

Arbuscular mycorrhizal and dark septate endophytic fungal associations in three *Dendrocalamus* species (Bambusoideae) of Manipur

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Bamboos are one of the most economically and ecologically important forest product. We examined the diversity of AMF in rhizospheric soils and the colonization patterns of AMF and DSE fungal structures in the roots of three *Dendrocalamus* species viz. *D. hamiltonii*, *D. latiflorus* and *D. longispathus* from Ngariyan hill, Imphal East, Manipur. Highest spore density of AMF was recorded in the soil samples of *D. longispathus*. Out of 18 AMF species isolated from seven genera i.e., *Acaulospora*, *Funneliformis*, *Glomus*, *Rhizophagus*, *Sclerocystis*, *Scutellospora* and *Septoglomus*, the most dominant genus was *Glomus* with seven species. Relative abundance (%RA) and Isolation frequency (%IF) were recorded to be maximum with the spores of *Funneliformis geosporum*. The examined root fragments of *D. hamiltonii*, *D. latiflorus*, and *D. longispathus* exhibited *Paris*- type of AM morphology. The highest percentage of total root length with AM colonization (%RLTC) was obtained in *D. longispathus*, while that of DSE fungal colonization (%RLDTC) was maximum in *D. latiflorus*. Thus, the present findings revealed that this region of Indo-Burma biodiversity hotspot harbours diverse species of AM fungi and indicates the possibility of utilizing them in eco-restoration and conservation of biodiversity.

Keywords : Arbuscular mycorrhizal fungi, colonization pattern, dark septate endophyte, Ngariyan hill, species richness,

INTRODUCTION

The gigantic grass, bamboo, belongs to the family Poaceae and subfamily Bambusoideae. After China, India has the second-highest genetic resources for bamboo (Bystrakova *et al.* 2003). Manipur being a part of Northeast India lies within the Indo-Burma mega biodiversity hot spot region and has 35 species of bamboos belonging to 10 different genera (Naithani *et al.* 2010). In comparison to other forest plants, bamboo is the most versatile forest product and one of the most significant renewable natural resources that can produce the highest biomass (Das and Kayang, 2010). Once regarded as the “wood of the poor,” this group of plants is now known as “green gold” and offers especially encouraging prospects for environmental challenges as well as a quickly expanding market (Sandhu *et al.* 2018). Therefore, it is necessary to create methods for

the rapid and economical propagation of bamboo species.

Beneficial microorganisms like AMF can be used to stimulate the growth and development of bamboo species (Zamora-Chacón *et al.* 2019; de Moura *et al.* 2019). In terms of soil erosion, land rehabilitation, water conservation, and carbon sequestration, bamboo forests serve both ecological and environmental purposes. They are also crucial for the preservation of biodiversity. (Forest Survey of India, 2011).

There are few studies that deal with the rhizospheric soil and soil biota of bamboo in general, and fewer studies that deal with the community composition and ecological interactions of the associated arbuscular mycorrhizal fungi (AMF) found in its rhizospheric soil (Jiang *et al.* 2013; Muthukumar and Udaiyan, 2006). Most of the research on bamboo has concentrated on evaluating its production potential (Shirasuna, 2012), its various uses, and

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its yield productivity (Chen *et al.* 2017; van Dam *et al.* 2018).

Arbuscular mycorrhizal fungi (AMF) are essential members of the soil microbial community that belongs to the phylum Glomeromycota. They establish symbiotic relationships with most land plants, developing *Arum*-, *Paris*-, or Intermediate-type AM morphology (Dickson, 2004). By improving the uptake of minerals like phosphorus (P), nitrogen (N), potassium (K), etc., AM fungi boost plant growth and productivity, particularly in weathered field soils in tropical and subtropical regions with low percentages of total and available phosphorus (Smith and Read, 2008; Smith and Smith, 2011; Bagyaraj *et al.* 2015). Understanding the ecological implications of AM fungal interactions can be aided by research on the distribution and activity of AM fungi (Sanders, 1990). Furthermore, there is mounting evidence linking the distribution and diversity of AM fungi to the structure of plant communities and the operation of ecosystems (van der Heijden and Sanders, 2002). Although there are only about 300 species of AMF, the majority of terrestrial plant species (82%) form the AMF symbiosis, which is common in most plant families and biomes on Earth (Brundrett and Tedersoo, 2018). According to Brundrett and Tedersoo, (2018), this symbiosis is thought to have been essential to plants' colonization of land and is currently present in the majority of commercially valuable plant species, including bamboo.

Another group of root colonizers that belong to the phylum Ascomycota are the dark septate endophytic (DSE) fungi. They are distinguished by the presence of septate melanized hyphae and a cluster of inflated, rounded, thick-walled cells called microsclerotial structures, which also coexist with AM (Jumpponen, 2001; Mandyam and Jumpponen, 2005). When DSE is present in plant roots, the host is protected against biotic and abiotic stresses in harsh environmental circumstances (Barrow, 2003). In contrast to temperate ecosystems, there are less reports on DSE fungal association in tropical environments, despite their distribution across various habitats and host plants.

Previous reports have documented the presence and dispersion of AMF in forest plant species,

such as bamboos, as well as its relationship with and dependence on bamboo species (Khan and Uniyal, 1999). Furthermore, it has been documented that several bamboo species exhibit distinct growth responses to mycorrhizal inoculation, as well as the synergistic interaction between AMF and plant growth promoting rhizobacteria (PGPR) (Muthukumar and Udaiyan, 2006).

Despite the fact that numerous researchers have documented AM and DSE associations in a variety of plants, the diversity of AM and DSE fungal colonization in the roots of bamboos growing in Manipur is still unknown. In order to ascertain the diversity of AM fungi, evaluate the AM morphology, and measure the colonization intensities by AM and DSE fungal structures in three *Dendrocalamus* bamboo species, the current study was conducted.

MATERIALS AND METHODS

Study sites and Materials

The soil and roots samples of *Dendrocalamus latiflorus*, *D. longispathus* and *D. hamiltonii* were collected from Ngariyan hill (24° 72' 32''N Latitude, 94° 02' 26''E Longitude) located at Imphal East, Manipur in the month of July 2019. The study site is located on the slope of the hill at an elevation between 790 to 900 m.a.s.l. The soil samples collected from the study site has sandy loam soil texture and were acidic in nature.

Sample collection

The soil and root samples of each species were collected by digging 0-20 cm depth of soil core from five different individual rhizome of each species. The samples were placed in polythene bags, labeled, and brought to the laboratory for further analysis. The soil samples were analyzed for pH and moisture content and then air dried under shade at room temperature. The air-dried soils samples were divided into two halves, one half for spore analysis and the other for soil physico-chemical properties and establishing trap culture. The collected root samples of each species were washed with water and fixed in FAA (formalin: glacial acetic acid: 70% ethyl alcohol,

5: 5: 90 mL, v: v: v) solution until further processing.

Spore Analysis

AMF spores were extracted by using wet sieving and decantation method (Gerdemann and Nicolson, 1963). One hundred grams of each soil sample of the bamboo species was dispersed in 1L of water and the suspension was decanted through a series of 710 to 37 μ m sieves. The residues in the sieves were collected by washing them into a beaker. They are then filtered through girdled filter paper and all the AMF intact spores were counted using microscope. Further, they were transferred on cleaned glass slides and mounted in Polyvinyl alcohol-Lacto glycerol with or without Melzer's reagents (Schenck and Perez, 1990). The AMF spores were identified based on the morphological and subcellular characteristics by comparing with the original descriptions established by Schüssler's website (www.lrz-muenchen.de/~schuessler/amphylo/amphylospecies.html). The morphology of the spores was also compared to the culture database established by International Culture Collection of Vesicular and Arbuscular Mycorrhizal Fungi, i.e., INVAM (<http://invam.cag.wvu.edu/>).

Analysis of AM and DSE colonization

AM and DSE fungal colonization were assessed using the method of Koske and Gemma (1989) and percentage root length by magnified intersection method (McGonigle *et al.* 1990). Fixed roots were washed to make them free of FAA under tap water, cut into 1cm segments and processed in 2.5% KOH at 90 °C until it becomes clear, acidified with 1N HCl and stained overnight in trypan blue-lactoglycerol (0.05%) at room temperature (Koske and Gemma, 1989). Stained root pieces were mounted on glass slides in Lactoglycerol and examined under compound microscope (Nikon Eclipse E100) for different AM and DSE fungal structures. Percentage of root length colonization was estimated according to magnified intersection method (McGonigle *et al.* 1990). AM morphology was characterized based on inter- or intracellular nature of fungal structures within the roots (Dickson, 2004). The percentage of root length colonized by AM fungal structures

and DSE was determined using the magnified intersection method (McGonigle *et al.* 1990).

Analysis of soil properties

The soil textures of the selected bamboo species were assessed by the Bouyoucos hydrometer method (Allen *et al.* 1974). Soil pH and electrical conductivity (EC) was determined in aqueous soil: water (1:1, v: v) using digital meter. Organic carbon (OC) was analyzed by rapid titration method (Walkley and Black, 1934). Total nitrogen (N) and available phosphorus (P) were determined by the methods as described by Jackson (1971) while exchangeable potassium (K) was assessed after extraction with ammonium acetate (Jackson, 1971).

Data Analysis

Spore density (SD), Species richness (SR), Relative abundance (%RA) and Isolation frequency (%IF) were calculated using the formula indicated by Dandan and Zhiwei (2007). For analyzing the significance of variations between spore densities, root colonizations and soil parameters of the three bamboo species, one way analysis of variance (ANOVA) was used. Further, Pearson's correlations were calculated to assess the relationship between soil physico-chemical properties and mycorrhizal and DSE colonization (IBM SPSS Statistics 26).

RESULTS AND DISCUSSION

Soil physico-chemical properties

The soil samples of the three bamboo species were found to be sandy loam in texture and moderately acidic in nature. Maximum concentration of EC, total N and available P were found in *D. longispathus*, while that of % OC was found in *D. latiflorus*. And maximum exchangeable K concentration was found in *D. hamiltonii*. (Table1).

Spore density and distribution of AM fungi

The maximum AMF spore densities observed in the examined soil samples were 246, 358 and 289 spores 100 g⁻¹ soil in *D. latiflorus*, *D.*

Table 1: Physical and chemical soil characteristics of the three bamboo species

Bamboo species	EC (dSm ⁻¹)	pH	OC (%)	N (Kg ha ⁻¹)	P (Kg ha ⁻¹)	K (Kg ha ⁻¹)
<i>D. latiflorus</i>	0.07±0.01a	5.32±0.03c	1.74±0.02c	81.2±0.23a	14.3±0.36a	90±1.53a
<i>D. hamiltonii</i>	0.22±0.02b	5.05±0.02a	1.58±0.02a	170.8±1.25b	15.1±0.25b	450±3.61c
<i>D. longispathus</i>	0.25±0.02c	5.17±0.02b	1.6±0.03a	174±2.04c	15.9±0.15c	425±3.79b

EC (electrical conductivity), soil pH, %OC (percentage organic carbon), N (available nitrogen), P (available phosphorus) and K (exchangeable potassium) respectively.

Means ±SE in a column followed by same letter(s) are not significantly different (P> 0.05) according to DMRT.

Table 2 : %Relative Abundance and %Isolation Frequency of AMF species of the three bamboo species.

AMF species	% Relative Abundance			% Isolation Frequency
	<i>Dendrocalamus latiflorus</i>	<i>Dendrocalamus longispathus</i>	<i>Dendrocalamus hamiltonii</i>	
<i>Acaulospora rehmsii</i> Sieverd and S. Toro	10.34	11.30	7.51	77.78
<i>Acaulospora</i> sp. 1	7.47	-	5.53	44.44
<i>Acaulospora</i> sp. 2	-	-	2.77	22.22
<i>Acaulospora</i> sp. 3	-	5.14	-	33.33
<i>Funneliformis geosporum</i> C. Walker and Schuessler	36.78	29.11	24.51	100
<i>Funneliformis monosporus</i> Oehl, G.A. Silva and Sieverd.	-	7.88	5.14	55.56
<i>Glomus macrocarpum</i> Tul. and C. Tul.	-	12.33	8.70	66.67
<i>Glomus magnicaule</i> I.R Hall	8.62	9.25	5.93	77.78
<i>Glomus multicaule</i> Gerd. and B.K. Bakshi	-	2.05	11.46	55.56
<i>Glomus</i> sp. 1	4.60	5.82	3.16	66.67
<i>Glomus</i> sp. 2	5.75	-	-	22.22
<i>Glomus</i> sp. 3	6.90	-	0.79	33.33
<i>Glomus</i> sp. 4	-	3.77	0.79	44.44
<i>Rhizophagus fasciculatus</i> C. Walker and Schuessler	-	7.53	15.42	55.56
<i>Sclerocystis sinuosa</i> Gerd. and B.K. Bakshi	7.47	-	3.16	44.44
<i>Scutellospora</i> sp. 1	4.02	-	-	22.22
<i>Scutellospora</i> sp. 2	8.05	4.45	5.14	77.78
<i>Septoglomus constrictum</i> Sieverd, G.A. Silva and Oehl	-	1.37	-	11.11
	100	100	100	

- indicate absence of AMF species

longispathus and *D. hamiltonii* respectively. The spore density of *D. hamiltonii* is comparatively lower than the reported 103 spores 25 g⁻¹ in *D. hamiltonii* from plantations of Meghalaya, Northeast India (Das and Kayang, 2010). The AM spore density in different crop soils were found to be influenced by the soil properties such as soil pH and nutrient availability therein

(Surendirakumar *et al.* 2019). A total of 18 AMF spore morphotypes belonging to seven genera i.e., *Acaulospora*, *Funneliformis*, *Glomus*, *Rhizophagus*, *Sclerocystis*, *Scutellospora* and *Septoglomus* were isolated from the soil samples of the three selected bamboo species of *Dendrocalamus* (Table 2, Fig. 1). Among the isolated AMF morphotypes, the genus *Glomus*

Table 3 : Summary of F values from one way Analysis of Variance (ANOVA) for spore densities, mycorrhizal and DSE root colonizations and soil parameters of the three bamboo species.

Variables	df	F values	Significance
SPN	2, 42	105.841	***
%AC	2, 42	19.161	***
%HC	2, 42	2.807	NS
%V	2, 42	0.181	NS
%DSH	2, 42	8.706	***
%MI	2, 42	15.928	***
%RLTC	2, 42	19.707	***
%RLDTC	2, 42	21.233	***
EC	2, 42	310.776	***
PH	2, 42	293.703	***
OC	2, 42	82.552	***
N	2, 42	10064.91	***
P	2, 42	62.031	***
K	2, 42	28603.65	***

*** Significant at $P < 0.001$, NS = non-significant.

recorded a maximum of 7 AMF species, followed by *Acaulospora* (4 AMF species) and 2 AMF species each were isolated from *Funneliformis* and *Scutellospora*. But, in case of, *Rhizophagus*, *Sclerocystis* and *Septoglomus*, only single species were isolated. Out of the 18 AMF spore morphotypes isolated, the genus *Glomus* was predominant. Similar findings was found by Sinha *et al.* (2018). There are also several reports of *Glomus* being the dominant genus occurring in Indian soil (Muthukumar and Udaiyan, 2000; Bhattacharya and Bagyaraj, 2002; Das and Kayang, 2009). The highest species richness was recorded from the soil samples of *D. hamiltonii* with 14 AMF species. Maximum relative abundance (%RA) and isolation frequency (%IF) were recorded with the spores of *Funneliformis geosporum* (Table 2). *Acaulospora* sp. 2 was found only in the rhizospheric soil of *D. hamiltonii*. While *Acaulospora* sp. 3 was exclusively found in *D. longispathus* soils. *Glomus* sp. 2 and *Scutellospora* sp. 1 were exclusively found in *D. latiflorus*. AMF morphotype *Septoglomus constrictum* was specially found in *D. longispathus*. Hence, the present findings revealed that the majority of the identified AMF species belong to Order Glomerales. This

suggests that the species under Glomerales can be considered as generalists that produce large number of spores with short period of time and show the ability to adapt wide range of plant communities in different ecosystems (Pandey *et al.* 2016).

AMF morphology and extent of AM and DSE fungal root colonization

Dual colonization of AMF and DSE fungi were seen in all the three species of bamboos (Fig. 2). The examined root fragments of *D. hamiltonii*, *D. latiflorus* and *D. longispathus* exhibited *Paris* type of AM morphology which is evidenced by the presence of associated hyphal coil (hc) and arbusculate coils (ac). Occurrence of *Paris* - type AM morphology in bamboo species corresponds with the study reports of Muthukumar and Prakash (2009).

Percentage of total root length colonization of AMF (%RLTC) of the three selected bamboos of *Dendrocalamus* ranges from 25.6 % to 65.07% and percentage total root length colonization of DSE (%RLDTC) ranges from 20.46% to 56.18%. Highest %RLTC was recorded from *D.*

Table 4 : Pearson Correlation between AMF and DSE colonization with soil properties

Variables	SPN	%AC	%HC	%V	%DSH	%MI	%RLTC	%RLDTC
PH	-.301*	-.535**	-0.265	0.049	.478**	.573**	-.535**	.623**
EC	.709**	.689**	.310*	0.024	-.499**	-.564**	.680**	-.630**
%OC	-.537**	-.628**	-.318*	0.066	.496**	.570**	-.632**	.631**
N	.675**	.675**	.338*	0.013	-.530**	-.642**	.684**	-.694**
P	.728**	.641**	0.195	0.158	-.366*	-.418**	.594**	-.464**
K	.612**	.669**	.338*	0.002	-.537**	-.651**	.678**	-.703**
SPN		.523**	0.266	0.071	-.372*	-.421**	.536**	-.470**
%AC			0.198	0.060	-.647**	-.609**	.854**	-.745**
%HC				-0.112	-.562**	-.537**	.673**	-.652**
%V					0.143	-0.254	0.066	-0.061
%DSH						.421**	-.770**	.849**
%MI							-.760**	.836**
%RLTC								-.908**
%RLDTC								

EC (electrical conductivity), soil pH, %OC (percentage organic carbon), N (available nitrogen), P (available phosphorus) and K (exchangeable potassium) respectively.

SPN (spore number), %AC (percentage arbusculate coil), %HC (percentage hyphal coil), %V (percentage vesicles).

%DSH (percentage dark septate hyphae) and %MI (percentage microsclerotia).

%RLTC (total root length colonization by AMF), %RLDTC (total root length colonization by DSE).

*means Correlation is significant at $P < 0.05$ and ** means Correlation is significant at the $P < 0.01$.

longispathus (65.07 ± 4.8) while highest %RLDTC was found in *D. latiflorus* (56.18 ± 2.98) (Fig. 3). Earlier reports have suggested that the AMF colonization of *D. hamiltonii* ranged between 38% and 100% in seasonal studies. In our study, %RLTC of *D. hamiltonii* was 59.31%, thus it falls within the range given by earlier workers. However, there are also reports of lesser %RLTC of *D. hamiltonii* than us such as 36% of %RLTC colonization reported by Das and Kayang, (2010).

The percentage total root length colonization of DSE (% RLDTC) in *D. hamiltonii* and *D. longispathus* species were lower than AMF root colonization which was in accord with the study of Das and Kayang, (2010). However, the percentage of total root length DSE colonization in *D. latiflorus* is found to be higher than AMF colonization in our study which does not corresponds with their findings. One way analysis

of variance (ANOVA) showed significant differences between spore densities, mycorrhizal and DSE root colonisations and soil parameters of the three bamboo species. Significant variations were observed among the studied bamboo species and their spore densities ($F_{2, 42} = 105.841$; $P < 0.001$). Overall significant variations were seen in all the root colonization structures i.e. %AC ($F_{2, 42} = 19.161$; $P < 0.001$), % RLTC ($F_{2, 42} = 19.707$; $P < 0.001$), %DSH ($F_{2, 42} = 8.706$; $P < 0.001$), %MI ($F_{2, 42} = 15.928$; $P < 0.001$) and %RLDTC ($F_{2, 42} = 21.233$; $P < 0.001$). However, percentage hyphal coil structure and percentage vesicular structure were non-significant among the studied bamboo species (Table 3).

The physico-chemical properties of soil are known to affect the functioning and colonization levels of AM fungi (Smith and Read, 2008). The findings of our present investigation showed that soil pH



Fig. 1: Arbuscular mycorrhizal spores isolated from the rhizosphere soils of studied bamboo species. a) *Acaulospora rehmlii*, b) *Acaulospora* sp. 1, c) *Acaulospora* sp. 2, d) *Acaulospora* sp. 3, e) *Funneliformis geosporum*, f) *F. monosporus*, g) *Glomus macrocarpum*, h) *G. magnicaule*, i) *G. multicaule*, j) *Glomus* sp. 1, k) *Glomus* sp. 2, l) *Glomus* sp. 3, m) *Glomus* sp. 4, n) *Rhizophagus fasciculatus*, o) *Sclerocystis sinuosa*, p) *Scutellospora* sp. 1, q) *Scutellospora* sp. 2, and r) *Septoglomus constrictum*. (Scale bars q = 10 μ m; a, b, c, d, k, m, o, p = 20 μ m; e, f, g, h, i, j, l, n, r = 40 μ m)

was significantly and negatively correlated with spore density ($r = -0.301$; $p < 0.05$), which contradicts with the reports of (Muthukumar and Muthuraja (2016) where they reported positive correlation for these variables while negative significance of soil pH with %RLTC ($r = -0.535$; $p < 0.01$) was in conformity with their reports. AMF spore density was significantly and negatively correlated with % OC ($r = -0.537$; $p < 0.01$), which was similar with the reports of (Singh *et al.* (2003) and (Surbala and Pandey (2020). Significant positive correlation of AMF spore density with %RLTC ($r = 0.536$; $p < 0.01$) was found in our study. (Wuen *et al.* (2002), (Isobe *et al.* (2008) and Sivakumar (2013) also found similar relationship of AMF spore density positively correlated to the percentage of root colonization in sugarcane. But our results contradict with the reports of negative correlation with these variables by Muthukumar and Muthuraja (2016); Surrendirakumar *et al.* (2019); Surbala and Pandey (2020). Significant negative correlation

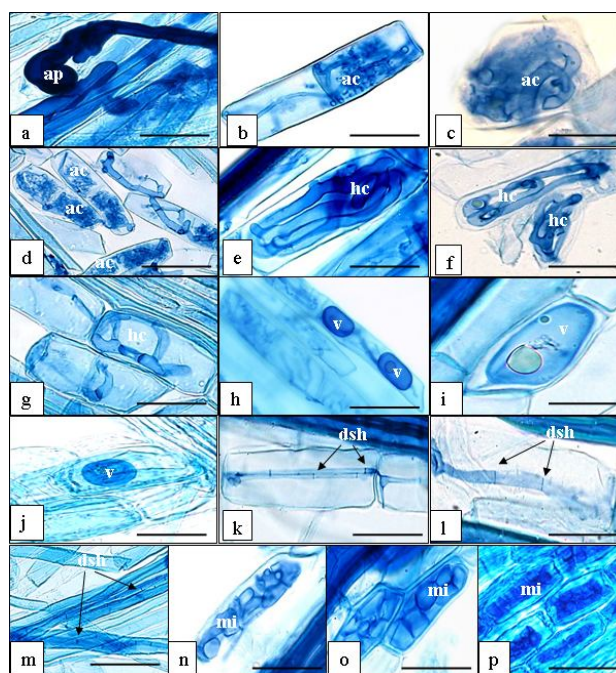


Fig. 2: Arbuscular mycorrhizal (AM) and dark septate endophyte (DSE) colonization in the roots of the three bamboo species. (Scale bar = 40 μ m). (a) Appressorium (ap); (b-d) arbusculate coils (ac) in *D. hamiltonii*, *D. latiflorus* and *D. longispathus*; (e-g) hyphal coils (hc) in *D. hamiltonii*, *D. latiflorus* and *D. longispathus*; (h-j) vesicles (v) in *D. hamiltonii*, *D. latiflorus* and *D. longispathus*; (k-m) dark septate hyphae (dsh) in *D. hamiltonii*, *D. latiflorus* and *D. longispathus*; (n-p) microsclerotia (mi) in *D. hamiltonii*, *D. latiflorus* and *D. longispathus*.

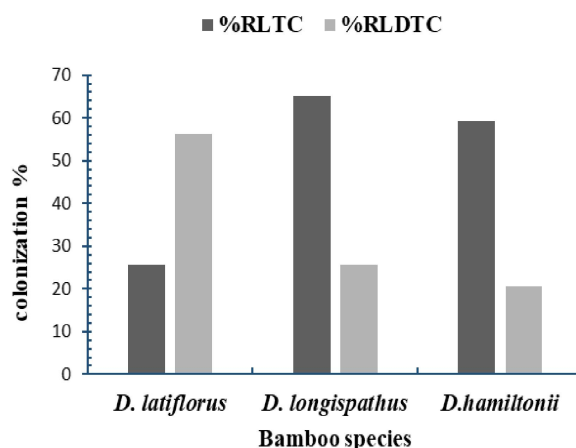


Fig 3: Percentage total root length colonization of AMF (%RLTC) and DSE (%RLDTC) of the three bamboo species

was observed between organic carbon (OC) and %RLTC ($r = -0.632$; $p < 0.01$) which was in conformity with the reports of (Lingfei *et al.* (2005) and (Pandey *et al.* (2016). In contrast to our results, Khade and Rodrigues (2008) and Lakshmiopathy *et al.* (2012) observed the positive correlation between AM colonizing structures and

soil %OC. Positive significant correlation was observed between available phosphorus (P) and %RLTC ($r = 0.594$; $p < 0.01$) which was not in accord with the reports of negative correlation with these variables by Lingfei *et al.* (2005). However, the %RLDTC was significantly and negatively correlated with available phosphorus ($r = -0.464$; $p < 0.01$) which was in conformity with the reports of Upson *et al.* (2009) but contradicts with the reports of positive correlation by Das and Kayang, (2010) and Muthukumar and Muthuraja (2016). A significant and negative correlation was found between %RLTC and %RLDTC ($r = -0.908$; $p < 0.01$) which was in accord with (Wu *et al.* (2009), however they suggested that there may be presence of AMF competition with DSE for econiche. Meanwhile Muthukumar and Muthuraja (2016) noticed a positive significant correlation between %RLTC and %RLDTC (Table 4).

CONCLUSION

The study clearly manifests the occurrence of AMF and DSE association in three bamboo species of *Dendrocalamus* and indicates the dynamic nature of root colonizing fungal communities in this region of NE India. Variation in edaphic, climatic and microclimatic factors might be responsible for the differences in AM and DSE fungal colonization in different species of bamboo and AMF spore density in respective rhizospheric soils. Prior to this study there were no reports of endorhizal associations in bamboos of Manipur. Therefore, it is suggested to evolve technology to combat exploitation of bamboo resources. With the proper knowledge about mycorrhizal diversity, there are possibilities of utilizing them in the eco-restoration and conservation of biodiversity and for exploration of different biological management programmes.

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DECLARATION

Conflict of Interest. Authors declare no conflict of interest.

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