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ORIGINAL RESEARCH ARTICLE

Comparative assessment of arbuscular mycoorhizal fungi (AMF) associated with *Oroxylum indicum* L. (Kurz.)- an ethno-medicinal plant of N. E. India.

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Abstract: *Oroxylum indicum* L. (Kurz.) is an ethno-medicinally important plant of North East India. In the present investigation, a comparative study was conducted to assess the AM fungal associations of *Oroxylum indicum* (L.) Kurz. each at two sampling locations of Jorhat district, Assam and Mon district of Nagaland, N. E. India. Comparatively higher root colonization (100±0) and maximum spore population (1239±13.4) were observed in the rhizospheric samples of Mon district, Nagaland, the region which is geographically more distinct and diverse than the other study location. The rhizospheric samples of Jorhat district showed relatively lower spore count and AM colonization. The Shannon-Weinner and Simpson diversity indices were measured as maximum (2.935 and 0.941 respectively) in samples of Mon district, Nagaland. The results of the present investigation indicated the effect of geographical variations, environmental selection as well as alterations of edaphic factors in changing the AMF populations associated with *Oroxylum indicum,* an ethno-medicinally important plant of NE India.

Key words: AM fungi; edaphic factors; ethno-medicinal plant; *Oroxylum indicum*; rhizosphere

Introduction

Mycorrhiza is the symbiotic association established in between the fungal hyphae and the roots of vascular plants. This is the most popular symbiotic associations reported among the soil microorganisms and the plant roots. According to Singh *et al.,* (2011) about 90% of the vascular plants are reported for the existence of mycorrhizal fungi. Ectomycorrhiza, ericoid mycorrhiza, arbutoid mycorrhiza, monotropoid mycorrhiza, orchid mycorrhiza, arbuscular mycorrhiza and ectendomycorrhiza etc. are the recently recognized mycorrhizal types (Bhattacharyya and Jha, 2015; Smith and Read, 2008). Arbuscular mycorrhizal fungi (AMF) are ubiquitous in plant taxa (Asmelash *et al.* 2016). This is the associations of phycomycetes septate fungus belonging to Glomeromycota that corresponds to five different genera such as *Glomus*, *Gigaspora*, *Acaulospora*, *Scutellospora* and *Sclerocystis* (Bhattacharyya and Jha, 2015). AMF consists of specialized structures called arbuscules and vesicles (Smith and Gianinazzi-Pearson, 1988). Arbuscules are dichotomously branched hyphal structures, the major site of nutrient exchange between the host and the mycorrhizal fungi. While, vesicles are globose to sub-globose hyphal structures that usually serves as temporary storage organs. The vesicles are well-known to provide drought resistance and assisting the host plant to develop an effective and more efficient root system. AMF plays important role in maintaining the soil fertility and plant nutrition (Bhardwaj *et al.,* 2014). They can make the event possible through enhancing the uptake and translocation of mineral nutrients like phosphorous, nitrogen, sulphur, potassium, calcium, iron, copper and zinc from soil to the host plants. AM fungi associated with the medicinal plants are also known to enhance the plant growth and improve the active metabolites (Chanda *et. al,* 2014).

India is an important mega biodiverse country (Bhattacharyya, 2012). North-East India being rich in medicinal plants and many other rare and endangered taxa has already sealed its position as an active centre of novel gene pools (Bhattacharyya *et al.,* 2011; Chatterjee *et al.,* 2006). Reports are there on the utilization of more than 1350 species of ethno-medicinal plants with diverse pharmacological research opportunities from this ecologically significant biodiverse zone (Chakraborty *et al.,* 2012). *Oroxylum indicum* L. (Kurz.) belongs to the family Bignoniaceae (commonly known as bhatghila, trumpet flower, broken bone plant, bhut-vriksha etc.) is a small tree with purple to violet flowers as well as enormous seed pods that hang from the branches.

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The plant is recognized for various medicinal properties such as anti-inflammatory, anti-microbial, anti-oxidant, anti-arthritic, anti-diabetic etc. (Deka *et al.,* 2013). A flavonoid, baicalein, extracted from the methanolic extract of *Oroxylum indicum* have been recorded for its efficacy to inhibit proliferation of cancer cell line *in vitro* (Roy *et al.,* 2007). Bark juice of the plant is also effective drug in the treatment of jaundice (Sarma, 2012). Ayurvedic preparations such as *Dasamula*, *Amartarista*, *Dantyadyarista*, *Brahma rasayana* etc. also requires *Oroxylum indicum* as one of the active ingredients (Preety and Sharma, 2016).

However, scarce information is available on the AMF populations associated with this economically important plant. Keeping the point under consideration, the present investigation has been undertaken to determine the AMF colonization in the rhizosphere of *Oroxylum indicum*, an ethno-medicinally important plant of N.E. India. The present investigation is the first report accounting the comparative assessment of AMF populations in the rhizosphere of *Oroxylum indicum* collected from two geographically distinct study locations of N. E. India.

Materials and Methods

Survey and sample collection

Rhizospheric soil samples and roots of *Oroxylum indicum* were collected from two different sampling points of Jorhat district (26°46' 52" N & 94°12' 30" E), Assam and Naginimora (26°49' 44.7" N & 94°46' 59.6" E), Mon district of Nagaland, N.E. India. The vegetation pattern of *Oroxylum indicum* is shown in Fig. 1. The sampling points were coded as JRT/BPS/OI-01 and JRT/BPS/OI-02 for two sampling points at Jorhat district, Assam and MON/NGM/OI-01 and MON/NGM/OI-02 for the two sampling points at Mon district, Nagaland respectively. The sampling points are shown in Fig. 2. The rhizospheric soil samples (approx. 500g) were collected randomly (three samples at each sampling point) from a depth of 1-15 cm (top soil). The collected soil was air-dried and used for mycorrhizal spore quantification purposes. The fine roots of the experimental plant were collected and preserved at 4°C to examine the root colonization.

Determination of mycorrhizal colonization

Arbuscular mycorrhizal colonization was determined by rapid clearing and staining method as suggested (Phillips and Hayman, 1970). For this, the roots were cut into small pieces (1.0 cm each) and thoroughly washed in sterile distilled water (SDW) and stored in 10% (w/v) KOH for overnight. This is followed by treatment with 1% HCl and finally stained with trypane blue. The roots were destained with lactophenol and mounted in lactic acid and glycerol and observed under trinocular light microscope for the possible existence of mycelium, arbuscules and vesicles. Mycorrhizal root colonization was estimated using the following formula

$$\text{Mycorrhizal root colonization} = \frac{\text{Total no. of infected root segments}}{\text{Total no. of root segments examined}} \times 100$$

Quantification of AM spores and identification

The arbuscular mycorrhizal spores in the soil samples were determined by wet sieving and decanting technique (Gerdemann and Nicholson, 1963). The collected spores were mounted on polyvinyl lactoglycerol (PVLG) to observe their spore characteristics and identification purposes. The quantitative analysis of spores was measured with modified quantitative analysis technique (Gaur and Adholeya, 1994) using stereobinocular microscope. The AM spores were identified based on diverse morphological characteristics such as colour, size, shape, wall structure, surface, ornamentation of spores, nature and size of subtending hyphae, bulbous suspensor, the number and arrangement of spores in the sporocarp etc. Taxonomic monographs (Morton and Benny, 1990; Morton and Reddcker, 2001; Schenck and Perez, 1990, Trappe, 1982, Walker, 1983) were used for the AM spore identification.

Species diversity indices

Shannon-Wienner diversity index and Simpson's index of diversity were calculated using the following formulae:

$$H_S = -\Sigma(pi)(\ln pi)$$

Where,

H_S = Symbol for the diversity in a sample of S species or kind

S = The number of species in the sample

pi = Measures the relative abundance of i^{th} species or kinds $= \frac{ni}{N}$

N = The total number of individuals of all kinds

ni = The number of individuals of i^{th} species

ln = log of base 2

Simpson's index of diversity = 1- D

Where, $D = \Sigma\left(\frac{n}{N}\right)^2$

n = The total number of organisms of a particular species

N = The total number of organisms of all the species

Results and Discussion

Table 1 represents the quantitative estimation of AM spores and percentage root colonization of *Oroxylum indicum* per 50 gm soil in different sampling locations. The present investigation indicated significant root colonization as well as spore counts at almost all the sampling points, although the percent root colonization and spore count varies with topographical variations. Geographical variations among the sampling locations played a significant role in affecting the AMF populations (Jansa *et al.,* 2014). Arbuscular infection was highest (up to 100%) in both the sampling points of Naginimora, Mon district, Nagaland, followed by JRT/BPS/OI-01 (75±7.07%) and JRT/BPS/OI-02 (65±7.07%)

respectively. Root colonization with arbuscles is shown in Fig. 3. Vesicular infections (Fig. 4) were recorded maximum in MON/NGM/OI-01 and MON/NGM/OI-02 (100±0% at each sampling points). Vesicular infection was minimum (up to 30±0%) at the sampling points in Jorhat district. However, all the sampling locations showed characteristically similar hyphal infections (up to 100±0%). Spore count was calculated as maximum (1239±13.4) in MON/NGM/OI-01 and minimum in JRT/BPS/OI-01 (20±1.41). The variations might be attributed to the soil edaphic factors as well as altitudinal variation and vegetation types. Jorhat and Naginimora lies at 116m and 312m AMSL (Above Mean Sea Level) respectively and high altitudinal gradient might be one of the reasons for such variations in the distributional pattern of AMF populations. Studies pertaining to the AMF associations with *Oroxylum indicum* have earlier reported by Zhao *et al.,* (2001), where they observed AMF root colonization with this economically important plant. Investigations pertaining to effect of altitudinal variations on species diversity and richness of AM fungi associated with 3 medicinal plants (*Catharanthus roseus*, *Ocimum* spp. and *Asparagus racemosus*) have been made by Gaur and Kaushik (2012), who observed some negative correlation of spore density and root colonization with altitude. There are variations in the relative occurrence of AMF populations at different study locations. Table 2 depicts the qualitative analysis of AMF in different sampling locations. It was observed that the AMF in the sampling point JRT/BPS/OI-01 belongs to 3 genera *Acaulospora* (*A. foveata, A. lacunosa, A. laevis, Acaulospora* sp.), *Glomus* and *Scutellospora*. Similarly, the sampling point JRT/BPS/OI-02 also indicates the existence of 3 genera such as *Acaulospora, Glomus* and *Scutellospora. Acaulospora foveata, A. lacunosa, Acaulospora* sp., *Glomus mosseae, Glomus* sp. and *Scutellospora* sp. are the species representatives under the observed categories. The AMF populations in the sampling point MON/NGM/OI-01 recorded the existence of 22 species belonging to 5 different genera such as *Acaulospora* (*A. foveata, A. lacunosa, A. bireticulata, A. mellea, A. laevis, A. rehmii, Acaulospora sp.*), *Glomus* (*G. clavisporum, G. reticulatum, G. macrocarpon, G. mosseae, G. claroideum, G. pansihalos, G. albidum, G. geosporum, G. epigaeum, G. tunicatus, Glomus* sp.), *Gigaspora* (*G. gigantis, Gigaspora* sp.), *Entrophospora* and *Scutellospora*. The sampling location MON/NGM/OI-02 also showed the existence of five different types of AMF genera such as *Acaulospora*, *Glomus, Gigaspora*, *Entrophospora* and *Scutellospora*. However, the species, *Acaulospora bireticulata* and *Gigaspora* sp. was absent in the sampling point. The photographs of some of the isolated AM spores have been shown in Fig. 5(A-D). Fig. 6 shows the percent occurrence of AM spores in the study locations. *Glomus* was the dominant AM species at all the sampling points followed by *Acaulospora*. The percent occurrence of *Glomus* is maximum in JRT/BPS/OI-02 (59.09%), followed by JRT/BPS/OI-01 (55%). Occurrence of *Glomus* was least in MON/NGM/OI-02(43.20%). Occurrence of *Acaulospora* is more in JRT/BPS/OI-01 (40%) and less in JRT/BPS/OI-02 (31.82%). There were also distinct variations in the occurrences of other genera like *Gigaspora*, *Entrophospora* and *Scutellospora* throughout the study locations. The result of the present investigation indicates that the target plants collected from the sampling points of Nagaland are much more dependent on AM fungi for their survival than those from Jorhat. Wu *et al.,* (2013) reported that the influence of vegetation type and forest composition as contributing factors for the existence of diverse fungal populations in a particular soil habitat. According to Imhof, (2010) monocots are best suited to develop myco-heterotrophy. The sampling points in Mon district, Naginimora were rich in monocots. Abundance in monocotyledonous vegetation in Mon district might influence the AM sporulation resulting in diverse AMF taxa as well as more AMF colonization in the sampling points.

Table 1: Percentage root colonization and spore count at different sampling points of N. E. India.

Geographical variations	Sampling points	Root colonization (%)	Hyphae infection (%)	Arbuscular infection (%)	Vesicular infection (%)	Quantification of AM spores
Jorhat district, Assam	JRT/BPS/OI-01	100±0	100±0	75±7.07	30±0	20±1.41
	JRT/BPS/OI-02	100±0	100±0	65±7.07	30±0	22±1.41
Mon district, Ngaland	MON/NGM/OI-01	100±0	100±0	100±0	100±0	1239±13.44
	MON/NGM/OI-02	100±0	100±0	100±0	100±0	1220±13.44

Table 2: Relative occurrence of AMF populations at different sampling points of N. E. India.

AMF populations	JRT/BPS/OI-01	JRT/BPS/OI-02	MON/NGM/OI-01	MON/NGM/OI-02
Acaulospora foveata	+	+	+	+
A. lacunosa	+	+	+	+
A. bireticulata	-	-	+	-
A. mellea	-	-	+	+
Acaulospora laevis	+	-	+	+
A. rehmii	-	-	+	+
Acaulospora sp.	+	+	+	+
Glomus clavisporum	-	-	+	+
G. reticulatum	-	-	+	+
G. macrocarpon	-	-	+	+
G. mosseae	-	+	+	+
G. claroideum	-	-	+	+

G. pansihalos	-	-	+	+
G. albidum	-	-	+	+
G. geosporum	-	-	+	+
G. epigaeum	-	-	+	+
G. tunicatus	-	-	+	+
Glomus *sp.*	+	+	+	+
Gigaspora gigantius	-	-	+	+
Gigaspora sp.	-	-	+	-
Entrophospora sp.	-	-	+	+
Scutellospora sp.	+	+	+	+

+; Present, -; absent.

Table 3: Shannon-Weiner and Simpson diversity indices for the sampling locations.

Sampling locations	Diversity indices	
	Shannon-Weiner	Simpson index
JRT/BPS/OI-01	1.373	0.65
JRT/BPS/OI-02	1.591	0.764
MON/NGM/OI-01	2.935	0.941
MON/NGM/OI-02	2.779	0.931

Fig. 1: A. General vegetation pattern of *O. indicum* in Jorhat district, Assam and **B.** Naginimora district of Nagaland, N. E. India.

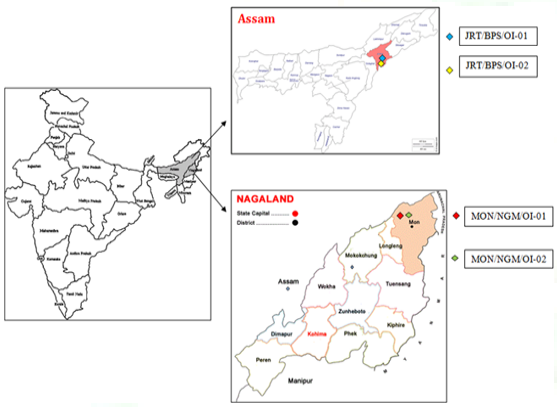

Fig. 2: Study area map showing the sampling locations.

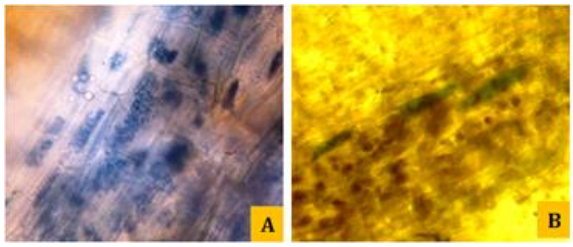

Fig. 3(A-B): Root colonization showing arbuscules.

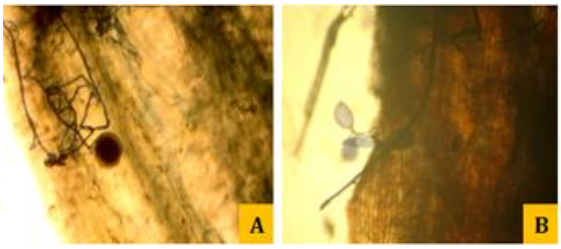

Fig. 4(A-B): Root colonization showing vesicles.

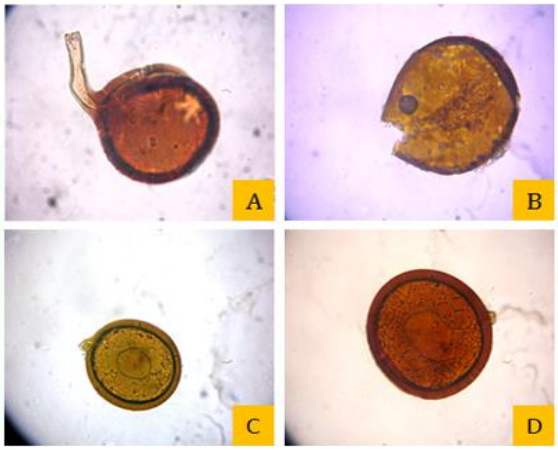

Fig. 5(A-D): Photographs of certain isolated spores. **A.** *Glomus mosseae* **B.** *Acaulospora sp.* **C.** *Glomus albidum* **D.** *Glomus macrocarpon.*

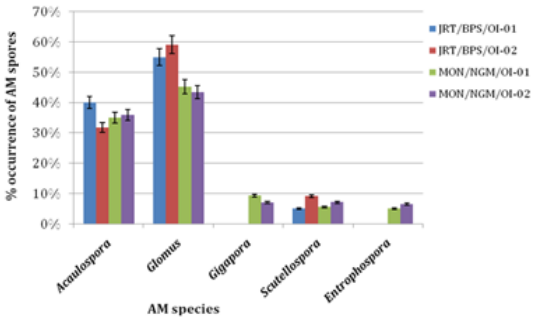

Fig. 6: Percentage occurrence of AM spores at different sampling points.

The Shannon Weiner and Simpson diversity indices also indicated distinct variations throughout the study locations (Table 3). Shannon Weiner and Simpson diversity indices were higher at sampling points MON/NGM/OI-01 and MON/NGM/OI-02, thus indicating high population diversity at those regions. More diverse spore populations and high species richness at these sampling locations might be due to the presence of more organic matter (Borah *et*

al. 2015) that may assist the mycorrhizal colonization in the rhizosphere of the target plant species.

Conclusion

During the present investigation, it is evident that *Oroxylum indicum* is colonized by diverse AMF populations. There are variations in the AMF colonization density which varied significantly with topographical alterations. AMF showed maximum colonization and highest spore density at the sampling points of Naginimora (Mon district, Nagaland) while, the samples collected from Jorhat district showed comparatively low AM colonizations as well as spore count. Further investigations on the isolation and characterization of AM spores as well as works on diversity and relative abundance of AM fungi associated with *Oroxylum indicum* in relation to alterations in altitude, soil type and geographical variations are need to be explored to understand the behavior of unexplored AMF populations associated with this economically important plant.

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