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**THE SOIL NUTRIENT CONTENT ALTERS ARBUSCULAR MYCORRHIZAL  
ASSOCIATION IN *OROXYLUM INDICUM* (L.) BENTH. EX KURZ  
GROWING UNDER DIFFERENT NATURAL HABITAT IN  
NORTH EAST INDIA**

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**ABSTRACT**

*The rhizospheric soil and roots were collected from five different sites of Northeast India, where *Oroxylum indicum* was naturally growing in its ecological habitat and were analysed. The study revealed that the roots of *O. indicum* were colonized with AM spores and the soil nutrient content plays a major role in the colonization of the AM spores. A total of 23 species of AM fungi belonging to four genera viz., *Glomus*, *Acaulospora*, *Gigaspora*, *Entrophospora* and *Pacispora* were recovered from the rhizosphere of *O. indicum*. The statistical analysis of the data revealed that the AM spore count is positively correlated with the soil nutrient status. AM spore count was maximum in forest fringe and agricultural farmland, hyphal and arbuscular colonization percentage was higher in agricultural farmland, while the vesicular colonization percentage was maximum in the rhizospheric soil of agricultural farmland and forest fringe areas. The AM spore count of *O. indicum* growing near the roadside showed positive correlation with root colonization. The correlation value of (0.766, 0.413, and 0.567) was obtained when root colonization was compared with soil organic carbon percentage, soil nitrogen and potassium content.*

**KEYWORDS:** *Oroxylum Indicum, Root Colonization, AM Spore & Soil Nutrient Content*

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**INTRODUCTION**

The rhizosphere is an area where the roots compete with the invading root systems of neighbouring plant species for space, water and mineral nutrients, and with soil-borne microorganisms, including bacteria, fungi, and insects feeding on an abundant source of organic material (Ryan and Delhaize, 2001). Soil acts as a habitat for a diverse group of microorganisms. Arbuscular mycorrhiza (AMÉVAM) is a symbiotic or mutualistic association between the roots of about 90% of the vascular species of plants. The AM fungi belong to the fungal phylum Glomeromycota (Schubler *et. al*, 2001). In such associations, both the partners share mutual benefits. The fungus derives nutrients via the root of the plant. In VA mycorrhizae the fungal hyphae develop some special organs called vesicles and arbuscules within the root called cortical cells. These vesicles are food storage organs of the fungus. However, the arbuscules are brush-like dichotomously branched (extensively) haustoria developed within the cortical cells. Mycorrhizal plants have the ability to explore larger soil volume beyond the rhizosphere through the external mycelium that assists in nutrient transport (Jakobsen *et. al*, 1992; Subramanian and Charest, 1999; Liu *et. al*, 2000). AMF benefit their host principally by increasing the uptake of relatively immobile

phosphate ions (Miransari *et. al*, 2009). Other benefits include: increased uptake of macronutrients, including N, K, and Mg (Veresoglou *et. al*, 2010), as well as some micronutrients (Kim *et. al*, 2009) increased resistance to soil pathogens (Lingua *et. al*, 2002) increased tolerance to salinity, heavy metals, and drought stress (Li *et. al*, 2011) regulation of the synthesis and distribution of plant hormones (Barker and Tagu, 2000) and maintenance of soil aggregate stability (Degens *et. al*, 1996).

*Oroxylum indicum* is a small or medium sized deciduous tree up to 12-15 m in height with soft light brown or greyish brown bark with corky lenticels (Dalal and Rai, 2004). *O. indicum* is being used as a medicinal herb for thousands of years without any known adverse effects (Ahad *et. al*, 2012). In Indian system of medicine, it has been used as a single drug or as a component of certain poly-herbal drug preparations. *O. indicum* is an indigenous tree of the Indian subcontinent, distributed throughout the country and is found up to an altitude of 1200 m mainly along the river banks or slopes of the hills, in damp region and moist places in the forests (Chauhan, 1999; Bennet *et. al*, 1992). It is distributed in the Himalayan foothills, Eastern and Western Ghats and North East India (Jayaram and Prasad, 2008). It was earlier reported by Pawaar and Kakde (2012) that the root of *O. indicum* does not show any type of mycorrhizal associations at all. However, the present study negates the previous report. It was found that the AM fungi are associated with the rhizosphere of *O. indicum*. The present research deals with the study of association of AM fungi along with the soil nutrient content of *O. indicum* along different natural habitats in North East India.

## MATERIALS AND METHODS

### Collection of the Rhizospheric Soil, Root and Plant Samples

The rhizospheric soil and plant samples were collected from five different sites of Northeast India, where *Oroxylum indicum* was naturally growing in its ecological habitat. The rhizospheric soil and root samples and pods were collected from Jorhat, Nalbari, Guwahati, Itanagar and North Lakhimpur. The samples of *O. indicum* were collected from trees showing fruiting from five different collection sites of a single eco-region (Brahmaputra Valley semi- evergreen forests), *i. e.* Jorhat, Nalbari, Guwahati, Itanagar and North Lakhimpur. The location, GPS coordinates and habitat are mentioned in the Table 1. Rhizospheric soil samples along with root segments were taken by digging out a small amount of soil (500g) close to the plant roots up to a depth 15-30 cm and these samples were kept in sterilized polythene bags for further processing in the laboratory for physicochemical analysis of soil, mycorrhizal colonization and spore quantification etc. In Jorhat the samples were collected from the tree growing along roadside. Rhizospheric samples of the selected plant species were collected from the plant growing along the riverside in Nalbari. From Guwahati, samples were collected from the tree growing in the forest fringe area. Samples from hill slope were collected from Itanagar, while, sample from agricultural farmland were collected from North Lakhimpur. The samples were collected from healthy trees which overall represented the region. Rhizospheric soil samples were collected from mature trees.

### Isolation of AM Spores

The AM spores were isolated by Wet sieving and decanting technique of Gerdemann and Nicolson (Gerdemann and Nicolson, 1963). Approximately 50 g of soil was added in 500 ml of water in a glass beaker and soil was allowed to settle overnight. The water was decanted through a series of sieves of different sizes (125µm, 90µ m, 63µ m and 45µm) arranged vertically in descending order. The spores trapped in the sieves were repeatedly washed and transferred later to Whatman filter paper No. 1. The spores thus collected were observed under stereo-binocular microscope. The spores were picked by hypodermic needle and then mounted in polyvinyl lactic acid glycerol (PVLG).

The quantitative estimation of the AM spores was done by Adholeya and Gaur's modified method (Adholeya and Gaur, 1994). The filter paper was divided into many small segments and the total numbers of spores were counted by adding the number of spores present in each segment under stereo-binocular microscope.

**Colonization of AM Fungi**

The root colonization was studied by Rapid clearing and staining technique (Phillips and Hayman, 1970). The root segments were washed repeatedly under running water. The cleaned roots were cut into small segments of 1cm length, and were kept in 10% KOH solution for 24 hours at room temperature. KOH was decanted and root segments were again acidified with 1% HCl for 10 minutes. The root segments were stained with 0.5% trypan blue for 24 hours and then destained with lactophenol. The root segments were then mounted in lactic acid: glycerol (1:1) for observation under microscope.

**RESULTS AND DISCUSSIONS**

The rhizospheric samples of *Oroxylum indicum* were collected from five different collection sites of a single eco-region of Northeast India (Brahmaputra Valley semi- evergreen forests), i. e. Jorhat, Nalbari, Guwahati, Itanagar and North Lakhimpur. In Jorhat the sampling was done along roadside, while, in Nalbari, the sampling was done along the riverside (Table 1). The samples were collected from the forest fringe zone in Guwahati collection site, while, soil samples from hill slope were collected from Itanagar site. In North Lakhimpur site the soil samples were collected from agricultural farmland. GPS coordinates of these sites were recorded. The highest elevation was observed in Itanagar (OI/ ITA/1) while the lowest (53 msl) was found in Nalbari (OI/NLB/1). The maximum soil temperature value (26°C) was recorded in North Lakhimpur (OI/LKP/1) while minimum soil temperature (19 °C) was observed in Nalbari (OI/ NLB/3). The humidity was found to be highest (89%) in Guwahati (OI/GHY/1) and lowest (51%) in Itanagar (OI/ ITA/1).

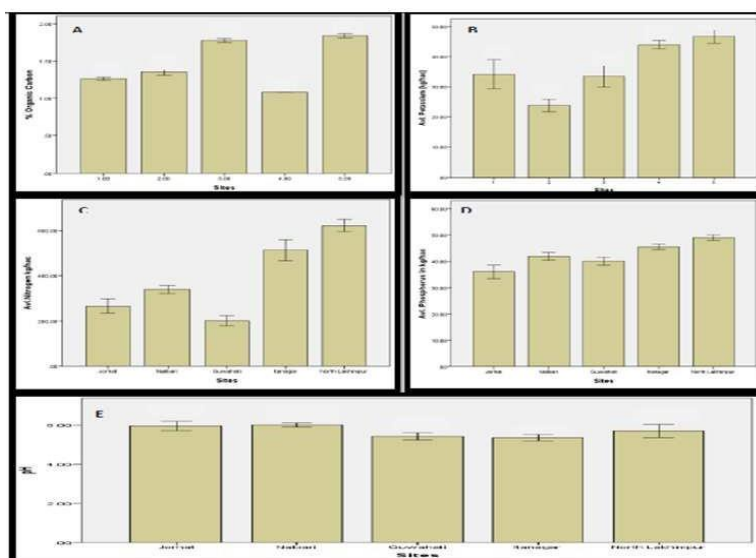
**Table 1: Survey and GPS Location of Different Collection Sites of *Oroxylum Indicum* (L.) Benth. ex Kurz**

SITE S	Locations	GPS Coordinates	Location	Height of plant appx. (ft)	Habitat	Elevation (msl)	Temperature (°C)	Humidity (%)
SITE 1	OI/ JRT/1	N 26° 47' 05.38" E 94° 17' 15.40"	Jorhat	10	Roadside	96	21	74
	OI/ JRT/2	N 26° 47' 15.70" E 94° 17' 35.97"	Jorhat	18	Roadside	99	21	70
	OI/ JRT/3	N 26° 46' 34 " E 94° 15' 34 "	Jorhat	21	Roadside	97	20	76
SITE 2	OI/ NLB/1	N 26° 27' 03.49 " E 91° 27' 36.81 "	Nalbari	13	Riverside	53	19	61
	OI/ NLB/2	N 26° 27' 03.14" E 91° 27' 36.30"	Nalbari	20	Riverside	54	20	57
	OI/ NLB/3	N 26° 27' 03.49" E 91° 27' 36.81"	Nalbari	17	Riverside	53	19	63
SITE 3	OI/ GHY/1	N 26° 05' 56" E 91° 47' 28.57"	Guwahati	18	Forest fringe	57	23	89
	OI/ GHY/2	N 26° 05' 57.93" E 91° 47' 17.49"	Guwahati	24	Forest fringe	56	22	83
	OI/ GHY/3	N 26° 05' 48.37" E 91° 47' 28.06"	Guwahati	25	Forest fringe	56	23	87
SITE 4	OI/ ITA/1	N 27° 05' 09.59" E 93° 36' 27.16"	Itanagar	15	Hill slope	783	24	51
	OI/ ITA/2	N 27° 05' 07.09" E 93° 36' 24.54 "	Itanagar	23	Hill slope	772	24	57
	OI/ ITA/3	N 27° 05' 07.24" E 93° 36' 24.26"	Itanagar	18	Hill slope	760	23	55
SITE 5	OI/ LKP/1	N 26° 57' 04.95" E 93° 51' 32.22"	North Lakhimpur	22	Paddy field	98	26	57
	OI/ LKP/2	N 26° 57' 05.63" E 93° 51' 30.40"	North Lakhimpur	27	Paddy field	100	24	62
	OI/ LKP/3	N 26° 57' 08.07" E 93° 51' 30.19"	North Lakhimpur	25	Paddy field	100	25	59

Table 2 shows the pH and nutrient status of the soil under naturally growing *O. indicum* tree species. The organic carbon content %, available Nitrogen (kg/hac), available Potassium and available Phosphorus (P) in kg/hac was determined. The nutrient status of soil varied along different collection sites. The soil status of the sample collected from Jorhat site has a pH value ranging between 5.89±0.11 to 6.2±0.03. The soil organic carbon percentage varied between 1.243±0.034 to 1.282±0.025, available Nitrogen (kg/hac) ranged between 242.69±0.001 to 301.27±0.001, and available Phosphorus (kg/hac) varied between 34.39±2.90 to 39±3.151, while available potassium (kg/hac) varied between 28.52±0.754 to 37.36±1.24. In Nalbari site the pH of the soil varied from 5.91±0.11 to 6.11±0.08. The organic carbon percentage was found to range between 1.320±0.033 to 1.397±0.034, the available nitrogen (kg/hac) varied between 326.37±0.002 to 359.85±0.002; available phosphorus (kg/hac) was between 40.78±3.15 to 43.61±0.81 and available potassium varied between 21.39±1.78 to 25.09±1.58. In Guwahati site the pH varied between 5.25±0.187 to 5.61±0.097, the organic carbon ranged between 1.760±0.050 to 1.808±0.033. The available nitrogen (kg/hac) varied between 175.74±0.0006 to 217.58±0.001; available phosphorus (kg/hac) was between 38.82±0.614 to 41.8±0.063 and available potassium varied between 29.37±1.24 to 36.22±1.02. The soil nutrient status of Itanagar site shows that the pH varied between 5.17±0.323 to 5.47±0.279, The organic carbon percentage was found to be 1.081±0.078, the available nitrogen (kg/hac) varied between 472.82±0.001 to 564.88±0.001, available phosphorus (kg/hac) was between 44.32±1.799 to 46.27±0.307 and available potassium varied between 42.49±1.50 to 45.34±1.48. In North Lakhimpur site the pH of the soil varied from 5.38±0.43 to 6.05±0.131. The organic carbon percentage was found to range between 1.808±0.033 to 1.865±0.049, the available nitrogen (kg/hac) varied between 594.17±0.003 to 648.56±0.004, available phosphorus (kg/hac) was between 48.22±0.639 to 50.17±0.639 and available potassium varied between 45.06 ± 1.99 to 49.34±1.50. (Figure 1A)

**Table 2: Soil Status of Different Collection Sites of *Oroxylum Indicum* (L.) Benth. ex Kurz**

Sample	pH	%OC	Available N (kg/hac)	Avl P (kg/hac)	Avl K (kg/hac)
OI/JRT/1	5.89±0.11	1.282±0.025	242.69±0.001	39±3.151	36.506±1.73
OI/JRT/2	6.2±0.03	1.243±0.034	251.05±0.002	34.39±2.90	28.52±0.754
OI/JRT/3	5.74±0.141	1.253±0.025	301.27±0.001	34.75±5.22	37.36±1.24
OI/NLB/1	6±0.173	1.339±0.025	330.56±0.001	40.78±3.15	21.39±1.78
OI/NLB/2	6.11±0.08	1.397±0.034	326.37±0.002	41.48±1.91	24.81±1.30
OI/NLB/3	5.91±0.11	1.320±0.033	359.85±0.002	43.61±0.81	25.09±1.58
OI/GHY/1	5.4±0.221	1.760±0.050	209.21±0.008	41.8±0.063	36.22±1.02
OI/GHY/2	5.25±0.187	1.760±0.074	175.74±0.0006	39.71±0.987	29.37±1.24
OI/GHY/3	5.61±0.097	1.808±0.033	217.58±0.001	38.82±0.614	34.79±1.99
OI/ITA/1	5.41±0.270	1.081±0.050	472.82±0.001	45.92±0.639	43.92±1.73
OI/ITA/2	5.47±0.279	1.081±0.078	502.11±0.001	44.32±1.799	45.34±1.48
OI/ITA/3	5.17±0.323	1.081±0.058	564.88±0.001	46.27±0.307	42.49±1.50
OI/LKP/1	5.65±0.174	1.808±0.033	594.17±0.003	48.22±0.639	49.34±1.50
OI/LKP/2	6.05±0.131	1.846±0.041	648.56±0.004	50.17±0.639	45.63±0.75
OI/LKP/3	5.38±0.43	1.865±0.049	623.46±0.001	48.58±0.639	45.06±1.99



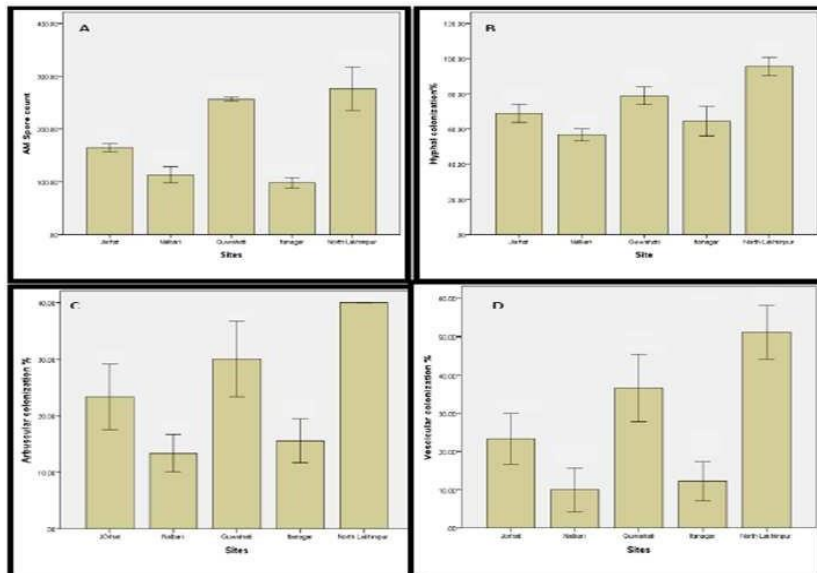
**Plate 1 A: DMRT analysis of soil status of different sites**  
**A: Organic Carbon % in different sites**  
**B: Available Potassium in kg/hac in different sites**  
**C: Available Nitrogen % in kg/hac in different sites**  
**D: Available Phosphorus in kg/hac in different sites**  
**E: pH of soil in different sites**

**Figure 1(a)**

Table 3 shows the AM spore quantification and root colonization of rhizospheric soil samples of *O. indicum* of different sites. The maximum ( $311.33 \pm 4.97$ ) AM spore count per 50 gm of soil, number was observed in the North Lakhimpur (OI/LKP/1) sample while minimum number ( $91.33 \pm 10.93$ ) was reported in Itanagar (OI/ITA/1) sample. Three types of root infection/ colonization was present in roots of *O. indicum* i. e. hyphal, arbuscular and vesicular colonization. The range of hyphal colonization in roots was from  $53.33 \pm 3.33$  in Nalbari (OI/NLB/2) to  $100 \pm 0$  in North Lakhimpur (OI/LKP/3) samples respectively. The observed arbuscular colonization ranged from  $10 \pm 0$  in Nalbari (OI/NLB/3) to  $40 \pm 5.77$  and  $40 \pm 10.00$  in North Lakhimpur (OI/LKP/1) and OI/LKP/3 sites respectively. Vesicular colonization was maximum ( $56.66 \pm 8.81$ ) in North Lakhimpur (OI/LKP/3) site minimum ( $6.66 \pm 3.33$ ) in Nalbari (OI/NLB/1 and OI/NLB/3) and Itanagar (OI/ITA/1) site. The analysis of the data revealed that the root colonization was positively correlated with the soil nutrient status of the samples collected from various collection sites. The correlation value of 0.766 was obtained when root colonization was compared with soil organic carbon percentage. The root colonization and soil nitrogen content also had positive correlation value of 0.413. Similarly the available phosphorus and potassium in the soil was positively correlated with root colonization, with correlation values of 0.387 and 0.567 respectively. The root colonization was found to be negatively correlated with soil ph. (Figure 1B).

**Table 3: Spore Count and Root Colonization of *Oroxylum Indicum* (L.) Benth. ex Kurz Collected from Different Sites**

Sample	Spore count	Hyphal colonization %	Arbuscular colonization %	Vesicular colonization %
OI/JRT/1	161.33±7.68	63.33±5.19	20±3.98	16.66±3.98
OI/JRT/2	173.66±5.84	70±5.77	20±0	23.33±3.33
OI/JRT/3	159±3.055	73.33±3.33	30±5.77	30±5.77
OI/NLB/1	131±3.78	60±4.36	16.66±6.41	6.66±3.33
OI/NLB/2	103.33±5.78	53.33±3.33	13.33±3.33	16.66±3.33
OI/NLB/3	105.33±4.17	56.66±8.81	10±0	6.66±3.33
OI/GHY/1	252.85±6.85	73.33±3.33	23.33±3.33	26.66±3.33
OI/GHY/2	260.33±4.37	83.33±3.33	30±5.77	40±5.77
OI/GHY/3	256.66±2.02	80±5.77	36.66±3.33	43.33±6.66
OI/ITA/1	91.33±10.93	56.66±3.33	20±5.77	6.66±3.33
OI/ITA/2	109.33±5.23	63.33±3.33	13.33±3.33	16.66±3.33
OI/ITA/3	93.33±4.37	73.33±3.33	13.33±6.66	13.33±6.66
OI/LKP/1	231±1.52	96.66±3.33	40±5.77	53.33±6.66
OI/LKP/2	286.33±4.63	90±5.77	40±0	43.33±8.81
OI/LKP/3	311.33±4.97	100±0	40±10.00	56.66±8.81



**Plate 1 B: DMRT analysis of AM Spore count and Root colonization of different sites**  
**A: AM Spore count in different sites**  
**B: Hyphal colonization % in different sites**  
**C: Arbuscular colonization % in different sites**  
**D: Vesicular colonization % in different sites**

**Figure 1(b)**

The DMRT analysis of different parameters was done to segregate different variables ( $p < 0.05$ ). The DMRT analysis of the pH status was higher in Jorhat and Nalbari, intermediate pH was found in North Lakhimpur site while minimum pH was found in Guwahati and Itanagar site. Overall, the pH of the soil under *O. indicum* is acidic. The % Organic Carbon was found to be highest in North Lakhimpur site while it was lowest in Itanagar site. Available Nitrogen was highest in North Lakhimpur, while it was minimum in Guwahati. Available Phosphorus was again the highest in North Lakhimpur site while it was lowest in Nalbari and Guwahati site. Available Potassium was highest in Itanagar and North Lakhimpur sites while it was lowest in Nalbari site. The DMRT analysis of the AM spore count shows that North Lakhimpur and Guwahati sites have highest AM spore count while, Itanagar and Nalbari sites have minimum

AM spore count. The hyphal colonization % was maximum in North Lakhimpur site and minimum in Nalbari site. The Arbuscular colonization % was again maximum in North Lakhimpur site and minimum in Nalbari site while, the Vescicular colonization % was maximum in North Lakhimpur and Guwahati sites while it was minimum in Itanagar and Nalbari sites(Table 4).

**Table 4: DMRT analysis of Soil Status and AM Spore Count, Hyphal Colonization %, Arbuscular Colonization % and Vescicular Colonization % of Different Sites**

Attributes	Sites				
	Jorhat(1)	Nalbari(2)	Guwahati(3)	Itanagar(4)	North Lakhimpur(5)
pH	5.9433 <sup>a</sup>	6.0067 <sup>a</sup>	5.420 <sup>b</sup>	5.350 <sup>b</sup>	5.693 <sup>ab</sup>
%OC	1.2593 <sup>d</sup>	1.3520 <sup>c</sup>	1.7760 <sup>b</sup>	1.081 <sup>e</sup>	1.8397 <sup>a</sup>
Available N (kg/hac)	265.00 <sup>d</sup>	338.926 <sup>c</sup>	200.84 <sup>e</sup>	513.27 <sup>b</sup>	622.06 <sup>a</sup>
Avl P (kg/hac)	36.0467 <sup>d</sup>	41.9567 <sup>c</sup>	40.1100 <sup>e</sup>	45.503 <sup>b</sup>	48.99 <sup>a</sup>
Avl K (kg/hac)	34.1287 <sup>b</sup>	23.7633 <sup>c</sup>	33.4600 <sup>b</sup>	43.916 <sup>a</sup>	46.6767 <sup>a</sup>
Spore count	164.663 <sup>b</sup>	113.2200 <sup>c</sup>	256.613 <sup>a</sup>	97.99 <sup>e</sup>	276.220 <sup>a</sup>
Hyphal colonization %	68.88 <sup>b</sup>	56.66 <sup>d</sup>	78.88 <sup>b</sup>	64.44 <sup>cd</sup>	95.55 <sup>a</sup>
Arbuscular colonization %	23.33 <sup>bc</sup>	13.330 <sup>d</sup>	29.996 <sup>b</sup>	15.553 <sup>cd</sup>	40.00 <sup>a</sup>
Vescicular colonization %	164.663 <sup>b</sup>	113.220 <sup>c</sup>	256.613 <sup>a</sup>	97.996 <sup>c</sup>	276.220 <sup>a</sup>

**Arbuscular Mycorrhizal (AM) Spore Diversity Associated with the Rhizospheric Soil Samples of *Oroxyllum Indicum* (L.) Benth. ex Kurz**

The AM diversity of all the rhizospheric soil samples was assessed. The study revealed that a total of 23 species of AM fungi belonging to 5 genera (*i. e. Glomus, Gigaspora, Acaulospora, Entrophospora, Pacispora*) were isolated from the rhizospheric soil of *O. indicum* as mentioned in Table 5. *Glomus* was found to be maximum in the rhizosphere of *O. indicum*, followed by *Acaulospora* and *Gigaspora* sp., *Pacispora* sp and *Entrophospora* sp. AM spores in Jorhat site comprised of *Acaulospora spinosa, Glomus geosporum, Acaulospora mellea, Gigaspora* sp., *Acaulospora rehmi, Entrophospora* sp. AM spores in Nalbari constituted of *Gigaspora decipience, Glomus* sp., *Acaulospora scrobiculata, Glomus palladium, Glomus geosporum*. AM spores in Guwahati *Gigaspora gigantea, Glomus geosporum, Acaulospora laevis, Glomus macrocarpum, Glomus mosseae, Gigaspora* sp., *Glomus microcarpum, Glomus* sp., *Acaulospora* sp. AM spores in Itanagar comprised of *Glomus fasciculatum, Glomus clarum, Gigaspora nigra, Glomus mosseae, Acaulospora bireticulata, Glomus geosporum*. AM spores in North Lakhimpur site comprised *Glomus mosseae, Acaulospora* sp., *Pacispora* sp, *Glomus multicaule, Glomus geosporum, Glomus aggregatum, Acaulospora laevis* only (Figure 2).



Table 5: Fungal Diversity Associated with *Oroxylum Indicum* (L.) Benth. ex Kurz

Sl. No.	Fungal Species	Family	Jorhat	Nalbari	Gawahati	Itanagar	N. Lakhimpur
1.	<i>Asidia</i> sp.	Cunninghamellaceae	-	-	-	+	+
2.	<i>Aspergillus</i> sp.	Trichocomaceae	+	+	-	+	+
3.	<i>Colletotrichum</i> sp.	Glomerellaceae	-	-	+	+	+
4.	<i>Cunninghamella</i> sp.	Cunninghamellaceae	-	+	-	-	-
5.	<i>Curvularia</i> sp.	Pleosporaceae	-	+	-	+	-
6.	<i>Fusarium</i> sp.	Nectriaceae	+	+	-	+	+
7.	<i>Geotrichum</i> sp.	Endomycetaceae	-	-	+	-	-
8.	<i>Gliocladium</i> sp.	Eypocraceae	-	-	-	-	+
9.	<i>Mucor</i> sp.	Mucoraceae	+	-	+	+	-
10.	<i>Pachybasium</i> sp.	Eypocraceae	+	+	+	+	+
11.	<i>Penicillium capsulatum</i> Raper & Fernell	Trichocomaceae	+	+	+	-	+
12.	<i>Penicillium citrinum</i> Thom	Trichocomaceae	+	+	+	-	-
13.	<i>Penicillium funiculosum</i> Thom	Trichocomaceae	+	-	-	-	-
14.	<i>Penicillium</i> sp. 1	Trichocomaceae	+	+	+	-	+
15.	<i>Penicillium</i> sp. 2	Trichocomaceae	-	+	-	-	-
16.	<i>Pythium</i> sp.	Pythiaceae	-	+	+	+	+
17.	<i>Rhizoctonia sclani</i> Kuhn	Ceratobasidiaceae	-	-	-	-	+
18.	<i>Rhizoctonia</i> sp.	Ceratobasidiaceae	-	-	+	+	+
19.	<i>Rhizomorpha</i> sp.	Mucoraceae	-	+	-	-	+
20.	<i>Scedosporium</i> sp.	Micrascoaceae	-	-	-	+	-
21.	<i>Trichoderma hamatum</i> (Benard.)	Eypocraceae	+	-	+	-	+
22.	<i>Trichoderma harzianum</i> Pers.	Eypocraceae	+	+	+	+	+
23.	<i>Trichoderma</i> sp.	Eypocraceae	-	-	+	-	-
24.	<i>Trichoderma viridae</i> Pers.	Eypocraceae	+	-	+	+	+
25.	<i>Verrucillum</i> sp.(Nees)	Plectospharellaceae	-	+	-	-	-

+ denotes present, - denotes absent

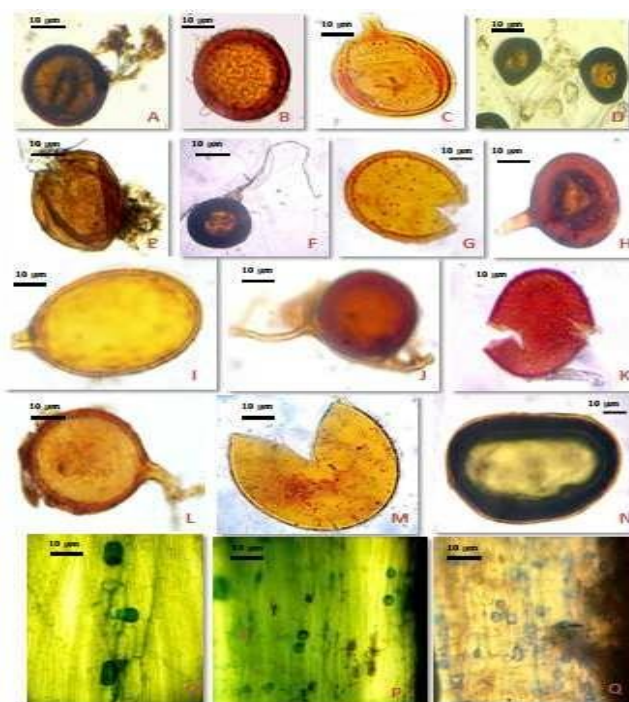


Plate 2. A: *Glomus decipiens*, B: *Acaulospora bisetellata*, C: *Glomus fasciculatum*, D: *Glomus aggregatum*, E: *Acaulospora spinosa*, F: *Glomus nigra*, G: *Acaulospora* sp. H: *Glomus microcarpum*, I: *Glomus multisetula*, K: *Glomus* sp., L: *Glomus* sp., M: *Glomus* sp., N: *Entrophospora* sp. O-Q: Network of hyphae, vesicles and arbuscules inside root

Figure 2

### Spore Morphology of AM Spores

The spore morphology of various AM spores is shown in Table 6. The identification was done on the basis of morphological characteristics. The micrometry of the spores was done to determine the size of the spores. The spore characteristics are mentioned below

Table 6: Spore Morphology of AM Fungi Associated with *O. Indicum*

Sl.No.	AM Species	Spore size (µm)	Spore shape	Spore colour	Wall layers	Ornamentation	Subtending hyphae
1.	<i>Acaulospora spinosa</i>	300×250	Sub globose to globose	Dirty brown to brown	Two wall layers	Surface ornamented with blunted spines	Absent
2.	<i>Acaulospora mellea</i>	130×110	Sub globose to ovoid	Reddish Brown to brown	Three wall layers	Concave pits present on papillae	Absent
3.	<i>Acaulospora rehmsii</i>	110×120	Round	Pale brown to dark brown	Wall layer not distinct	Labyrinth form of channels on wall layers	Absent
4.	<i>Acaulospora</i> sp.	130×130	Globose to sub globose	Reddish brown to Dark brown	Smooth wall layer	Presence of concave pits	Present
5.	<i>Acaulospora laevis</i>	300×300	Ellipsoid to sub ellipsoid	Yellowish-brown to honey brown	Smooth wall layer	Smooth surface	Absent
6.	<i>Acaulospora bireticulata</i>	90×90	Globose to sub globose	Dark greyish brown to brown	Three wall layers	Polygonal reticulum network on surface	Present
7.	<i>Acaulospora scrobiculata</i>	400×370	Globose to sub globose	Brown to Dark brown	Unit wall layer	Tiny multiple Concave pits over surface	Absent
8.	<i>Glomus macrocarpum</i>	150×150	Globose to sub globose to ovoid	Dark brown	Multiple layered	Cross channels present on walls	Absent
9.	<i>Glomus palladium</i>	200×200	Globose to sub globose	Light yellowish brown	Two wall layer	Sporocarp contain peridium	Absent
10.	<i>Glomus microcarpum</i>	70×100	Globose to sub globose to ellipsoid	Dark yellowish brown to brown	One wall with two layers	Smooth with laminations	Present
11.	<i>Glomus aggregatum</i>	130×130 (Size of each zygospore)	Globose to Subglobose to ovoid	Dirty yellowish brown to brown	One wall with three layers	Laminated wall layer	Present aggregated
12.	<i>Glomus</i> sp.	150×150	Globose to sub globose	Yellowish brown to brownish yellow	Single wall layer	Sporocarp present with warts	Absent
13.	<i>Glomus geosporum</i>	170×150	Globose to sub globose	Yellowish honey brown	Three wall layers	Smooth	Absent
14.	<i>Glomus fasciculatum</i>	100×100	Globose to sub globose	Yellowish brown to brown	Three wall layers	Perforated with thickened inward projections	Absent
15.	<i>Glomus clarum</i>	180×140	Globose to Sub globose	Hyaline to pale yellow colour	Single wall with three layers	Laminated spore wall	Absent
16.	<i>Glomus multicaule</i>	150×150	Globose to sub globose to ovoid	Dark brown to brown	Single thick wall layer	Very thick ornamentation	Multiple attached hyphae
17.	<i>Glomus mosseae</i>	100×150	Globose to Sub globose	Honey brown to yellowish brown	Single wall layer	Hyaline outer wall	Absent
18.	<i>Gigaspora</i> sp.	450×500	Sub globose to ovoid	Dirty yellowish brown to brown	Single wall layer	Presence of warts irregular	Absent
19.	<i>Gigaspora nigra</i>	550×550	Globose to sub globose	Dirty Yellowish-brown to Dark brown	Single wall layer	Irregular warts	Present
20.	<i>Gigaspora gigantea</i>	750×750	Globose to sub globose	Yellowish brown to brown	Double wall layer	Ornamented with evenly distributed warts	Absent
21.	<i>Gigaspora decipiens</i>	300×300	Globose to sub globose	Pale yellow to brownish yellow	Three layered wall	Warty	Present
22.	<i>Entrophospora</i> sp.	300×100	Flattened ellipsoid	Yellowish brown	Single wall with multiple layer	Hyaline	Absent
23.	<i>Pactispora</i> sp. (carapase)	130×100	Globose to sub globose to ovoid	Dirty Yellowish brown to Yellowish brown	Single wall layer	Presence of warts irregular	Absent

#### *Acaulospora scrobiculata* (Trappe)

The spore size varies between 400×370 (µ m). The spore shape is sub globose to globose. The colour of the spore is brown to dark brown. The spore has a single wall layer with surface ornamented with tiny multiple concave pits.

#### *Acaulospora spinosa* (Walker and Trappe)

The spore size ranges between 300×250 (µm). The spore shape is sub globose to globose. The colour of the spore is dirty brown to brown. The spore has two wall layers with surface ornamented with blunted spines.

#### *Acaulospora mellea* (Spain and Schenck)

The spore size ranges between 130×110 (µm). The spore shape is sub globose to ovoid. The colour of the spore is reddish brown to brown. The spore has three wall layers with concave pits present on papillae

#### *Acaulospora rehmsii* (Sieverd. and S. Toro)

The spore size ranges between 110×120 (µ m). Round spore shape. The colour of the spore is pale brown to dark brown. The wall layer is not distinct. The surface is ornamented with a labyrinth form of channels on wall layers.

#### *Acaulospora* sp.

The spore size ranges between 130×130 (µm). The spore shape is globose to sub globose. The colour of the spore is reddish brown to dark brown. The spore has smooth wall layers with the presence of concave pits. Subtending hypha is

present.

***Acaulospora laevis* (Gerdemann and Trappe)**

The spore size ranges between 300×300 (μ m). The spore shape is ellipsoid to sub ellipsoid. The colour of the spore is yellowish-brown to honey brown. The spore has a smooth wall layer with smooth surface.

***Acaulospora bireticulata* (Rothwell and Trappe)**

The spore size ranges between 400×370 (μ m). The spore shape varies between globose to sub globose. The colour of the spore is brown to dark brown. The spore has unit wall layer with ornamented with tiny multiple concave pits. Subtending hypha is present.

***Glomus macrocarpum* (Tulasne and Tulasne)**

The spore size ranges between 150×150 (μm). The spore shape varies between globose to sub globose to ovoid. The spore is dark brown in colour. Multiple layered spore wall with cross channels present on walls.

***Glomus palladium* (Hall)**

The size of the spore is between 200×200(μm). The spore shape varies between globose to sub globose. The colour of the spore is light yellowish brown. It has two wall layers with sporocarp containing peridium.

***Glomus macrocarpum* (Tul. and C. Tul.)**

The size of the spore is between 70×100 (μ m). The spore shape varies between globose to sub globose to ellipsoid. The colour of the spore varies from dark yellowish brown to brown. The single wall layer is present with two layers. The surface of the wall is smooth with laminations. Subtending hypha is present.

***Glomus aggregatum* (Schenck and Smith emend. Koske)**

The size of each zygosporangium is between 130×130(μ m) which are aggregated together. The spore shape varies between globose to sub globose to ovoid. The colour of the spore varies from dirty yellowish brown to brown. The spore has single wall with three layers. The spore wall is laminated. There is presence of aggregated hyphae.

***Glomus* sp.**

The size of the spore is between 150×150(μm). The spore shape varies between sub globose to globose. The colour of the spore varies from yellowish brown to brownish yellow. There is the presence of a single wall layer, with sporocarp present with warts.

***Glomus geosporum* (Nicol. and Gerd.) Walker**

The size of the spore is between 170×150(μm). The spore shape varies between sub globose to globose. The colour of the spore is yellowish honey brown. The surface is smooth with three wall layers.

***Glomus fasciculatum* (Thaxter sensu Gerd.) Gerd. and Trappe**

The size of the spore is between 100×100(μm). The spore shape varies between globose to sub globose. The colour of the spore is yellowish brown to brown. Three wall layers with surface perforated with thickened inward projections.

The size of the spore is between 180×140(μm). The spore shape varies between globose to sub globose. The colour of the spore varies from hyaline to pale yellow colour. There is single wall with three wall layers. The spore wall is laminated.

***Glomus multicaule (Gerd. and Baskhi)***

The size of the spore is between 150×150(μm). The spore shape varies between globose to sub globose to ovoid. The colour of the spore varies from dark brown to brown. Presence of single thick wall layer with very thick ornamentation. Multiple attached hyphae are present.

***Glomus mosseae (Nicol. and Gerd.) Gerd. and Trappe***

The size of the spore is between 100×150 (μ m). The spore shape varies between globose to sub globose. The colour of the spore varies from honey brown to yellowish brown. Presence of single wall layer. The wall layer is hyaline.

***Gigaspora sp.***

The size of the spore is between 450×500(μ m). The spore shape varies between sub globose to ovoid. The colour of the spore varies from dirty yellowish brown to brown. Presence of single wall layer with surface ornamented with multiple irregular warts.

***Gigaspora nigra (J. F. Redhead)***

The size of the spore is between 550×550(μm). The spore shape varies between globose to sub globose. The colour of the spore varies from dirty yellowish brown to dark brown. Presence of single wall layer with warts. Subtending hypha is present.

***Gigaspora gigantea (Nicol. and Gerd.) Gerd. and Trappe***

The size of the spore is between 750×750(μm). The spore shape varies between globose to sub globose. The colour of the spore varies from dirty yellowish brown to brown. Presence of double wall layer with surface ornamented with evenly distributed warts.

***Gigaspora decipience (Hall and Abbott)***

The size of the spore is between 300×300 (μm). The spore shape varies between globose to sub globose. The colour of the spore varies from pale yellow to brownish yellowish. Presence of three wall layers, the wall layer is warty. Subtending hypha is present.

***Entrophospora sp.***

The size of the spore is between 300×100 (μm). The spore shape is flattened ellipsoid. The colour of the spore is yellowish brown. Single wall with multiple layers. Hyaline spore wall.

***Pacispora* sp.**

The size of the spore is between 130×100 (µ m). The spore shape varies between globose to sub globose to ovoid. The colour of the spore is dirty yellowish brown to yellowish brown. Presence of single wall layer with irregular warts.

Soil is the reservoir of the essential nutrients which are required for the plant growth and development. The present investigation shows that there was variation in the soil nutrient status along different collection sites. Changes in the soil properties vary due to the variation in biotic as well as abiotic factors prevalent in nature. The results show that the samples collected from agricultural farmland had higher organic Carbon percentage, Available Nitrogen (kg/hac), Available Phosphorus (kg/hac) as well as Available Potassium (kg/hac). The pH of the soil was found to be higher along the roadside as well as riverside. The pH of the soil influences almost every characteristic of soil that is affected by multiple biological interactions within the rhizosphere. The influencing effects of pH in the rhizosphere are crucial in supporting a biologically diverse microbial community. Manipulation of the pH by plant roots is constantly occurring and, it is usually believed that plants manipulate the soil pH in order to change the availability of nutrients; it also undoubtedly influences the abundance and diversity of associated microorganisms (Kent and Triplett, 2002). Soil fertility is *inter alia* dependent on the soil organic content. The organic carbon releases nutrients for plant growth and helps in promoting the structural, biological and physical health of the soil. The amounts of soil organic carbon present in the soil can vary according to the changes in temperature, rainfall, land management, soil nutrition and soil type and landscape. Carbon stored in the soil as organic carbon also aids in carbon sequestration. Phosphorus is an essential macro-nutrient which is required for plant nutrition. It contributes in various metabolic processes such as photosynthesis, energy transfer, and synthesis and breakdown of carbohydrates. It is available in both organic well as inorganic form in the soil. However, the amount of readily available phosphorus is very low compared with the total amount of phosphorus in the soil. Nitrogen is also an important plant nutrient for crop production. Nitrogen is a macronutrient responsible for plant growth and nutrition. It is also a constituent of the building blocks of almost all plant structures and an essential component of chlorophyll, enzymes, proteins, etc. Nitrogen occupies a unique position as a plant nutrient because rather high amounts are required compared to the other essential nutrients. It stimulates root growth and crop development as well as uptake of the other nutrients. Potassium is another essential plant nutrient and is required in large amounts for proper growth and reproduction of plants. Rhizosphere is a narrow zone of soil affected by the presence of plant roots. The rhizosphere is known to be a hot spot of microbial activities. Plants live in association with a rich diversity of microorganisms during their entire development (Johnson *et. al*, 1997). This is caused by an increased nutrient supply for microorganisms, since roots release a multitude of organic compounds (e. g. exudates and mucilage) derived from photosynthesis and other plant processes (Brimecombe *et. al*, 2007). Mycorrhizal symbiosis plays a key role in nutrient cycling in the ecosystem and also protects plants against environmental and cultivation stress (Carvalho *et. al*, 2004) Certain soil microbes like beneficial bacteria and fungi can improve plant performance under stress environments and consequently, enhance yield (Kaligari *et. al*, 2008).

Soil microbes act as an essential component of plant community variety and productivity (Wardle *et. al*, 2004). The environmental factors such as the soil pH, moisture, temperature, organic carbon and nitrogen play an important role in the distribution of microorganisms (Gaddeyya *et. al*, 2012). These are the main factors affecting the microbial population and diversity. A total of 23 species of AM fungi belonging to four genera viz., *Glomus*, *Acaulospora*, *Gigaspora*, *Entrophospora* and *Pacispora* were recovered from the rhizosphere of *O. indicum*. Amongst the various AM

fungi harboured in the soils of study sites, *Glomus* sp. was mostly associated in the rhizosphere of *O. indicum*. The study shows that the *Glomus* sp. was mostly present in the rhizosphere of *O. indicum* followed by *Acaulospora* sp. and *Gigaspora* sp. (Manoharachary *et. al.*, 2002) also reported that *Glomus* as the dominant species in various ecosystem of India. The *Glomus* spp. might be dominant due to their sporocarpic or clustered nature that helps in sporulation more frequently than rest of the genera as they appear singly in nature. Similarly, Das and Highland (2010) also reported the dominance of *Glomus* spp. associated with *Michelia champaca* L. in north-east India. Dhar and Mridha (2006) also reported the same reason for the dominance of *Glomus* spp. in their study.

The roots of *Oroxylum indicum* were colonized by AM fungi in all the sites. It was earlier reported by Pawaar and Kakde (2012) that the root of *O. indicum* does not show any type of mycorrhizal associations at all. But this study shows the presence of hyphal, arbuscular as well as vesicular colonization in the roots of the target plant species which is also in confirmation with (Reddy *et. al.*, 2015). Based on literature review, five different habitats were classified mainly roadside, riverside, forest fringe, hill slopes and agricultural farmland for this study. As the symbiotic association between micro-organisms and the medicinal plants is of great significance, the study shows the degree of dependence of medicinal plants on the fungal symbionts varies with the prevailing environmental conditions, particularly soil fertility conditions (Debi and Parkash, 2017). In the present study, it was found that AM spore count was maximum in forest fringe and agricultural farmland. The hyphal and arbuscular colonization percentage was again higher in soil samples collected from agricultural farmland, while vesicular colonization percentage was maximum in agricultural farmland and forest fringe areas. The AM spore count of the rhizospheric soil sample of *O. indicum* growing near roadside showed positive correlation with root colonization. AMF behaviour is affected by soil pH and influence mycorrhizal establishment and growth. The root colonization and the AM spore count were also positively correlated which is in confirmation with earlier finding (Sivakumar, 2013; Carrenho, 2007). It may limit the availability of nutrients; influence the pattern of absorption of nutrient and their exchange in root zone and even distribution of AMF species. The statistical analysis of the data revealed that the AM spore count is positively correlated with the soil nutrient status of the samples collected from various collection sites. The correlation value of (0.766, 0.413, and 0.567) was obtained when root colonization was compared with soil organic carbon percentage, soil nitrogen and potassium content. Similar findings were also reported by Basumatary *et. al.*, 2014. Similarly the available phosphorus in the soil were positively correlated with root colonization, with correlation values of 0.387 and respectively. This is in negation to similar earlier studies by other researchers (Aliasgharzadeh *et. al.*, 2001; Khakpour and Khara, 2012) where negative correlation values were observed between mycorrhizal root colonization (%) and available soil phosphorus. The positive correlation between soil P and AM colonization, contrary to early observations, could be due to the fact that an increase in P levels in P deficient soils can enhance mycorrhization before an expected decrease occurs (Bolan, 1991). The trees growing along riverside showed negative correlation values when soil pH, Org. Carbon (OC), and N, P, K status were correlated with AM spore count ( $R^2 = -0.12, -0.34, -0.33, -0.581, -0.99$ ), while AM spore count was positively correlated with colonization. The *O. indicum* trees growing along forest fringe area, AM spore count was positively correlated with soil Org. Carbon while negative correlation values were obtained when AM spore count was compared with soil pH and N, P, K, status. There are several reports that soil N could suppress root colonization by AM fungi (Chambers *et. al.*, 1980; Buwalda and Goh, 1982; Johnson, 1984). The suppressive effect of soil N on AM fungi has been attributed primarily to pH changes associated with variations in soil N (Thompson, 1986). Fluctuations in the number of AM fungal spores would be expected to occur if they were lost during periods of mycorrhizal formation, or as a result of predation by soil micro-organisms, as well as due to the impact of adverse soil

conditions during periods of inactivity (Brundrett, 1991). The trees growing along hill slopes showed positive correlation values for soil pH, Org. Carbon, soil Potassium and AM spore count while negative correlation values were obtained when AM spore count were compared with soil N and P status and root colonization. While slight negative correlation value was obtained when root colonization as well as spore count were compared with pH of soil. Ghorbani et. al, (2012) and Khakpour and Khara (2012) also reported similar findings. The negative correlation between mean spore density and available soil P recorded in the hill slope is in agreement confirmation with the findings of Nemeč *et al* (1990) and Khade and Rodrigues (2009), who reported similar findings while studying the distribution and ecology of AM fungi in Citrus nurseries and spatio-temporal variations of AM fungi in papaya plantations. In trees growing along agricultural farmland the AM spore count was positively correlated with soil OC and N, P status while it was negatively correlated with soil pH and K status. Soil K is often reported to have a stimulatory effect on mycorrhization (Furlan and Bernier-Cardou, 1989) and a minimum soil K is often a prerequisite for mycorrhization in some plant species (Ouimet *et. al*, 1996). Further studies are in progress to study and characterize different AM species associated with medicinal plants and their application as effective bioinoculants.

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