

*et al.*, 2009); heavy metal sequestration (Tonin *et al.*, 2001); and showed diversity of structural colonization (Chaudhry, *et al.*, 2012) etc. Fungi play a central role in many microbiological and ecological processes, influencing soil fertility, decomposition, cycling of minerals and organic matter, as well as plant health and nutrition (Finlay, 2008). So, it is prominently important to encourage AMF relationship with plant root and utilization of Biofertilizer. Few works on Mycorrhiza fungi in Bangladesh has been done but no work has been performed in Bangladesh Council of Scientific and Industrial Research (BCSIR). Some works on Mycorrhizal diversity in different forest species (e.g woody plants, spices plants, ornamental plants etc.) in different region of Bangladesh has also been investigated (Mridha and Dhar, 2007, Dhar and Mridha, 2006, Dhar et al., 2005). Dhar and Mridha, (2012) studied Arbuscular mycorrhizal associations in different forest tree species of Hazarikhil forest of Chittagong Bangladesh. But still now research on Medicinal plants and mycorrhiza colonization is in dark. Also no work on relationship between soil properties and Mycorrhiza colonization has been studied. So, relationship between root Medicinal plants root colonization by Mycorrhiza fungi and rhizosphere soil properties is very important for fungal growth which also assists Medicinal plant growth. Information on the arbuscular Mycorrhizal status in Medicinal plants root and relationship with the soil properties are limited. Keeping these in minds our present work was conducted to evaluate the diversity of Mycorrhiza colonization in different Medicinal plant roots of Chittagong BCSIR forest, Bangladesh and relationship with rhizosphere soil characteristics.

## MATERIALS AND METHODS

### Study area and root sample collection

The studied area was located in BCSIR laboratory Chittagong at 22°24'35.4"N 91°49'00.6" which is located in the south east part of Bangladesh, approximately 100 acres. Minimum and maximum temperature of these months (March and April) was from 30°C to 38 °C respectively. Maximum rainfall was range from 0.02 to 3.31mm and average 0.07mm. Name and family name of selected 15 highly medicinal plants are given below

Table 1.

Root samples of 12 abundant family plants were collected from 0-30cm depth of each plant rhizosphere at different location of BCSIR forest areas. After the collection of root it was carried out to laboratory for analysis. Then the fresh root samples were washed by distilled water and preserver in 5% formalin. Roots preserved in 5% formalin were washed well to remove the formalin and chopped into 1cm pieces. Clean root samples were cleared by 10% KOH solution for 10 min at 85-90°C and deeply pigmented roots were treated with 10% H<sub>2</sub>O<sub>2</sub> at room temperature for 10 min, stained with 0.05% aniline blue solution at 90°C for 90 min, and then stored in glycerol solution (Phillips and Hayman 1970) with some modifications. A total of 100 segments from each species were examined. Roots segments were observed by a compound microscope at 10×10 magnification. Percent root colonization was calculated (Dhar and Mridha, 2003). Presence of mycelium was regarded as the AM positive and total mycelial colonization was treated as the total RC (Root Colonization)/PRC (Percent Root Colonization) colonization. The root colonization was calculated by using following formula.

$$\text{Root colonization (\%)} = \frac{\text{Number of AM positive segments}}{\text{Total number of segments observed}} \times 100$$

### Soil Sample collection and sample preparation

Soil samples were collected from rhizosphere zone of selected 15 highly medicinal plants (name is given above Table 1) at BCSIR, Chittagong forest areas. Each soil sample was replicated in three times. Soil sample brought to soil science laboratory of BCSIR. At first the soil samples were grinding to smaller ones then the foreign material were removed from soil. At last the soil samples were sieved at 2mm sieve and finally preserved in polythene bags.

### Soil samples analysis

Soil pH (soil: water ratio 1:2.5) and EC (soil: water ratio 1:2.5) was determined on a soil/water ratio (1/2.5) and available P (Jackson, M.L. 1973), SOM (Jackson, M.L. 1973), Available K, Ca, Na (Jackson, M.L. 1973), Soil moisture were determined by gravimetric method.

### Statistical analyses

Correlation analysis was performed to evaluate the relationships between different soil

of AMF colonization of different medicinal plants of BCSIR forest is shown below figure 1.

### Response of AMF Colonization to rhizosphere soil P concentration

Highest P concentration (mg/kg) was found in rhizosphere soil of *O. basilicum* L. var. *purpurescence* the value was 64.6 (mg/kg) compared to other medicinal plant's rhizospheric soil. But the RC (Root Colonization) of AMF was lowest in the plant *O. basilicum* L.

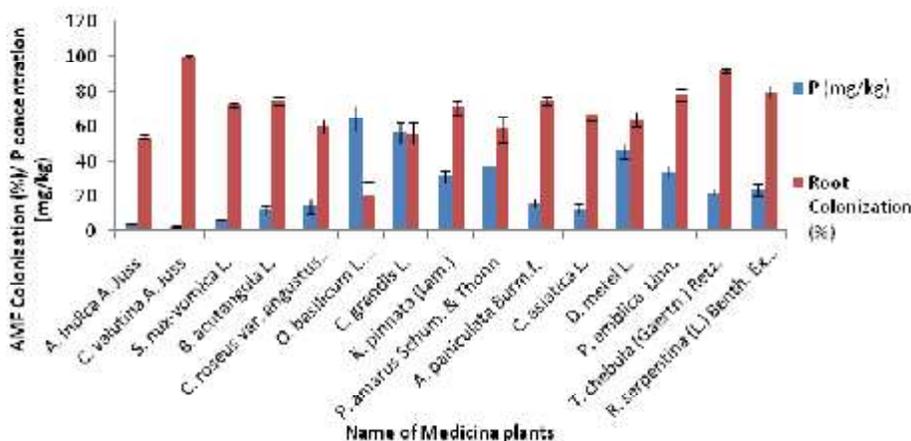
**Table 2.** Comparison between K concentration (mg/kg) of some medicinal plants rhizosphere soil and percent AMF colonization of some medicinal plants in BCSIR forest. Mean  $\pm$  S.D (Standard deviation), n=3.

Plant name	Colonization %	K concentration ((mg/kg))
<i>A. paniculata</i> .	75.00 $\pm$ 7.37	92.51 $\pm$ 7.31
<i>A. indica</i>	54.10 $\pm$ 5.23	48.13 $\pm$ 4.32
<i>B. acutangula</i> .	75.00 $\pm$ 11.2	55.00 $\pm$ 3.76
<i>C. roseus</i>	60.23 $\pm$ 4.57	52.43 $\pm$ 2.35
<i>C. asiatica</i>	66.67 $\pm$ 1.27	54.78 $\pm$ 5.82
<i>C. valutina</i>	100.00 $\pm$ 7.1	101.42 $\pm$ 3.56
<i>C. grandis</i> .	56.00 $\pm$ 7.9	50.32 $\pm$ 2.31
<i>D. metel</i> .	64.00 $\pm$ 4.57	67.04 $\pm$ 2.45
<i>K. pinnata</i>	70.83 $\pm$ 3.21	78.21 $\pm$ 3.25
<i>O. basilicum</i>	20.83 $\pm$ 8.91	30.34 $\pm$ 3.68
<i>P. amarus</i>	58.33 $\pm$ 2.48	45.52 $\pm$ 8.32
<i>P. emblica</i>	77.78 $\pm$ 6.12	92.45 $\pm$ 4.97
<i>R. serpentina</i>	80.00 $\pm$ 9.21	93.43 $\pm$ 1.57
<i>S. nux-vomica</i>	72.32 $\pm$ 5.76	82.82 $\pm$ 5.36
<i>T. chebula</i> .	91.67 $\pm$ 7.32	94.93 $\pm$ 5.26

var. *purpurescence* as 20.83 $\pm$ 8.91%. The lowest concentration of P was observed in rhizospheric soil of *C. valutina* A. Juss the value was 2.59 (mg/kg) but RC of AMF was reached peak at that plants root. High P concentration reduces AMF colonization. Our finding was identical with the other researchers (Abbott & Robson 1991, Aliasgharzadeh *et al.*, 2001-, Rosilaine Carrenho *et al.*, 2007). Statistically P concentration was negatively and significantly correlated ( $r = -0.609^*$ ,  $p < 0.05$ ) with RC (Root colonization) of AMF

**Table 3.** Comparison between percentage root colonization of AMF and percent soil moisture of rhizosphere soil of medicinal plants at BCSIR forest. Mean  $\pm$  S.D (Standard deviation), n=3.

Plant name	Colonization %	Moisture
<i>A. indica</i>	54.10 $\pm$ 5.23	7.2
<i>A. paniculata</i>	75.00 $\pm$ 7.37	6.4
<i>B. acutangula</i>	75.00 $\pm$ 11.2	6.7
<i>C. grandis</i> .	56.00 $\pm$ 7.9	7.3
<i>C. asiatica</i>	66.67 $\pm$ 4.57	8.1
<i>C. roseus</i>	60.23 $\pm$ 4.57	7.1
<i>C. valutina</i>	100.00 $\pm$ 7.1	9.7
<i>D. metel</i> .	64.00 $\pm$ 6.12	7.3
<i>K. pinnata</i>	70.83 $\pm$ 3.21	8.2
<i>O. basilicum</i>	20.83 $\pm$ 8.91	2.3
<i>P. amarus</i>	58.33 $\pm$ 2.48	6.2
<i>P. emblica</i>	77.78 $\pm$ 7.32	7.3
<i>R. serpentina</i>	80.00 $\pm$ 9.21	8.7
<i>S. nux-vomica</i>	72.32 $\pm$ 5.76	6.2
<i>T. chebula</i>	91.67 $\pm$ 7.32	8.6



**Fig. 2.** Prevalence of AMF colonization (%) in medicinal plants root and P concentrations (mg/kg) of different medicinal plant rhizospheric soil at BCSIR forest in Bangladesh. Error bars represent the standard deviations (SDs).

$p < 0.01$ ), soil salinity ( $-0.657^{**}$ ,  $p < 0.01$ ) also significantly correlated with K concentration. So, K concentration is a dominant soil property to control AMF infection and also other soil property.

#### Response of Mycorrhiza Root Colonization to rhizosphere soil Na concentration

Sodium concentration was more or less same without some exceptional values throughout all rhizosphere soil. Range of Na varied from 76.85 mg/kg to 41.61 mg/kg which retained highest and lowest AMF colonization respectively and differed within a narrow extent. Same family like as *Meliaceae* had two representatives as *C. valutina* and *O. basilicum* but differed each other in AMF colonization that might be due to the different soil characteristics. The highest Na percentage with low AMF colonization was observed in *O. basilicum*

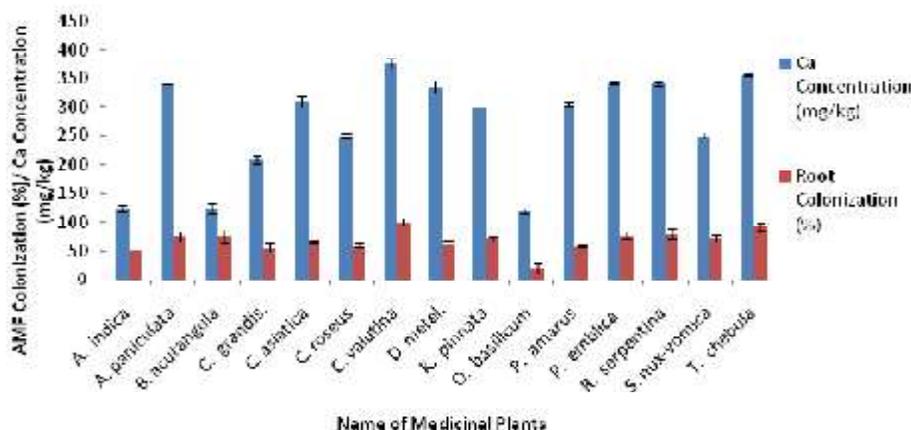
but *C. valutina* showed highest root colonization but lowest sodium concentration. Statistically Na concentration was significantly correlated with the prevalence of AMF colonization ( $r = -0.824^{**}$ ,  $p < 0.01$ ), Ca concentration ( $r = -0.574^*$ ,  $p < 0.05$ ), K concentration ( $r = -0.552^*$ ,  $p < 0.05$ ), P concentration ( $r = -0.659^*$ ,  $p < 0.05$ ), moisture percentage ( $r = -0.832^{**}$ ,  $p < 0.01$ ), EC ( $r = 0.640^*$ ,  $p < 0.05$ ). So, the prevalence of AMF colonization was reduced with addition or rising of Na concentration in rhizosphere soil. Our findings agreed with the findings of other research (Ying-Ning and Qiang-Sheng, 2011; Khaliel *et al.*, 2011). But our work was contradictory with the findings of other researchers (G. Feng *et al.*, 2002). G. Feng *et al.*, (2002) showed that the extent of mycorrhizal colonization was not significantly affected by Na salt treatments. The Na concentration of

**Table 5.** Pearson correlation co-efficient of AMF colonization with soil properties of some medicinal plants rhizosphere at BCSIR forest in Bangladesh

	RC	K	Ca	Na	Moisture	SOM	P	EC	pH
RC	1								
K	.869**	1							
Ca	.692**	.760**	1						
Na	-.824**	-.552*	-.574*	1					
Moisture	.865**	.668**	.713**	-.832**	1				
SOM	.307	.361	.535*	-.331	.325	1			
P	-.609*	-.414	-.141	.659**	-.549*	.029	1		
EC	-.722**	-.657**	-.605*	.640*	-.481	-.610*	.368	1	
pH	-.277	-.291	.124	.157	-.191	-.096	.409	.186	1

\*\* . Correlation is significant at the 0.01 level.  $P < 0.01$

\* . Correlation is significant at the 0.05 level.  $P < 0.05$



**Fig. 4.** Prevalence of AMF colonization at different medicinal plants root and Ca concentrations of mycorrhiza colonize root rhizosphere soil of medicinal plants at BCSIR forest. Error bars represent the standard deviations (SDs)

reached at peak compared to all other samples but prevalence of AMF colonization was relatively higher compared to *O. basilicum* AMF colonization which is shown in below figure 5. This might be due to the presence of fungitoxic compounds in root cortical tissue or in root exudates that may reduce susceptibility of plants to mycorrhization in *O. basilicum* (Tester et al. 1987). So, statistically soil salinity significantly correlated (Mostly negative) with AMF colonization ( $r=-0.722^{**}$ ,  $p<0.01$ ), K concentration ( $r=-0.657^{**}$ ,  $p<0.01$ ), Na concentration ( $r=0.640^{*}$ ,  $p<0.05$ ), Ca concentration ( $r=-0.605^{*}$ ,  $p<0.05$ ), and SOM (Soil Organic Matter) ( $r=-0.610^{*}$ ,  $p<0.05$ ). So, AMF colonization was attenuated with increasing soil salinity. Our findings agreed with the other researchers (Ying-Ning and Qiang-Sheng, 2011; Khalil et al., 2011). But this result was contradicted with the findings of G. Feng et al., (2002). Comparison between prevalence of AMF colonization in medicinal plants root and EC are given below figure 5.

#### **Response of AMF colonization in root with rhizosphere soil moisture of medicinal plants**

Soil moisture was varied with in a narrow range from 2.3% to 9.7%. High percentage of soil moisture was observed in *C. valutina* (9.7%) plants rhizosphere soil followed by *R. serpentina* (8.7%) and root colonization also high in *C. valutina* plants root which is presented in Table 3. Without these marginal values the root colonization of AMF and moisture percentage were varied slightly random way. But most of the root colonization of AMF positively differed with moisture percentage. David J. Burke et al., (2009) showed that pH and moisture positively varied with mycorrhiza colonization which was identical with our observation. Variation of AMF colonization with moisture percentage is depicted in Table 3. Statistically moisture percentage significantly correlated with prevalence of AMF colonization in root ( $r=0.865^{**}$ ,  $p<0.01$ ), Na ( $r=-0.832^{**}$ ,  $p<0.01$ ), Ca ( $r=0.713^{**}$ ,  $p<0.01$ ), K ( $r=0.668^{**}$ ,  $p<0.01$ ) and soil P concentration ( $r=-0.549^{*}$ ,  $p<0.05$ ).

#### **Response of AMF colonization to medicinal plants rhizosphere soil pH and SOM (soil Organic Matter) at BCSIR forest areas.**

pH in the soil of medicinal plants rhizosphere zone differed with in a short range from 5.2 to 6.3 (Table 4). The entire sample's soil pH is

moderately acidic.  $100.00 \pm 7.1\%$  AMF colonization was occurred in *C. valutina* but pH of that soil was 5.2 and lowest root colonization was observed in pH 5.8 in plant *O. basilicum* but our study was opposed by the work of A. Sreevani and B. N. Reddy (2004). They observed that mycorrhizal fungi grow well in slightly alkaline soil. pH randomly differed with the prevalence of AMF colonization also other nutrients element. Statistically there was no relationship with any other parameters as well as AMF colonization. Soil organic matter percentage also varied within a short range from 1.94 to 3.15. SOM percentage varied unpredictable way with AMF colonization but have a significant relationship with EC ( $r=-0.610^{*}$ ,  $p<0.05$ ) and Ca concentration of soil. Pearson correlation coefficient of response of prevalence of AMF colonization with rhizosphere soil properties is presented in Table 5.

### **CONCLUSION**

Prevalence of AMF colonization in medicinal plants root was significantly correlated with rhizosphere soil properties. K, Ca and Moisture content had a significant positive correlation with percentage of AMF colonization. But Na, P and soil salinity had a significant negative correlation with AMF colonization. The result of pH had a controversy with other researchers but SOM varied randomly with AMF colonization.

### **ACKNOWLEDGEMENTS**

The author gratefully acknowledges to the Soil Management and Agronomical Research Division, BCSIR laboratories, Chittagong, Bangladesh. We all are grateful to the Director for helping all of our research work.

### **REFERENCES**

1. Abbott, L.K. & Robson, A.D. Field management of VA mycorrhizal fungi. Pp.355-362. In: D.L. Keister. (ed.). The rhizosphere and plant growth. Kluwer Academic Publishers, Dordrecht 1991.
2. Aliasgharzadeh N, Saleh Rastin N, Towfighi H, Alizadeh A, Occurrence of arbuscular mycorrhizal fungi in saline soils of the Tabriz

- Mature Beech-Maple Forest, *Applied and Environmental Microbiology*, 2009; **75**: 247639-7648.
25. G. Feng · F. S. Zhang · X. L. Li · C. Y. Tian · C. Tang Z. Rengel, Improved tolerance of maize plants to salt stress by arbuscular mycorrhiza is related to higher accumulation of soluble sugars in roots, *Mycorrhiza*, 2002; **12**:185–190,
  26. Khaliel A. S., Shine K. and Vijaya kumar K. Salt tolerance and mycorrhization of *Bacopa monneiri* grown under sodium chloride saline conditions. *African Journal of Microbiology Research*, 2011; **5**(15) : 2034-2040.
  27. Ying-Ning ZOU and Qiang-Sheng WU, Sodium Chloride Stress Induced Changes in Leaf Osmotic Adjustment of Trifoliolate Orange (*Poncirus trifoliata*) Seedlings Inoculated with Mycorrhizal Fungi. *Not Bot Horti Agrobo*, 2011; **39**(2):64-69.
  28. Tester M, Smith SE, Smith FA. The phenomenon of non mycorrhizal plants. *Canadian Journal of Botany*, 1987; **65**: 419–431.
  29. Proctor. J, & Woodell, S. The ecology of serpentine soils. *Advances Ecol. Res.*, 1975; **9**: 255-366.
  30. Mengel K. and E.A. Kirkby. Potassium Crop Production. *Adv. Agron*, 1980; **33**: 59-110.
  31. Sreevani and B. N. Reddy, Arbuscular Mycorrhizal Fungi Associated with Tomato (*Lycopersicon esculentum* Mill.) as Influenced by Soil Physico-Chemical Properties, *Philippine Journal of Science*, 2004; **133** (2): 115-129.