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EFFECTS OF EDAPHOCLIMATIC FACTORS ON ARBUSCULAR MYCORRHIZA FUNGI COLONIZATION IN CHITTAGONG BCSIR RESERVE FOREST, BANGLADESH

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Abstract

The present experiment was conducted for exploring, selected plant species, the edaphic and climatic variability of Arbuscular Mycorrhiza Fungi (AMF) colonization for the first time in Bangladesh. The highest colonization was obtained from *Phyllanthus emblica* (100%) followed by *Cynodon dactylon* (90%) and the lowest was in *Catharenthus roseus* (15.38%) in top soil but in sub soil the highest colonization was noted in *Plumbago auriculata* (66.67%) followed by *C. dactylon* (50%) and the lowest in *C. roseus* (9%) but no colonization was obtained in *Paederia fotieda* and *Strychnos nux-vomica*. AMF colonization was the highest in rainy season but varied randomly in summer and winter. Results exhibit AMF colonization variation depending on edaphoclimatic factors which might be very effective tool for management of sustainable agriculture.

Introduction

Arbuscular mycorrhizal (AM) fungi are the most important soil beneficial organisms and form symbiotic associations with the majority of terrestrial plant species (Smith and Read 2008). This relationship of host plant and fungi is naturally mutualistic. The benefits of this mutualism is related to the fungus deriving carbon from the host and the plants, in turn obtains numerous potential benefits (Harley and Smith 1983), mostly enhanced uptake and transport of relatively immobile soil nutrients (especially phosphorus), improved water relations, reduced pathogenic infections (Muthukumar and Udaiyan 2002). Ecological studies on the community structure of AMF are generally restricted to the top 20 cm of soil, where most of the root biomass is concentrated (Brundrett 1991). The distribution and functionality of AM in natural ecosystems are not clearly understood, but information in their prevalence and importance in natural ecosystem is limited and often contradictory (Muthukumar and Udaiyan 2002). Only a few studies on mycorrhizal fungi colonization including the subsoil have been conducted. Mycorrhizal colonization (Rillig and Field 2003), extra-radical mycelium decreases with increasing soil depth. Edaphic factors or soil nutrient status are claimed to be implicated in the patterns and timing of the development of AM fungi (Mullen and Schmidt 1993). Edaphic factors such as soil pH, electrical conductivity, soil depth, soil phosphorous, potassium, nitrogen, sulfur, organic matter, calcium, magnesium, iron concentration etc. and climatic factors indicate soil moisture, temperature, rainfall etc. Favorable condition of edaphoclimatic factors always favors in growth of agricultural production and ecosystem processes including microbial activity. Conditions of the soil moisture are known to affect root development and AM colonization. Reduction in soil moisture may lead to reduce nutrient availability and may

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be favorable to AM colonization patterns of mycorrhizal dependent species which are closely adapted to the growth stages of the host plants (Mullen and Schmidt 1993). So far no report is available in Bangladesh in respective plant species of the AMF colonization variability with different edaphoclimatic factors. Thus an attempt was taken to explore the Arbuscular Microrhiza Fungi (AMF) colonization dependency due to edaphic factors and climatic variability in BCSIR reserve forest of Chittagong, Bangladesh.

Materials and Methods

Samples were collected from Bangladesh Council of Scientific and Industrial Research (BCSIR) reserve forest, Chittagong. It is situated at 22°24'35.4"N 91°49'00.6"E in the south-eastern part of Bangladesh. List of different plant species studied for colonization dependency with soil depth is presented in Table 1.

Table 1. List of different plant species which are used for the study of colonization dependency and edaphic factors.

Sl. No.	Scientific name	Family name	Habit
1	*Aloe indica* (L.) Burm.f.	Xanthorrhoeaceae	Herb
2	*Catharenthus roseus* (L.) G. Don	Apocynaceae	Herb
3	*Centella asiatica* (L.) Urban	Apiaceae	Herb
4	*Cynodon dactylon* (L.) Pers.	Poaceae	Herb
5	*Elettaria cardamomum* (L.) Maton	Zingiberaceae	Herb
6	*Kalanchoe pinnata* (Lam.)	Crassulaceae	Sub shrub
7	*Ocimum sanctum* L.	Lamiaceae	Multi-branched shrub
8	*Paederia foetida* L.	Rubiaceae	Shrub
9	*Piper betel* L.	Piperaceae	Climber/creeper
10	*Plumbago auriculata* (Lam.)	Plumbaginaceae	Herb
11	*Phyllanthus emblica* L.	Phyllanthaceae	Ttree
12	*Strychnos nux-vomica* L.	Loganiaceae	Tree

For measurement of root colonization, root samples were collected from rhizosphere zone of studied plants of BCSIR reserve forest. Soil was vertically dug and very fine root and soil samples in each plant were collected from 0 to 15 cm and 15 to 30 cm along the vertical soil profile separately. Clean and preserved roots were stained following destained with 50% glycerol solution to remove excess stain by following method (Phillips and Hayman 1970). Root colonization (%) was calculated by using following formula.

$$\text{Per cent colonization} = \frac{\text{Number of AM positive segments}}{\text{Total number of segments observed}} \times 100$$

To determine climatic variability AMF colonization was measured in winter (December-January, rainfall (RF): 11.9 to 25.18 mm), rainy (July-August, RF: 727.0 to 530.6 mm) and dry (April-May, RF: 147.4 to 298.6 mm) seasons, respectively. List of various plant species selected to determine climatic variability randomly is presented in Table 2.

From each soil sample, three subsamples were analyzed to study the different soil properties. Soil pH (soil: water = 1 : 2.5), EC (soil : water = 1 : 2.5), available soil nutrients (Na, K, P), soil

organic carbon and soil organic matter were determined using the method described by Imamul Huq and Alam (2005). Intensity of AMF colonization variation due to climatic factor was determined by collecting root samples from top soil at different seasons (dry, rainy, and winter). Turkey's test (SAS 6.0) method (at $p < 0.05$ level) was used to determine the significance difference of AMF colonization due to climatic variability. ANOVA was also done for DMRT at 0.05 level of significance. Pearson correlation (SPSS) coefficient (at $p < 0.01$ and $p < 0.05$) was also performed to predict the relationship between the edaphic factors and AMF colonization of host plant species.

Table 2. List of different plant species which are used for the study of climatic variability.

Sl. No.	Scientific name	Family name	Habit
1	*Acacia concinna* (Willd.) DC.	Fabaceae	Climbing shrub
2	*Amomum aromaticum* Roxb.	Zingiberaceae	Herb
3	*Bryophyllum calycium* Salisb	Crassulaceae	Herb
4	*Butea monosperma* (Lam.) Taub.	Fabaceae	Tree
5	*Coccinia grandis* (L.) Voigt	Cucurbitaceae	Sub shrub
6	*Cymbopogon nardus* (L.) Rendle	Poaceae	Herb
7	*Mimosa pudica* L.	Fabaceae	Under shrub
8	*Piper betel* L.	Piperaceae	Shrub
9	*Paederia foetida* L.	Rubiaceae	Vines shrub or sub shrub
10	*Scoparia dulcis* L.	Scrophulariaceae	Much-branched herb

Results and Discussion

AMF colonization widely differed not only among the plant species but also throughout the soil profile. In case of top soil highest colonization was recorded in *P. emblica* as 100 ± 1.26% followed by *C. dactylon* as 90 ± 12.45% and least percentage was recorded in *C. roseus* as 15.38 ± 4.23. But in *P. foetida* no colonization was observed in both top soil and sub soil. Otherwise in sub soil highest colonization was obtained in *P. auriculata* as 66.67 ± 11.31% followed by *O. sanctum, P. emblica* as 50 ± 6.74%, 50 ± 4.37%, respectively. *S. nux-vomica* was also absent for colonization in sub soil like *P. foetida.* Except *A. indica,* all other plants root AMF colonization decreased with increasing vertical soil depth (Fig. 1). The present result is consistent with the results of previous workers (Vyas and Gupta 2014), who measured both colonization percentage where intensity decreased with the increase of depth in Tallgrass or True prairie species. Abbott and Robson (1991) suggested that there is an exponential decrease of both mycorrhizal colonization and spore number according to soil depth. This might be due to the oxygen diffusion.

Soil nutrients status varied vertically along with the soil profile (Tables 3 and 4). The study sites pH ranged from strongly to slightly acidic and the pH values attenuated with increasing soil depth. Edaphic factors like as P (mg/kg), % OM, EC (µS), Na (mg/kg) and K mg/kg) decreased with increasing vertical soil depth in rhizospheric zone.

AMF colonization as well as measured edaphic factors like soil pH, P (mg/kg), %OM, EC (µS), Na (mg/kg), %OC and K (mg/kg) decreased with increasing soil depth. ANOVA ($p < 0.05$) among the sub and top soil properties are presented in Tables 3 and 4, respectively. Significant bivariate correlation among some soil properties and AMF characteristics in sub soil also existed as pH with mycelium, Na and SOM with vesicle, arbuscules with EC ($p < 0.01$) as well as P with vesicle and Na with arbuscules ($p < 0.05$). Significant bivariate correlations between top soil properties with AM properties were also noticed as mycellium with pH, SOM and vesicle with K ($p < 0.01$) but vesicle with P and mycelium with K ($p < 0.05$).

Table 3. Soil properties of subsoils layer in study area of BCSIR reserve forest of Chittagong, Bangladesh.

Plant species	pH	EC (µ/cm)	OM (%)	OC (%)	Na (mg/kg)	K (mg/kg)	P (mg/kg)
A. indica	5.3 ± 0.02f	43 ± 1.53j	1.2 ± 0.07c	0.7 ± 0.02c	69.4 ± 0.8d	11.3 ± 1.5g	11.56 ± 1b
C. roseus	5.3 ± 0.01ef	81 ± 2.52g	1 ± 0.04d	0.57 ± 0.04d	70.12 ± 1c	12.5 ± 0.8g	4.34 ± 0.4c
C. asiatica	5.8 ± 0.01c	63 ± 1.00h	1.1 ± 0.10c	0.66 ± 0.02	71.2 ± 0.8c	19.7 ± 2cd	12.7 ± 0.7a
C. dactylon	5.8 ± 0.02d	137 ± 1.15a	0.8 ± 0.04ef	0.4 ± 0.02e	69.4 ± 1cd	17.5 ± 0.5ef	2.5 ± 0.5def
E. cardamomum	4.4 ± 0.03i	54 ± 1.53i	0.8 ± 0.04ef	0.5 ± 0.04d	70.4 ± 1.4bc	21.2 ± 1bc	1.29 ± 0.4f
K. pinnata	6.1 ± 0.04b	102 ± 3c	0.87 ± 0.02e	0.5 ± 0.04d	73.5 ± 0.8ab	23.6 ± 0.3b	3.25 ± 0.4d
O. sanctum	6.85 ± 0.006a	132 ± 3b	0.7 ± 0.02ef	0.4 ± 0.02e	69.4 ± 1cd	18.2 ± 1de	2.5 ± 0.5de
P. foetida	4.13 ± 0.02*l*	107 ± 1.5c	0.7 ± 0.02ef	0.43 ± 0.01ef	72.3 ± 1.5ab	19.3 ± 1.2cd	1.7 ± 0.5ef
P. betel	5.15 ± 0.02g	97 ± 2.08d	2.1 ± 0.01b	1.24 ± 0.01b	69.6 ± 1.5c	17.1 ± 1ef	11.5 ± 0.3b
P. auriculata	5.33 ± 0.03e	93 ± 1.53e	2.45 ± 0.06a	1.4 ± 0.06a	74.8 ± 0.5a	16.5 ± 0.4f	11 ± 0.61b
P. emblica	4.75 ± 0.01h	53 ± 2.00i	0.8 ± 0.01ef	0.44 ± 0.02e	69.4 ± 1.6c	20.8 ± 1bc	1.4 ± 0.8ef
S. nux-vomica	4.26 ± 0.05k	87 ± 2.08f	0.76 ± 0.03f	0.44 ± 0.03ef	74.7 ± 1.7a	25.1 ± 1.1a	2.5 ± 0.5de

Mean ± Sd., different letters indicate significant difference as shown by the DMRT ($p < 0.05$).

Table 4. Soil properties of top soils in study area of the BCSIR reserve forest of Chittagong, Bangladesh.

Plant species	pH	EC (µ/cm)	OM (%)	OC (%)	Na (mg/kg)	K (mg/kg)	P (mg/kg)
A. indica	4.79 ± 0.03i	87.00 ± 1.53e	2.21 ± 0.01c	1.28 ± 0.18b	90.74 ± 0.89c	39.85 ± 0.69d	18.49 ± 0.33a
C. roseus	5.56 ± 0.01f	93.00 ± 1.53d	1.92 ± 0.04d	1.11 ± 0.05c	74.73 ± 1.04e	40.85 ± 1.21d	12.47 ± 0.47ab
C. asiatica	6.12 ± 0.03d	86.00 ± 2.00e	2.91 ± 0.05b	1.69 ± 0.04a	74.73 ± 0.95e	29.45 ± 0.91f	2.73 ± 0.29c
C. dactylon	5.73 ± 0.01e	153.00 ± 1.52b	3.12 ± 0.03a	1.8 ± 0.04a	85.4 ± 0.7d	23.72 ± 2g	6.34 ± 0.1b
E. cardamomum	4.62 ± 0.03j	86.00 ± 2.52e	2.12 ± 0.04c	1.23 ± 0.05bc	101.41 ± 1.51a	47.18 ± 0.53c	1.57 ± 0.55c
K. pinnata	6.54 ± 0.02b	93.00 ± 2.08d	0.97 ± 0.07h	0.56 ± 0.09d	95.73 ± 0.92b	23.49 ± 1.41g	15.34 ± 0.80a
O. sanctum	6.42 ± 0.02c	56.00 ± 2.08g	2.97 ± 0.1b	1.72 ± 0.03a	85.40 ± 1.03d	29.45 ± 2.23f	1.18 ± 0.24c
P. foetida	4.57 ± 0.03k	125.00 ± 1.0c	1.23 ± 0.07f	0.71 ± 0.02d	75.23 ± 1.89e	34.65 ± 1.42e	3.46 ± 0.84c
P . betel	5.12 ± 0.02h	89.00 ± 2.89e	1.76 ± 0.04e	1.02 ± 0.16c	76.12 ± 1.43e	23.79 ± 0.84g	1.38 ± 0.14c
P. auriculata	6.94 ± 0.04a	519.00 ± 3.79a	3.06 ± 0.11a	1.77 ± 0.05a	101.41 ± 2.13a	52.84 ± 1.31b	24.44 ± 0.30a
P. emblica	5.24 ± 0.04g	66.00 ± 2.51f	1.00 ± 0.08g	0.58 ± 0.1d	74.73 ± 1.84e	77.96 ± 1.70a	1.56 ± 0.09c
S. nux-vomica	4.86±0.01i	94.00±2.08d	0.48±0.02i	0.28±0.12e	74.73±1.41e	52.84±1.65b	3.78±0.73c

Mean ± Sd., different letters indicate significant difference as shown by the DMRT ($p < 0.05$).

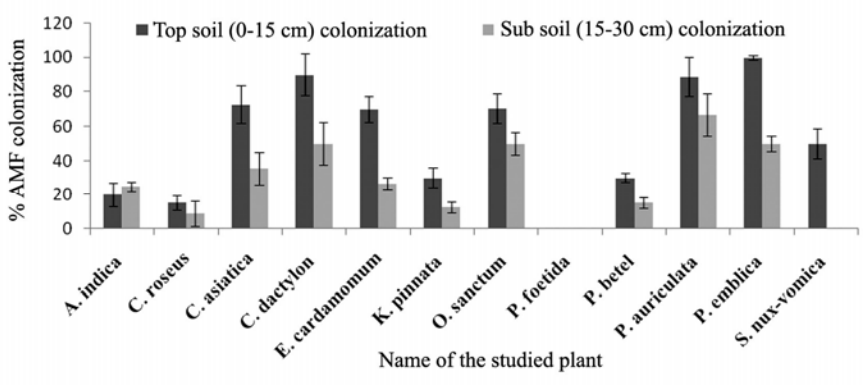

Fig.1. Comparison of AMF colonization in the roots from top soil and subsoil layers

Hyphal colonization of mycorrhiza fungi means presence of mycelia in plant's root samples (Fig. 2). In rainy season, 60% higher values of hyphal colonization were observed but in summer and winter about 30 and 10% covered, respectively. But one remarkable result was that all the studied samples except *A. aromaticum* varied significantly in respect of hyphal colonization throughout the year (Fig. 2). It was suggested that the mycorrhizal associations were well established and functional with time required higher nutrients allocations to support their enhanced metabolic activities synchronized with higher water availability and lower ambient temperature (Bohrer *et al.* 2004). In rainy season soil of BCSIR holds maximum amount of soil moisture which rises nutrient availability and metabolic activity for medicinal plants (Halder *et al.* 2015). Present data are consistent with the work of Bohrer *et al.* (2004) and Halder *et al.* (2015).

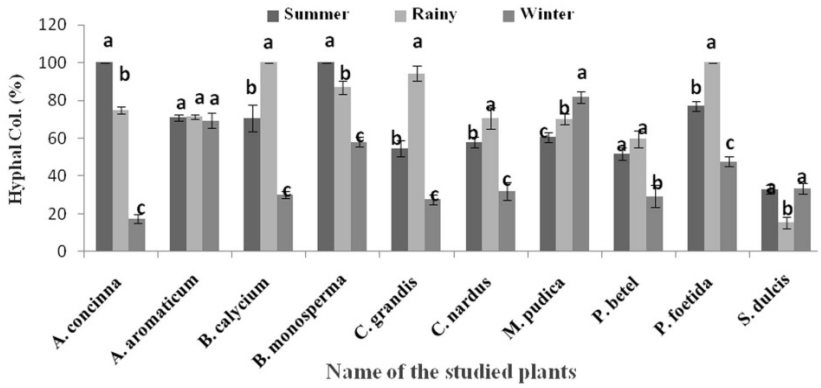

Fig. 2. Seasonal variability of hyphal colonization in selected plants roots at BCSIR forest. Different letters within three seasons belong to one plant species indicate significant difference at $p < 0.05$. (Col. = Colonization).

Arbuscular colonization in studied plants, 70% of the studied medicinal plants reached peak in rainy season and significantly ($p < 0.05$) varied with the other season (Fig. 3). It was reported that AMF colonization could be coordinated with growth stages of plants (Kennedy *et al.* 2002). Rainy season is the growing season of the medicinal plants of BCSIR (Halder *et al.* 2015) which might be

the reason of higher percentage of Arbuscules in studied medicinal plants of BCSIR. AMF colonization in some of the medicinal plants (*M. pudica*, *B. calycium*) varied randomly. This finding agreed with the view that AM symbiosis was considered to be probably species-specific (Ruotsalainen *et al.* 2002).

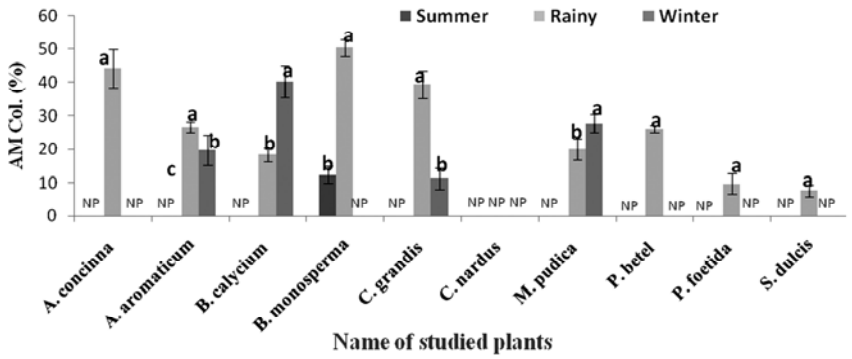

Fig. 3. Seasonal variability of Arbuscular colonization in different studied plants roots at BCSIR forest. Different letters within three seasons belong to one plant species indicate significant difference at 0.05 level. (AM = Arbuscular mycorrhiza; Col. = Colonization; NP = Not Present).

Percentage of vesicle colonization was very poor in comparison to Arbuscule and mycelia colonization (Fig. 4). Only 30% plants were free from vesicle formation but the rest 70% plants formed vesicle (Fig. 4). The 70% studied medicinal plants species showed vesicle colonization and mostly colonization occurred in summer season. Arbuscule formation follows a cyclic pattern where it ceases at the end of growing season when vesicle formation increases (Alexander *et al.* 1988).

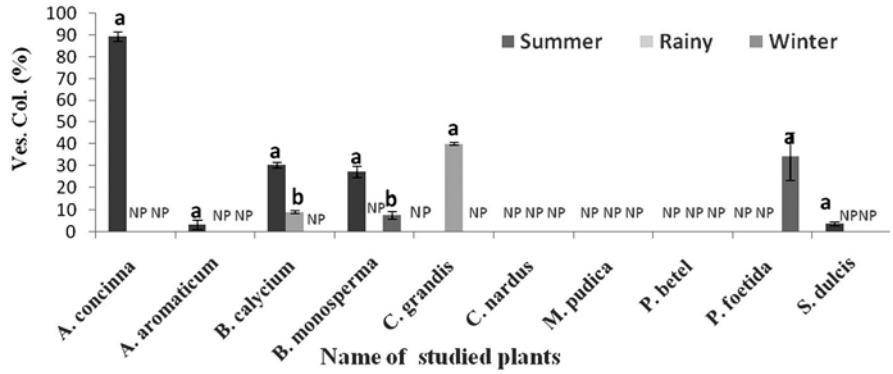

Fig. 4**.** Seasonal variability of vesicle colonization in studied plants roots at BCSIR forest. Different letters within three seasons belong to one plant species indicate significant difference at 0.05 level. (Ves. = Vesicle, Col. = Colonization; NP = Not Present).

Vesicle colonization was found to be higher in dry season. This might be due to higher oxygen diffusion rate as well as reduction of redox potential (Tonner and Clayton 1985). The result is in agreement with previous data (Alexander *et al.* 1988).

AMF colonization intensity and edaphic factors such as P (mg/kg), %OM, EC (µS), Na (mg/kg), %OC and K(mg/kg) of studied samples decreased with increasing vertical soil depth otherwise soil temperature, soil moisture opposed the earlier trends. Relationship of AMF properties with edaphic factors like as P, K, Na, pH, soil temperature and soil moisture was remarkable as well as significant but other measured soil properties were independent from AMF properties. But climatic factors also influence on AMF properties colonization as rainfall influence on colonization positively but colonization in winter and summer differed randomly. The current research result has established that AMF colonization varies with the edaphoclimatic factors of any fungi living habitat which is very important for sustainable agriculture and ecosystem process management.

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