Ann. For. Res. 55(2): 207-216, 2012

ANNALS OF FOREST RESEARCH
www.e-afr.org

Reforestation of bauxite mine spoils with *Eucalyptus tereticornis* Sm. seedlings inoculated with arbuscular mycorrhizal fungi

A. Karthikeyan, N. Krishnakumar

Karthikeyan A., Krishnakumar N., 2012. Reforestation of bauxite mine spoils with *Eucalyptus tereticornis* Sm. seedlings inoculated with arbuscular mycorrhizal fungi. Ann. For. Res. 55(2): 207-216, 2012.

Abstract. Open cast mining for bauxite at Yercaud hills (India) resulted in degradation of forest ecosystem and production of large quantities of waste rocks (called mine spoils). To ameliorate mine spoils, topsoil is used to spread over before the planting of tree species, conventional method as the topsoil has a good structure, water holding capacity and beneficial microbes like Arbuscular Mycorrhizal (AM) fungi essential for plant growth. However, the use of top soil is expensive and in this study bauxite mine spoils were reforestated with AM fungi instead of it. The beneficial microbes AM fungi (*Glomus aggregatum* Schenck & Smith, *G. fasciculatum* (Thatcher) Gerd. & Trappe emend. Walker & Koske, *G. geosporum* (Nicol. & Gerd.) Walker) were isolated, cultured and inoculated into the seedlings of *Eucalyptus tereticornis* Sm. and grown in bauxite mine spoils as potting medium under nursery conditions. Then, the biomass improved seedlings of *E. tereticornis* with inoculation of AM fungi were directly transplanted at bauxite mine spoils. After transplantation of the seedlings at bauxite mine spoils, the growth and survival rate were monitored for two years. The AM fungi inoculated seedlings of *E. tereticornis* showed 95% survival over the control seedlings and their growth was also significantly higher. Tissue nutrients (N, P, K) were also found higher in AM fungi inoculated *E. tereticornis* than un inoculated control seedlings.
Keywords AM fungi, *Eucalyptys tereticornis*, bauxite, biomass, reforestation, mine spoils.

Authors. A. Karthikeyan (karthika@icfre.org), N. Krishnakumar - Institute of Forest Genetics and Tree Breeding Coimbatore, 641 002 Tamilnadu, India.
Manuscript received March 13, 2012; revised July 16, 2012; accepted July 26, 2012; online first October 15, 2012.

Introduction

Mining activities have created wide spread environmental damage that severely affected not only the landscape but also social well-beings. Mining also has led to discharge of millions of tons of waste rock and soils called 'mine spoils' are dumped over millions of hectares as wastelands in India. Reforestation of these stressed environment lands is very essential to overcome the health hazards and ecological problems. But, reforestation of these stressed environment lands i.e. mine spoils is difficult because the mine spoils have low water – holding capacity, lacks organic matter, deficient in Nitrogen and Phosphorus. Using conventional reforestation techniques of grading, res-soiling, and fertilizing of mine spoils are economically not always feasible. However, planting of trees directly on mine spoils is an excellent alternative method for reforestation. This method will help to stabilization of mine spoils so that the mined out wastes could be sealed away from the stressed environment. But to plant a tree the topsoil is considered as an important material because the topsoil is always having good structure and beneficial microbes compared to subsoil. The topsoil is enriched with organic matter, minerals and has characteristics of nutrient supply and water uptake to the plants. Hence, it is more hospitable to plant growth. To deliver it's full potential for improved plant growth it must retain the advantageous chemical, physical and biological properties such as beneficial microbes (Smith & Read 1997). Studies on reclaimed bauxite mine spoil surface with topsoil in Australia also suggest that recovery of biological and microbial activity as a good indicator of reclamation success (Jasper et al. 1998). Further, essential nutrients content of mine spoils is significantly lower due to lack of microbes (Abdul Kareen & Mc Rae 1984). All these characters lead to reduced soil quality, nutrient cycling and lower availability of nutrients in mine spoils. Moreover, the process of opencast bauxite mining scars the landscape and destroys microbial communities. Therefore, it is essential to introduce the beneficial microbes in mind out areas along with the suitable trees for better reforestation. The activity of soil beneficial microbes is important in nutrient transfer and establishment of tree seedlings in these stressed environment lands. Arbuscular mycorrhizal (AM) fungi is one of the beneficial soil microorganisms associated with the majority of higher plants in natural and semi natural and agro-eco systems as they improve plant water relations and nutrient up take under stressed conditions (Smith & Read 1997). AM fungi reported as potential micro organism for rehabilitation and reclamation of coal mine spoils and fly ash dumps (Kumar et al. 2010, Babu & Reddy 2011). AM fungi enhances the success of seedlings under poor soil conditions (Visser et al. 1984) and improve the nutrient uptake especially phosphorus (P) of the host plants (Jakobsen 1999). The extra radical mycelia of AM fungi may act as extensions of the root systems (Muthukumar & Udaiyan 2010) and have a high nutrient mobilizing potential. Banning et al (2010) reported that successful rehabilitation must involve the development of microbially driven nutrient cycling for the long term provision of nutrient to the planted seedlings. Hence, in this study AM fungi were attempted to enhance nutrient uptake in *Eucalyptus tereticornis* tree seedlings at bauxite mine spoils for reforestation. Further for any reforestation and rehabilitation programme it must involve income generation with aim of poverty alleviation (Bognounou et al. 2010). *E. tereticornis* is an economically important tree crop used in paper and pulp industries. Therefore, in this study *E. tereticornis* was selected for reforestation of bauxite mine spoils.

Material and methods

Study site. The study site selected for reforestation is located at Yercaud Hill, Salem Dis-

trict, Tamil Nadu, India (11˚48' to 11˚50' and 78˚13' to 78˚14' E) with an elevation of 1640 m.s.l where the Madras Aluminium Company (MALCo) has acquired about 190 ha of land for open cast bauxite mining. The site receives 1500 mm average rainfall and has a wide diversity of plant species around the bauxite mined out areas. The bauxite mine spoils were collected from the mined out area and used for nursery experiments. Within the area adjacent to the MALCo bauxite mines *Syzgium cumini* L. was identified as the dominant species.

Plant material. The seeds of *E. tereticornis* were collected from the clonal seed orchard of Eucalyptus established by Institute of Forest Genetics and Tree breeding (IFGTB) at Coimbatore, India.

Isolation and culture of AM fungi. The AM fungi (*Glomus aggregatum* Schenck & Smith, *G. fasciculatum* (Thatcher) Gerd. & Trappe emend. Walker & Koske, *G. geosporum* (Nicol. & Gerd.) were isolated from the rhizosphere of *S. cumini* by the method of Gerdemann & Nicolson (1963) and identified with Schenck & Perez manual (1990). The freshly collected AM fungal spores were then multiplied and maintained in sterile soil media (alfisoil: sand) with *Sorghum bicolor* (L.) Moench (as host) under laboratory conditions for six months in clay pots.

Analysis of mine spoils. Sieved bauxite mine spoils were analyzed for nutrient status and soil pH and electric conductivity (EC). Soil pH was determined (1:1, soil: water) as soon as the samples were brought to the laboratory and EC was measured using a bridge meter. The total nitrogen (N) and available Phosphorus (P) were determined respectively by micro-kjeldahl and molybdenum blue methods of Jackson (1973). Exchangeable K was extracted from the soil in ammonium acetate solution (pH 7) and measured with a digital flame photo meter (Jackson, 1973). Physical and chemical properties of the bauxite mine spoils are given in Table.1.

Seed germination of *E. tereticornis*. The *E. tereticorniss* seeds were directly applied in the nursery beds containing pure sand with sufficient water spray. The germinated seedlings after 10 days were transplanted to polythene bags (14 x 27 cm) containing sieved bauxite mine spoils.

Inoculation of AM fungi. The AM fungal inoculum along with soil from pot cultures of *S. bicolor* comprising chopped roots, hyphal segments and chlamydospores was used for inoculations. Ten gram of inoculum was placed 5 cm below the soil surface of each polythene bag of all tree seedlings. Thereafter the seedlings were maintained under nursery conditions for 2 months and watered regularly.

Harvest and assessment of seedlings. Sixty days after emergence the *E. tereticornis* seedlings were harvested with their entire root system intact. The root length, shoot length, number of branches and collar diameter of each seedling was measured.

Transplantation of seedlings. The beneficial microbes inoculated seedlings of E. tereticornis were transplanted in bauxite mine spoils in 1 ft. deep pits at an espacement of 1.5 x 1.5 m in a randomized block design with un inoculated control seedlings. The growth and survival of all the seedlings were monitored regularly at 30 days interval. The data on shoot height, collar diameter and number of leaves

Table 1 Physical and chemical properties of sieved bauxite mine spoils (mean of three replicates)

Sl. No.	Bauxite mine spoils	pH	E.C mS	N (mg Kg^{-1})	P (mg Kg^{-1})	K (mg Kg^{-1})
1.	Before planting	6.0 (± 1.24)	0.08 (± 0.02)	0.30 (± 0.25)	1.30 (± 0.68)	4.0 (± 1.56)
2.	After planting	6.8 (± 1.36)	0.28 (± 0.12)	1.50 (± 0.85)	6.40 (± 1.58)	9.5 (± 1.78)

Note: In paranthesis is the standard error of mean.

were collected at different months after planting.

Root colonization of AM fungi. Planted seedlings of *E. tereticornis* root samples of feeder roots were collected after 2 years of planting and washed with sterile water. Roots were cut in to 1 cm segments, cleared and stained for rapid assay of mycorrhizal colonization. The cleared segments were washed in distilled water acidified with 5N HCl and stained in trypan blue (0.05% in lactophenol; Phillips and Hayman, 1970). Stained root segments were examined under a research microscope for the presence of AM fungal structures. The percentage root colonization (% hyphae + % arbuscules + % vesicles) was determined according to the root slide technique of Read et al. (1976).

Tissue nutrient analyses. Constant weights of (10g) of leaves of *E. tereticornis* treated with AM fungi were collected from the reforestation plot. After drying in a hot air oven for 72 hr the leaves were assessed for nutrient content of N, P and K. The total N was determined on a kjeltec auto analyser (1030), P determination was done by vanadomolybdate phosphoric yellow colour method and K content was determined by flame photometer (Jackson, 1973).

Statistical analyses. Each measured variable in the nursery and field experiments were subjected to analyses of variance and mean separations has been done using Duncan's Multiple Range Test (SPSS. Ver. 10.)

Results

Physical chemical properties of bauxite mine spoils

The soil nutrients pH and E.C were increased after transplantation of *E. tereticornis* at bauxite mine spoils (Table 1).

Nursery experiments. AM fungi inoculated *E. tereticornis* seedlings showed significantly improved growth, nutrient content (NPK), bio mass rather than non inoculated control seedlings (Table 2, Fig.1).

Field experiments. After transplantation of *E. tereticornis* seedlings at bauxite mine spoils their growth and survival were monitored at regular monthly interval up to 2 years. AM fungi inoculated *E. tereticornis* seedlings showed improved growth and collar diameter at the age of three months after planting. At the age of two years after planting the AM fungi inoculated seedlings increased the growth in three folds over control and also increased the collar diameter. The tissue nutrient contents of N, P and K were also improved significantly ($p < 0.05$) in AM fungi inoculated seedlings than control seedlings (Table 3, Fig. 2)

Survival performance. AM fungi inoculated *E. tereticornis* showed 95 % survival. The non inoculated control seedlings showed only 40% survival at the age of two years after planting (Fig. 3).

AM fungal root colonization and spore population. AM fungi inoculated seedlings of *E. tereticornis* planted at bauxite mine spoils showed 75 per cent of root coloni-

Table 2 Growth responses of *E. tereticornis* seedlings with (+) without (-) AM fungi inoculation (mean of 5 replicates)

Treat ments	Shoot length (cm)	Root length (cm)			Root collar diameter (mm)	Root Dry weight (g/plant)	Shoot Dry weight (g/ plant)	Root/Shoot ratio
		I	II	III				
+ AMF	52^b	18.4^b	12.3^b	4.1^b	4.28^b	0.026^b	0.296^b	0.08^a
- AMF	28^a	11.8^a	5.4^a	1.2^a	2.35^a	0.012^a	0.116^a	0.10^b

Note: Abreviations for: root length; I: primary; II: secondary; III: tertiary roots. Means followed by same letter are not significantly different at $p < 0.05$ of Duncan's Multiple Range Test.

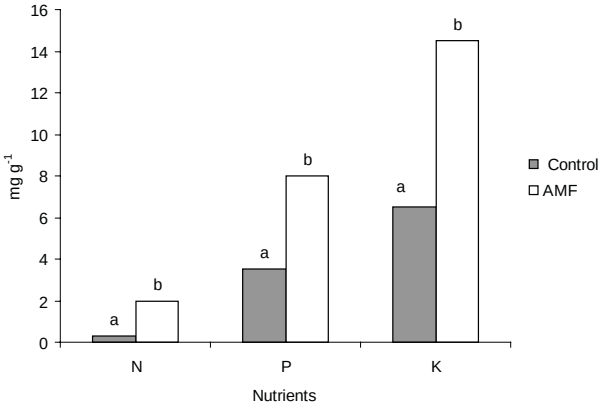

Figure 1 Influence of AM fungal inoculation on nutrient concentration of *E. tereticornis* seedlings at nursery conditions. The bars are means; when followed by same letter are not significantly different at $p < 0.05$ of Duncan's Multiple Range Test

Table 3 Growth performances of *E. tereticornis* seedlings inoculated with (+) and without (-) AM fungi at bauxite mine spoils (mean of ten replicates)

	Height (cm)		Collar Diameter (mm)		No. of branches/plant	
Treatments	3 months after planting	2 years after planting	3 months after planting	2 years After planting	3months after planting	2 years after planting
- AMF	35. 2[a]	38.77[a]	4.49[a]	6.87[a]	2.33[a]	6.37[a]
+ AMF	38.5[a]	96.22[b]	4.34[a]	11.42[b]	2.30[a]	16.14[b]

Note: Means followed by same letter are not significantly different at $p < 0.05$ of Duncan's Multiple Range Test

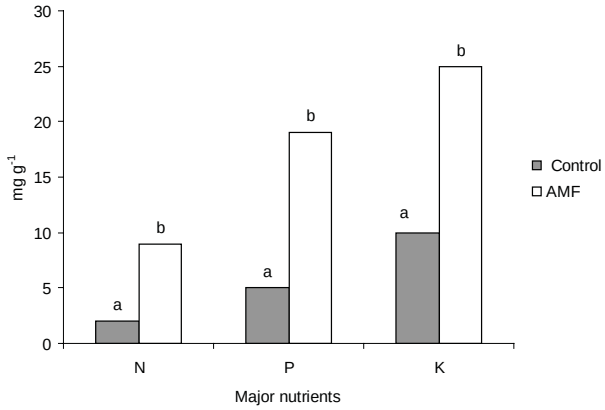

Figure 2 Tissue nutrient content of *E. tereticornis* seedlings inoculated with AM fungi under field conditions. The bars are means; when followed by same letter are not significantly different at $p < 0.05$ of Duncan's Multiple Range Test

zation and has an average of 8.3 spores g^{-1} soil after planting of three months. Two years after planting the seedlings showed 92% of root colonization and 6.6 spores g^{-1} soil (Fig. 4).

Discussion

The excessive use of chemical fertilizer and pesticides has generated several environmental problems including the green house effect, ozone layer depletion and acidification of water. These problems can be tackled by use of biofertilizers which are natural, beneficial and ecofriendly. The biofertilizers provide nutrients to the plants and maintain soil structure. In this study of AM fungi, the biofertilizer is used for the remediation of stressed environment sites of bauxite mine spoils.

AM fungi are important microbes of soil that form symbiotic association with most of the terrestrial plants on the earth. These fungi are chiefly responsible for P uptake. Early inoculation of AM fungi at the seedlings stage has been proven beneficial in nutrient uptake in many studies (Muthukumar & Udaiyan 2006; 2010). Auge et al. (1992) reported that increased P absorption and plant growth ascribed to AM fungi. AM fungi inoculated seedlings of *E. tereticornis* in this study showed improved root and shoot growth and biomass due to AM fungi colonization that alter the host root morphology and physiology. Effects of AM fungi on secondary and tertiary roots were most significant with their length and branching. It may be due to low nutrient availability in the mine spoils that increased root length in presence of AM fungi (Fitter 1987). Presumably, the higher root length allows maximum

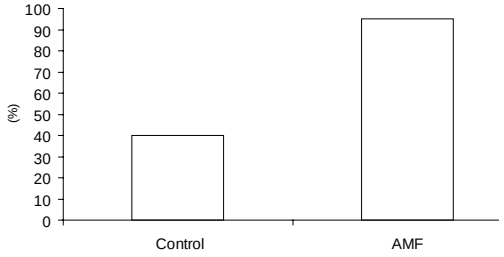

Figure 3 Survival performance of AM fungi inoculated seedlings of *E. tereticornis* at bauxite mine spoils after two years

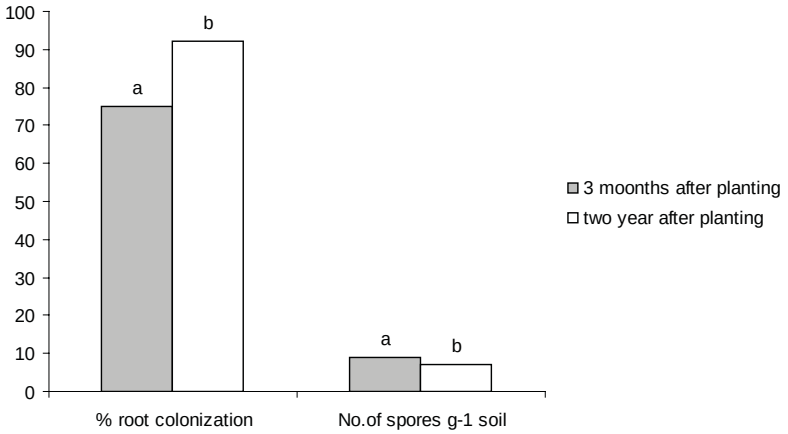

Figure 4 Root colonization and sporulation of AM fungi in *E. tereticornis* planted at bauxite mine spoils (Bars indicated as means and followed by same letter are not significantly different at $p < 0.05$ of Duncan's Multiple Range Test)

surface area for nutrient uptake. These effects may be due to colonization of AM fungi resulted that some changes in root morphology. The changes in host root meristematic activity may result in root changes (Berta et al. 1990) and secretion of cytokinin like substances in AM fungi (Barea & Azcon–Aguilar 1992) may also be involved. Similar effect on root system architecture has demonstrated in another tree *Prunus cerasifera* inoculated with AM fungi G. intraradices and G. mosseae (Hooker & Atkinson 1996).

The physical and chemical properties of the soil collected from bauxite mine spoils showed that the soil is acidic and poor in major nutrients because of the topsoil is stripped off during mining. However, after a period of time the soil nutrient status was improved due to planting of *E. tereticornis* with AM fungi. The physical and chemical properties were reported as improved in fly ash dumps due to inoculation of AM fungi with Dendrocalamus strictus (Babu & Reddy 2011). The inoculated AM fungi mobilized the available nutrients to the seedlings and helped to increase the uptake of number of ions such as K^+ and $NH4^+$ (Bowen 1990). The increased nutrient status in bauxite mine spoils is due to activity of AM fungal populations. The improvement of soil nutrients due to AM fungi is the reason for soil quality and soil aggregation that gives adequate aeration for plant growth (Wheeler et al. 2000). This is the one of the reasons the seedlings of E. tereticornis s are surviving successfully in bauxite mine spoils. Similar studies were also reported worldwide on disturbed sites. Field inoculation of Prosopis juliflora with AM fungi in a study conducted on semi arid wasteland significantly increased plant bio mass and soil nutrient after six years of growth (Bhatia et al. 1998). Similarly it was reported that Acacia nilotica and Pongamia pinnata responded well with inoculation of AM fungi in bauxite mines residue (Chauhan & Silori 2011). In this study the significant growth enhancement of *E. tereticornis* with AM fungi was due to increased N, P and K accumulation and root colonization. Similar effect was also reported by Jeffries et al (2003) and Jasper et al. (1998) at bauxite mine spoils. Higher AM fungal colonization and low sporulation in the planted seedlings of *E. tereticornis* after two years of planting were found in this study. AM fungal sporulation generally occurs when root growth slows down or ceases (Abbott & Robson 1991). Under stressed environmental conditions like mined out areas where root growth is continuous, the vegetative phase of AM fungi may be actively involved in initiating infection and spreading infection to new roots (Udaiyan et al. 1996). These effects may improve nutrient utilization efficiency of planted seedlings that gives better growth improvement and out plant survival. Few earlier studies have also showed that colonization by AM fungi enhances plant survival and growth by decreasing phosphorus deficiency and water stress (Rouseeau et al. 1994) and improving membrane infectivity (Graham et al. 1981). This is the reason the AM inoculated seedlings showed the survival rate of *E. tereticornis* in the stressed environment conditions at the average of 95%. Ottega-Larocea et al. (2010) also reported that among soil microorganisms AM fungi play nutrient roles for establishment, survival of plants and improved soil properties in stressed environments.

Conclusions

The results from this study support the general conclusion that the introduction of plants an stressed environment sites like mine spoils inoculated with AM fungi is a beneficial bio technological tool to aid the recovery of an degraded ecosystem. The AM fungi have the potential to increase the efficiency of the plant system by providing the seedlings with essential levels of P and other nutrients for growth. Therefore, tree seedlings could be inoculated with symbiotic microorganisms like AM fungi before planting to restore stressed sites.

Acknowledgements

The authors thanksd to Indian Council of the Forestry Research and Education, India for financial support for this study.

References

Abbott L.K., Robson A.D., 1991. The distribution and abundance of VA endophytes in some west Australian soils. Australian Journal of Botany 25: 515–522.

Abdul–Kareen A.W., Mc Rae S.G., 1984. The effects on topsoil of long term storage in stockpiles. Plant and Soil 76: 357–363.

Auge R.M., Stodola A.J., Brown M.S., Bethlenfalvay G.J., 1992. Stomatal response of mycorrhizal cow pea and soybean to short term osmotic stress. New Phytologist 120: 117–125.

Babu G.A., Reddy M.S., 2011. Dual inoculation of Arbuscular mycorrhizal and phosphate solubilising fungi contributes in sustainable maintenance of plant health in fly ash ponds. Water, Air and Soil Pollution 219: 3-10.

Barea J.M., Azcon–Aguilar C., 1992. Production of plant growth relating substances by vesicular – arbuscular mycorrhizal fungus *Glomus mosseae*. Applied Environmental Microbiology 43: 810–813.

Banning N.C., Phillips I.R., Jones D.L.,Murphy D.V., 2010. Development of microbial diversity and functional potential in bauxite residue sand under Rehabilitation. Restoration Ecology 17: 350-358.

Berta G., Fusconi A., Trotta A., Scannerini S., 1990. Morphogenetic modifications induced by the mycorrhizal fungus *Glomus strain* E3 in the root system of *Allium porum* L. New Phytologist 114: 207–215.

Bhatia N.P., Adholeya A., Sharma A., 1998. Biomass production and changes in soil productivity during long term cultivation of *Prosopis julifera* (Swartz) DC inoculated with VA mycorrhiza and *Rhizobium* sp. in a semi arid wasteland. Biology and Fertility of Soils 26: 208–214.

Bognounou F., Thiombiano A., Oden P.C., Guinko S., 2010. Seed provenance and latitudinal gradient effects on seed germination capacity and seedling establishment of five indigenous species in Burkino Faso. Tropical Ecology 51: 207-220.

Bowen G.D., 1990. Mycorrhizal roles in tropical plants and ecosystems. In: Mikola, P., (ed.) Tropical mycorrhizal research. Oxford University Press. Oxford, pp. 148–179.

Chauhan S., Silori C.S., 2011. An evaluation of successful reclamation of bauxite residue through afforestation activities in South India. Journal of Horticulture and Forestry 3: 214–224.

Fitter A.H., 1987. An architectural approach to the comparative ecology of plant root systems. New Phytologist 106 : 61–77.

Gerdemann J.W., Nicolson T.H., 1963. Spores of mycorrhizal Endogone species extracted from soils by wet sieving and decanting. Transactions of British Mycological. Society 46: 235–244.

Graham J.H., Leonard R.T., Menge J.A., 1981. Membrane – mediated decrease in root exudation responsible for Phosphorus inhibition of vesicular arbuscular mycorrhiza formation. Plant Physiology 68: 549–552.

Hooker J.E., Atkinson D., 1996. Arbuscular mycorrhizal fungal induced alteration to root architecture in straw berry and induced resistance to the root pathogen *Phytopthora fragaria*. Plant and Soil 159: 229-234.

Jackson M.L, 1973. Soil chemical analysis Prentice Hall, New Delhi, India 498p.

Jakobsen I., 1999. Transport of phosphorus and carbon in arbuscular mycorrhiza. In: Varma, A. & Hock, B., (eds.), Mycorrhiza: structure, function, molecular biology and bio technology, 2nd edn. Springer-Verlag, agriculture and natural eco systems. Birhauser Verlag, Basel, Switzerland, pp 101-115.

Jasper D.A., Sarwasa Y., Ward S.C., 1998. Indicators of reclamation success- recovery patterns of soil biological activity compared to remote sensing of vegetation. In: Fox H.R., Moore H.M., Mc Intosh A.D., (eds), Land reclamation achieving sustainable benefits. Proceedings of the fourth international conference of the international application of land reclamation Nothnigham U.K 7-11, Seplso, 1998. Rotherdam, Balkam, pp. 21-24.

Jefries S.P., Gianinazzi S., Perotto K.,. Turnau T., Barea J.M., 2003. The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. Biology and Fertility of Soils 37: 1-16.

Kumar A., Raguvanshi R., Upadhyay R., 2010. Arbuscular mycorrhizal technology in reclamation and revegetation of coal mine spoils with various revegetation model. Engineering 9: 683-689.

Muthukumar T., Udaiyan K., 2006. Growth of nursery grown bamboo inoculated with arbuscular mycorrhizal fungi and plant growth promoting rhizobacteria in two tropical soil types with and without fertilizer application. New Forests 31: 469-485.

Muthukumar T., Udaiyan K., 2010. Growth response and nutrient utilization of *Casuarina equisetifolia* seedlings inoculated with bio inoculants under tropical nursery conditions. New Forests 40: 101-118.

Ortega-Larrocea M.P., Xoconostle-Cazares B., Maldonado Mendoza I., Carrilo-Gonzalez R., Hernandez-Hernandez J., Diaz Gardunno M., 2010. Plant and fungal bio diversity from metal mine wastes under remediation at Zimapan, Hidalgo, Mexico. Environmental Pollution, 158: 1922-1931.

Philips J.M., Hayman D.S., 1970. Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of British Mycological Society 55: 158-160.

Read D.J., Koucheki H.K., Hodgson J., 1976. Vesicular

– arbuscular mycorrhiza in natural vegetation systems. The occurrence of infection. New Phytologist 77: 641-653.

Rousseau J.V.D, Sylvia D.M., Fox A.J., 1994. Contribution of ectomycorrhiza in the potential nutrient absorbing surface of pine. New Phytologist 128: 639-644.

Sanchez- Diaz M., Honrubia M., 1994. Water relation and alleviation of drought stress in mycorrhizal plants. In: Gianninazzi S., Schuepp H., (eds.), Impact of arbuscular mycorrhizas on sustainable agriculture and natural eco systems. Birhauser, Basel, Switzerland, pp. 167-178.

Schenck N.C., Perez Y., 1990. Manual for the identification of VA mycorrhizal fungi. Synergistic, Gainesville, FL. 605 p.

Smith S.A., Read D.J., 1997. Mycorrhizal symbiosis Academic Press. San Diego. 605 p.

Udaiyan K., Karthikeyan A., Muthukumar T., 1996. Influence of edaphic and climatic factors on dynamics of root colonization and spore density of vesicular arbuscular mycorrhizal fungi in *Acacia farnesiana* Willd and *A. planifrons* W.et.A. Trees Structure and Function 11: 65-71.

Visser S., Fujolava., C.L., Griffith S., Parkinson D., 1984. Effect of top soil storage on microbial activity, primary production and decomposition potential. Plant and Soil 82: 41–50.

Wheeler C.T., Tilak C., Scrimgeons M., Hooker J.E., Hardly L.L., 2000. Effects of symbiosis with Frankia and arbuscular mycorrhizal fungus in the natural abundance of ^{15}N in four species of *Casuarinas*. Journal of Experimental Botany, 51: 287-297.

Wright S.F Upadhyaya A., 1998. Survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. Plant and Soil 198: 97–107.