Global Journal of Environmental Research 4 (3): 192-198, 2010
ISSN 1990-925X

Performance of Native Arbuscular Mycorrhizal Plant Species and Soil Amendments in Revegetation of Coalmine Overburden

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Abstract: Growth of a few native mycorrhizal plant species was studied in a field experiment along with application of arbuscular mycorrhizal fungi (AMF) inoculum and other amendments in a 5-year old coalmine overburden dump (OBD) of Tikak Colliery, Margherita, Assam during 1999-2001. Seeds mixture of three leguminous species (*Mimosa pudica*, *Crotalaria striata* and *Cassia seamea*), a native shrubs species (*Melastoma malabathricum*), a herb (*Ageratum conyzoides*) and a grass species (*Axonopus compressus*) in micro-plots in randomized block designed (RBD). Application of lime improved the percent AM colonization in Colliery spoils in the field experiment and Lime+ AM + FYM treatment percent colonization was poor in comparison to other treatments. However, exhibited highest in Lime+ AM + FYM treatment and lest in topsoil 10-year OBD amended treatment. Soil attributes such as pH, total nitrogen and available phosphorus levels of the spoils increased over initial values under all treatments. Biomass production of these plant species mixture was enhanced due to application of Lime + AMF + FYM compared to the biomass production in control plot and was the best among different treatments applied. Amendments of 5-year OBD field with topsoil from 10-year OBD and natural forest were also effective in enhancing plant growth. Thus, this result identified two important treatments i.e. combination of Lime, AMF and FYM and topsoil from either 10-year or natural forest for enhancing vegetation cover in 5-year OBD spoil.

Key words: Arbuscular mycorrhizal plant · Soil amendment · Coalmine overburden dump (OBD) · Revegetation

INTRODUCTION

Opencast coalmining is a major problem of present days, which creates hectares barren overburden dumps (OBDs) and needs to revegetate for eco-restoration. The aim of ecosystem restoration is to return degraded biological communities to their original state [1]. Considerable research has been published showing that the success of restoration directly depends on the establishment of mycorrhizas. Reestablishment of arbuscular mycorrhizal fungi (AMF) in degraded area where indigenous populations have been reduced by mining activities or other disturbances may be critical to plant community restoration [2,3,4,5]. Almost any ecosystem perturbation to reduce the mycorrhizal population of disturbed land depends on the restoration of the mycorrhizal symbiosis [6]. The rate of restoration or plant succession may enhance by manipulation of the mycorrhizal fungus population or inoculation programmes [7-9]. The selection more efficient strains of AMF and inoculation with plants may improve plant growth [10-15,4]. Many environmentalist of the present era have given importance in rehabilitation of degraded lands with native plant species to regenerate plant communities that found before the disturbance to sustain the ecosystem [16-19]. Contribution of arbuscular mycorrhizal inoculation alone or with other soil beneficial microorganisms and substrate amendment for rehabilitation of mining sites has also been studied well in a series of pot-experiments and in fields in India and abroad [20,13,21,22]. However, no work has been done with native plant species in the coalmine overburden dumps (OBDs) of Assam, India. Performance of different treatments and amendments that recorded in pot-experiments are necessarily importance for examine under field condition for the success of large-scale revegetation programme. Therefore,

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this field trial experiment attempts to evaluate suitability of the use of the AM fungi adopted relationships with native plant species in a 5-year old OBD after dumping for utilizing in different coalmine OBDs of Assam.

MATERIALS AND METHODS

Study Site: A 5-year coalmine overburden dump with hostile environment for plant growth was chosen for the field experiment. The site was located at about 800m from the adjacent natural forest. There was no initial vegetation cover on the OBD, almost flat on the north edge of the Colliery road. Towards southern side of the plots, there was the 1-year OBD located and thereafter continued the coal exploration seam and natural forest of Patkai Range. Eastern side of the plot was the 1-year OBD. On the west the plot there was the Colliery road.

Experimental Deign: The experiment was established in May 1999. The total area of the experimental plot was 0.05 ha. Of which, about 277.50m^2 area was used for the treatment plots. The experiment was plotted in randomized block design (RBD). Twenty (20) small blocks (micro plots) of 2.5 x 2.5m^2 area which was spaced by a buffer zone of 1.0 m between a plot. There were five (5) treatments: T_1.= Control (Without AM fungi), T_2. = Topsoil of natural forest, T_3. = Topsoil of 10-year OBD, T_4. = Lime + AMF and T_5. = Lime + AMF+ FYM. Each treatment had four (4) replica plots.

Experimental Plants: Seeds of three leguminous plant such as *Mimusa pudica*, *Crotalaria striata* and *Casia siamea* And A shrub of the locality *Melastoma malabathricum* and a herb *Ageratum conyzoides* and a grass species (*Axonopus compressus*) were used to sow in the plots under different treatment combinations. All the plant species used for the trial were arbuscular mycorrhizal. Plant species were selected based on performance of two-conjucutive pot culture experiments on germination, growth, survival and biomass production, mycorrhization, and nodulation responses in shed house condition [13].

Application of AMF Inoculum and Amendments: Freshly collected topsoils from revegetated 10-yr OBD and natural forest were collected in bulk and used to spread over the respective plots. The thickness of the topsoil cover was maintained at approximately 1 inch on the surface. This topsoil contained AMF inoculum and other microbial agents. For other treatments AMF inoculum was obtained from 3 kg topsoil collected from 10-year OBD by wet sieving and decanting method [23]. Approximately 13,600 numbers of AMF spores were added in 6.25m^2 area. These spores were suspended in water, distributed on the 6.25m^2 plots and mixed by hoeing. Agricultural lime was added @ 215g /m^2 area. Farmyard manure (FYM) was added @ 20 ton ha^{-1}. The experiment was conducted during May'99-June'2001. To prevent washing and removal of seeds and topsoil layer due to rain a thin layer of straw of *Saccharum spontaneum* was spread over the plots.

Record of Plant Biomass and AMF Colonization: Plant samples were collected at random after 1 year of seed sowing from the respective treatments plots by uprooting from an area of 1ft^2. Plants were uprooted carefully from the area within the frame, washed to clean adhered materials, and kept separately in polythene packets for plant biomass and AMF infection in roots. Plant biomass recorded for each replica of the treatments after samples were oven dried by weight of roots and shoots separately in an electronic balance.

Roots were processed and examined for determination of AMF colonization. Dried roots of the samples were cut into 1 cm pieces, soaked in tap water, and kept in a 10 per cent KOH solution for 24 h. It was then washed in tap water for several times and bleached with 10 per cent H_2O_2, slightly acidified with 0.01N HCl at room temperature and stained in 0.05 per cent Trypan blue in lecto-glycerol [3,24]. After 24 h the roots were transferred to 50 per cent glycerol and kept until per cent AMF colonization determined. Stained root segments were examined under a compound microscope (Leitz- Laborlux 11 POL compound microscope, (100x) for AMF structures (vesicles, arbuscules, hyphae etc.) and hundred such root segments were examined for per cent AMF colonization. Per cent AMF colonization was quantified by gridline intersect method [25].

Record of Soil Sample for AMF Spore and Soil Analysis: Soil samples were collected from top 15 cms of the soil surface of each replicate plots under different treatments at the time of harvest. AMF spores were isolated from soil by wet sieving and decanting technique [23] and number were counted under stereomicroscopes. Spore numbers were expressed on dry wet basis of OBD spoils.

Soil samples were air-dried and sieved through 2 mm sieve. Soil pH was determined by following standard methods (1:2; soil: water). Total nitrogen (TN) of the soil samples were determined by regular Kjeldahl method [26] available phosphorus by Bray's-1 method [27] and Bray's-I method [28] available K by ammonium acetate extraction and flame photometric determination method as described in [29]. Effect of different treatment combinations on restoration and improvement of soil quality was evaluated by comparing values of pH, available phosphorus (P) kg ha^{-1}, total nitrogen (TN) kg ha^{-1} and available potassium (K) kg ha^{-1}.

Statistical Analyses: ANOVA was analyzed for shoot and root dry biomass, AMF spore number 100^{-1}g soils, root-length colonization by AMF, root nodule numbers, soil pH and available P of different treatment of the experiment. A correlation between root biomass and root length infection of two plant species due to AMF inoculation in different OBD spoils during pot culture was also studied. Single factor ANOVA, standard deviation and correlation were analyzed by using statistical package of Window'2000, MS Office-Excel. Least significant different (LSD) of the data were calculated at 5 per cent level.

RESULTS

Effect of Treatment and Amendment on Percent AM Colonization and AM Sporulation: Significant treatment effect was analyzed for AM fungal colonization in roots of mixed plant species in micro-plots of the field experiment (Table 1). The roots of the plants sampled for different treatments attained AMF colonization levels to the extent of 44 to 63 per cent. Highest AM colonization was recorded in roots of plant species of Lime + AM treatment (63%) followed by treatment with Topsoil of 10-year OBD (53%), Topsoil NF (45%), Lime + AM + FYM (44%) and very interestingly in control treatment no AM fungal infection was detected in root sampled studied. It was also recorded that application of lime improved the percent AM colonization in Colliery spoils in the field experiment (Table 1).

AM spore production in micro-plots soil of different treatments was studied for ANOVA and result was found statistically significant (Table 1.). Average data of AM spore isolated from 100g soils of different treatments exhibited highest in Lime + AM + FYM treatment 209 (100^{-1}g soil) and least in topsoil 10-year OBD amendment treatment 84 (100^{-1}g soil). AM fungi sporulation in Topsoil NF amendment treatment 115(100^{+} g soil) was comparatively more superior than in Topsoil 10-year OBD amended one 84 (100^{-1}g soil). Application of Lime may improve more AM spore production, which was recorded better in lime amendment treatments over the topsoil amendment treatments. There was apparent relationship between percent AMF colonization and AMF spores.

Effect of Treatment and Amendment on Plant Dry- Biomass Production: Different treatments in the field experiment had variation in plant biomass production that was statistically significant (Table 2). The highest biomass production attributed in Lime + AM+ FYM treatment plots (88.60 g/ ft^2 area) with 80.02% treatment contribution over the control and least in control plots (17.69g. / ft^2 area). However, Lime + AM treatment with a root shoot ratio about 1:208 produced highest root biomass. However, root shoot ratio in Lime + AM + FYM treatment had 1: 3.83. Topsoil amendment from revegetated 10-year OBD was superior in biomass production about 2.7 folds more with an 73.2% treatment contribution over control than that of topsoil natural forest amended treatment. Application of lime with AM fungi performed better in biomass production over the rest and was equivalent to topsoil 10-year OBD amended treatment. It was also revealed that application of 20 ton ha^{-1} farm yard manure (FYM) can improve about 10.32% in biomass production while compared to the biomass of lime + AM treatment plots (Table 2).

Among different treatments, amendments of the 5-yr OBD in field with Lime + AMF + FYM produced most striking result in above ground biomass production of the mixed plant species (Table 2.). The amount of above ground dry biomass under Lime + AMF+ FYM treatment was highest (70.23g/ft^2) followed by that of Lime + AMF (38.4g/ ft^2) and amendment with natural forest Topsoil (49.50g/ft^2). Topsoil amendment was as effective as lime + AMF or Lime +AMF + FYM treatment combinations in stimulating root growth (Table 2.). FYM treatment was a very important amendment in increasing biomass production (Table 2).

Effect of Treatment and Amendment on Physicochemical Properties: Effect of different treatment and amendment in restoration and improvement of soil quality was studied (Table 3). pH of the experimental plot before treatment was recoded as 3.52 and at the time of harvest it was reduced to 3.37 which was recoded from control micro plots. Even after 1 year of applying lime pH values showed almost consistent results only reducing slightly the value from 6.5 to 6.13or 6.16 (Table 3). Topsoil amended treatments were poor in improvement of pH than lime applied treatments. Available phosphorus (P) of the site was 7.84 kg ha^{-1} before treatments. In control plots and topsoil NF treatment plots, P availability was recoded reduce than before treatment level. Addition of lime with AM fungi could enhance the availability of P in spoils of the OBD. In Lime+ AM+ FYM treatment had an improvement of available P status about 1 fold to that of available P of the site before treatment. Total nitrogen content of the spoil materials was found to increase due to treatments. Even in the control plots total nitrogen content was found to increase about 103.5 kg ha^{-1}over the amount of total nitrogen content recorded before treatment of the plot. In the context of lime application with AM there was evident about 3 folds increase in TN content. Contribution of FYM applied @ 20 t ha^{-1} over lime and AM treatment was about 66 kg ha^{-1} to the TN content. Single factor ANOVA showed the treatments of the experiments were statistically significant for total nitrogen content. Available K of the spoil materials was also increased significantly due to treatment effect (Table 3). In control plots available K was 52.05 kg ha^{-1} at the time of harvest the experiment. Lime+ AM treatments were more superior in improving available K status over topsoil-amended treatments. It was also observed that FYM application improve about 5.67 kg ha^{-1} available K over Lime + AM treatment.

Table 1: AMF colonization in roots and AMF spore in rhizophere soils under different treatment combination of mixed plants grown in 5-year OBD spoils in field experiment at hervest

Treatment	Per cent AMF colonization	AMF spores /100g soil
1.Control	0.0	0.0
2.Topsoil NF	45.0	115.0
3.Topsoil 10 -yr OBD	53.0	84.0
4.Lime+ AMF	63.0	143.0
5.Lime+ AMF+FYM	44.0	209.0
SEm	± 3.7	±32.5
LSD (*P*=0.05)	11.1	97.9

DISCUSSION

Reclamation and revegatation success that measured by improvement in soil physicochemical properties and plant biomass production was largely determined by the amendment made by farmyard manure (FYM), lime and AM fungal inoculum. Consistency of the result of this treatment combination with legume plant also found to support the findings of two other pot culture experiments in 1- and 5-year OBD spoils [20,13]. Therefore, this treatments combination with native leguminous and other plant species may have successful impact for revegetation practices. The experiment reveals that freshly collected topsoil (not stockpiles) amendment may be viable alternative in restoration of biological activities including establishment of vegetations. Stockpiling topsoil for 1 to 3 years will decrease AM fungal inoculum to less than 10% of its original density; longer storage can reduce survival to near zero [30]. Accordingly, application of AM fungi inoculum may still be necessary even after stockpiles natural topsoil is used. Moreover, freshly collected topsoil contains some beneficial microorganisms such as fluoresent pseudomonads, other bacteria and fungi in addition to AM fungi, which had positive impact in better plant biomass production [20]. Also advocated the application of topsoil is essential for re-establishment of mycorrhizas [31] as topsoil represents the primary source of mycorrhizal inoculum. Similar observation reported [32] that topsoil addition can promote both the development of mycorrhizas and vegetation. Without topsoil, the development of mycorrhizae on spoils or tailings can be quite slow [31] and that can be boost up by application of AM fungi inoculum and lime which was quite evident particularly in lignite coalmine area of Assam, India. Application of lime was found to beneficial which enhanced mycorrhizal status of Colliery spoils and plant biomass production. Nurlaney *et al.* [33] also observed that application of lime increased shoot-dry

Table 2: Effect of different amendments on biomass production of mixed plants grown in 5 -year OBD in field experiment at Tikak Colliery, Margherita

Treatment	Plant dry Biomass (g/ ft^2area) Root	Shoot	Total	Per cent increase in total biomass over control
1.Control	3.8	13.9	17.7	0.0
2.Topsoil NF	6.2	18.4	24.6	28.1
3.Topsoil 10 -yr OBD	16.6	49.5	66.0	73.2
4.Lime + AMF	18.4	38.4	58.4	69.7
5.Lime + AMF+FYM	18.3	70.2	88.6	80.0
SEm	-	-	±0.85	
LSD (*P*=0.05)	-	-	2.57	

Table 3: Changes in chemical properties of 5-yr OBD spoils due to growth of plant introduction under different treatment combinations at harvest in field experiment

Treatments	pH	Total N (kg ha^{-1})	Available P (kg ha^{-1})	Available K (kg ha^{-1})
Control	3.37	413.5	5.19	52.07
Topsoil NF	4.36	445.0	6.05	116.53
Topsoil 10-yr OBD	5.11	678.0	8.12	162.66
Lime+ AMF	6.13	939.0	11.05	169.43
Lime+ AMF+ FYM	6.16	1005.0	14.88	175.10
Sem	±0.12	±107.1	±1.035	±6.71
LSD (*P=0.05*)	0.35	322.8	3.12	9.498

The initial values of pH (3.52), total N (310 kg ha^{-1}), available P (7.84 kg ha^{-1}) and available K (59.72 kg ha^{-1})

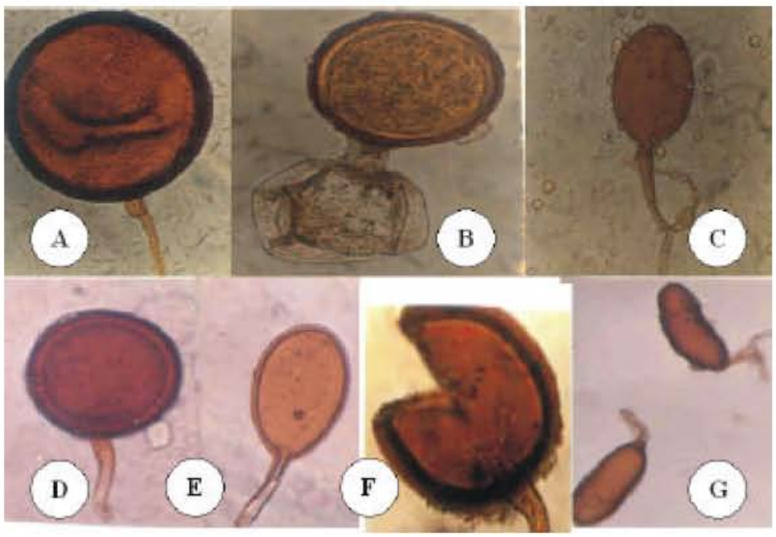

Fig. 1: Photomicrographs of arbuscular mycorrhizal fungi (AMF) inoculum for field- experiment isolated from revegetated 10-year-old OBD of Tikak Colliery, Margherita (A) *Gigasopra sp.*, (B) *Entrophospora sp.*, (C) to (G) *Glomus spp.*

weight, total root-length and AMF colonization in maize and soybean grown in two tropical acid soils. Jordan and McGraw [34] amended organic matter in absence of topsoil to enhance the mycorrhiza formation. Norland *et al.* [35] successfully increased plant cover and recorded better crop biomass by applying locally available sources of organic matter such as compost, yard waste and municipal waste. They also reported that these amendments even improved soil reaction, available nitrate, ammonia, phosphate as well as cation exchange capacity (CEC) of mine spoils. There is a role of legume plant species in enhancement of nitrogen in Colliery spoils. Amount of total nitrogen found to be increase in all the treatment even in control also might be because of nodulation of rhizobium in native legume plant species that were used for revegatation. Similar findings had also described by [24] in open-cut coal mining at Collie in southwest Western Australia and to recover desertified Mediterranean ecosystems [36].

It was also seen that AM fungi alone could support plant growth to some extant and allow native plant species to invade naturally on the OBDs [20]. Allen [6] described the mycorrhizal symbiosis as a part of the restoration of disturbed land. The rate of restoration or plant succession may be hastened by manipulation of mycorrhizal fungal population or inoculation programmes [19,7]. The spore pellet technology for establishment of direct seeded grasses, flowers, forbs and shrubs and the technology to custom-inoculate seedlings of native trees and shrubs in nurseries are now operational for mined land revegetation Although, plant species that seed were broadcasted colonized well in AM added treatments and speculated that Lime + AM + FYM also support best in increasing native biodiversity. But an one/two year is not enough to conclude that treatment combination may provide further support in increase above ground biodiversity of the OBD more sufficiently. The necessity to monitor the soil micro biota, including mycorrhizal fungi and their activities for the success of strategies to restore or reclaim degraded land was well emphasized [37,38]. Therefore, further extensive and long-term programmes of revegetation trial in OBDs should be taken up to have a meaningful package of practice for revegatation with these findings and earn more authenticity to boost up and strengthen scientific knowledge of the nature to protect her more carefully. However, the results of field experiment again indicated that plant growth could be achieved in the harsh environment of 5-year coalmine OBD by using lime, AMF and topsoil as amendment.

ACKNOWLEDGEMENT

Authors are thankful to the North Eastern Coalfields, Margherita; Rain Forest Research Institute, Jorhat; Soil Microbiology Laboratory, Assam Agricultural University, Jorhat and FRI University, DehraDun, Uttarakhand for timely permission and facilities provided to complete this study.

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