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REFORESTATION IN BAUXITE MINE SPOILS WITH *CASUARINA EQUISETIFOLIA* FROST. AND BENEFICIAL MICROBES[1]

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ABSTRACT

Reforestation in mine spoils difficult because the mine spoils are low or lack in beneficial microbial populations. To overcome this problem a field experiment was conducted on bauxite mine spoils with *Casuarina equisetifolia* Frost and beneficial microbial inoculants (arbuscular mycorrhizal fungi, *Frankia* and phosphobacterium) for reforestation. Under nursery experiments the cultured arbuscular mycorrhizal (AM) fungi *Glomus aggregatum* Schenck & Smith emend. Koske, Phosphobacterium (PSB) and root nodule extract of *Frankia* have inoculated to *C. equisetifolia* individually and combinations at nursery level. The mine wastes of bauxite called bauxite mine spoils were collected and used as potting media to grow *C. equisetifolia* seedlings along with these microbial inoculants. From the nursery experiments it was found that the AM fungi and other beneficial microbial inoculants improved the seedlings in terms of bio mass and growth. The seedlings thereafter were transplanted at bauxite mine spoils and the growth and survival of seedlings were monitored for two years. In field conditions, AM fungi, PSB and *Frankia* inoculated seedlings of *C. equisetifolia* showed 90 to 100% survival over the control seedlings. Their growth was also significantly higher than the control seedlings. The nutrient uptake (N, P, K) was also increased in the trees inoculated with AM fungi, *Frankia* and PSB. From this study it was understood that *C. equisetifolia* inoculated with beneficial microbes is a suitable tree species for reforestation in bauxite mine spoils

Key words: Casuarina equisetifolia, AM fungi, Phosphobacterium, *Frankia,* Mine spoils

INTRODUCTION

Open cast mining for bauxite in Yearcaud (Tamilnadu, India) results degradation of forest eco system and production of large quantities of waste rocks called ‘mine spoils’. The ecological problems found at these mining sites posing health hazards to nearby residents in asthma and stomach ailments due to the mining dust and contamination of water (Karthikeyan, 2003). Reforestation of these mine spoils is very essential to over come the health hazards and ecological problems. But, reforestation in mine spoils is difficult because the mine spoils

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have low water – holding capacity, lacks organic matter, deficient in nitrogen (N) and phosphorus (P). Using conventional rehabilitation or reclamation techniques of grading, re-soiling, and fertilizing of mine spoils is economically not always feasible. Further, during mining activities topsoil is stripped off and stored as stockpiles until the site is ready for restoration, which may take many years. Studies on rehabilitated bauxite mine spoils with topsoil in Australia also suggest that recoveries of biological and microbial activity are the good indicators of rehabilitation success (Jasper *et al.,* 1998; Jeffries *et al.,* 2003). But in stored mine spoils the microbial populations are reduced when compared to undisturbed sites (Miller and Cameron, 1976). Carbon content of stored mine spoils is also significantly lower due to lack of microbes (Abdul-Kareen and McRae, 1984). All these characters lead to reduced soil quality, nutrient cycling and lower availability of nutrients in mine spoils. However, suitable trees with beneficial microbial inoculants are always successful for reforestation in mine spoils.

A recent study by Dupponnois *et al.* (2007) indicated that mycorrhizal inoculation significantly improves the growth of *Acacia holosericea* seedlings in degraded soils. In this present study *C. equisetifolia* was selected for reforestation in bauxite mine spoils. In South India large scale of planting of *C. equisetifolia* was occurred for the past few years for stabilization and restoration of degraded soils. This fast growing tree is enormously utilized as poles, pulpwood and firewood (Diem and Dommergues, 1990). It is also tolerant to salt and used for windbreak and erosion control in coastal areas. It is also used as agroforestry crop and the uses are scaffolding for building construction, ornamental, and soil improvement. In India, 800,000 ha are planted with *C. equisetifolia* and the annual production of pulpwood alone is 10 million tonnes worth Rs. 200 crores. After the devastation of coastal areas by a tsunami in the year 2004 *C. equisetifolia* gained much importance as it was reported that the mature trees up to 20 m tall successfully reduced the force of the tsunami and saved the coastal areas in few parts of Tamilnadu (India). This tree is having the ability to fix atmospheric nitrogen through the symbiotic nitrogen fixing actionomycete *Frankia*. For the successful field performance in degraded areas like mine spoils this tree would be suitable as it have the ability of drought tolerant. However, it is essential to introduce the beneficial microbes such as mycorrhizal fungi, nitrogen fixing actinomycete *Frankia* and Phosphobacterium (PSB) for better out plant survival in degraded areas like mine spoils.

MATERIALS AND METHODS

Study site

The study site selected for reforestation is located at Yercaud Hill, Salem District, Tamil Nadu, India (116° 48' to 11° 50' and 78° 13' to 78° 14 E') at an elevation of 1640 m a.s.l where the Madras Aluminium Company (MALCo) has acquired

about 190 ha of land for open cast mining for bauxite. The site receives 1500 mm average rainfall and has a wide diversity of plant species around the bauxite mined areas. The bauxite mine spoils were collected from the mined area was used for nursery experiments at Institute of Forest Genetics and Tree Breeding, Coimbatore, India. The physical and chemical properties of bauxite mine spoils were assessed according to Jackson (1973) and showed that the bauxite mine spoils had a pH 6.0 ($\pm$ 1.24), electrical conductivity 0.08 mS ($\pm$ 0.02), nitrogen 0.30 mg Kg^{-1} ($\pm$ 0.068), phosphorus 1.30 mg Kg^{-1} ($\pm$ 0.68) and potassium 4.0 mg Kg^{-1} ($\pm$ 1.56). ($\pm$ is Standard Error of the Mean).

C. equisetifolia

The seeds of *C. equisetifolia* were procured from the Seed Technology Division of Institute of Forest Genetics and Tree Breeding, Coimbatore. *C. equisetifolia* seeds were directly sown in the nursery beds containing pure sand with sufficient water spray. Ten days old seedlings were transplanted to polythene bags (14 × 27 cm) containing sieved bauxite mine spoils.

Isolation and Culture of AM fungi

In areas adjacent to the MALCo bauxite mines, *Syzgium cuminii* L. trees are found dominant. The AM fungi *Glomus aggregatum* Schenck & Smith emend. Koske was isolated from the rhizosphere of *S. cumini* by the method of Gerdemann and Nicolson (1963) and identified with Schenck and Perez manual (1990). The freshly collected *G. aggregatum* spores were then multiplied and maintained in sterile soil media (alfisoil:sand) with *Sorghum bicolor* (L.) Moench. (as a host) under laboratory conditions at 1–23°C and 40–45% relative humidity (RH) for six months in clay pots.

Isolation of Phosphobacterium (PSB)

Three 0.1 ml aliquots of rhizosphere soil diluted in sterile water (10^{-3} to 10^{-6}) were spread on the standard medium for PSB culture (Wollum, 1982). The PSB medium composition per liter of distilled water is as follows. 10 g sucrose, 5.0g Ca_3 (PO_4), 0.27g NH_4 NO_3, 0.2g KCl, 0.1g $MgSO_4$. $7H_2O$, 0.1g Yeast extract, 1.0mg $MnSO_4.6H_2O$, 1.0mg $FeSO_4.7H_2O$, 0.1g Yeast extract, and 15g agar. The plates were incubated at 27°C for 3–5 days. The colonies in PSB medium that formed a clear zone on the medium were observed. The PSB was identified as *Pseudomonas fluorescens* as shown yellowish green fluorescent colonies appearance under ultra violet light.

Isolation of Frankia

Root nodules were collected from a field grown *C. equisetifolia* tree and surface sterilized with 1% $HgCl_2$. The root nodules were crushed with sterile water and centrifuged in cooling centrifuge @1000 rpm for 5 minutes. Then the supernatant was collected (1 ml) and made up to 100 ml with sterile water and stored in deep freezer at 4°C.

Inoculation of *G. aggregatum* and other microbes

The AM fungal inoculum of *G. aggregatum* along with soil from pot cultures of *S. bicolor* comprising mycorrhizal roots, soil hyphae and spores was used for inoculations. 10 g of inoculum at the rate of 1367 propagules/g of inoculum (Karthikeyan, 1997) was placed 5 cm below the soil surface of each polythene bag of seedlings. Thereafter the seedlings were maintained under nursery conditions for 2 months and watered regularly. 15 ml of inoculum of PSB cultured in nutrient medium broth and 10 ml of root nodule extract of *Frankia* were applied to each seedling of *C. equisetifolia* individually and in combinations with AM fungi. Hence 6 treatments were applied along with a control.

(i) Control
(ii) AM
(iii) PSB
(iv) *Frankia*
(v) AM + *Frankia*
(vi) AM + PSB + *Frankia*

Each treatment replicated at fifteen times and each replicate contained five seedlings arranged in a randomized block design. The seedlings were maintained under green house conditions at 31.6°C (± 2.8), 72% (± 5.2) RH and watered regularly.

Harvest and assessment of seedlings

Sixty days after transplantation five replicates of seedlings were harvested destructively with their entire root system intact. The root length, shoot length, no. of branches and collar diameter of each seedlings were measured.

Survival percentage of trees

Tree survival percentage was calculated by the following method. for each treatment.

$$\% \text{ survival} = \frac{\text{No. of survival trees /plot}}{\text{Total number of planted trees/ plot}} \times 100$$

Transplantation of seedlings

The microbial inoculants inoculated seedlings of *C. equisetifolia* (five replicates) were transplanted in bauxite mine spoils in 40 cm deep pits at an espacement of 1.5 × 1.5 m. The growth and survival of all the seedlings were monitored at regularly at monthly intervals. The data on plant height, collar diameter and no. of cladophylls were collected at different months after planting. To estimate cladophyll biomass the constant weight and uniform length of cladophylls (10g fresh weight; 15cm length) were collected from the transplanted seedlings of *C. equisetifola* of each treatment and oven dried at 70° C for 48 hours.

AM fungal infection assessment

The root samples were collected from the AM fungi inoculated trees of *C. equisetifolia* from reforestation plots bauxite mine spoils and processed for microscopic observation by procedure of Phillips and Hayman (1970). The percent of AM fungal infection was determined by the root slide method of (Read *et al.,* 1976).

Tissue nutrient analysis

Constant weights of (10g) of cladophylls of *C. equisetifolia* treated with beneficial microbial inoculants were collected from the reforestation plot. After drying in a hot air oven at 80ºC for 72 hr the cladophylls were assessed for nutrient contents of N, P and K. The total N was determined on a kjeltec auto analyzer (1030), P determination was done by vanadomolybdate phosphoric yellow colour method and K content was determined by flame photometer (Jackson, 1971).

Statistical analyses

Each measured variable in the nursery and field experiments were subjected to analyses of variance and means were separated using Duncan's Multiple Range Test (SPSS. Ver. 10).

TABLE 1

Growth response of *Casuarina equisetifolia* to inoculations of AM fungi and other beneficial microbes under nursery conditions (mean of 5 replicates).

Sl.No.	Treatments	Root length (cm)	Shoot length (cm)	Collar diameter (mm)	Nodules/ Plant	Branches/ Plant	Root dry(cm) weight (g/plant)	Shoot dry weight (g/plant)
1.	T_1	5.70^{a}	4.20^{a}	0.34^{a}	–	–	0.0026^{a}	0.0076^{a}
2.	T_2	6.80^{b}	4.10^{a}	0.46^{a}	–	–	0.0080^{a}	0.0130^{b}
3.	T_3	7.80^{b}	6.30^{a}	0.46^{a}	1^{a}	–	0.0056^{a}	0.0140^{b}
4.	T_4	8.70^{bc}	7.45^{b}	0.60^{b}	–	–	0.0070^{a}	0.0100^{b}
5.	T_5	11.76^{c}	8.50^{b}	0.70^{b}	2^{a}	–	0.0110^{b}	0.0160^{b}
6.	T_6	16.16^{d}	11.20^{c}	0.83^{c}	1^{a}	1	0.0120^{b}	0.0230^{b}

T_1: Control; T_2: AM; T_3: *Frankia*; T_4: PSB; T_5: AM + *Frankia*; T_6: AM + *Frankia* + PSB
Means followed by same letter are not significantly different at $p < 0.05$ of Duncan's Multiple Range Test.

TABLE 2

Growth performance of *C. equisetifolia* seedlings in oculated with AM fungi and other beneficialmicrobes at bauxite mine spoils (mean of five replicates)

a. Three months after planting:

Sl No.	Treatments	Height (cm)	Collar diameter (mm)	No. of branches/plant	Per cent of AM fungal infection
1.	T_1	41.10[a]	2.85[a]	4.50[a]	–
2.	T_2	44.60[a]	4.04[b]	7.20[a]	75[a]
3.	T_3	55.30[b]	5.13[bc]	7.33[bc]	–
4.	T_4	42.11[a]	4.20[b]	7.11[a]	–
5.	T_5	47.80[a]	4.36[bc]	6.70[a]	76[a]
6.	T_7	58.70[b]	5.65[bc]	10.22[b]	78[a]

b. Two years after planting:

Sl No.	Treatments	Height (cm)	Collar diameter (mm)	No.of branches/plant	Percent of AM fungal infection
1.	T_1	64.80[a]	10.65[a]	12.00[a]	–
2.	T_2	79.20[a]	14.32[b]	16.20[b]	88[a]
3.	T_3	94.60[b]	14.80[b]	15.77[b]	–
4.	T_4	78.30[a]	14.18[b]	17.11[b]	–
5.	T_5	93.77[b]	14.85[b]	15.00[a]	91[a]
6.	T_7	100.60[c]	16.90[c]	18.05[b]	91.5[a]

T_1: Control; T_2: AM; T_3: *Frankia*; T_4: PSB; T_5: AM + *Frankia*; T_6: AM + *Frankia* + PSB
Means followed by same letter are not significantly different at $p < 0.05$ of Duncan's Multiple Range Test.

Results

Nursery experiment

Single and combined microbial inoculants inoculated seedlings were significantly ($p < 0.05$) increased in their growth and bio mass over un inoculated control seedlings. The triple microbial inoculants (AM + PSB + *Frankia*) treated seedlings showed significantly increased growth and biomass, nodule number and collar diameter than control and other treatments (Table 1).

Field experiment

Frankia and AM + PSB + *Frankia* inoculated seedlings were increased in their growth and collar diameter significantly ($p < 0.05$) at the age of three months after planting. Whereas at the age of two years after planting the triple inoculants inoculated seedlings significantly increased ($p < 0.05$) the growth in two folds over control (Table 2a & b).

AM fungal infection

AM fungi inoculated trees showed 75% of infection as confirmed by microscopic observation of AM fungal structures (Vesicles, arbuscules and hyphae). AM fungi with other microbial inoculants showed 76 and 78% of infection in three months after planting. Two years after planting the *C. equisetifolia* tree showed increased AM fungal infection ranged from 88–91.5% (Table 2a & b).

Survival performance

The individual microbial inoculants inoculated seedlings showed 90–95% survival at bauxite mine spoils. The triple inoculants inoculated seedlings showed 100 % survival and the un inoculated seedlings showed 35% survival at bauxite mine spoils (Figure 1).

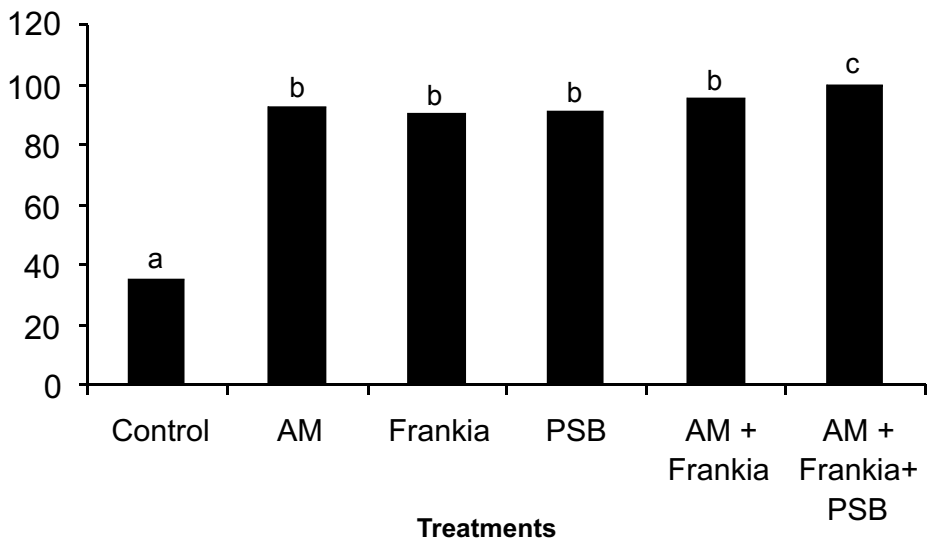

Figure 1. Per cent survival of *C. equisetifolia* at bauxite mine spoils (2 years after planting)

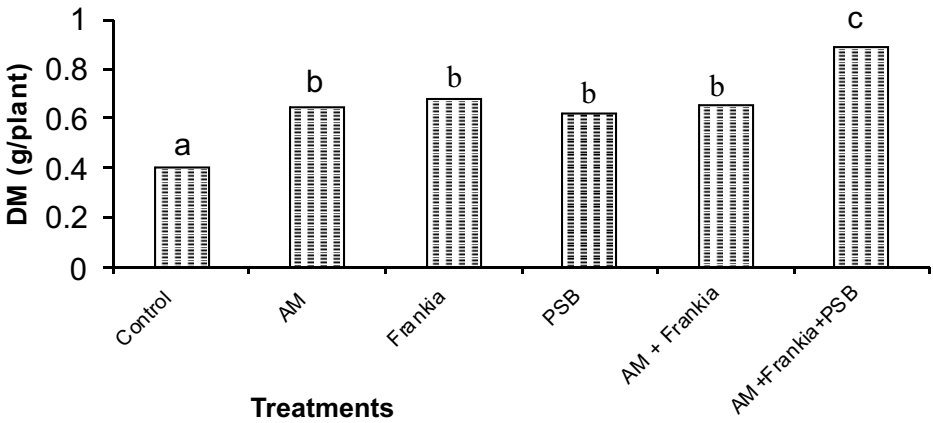

Figure 2. Cladophyll Biomass of *C. equisetifolia* at bauxite mine spoils (2 years after planting)

Values of Bars (mean of 5 replicates) having same letter od not differ significantly (Duncan's Multiple Range Test, $p < 0.05$).

Cladophyll biomass

Significantly increased leaf biomass showed in AM, *Frankia,* PSB, AM + *Frankia* and AM + *Frankia* + PSB inoculated plants over control (Figure 2).

Nutrient status in Cladophylls

Significantly increased N, P and K content were showed in AM + *Frankia* and AM + *Frankia* + PSB inoculated trees than control and individual inoculated treatments (Figure 3).

DISCUSSION

Among microbial inoculants 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. PSB are also immensely important, as they have been reported to increase uptake of P by converting insoluble from soluble ones. *Frankia* is the genus of N_2 fixing actinomycetes that are capable of infecting and nodulating a group of eight families of woody plants including *C. equisetifolia.* These microbial inoculants are used in many studies for growth enhancement of *C. equisetifolia* (Wheeler, *et al.,* 2000; Vasanthakrishna, *et al*., 1995). In the present study these microbial inoculants were used for *C. equisetifolia* for reforestation in bauxite mine spoils.

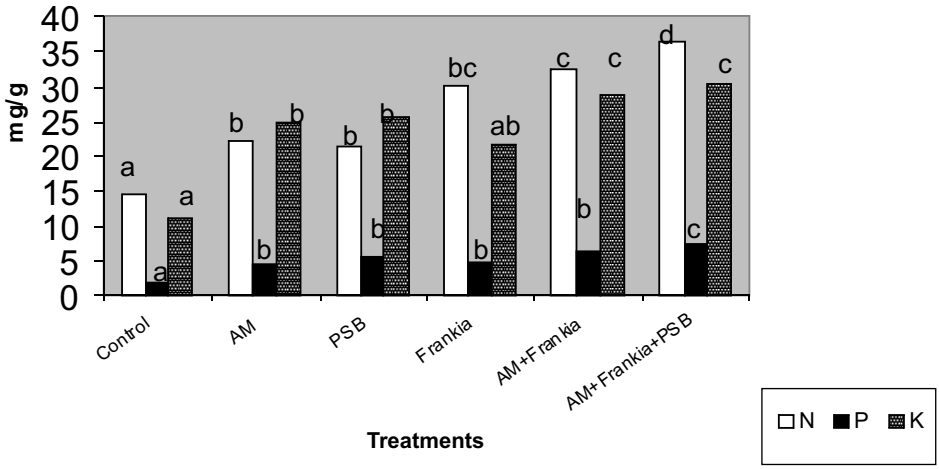

Figure 3. Nutrient status of *Casuarina equisetifolia*cladophylis inoculated with AM fungi, PSB and *Frankia* in field conditions.

Values of Bars (mean of 5 replicates) having same letter do not differ significantly (Duncan's Multiple Range Test, $p < 0.05$).

The physico–chemical properties of the soil collected from bauxite mine spoils showed that the soil is acidic and poor in major nutrients. However these soils were used as potting media for *C. equisetifolia* seedlings in the nursery experiments with microbial inoculants of AM fungi, PSB and *Frankia*. The seedlings inoculated with these microbial inoculants showed better performance than the uninoculated control plants. Though the nutrient status poor in soil the inoculated microbial inoculants mobilized the available nutrients to the seedlings and helped to increase the uptake of number of ions such as K^+ and NH_4^+ (Bowen, 1980). Several earlier studies have also shown that growth, nitrogen and phosphorus accumulation in seedlings were enhanced by inoculation with effective microbial symbionts in *C. equisetifolia* (Sampavalan, *et al.,* 1996; Subba Rao and Rodriquez Barrueco, 1995). PSB are also known to play a major role in the solubilization of unavailable forms of soil P and the uptake of its native and applied forms (Sperber, 1998). They produce organic acids, phosphatases, chelating compounds and mineral acids to play a vital role in P solubilization (Singh *et al.,* 1989).

AM + PSB + *Frankia* inoculated seedlings showed significantly improved the growth and biomass of *C. equisetifolia* seedlings in the present study. Muthukumar *et al*., (2001) found similar results in *Azadirachta indica* at nursery conditions. The fact is combined inoculation always promotes the plant growth in multiple ways such as P and N uptake. The flow of carbon from shoot to root may be increased by AM infection in accord with Muthukumar and Udaiyan (2000) that may alter the carbon availability for microbial inoculants.

Further, multiple inoculations AM + PSB + *Frankia* stimulated better activity of plants and these microbial inoculants interacted with each other to improve soil nutrients (Rajendran and Devaraj, 2004). This is the way the beneficial microbes improving the bioavailability of major nutrients.

In the present study of field experiments at bauxite mine spoils the growth and survival performance of AM, PSB and *Frankia* inoculated *C. equisetifolia* seedlings were significantly higher than uninoculated controls. Similar results found in field inoculation of *Prosopis juliflora* with AM and *Rhizobium* sp. in a semi arid wasteland significantly increased plant biomass and soil nutrient after six years of growth (Bhatia, *et al*., 1998). In earlier studies also the growth of *Alnus cordata* inoculated with AM + *Frankia* was significantly improved in mine spoils one year after planting (Lumini, *et al.,* 1994). In the present study the inoculation with AM + *Frankia* increased the growth and survival rate at bauxite mine spoils due to the nutrient uptake of N and P through bio fertilizers.

Few earlier studies showed that colonization by AM fungi enhances plant survival and growth by decreasing phosphorus deficiency (Joner and Leyval, 2001) water stress (Sanchez-Dinz and Honrubia, 1994), improving membrane infectivity (Graham *et al*., 1981) or by stimulation of oxidative enzyme produce (Salzer *et al*., 1999). Similarly, in a recent study PSB strain had significantly increased the plant biomass and total P in winter wheat under both and field conditions (Chen *et al*., 2008). This is the reason the AM inoculated seedlings either singly or with PSB and *Frankia* showed the survival rate at the average of 90%. Similarly, Karthikeyan and Surya Prakash (2008) found 95% survival in planted *Eucalyptus camaldulensis* at bauxite mine spoils pre inoculated with AM fungi, *Azospirillum* sp. and PSB. The significant growth enhancement of *C. equisetifolia* with AM + *Frankia* or AM + PSB + *Frankia* is may be due to increased population of nitrogen fixation, phosphorus mineralization and AM colonization. These effects improve nutrient utilization efficiency of planted seedlings that gives increases plant survival at bauxite mine spoils.

CONCLUSION

The results from this study supports the general conclusion that introduction of plants to mine spoils with microbial inoculants is a beneficial biotechnological tool to aid the recovery of degraded eco systems. The AM fungi and the other beneficial microbes have the potential to increase the efficiency of shoot and root system in providing the seedlings with essential levels of P and N for growth. In this study, a portion of degraded land was successfully reforestated due to application of microbial inoculants with *C. equisetifolia*. The survival percent of planted *C. equisetifolia* trees with beneficial microbes was also significantly increased (90%) over the control. The results of this experiment assesses the role of beneficial microbes in enhancing plant survival on the degraded lands to increase forest productivity.

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REFERENCES

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

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

Bowen G.D. 1980. *Mycorrhizal roles in tropical plants and ecosystems* In: Mikola P. (ed) Tropical mycorrhizal Research. Oxford University Press, Oxford, pp. 148–179.

Chen Z., Ma S. and Liu L. 2008. Studies on phosphorus solubilizing activity of a strain of phosphobacteria isolated from chestnut type soil in China. *Bioresource Technology* **99**: 6702–6707.

Diem H.G. and Dommergues Y.R 1990. Current and potential uses and management of Casuarinaceae in the tropics and sub tropics. In: Schwintzer C.R. Trepkana J.D. (eds) The history of *Frankia* and actinorhizal plants San Diego, Academic press, pp. 317–342.

Dupponnois R., Plenchette C., Prin Y., Ducousso M., Kisa M., Ba A.M, Gallina A. 2007. Use of mycorrhizal inoculation to improve reafforestation process with Australian Acacia in sahelian eco zones. *Ecological Engineering* **29**: 105–112.

Gerdemann J.W. and 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. and Menge J.A. 1981. Membrane–mediated decrease in root exudation responsible for Phosphorus inhibition of vesicular arbuscular mycorrhiza formation. *Plant Physiology* **68:** 549–552.

Jackson M.L 1973. *Soil Chemical Analysis*. Prentice Hall, New Delhi.

Jasper D.A., Sarwasa Y., Gaunt E., Ward S.C 1998. Indicators of reclamation success – recovery patterns of soil biological activity compared to remote sensing of vegetation In: *Land reclamation: Achieving sustainable benefits* H.R. Fox, H.M. Moore and A.D McIntosh (eds). Proceedings of the fourth international conference of the international application of land reclamations Nottingham, UK 7–11 September 1998. Rotherdam, Balkema, pp. 21–24.

Jeffries P., Gianinazzi S., Perotto S., Turnau K, 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.

Joner E.J. and Leyval C. 2001 Influence of arbuscular mycorrhizae on clones and ryegrass grown together in a soil spiked with polycylic armotic hydrocarbons. *Mycorrhiza* **10**: 155–159.

Karthikeyan A. 1997. Studies on Arbusucular mycorrhizal fungi associated with *Camellia sinensis* (L.) O. Kuntze and co-existing shade plants. Ph. D. Thesis, Bharathiar University, Coimbatore, India

Karthikeyan A. 2003. Reclamation of mine spoils using Arbuscular mycorrhizal fungi: A socio – environment friendly method. *South Asian Journal of socio political studies* **12**: 110–114.

Karthikeyan A. and Surya Prakash M. 2008. Effects of arbuscular mycorrhizal fungi, Phosphobacterium and *Azospirillum* sp on the successful establishment of *Eucalyptus camaldulensis* Dehn. In bauxite mine spoils. *Forests Trees and Livelihoods* **18**: 183–191.

Lumini E., Bosco M., Puppi G., Isopi R., Fralergrani M., Bureti E. and Faviki F. 1994. Field performance of *Alnus cordata* Loisel (Italian alba) inoculated with *Frankia* and VA mycorrhizal strains in mine spoil afforestation plots. *Soil Biology and Biochemistry* **26**: 659–661.

Miller R.M. and Cameron R.E. 1976. Some effects in soil microbiota of top soil storage during surface mining In: *Transactions of the 4th symposium on surface mining and reclamation,* pp 131–139. Washington D.C., National Coal Association

Muthukumar T. and Udaiyan K. 2000. Influence of organic manures on arbuscular mycorrhizal fungi associated with *Vigna unguigulata* (L.) Walp. in relation to tissue nutrients and soluble carbohydrates in roots under field conditions. *Biology and Fertility of Soils* **31**: 114–120.

Muthukumar T., Udaiyan K. and Rajeshkannan V. 2001. Response of Neem (*Azadirachta indica* A. juss) to indigenous arbuscular mycorrhizal fungi, phosphate solubilizing and asymbiotic nitrogen fixing bacteria under tropical nursery conditions. *Biology and Fertility of Soils* **34**: 417–426

Phillips J.M, and Hayman D.S 1970 Improved procedures for clearing roots and staining parasitic VAM fungi for rapid assessment of infection. *Transactions of British Mycological Society* **55**: 158–161.

Rajendran K. and Devaraj P. 2004. Biomass and nutrient distribution and their return of *Casuarina equisetifolia* inoculated with bio fertilizers in farmland. *Biomass and Bioenergy* **26**: 235–249

Read D.J., Kouchei H.K. and Hodgson J. 1976. Vesicular arbsucular mycorrhiza in natural vegetation systems. *New Phytologist* **77**: 641–653.

Salzer P., Corberer H. and Boller T. 1999. Hydrogen peroxide accumulation in *Medicago truncatula* roots colonized by the arbuscular mycorrhiza forming fungus *Glomus intraradices*. *Planta* **208:** 319–325

Sampavalan J., Wheeler C.T. and Narayanan R. 1996. The isolation and characterization of *Frankia* from nodules of *Casuarina equisetifolia* from Tamilnadu. *Indian Journal of Microbiology* **36**: 149–151

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

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

Singh J.S., Raghabanshi A.S., Singh R.S. and Srivastava S.C. 1989. Microbial bio mass acts as a source of plant nutrients in dry tropical forest and savanna. *Nature* **338:** 499–500.

Sperber J.I. 1998. The incidences of apatite solubilizing organisms in the rhizosphere and soil. *Australian Journal of Agricultural Research* **9**: 778–781.

Subba Rao N.S.and Rodruquez Barrueco C. 1995. *Casuarinas*. Oxford & IBH. New Delhi.

Vasanthakrishna M., Jospeh D., Bagyaraj D.J. and Nirmalnath J.P 1995. Selection of efficient VA mycorrhizal fungi for *Casuarina equisetifolia* – second screening. *New Forests* **9**: 157–167

Wheeler C.T., Tilak M., Srinngeour C.M., Hooker J.E. and Hardley l.L. 2000. Effects of symbiosis with *Frankia* and arbuscular mycorrhizal fungus with natural abundance of 15^N in four species of Casuraina. *Journal of Experimental Botany* **51**: 287–297

Wollum A.G. (1982) Cultured methods for soil microorganisms. In: Page A.L. Miller and R.H. Kucey D.R. (eds), (Methods of soil analysis Part 2. Chemical and microbiological properties). *American Society of Agronomy*, Medison, pp. 781–802.