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Distribution and Diversity of AM Fungi in the Rhizospheric Soils of Naturally and Artificially Growing *Aquilaria malaccensis* Lamk. Trees in Arunachal Pradesh and Assam States of North East India

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ABSTRACT

An investigation was conducted in five locations of *Aquilaria malaccensis* Lamk. growing areas of Arunachal Pradesh and Assam, north-eastern India to determine distribution and diversity of symbiotic arbuscular mycorrhizal (AM) fungi in relation to physico-chemical properties of rhizospheric soil. The results showed a well distributed (qualitatively and quantitatively) AM fungal associates in the soils supporting *A. malaccensis* trees as well as in their roots. Chemical properties of soils reveal that all the locations were moderately acidic in nature. The soil samples from naturally grown sites in Golaghat, Assam were recorded with highest in moisture content (22.5%) and nitrogen (3.21%) percentage whereas lowest in phosphorus (0.54%) and potassium (25.9 ppm) contents however, association of maximum number of AM spores 887/100g of soil as compare to commercially cultivated location of Hojai, Assam with average physico-chemical properties harbouring minimum number of AM spores (448)/100g of soil. A total of 20 known different AM fungal species belonging to five genera viz; *Acaulospora* (2 species), *Gigaspora* (3 species), *Glomus* (10 species), *Sclerocystis* (2 species) and *Scutellospora* (1 species) were recorded with few unknown genera. *Glomus* species was most dominating species, of which *G. fasciculatum* individually contributed the maximum percentage (15.3%) of the total AM fungi recorded during the whole study period. Similarly, AM fungal colonization in roots were also maximum in the natural forest of Golaghat (92.80%) as compare to Nirjuli A (81.60%), Nirjuli B (74.40%) and Itanagar (52.80%) whereas recorded lowest in plantations of Hojai (42.40%) in AM root colonisation of *A. malaccensis.* Presence or absence of AM hyphae, arbuscules and vesicle in the root samples varied with area to area. The Jaccard index of similarity was high in sites located geographically in different region [Itanagar to Golaghat (0.421) & Hojai (0.433)] while same site and region has much lower. The percentage contribution of AM families, Simpson's dominance and Shannon diversity index were calculated as highest in Glomeraceae as compared to other families.

Keywords: *Aquilaria malaccensis,* AM fungi, distribution, diversity, different locations

INTRODUCTION

Northeast India, a part of eastern Himalayas are covered with tropical evergreen, subtropical, temperate, and alpine forests and known as store house for biodiversity. Many important tree species were distributed under natural conditions (Bhuyan et al., 2003). *Aquilaria malaccensis* is one of the most valued tree species grows in mixed forest habitat of the foothills Arunachal Pradesh and Assam. The tree occurs in the rain forest of north east India (Barden et al., 2000; IUCN, 2002; Donovan and Puri. 2004) and distributed naturally particularly in Arunachal Pradesh. Current levels of exploitation for raw materials may lead it to the threat of extinction. The trees have been the principle

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source of Agarwood products traded internationally.

Plant community is determined by AM (arbuscular mycorrhizal) association and AM fungi are essential for any community (Francis and Read 1994). AM colonization and spore density also differ with season, edaphic conditions, and plant species (Ghosh and Verma 2002, 2004). The association of AM fungi with tree species have been well accounted. Arbuscular mycorrhizal (AM) are important symbiotic fungi of natural eco-system and develop mutualistic relationship with plant community (Bever et al., 2002) of medicinal and aromatic plant (Gupta et al., 1994/95; Soni and Vyas, 2007). Diversity and spore abundance of mycorrhizal fungi in soils of forest plants (Chris 2000; Schalamuk et al., 2006) were highly dependent on symbiosis nature, broad association (Harley and Smith 1983) and geographical variations. Some mycorrhizal communities are site specific and each mycorrhizal community were affected by nature of ecosystems (Eom et al., 2000).

Knowledge about the presence and diversity of arbuscular mycorrhizal fungi (AMF) in aquilaria tree under natural condition is the first essential step for utilizing these fungi in any application. Since mycorrhizal fungi colonized the root of host plant and help to obtain essential nutrients like N, P, K, Ca etc (Panwar and Vyas. 2002) from the surrounding soil to enhance plant growth and development (Abbott and Robson. 1984). AM fungi were not only act as biofertilizers but they also protect against root pathogens and environment degrading agents. The present study deals with distribution, percentage of occurrence contributed by individual specie (% O), Shannon diversity (H) and index of dominance (C) of different species of mycorrhizae in the vicinity of aquilaria plant in different region in relation to physioco-chemical properties of rhizospheic soil.

In the absence of spatial data on the distribution and abundance of AM fungal species, it is difficult to assess the prospect of conservation of aquilaria in the immediate future.

MATERIALS AND METHODS

Five locations such as Golaghat, Hojai, Itanagar, Nirjuli A and Nirjuli B were selected from north east India to study physico-chemical properties and AM fungi distribution in the rhizospheric soil of *Aquilaria malaccensis* tree. The soil along with root samples was collected from a depth of 10–15 cm in the month of September 2005 from two locations of Assam and in the month of October 2006 from three locations of Arunachal. It is packed in sterile poly bag, well levelled and brought to the lab for analysis. Fine lateral root of each site were graded out, washed with tape water and preserved in FAA solution for further analysis of root infection. Various physicochemical properties like pH, EC, moisture content, organic carbon, total nitrogen, available phosphorus and available potassium of the rhizospheric soil of the trees were analysed following the standard methods of Gupta (2004)*. AM spores were recorded from each 100g composite soil samples by following the wet sieving and decanting methods (Gerdemann and Nicolson, 1963). The spores were picked up by needle/brush on glass slide, counted, separated and mounted in glycerine for identification. The spores were observed in light microscope 100X and identified using reference cultures from the International Collection of Vesicular and Arbuscular Mycorrhizal Fungi (INVAMF) (Morton et al., 1993) and species description of Schenck and Perez (1990) and recent literature available in the internet. The percent of root length colonized by AM fungi was determined using methods of Gehring et al. (2002). To compare different study sites, the Jaccard index of similarity (Jaccard 1912) was calculated from the morphological determination of spores:

$IS = c/(a+b+c)$

where a is the number of species occurring only at site A, b is the number of species occurring only at site B, and c is the number of species occurring at both site A and site B.

The percentage of occurrence contributed by individual specie (%), Shannon diversity (H) and index of dominance (C) of species are determined using following formula:

$$\% \text{ contribution} = \frac{\text{Total count of individual AM fungi over 1 month/year}}{\text{Total fungal count over 1 month/year}} \times 100$$

$H = -\sum (ni/N)) \log (ni/N)$

$C = \sum (ni/N)^2$

Where, ni = total number of individual species,
N= Total number of all species.

RESULTS AND DISCUSSION

The physico-chemical properties of soil differ significantly with the locations. In all the locations, pH value was found to be acidic which give rise to low electrical conductivity (EC) of the soil. The pH value and EC of soil collected from Golaghat, a undisturbed protected forest was much acidic (pH-5.2) and lower (EC-0.02mm ohs/cm) as compare to soils of other locations disturbed for cultivation. However, percent moisture content (22.5%), organic carbon (1.23%) and total nitrogen (3.21%) in the soil of Golaghat were at par with other locations. The level of available phosphorus and potassium were better in soil disturbed for cultivation. Phosphorus was highest in Nirjuli A (1.38%) followed by Nirjuli B (1.36%), Itanagar (0.90%), Hojai (0.62%) and lowest in Golaghat (0.54%) but potassium was recorded better in Hojai (44.4 ppm) followed by 37.8 in Nirjuli B, 36.3 in Nirjuli A, 27.3 in Itanagar and lowest of 25.9 ppm in Golaghat. The AM fungi of all the locations showed variables with maximum of 887 spores/ 100g in Golaghat (natural vegetation) followed by Nirjuli A (634), Nirjuli B (621), Itanagar (518) and lowest in Hojai (448). (Table 1)

A total of 21 distinct spore types were recorded from the rhizospheric soils of five different locations, a few similar species that was difficult to distinguish were round up to particular genus (Plate1). In an individual representation of species, except for *Glomus fasciculatum, G. macrosporum, Sclerocystis clavispora* and *Scutellospora coralloidea* all others species were either found or missing in one or other locations showing wide variation between the locations. The highest individual percent was recorded in *G. fasciculatum* (15.3%) much higher than other species except for *G. aggregatum* (10.3 %) which got 5.0 % behind and lowest was recorded in *Sclerocystis sinuosa* (1.0 %). All the location shows the presence of AM spores where the highest was recorded in Golaghat with total No. of 868 spores and lowest in Hojai with 436 (Fig.1 and Table 2). However, all AM species recorded belongs to five families viz; *Acaulosporaceae, Gigasporaceae, Glomeraceae, Sclerocystaceae* and *Scutellosporaceae*. Of which family *Glomeraceae* with 71.89 % is dominating followed by *Gigasporaceae* (9.34 %), *Sclerocystaceae* (7.04 %), *Scutellosporaceae* (4.52 %) and *Acaulosporaceae* (4.84 %). The Glomeraceae was having highest percent contribution of 0.7189 %, Simpson's dominance of 0.5168 and Shannon diversity of 0.103 followed by Gigasporaceae, Sclerocystaceae,

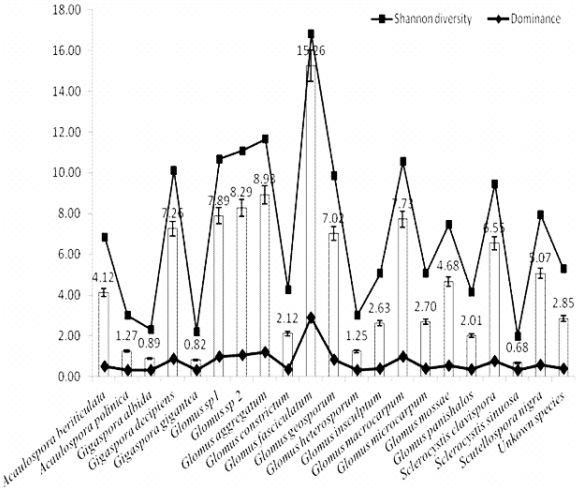

Fig 1: Contribution (%), Dominance and Shannon diversity of AM species (Vertical bar represent Standard Error)

Table 1: Physico-chemical properties and AM fungi of the rhizospheric soil of *Aquilaria malaccensis* tree

Treatments	Arunachal Pradesh			Assam	
	Itanagar	Nirjuli A	Nirjuli B	Golaghat	Hojai
pH	5.7±0.12	6.0±0.17	6.3±0.25	5.2±0.20	5.5±0.29
EC (mm ohs/cm)	0.04±0.02	0.07±0.01	0.09±0.01	0.01±0.00	0.02±0.00
Moisture Content (%)	12.4±0.69	15.1±1.13	14.7±0.90	22.5±1.20	17.1±0.52
Organic Carbon (%)	0.42±0.06	0.64±0.00	0.65±0.00	1.23±0.22	0.97±0.15
Total N (%)	1.29±0.12	2.72±0.52	2.80±0.36	3.21±0.27	1.10±0.21
Available P (%)	0.90±0.10	1.38±0.22	1.36±0.26	0.54±0.05	0.62±0.08
Available K (ppm)	27.3±1.23	36.3±0.90	37.8±0.55	25.9±0.92	44.4±1.23
Number of AM spores per 100 g rhizospheric soil ± SE (n-5)	518±9.01	634±8.66	621±7.37	887±8.71	448±7.75

Table 2: AM fungal species identified from the rhizospheric soil of *Aquilaria malaccensis* tree in different part of Arunachal Pradesh and Assam

AM species	Arunachal Pradesh			Assam		Species %
	Itanagar	Nirjuli A	Nirjuli B	Golaghat	Hojai	
Acaulospora beriticulata	18±2.0	28±6.5	22±3.2	25±5.6	-	3.0
Acaulospora polinica	-	-	-	34±3.6	25±2.0	2.0
Gigaspora albida	-	-	-	40±5.8	-	1.3
Gigaspora gigantea	37±4.7	-	-	-	-	1.2
Gigaspora margarita	-	50±4.6	44±5.7	71±8.3	48±4.1	6.9
Glomus sp1	51±7.7	33±6.8	41±6.5	88±5.7	-	6.9
Glomus sp 2	-	32±3.3	46±7.1	95±4.2	63±7.0	7.6
Glomus aggregatum	82±8.1	78±7.4	67±5.2	94±4.9	-	10.3
Glomus constrictum	-	49±5.7	46±7.1	-	-	3.1
Glomus fasciculatum	94±4.6	99±2.9	97±7.8	105±3.1	79±3.3	15.3
Glomus geosporum	45±3.2	62±6.4	61±4.7	-	-	5.4
Glomus heterosporum	-	-	-	56±7.5	-	1.8
Glomus insculptum	33±2.6	43±5.6	42±9.1	-	-	3.8
Glomus macrocarpum	46±8.1	57±6.7	53±4.1	-	48±5.9	6.6
Glomus microcarpum	-	-	-	68±7.7	53±4.9	3.9
Glomus mossae	-	-	-	88±7.2	49±7.3	4.4
Glomus panishalos	34±3.8	26±3.6	30±5.3	-	-	2.9
Sclerocystis clavispora	40±7.5	33±4.9	32±4.1	44±6.7	39±4.5	6.1
Sclerocystis sinuosa	-	-	-	30±5.6	-	1.0
Scutellospora coralloidea	26±5.5	25±2.7	26±4.2	32±5.9	32±4.2	4.5
Unkown species	12±1.6	19±3.0	14±1.7	17±2.6	12±1.1	2.4
Total	506	615	607	868	436	
± SE (n-5)						

Scutellosporaceae and Acaulosporaceae. The percent member of the family Glomeraceae was dominant (71.89 %), followed by Gigasporaceae (9.34 %), Sclerocystaceae (7.04 %), Scutellospraceae (4.52 %), Acaulosporaceae (4.84 %) and Unknown species (2.38 %). (Table 3)

The data in table 4 shows presence or absence of hyphae, arbuscular, vesicle and percent root colonization. The hyphae and arbuscules structures of AM fungi were either present or absent in the root segments of randomly selected samples of each location, however, the presence of vesicles were

Table 3: Percent occurrence contribution, Dominance index and Shannon diversity of AM fungi family in the rhizosperic soil of *A. malaccensis*

Families	Contribution (%)	Simpson's Dominance	Shannon Diversity (H)	%
Acaulospraceae	0.0484	0.0023	0.064	4.84
Gigasporaceae	0.0934	0.0087	0.096	9.34
Glomaraceae	0.7189	0.5168	0.103	71.89
Sclerocystaceae	0.0704	0.0050	0.081	7.04
Scutellospraceae	0.0452	0.0020	0.061	4.52
Unknown species	0.0238	0.0006	0.039	2.38

Table 4: Extent and colonization of AM fungi in the roots of *Aquilaria malaccensis* in different locations

Locations	Hyphal	Arbuscules	Vesicles	Root Colonization (%)
Golaghat	+	±	+	92.80
Hojai	±	±	+	42.40
Itanagar	+	-	+	52.80
Nirjuli A	+	-	+	81.60
Nirjuli B	+	-	+	74.40

(-) Absence, (+) Present and (±) Present or Absence

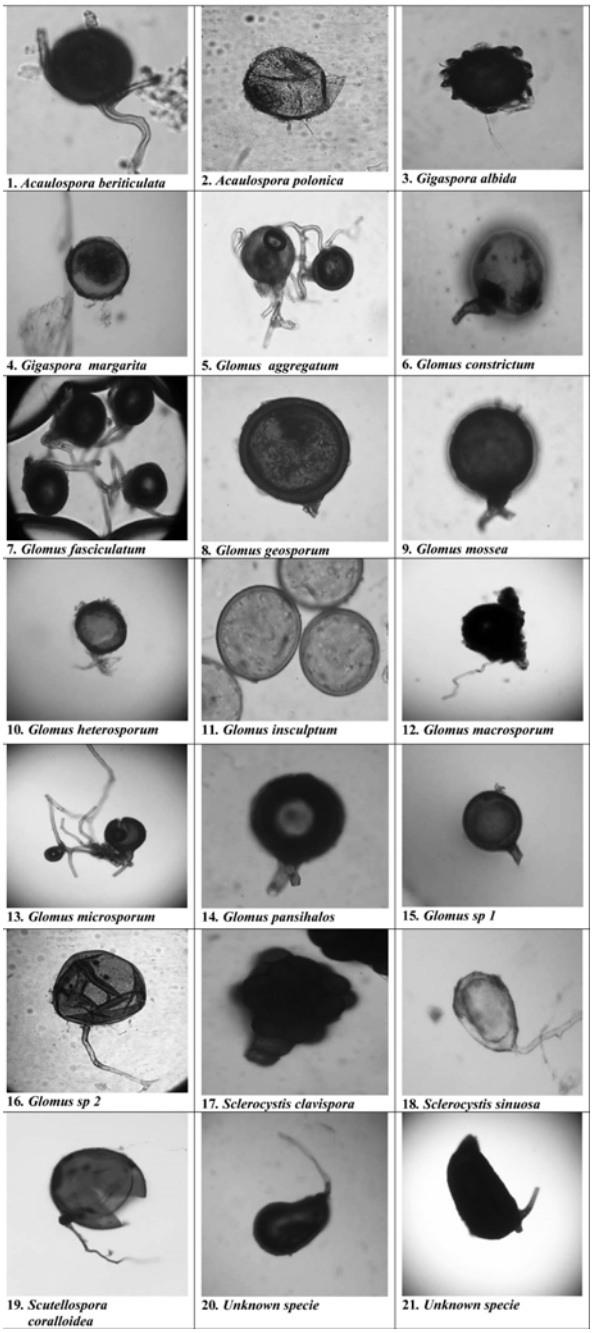

Plate 1: AM fungi recorded from different locations of Assam and Arunachal Pradesh

found in the soil of all the locations irrespective of different geographical region. The entire samples collected from different locations showed AM fungi infections on the roots of *A. Malaccensis* but percentage of colonization were found differ among the locations. The highest percent of root colonization was recorded in Golaghat (92.80%) followed by Nirjuli A (81.60%), Nirjuli B (74.40 %), Itanagar (52.80 %) and least with Hojai (42.40%).

The study sites located in the same geographical region have a comparable Jaccard index of similarity (Table 5). Nirjuli A-B has an ISJ of 0.333, while Itanagar-Nirjuli A and Itanagar-Nirjuli B have 0.297 each. Comparably, area located in different geographical region have low index of similarity. Itanagar-Golaghat showed lowest index of similarity (0.185) as compare to others. It may be due to location of Itanagar at higher altitude than the Golaghat. A notable exception of same geographical region of Golaghat-Hojai that has an ISJ of 0.265 was much lower as that calculated for other same region.

Table 5: Jaccard index of similarity for spores of AM fungi between two locations

Origin	Index of similarity
Itanagar-Nirjuli A	0.297
Itanagar-Nirjuli B	0.297
Itanagar-Golaghat	0.185
Itanagar-Hojai	0.206
Nirjuli A-Nirjuli B	0.333
Nirjuli A-Golaghat	0.226
Nirjuli A-Hojai	0.237
Nirjuli B-Golaghat	0.226
Nirjuli B-Hojai	0.237
Golaghat-Hojai	0.265

The results showed that variability in soil physicochemical properties of each location affect the species distribution and diversity of AM fungi. Its diversity has direct or indirect impact on the root colonization. Further analyses were needed to see the effect of AM fungi on the growth of *A. malaccensis* tree species.

The data presented supports the availability of AM fungi in the rhizosphere soil of *Aquilaria malaccensis* in the different locations of northeast India. It appears as a regular component of the soil microflora but there were differences in the species distribution, diversity and root colonization percent among the location. The variability in the distribution of AM fungi from 488 spores/100g soil of Hojai to 777 spores/100g of Golaghat within Assam as compared to different location of Arunachal Pradesh might be the result of disturbances cause to the land through manipulation and chemical fertilization in Hojai that leads low fertility and acidic reaction in the soil whereas natural forest condition preserved in Golaghat might have influence more on AM fungi population.

The variability in AM fungi in the rhizosphere soils of many medicinal plants under different sites/soil condition were also reported (Gupta and Janardhanan 1991; Zhao et al. 2001). This is also agreement with the observations made on some natural forest tree of other species (Raman and Sambandan, 1998; Wubeta et al. 2003). The diversity in AM fungi communities within location of each rhizospheric soil of *A. malaccensis* tree also varies depending upon the magnitude of disturbance and the physicochemical properties of soil such as soil pH (Nibha et al., 2003), EC, moisture content, organic carbon, total nitrogen (Beena et al., 2000), phosphorus levels (Selvaraj et al., 2001), potassium levels and vegetation cover etc of the locations (Tholkappian, 1986; Claudia et al., 2004; Elisabeth et al., 2004; Schalamuk et al., 2006). In recent years several studies have shown the harmful effects of volatile substances and chemical residue on microbial diversity and activity in soil (Koske, 1987; Del Val et al., 1999.) causing changes in the soil properties through acidification, which increases the availability of metal in the soil solution to toxic levels for long periods. The soil degradation usually produces changes in the diversity and abundance of AMF populations (Jasper, D. A.; Abott, L. and Robson, A. D. 1991). The effects of low P to AM fungi population in the soil of economically important plants have been well explained (Huat et al., 2002). Other ecological factors like seasonality, host specific and sporulation capability of the AM fungi and root exudation of *A. malaccensis* could have also affected distribution and diversity of AM fungi (Guadarrama and Alvarez-Sanchez 1999; Buee et al., 2000).

Most of the identified AM fungi species in the northeast states belonged to *Acaulosporaceae, Gigasporaceae, Glomeraceae, Sclerocystaceae* and *Scutellosporaceae* families where Glomeraceae was dominating in all the locations. The dominance of Glomeraceae in other medicinal plants was also reported (Shi et al., 2006; Younes et al., 2006; Soni and Vyas, 2007). Among the identified species *Glomus fasciculatum, G. macrosporum, Sclerocystis clavispora* and *Scutellospora coralloidea* were common in all the locations. However, Glomus species was dominating in an individual representation. Val et al., (1999) reported that the host plants exerted a differential effect on AMF diversity especially Glomus species in their rhizospheric soil. This may be the reason for Glomus species dominance. The highest AM spores in Golaghat and lowest in Hojai might be due to the reason best known for natural forest system existing in Golaghat and land over manipulation by human in Hojai. The AM fungal communities belonging to Glomeraceae family have shown percent contribution of 0.6923%, Simpson's dominance index of 0.4792 and Shannon diversity of 0.103. The AM fungal diversity was higher in Golaghat than in other locations which were similar for dominance and percent contribution too. The edaphic features of the soil might be the limiting factors in other locations which may have resulted in poor species diversity of AM fungi (Beena et al., 2000). These results support the theory of reduction of AM fungal propagation under severe disturbance which affects the natural plant community structure, leading to the AM fungi instability (Beena et al., 2000)

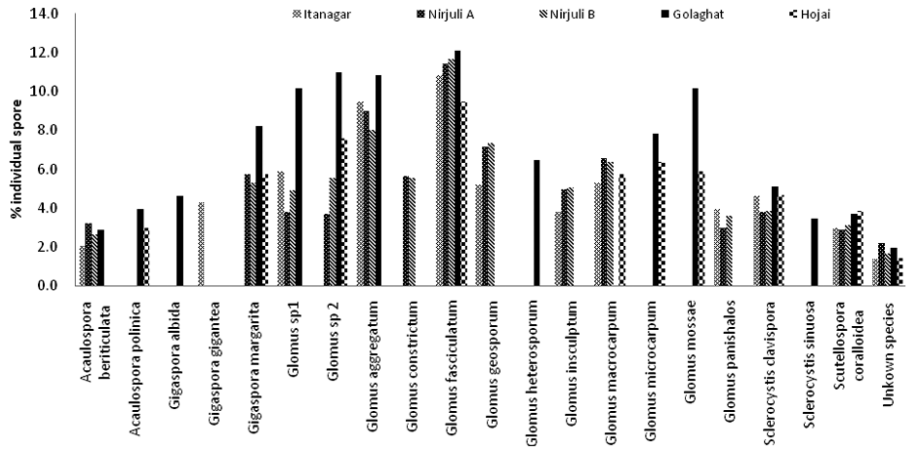

Fig 2: Percent individual spore of AM fungi in different location of Arunachal Pradesh and Assam

The occurrence of arbuscules and hyphal were lower than that of vesicles. However, percent root colonization of AM fungi of was different between the locations. Among the locations, Golaghat showed maximum colonization (92.80%), representing 13 types of AM fungal species. This difference in AM fungal root colonization may be attributed due to the quantity, type of AM strains and increasing age of the plant (Sylvia, 1986 Chandra & Jamaluddin, 1995). Allen et al (2003) have pointed out that AMF root colonization is mediated by interspecific fungal interactions, such as competition, antagonism and dominance.

According to the Jaccard index of similarity, all locations have different communities of AMF spores Uhlmann et al., (2004) states that for AM communities belonging to the different location, the Jaccard index is usually between 0.25 and 0.5. Keeping this in mind, the indices of similarity seems to be surprisingly low, but as the number of samples is too small to deal adequately with the distribution of AM spores, the indices of similarity probably have to be considered as low.

In the present study, the edaphic features of the soil and the structure of natural plant community of different locations might be the reason for AM fungi instability that leading to poor species diversity of AM fungi in the region.

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