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A STUDY OF THE MYCORRHIZAL ASSOCIATION WITH VEGETATION ON COAL MINES SPOIL

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ABSTRACT

The paper deals with the mycorrhizal association with plant species occurring over 3 and 6 yr old coal mine spoil overburden dumps from Orissa. Percentage of mycorrhizal infection among the colonising grass species were recorded to be the maximum (95-96%) in *Cynodon dactylon* and minimum (25-47%) in *Cyperus metzii*. Except *Blumea lacera* all the nongrass herbaceous species showed mycorrhizal infection (88-92%) in the roots. Mycorrhizal infection were catagorised with 3 types *i.e.* hyphae, Arbuscule and vesicules,out of which incidence of hyphal infection was recorded to be relatively high (>80%). From statistical analysis it is confirmed that percentage of mycorrhizal root infection among different overburden with respect to age was significant. However percentage of infection with respect to different species in different overburden didn't show significant variation. The study analysis the implications of mycorrhizal association with respect to vegetational colonisation and mine spoil reclamation.

INTRODUCTION

Open cast coal mining produces huge amounts of spoils, which are usually deposited in form of overburden dumps adjacent to the mining sites. Such dumps have harsh physico-chemical and nutrient deficient properties (Jha and Singh, 1991; Dutta and Agarwal, 2000) and poses problem for the re-establishment of vegetation through succession (Brenner, 1979; Chaney *et al.*, 1995) .However, it is desirable to develop vegetation on overburden dumps for the purpose of reclamation and environmental stability of the area (Roberts *et al.*, 1981; Singh and Jha, 1992). In this context, micro-organisms are expected to play critical role specifically for the nutrient supply to the roots of the colonizing plants (Montesinos, 2003). Role of mycorrhiza in re-establishment of vegetation on harsh habitat (Turnau and Haselwandter, 2002) and in the coal mines spoil has been well emphasized by several workers (Jasper *et al.*, 1988; Johnson and McGraw, 1988). Mycorrhiza often causing root infection, can extend their hyphae out several centimetres from the root and absorb essential nutrients (Benthlenfalvay and Linderman, 1992; Smith and Read, 1997; Marschner and Dell, 1994) required for the survival and growth of the plants (Sylvia and Williams, 1992). Increase in nutrient uptake by plant root due to the mycorrhizal infection has been reported by several workers (Marschner, 1995; Leyval *et al.*, 1997; Smith and Read, 1997). Mycorrhizal association on the roots of different plant species growing on coal mine spoil has been reported by Gould *et al.*, (1996) and Kumar *et al.*, (2003). In the present study, we attempted to investigate the mycorrhizal association with the roots of the overlying vegetation during the reclamation of coal mine spoil overburden in one of the open cast coal mine area from Orissa.

Study sites

The study was conducted in the Basundhara coal mines of Mahanadi coal field Limited, in the district of Sundargarh, Orissa. The mining area is 48 Km away from the district head quarter township of Sundargarh. Geographically it is located between 22°03′ 32″ and 22°04′11″ NL and 83°42′ 18″ and 83°44′ 08″ EL. The area experiences seasonal tropical climate with three distinct seasons *i.e.* summer (March-June), rainy (July-October) and winter (November -February). The annual rainfall received by this area is approximately 1400mm, of which 85% fall during rainy season. During the summer, the area experiences a maximum temperature of 50°C and during winter the average minimum temperature remains around 10°C. The mining is continuing in this area since 1993 over 459 ha of land, of which the overburden dumps covers, approximately an area of 40 ha.

MATERIALS AND METHODS

In the present study, we attempted to look in to the mycorrhizal association in different age series mine spoil overburden dumps, such as: fresh overburden (OB_0), 3 year old overburden (OB_3) and 6 year old overburden (OB_6). These overburden dumps were botanically explored for the record of plant species which includes both herbaceous grass and non-grass and lignaceous species on it. Subsequently then plant species were randomly sampled out along with their fine roots and rhizospheric soil. The collected fine root samples along with the rhizospheric soil were separated carefully and brought to the laboratory in a plastic bag for mycorrhizal study. The separated, root samples were cut into pieces of 1cm. length and these pieces were subjected to cleaning by exposing the root materials to running tap water. The cleaned root materials were subjected to 2N KOH treatment at 80ºC for 30 minutes after which treated root samples were washed in running tap water .Then the root materials were treated with 3.5% HCl (v/v) in a glass beaker at 80ºC for 30 minutes (Bevege, 1968, Kormanik and McGraw, 1982).The root materials were subsequently subjected to repeated water washing and were stained with tryphan blue (0.05%) for a period of 30 minutes at 80ºC. The stained root samples were observed under light microscope for the detection of mycorrhizal infection. After detecting mycorrhizal infection on the root, mode of infection mostly in form of hyphae, arbuscule and vesicles were noted. From the microscopic observation of the root sample, percentage of mycorrhizal infection was estimation following the equation:

$$\text{Mycorrhizal infection (\%)} = \frac{\text{No. of mycorrhizae infected roots}}{\text{Total no. of roots inspected}} \text{X100}$$

Occurrence (%) of different categories of infection was calculated as:

$$\text{Percent occurrence} = \frac{\text{No. of times, the category of infection detected on the sample}}{\text{Total number of samples observed}} \text{X100}$$

The percentage of root infection data with respect to different age series of overburden is statistically analysed (ANOVA: Two-Factor without replication) to test the significance by using Microsoft office Excel 2007.

RESULTS

Since the fresh mines spoil overburden (OB_0) was without any vegetation, there was no scope for studying the mycorrhizal association in it. However, 3 and 6 years old overburden dumps (OB_3 and OB_6) were associated with vegetation, which includes herbaceous (grass and non-grass) and lignaceous species (Table 1 to 3). Study of the mycorrhizal infection indicated that all the recorded 9 grass species were with mycorrhizal infection (Table 1). The percentage of infection varied from 47 to 95% in OB_3 and 25 to 96% in OB_6 dump. In both the dumps *Cynodon dactylon* was the grass species showing highest percentage of mycorrhizal infection. Further study of infection revealed the presence of three types of mycorrhizal infection *i.e.* hyphae, arbuscules and vesicles on different grass root samples. The occurrence of hyphal infection was noted to be prevalent in both OB_3 and OB_6 dumps. However the occurrence of arbuscule was noted to be confined to three species *i.e. Cyperus metzii, Eragrostis tennela and Heteropogon contortus*. Similarly, roots of 6 species exhibited vesicular infection and in species like *Aristida setaceae, Dichanthium annulata* and *Heteropogon contortus*, vesicular infection were marked to be absent.

Percentage of infection on the non-grass herbaceous species (Table 2) showed a range of 40-92 % in OB_3 and 27-88 % in OB_6 dumps. In both OB3 and OB6 dump the highest percentage of mycorrhizal infection was noted in the root samples of *Achyranthes aspera*. Further, the study of the category of infection in both the dumps indicates the hyphal infection to be the prevalent category of infection. Numbers of species showing the arbuscules in the roots were recorded to be 9 in OB_3 and 10 in OB_6 dumps. Similarly number of species showing vesicular infection was 6 in OB_3 and 10 in OB_6 dumps. Incidence of both arbuscule and vesicular infection were marked to be comparatively much lower than the hyphal infection. It is interestingly to note that the species *Blumea lacera* was with out any mycorrhizal infection.

On OB_3 only one lignaceous species *Cassia siamea* was recorded and the root samples of the species showed 96 % mycorrhizal infection (Table 3). However on OB_6 four lignaceous species were noted and among this *Cassia siamea* exhibited maximum mycorrhizal infection .With respect to category of infection it was observed that, the occurrence of arbuscular and vesicular infection was comparatively less than that of hyphal type.

The ANOVA indicated that mycorrhizal root infection with respect to grass (F value; 122.47; $p<0.05$); non-grass (F value; 34.69; $p<0.05$) and for lignaceous species (F value; 8.56; $p<0.05$), between different age series (OB_0, OB3 and OB_6) of overburden. However among different species the mycorrhizal infection did not show any significant difference.

Table 1: Mycorrhizal infection in different grass species recorded from 3 yr. and 6 yr. old coal mine spoil overburdens.

S.N.	Species name	Family	3 years old overburden dump (OB_3)				6 years old overburden dump (OB_6)			
			Infection (%)	Category of infection*			Infection (%)	Category of infection*		
				H	A	V		H	A	V
1	*Aristida setacea,* Retz	Poaceae	85±2	100	0	0	83± 2	100	0	0
2	*Cynodon dactylon,* Pers.	Poaceae	95±1	96	0	28	96±1	88	0	41
3	*Cyperus metzii,* Vahl	Cyperaceae	47±2	82	34	23	25±2	80	36	28
4	*Dichanthium annulata,* Stapf.	Poaceae	67±4	100	0	0	62±4	100	0	0
5	*Eragrostis coarctata,* Stapf.	Poaceae	56±3	98	0	23	48±3	90	0	23
6	*Eragrostis tenella,* Stapf.	Poaceae	78± 3	94	40	26	76± 3	92	30	43
7	*Eragrostis tremula,* Hochst.	Poaceae	76± 4	100	0	28	67± 3	98	0	78
8	*Heteropogon contortus,* Roem.	Poaceae	68± 4	93	24	0	57± 3	96	33	0
9	*Pennisetum pedicellatum.*Trin.	Poaceae	81± 2	96	0	46	78± 2	99	0	36

*values represent percentage of occurrence; H = Hyphae; A = Arbuscules; V = Vesicles.

Table 2: Mycorrhizal infection in different non-grass species recorded from 3 yr. and 6 yr. old coal mine spoil overburdens.

S.N.	Species name	Family	3 years old overburden dumps(OB_3) Infection (%)	Category of infection* H	A	V	6 years old overburden dumps(OB_6) Infection (%)	Category of infection* H	A	V
1	*Alysicarpus monilifer,* D C.	Papilionaceae	72±2	100	0	0	61±3	100	0	0
2	*Achyranthes aspera,* L.	Amaranthaceae	92±1	100	15	12	88±2	86	10	35
3	*Alternenthera sessilis,* R.Br .	Amaranthaceae	87±2	93	18	14	75±3	89	12	16
4	*Andrographis paniculata,* Nees,	Amaranthaceae	40±2	100	0	0	39±2	100	0	0
5	*Blumea lacera,* DC.	Compositae	-	-	-	-	0	0	0	0
6	*Celosia argentea,* L.	*Amaranthaceae*	-	-	-	-	86±3	89	0	38
7	*Evolvulus alsinoides,* L.	Convolvulaceae	84±2	96	29	0	77±3	90	22	0
8	*Evolvulus numularis,*L.	Convolvulaceae	80±2	92	41	0	74±1	89	42	0
9	*Euphorbia hirta,* L.	Euphorbiaceae	73±3	100	22	0	59±2	100	34	0
10	*Hemidesmus indicus,*R.Br ,	Asclepidaceae	52± 2	90	40	13	47± 2	87	19	40
11	*Lantana camara,* L.	Verbenaceae	-	-	-	-	53±2	91	15	40
12	*Ocimum basilicum,* L.	Lamiaceae	77±4	87	12	0	73±3	86	18	0
13	*Ocimum canum,* Sims.	Lamiaceae	67±3	91	31	22	63±3	90	25	33
14	*Phyllanthus fraternus,* Web.	Compositae	-	-	-	-	43±2	100	0	0
15	*Rungia parviflora,* Nees.	Acanthaceae	-	-	-	-	27±3	100	0	0
16	*Solanum surattene,*Burm.f.	Solanaceae	43±2	95	21	30	41±2	90	29	41
17	*Tridex procumbens,* L.	Compositae	67±2	94	0	19	41±2	95	0	37
18	*Tephrosia purperia,* Pers	Papilionaceae	-	-	-	-	50±1	94	0	42
19	*Tephrosia villosa,* Pers	Papilionaceae	-	-	-	-	33±3	94	0	33

*values represent percentage of occurrence; H = Hyphae; A = Arbuscules; V = Vesicles.

Table 3: Mycorrhizal infection in different Lignaceous species recorded from 3 yr. and 6 yr. old coal mine spoil overburdens.

S.N.	Species name	Family	3 years old overburden dumps(OB_3) Infection (%)	Category of infection* H	A	V	6 years old overburden dumps(OB_6) Infection (%)	Category of infection* H	A	V
1	*Acacia nilotica,* L.	Mimosaceae	–	-	-	-	63±3	81	37	21
2	*Acacia leucophloea,* L.	Mimosaceae	–	-	-	-	65±3	89	29	57
3	*Butea monosperma* (Lam.)Taub.	Fabaceae	–	-	-	-	57±3	82.	0	51
4	*Cassia siamea,* L.	Caesalpiniaceae	96±1	91	33	22	96±1	84	29	43

*values represent percentage of occurrence; H = Hyphae; A = Arbuscules; V = Vesicules.

DISCUSSION

Relatively high percentage of mycorrhizal infection in the roots of the herbaceous species, as noted in the present study is in confirmation with the observation of Javaid *et al.,* (2007) and Leung *et al.,* (2007) and such observation highlights the effective role of grass species in the reclamation and subsequently vegetational succession on inhospitable mines spoil. Susceptibility of *Cynodon dactylon* roots samples for mycorrhizal infection has been noted by Javaid *et al.,* (2007) and Leung *et al.,* (2007). Hence, the highest percentage of infection in root samples of *C.dactylon,* as observed in this study confirms their observation. Among the non-grass herbaceous species, no mycorrhizal association was noted in the roots of *Blumea lacera* and this is in agreement with the observation of Kumar *et al.,* (2003).Besides relatively high percentage of mycorrhizal infection of *Cassia siamea* in comparison to other Lignaceous species reflects its better susceptibility for interaction with mycorrhizal fungi and emphasises the reclamatory role of the species for the disturbed habitat (McKell, 1989; Mehrotra, 1998).

Three categories of mycorrhizal infection, has been reported by earlier workers (Kumar *et al.*, 2003; Pezzani *et al.*, 2006). Among this, hyphal infection has been explained to serve as transport of essential nutrients from the surrounding to the plant roots (Pearson and Jakobsen, 1993; Newman *et al.*, 1994; Fitter *et al.*, 1998; Sen, 2000; Nasim, 2005). Thus relatively higher incidence of hyphal infection on mine spoil overburden dumps is an indication of the establishment of a mycorrhizal transport mechanism for the supply of nutrients to the colonising plants as such arbuscular and vesicular structure as explained by Kumar *et al.,* (2003). As the result of long and sustaining interaction of mycorrhizal fungi with the plant. An establish hyphal interaction may lead to the development of intercellular arbuscular and vesicular structures of the plant. Since the vegetational development on the spoil of the dumps are in the early stage, roots of colonising plants during mature stage of vegetational development on spoil may record relatively higher incidence of arbuscules and vesicles.

Mycorrhizal association is well known to enhance vegetational growth by increasing nutrient uptake (Bethlenfalvay and Linderman, 1992; Marschner and Dell, 1994; Al-Karaki, 2002) .Since the mine spoil represents a nutrient deficient situation, possible establishment of mycorrhizal association is quite essential for proper vegetational colonisation and development and consequential spoil reclamation. Besides, mycorrhizal association is also expected to reduce the quantum of stress associated with the plant species while growing on an inhospitable mine spoils environment (Gonzal *et al.*, 1996; Robertson *et al.*, 2007; Roy *et al.*, 2007). Thus proper mycorrhizal colonisation on mine spoil is a pre-requisite for the survival and growth of vegetation.

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