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RESEARCH ARTICLE

Seasonal variation of arbuscular mycorrhiza fungi colonization with some medicinal plant species of Chittagong BCSIR forest

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Abstract

This study aimed to evaluate the effects of seasonality on mycorrhizal colonization characteristics as hyphal, vesicular and arbuscular colonization with some medicinal plants species of Chittagong BCSIR forest of Bangladesh. Ten highly valued medicinal plants were selected randomly from Chittagong BCSIR forest to conduct the research. Root samples were collected and examined to determine fungal colonization in three times (Dry, Rainy and Winter) during the year in 2014. The result revealed that the medicinal plants of BCSIR forest were mycorrhizal. The highest hyphal colonization was obtained during rainy season of the corresponding year but decreased during dry and winter season. Vesicular colonization was attenuated during rainy season but increased in dry and winter season but arbuscular colonization was higher proportion in rainy and winter season whereas arbuscular colonization reduced in dry season. This result indicates that AMF colonization varies seasonally as well as depending on some factors like as climate, edaphic, plant host relationship and species diversity.

Keywords: Arbuscular mycorrhiza; Mycorrhizal fungi; Medicinal plant; Seasonal variation

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Introduction

Medicinal plants have been widely recognized as having high healing properties although it has minor toxic side effects. Cultivation of medicinal plants has been encouraged as the scarcity and increasing demand for medicinal plants and their products. Currently, medicinal plants cultivation systems have been increased due to the unstable quality of the products. Arbuscular mycorrhiza (AM) expediting secondary metabolism and the development of active ingredients of medicinal plants which influence the quality of herbal medicines (Zeng *et al.,* 2013). Thus we can use AMF as Biofertilizer instead of inorganic fertilizer by providing suitable environment which helps production and accumulation of important active ingredients of medicinal plants such as terpenes, phenols, and alkaloids and thus ultimately improving the quality of herbal materials. AMF are the most common soil fungi (Gerdemann and Nicolson, 1963) associated with most of the plants' root in a variety of natural and agricultural ecosystems (Tao and Zhiwei, 2005), including arid and semiarid areas (Carrillo-Garcia *et al.,* 1999). Their extraradical mycelia can transport nutrients from one host to another through a common mycelial network (Graves *et al.,* 1997; Robinson and Fitter, 1999). AMF can stimulate plant growth by mitigating nutrient-deficient

and water stresses (Thompson, 1996; Mohammad *et al.,* 1998; Auge, 2001), developing soil stabilization (Wright and Upadhyaya, 1998; Bearden and Petersen, 2000) and assist plants resistance to soil-borne pathogens (Gange *et al.,* 2003; Idoia *et al.,* 2004), helps in soil aggregation (Rillig *et al.,* 2002), tolerance to drought and improvement of salinity (Karaki *et al.,* 2004), deter to toxic heavy-metal uptake (Leyval *et al.,* 1997), maintenance of soil structure (Smith and Read, 1997; Wright and Upadhyaya, 1998). Oliveira (2001) and Oliveira and Oliveira (2003) suggest that both AMF spore numbers and AMF colonization are influenced by pH, Mg, K, Fe, Al, Ca, and Mn availability. However, we have little information about the influences of seasonal variation on the abuscular mycorrhiza fungi colonization characteristics. Development and seasonal fluctuations in AMF colonization and sporulation have been investigated for several plant species growing under different ecosystems throughout the world (Beena *et al.,* 2000; Ruotsalainen *et al.,* 2002; Bohrer *et al.,* 2004). Liu *et al.* (2013) conducted an experiment under green house condition and suggested that community structure of Mycorrhiza fungi was influenced by seasonality and soil depth. Seasonal fluctuations and development of AMF fungus colonization has been investigated in several plant species and in several countries, including the Portugal (Carvalho *et al.,* 2001), Great Britain (Merryweather and Fitter, 1998), Israel (He *et al.,* 2002) and India (Muthukumar and Udaiyan, 2002). In contrast, this type of study is scarce in Bangladesh (Dhar and Mrida, 2003, 2005, 2006, 2012; Halder *et al.,* 2015). Halder *et al.* (2015) investigated mycorrhizal status in medicinal plants of BCSIR forest and identified relationships to edaphic factors. Dhar and Mridha, 2003, 2006, 2012 conducted some research on forest trees in different parts of Bangladesh and determined AMF status, diversity and community structure of mycorrhiza fungi, spore population etc. The current study deals with the status of AMF colonization with medicinal plants of BCSIR as well as effects of seasonality on Mycorrhiza fungi colonization characteristics.

Materials and Methods

Study site

Chittagong BCSIR laboratory is a government research organization under the Ministry of Science and Technology, People's Republic of Bangladesh. BCSIR forest is the reserve forest as specialized for research on medicinal and aromatic plants. It is situated at 22°24'35.4"N 91°49'00.6"E in the south-eastern part of Bangladesh, with an area of approximately 100 acres. More than 1600 species of medicinal plants are being grown in the campus (Anonymous, 2014).

Root sample collection and preparation

Roots samples of the plants were collected during the year of 2014 but the whole year was divided into three seasons as Dry (April-May, average rainfall about 147.4 to 298.6 mm), Rainy (July-August, average rainfall about 727.0 to 530.6 mm) and winter (December-January, average rainfall about 11.9 to 25.18 mm) as well as samples were collected respectively. List of various medicinal plant species selected randomly were included in the Table 1.

Roots were separated from the soil, washed in the tap water and preserved in 50% ethyl alcohol for future use. Roots were washed well to remove the alcohol and chopped into 1cm pieces. Clean root samples were stained by following the procedures of Phillips and Hayman (1963). Preserved roots were heated in 10% KOH solution for 10 min at 80-85°C and deeply pigmented roots were treated in 3% H_2O_2 at room temperature for overnight. Roots treated with 3% H_2O_2 were acidified in 1% HCl solution for 30 minutes and heated at 80-85°C for 15-20 minutes with 0.05% aniline blue solution. Stained roots were destained in 50% glycerol solution to remove excess stains and preserved in 50% glycerol solution. A total of 25 segments of the roots were mounted on the microscopic slides with 50% glycerol and smashed softly after placing a cover glass on the root pieces. Roots segments were observed by a compound microscope at 40×10 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 percentage root colonization. The intensity of AM structures *i.e.* mycelial, vesicular and arbuscular colonization were recorded and calculated percent hyphal colonization, percent vesicles colonization, and percent arbuscules colonization. Percent root colonization was calculated by using following formula.

$$\text{Percent colonization (\%)} = \frac{\text{Number of AM positive segments}}{\text{Total number of segments observed}} \text{X100}$$

Table 1. List of different randomly studied medicinal plants, with their family as well as habit, of BCSIR forest, Chittagong.

Plants Name	Family Name	Habit
Asparagus racemosus Wild.	Asparagaceae	Perennial climber
Azadirachta indica A. Juss.	Meliaceae	Tree
Catharanthus roseus (L.) G.Don	Apocynaceae	Herb
Centella asiatica (L.) Urban	Apiaceae	Herb
Cynodon dactylon (L) Pers	Poaceae	Herb
Datura metel L	Solanaceae	Shrub
Ocimum tenuiflorum L.	Lamiaceae	Multi-branched shrub
Strychnos nux-vomica L	Loganiaceae	Tree
Terminalia bellirica (Gaertn.) Roxb.	Combretaceae	Tree
Terminalia chebula Retz.	Combretaceae	Tree

Table 2. Soil chemical properties in three season of BCSIR forest study area during the year in 2014.

Studied soil chemical properties	Soil properties in winter season	Soil properties in dry season	Soil properties in rainy season
pH	5.35± 0.88	6.12± 0.18	5.35± 0.88
EC	110.63± 25.16 µS	132.13± 21.34 µS	105.34± 19.37 µS
CEC	19.30 meq/100g soil	15.13meq/100g soil	21.12 meq/100g soil
Moisture content	0.98%	1.12%	23%
Available K	40.14±8.69 mg/kg	44.17±5.29 mg/kg	36.37 ± 7.21 mg/kg
Available P	7.09± 4.09 mg/kg	9.09± 3.21 mg/kg	6.95 ± 5.28 mg/kg
Available Ca	270 mg/kg	272 mg/kg	257 mg/kg
Available Mg	0.061%	0.072%	0.058%
Available Na	81.66± 5.65 mg/kg	87.66± 7.31 mg/kg	76.27± 3.76 mg/kg
Available S	15.01± 4.9 mg/kg	16.01± 4.57 mg/kg	13.01± 5.18 mg/kg
Soil Organic Matter	1.06 ± 0.51%	0.97 ± 0.15%	1.25 ± 0.12%

Table 3. Vesicles colonization intensity in mycorrhiza fungi colonize medicinal plant's root due to seasonal variability during the year in 2014. Mean ± SD (Standard Deviation), n=3

	Percentage of Vesicles		
Plants Name	Dry Season	Rainy Season	Winter Season
A. racemosus	8.7±4.23	8.33±1.27	0. 00
A. indica	8±3.7	0.00	80±16.82
C. roseus	0.00	0.00	0.00
C. asiatica	0. 00	0.00	9.09±1.1
C. dactylon	0. 00	40±6.77	0. 00
D. metel	20±5.65	0.00	0.00
O. tenuiflorum	0.00	0.00	6.67±2.31
S. nux-vomica	0.00	0.00	0.00
T. bellirica	9.68±2.13	0.00	55.55±5.73
T. chebula	40.91±12.43	41.66±10.32	30±8.92

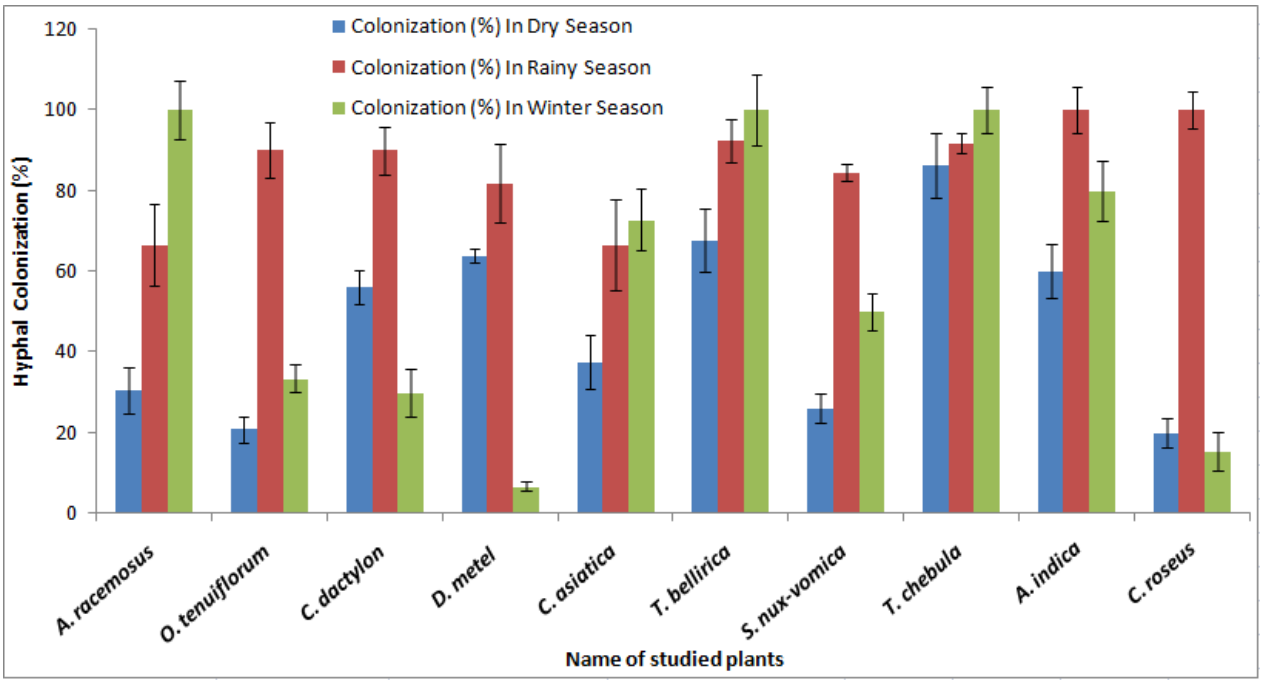

Fig.1. Mycorrhizal colonization status in medicinal plant's root due to seasonal variability during the year in 2014. Error bars indicate the standard deviation (SD). n=3

Soil sample collection and analysis

Soils samples were collected from studied medicinal plants cultivation area to determine the soil chemical properties as three times of in the corresponding tenure. Three replicated samples were studied for analyzing the soil chemical characteristics. Soil pH (soil: water = 1:2.5) and EC (soil: water =1:2.5) available P, K, SOM, and S (Jackson, 1973) and soil moisture content were determined by using different prescribe method. The chemical properties of soil of studied area are given in Table 2.

Statistical analyses

Statistical analyses were accomplished using MS Excel 2007 software. The MS Excel 2007 software was used for calculating mean value (n=3) and standard deviations.

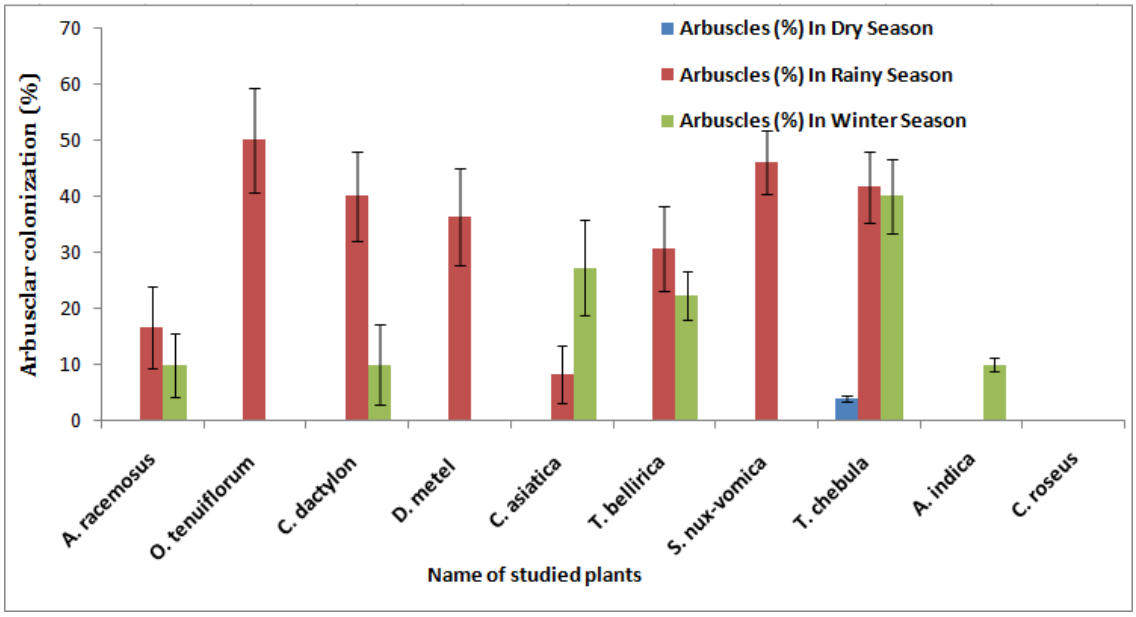

Fig. 2. Arbuscles colonization status in medicinal plant's of BCSIR forest under different season during in 2014. Error bars indicate the standard deviation (SD); n=3.

Result and Discussion

Effects of seasonality on hyphal colonization in studied medicinal plant's rhizosphere

All survey plants were infected heavily by mycorrhiza fungal hyphae and some root samples formed typical arbuscular mycorrhizal (AM) structures. Mycelia were present in all root samples which characterized as colonization of mycorrhiza fungi (Fig. 1). Intra- and intercellular hyphae, vesicles, arbuscules, hyphal coils were observed in most of the medicinal plants root tissues but occasionally varied with season. Hyphal colonization of each species reached peak in different time of the year (Fig. 1). Hyphal colonization was often reached above 20%, occasionally, even beyond 95% in *A. racemosus*, *A. indica*, *C. roseus, T. bellirica, T. chebula*. The percent of root colonization ranged between 20±3.54% and 86.36±8.12% in dry season, 66.66±10.12% and 100±3.53% for rainy season but 6.67±1.23% and 100±5.53% for winter season. This symbiotic relationship has been previously demonstrated (Halder *et al.,* 2015). Minimum root colonization levels were obtained in the dry season but maximum levels were observed during the wet season (Fig. 1). It is suggested that the mycorrhiza fungi symbiosis 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 (Anderson *et al.,* 1984; Sanders and Fitter, 1992; Bohrer *et al.,* 2004). Such a result was consistence with data published from several other research works on different plant species from different habitats (Lingfei *et al.,* 2005, Bohrer *et al.,* 2004).

Effects of seasonality on arbuscular colonization in studied medicinal plant's roots

Intensity of Arbuscule was not equal in all samples as well as Arbuscle was totally absent in some medicinal plant's root samples. In dry season all the studied samples were totally free from formation of any arbuscles except *T. chebula* (Fig. 2). Arbuscular colonization reached its maximum and minimum in different season (Fig. 2) for different plants. In rainy season most of the plant species, except for *A. indica, C. roseus*, were highly infected by AMF followed by winter season. Arbuscular colonization ranged from 0 to 4 ± 0.57% in dry season and 0 to 40 ± 6.71% in winter season but reached peak in rainy season as 0 to 50 ± 9.37%. Arbuscules were observed in 10% medicinal plants root in dry season whereas highest 80% medicinal plants showed arbuscles in rainy season followed by 60% in winter season. So, the intensity order of arbuscle formation was dry<winter<rainy season. It has been reported that AMF colonization could be coordinated with growth stages of plants (Kennedy *et al.,* 2002). In this study, higher arbuscular colonization occurred from rainy to winter season of 2014, as this period was the growth stages for most of the medicinal plants of BCSIR forest that was consisted with study of Lingfei *et al.* (2005). Arbuscular colonization in some of the medicinal plants varied randomly which agreed with the view that AM symbiosis was considered to be probably species-specific (Ruotsalainen *et al.,* 2002). Nutrient status of studied area was varied within a little extent (Table 2) but EC and Na concentration were varied little bit large extent. Mycelia colonization were decreased in dry season that might be due to high soil EC and Na concentration in dry season

(Halder *et al.,* 2015). Climatic factors and soil nutrient availability have temporal and spatial dynamics. Bohrer *et al.* (2004) concluded that abiotic factors had minimal influence on AMF colonization variation. So, AM seasonal dynamics were in response to plant phenology. Moisture content of soil of study area was more or less similar in dry (0.98%) and winter season (1.12%) but variation of arbuscular formation was varied large extent which might be due to growth stage of medicinal plants.

Effects of seasonality on Vesicular colonization in studied medicinal plant's roots

Vesicle colonization was very low during the whole year of 2014 in compare to hyphal and arbuscular colonization. The time of maximum and minimum vesicle colonization for each species appeared different (Table 3) in compare to different season. The vesicular colonization rate in studied medicinal plant species detected in dry and winter season was differed in compare to rainy season. The growing season of medicinal plants of BCSIR is rainy season (July-August). Alexander *et al*. (1988) have explained that arbuscule formation follows a cyclic pattern where it ceases at the end of growing season when vesicle formation increases. Vesicle colonization was higher percentage in medicinal plant's roots during dry spring as well as winter season that might be due to the favorable condition for plant respiration and oxyzen diffusion rate in plant rhizosphere zone. Such result is consistence with the previous published data (Shamim *et al.,* 1994; Khade and Rodrigues, 2008; Bajwaya *et al.,* 2001). But VAM formation was reduced at rainy season in studied medicinal plants. The reduction of VAM infection in rainy season may be due to the inhibition of spore germination at low oxyzen tension (Tacon *et al.,* 1983) as well as reduction of redox potential (Tonner and Clayton, 1985). Furthermore, Reid and Bowen (1979) demonstrate that low number of entry points for VAM on roots epidermis in wet soils help to reduce VAM infection.

Conclusion

Mycorrhizal, arbuscles, vesicles colonization were differed not only seasonally but also varied regarding to medicinal plant species to species. Arbuscle and hyphal colonization were higher in rainy season might be due to growth stage, host plant relationship of medicinal plants in BCSIR forest as well as moisture availability, nutrient availability and allocation, ambient temperature respectively. Vesicle colonization was increased in dry and winter season in comparison to rainy season that might be due to oxyzen diffusion rate, redox potential. The study highlights the facts that the existence of a seasonal pattern of arbuscular mycorrhizal fungi and is indicated by hyphal colonization, vesicular formation and arbuscular mycorrhizal structures which is subjected to the some factors like as growth stage, species diversity, edaphic factors, and climatic condition. This research is restricted to few plants of diverse group; therefore it does not reflect the actual situation. Finally, further research is required by selecting with greater number of plant species growing in diverse habitat to explore the real fact regarding to medicinal plants species.

Competing interests

The authors declare that they have no competing interests.

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References

Alexander, T., R. Toth, R. Meier, H. C. Weber. 1988. Dynamics of arbuscule development and degeneration in onion, bean and tomato with reference to vesicular-arbuscular mycorrhiza in grasses. *Canadian Journal Botany* 67: 2505-513. http://dx.doi.org/10.1139/b89-320

Anderson, R. C., A. E. Liberta and L. A. Dickman. 1984. Interaction of vascular plants and vesicular-arbuscular mycorrhizal fungi across a soil moisture-nutrient gradient. *Oecologia* 64: 111-117. http://dx.doi.org/10.1007/BF00377552

Anonymous. 2014. Annual Report, BCSIR Chittagong, Bangladesh.

Auge, R. M. 2001. Water relations, drought and vesicular–arbuscular mycorrhizal symbiosis. *Mycorrhiza* 11: 3–42. http://dx.doi.org/10.1007/s005720100097

Bajwaya, R., A. Yaqoob and A. Javaid. 2001. Seasonal Variation of in VAM in wetland plants. *Pakistan Journal of Biological Science* 4(4): 464-470. http://dx.doi.org/10.3923/pjbs.2001.464.470

Bearden, B. N. and L. Petersen. 2000. Influence of arbuscular mycorrhizal fungi on soil structure and aggregate stability of a vertisol. *Plant and Soil* 218: 173–183. http://dx.doi.org/10.1023/A:1014923911324

Beena, K. R., N. S. Raviraja and K. R. Sridhar. 2000. Seasonal variations of arbuscular mycorrhizal fungal association with Ipomoea pes-caprae of coastal sand dunes, Southern, India. *Journal of Environmental Biology* 21: 341-347.

Bohrer, K. E., C. F. Friese and J. P. Amon. 2004. Seasonal dynamics of arbuscular mycorrhizal fungi in differing wetland habitats. *Mycorrhiza* 14: 329-337. http://dx.doi.org/10.1007/s00572-004-0292-7

Carrillo-Garcia, Á., J. L. Leó n de la Luz, Y. Bashan and G. J. Bethlenfalvay. 1999. Nurse plants, mycorrhizae, and plant establishment in a disturbed area of the Sonoran Desert. *Restoration Ecology* 7: 321-335. http://dx.doi.org/10.1046/j.1526-100X.1999.72027.x

Carvalho, L. M., Caçador, I., M. A. Martins-Loução. 2001. Temporal and spatial variation of arbuscular mycorrhizas in salt marsh plants of the Tagus estuary (Portugal). *Mycorrhiza* 11: 303-309. http://dx.doi.org/10.1007/s00572-001-0137-6

Dhar, P. P. and M. A. U. Mridha, M. K. U. Bhuiyan, M. Mohiuddin. 2005. Status of arbuscular mycorrhizal fungi in Gamari (*Gmelina arborea* Roxb.) growing in different parts of Bangladesh. *The Chittagong University Journal of Science* 29: 65-74. http://dx.doi.org/10.1007/s11676-006-0047-8

Dhar, P. P. and M. A. U. Mridha. 2003. Status of biodiversity of arbuscular mycorrhizal fungi in different tree species growing in Betagi community forests. *The Chittagong University Journal of Science* 27: 13-19.

Dhar, P. P. and M. A. U. Mridha. 2006. Biodiversity of arbuscular mycorrhizal fungi in different trees of Madhupur forest, Bangladesh. *Journal of Forest Research* 17: 201–205.

Dhar, P. P. and M. A. U. Mridha. 2012. Arbuscular mycorrhizal associations in different forest tree species of Hazarikhil forest of Chittagong, Bangladesh. *Journal of Forest Research* 23: 115–122. http://dx.doi.org/10.1007/s11676-012-0241-9

Gange, A. C., V. K. Brown, and D. M. Aplin. 2003. Multitrophic links between arbuscular mycorrhizal fungi and insect parasitoids. *Ecology Letters* 6: 1051–1055. http://dx.doi.org/10.1046/j.1461-0248.2003.00540.x

Gerdemann, J. W., T. H. Nicolson. 1963. Spore density of Endogone species extracted from soil wet sieving and decanting. *Transactions British Mycological Society* 46: 235-244. http://dx.doi.org/10.1016/S0007-1536(63)80079-0

Graves, J. D., N. K. Watkins, A. H. Fitter, D. Robinson and C. Scrimgeour. 1997. Intraspecific transfer of carbon between plants linked by a common mycorrhizal network. *Plant Soil* 192: 153–159. http://dx.doi.org/10.1023/A:1004257812555

Halder M., S. Akhter, A. S. M. Mahmud, F. Islam, R. Mullick, J. C. Joardar, M. S. Amin, R. Karim, H. Talukder and M. S. Rahman. 2015. Prevalence of Arbuscular Mycorrhiza Fungi (AMF) Colonization in Medicinal Plant Root and Response of Prevalence with Some Selected Medicinal Plants Rhizosphere Soil Properties in BCSIR Forest, Chittagong, Bangladesh. *Journal of Pure and Applied Microbiology* 9(1): 131-140.

He, X., S. Mouratov and Y. Steinberger. 2002. Temporal and spatial dynamics of vesicular-arbuscular mycorrhizal fungi under the canopy of *Zygophyllum dumosum* Boiss. in the Negev Desert. *Journal of Arid Environment* 52: 379-387. http://dx.doi.org/10.1006/jare.2002.1000

Idoia, G., G. Nieves and A. Jone. 2004. Plant phenology influences the effect of mycorrhizal fungi on the development of Verticillium-induced wilt in pepper. *European Journal of Plant Pathology* 110: 227– 238. http://dx.doi.org/10.1023/B:EJPP.0000019790.45397.90

Jackson M. L. 1973. *Soil Chemical Analysis*. 2nd Edition. Prentice-Hall Inc., Englewood Cliffs, NJ.

Karaki, G. A., B. McMichael and J. Zak. 2004. Field response of wheat to arbuscular mycorrhizal fungi and drought stress. *Mycorrhiza* 14: 263-269. http://dx.doi.org/10.1007/s00572-003-0265-2

Kennedy, L. J., R. L. Tiller and J. C. Stutz. 2002. Associations between arbuscular mycorrhizal fungi and *Sporobolus wrightii* in riparian habitats in arid South-west North America. *Journal of Arid Environment* 50: 459–475. http://dx.doi.org/10.1006/jare.2001.0899

Khade, S. W. and B. F. Rodrigues. 2008. Ecology of arbuscular mycorrhizal fungi associated with *Carica papaya*. In: agro-based ecosystem of Goa, India. *Tropical and Subtropical Agroecosystems* 8: 265–278.

Le Tacon, F., F. A. Skinner and B. Mosse. 1983. Spore germination and hypal growth of vesicular arbuscular mycorrhizal fungus, Glomus Mosseae (Gerdman and Trappe) under reduced oxyzen and increased canbon dioxyde concentrations. *Canadian Journal of Microbiology* 29: 1280-1285. http://dx.doi.org/10.1139/m83-200

Leyval, C., K. Turnau and K. Haselwandter. 1997. Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. *Mycorrhiza* 7: 139–153. http://dx.doi.org/10.1007/s005720050174

Lingfei, L., Y. Anna and Z. Zhiwei. 2005. Seasonality of arbuscular mycorrhizal symbiosis and dark septate endophytes in a grassland site in southwest China. *FEMS Microbiology Ecology* 54: 367–373. http://dx.doi.org/10.1016/j.femsec.2005.04.011

Liu R., L. An, D. Zhi-Kai, LIM in and L. Xian-Gui. 2013. Effects of Soil Depth and Season Variation on Community Structure of Arbuscular Mycorrhizal Fungi in Greenhouse Soils Planted with Watermelon. *Pedosphere* 23: 350–358. http://dx.doi.org/10.1016/S1002-0160(13)60026-0

Merryweather, J. and A. Fitter. 1998. The arbuscular mycorrhizal fungi of Hyacinthoides non-scripta. II. Seasonal and spatial patterns of fungal populations. *New Phytologist* 138: 131-142. http://dx.doi.org/10.1046/j.1469-8137.1998.00889.x

Mohammad, M. J., W. L. Pan and A. C. Kennedy. 1998. Seasonal mycorrhizal colonization of winter wheat and its effect on wheat growth under dryland field conditions. *Mycorrhiza* 8:139–144. http://dx.doi.org/10.1007/s005720050226

Muthukumar, T. and K. Udaiyan. 2002. Seasonality of vesicular-arbuscular mycorrhizae in sedges in a semi-arid tropical grassland. *Acta Oecologica* 23: 337-347. http://dx.doi.org/10.1016/S1146-609X(02)01165-7

Oliveira, A. N. 2001. Fungos micorrízicos arbusculares e teores de nutrients em plantas de cupuaçu e guaraná de um Sistema Agroflorestal na região de Manaus, AM. Manaus, (Dissertação de Mestrado. Universidade Federal do Amazonas. UFAM), pp.150.

Oliveira, A. N. and L. A. Oliveira. 2003. Características químicas do solo, esporulação e colonização micorrízica em plantas de cupuaçuzeiro e de pupunheira na Amazônia Central. *Revista de la Facultad de Ciencias Agrarias* 40: 33-44.

Phillips, J. M., and D. S. Hayman. 1970. Improved procedures for clearing roots for rapid assessment of infection. *Transactions of the British Mycological Society* 55: 158-161. http://dx.doi.org/10.1016/S0007-1536(70)80110-3

Reid, C. P. P and G. Bowen. 1979. Effects of moisture on VA mycorrhizae formation and root development in medicago. In: The soil root interface (J. R. Harley and R. S. Russel eds.), Academic press, London. pp. 219. http://dx.doi.org/10.1016/B978-0-12-325550-1.50023-9

Rillig, M. C., S. F. Wright and V. T. Eviner. 2002. The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: comparing effects of five plant species. *Plant and Soil* 238: 325-333. http://dx.doi.org/10.1023/A:1014483303813

Robinson, D. and A. Fitter. 1999. The magnitude and control of carbon transfer between plants linked by a common mycorrhizal network. *Journal of Experimental Botany* 50: 9–13. http://dx.doi.org/10.1093/jxb/50.330.9

Ruotsalainen, A. L., H. Väre and M. Vestberg. 2002. Seasonality of root fungal colonization in low-alpine herbs. *Mycorrhiza* 12: 29-36. http://dx.doi.org/10.1007/s00572-001-0145-6

Sanders, I. R. and A. H. Fitter. 1992. The ecology and functioning of vesicular-arbuscular mycorrhizas in co-existing grassland species II. Nutrient uptake and growth of vesicular arbuscular mycorrhizal plants in a semi natural grassland. *New Phytologist* 120: 525-535. http://dx.doi.org/10.1111/j.1469-8137.1992.tb01802.x

Shamim, D. T., Ahmed and N. Ayub. 1994. Influence of seasonal variations on VAM infection in perennial plants. *Pakistan Journal of Phytopathology* 6: 77-86.

Smith, S. E. and D. J. Read. 1997. Mycorrhizal Symbiosis. Academic Press, San Diego, pp. 605.

Tao, L. and Z. Zhiwei. 2005. Arbuscular mycorrhizas in a hot and arid ecosystem in southwest China. *Applied Soil Ecology* 29: 135-141. http://dx.doi.org/10.1016/j.apsoil.2004.11.005

Thompson, J. P. 1996. Correction of dual phosphorus and zinc deficiencies of linseed (*Linum usitatissimum* L.) with cultures of vesicular–arbuscular mycorrhizal fungi. *Soil Biology and Biochemistry* 28: 941–951. http://dx.doi.org/10.1016/0038-0717(95)00185-9

Tonner, C. C. and J. S. Clayton. 1985. Vesicular Arbuscular Mycorrhiza studies with submerged aquatic plants. *Transactions of the British Mycological Society* 86: 683-688. http://dx.doi.org/10.1016/S0007-1536(85)80264-3

Wright, S. F. and A. Upadhyaya. 1998. A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. *Plant Soil* 198: 97–107. http://dx.doi.org/10.1023/A:1004347701584

Zeng, Y., L. Guo, B. Chen, Z. Hao, J. Wang, L. Huang, G. Yang, X. Cui, L. Yang, Z. Wu, M. Chen and Y. Zhang. 2013. Arbuscular mycorrhizal symbiosis and active ingredients of medicinal plants: current research status and prospective. *Mycorrhiza* 23: 253–265. http://dx.doi.org/10.1007/s00572-013-0484-0