Arbuscular Mycorrhizal Colonization in Five Tropical Forest Tree Legumes of Chittagong University Campus in Bangladesh

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Abstract: Arbuscular mycorrhizal (AM) colonization in five tropical forest tree legumes (*Gliricidia sepium*, *Dalbergia sissoo, Indigofera tysmanii, Delonix regia* and *Samanea saman)* was investigated in Chittagong University (CU) campus. The results of the present study clearly suggests that, Vesicular AM fungi (VAM) are common in all the studied forest tree species, and that the studied forest tree species differ in their rates of AM formation. The intensity of colonization is maximum (98%) in *I. tysmonii* followed by *D. sissoo* (95%), *D. regia* (63%), *S. saman* (59%) and *G. sepium* (52%). Coiled structures of hyphae were recorded in *D. regia*. Arbuscule formation was recorded in *D. sissoo* and in *I. tysmonii*. Mycorrhizal spores were found in rhizosphere soils of all the sites. *Glomus, Acaulospora, Entrophospora* and *Gigaspora* spores were identified in all the hosts. Forest plants differ in their rates of AM formation. Edhapic conditions like moisture content, soil pH also influence the extent of root colonization.

Keyword: Mycorrhizae, Legume, Colonization, Edhapic condition, Rhizosphere, Underground networking.

INTRODUCTION

Apparently, as can be seen aboveground, plants compete for life-giving sunlight, but below the soil surface a more complex situation exists in some plants. Higher plants can take advantage of the symbiotic fungal network present in their roots. However, yet it remains contentious whether the flow of nutrients between plants *via* fungi is a general and significant feature of the 'wood-wide web' of forest ecosystems [1]. Mycorrhizal symbioses- the union of roots and soil fungi- are universal in terrestrial ecosystems and may have been fundamental to land colonization by plants [2]. So far, little attention has been paid to the effects of microbe–plant interactions, particularly the mycorrhizal symbiosis, on ecosystem variability, productivity and plant biodiversity [3]. Belowground diversity of arbuscular mycorrhizal fungi (AMF) is a major factor contributing to the maintenance of plant biodiversity and to ecosystem functioning [4]. Mycorrhizal fungi can also provide resistance to stress, drought [5] and in some cases to soil pathogens [6]. In the dry regions where forest shades into grassland, water can also move between trees *via* fungi. Mycorrhizae are more important in more stressful climates. By helping plants cope with stress, and by helping seedlings survive, it is thought that fungal networks make plant communities more stable in the face of environmental stress, and quicker to recover from damage. By distributing resources between different species, fungi can preserve a variety of plant partners and insure against the effects of plant disease or herbivores. If the fungus can form a bigger or more diverse network, it's chances of survival are better [1]. Fungi might help young plants to get established because they help them compete with other fungi in the soil - nourishing an existing partnership might be a more effective strategy than seeking out new hosts. Alternatively, it could be that some plants provide trace amounts of vitamins or even hormones in return for fungal carbon [1].

Fungal networks may allow trees to support their own seedlings, perhaps providing the trees with an evolutionary benefit. There is lots of evidence that mature trees facilitate the growth of conspecific seedlings beneath them, and the evidence is growing that such network is important [5, 7]. Although endomycorrhizal association is beneficial for tropical plant species, considerable work has not been done on the different aspects such as physiology, ecology and taxonomy of endomycorrhizae as well as AM association. Again, though many works have been done on mycorrhizal colonization in other tropical countries such as India, Malaysia, Indonesia very little research work has been reported from Bangladesh [8]. Finally, the basic knowledge of mycorrhizal research is to be incorporated in the practical field of forestry with particular reference to Bangladesh. The selection of the most appropriate plant-fungus association for each specific environmental and ecological situation is one of the main challenges in current research on AM. Existing literature shows that very little work has been

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done on AM occurrence both in soil and trees of Bangladesh [9, 10, 11, 12]. *Dalbergia sissoo, Gliricidia sepium, Indigofera tysmonii, Delonix regia* and *Samania saman* are five leguminous tree species that are widely used for plantation in Bangladesh. But the status of mycorrhizal association of these tree species in plantations of Bangladesh has never been investigated before. Therefore, the present study is an attempt to assess the status of AM fungi in the roots and rhizosphere soils of the five leguminous forest tree species in plantations of Chittagong University campus.

MATERIAL AND METHODS

This experiment was carried out in the nursery of the Institute of Forestry and Environmental sciences, University of Chittagong, Chittagong (IFESCU), Bangladesh (91°50′ E latitude and 22°30′ N longitude). Roots and rhizosphere soil samples of trees were collected from plantations of G*liricidia*, *Sissoo, Indigofera, Samanea* and *Delonix* that occurred in various sites in the vicinity of the premises of the Institute of Forestry and Environmental Sciences of Chittagong University campus. Rhizosphere soils, fine and feeder roots were collected from depths of 25-30 cm.

The soils and roots were separated without delay in the laboratory. Collected root samples were washed carefully and fine roots were cut into small segments of approximately 1 cm for determination of AM colonization rate. The root pieces were cleaned and stained using aniline blue according to the method of Phillips and Hayman [13] supplemented by that of Mridha *et al*. [14]. The root pieces were boiled in 10% KOH solution for 15-20 minutes at 90^0C temperature. Then they were rinsed in tap water several times and acidified with 1% HCl solution for 1 hour. When necessary, heavily pigmented roots were bleached in H_20_2 for about 15-20 minutes and again washed with water. Again these segments were boiled in 0.05% aniline blue at 90^0C for about 10 minutes and subsequently the roots were destained at room temperature in acidic glycol. The roots were then observed under the compound microscope at 10x10 magnifications. Percent root colonization was calculated using the following formula:

$$\% \text{ Colonisation} = \frac{\text{Total number of AM positive segments}}{\text{Total number of segment studied}} \times 100$$

Presence of mycelium, vesicles and arbuscules was treated as the AM positive. The intensity of root colonization i.e. intensity of mycelium, vesicle and arbuscule of AM fungi was estimated as poor (P) when < 10% of the root area was infected, moderate (M) when <25% of the root area was infected and abundant (A) when > 25% of the root area was infected.

From the rhizosphere soil samples 100g soil was mixed with one litre of water and the suspension was left for few minutes for settlement of sands and other heavy particles. The suspension was passed through the ASTM-60, ASTM-100, ASTM-240 and ASTM-400 sieves gradually to extract the spores followed by wet sieving and decanting method [15]. The residues of the sieves were filtered with the Whatman filter paper No-1. Squares of intersecting gridlines were drawn earlier on the filter paper for easy counting of spores. After water filtration the paper was examined under the stereo-binocular microscope at 2.5.10 magnification and the number was recorded. Spores were separated on the basis of morphological characters and then they were observed under compound microscope mounting on PVLG and Melzer's reagent to determine the proper genus by following the established literature [16]. Percent population of individual genus was calculated by the following formula:

$$\% \text{ Genus} = \frac{\text{Number of individual genus}}{\text{Total number of species}} \times 100$$

Soil samples were analyzed for pH and moisture content to facilitate the understanding of spore dynamics of fungal culture. The soil samples were dried in an oven for 8 hours at temperature 105^0C. Moisture content was calculated from the following relationship:

$$\% \text{ Moisture} = \frac{b - c}{b - a} \times 100$$

Where, *a* stands for weight of the petriplates; *b* stands for weight of the petriplates + moist soil; *c* stands for weight of the petriplates + dry soil; *b-c* denotes amount of moisture lost and *b-a* means amount of soil taken.

The calculated % Moisture content is then counted with the help of moisture meter. Soil pH was measured using a low conductivity glass calomel electrode in a soil suspension prepared by mixing 10g of moist soil with 20 ml of distilled water. The pH meter was standardized using pH 4 and 7 buffer solution.

RESULTS

The root and soil samples collected from the five plantation tree species were assessed for percentage of root colonization, intensity of root colonization and number of spores/100gm dry soil. Number of spores in each genus along with the occurrence of AM Fungi in forest trees is shown in Table **1**.

The intensity and percentage of colonization in root samples of the studied forest tree species varied. The intensity of colonization was maximum (98%) in *Indigofera tysmonii* followed by *Dalbergia sissoo* (95%) at plantation age of 10 years. Moderate (only hyphae and vesicle present) colonisation was present in *Delonix regia* (63%), *Samania saman* (59%) and *Gliricidia sepium* (52%) in ten year old plantations of the tree species. Coiled structures of hyphae were recorded in *Delonix regia*. Arbuscule formation was recorded in *Dalbergia sissoo* and *Indigofera tysmonii*. Most of the studied plant species had prominent AM fungal association in the forms of vesicular and arbuscular structures or both. The vesicles observed in different plants varied in shape and size (Plate **9-12**).

Though the vesicles and arbuscules were present in the roots of different plant species, it was difficult to identify the fungi. With a view to identify as many AM fungi as possible, the rhizosphere soil samples of all the seedlings were processed for isolating different AM fungal propagules. The rhizosphere samples of all the tree seedlings contained AM fungal spores. Analysis of the rhizosphere soils showed that mycorrhizal spores were present in all locations from where the plant roots were collected. The numbers of spores found in 100 g dry soils ranged between 60 and 209 (Table **1**). The highest (209) spore population was found in *Indigofera tysmonii* while the soils of *Gliricidia sapium* contained the lowest (60). Two to four different types of spores were identified in the soils under each genus for the host plants (Table **2**). Based on spore characters, the identified genera of the spores for all the hosts were *Glomus* (Plate **5-6**), *Acaulospora* (Plate **2-4**), *Entrophospora* (Plate **7-8**) and *Gigaspora* (Plate **1**).

Among these, *Glomus*, *Acaulospora* and *Entrophospora* were dominant and these three genera were common in all the hosts. *Entrophospora* were found as a pure form in the studied samples of *Dalbergia sissoo*.

The results of the assessment of root colonisation indicate that all the studied forest tree species are highly mycorrhizal and a wide range of variation in the percentage and intensity of AM root colonization was recorded. The intensity of root colonisation was related to the percentage of root colonisation. Recorded Moisture content (MC) and soil pH of the collected rhizosphere soils of *Dalbergia sissoo, Gliricidia sepium, Indigofera tysmonii, Delonix regia* and *Samanea saman* in relation to fungal factors have been presented in Table **3**.

In the studied areas, Soil pH ranged between 6.7 and 5.8 indicating soils of acidic nature. The soil of *Dalbergia* and *Gliricidia* showed similar pH level but the percentage of MC varied and the percentage colonization also varied significantly. Huge *Entrophosphora* spores occurred in *Dalbergia*, where as, number of spore populations was the least in case of *Gliricidia*. The highest percentage of colonization and spore population occurred in *Indigofera tysmonii* in soil pH 6.7, which appeared to be the highest pH level among the studied samples. Highest percentage of MC was recorded in case of *Delonix regia* whilst the lowest was in case of *Dalbergia sissoo*. The results of the present study clearly suggest that forest plants differ in their rates of AM formation. Additionally, edaphic conditions affect the formation of AM or influence the extent of root colonization.

Table 1: Occurrence of AM fungi in Forest Plantation Tree Species at Chittagong University Campus

Species	Percentage of colonization	Colonization (a)	Intensity of colonization (b)	Total species 100^{-1}g dry soil
Dalbergia Sissoo	95	+	**	178
Gliricidia sepiulm	52	+	**	60
Indigofera tysmonii	98	+	***	209
Samanea saman	59	+	**	72
Delonix regia	63	+	**	84

a) Colonization => + = Present; -- = absent.
b) Intensity of colonization => * = Only hypae present; ** = Mycelium and vesicle present *** = Mycelium, vesicle and arbuscule present (coiled mycelium also).

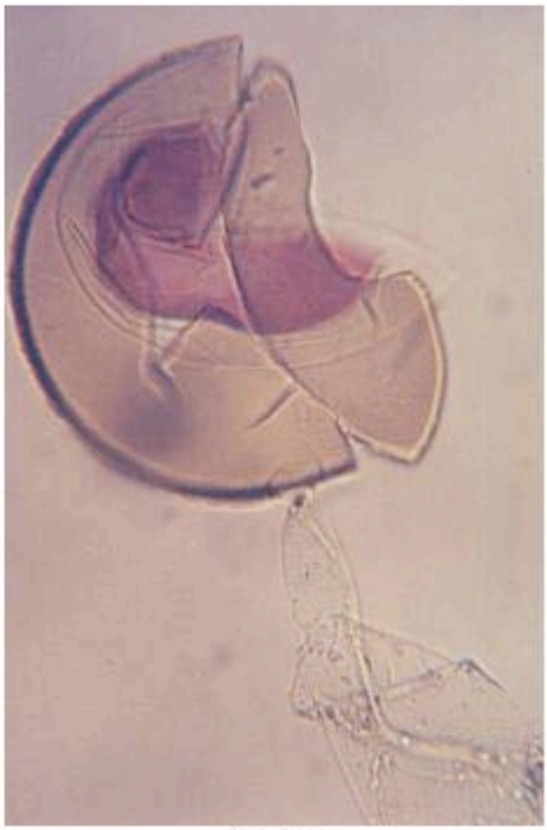
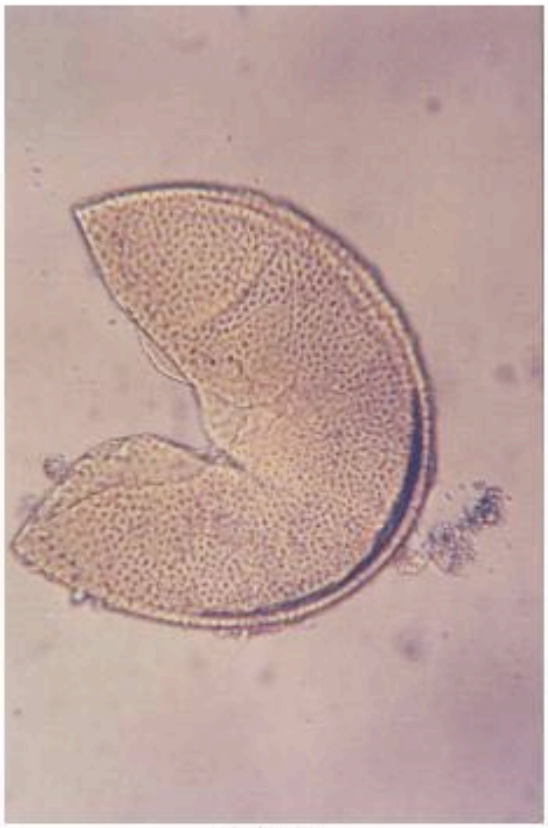


Plate 1



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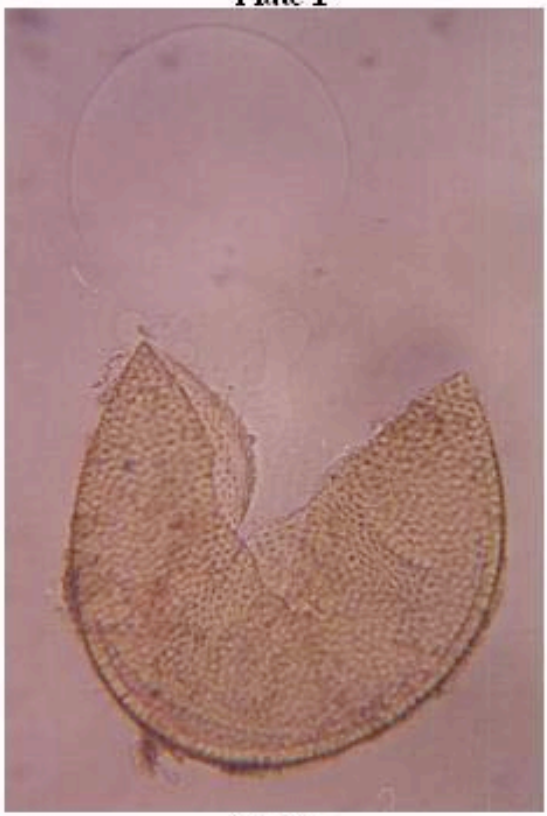

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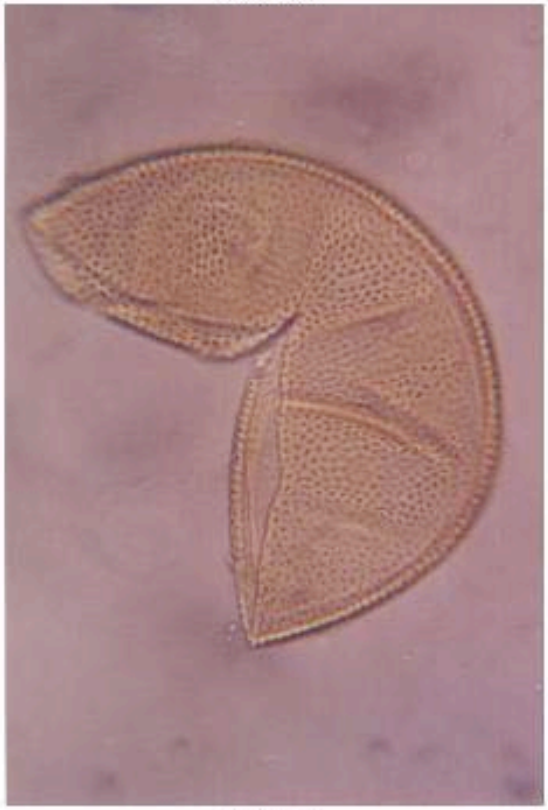


Plate 4

Plate 1: Spore of *Gigaspora* with subtending hyphae. **Plate 2:** Spore ornamentation in *Acaulospora*. **Plate 3:** Spore of *Acaulospora* showing wall layers and **Plate 4:** Crushed spore of *Acaulospora*.

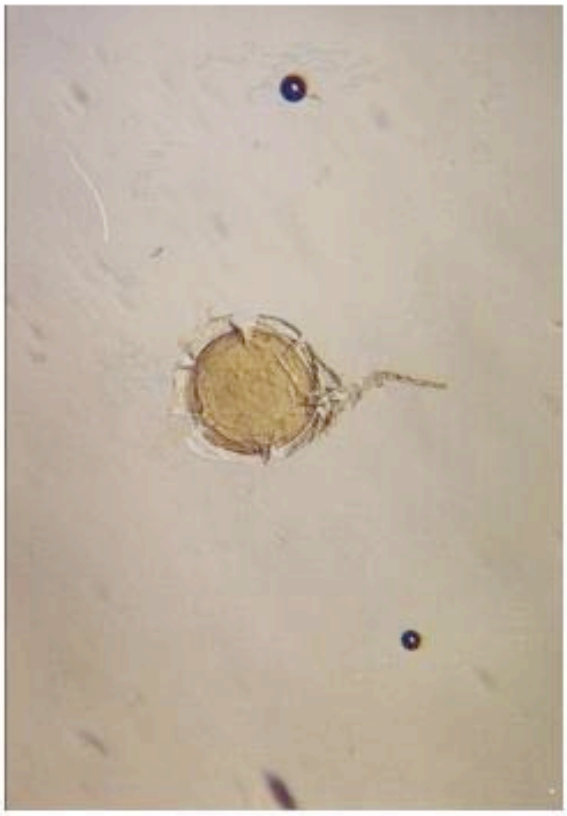
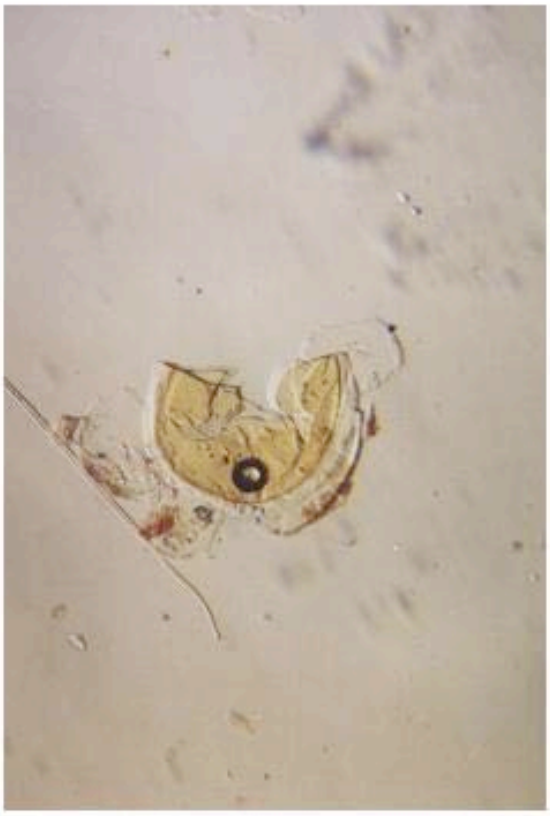


Plate 5



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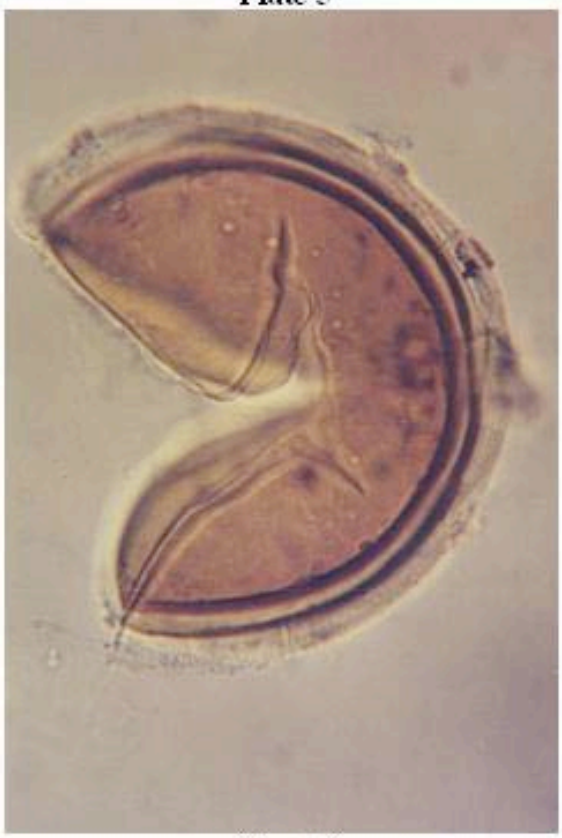

Plate 7

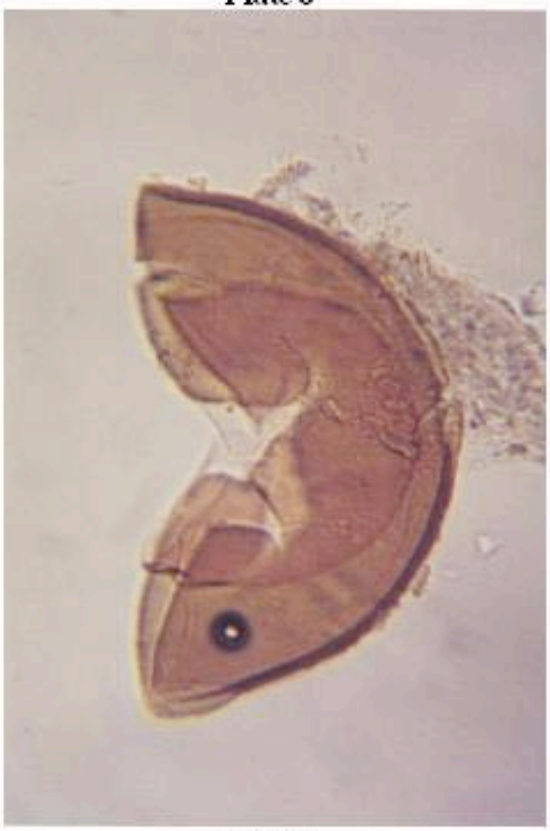

Plate 8

Plate 5: Spore of *Glomus* with subtending hyphae. **Plate 6:** Crushed spore of *Glomus* showing thin wall layers. **Plate 7:** Spore of *Entrophospora* showing thick wall layers and **Plate 8:** Crushed spore of *Entrophospora*.

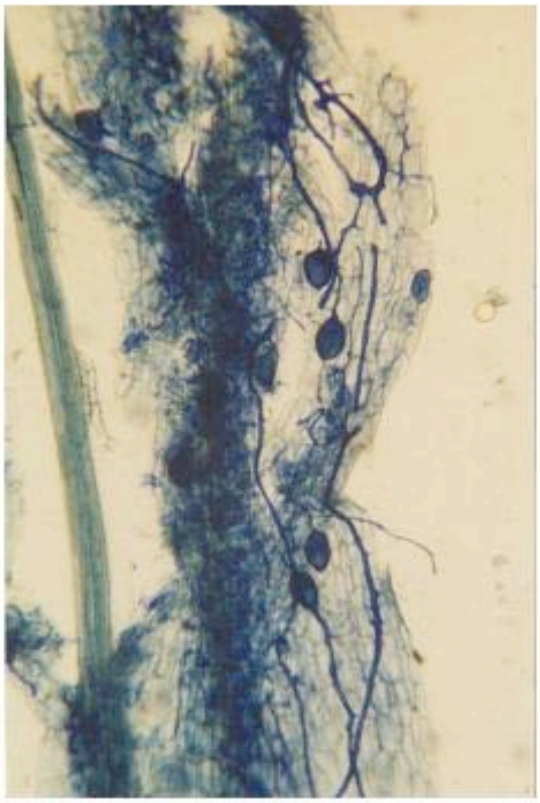

Plate 9

Plate 10

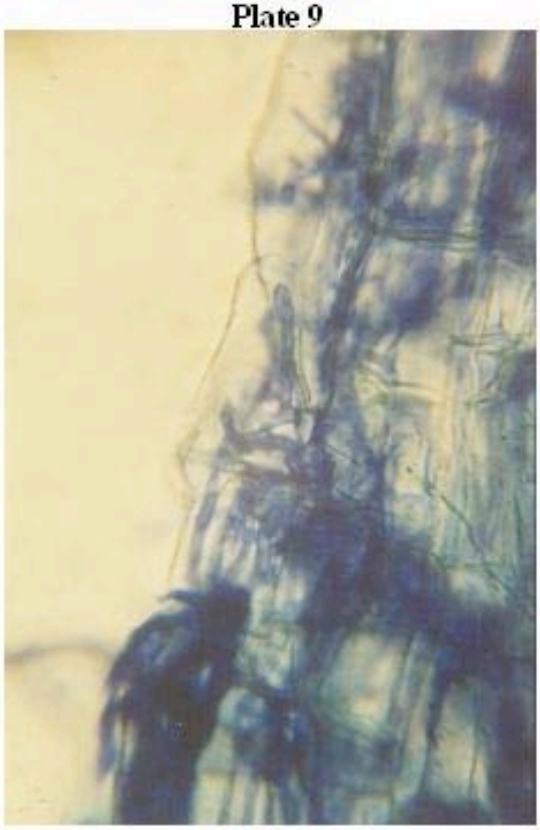

Plate 11

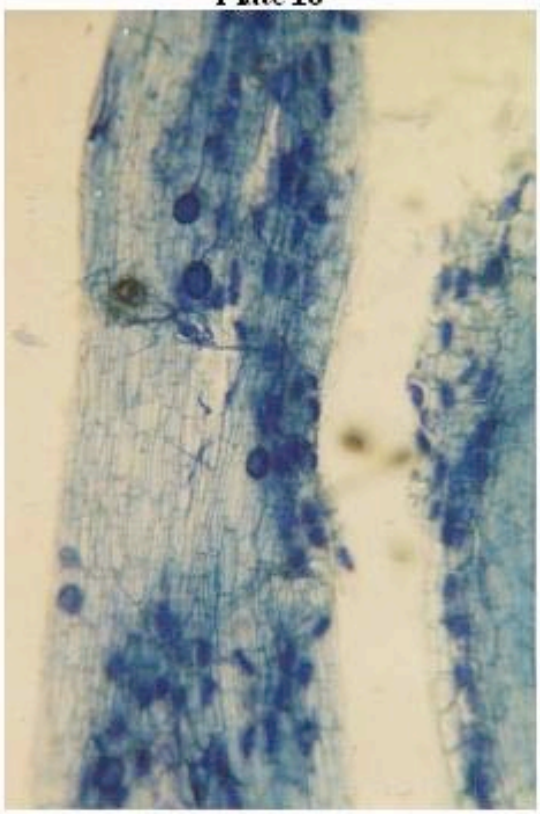

Plate 12

Plate 9: Glomus AM colony with darkly staining hyphae and vesicles in the root cortex. **Plate 10:** Acaulospora VAM colony with characteristic branching pattern of hyphae and vesicles. **Plate 11:** Gigaspora VAM colony with thicker walled hyphea layers and **Plate 12:** AM Colony showing hyphae vesicle and arbuscule.

Table 2: Distribution of Different AM Genera in the Rhizosphere Soils of Various Tree Species in Plantations of Chittagong University Campus

Species	Number of identified spore per genus				Total spores
	Glomus	*Acaulospora*	*Entrophospora*	*Gigaspora*	
Dalbergia sissoo	35	13	130	-	178
Gliricidia sepium	22	18	20	-	60
Indigofera tysmonii	10	116	58	25	209
Samanea saman	19	17	28	08	72
Delonix regia	15	34	11	24	84

Table 3: Fungal Factors in Relation to Soil Factors of Rhizosphere Soils Collected from Chittagong University Campus

Location	Species	Fungal factors		Soil factors	
		Total colonisation (%)	Spore population	MC (%)	pH
Site - 1	*Dalbergia sissoo*	95	178	18.7	5.8
Site - 2	*Gliricidia sepium*	52	60	19.5	5.8
Site - 3	*Indigofera tysmonii*	98	209	23.85	6.7
Site-4	*Samanea saman*	59	72	60.0	6.4
Site-5	*Delonix regia*	63	84	82.3	6.1

Here,
Site -1 => *Dalbergia sissoo* plantation beside IFESCU Ladies Hostel.
Site -2 => *Gliricidia sepium* plantation beside IFESCU Nursery boundary.
Site -3 => *Indigofera tysmoni* plantation beside IFESCU Hostel.
Site -4 => *Samanea saman*, Beside the entrance of IFESCU and
Site -5 => *Delonix regia*, Beside the Paharika Housing Estate, CU.
MC(%) => Percentage moisture content.

DISCUSSION

The present investigation reveals that AM fungi occur in the five forest tree species studied in various plantations of Chittagong University Campus. Of the AMF species recorded in the study, *Glomus* spp., *Acaulospora* spp. and *Entrophospora* spp. are most common. Gerdemann and Trappe [17] and Blaszkowski [18] reported *Glomus* spp. as the most common AMF in the nursery seedlings. The difference in species distribution may be attributed to edaphic factors and host plant interactions [19]. The occurrence and abundance of AMF in plantation trees depends on soil pH. *Acaulospara* are most tolerant to acidic soils, whereas *Glomus* species favor neutral to alkaline soils. In this study *Acaulospora* and *Entrophospora* occur frequently along with *Glomus* spp. This may be because the soil of the studied area is mostly acidic (with pH ranging between 5.4 and 6.7). The investigation reveals that commonly occurring AM fungi are present in the tree plantations of the Chittagong University campus. This finding is in agreement with the findings of Rahman [20]. Vesicles were observed more frequently than arbuscules. Al-Agely *et al*. [21] found a strong positive correlation between mycorrhiza formation and the amount of above-ground plant cover, but a strong negative correlation between mycorrhiza formation and increases in initial soil depth, moisture and pH. Muthukumar *et al*. [22] reported soil moisture contents negatively correlated with the plantation. In the present study, edaphic factors did not show any smooth relation with the AM colonization. Both species richness and spore density of AMF depend upon the size of the area sampled, season sampled and yearly variation in precipitation and temperature [23]. The factors like edaphic or climatic conditions; host fungus compatibility, root properties and soil microorganisms might influence the abundance of spore population and mycorrhizal association with a particular tree species or nursery soil. The tree seedling and grown up trees must possess adequate amounts of mycorrhizal

colonization in order to survive better and perform well in adverse plantation sites.

CONCLUSION

The investigation reveals that, commonly occurring AM fungi were present in all the five leguminous tree species studied that occurred in the plantations of Chittagong University campus. All these tree species had mycorrhizal association at seedling stage in the nursery [24] as well as in the established plantations at age of 10 years. The variation in AM spore population, the percentage of root colonization and the intensity of colonization as reported in the present study may have been affected by presence of indigenous AM fungal species, the studied host tree species and their growth conditions such as various soil factors etc. High mycorrhizal association as recorded in some samples of the present study indicate that the available indigenous AM fungal species can be managed and manipulated for a better uptake of nutrient by the host plants and improved growth of trees in forest plantations of Chittagong University campus. However, more studies are needed to select suitable indigenous AM fungal strains for the production of quality seedlings in nursery stage as well as for good growth performance of transplanted seedlings in plantations.

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