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ORIGINAL ARTICLE

Reclamation of coalmine overburden dump through environmental friendly method

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Abstract Coal mine spoils (-the previous overburden of coal seams, inevitable by-product in the mining process) which are usually unfavorable for plant growth have different properties according to dumping years. The reclamation of overburden dumps (OBDs) through plantation by using efficient microbes with suitable bio-inoculants is an environmental friendly microbial technique for significant improvement in fertility status and biological activities of the OBD soil. A systematic greenhouse pot experiment program followed by field trial was conducted to investigate the influence of *arbuscular mycorrhizal* (AM) and NFB on the performance of plant growth which have resulted in the development of environmental friendly bio-inoculant package for soil reclamation of abandoned mine land by revegetation.

1. Introduction

Coal (-the most abundant fossil fuel resource) provides around 30% of global primary energy needs, generates 41% of the world's electricity and is used in the production of 70% of the world's steel (http://www.worldcoal.org/). During opencast mining, the overlying soil is removed and the fragmented rock is heaped in the form of overburden dumps (Ghosh, 2002). These dump materials are left over the land, occupy a large amount of land, which loses its original use and generally gets soil qualities degraded (Barapanda et al., 2001). As the dump materials are generally loose, fine particles from it become highly prone to blowing by wind. These get spread over the surrounding fertile land plants; disturb their natural quality, and growth of fresh leaves. It has been found that overburden dump top materials are usually deficient in major nutrients. Hence, most of the OBD do not support plantation. The physic-chemical properties of OBD materials are site specific and differ from one dump to another due to different geological deposits of rocks (Lovesan et al., 1998). Thus, the opencast coal mining particularly releases a huge amount of mining wastes to the upper part of the land surface as OBD materials which raises a number of environmental challenges, including soil erosion, dust, water pollution, loss of nutrient qualities and microbial activities of the soil system, and ultimately impacts on local floral diversity.

The soil reclamation of abandoned mine has been focused in several studies (see Sheoran et al., 2010) since, the

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physic-chemical properties of OBD materials are site specific (Lovesan et al., 1998). In the present study, the reclamation of coalmine OBD was undertaken through environmental friendly method in order to select the efficient photosynthetic and soil conserver plant species suitable for growing on OBD with the ability to grow on poor and dry soils and develop the vegetation cover in a short time and to accumulate biomass rapidly. Further, the plant should have ability to bind soil for arresting soil/water erosion and checking nutrient loss/water runoff, and improvement of the soil organic matter status and soil microbial biomass, thereby enhancing the supply of available nutrients for plants.

2. Materials and methods

The study site (ECL, Coal field, Lalmatia, Jharkhand, India) was surveyed in order to collect the data on general topography, meteorological data, enumeration and documentation of native vegetation, and quantitative structures (density and frequency) of plant community in core zone. 5, 10 and 15 year (yrs) old OBDs were selected. The soil samples along with fine feeder roots were collected for assessment of physico-chemical characteristics (i.e. pH of OBD soil, water holding capacity, bulk density, porosity, soil texture, available minerals (phosphorous, potassium, nitrogen), organic carbon, and rhizosphere microflora (VAM spore population, VAM root colonization, estimation of N_{2-} fixing bacteria i.e. isolation of Rhizobia from root nodules, isolation of *Azospirillum*, and estimation of phosphate solubilizing bacteria). VAM spores were isolated by wet sieving and decanting techniques (Gerdemann and Nicolson, 1963), the spores were filtered in a line grid marked filter paper. The number of spores per 5 g soil sample was identified following the key manual (Schenck and Perez, 1987). Maize plants were used as a host for the mass multiplication of bio-inoculants (i.e. VAM fungi, *Rhizobium* and *Azospirillum*).

The collected OBD soil was sterilized. A total number of 10 plant species viz. *Acacia mangium*, *Acacia cracicarpa*, *Cassia siamia*, *Dendrocalamus strictus*, *Dalbergia sissoo*, *G. sepium*, *Pterocarpus santalinum*, *Sesbania grandiflora*, *Stylo hamata* and *Stylo scabra* were selected for the evaluation of growth performance in pot and filed condition. The seeds were also surface sterilized by immersing them into 1% $HgCl_2$ solution for 1 h and were subsequently washed with sterilized tap water followed with sterilized distilled water for 5–6 times. The seeds were then allowed to germinate on to sterilized OBD soil. The surface disinfectant seeds were left in the germination chamber for 36 h maintaining optimum temperature and humidity for their sprouting. The very next day after the emergence of radicle and plumule, saplings were transferred to the polybags (22 × 30 cm) containing admixture of sterilized OBD soil and vermicompost along with 10 g VAM infected chopped root bits or N_2FB carrier medium separately or in combination. A total number of nine different treatments including control {T_1 – OBD soil (Control), T_2 – OBD soil (1 KG) and vermicompost (1 KG), T_3 – OBD soil (2 KG) and vermicompost (1 KG), T_4 – OBD soil (1 KG) and vermicompost (1 KG) + VAM, T_5 – OBD soil (2 KG) and vermicompost (1 KG) + VAM, T_6 – OBD soil (1 KG) and vermicompost (1 KG) + VAM, + N_2FB, T_7 – OBD soil (2 KG) and vermicompost (1 KG) + VAM + N_2FB, T_8 – OBD soil (1 KG) + VAM, T_9 – OBD soil (1 KG) + N_2FB} were set in poly bags meant for assessment of their effect on growth performance of raised plants under green house condition. The population of bio-inoculants used for each experiment set was also observed. The growth parameters in terms of shoot height and number of branches were recorded at an interval of 30 days till the saplings attained an age of 3 months.

After the evaluation of growth parameters in green house, all the plants were shifted to the OBD site taking utmost care in transportation. The pits of 1.5ft in depth and 2ft in diameter were dug out keeping 2 m distance between them. The poly bags were removed with care and finally all the test plant species were transplanted on the OBD site ($80 \times 80\ m^2$) keeping their natural habit in consideration i.e. big tree plants were at the inner row, herbs at outer making a circular row and the bushy at outer most circle. Irrigation at the time of plantation and thereafter at the interval of 15 days till three months was done to maintain the optimum moisture of the rhizoplane. Growth parameters in terms of plants height and number of branches were also observed till one year at an interval of three months.

2.1. Statistical analysis

All the results were mean of the five replicates. Data were analyzed {standard error (SE), Critical Difference (CD) and *F*-ratio at 5% and 1% of significant level} statistically using MS Excel. ANOVA (analysis of variance) and two way mean value were also calculated for the evaluation of growth performance, treatment and time factors.

3. Results and discussion

3.1. Plant community and quantitative structures

The composition (diversity, density and frequency) of native vegetation growing on coalmine waste (OBD soil) of study site was studied. The results as depicted in Tables 1 and 2 clearly reveal that altogether 102 (belonging to 37 families, 53 herbaceous, 11 shrubs and 38 trees; Fig. 1) angiospermic plants of diverse nature were found to grow in core and periphery zone. Herbaceous plants were more prevalent in the core zone due to settlement of new soil with changed microbial niche. However, density of flora varied with the age of the OBD. 10 and 15 year old OBDs showed the highest number of plants (61) of different habits whereas 5 year old overburden dump was found to cover only herbaceous plants species viz. *Saccharum spontaneum*, *Croton bonaplanadium*, *Xanthium strumarrium*, *Alternanthera sessilis*, *Launea nudicaulis*, *Cyandon dactylon* and *Chrysopogon asciculatus*. While *D. sissoo*, *Tectona grandis*, *Acacia nilotica*, *Acacia angustaifolia*, *Terminalia alba*, *Alstonia scholaris*, *Agele marmelous*, *Artocarpus lachoa*, *Semicarpus anacardium*, *Terminalia bilarica*, *Sterculia urens*, *Ficus bengalensis*, *Ficus glomerata*, *Ficus religiosa*, *Madhuca latifolia*, *Bombex cieba*, *Bauhinia variegata*, *Butea monosperma*, *Holarhanea pubescence*, *Shorea robusta*, etc. were observed as sparse vegetation (natural or by plantation) in the periphery zone of the mining wasteland, *Ficus benghalensis*, *A. sessilis*, *Artocarpus lakoocha*, *T. grandis*, *S. robusta* and *Mangifera indica* constitute dominant flora in the periphery zone.

Table 1 Taxon growing on different age group OBDs in the Core zone. [+ (presence), – (absence), H (Herbs), S (Shrub), T (Tree)].

Taxon	Family	Habit	Zone		Age of OBD (in years)		
			Periphery	Core			
					5	10	15
Acacia farnesiana (L.) Willd.	Mimosaceae	T	–	+	+	+	+
Acacia arabica. Ruct.	Mimosaceae	S	–	+	–	+	+
Acacia nilotica (L.) willd.	Mimosaceae	T	+	–			
Acacia angustifolia L.	Mimosaceae	T	+	–			
Aegle marmelos (L.) Coorea.	Rutaceae	T	+	–			
Alstonia scholaris (L.) R. Br.	Apocynaceae	T	+	–			
Aerva sanguinolenta L.	Amaranthaceae	H	–	+	–	+	+
Altantia monophylla	Rutaceae	T	+	–			
Alternanthera sessilis L.	Amaranthaceae	H	+	+	+	+	–
Alternanthera aronychoides St. Hill	Amaranthaceae	H	–	+	–	+	+
Amaranthus spinosus L.	Amaranthaceae	H	–	+	–	+	+
Andropogon pumilus Roxb.	Poaceae	H	–	+	–	+	+
Annona squamosa L.	Annonaceae	T	+	–			
Annona reticulata L.	Annonaceae	S	+	–			
Argemone mexicana L.	Paparveraceae	H	–	+	–	+	+
Artocarpus lakoocha Roxb.	Moraceae	T	+	–			
Artocarpus heterophyllus Lam.	Moraceae	T	+	–			
Atylosia scarabaeoides (L.) Benth.	Asteraceae	H	–	+	–	+	+
Azadirachta indica A. Juss.	Melliaceae	T	+	–			
Bauhinia variegata L.	Caesalpiniaceae	T	+	–			
Blumea mollis Merr.	Asteraceae	H	–	+	–	+	+
Blumea lacera Burn.f	Asteraceae	H	–	+	–	+	+
Boerhaavia diffusa L.	Nyctaginaceae	H	–	+	–	+	+
Bombax ceiba L.	Bombacaceae	T	+	–			
Borassus flabellifer L.	Arecaceae	T	+	–			
Butea monosperma Lam.	Fabaceae	T	+	–			
Calotropis procera Aiton.	Asclepiadaceae	S	+	–			
Cassia cordata L.	Caesalpiniaceae	S	–	+	–	+	+
Cassia occidentalis L.	Caesalpiniaceae	S	+	+	–	+	+
Calotropis gigantea L.	Asclepiadaceae	S	+	+	–	+	+
Cassia fistula L.	Caesalpiniaceae	T	+	–			
Cassia hirsuta L.	Caesalpiniaceae	S	+	–			
Cassia sophera L.	Caesalpiniaceae	S	+	–			
Convolvulus alsenoides L.	Convolvulaceae	H	–	+	–	+	+
Costus speciosus (J. Koenig)	Zingiberaceae	S	+	–			
Chromolaena odorata L.	Asteraceae	H	–	+	–	+	+
Chrysopogon aciculatus (Retz.) Trin	Poaceae	H	–	+	+	+	+
Crotalaria juncea L.	Fabaceae	H	–	+	–	+	+
Croton bonplandianum Bail.	Euphorbiaceae	H	+	–			
Cyperus rotundus L.	Cyperaceae	H	–	+	–	+	+
Cynodon dactylon L	Poaceae	H	–	+	+	+	+
Dalbergia sissoo Roxb.	Papilonaceae	T	+	–			
Desmodium triflorum L.	Oxalidaceae	H	–	+	–	+	+
Entada pursaetha	Leguminaneae	T	+	–			
Eclipta alba (L.) Hassk	Asteraceae	H	–	+	–	+	+
Eragrostis coarceata Stap f.	Poaceae	H	–	+	–	+	+
Euphorbia hirta L.	Euphorbiaceae	H	–	+	–	+	+
Euphorbia prostrata Aiton.	Euphorbiaceae	H	–	+	–	+	+
Evolvulus alsenoides L.	Convolvulaceae	H	–	+	–	+	+
Ficus benghalensis L.	Moraceae	T	+	–			
Ficus glomerata Roxb.	Moraceae	T	+	–			
Ficus religiosa L.	Moraceae	T	+	–			
Hygrophila auriculata Heine	Acanthaceae	H	–	+	–	+	+
Holarhaena pubescence	Fabaceae	T	+	–			
Indiogofera latifolia Retz.	Fabaceae	H	–	+	–	+	+
Ipomea cairica L.	Convolvulaceae	H	–	+	–	+	+
Jatropha gossypifolia L.	Euphorbiaceae	H	–	+	–	+	+
Justicia diffusa Willd.	Acanthaceae	H	–	+	–	+	+
Jatropha curcas L.	Euphorbiaceae	H	–	+	–	+	+
Launea nudicaulis Plur	Asteraceae	H	–	+	+	+	+

(*continued on next page*)

Table 1 (*continued*)

Taxon	Family	Habit	Zone		Age of OBD (in years)		
			Periphery	Core			
					5	10	15
Lantana camara L.	Verbenaceae	S	–	+	–	+	+
Leucas aspera Willd.	Labiatae	H	–	+	–	+	+
Leucas cephalotes Spreng.	Labiatae	H	–	+	–	+	+
Madhuca latifolia Macbr.	Sapotaceae	T	+	–			
Mangifera indica L.	Anacardiaceae	T	+	–			
Melia azedarach L.	Melliaceae	T	+	+	–	+	+
Mecardonia procumbens L.	Scrophulariaceae	H	–	+	–	+	+
Mollugo pentaphylla L.	Verbenaceae	H	–	+	–	+	+
Pentanema indicum L.	Asteraceae	H	–	+	–	+	+
Pithecellobium dulce Roxb.	Minosaceae	T	+	–			
Phyllanthus niruri L.	Euphorbiaceae	H	–	+	+	+	+
Phyllanthus urinaria L.	Euphorbiaceae	H	–	+	–	+	+
Pyhllanthus simplex Retz.	Euphorbiaceae	H	–	+	–	+	+
Phyla nodiflora L	Verbenaceae	H	–	+	–	+	+
Phoenix sylvestris (L.)	Arecaceae	T	+	+	–	+	+
Pongamia pinnata (L.) Pierre	Fabaceae	T	+	–			
Rungia pactinata (L.) Nees	Acanthaceae	H	–	+	–	+	+
Socparia dulcis L.	Scrophulariaceae	H	–	+	–	+	+
Scripus arteculatus L.	Cyperaceae	H	–	+	–	+	+
Semecarpus anacardium L.F.	Anacardiaceae	T	+	–			
Sida cordifolia L.	Malvaceae	H	–	+	–	+	+
Saccharum spontaneum L.	Poaceae	H	–	+	+	+	+
Shorea robusta Roxb.	Dipterocarpaceae	T	+	–			
Siphonodon celastrineus Griff.	Celastraceae	T	+	–			
Solanum xanthocarpum Schrad. & Wendl.	Solanaceae	H	–	+	–	+	+
Sporobolus diander (Retz.)	Poaceae	H	–	+	–	+	+
Sterculia urens Roxb.	Sterculiaceae	T	+	–			
Streblus asper Lour.	Moraceae	T	+	–			
Tacca leontopetaloides (L) Kuntze	Taccaceae	T	+	–			
Tephrosia purpurea (L.)	Fabaceae	H	–	+	–	+	+
Terminaia alata Roth	Taccaceae	T	+	–			
Terminalia arjuna Roxb.	Combretaceae	T	+	–			
Terminalia bellenica Roxb	Combretaceae	T	+	–			
Tectona grandis L.	Fabaceae	T	+	–			
Tragia involucrate L.	Euphorbiaceae	H	–	+	–	+	+
Tridax procumbens L.	Asteraceae	H	–	+	–	+	+
Croton bonplandianum Bail.	Euphorbiaceae	H	–	+	+	+	+
Vinca rosea L.	Asteraceae	H	–	+	–	+	+
Vitex negundo L.	Verbenaceae	S	+	+	+	+	+
Vernonia cinerea (L.) Less.	Asteraceae	H	–	+	–	+	+
Xanthium strumarium Boiss	Asteraceae	H	–	+	+	+	+
Zizyphus mauritiana Lam.	Rhamnaceae	T	+	–			

The quantitative enumeration of native vegetation on different aged OBDs reveals that the newly generated OBD did not have any vegetation but it was found to come up with slow primary succession and proceeded predominantly with the age of OBD. The 5 years old OBD had the least number of plants whereas 10 and 15 year old OBDs were found to favor the growth of several herbaceous and shrubby plants. *S. spontaneum* constitutes a dominant flora over other communities. However, the number of plant species was found almost similar in both on 10 and 15 year old OBDs as evinced by the general sparseness of plant and low plant cover (Table 2).

The natural vegetation represents actual successional stage toward the native vegetation progressing at a very slow pace. The richness in the species diversity and sparseness of some plants are suggestive of the fact that succession has proceeded predominantly by enrichment with native taxa over a period of time. Ekka and Behera (2011) and Dugaya et al. (1996) also observed similar tree species composition and diversity on an age series of coal mine spoil in an opencast coalfield in Orissa and Madhya Pradesh of India, respectively.

3.2. Population of bio-inoculants

VAM spore population in rhizosphere soil (No./5 g OBD soil) and percent root colonization were observed in case of 90 day old plants under study. Out of 10 plants studied under poly bag experiment, spore population in *G. sepium* and *S. grandiflora* were maximum 52 and 23 respectively, followed by *A. cracicarpa* (8) and *Cassia siamea* (7). It was also noticed that *S. grandiflora* and *G. sepium* showed highest root colonization

Table 2 Density of plant species found on the core zone of mine area.

Taxon	Density of plant community on OBDs			Frequency (%) of plant community on OBDs		
	5 years	10 years	15 years	5 years	10 years	15 years
Acacia fernesiana	00	1.6	2.8	00	20	25
Alternanthera sissilis	00	2.1	5.3	00	30	40
Coroton bonplandianum	00	3.1	6.3	00	35	56
Chrysopogon acieolatum	1.9	2.8	4.9	25	38	47
Cynodon dictylon	2.6	3.8	5.1	28	39	49
Lantana camara	1.8	4.2	6.4	20	50	58
Launia nudicaulis	1.4	3.1	7.2	25	55	70
Phyllanthus niruri	00	2.3	5.3	00	27	51
Saccharum spontaneum	3.6	6.4	9.2	60	80	90
Xanthium strumarium	1.3	2.7	6.5	30	35	69

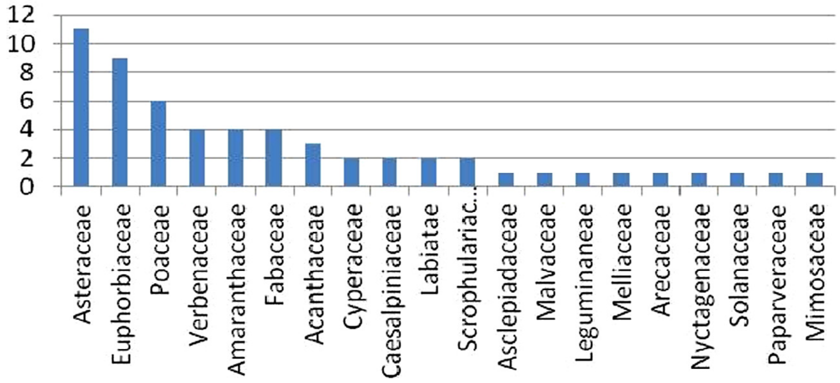

Figure 1 Dominant plant families in core zone.

i.e. 52% and 49% in OBD soil respectively followed by *D. sissoo* (37%), *D. strictus* (35%), *A. mangium* (28%), *Cassia siamea* (23%); however, less VAM infection in *S. hamata* (9%) and *S. scabra* (12%) was recorded. The maximum number of nodules of *Rhizobium* i.e. 76 and 67 was recorded in the case of *G. sepium* and *S. grandiflora* respectively followed by *D. sissoo* (56), *D. strictus* (48) and the least (13) were recorded in the case of *S. hamata* and *A. mangium* (Table 3). The present results clearly indicate that there is a cumulative effect of bio-inoculant on plant growth performance and further, it is also evident that there is a direct correlation between microbial population and growth performance of plant species. The effects of mine spoil substrate on mycorrhizal colonization varied among AMF species (Rydlová et al., 2011). The existence of a certain degree of selectivity between AMF species and mine spoil substrates may result in different root colonization rates of host plants (Malcová et al., 2001; Gryndler et al., 2008).

3.3. Growth performance under green house and field condition

The morphometeric assessment in terms of shoot height and number of branches under the influence of treatment ($T_{1\text{-}9}$) indicates that the effect of different treatments on growth of

Table 3 Spore population, root colonization and nodulation in raised plants (90 DAP) in poly bags under green house (Significant marked with bold).

Taxon	Spore population per 5 g of dry soil		Percent root colonization		Nodule per plant	
	90 DAP	15 years old OBD	90 DAP	15 years old OBD	90 DAP	15 years old OBD
Acacia mangium	06	16	28	48	13	24
Acacia cracicarpa	08	18	17	27	30	33
Cassia siamea	07	27	23	33	38	54
Dendrocalamus strictus	09	29	35	43	48	57
Dalbergia sissoo	07	37	27	47	56	78
Gliricidia sepium	**52**	**62**	**49**	**97**	**76**	**93**
Pterocarpus santalinum	02	22	11	21	43	43
Sesbania grandiflora	**23**	**43**	**52**	**98**	**67**	**84**
Stylo hamata	03	13	9	19	16	37
Stylo scabra	05	15	12	18	13	34

the raised plant over the control (T_1) in poly bags under green house was appreciable (data not shown). All the plants showed maximum increase in shoot height and number of branches in tripartite combination i.e. with T_6 against T_1 (control), whereas *S. grandiflora* and *G. sepium* achieved highest level of increase. Further height followed slow pace up to three month in case of each raised plant species. Analysis of variance (data not shown) shows that the main effect of treatment (T) and Duration (D) and its interaction effect with shoot height and number of branches were found significant at 5% and 1% level of significance in all the plant species. In all the cases, value of F ratio was 2.10 (8, 52 df), and 2.82 (8 df). From the mean table of treatments, it is clear that T_6 was the best among the treatments given to the raised plant species followed by T_5, T_4, T_3, T_2 and the least effect was shown with T_1 (control). This indicates that the plants were suitable for the biological reclamation of OBD and were transplanted to a 15 year old OBD experimental field.

A total number of 11 species under four genera viz. *Glomus* (*G. mossesae*, *G. fasciculatam*, *G. macrocarpum*, *G. epigeum*, *G. microcarpum*, *G. invermarium*, *G. lenue*, *G. caledonium*) *Gigaspora calospora*, *Sclerocystis sinuosa*, *Scutellospora clavispora*) were isolated from 15 year old OBD soil. The density of VAM spore was found maximum in 15 year old OBD. On screening of various OBD soils, altogether 10 different colonies of bacteria were isolated out of which the highest population of *Agrobacterium*, *Xanthomonas* and *Rhizobium* was recorded in 15 year old OBD soil samples. The increase in plant dry weight was attributed to the stimulatory effect of spore germination and establishment of AMF, contributing to the improved nutrition and development of AMF–plant–soil system (Jin et al., 2005; Kim et al. (2010). The influence of fungal strains on plant development in mine wastes varied according to their ecotypes (Orłowska et al., 2005). The different response of AMF to plant growth may result from different morphological and molecular features and the adaptation to coal mine spoils. Moreover, the effect of AMF on plant growth was not necessarily correlated with the level of colonization by the AMF. Enkhtuya et al. (2000, 2005) found that the mycorrhizal growth response of plants was very low even when the development of all AMF was successful in coal mine spoils. However, in accordance with previous studies (Püschel et al., 2008; Redon et al., 2009), plant growth is positively correlated with the level of AMF colonization.

The spore populations per 5 g of dry soil, percent root colonization of VAM fungi and root nodules were observed at the interval of three months to study the acclimatization process of the plants with bio-inoculants under the influence of desired treatments. The maximum number of spore was found in *G. sepium* (62) and *S. grandiflora* (43) followed by *D. sissoo* (37) and *D. strictus* (29), and the least were recorded from *S. hamata* (13). Maximum infection was recorded in *S. grandiflora* (98%) followed by *G. sepium* (97%), *A. mangium* (48%), *D. strictus* (43%), *D. sissoo* (47%) and *A. cracicarpa* (27%). The maximum numbers of nodules were observed in *G. sepium* (93) and *S. grandiflora* (84) followed by *D. sissoo* (78) and *D. strictus* (57), and the least number of nodules were observed in case of *Acacia crascicarpa, A. mangium*, *S. hamata* and *S. scabra*.

Growth parameters were taken into consideration after a gap of three months i.e. at 6, 9 and 12 months after plantation in terms of shoot height and number of branches under the influence of treatment ($T_{1\text{-}9}$). Effects of different treatments on growth of the raised plant over the control (T_1) in field condition were appreciable and followed the similar trends as in green house condition (data not shown). Although all the raised plant species were found to have appreciable increase in plant height and number of branches, and well flourishing *D. sissoo*, *D. strictus* and *S. grandiflora* among woody species were quite suitable and dominating in respect of their canopy on the 15 year old OBD site. The mortality rates in field condition were also estimated at 6, 9 and 12 months of the plantation and were found in the range of 5–25%. The mortality rates in the leguminous plant species were less. Initially (within 6 months) the mortality rate was higher, but subsequently it was followed by 9 and 12 months. The initial high mortality rate might be correlated with the acclimatization of plant in new ecological niche. It was noticed that the overall growth condition of all the planted species was luxuriant and in good condition and their survival rate was in the range of 86–94% (Fig. 2).

All the plants showed maximum increase in shoot height and number of branches in tripartite combination i.e. with T_6 against T_1 (control), where as *S. grandiflora* and *G. sepium* achieved highest level increase in both growth as well as in nodulation, spore population and percent root colonization (Table 4). Further height followed slow pace up to six months in case of each raised plant species. The treatment T_6 was the best (statically significant) among the treatments given to the raised plant species in field also, followed by T_5, T_4, T_3, T_2 and the least effect was shown with T_1 (control). So, it is clearly evident from the results that all the treatments were found to have variable effects on plant growth; however, the leguminous plants showed better performance. Legume roots growing in non-sterile soil become naturally infected with indigenous AM (Ganry et al., 1982, 1985; Subba Rao et al., 1986; Barea et al., 1987; Shivaram et al., 1988; Goss and de Varennes, 2002). Physical disruption of soil has been successfully employed as a means to generate differences in indigenous AM colonization potential (Goss and de Varennes, 2002; Antunes et al., 2006). Inoculation with *Rhizobium* increased nodulation in the +AM compared with the -AM treatment (Chalk et al., 2006). Agronomically significant increases in the amounts of fixed N were obtained in +AM compared with −AM treatments in the presence of *Rhizobium* inoculation in soybean (Ganry et al., 1982, 1985), and *Hedysarum coronarium* (Barea et al., 1987).

The physical profile i.e. sand, silt, clay, bulk density, water holding capacity (WHC) and pH were found to vary with the age of OBD soil. Most of the factors were improved with the successional growth of vegetation. The pH which was initially 8.02, gradually moved toward neutrality, favorable for plant growth. Similar observations were also recorded by Maiti et al. (2002). The recycling of biomass in OBD soil might be one of the possible reasons for the change in WHC, porosity, clay and other physical factors. The chemical analysis of soil including P, K and Ca was also found to increase gradually with the age of OBD soil, which might be due to microbial mineralization. The diversity in microbial population on different aged OBD sites was also recorded; however, the main emphasis was given on N_2 fixing bacteria and phosphate solubilizing fungi (VAMF) which have established role for biological rejuvenation in stressed land soil. Positive effects of AMF on plant biomass have been previously reported,

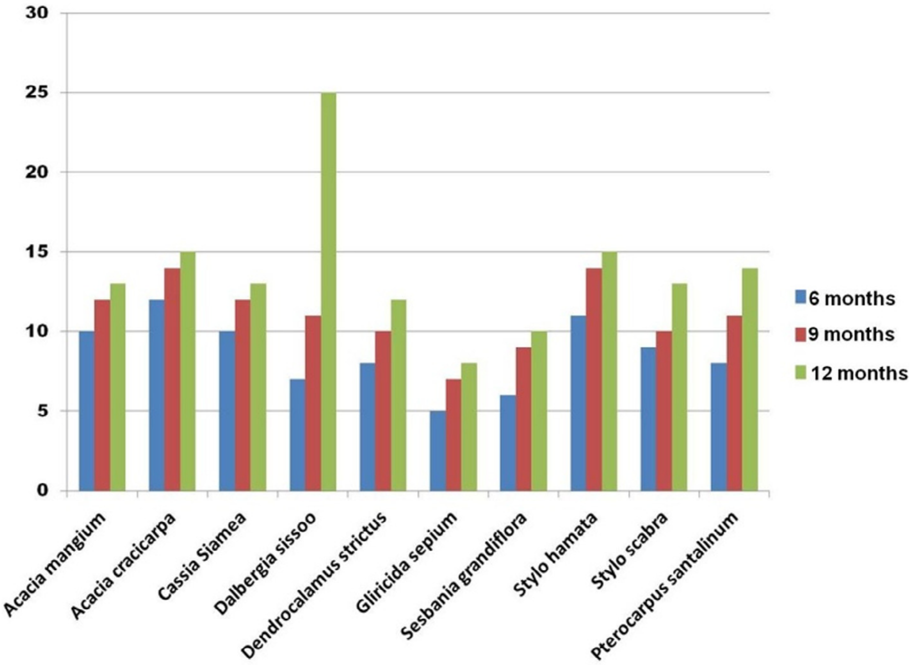

Figure 2 Mortality rate on field condition.

Table 4 Nodulation, spore population and percent root colonization at 6, 9 and 12 months in two representative plant species (Significant marked with bold).

Taxon	Treatment	Average nodulation			Average spore population/5 gm dry OBD soil			Percent root colonization		
		Period (in month)			Period (in month)			Period (in month)		
		6	9	12	6	9	12	6	9	12
Sesbania grandiflora	T_1	00	6.5	13.4	19.4	25.4	28.5	10	13	15
	T_2	17.6	28.3	30.3	27.6	37.6	44.6	14	16	19
	T_3	18.7	33.5	38.6	26.3	36.3	56.6	34	36	37
	T_4	19.3	53.5	56.7	24.4	46.4	57.4	43	54	65
	T_5	21.3	48.9	63.3	28.2	48.2	65.4	35	36	39
	$\mathbf{T_6}$	**24.7**	**60.5**	**79.4**	**42.8**	**52.8**	**76.4**	**45**	**46**	**68**
	T_7	33.1	76.5	124	38.9	48.9	65.7	46	48	52
	T_8	36.4	67.7	98.6	38.4	48.8	61.3	34	48	51
	T_9	31.1	48.3	87.5	34.8	44.8	64.6	36	38	41
Gliricida sepium	T_1	12	14	28	16.4	35.4	38.5	20	33	35
	T_2	15	17	29	28.6	37.6	44.6	44	46	59
	T_3	17	19	28	28.3	36.3	56.6	48	56	57
	T_4	18	21	26	25.4	46.4	57.4	59	54	65
	T_5	19	20	25	26.2	48.2	65.4	43	36	69
	$\mathbf{T_6}$	**23**	**28**	**31**	**44.8**	**52.8**	**76.4**	**65**	**46**	**78**
	T_7	24	29	34	35.9	48.9	65.7	66	48	72
	T_8	22	27	35	37.4	48.8	61.3	55	48	71
	T_9	25	27	34	37.8	44.8	64.6	66	38	71

AMF decreased the biotic and abiotic stresses exerted by coal mine wastes (Taheri and Bever, 2011; Qian et al., 2012).

In conclusion, all the treatments in relation to growth performance over control were found statistically significant; however, treatment T_6 showed highly significant, therefore it was considered as the best combination for the development of bio-inoculant package for soil reclamation of abandoned mine land by revegetation. Though, the interactions between mycorrhizal fungi and NFB have not been well understood cell to cell interaction might be one of the important factors. NFB are found capable of adhering to VAMF spore in soil. The mycorrhizal infected plants create a congenial climate for the greater activity of Rhizobial cells and both acted synergistically (Chalk et al., 2006; Cardoso and Kuyper, 2006). The vermicompost component of the treatments might be helpful in amending OBD soil texture as well as also provide nutrients for rapid growth of VAM fungi and multiplication of Rhizobial cells for the development of nodulation in plants. Thus, increase in phosphate solublization and mobilization by VAMF and fixation of atmospheric nitrogen by Rhizobia might be the possible reasons for influencing best plant growth performance both under poly bag and field experiments.

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