizosphere soils influenced by medicinal plants (Turnau *et al*, 2001).

Colonisation of plant roots by AM fungi also revealed significant impact of plant species rather than seasonal variations. In this study higher percentage of root colonization was recorded in the rhizosphere of two medicinal plants, *B. balsamifera* and *S. nigrum*, followed by *Z. acanthopodium* and *A. vasica* while lowest colonization was recorded in *Alpinia zerumbet* in all four seasons. These findings suggest that different plant species influence AM fungi through the release of diverse phytochemicals in the rhizosphere zones which selectively allows infection of AM fungi in their root systems. The lowest percentage of root colonization in *A. zerumbet* could be the result of presence of toxic phytochemical substances (antimicrobial and antifungals) which inhibits infection and growth of AM fungi in the root tissue. Presence of antimicrobial and antifungal substances (essential oils and other phytochemicals) have been reported in the rhizome and leaves of *A. zerumbet* and other species of the family (Zingiberaceae) could be the primary reason for lower colonization percentage of AM fungi in this plant species (Kader *et at*, 2015, Wong and Omar, 2009,Xuan and Teschke, 2015).

There were significant positive correlation between spore population and percentage root colonisation in two medicinal plants, *A. vasica* and *B. balsamifera* though other three plant species exhibited no positive correlations between the two AM fungal parameters. Wang and Jiang (2015) have also shown no significant positive correlation between spore population and root colonisation in 20 medicinal plants from Southeast China. One of the reasons for insignificant correlation of spore population and percentage colonisation in some plants might be due to reduced sporulation of AM fungi in the soil even after infection in the plant root tissues (Tian *et al*., 2009).

Glomus and *Acaulospora* were the most common groups of AM fungi recorded in this study with highest number of species while *Gigaspora* was least common genus. Similar pattern of highest species distribution of *Glomus* have been reported from the rhizosphere of various plants species across the world (Blaszkowski 1989; Ferdousee *et al*, 2012, Talukdar and Germida 1993, Wang and Jiang, 2015). One of the primary reasons for highest occurrence percentage and species abundance of the members of Glomeraceae could be ecological plasticity of these species that favours them to tolerate nutrient and water stress in disturbed soil systems as well as capacity to neutralize toxic phytochemicals in the rhizosphere of diverse plant species (Jansa *et al*, 2014, Schneider *et al*, 2015, Varela-Cervero *et al*, 2016). Highest percentage occurrence of *Acaulospora delicata* in the rhizosphere soils of the five medicinal plants in all seasons suggests the potential scopes for application of this AM fungus in large scale cultivation of medicinal plants in a sustainable way which otherwise have been focussed on use of few *Glomus* species in the past.

The present study concludes that ethnomedicinal plants in wild habitat are closely associated with different species of AM fungi in both rhizosphere region as well as in the root systems through endomycorrhizal symbiosis. There was significant variation in spore density and root colonization of AM fungi between and among five plant species. The influence of season on these parameters of AM fungi were not stable but dynamic and therefore no clear pattern of impact was observed. The medicinal plant species, *B. balsamifera* and *S. nigrum* harboured highest number of species and percentage root colonization while *A. zerumbet* harbours lower number of species and exhibited lowest percentage of root colonization by of AM fungi. *Glomus* species were dominant AM fungi recorded in this study from all plant species however *A. delicata* was found to occur with highest percentage of occurrence being distributed in the rhizospehere soils of all medicinal plants and in all the seasons. Therefore, cultivation practices for conservation and commercial exploitation may include three species of AM fungi (*A. delicata, G.macrocarpum* and *G.microaggregatum*) in the soil management activities for improving biomass production and growth performance of of ethnomedicinal plants, especially the five species reported in this study.

Table 1. Details of medicinal plants and their collection sites.

S. No.	Name of Plant (Family)	Local Name (*Manipuri*)	Collection Sites	District	Latitude (N)	Longitude (E)	Elev. (m)
1.	*Adhatoda vasica* (Acanthaceae)	Nongmang-kha-angouba	Keithelmanbi	Senapati	24°41′54.19′′	93°43′27.12′′	980
			Langol	Imphal West	24°50′07.93′′	93°54′58.25′′	772.12
			Khumbong	Imphal West	24°45′25.63′′	93°50′08.64′′	771.21

2.	*Zanthoxylum canthopodium* (Rutaceae)	Mukthrubi	Uyumpok	Imphal East	24°55′48.34′′	94°02′47.47′′	794.24
			Keithelmanbi	Senapati	24°41′54.19′′	93°43′27.12′′	980
			Langol	Imphal West	24°50′07.93′′	93°54′58.25′′	772.12
3.	*Solanum nigrum* (Solanaceae)	Leipung-khanga	Keithelmanbi	Senapati	24°41′54.19′′	93°43′27.12′′	980
			Khumbong	Imphal West	24°45′25.63′′	93°50′08.64′′	771.21
			Langol	Imphal West	24°50′07.93′′	93°54′58.25′′	772.12
4.	*Blumea balsamifera* (Asteraceae)	Langthrei	Uyumpok	Imphal East	24°55′48.34′′	94°02′47.47′′	794.24
			Keithelmanbi	Senapati	24°41′54.19′′	93°43′27.12′′	980
			Khumbong	Imphal West	24°45′25.63′′	93°50′08.64′′	771.21
5.	*Alpinia zerumbet* (Zingiberaceae)	Kangngoo/ Kanghoo	Uyumpok	Imphal East	24°55′48.34′′	94°02′47.47′′	794.24
			Keithelmanbi	Senapati	24°41′54.19′′	93°43′27.12′′	980
			Khumbong	Imphal West	24°45′25.63′′	93°50′08.64′′	771.21

Table 2. Seasonal distribution of AM fungal species in the rhizosphere soil of five medicinal plants

S. No.	VAM Species	*A. vasica*				*Z. acanthopodium*				*S. nigrum*				*B. balsamefera*				*A. zerumbet*			
		S1	S2	S3	S4	S1	S2	S3	S4	S1	S2	S3	S4	S1	S2	S3	S4	S1	S2	S3	S4
1	*Acaulospora capsicula*	−	−	−	−	−	−	−	−	−	−	+	−	−	−	+	−	−	−	−	−
2	*A. delicata*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	*A. denticulata*	−	+	−	+	−	−	−	−	−	−	−	+	+	−	+	+	−	+	−	−
4	*Gigaspora decipiens*	−	−	+	+	−	−	−	+	−	+	+	+	+	−	+	−	+	−	+	+
5	*Glomus aggregatum*	+	+	+	+	−	+	−	+	+	+	+	+	−	+	+	+	+	+	+	+
6	*G. claroideum*	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−
7	*G. fasciculatum*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	−	+	+
8	*G. fulvum*	+	+	−	+	−	+	−	+	+	+	−	+	+	+	−	−	+	+	+	−
9	*G. glomerulatum*	+	+	+	+	+	−	−	+	+	−	+	+	+	+	+	−	+	+	+	−
10	*G. intraradices*	+	+	−	+	+	−	+	+	+	−	−	+	−	+	−	+	+	−	+	−
11	*G. macrocarpum*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+
12	*G. maculosum*	−	−	−	−	−	+	+	+	−	+	+	+	−	−	+	+	−	−	+	+
13	*G. microaggregatum*	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
14	*G. microcarpum*	−	+	−	−	+	+	+	−	−	+	+	−	−	+	−	−	+	+	+	−
15	*G. mosseae*	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+
16	*G. pulvinatum*	+	+	−	+	+	+	−	+	+	−	+	+	+	−	−	+	+	+	+	+
17	*G. reticulatum*	−	−	−	+	+	−	+	+	+	−	−	+	+	−	−	+	+	−	+	+
18	*G. tenebrossum*	−	−	−	+	−	+	−	+	−	−	+	+	+	−	−	+	−	−	−	+
19	*Sclerocystis dussii*	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+	−	−	−	−
20	*Scutellospora gregaria*	−	−	−	+	−	−	+	+	−	−	+	+	−	+	+	+	+	+	+	+
	No. of species	10	12	8	16	11	12	11	16	12	11	15	17	14	12	12	14	14	12	15	12

[Presence of AM fungal species (+) and absence (-)]

REFETRENCES

1. Aziz, T. and Sylvia, D.M. 1995. Activity and species composition of arbuscular mycorrhizal fungi following soil removal. Ecological Applications, 5(3): 776–784.
2. Blaszkowski, J. 1989. The occurrence of the Endogonaceae in Poland. Agril Ecosy Envirn 29: 45-50.
3. Chanda, D. and Sharma, G.D. 2012. Arbuscular Mycorrhiza in the cultivation of medicinal plants, In: Researches in Medicinal and Aromatic Plants (Eds. Choudhury, M.D., Sharma, G.D., Talukdar, A.D. and Choudhury, S.), Swastik Publications, New Delhi
4. Chanda, D, Sharma G. D, Jha D. K. 2014. The Potential Use of Arbuscular Mycorrhiza in the Cultivation of Medicinal Plants in Barak Valley, Assam: A Review. Current World Environment, 9 (2). 544-551.
5. Chandel, S. 2016. Diversity Status of Arbuscular Mycorrhizal (AM) Fungi from Rhizospheric Soils of Medicinal and Aromatic Plants in Himachal Pradesh. The Indian Forester, 142(7).
6. Chen, Y.L., Li, J.X., Guo, L.P., He, X.H. and Huang, L.Q. 2014 Application of AM Fungi to Improve the Value of Medicinal Plants. In: Mycorrhizal Fungi: Use in Sustainable Agriculture and Land Restoration (Eds. Solaiman, Z.M., Abbott, L.K. and Varma,A), Springer-Verlag Heidelberg, pp171-187.
7. Chatterjee, S., Chatterjee, S. and Dutta, S. 2010. A survey on VAM association in three different species of Cassia and determination of antimicrobial property of these phytoextracts. Journal of Medicinal Plants Research, 4(4): 286-292.
8. Debnath, A., Karmakar, P., Debnath, S., Roy Das, A., Saha, A. K. and Das, P. 2015. Arbuscular mycorrhizal and dark septate endophyte fungal association in some plants of Tripura, North-East India. Current Research in Environmental & Applied Mycology, 5 (4): 398–407.
9. Ferdousee, N., Misbahuzzaman, K. and Rafiqul Hoque, A. T. M. 2012. Arbuscular Mycorrhizal Colonization in Five Tropical Forest Tree Legumes of Chittagong University Campus in Bangladesh. Journal of Basic & Applied Sciences 8: 353-361.
10. Gaur, S. and Kaushik, P. 2012. Effect of Seasonal Variation on Mycorrhizal Fungi Associated with Medicinal Plants in Central Himalayan Region of India. American Journal of Plant Sciences, 3: 618-626.
11. Gerdemann, J.W. and Nicolson, T. H. 1963. Spores of mycorrhizal Endogone species extracted from soil wet sieving and decanting. Transactions of British Mycological Society, 46: 235-244.
12. International Culture Collection of Arbuscular & Vesicular-Arbuscular Mycorrhizal Fungi (INVAM). http://invam.wvu.edu.
13. Jansa, J., Erb, A., Oberholzer, H. R., Smilauer, P. and Egli, S. 2014. Soil and geography are more important determinants of indigenous arbuscular mycorrhizal communities than management practices in Swiss agricultural soils. Mol. Ecol, 23: 2118-2135.
14. Kader, G., Nikkon, F., Rashid, M. A. and Yeasmin, T. 2011. Antimicrobial activities of the rhizome extract of Zingiber zerumbet Linn. Asian Pac J Trop Biomed, 1(5): 409-412.
15. Koul, K.K., Agarwal, S. and Rafiq, L. 2012. Diversity of Arbuscular Mycorrhizal Fungi Associated With the Medicinal Plants from Gwalior-Chambal Region of Madhya Pradesh-India. American-Eurasian J. Agric. & Environ. Sci, 12 (8): 1004-1011.
16. Kormaik, P. P. and McGrew, A. C. 1982. Quantification of Vesicular-arbuscular Mycorrhizae in Plant Roots. In Methods and Principles of Mycorrhizal Research. Ed. N.C. Schenck. The American Phytopathological Society, pp. 37-36.
17. Mondal, T and Dutta, S. 2017. Study of arbuscular mycorrhizal association in some medicinal herbs of Acanthaceae family from Darjeeling district, West Bengal, India. Int J Pharm Bio Sci. 8(1): 137–142.
18. Moreira, M., Baretta, D., Tsai, S. M. and Cardoso, E. J. B. N. 2006. Spore density and root colonisation by arbuscular mycorrhizal fungi in preserved or disterved Araucaria angustifolia (Bert.) O. Ktze. Ecosystem Sci. Agric. (Piracicaba, Braz.), 63(4): 380-385.
19. Radhika, K. P. and Rodrigues, B. F. 2015. Arbuscular mycorrhizal fungal diversity in some commonly occurring medicinal plants of Western Ghats, Goa region. Journal of Forestry Research, (2010) 21(1): 45–52.
20. Rajkumar, H. G., Seema, H. S and Kumar, C. P. S. 2012. Diversity of arbuscular mycorrhizal fungi associated with some medicinal plants in Western Ghats of Karnataka region, India. World Journal of Science and Technology, 2(1):13-20.
21. Setua, G.C., Ghosh, J.K., Das, N.K. and Saratchandra, B. 2001. Response of direct inoculation of VAM on growth, leaf yield and phosphorus uptake in Mulberry (Morus alba). Indian J Agric Sci, 69: 444-448.
22. Schneider, K. D., Lynch, D. H., Dunfield, K., Khosla, K., Jansa, J. and Voroney, R. P. 2015. Farm system management affects community structure of arbuscular mycorrhizal fungi. Appl Soil Ecol, 96, 192-200.
23. Schenck, N.C. and Perez, Y. 1987. A manual for identification of Vesicular-arbuscular mycorhizal fungi. INVAM, University of Florida, Gainesville.
24. Sinegani, A.A.S. and Yeganeh, M.E. 2017. The occurrence of arbuscular mycorrhizal fungi in soil and root of medicinal plants in Bu-Ali Sina garden in Hamadan, Iran, Biological Journal of Microorganism, 5(20) : 43-59
25. Singh, S.S. and Tiwari, S.C. 2001. Modified wet sieving and decanting technique for enhanced recovery of spores of vesicular-arbuscular mycorrhiza (VAM) fungi in forest soilos. Mycorrhiza News, 12:12-13.
26. Smith, S.E. and Read, D.J. 1997. Mycorrhizal Symbiosis, New York: Academic Press.
27. Sundar, S. K., Palavesam, A. and Parthipan, B. 2011. AM Fungal Diversity in Selected Medicinal Plants of Kanyakumari District, Tamil Nadu, India. Indian J Microbiol, 51(3):259–265.
28. Talukdar, N. C. and Germida, J. J. 1993. Occurrence and isolation of vesicular–arbuscular mycorrhizae in cropped field soils of Saskatchewan, Canada. Can J Microbiol, 39: 567-575.
29. Tian, H., Gai, J.P., Zhang, J.L., Christie, P. and X.L. Li, X.L. 2009. Arbuscular mycorrhizal fungi associated with wild forage plants in typical steppe of eastern Inner Mongolia. European Journal of Soil Biology, 45(4): 321–327.
30. Turnau, K., Ryszka, P., Gianinazzi-Pearson, V. and Van Tuinen, D. 2001. Identification of arbuscular mycorrhizal fungi in soils and roots of plants colonizing zinc wastes in southern Poland. Mycorrhiza, 10(4): 169–174.
31. Varela-Cervero, S., Lopez-García, A., Barea, J. M. and Azcon-Aguilar, C. 2016. Differences in the composition of arbuscular mycorrhizal fungal communities promoted by different propagule forms from a Mediterranean shrubland. Mycorrhiza, 26: 489-496.
32. Wang, M and Jiang, P. 2015. Colonization and Diversity of AM Fungi by Morphological Analysis on Medicinal Plants in Southeast China. The Scientific World Journal, Volume 2015, Article ID 753842, 7 pages
33. Willmann, M., Gerlach, N., Buer, B., Polatajko, A., Nagy, R., Koebke, E., Jansa, J., Flisch, R., and Bucher, M. 2013. Mycorrhizal phosphate uptake pathway in maize: Vital for growth and cob development on nutrient poor agricultural and greenhouse soils.

Front. Plant Sci, 4: 533.
34. Wong, L. F., Lim, Y. Y. and M. Omar, 2009. Antioxidant and Antimicrobial activities of some Alpinia species. Journal of Food Biochemistry, 33: 835–851.
35. Xuan, T. D. and Teschke, R. 2015. Dihydro-5,6-dehydrokavain (DDK) from Alpinia zerumbet: Its Isolation, Synthesis, and Characterization. Molecules, 20: 16306-16319.
36. Zeng, Y., Guo, L.P., Che, D.B., Hao, Z.P., Wang, J.Y., and Huang, L.Q. 2013. Arbuscular mycorrhizal symbiosys and active ingredients of medicinal plants: Current research status and prospectives. Mycorrhiza, 23: 253–265.