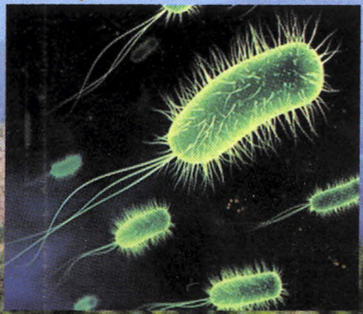
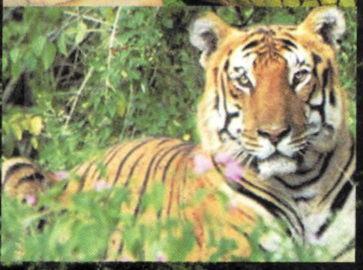
STATUS AND CONSERVATION OF BIO-DIVERSITY IN NORTH EAST INDIA





M.D. CHOUDHURY
G.D. SHARMA
S. CHOUDHURY
A.D. TALUKDAR

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Arbuscular Mycorrhizal Fungi from the Acidic Soils of Meghalaya, Northeast India

Panna Das [1] and Highland Kayang [2]*

Abstract

The soils of Khasi and Jaintia Hills of Meghalaya are characterized of having acidic pH. The soil samples from several sites such as abandoned coalmine, coalmine roadside, potato field and pine forest were examined for arbuscular mycorrhizal fungi (AMF). The soils were analysed for spore extraction and soil physico-chemical properties. Soil-physico-chemical properties such as moisture content (%), texture (%), pH and available phosphorus (%) differed significantly. Spore density counts revealed that maximum spores were recovered from coalmines. Interestingly, spore richness was more to be in potato field as well as in coalmines. PCA exhibit least variation in spore density compared to other environmental factors. Cluster analysis shows less dissimilarity between pine forest and abandoned coalmine

1 Microbial Ecology Laboratory, Department of Botany, North Eastern Hill University, Shillong- 793 022, India
2 Microbiology Laboratory, Department of Botany, Tripura University, Suryamaninagar- 799 130, Tripura, India
* Author for Correspondence Email: panna11d@yahoo.com

and maximum dissimilarity between pine forest and coalmine. Morphotypes from *Glomus, Gigaspora, Acaulospora, Entrophospora, Pacispora* and *Scutellospora* were identified.

Keywords: arbuscular mycorrhizal fungi, dissimilarity, morphotypes, pH, spore density

Introduction

Arbuscular mycorrhizal fungi (AMF) form associations with the majority of terrestrial plant species and have been shown to improve the growth and nutrition of individual plants (Smith & Read, 1997), defends plants against pathogens (Rabie, 1998), alleviates environmental stresses on plants (Ruiz-Lozano et al., 2001), improves plant tolerance to drought and polluted environments (Auge´, 2001; Vivas et al., 2003) and accelerates plant establishment (Caravaca et al., 2003). At present, AMF are considered as an important component in the restoration and reestablishment of the vegetation in fragile or degraded ecosystems, and in the maintenance of plant biodiversity and ecosystem functioning (van der Heijden et al., 1998; Dhillion & Gardsjord, 2004).

The acidity, low P content, and high P sorption capacity (Arines & Sainz, 1987) advice liming and phosphate fertilization as a common practice to improve plant productivity, and these treatments are known to affect the fungal populations in soils (Azcón -Aguilar et al., 1985). The low productivity of acidic soils is often related to chemical constraints including deficiency of phosphorus, nitrogen, calcium, and other nutrients and toxicity of manganese and aluminum (Sanchez, 1976; Sanchez & Salinas, 1981). The soils developed on sedimentary and metamorphic rocks of north-eastern region are acidic (pH 4.0 to 5.6) and highly leached, having poor base saturation and low exchange capacity (Sen et al., 1997).

Our aim is to determine the inoculum density and species compostion of the acidic soils from Meghalaya. In addition to compare the edaphic factors of different sites such as coalmine,

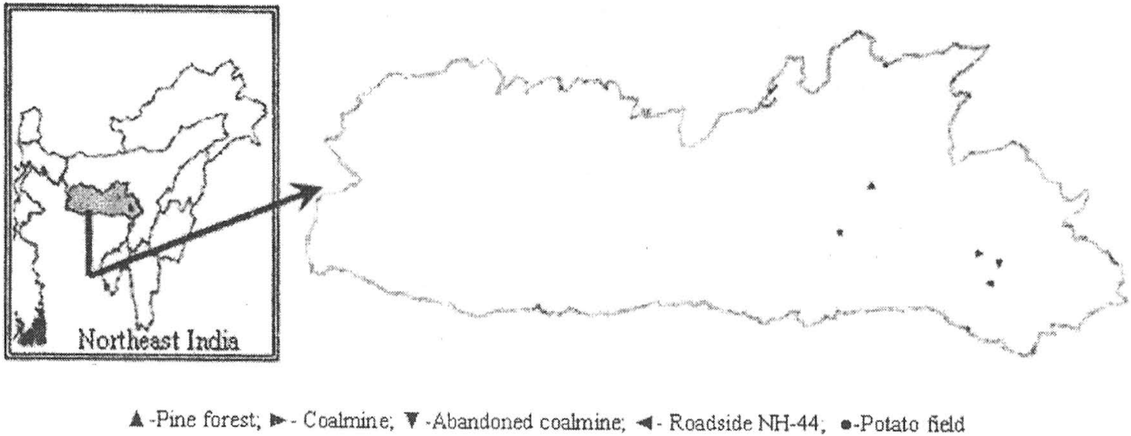

Figure 1: Map of northeast India showing Meghalaya

▲Pine forest; ▼ Coalmine; ►-Abandoned coalmine; ◄ - Roadside NH-44; ●-Potato field abandoned coalmine, potato field, roadside and pine forest.

Materials and methods

The soil samples were collected from Shillong, Swer and Khliehriat, Meghalaya, India. In Khliehriat disturbed areas such as coalmine, abandoned coalmine and road side (NH-44) are included in this study. In Swer, a potato field was selected and a sub-tropical pine forest was selected in Shillong (Fig. 1)

Spores were extracted by modified wet sieving and decanting method (Muthukumar et al. 2006). 100 g of soil was dispersed in 1 l water and decanted through a series of 710 to 38 l sieves. The residues were filtered through gridded filter papers and all whole spores were counted using a dissection microscope at 40X magnification. Sporocarps and spore clusters were considered as one unit. AMF spores were mounted in polyvinyl alcohol-lactoglycerol with or without Meltzer's reagent for identification using keys from Oehl & Sieverding 2004; Blaszkowki 1989; http://www.invam.caf.wvu.edu. The photography of the spores and the root segment colonized by AMF was done with the help of Leica EC 3 camera attached in Leica dm 1000 microscope. Spore density was expressed as number of AM fungal spores per soil sample. Species richness is the number of identified AMF species per soil sample.

The soil physical characteristics such as texture and moisture content (%). Soil texture was analyzed using sodium hexametaphosphate method (Allen et al. 1974). For moisture content (%), 10 g sub sample was oven dried to constant weight was determined. The soil was analyzed for soil chemical parameters such as pH, organic Carbon and available Phosphorus. Measurement of pH was done using Microprocessor - based Pocket pH testr 2 (Eutech Instruments) Available phosphorus of soil was determined following standard methods molybdenum-blue method (Allen et al. 1974). The soil organic carbon was estimated using colorimetric method (Anderson & Ingram 1993)

One sample t test was conducted to analyze the variation in the soil physic-chemical properties and spore density. Principal component analysis (scatter plot) was used to examine whether the five sites differ on the basis of the eight variables. To represent the complex multivariate relationships among the variables, agglomerative hierarchical cluster analysis was performed and results were expressed as a dendrogram.

Result

The physico-chemical properties are presented in Table 1. The percentage of sand in roadside was highest and lowest in clay and vice-versa applied for the pine forest. The silt percentage was maximum in coal mine and lowest in abandoned coal mine. The moisture content was highest in coalmine and minimum in roadside. The pH was lowest in abandoned coal mine and maximum in pine forest. The organic carbon and available phosphorus was lower values in abandoned coal mine and higher in potato field. The spore density per 25 g of soii was presented in Table 1. The variation of spore density is shown in Figure 2.

Table 1: Spore density and selected soil physico-chemical properties of five sites

Site	*Spore density/ 25g*	*Moisture (%)*	*Sand (%)*	*Silt (%)*	*Clay (%)*	*pH*	*Organic C (%)*	*Phos-phorus (mg/kg)*
Pine forest	197	23.13	75.36	2.56	22.08	6.4	1.0	2.38
Roadside NH-44	33	13.16	90.06	1.72	8.22	5.4	0.64	2.33
Abandoned coalmine	143	20.23	89.3	0.42	10.28	4.4	0.51	2.33
Coalmine	567	32.9	84.1	4.98	10.92	4.8	1.52	2.88
Potato field	102	28.21	82. 36	3. 56	14. 08	5.37	11.45	5.94

Spore density and Organic C does not differ significantly between the five sites and other soil physico-chemical properties significantly differ at $p<0.05$.

Principal component analysis reveals least variation in spore

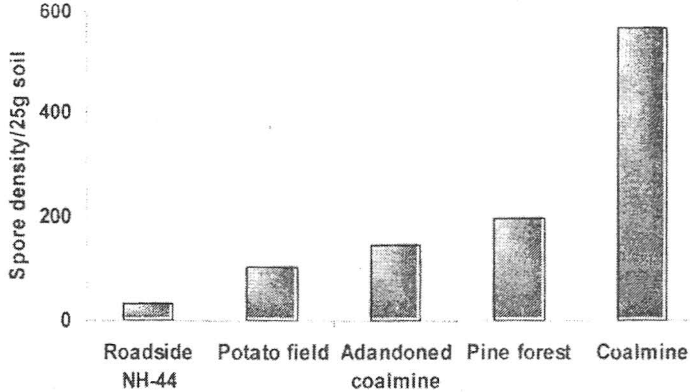

Figure 2: Spore density of different sites

density in all the sites as compared too the environmental factors (Figure 3). Cluster analysis exhibit highest dissimilarity between pine forest and coalmine and low between pine forest and abandoned coalmine (Figure 4).

Seventeen morphotypes were recovered from all the sites (Table 2 & Figure 5). Isolation frequency is presented in Table 2. Species richness in the sites follows the trend potato field > coalmine > pine forest > roadside > abandoned coalmine. *Glomus* was the richest species of all the genera (Figure 6). Sorenson's coefficient shows higher values in all the disturbed sites than between pine forest and disturbed sites (Figure 7).

The AMF community composition similarity as assessed by Sorenson's coefficient between the pine forest and roadside, abandoned coalmine and coalmine were 0.4, 0.25 and 0.31, respectively. Sorenson's coefficient between roadside and abandoned coalmine and between roadside and coalmine were 0.67 and 0.72, respectively. The similarity between coefficients was 0.44 in between abandoned coalmine and coalmine. Sorenson's coefficient between potato field and pine forest, roadside, coalmine

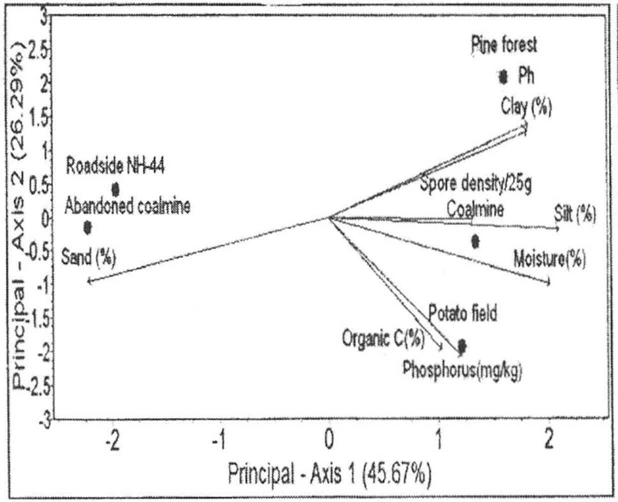

Figure 3: Principal Component Analysis for the ecological attributes in coalmine, abandoned coalmine, Roadside (NH-44), sub-tropical pine forest and potato field.

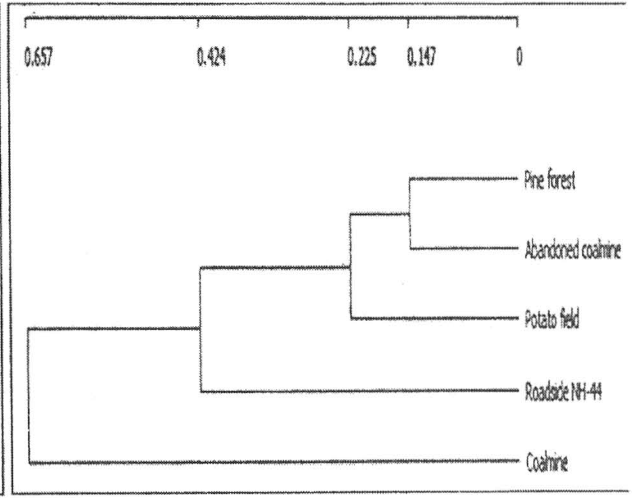

Figure 4: Dendrogram representing cluster analysis of different sites.

Table 2: AMF species composition of all the five sites

AMF species	*Pine forest*	*Roadside NH-44*	*Abandoned coalmine*	*Coalmine*	*Potato field*	*Frequency*
Acaulospora scrobiculata	+	+	-	+	-	60
Acaulospora sp 1	+	-	-	-	-	20
Acaulospora sp 2	-	+	-	-	-	20
Acaulospora sp 3	-	-	-	-	+	20
Enthophosphora sp	-	-	-	+	-	20
Gigaspora sp 1	-	-	-	+	+	40
Gigaspora sp 2	-	-	-	-	+	20
Glomus sp1	+	+	+	+	+	100
G. sp2	-	+	+	+	-	60
G.aggregatum	-	+	-	+	+	60
G. caledonian	+	-	-	-	-	20
G. clavispora	+	-	-	-	+	40
G. rubiformis	+	-	-	-	+	40
G. tortuosum	-	-	-	-	+	20
Pacispora boliviana	-	-	-	-	+	20
Pacispora sp 1	-	-	-	-	+	20
Scutellospora fulgida	-	-	-	+	+	20

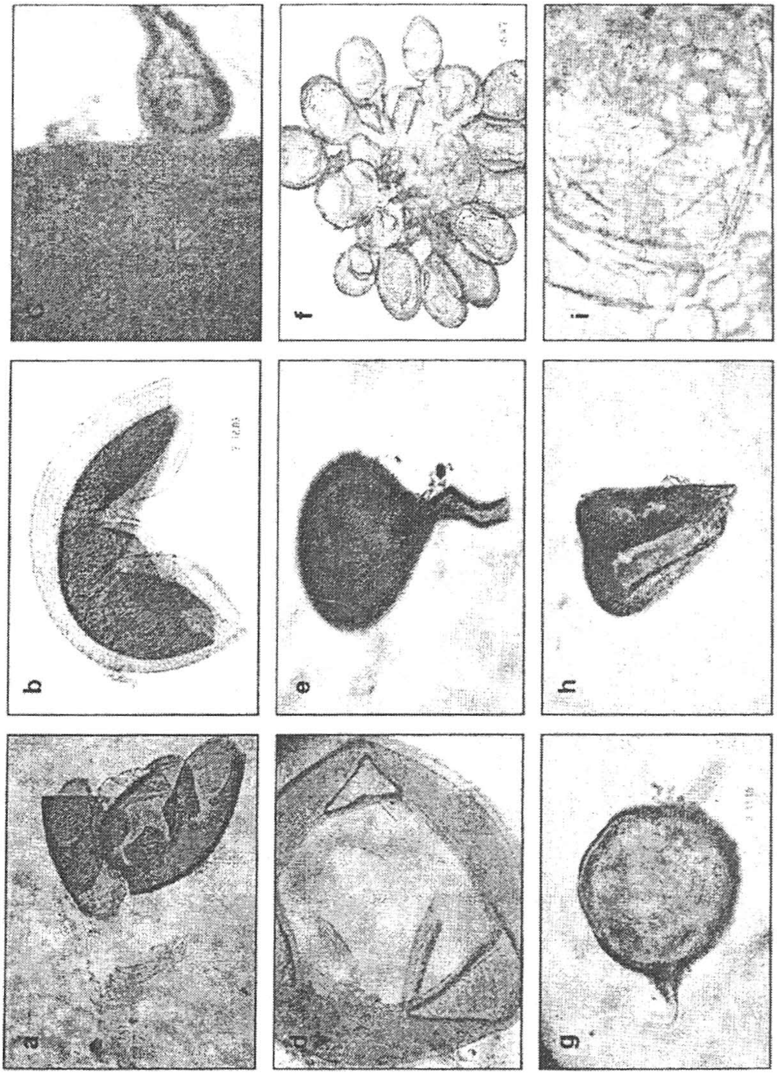

Figure 5: Arbuscular mycorrhizal fungi isolated from the acidic soils (a & b) *Acaulospora* species isolated from different; (c & d) *Gigaspora* species; (e-h) *Glomus* species isolated from different sites and (i) *Pacispora* species.

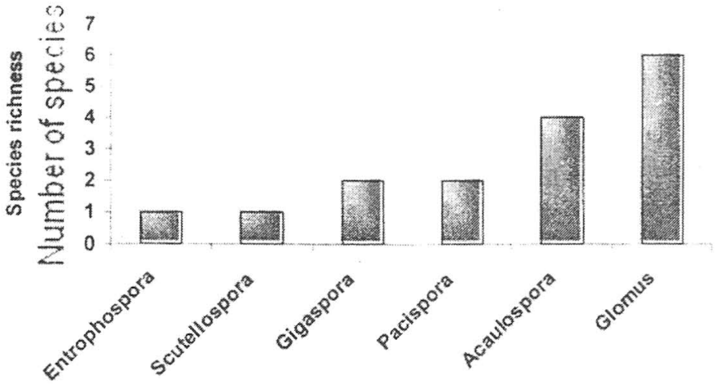

Figure 6: Species richness of different AMF species isolated from acidic soils

and abandoned coalmine are 0.18, 0.13, 0.08 and 0.22, respectively.

Discussion

The features favoring the higher population may either be the conducive to edaphic conditions for sporulation like low nutrient status, high aeration and optimum moisture or the undisturbed conditions of the soils which allowed sufficient time for the build up of mycorrhizal spores (Chulan and Omar, 1991). However, in this study coalmine is found to be very high comparatively to other sites. AMF distribution and mycorrhizal efficiency might be influenced by soil pH, soil humidity level, etc (Abbott and Robson, 1991; Moreira-Souza *et al.*, 2003). Sharma *et al.*, (1986) reported that the incidence of AMF species in the subtropical forest of Meghalaya (India), are controlled to a great scale by the soil pH, organic matter, moisture and nutrients status of forest floor. The soil pH is known to control the availability of nutrients from the soils to plants thereby regulating the status of mycorrhiza (Baylis, 1967). In an undisturbed ecosystem, higher spore population was quite natural as AMF spores tended to be higher and the population diversity was higher in native undisturbed forests than the disturbed and replanted areas (Sieverding, 1991).

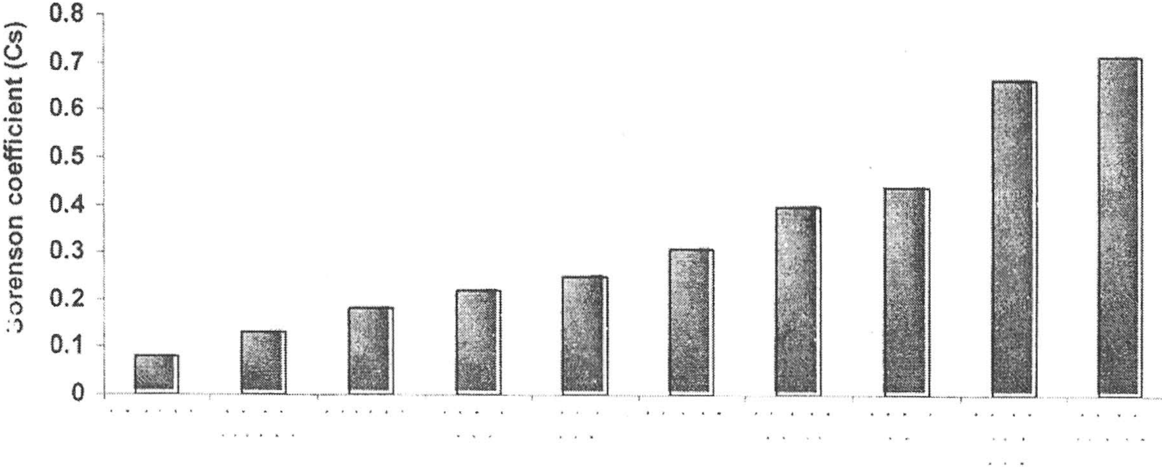

Figure 7: Sorenson's coefficients between various sites where PF= pine forest, RNH = roadside national highway, ACM = abandoned coalmine and CM = coalmine and PCF = potato cultivation field

Patterns of spore production, spore quantity etc are closely related to the plant phenology, root phenology and root production (Brundrett, 1991). Every life history of a mycorrhizal fungus is subjected to be influenced by plant roots. Spore germination, germination rate, direction of germ tubes hyphal branching recognition of the host root penetration establishment intensity of colonization growth of hyphae into soils and sporulation of the AMF were reported to be affected by the plant roots (Bhatia *et al.*, 1996). Different life duration of the different host plants had been reported to be the controlling factors of the species composition of AMF (Muthukumar and Udaiyan, 2000), thus the variation of diversity indices might be resulted in. There was also great diversity of mycorrhizal fungi often associated with the same plant (Alen and Boosalis, 1983). Disturbance might also responsible for distribution of AMF (Jasper *et al.*, 1989).

Sharma *et al.*, (1984 and 1986) reported the dominance of the *Glomus* in northeast India. They described the wider adaptation of the taxon in varied soil conditions. The sporulation pattern of *Glomus* might bring about the dominance of the taxon. Spores of *Glomus* are grown in cluster and sporulate more frequently while other like *Gigaspora, Scutellospora* etc sporulated singly. Thus, less population of Gigasporineae might be quite expectable as also reported (Dhar & Mridha, 2006). *Acaulospora* species are often associated with acidic soils (Abbott and Robson, 1991), whereas the soils studied were also acidic which supports the occurrence of two species of *Acaulospora*. *Pacispora boliviana* may favour these sub-tropical region and also ubiquitous as reported by (Oehl and Sieverding, 2004).

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REFERENCES

Abbott LK, Robson AD. 1991. Factors influencing the formation of arbuscular mycorrhiza. Agriculture Ecosystem and Environment. 35,121"150.

Allen SE, Grinshaw HM, Parkinson JA, Quaramby C. 1974. Chemical analysis of ecological materials. Blackwell Scientific Publications, Oxford.

Allen MF, Boosalis MG. 1983. Effects of two species of vesicular-arbuscular mycorrhizal fungi on drought tolerance of winter wheat. New Phytologist. 93, 67"76.

Anderson JM, Ingram JSI. 1993. Tropical Soil Biology and Fertility. A handbook of methods. CAB International, Oxford.

Arines J, Sainz M. 1987. Phosphorus sorption by acid soils: comparative study of some parameters. Journal of Agricultural Science. 109, 87-94.

Auge´ RM. 2001. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza. 11, 3-42.

Azcon-Aguilar C, Barea JM, Roldan-Fajardo BE. 1985. Avances recientes en el estudio de las micorrhizas VA. II. Factores que afectan su formaci6n y aplicaciones prfi.cticas en agricultural (1). Anales de Edafologia y Agrobiologia. 44, 943-958.

Baylis GTS. 1967. Experiments on ecological significance of phycomycetous mycorrhizas. New Phytologist. 66, 231-243.

Bhatia NP, Sundari K, Adholeya A. 1996. Diversity and selective dominance of vesicular arbuscular mycorrhizal fungi. In: Mukerji, K.G. (ed.), Concepts in Mycorrhizal Research. Netherlands: Kluwer Academic Publishers.

Blaszkowki J. 1989. Acaulospora cavernata (Endogonaceae) - a new species from Poland with pitted spores. Cryptogamic Botany. 1: 201-207.

Brundrett M. 1991. Mycorrhizas in natural ecosystems. Advanced Ecological Research. 21, 271"315.

Caravaca F, Barea JM, Palenzuela J, Figueroa D, Alguacil MM, Rolda´n A. 2003. Establishment of shrub species in a degraded semiarid site after inoculation with native or allochthonous arbuscular mycorrhizal fungi. Applied Soil Ecology. 22, 103-111.

Chulan A, Omar M. 1991. Incidence of VAM spores in some Malaysian soils [J]. Pertanika. 14, 133"137.

Dhar, Mridha. 2006. Biodiversity of arbuscular mycorrhizal fungi in different trees of madhupur forest, Bangladesh Journal of Forestry Research. 17(3), 201"205

Dhillion SS, Gardsjord TL. 2004. Arbuscular mycorrhizas influence plant diversity, productivity, and nutrients in boreal grasslands. Canadian Journal of Botany. 82, 104–114.

Jasper DA, Abbott LK, Robson AD. 1989. Soil disturbance reduces the infectivity of external hyphae of vesicular arbuscular mycorrhizal hyphae. New Phytologist. 112, 93"99.

Moreira-Souza M, Trufem SFB, Gomes-da-Costa SM, Cardoso EJBN. 2003. Arbuscular mycorrhizal fungi associated with *Araucaria angustifolia* (Bert.)O. Ktze [J]. Mycorrhiza. 13, 211"215.

Muthukumar T, Udaiyan K. 2000. Arbuscular mycorrhizas of plants growing in the Western Ghats region, Southern India. Mycorrhiza. 9, 297"313.

Muthukumar T, Senthilkumar M, Rajangam M, Udaiyan K. 2006. Arbuscular mycorrhizal morphology and dark septate fungal associations in medicinal and aromatic plants of Western Ghats, Southern India. Mycorrhiza 17, 11–24.

Oehl F, Sieverding E. 2004 *Pacispora*, a new vesicular arbuscular mycorrhizal fungal genus in the Glomeromycetes. Journal of Applied Botany. 78, 72-82.

Rabie GH. 1998. Induction of fungal disease resistance in *Vicia faba* by dual inoculation with *Rhizobium leguminosarum* and vesicular-arbuscular mycorrhizal fungi. Mycopathologia. 141, 159–166.

Ruiz-Lozano JM, Collados C, Barea J.M, Azcón R. 2001. Arbuscular mycorrhizal symbiosis can alleviate drought induced nodule senescence in soybean plants. New Phytologist. 151, 493–502.

Sanchez PA. 1976. Properties and management of soils in the tropics. Wiley, New York.

Sanchez PA, Salinas JG. 1981. Low input technology for managing oxisols and ultisols in tropical America. Advances in Agronomy. 34, 279-406.

Sen TK, Nayak DC, Singh RS, Dubey PN, Maji AK, Chamuah GS, Sehgal J. 1997. Pedology and edaphology of benchmark acid soils of north-eastern. Journal of the Indian Society of Soil Science. 45, 782-790.

Sharma SK, Sharma GD, Mishra RR. 1984. Endogonaceous Mycorrhizal Fungi in a sub-tropical forest of N.E. of India. Journal of Tree Science. 2, 10-14.

Sharma SK, Sharma GD, Mishra RR. 1986. Status of mycorrhizae in sub-tropical forest ecosystem of Meghalaya. Acta Botanica Indica.14, 87-92.

Sieverding E. 1991. Vesicular arbuscular mycorrhizae management in tropical agro systems. Deutsche Gesellschaft fur technische Zusammenarbeit (GTZ) GmBH, Eschh boran.

Smith SE, Read DJ. 1997. Mycorrhizal symbiosis. London. Academic Press.

van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders LR. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature. 396, 69–72.

Vivas A, Vo¨ro¨s I, Biro´ B, Campos E, Barea JM, Azcón R. 2003. Symbiotic efficiency of autochthonous arbuscular mycorrhizal fungus (*G. mosseae*) and *Brevibacillus* sp. isolated from cadmium polluted soil under increasing cadmium levels. Environment and Pollution 126, 179–189.