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Distribution and Diversity of Arbuscular Mycorrhizal Fungi in Four Coalmine Soils of Telangana State, India

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Abstract

Coal mine sites are highly disturbed due to unearthing and dumping of underground soil. Both physicochemical and biological properties of soils are highly disturbed. Native mycorrhizal population, beneficial for plant establishment and growth, is depleted. In the present investigation, an attempt has been made to assess the status of arbuscular mycorrhizae in terms of occurrence and diversity in four coal mine disturbed sites of Telangana state. Thirty plant species commonly growing in all the four sites were selected and the mycorrhizal infection percentage, nature of infection was determined. Mycorrhizal infection percentage varied with the coal mine site and plant species. Distribution, diversity and abundance of AM fungal species varied with the coalmine location. *Glomus* species were frequently observed in all the four locations followed by *Acaulospora* and least *Entrophospora.* Species wise distribution also varied with the test location. No single species was recorded from all the four locations. Among four different locations, Yellandu JK-5 OCP was found to contain a greater number of AM fungal species. We conclude that even in depleted state, native AMF species can be exploited for revegetation of these disturbed locations because they are well adopted to the local edaphic conditions.

Keywords

Arbuscular mycorrhizal fungi, Diversity, Distribution, Coal mine spoils, seasonal variation.

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INTRODUCTION

Natural soils developed over millions of years are mostly ideal for the growth of a variety of plants. They also support the microfauna and rich microflora that include bacteria, actinomycetes, fungi and thus facilitating a wide range of biogeochemical cycles. Soil influences vegetation development supported by microbial growth and mineralization, decomposition of organic matter (1-2) and there by undergoing pedogenesis leading to soil fertility and physicochemical changes. However, due to mining, urbanization, industrialization, laying of roads and railway tracks etc. There is an unprecedented degradation of terrestrial habitats resulting in loss of natural ecosystem with associated biodiversity and leading to geo environmental disasters. Mining especially for coal is a major activity throughout the world including India. Millions of natural soils are disturbed and degraded. Mining disrupts the aesthetics of the landscape and along with it disrupts

soil components such as soil horizons, structure, soil microbe population, sand nutrient cycles those are crucial for sustaining health of ecosystem and hence results in the destruction of existing vegetation and soil profile (3). The severely disturbed coalmine soils commonly known as coalmine spoils are poor representatives of microorganisms especially beneficial organisms like nitrogen fixers, phosphate solubilizers and mycorrhizal fungi.

AM fungi forms the fundamental linkage between the biotic and abiotic components of the ecosystem in addition to their being the primary colonizers of coal mine spoils. (4-5). Their contribution to soil aggregate formation and establishment of primary plant colonizers has been proved (6). The qualitative and quantitative distribution of arbuscular mycorrhizal indicates suitability of coalmine spoils for reclamation. The assessment of mycorrhizal fungi in coalmine disturbed soils is useful in assessing the extent of disturbance and also to exploit the indigenous fungi in the revegetation programs.

In the present investigations, an attempt has been made to assess the mycorrhizal fungi in terms of diversity inBhupalpallyKtK5 incline, Yellandu JK-5 OCP, Mancherial RK5 and Ramagundam (OCP2**)** coalmine disturbed locations (Fig. 1) of Telangana state.

Fig: 1. Map showing the location of study sites

MATERIAL AND METHODS

Sampling

The rhizosphere soil samples along with the root bits of test plants were collected from the Bhupalpally (Ktk5 incline), Yellandu (JK-5 OCP), Mancherial (RK5) and Ramagundam (OCP2) coal mine dumping area. Soil samples from 0-15 cm depth were collected in polythene bags and air dried. About 300g of the soil samples were crushed and passed through sieve sets before analysis. A part of the samples was used for spore count and taxonomic analysis. The root samples were cut into 1 cm pieces, washed with tap water, preserved in 70% ethanol and stored at 4^0C for further analysis.

Staining of roots for arbuscular mycorrhizal fungi colonization

Staining of mycorrhizal roots was made according to (7), The root samples were carefully washed several times with tap water. Then are cut roots in to 1 cm pieces and treated the root samples in 10% KOH at 121^0C for 10 minutes in an autoclave at 90 ^{0}C for one hour, the KOH solution clears the host cytoplasm and nuclei and readily allows strain penetration, Then remove KOH solution and rinse the roots with several

changes of tap water to remove traces of KOH. After KOH treatment the roots were then treated with 1% HCl (v/v) for 15-20 minutes at room temperature and finally stained with 0.05% w/v trypan blue in lactoglycerol (1:1:1; lactic acid, glycerol and water) at 90^{0}C for 30 minutes in a water-bath**.** Then except HCl treatment samples were drained and washed thoroughly with distilled water at the end of every step. The root samples were then left overnight in the lacto glycerol destaining solution (1:1:1; lactic acid, glycerol and water) in a dark room to remove colour from root cells. Finally, roots were mounted in PVLG mounting on microscopic slides and covered with 40×22 mm cover slips. Slides showing clear AM fungal colonization are sealed with DPX for permanent mounting

Quantification of AMF root colonization

AM colonization was assessed from cleared and stained roots according to McGonigle *et al.* (1990) (8). A total of 100 intersections were taken for each subsample to estimate percent of AM root colonization under a compound microscope at a magnification of 40×.

Quantification of the root colonization is evaluated by presence or absence of arbuscules, vesicles and internal hyphae in the root tissue. Percentage of root colonization was calculated by the following formula.

Percentage of root colonization

$$= \frac{\text{Number of root segments colonized}}{\text{Total number of root segments observed}} \times 100$$

Isolation of arbuscular mycorrhizal (AM) fungal spores from rhizosphere soil

Spore extraction

Soil were air-dried and sieved through a 2-mm sieve to remove coarse debris before extracting, counting and identifying AM fungal spores. 100 grams of dry soil was taken to extract the spores using the wet sieving and decanting method (9), followed by centrifugation in water and 50% sucrose solution (10). Each soil sample was mixed in a substantial volume of water and decanted through a series of sieves of 500, 250 and 50 µm. The contents from 250 and 50 µm sieves were mixed with water and centrifuged for 5 minutes at 1500 RPM. After having discarded the supernatant, the pellets were re-suspended in 50% sucrose solution and centrifuged for 1 minute. The supernatant was carefully poured through a 50 µm sieve and washed with water to remove the sucrose.

Finally spores, spore clusters and sporocarps were carefully washed and transferred to a Petri dish for spore counting under the Nikon stereo microscope at ×4. Enumeration of spore numbers per gram of dry soil was undertaken according to INVAM.

Identification and characterization of spores

About 50-70% of healthy spores were picked with brush and mounted on slides in polyvinyl-lactic acid-glycerol (PVLG), (11) or in PVLG mixed with Melzer's reagent (1:1 v/v) (12). Spores were examined under a compound microscope magnification of ×400 and identified to the species level or to a specific morphotype by using standard manuals.

Estimation of isolation frequency

The samples collected were screened for the presence of AM fungi genera by wet sieving and decantation method and calculated the isolation frequency (IF) as given below

$$\textbf{Isolation frequency (IF) (\%)} = \frac{\textbf{Number of samples consisting a AMF species}}{\textbf{Total number of samples collected}}$$

RESULTS

In the present investigations geographical, climatic features of four selected locations were recorded. Similarly, soil physicochemical characteristics were determined, and the results are presented in Table 1 and Table 2. All the four test locations are found to be on the same latitude and longitude. However, the altitudinal variations are noticed Yellandu JK-5 OCP is in highest altitudinal location, whereas Mancherial RK5 is in lowest altitude. The average annual rainfall varied between 977mm and 1099 i.e. about 100mm difference. In all the locations humidity was highest in rainy season followed by winter and summer. Highest temperature recorded in summer varied with the location: highest for Bhupalpally and low in Mancherial RK5. Winter seasons recorded less temperatures i.e. around 16^{o}C Rainy temperatures ranged between 18.2 and 21.5^{0}C

A perusal of the Table2 reveals that soils of four locations distinctly varied in physio chemical characteristics. Bhupalpally soils and Mancherial soils are sandy loam and silt loam respectively, whereas Yellandu and Ramagundam soils are clay loam soils. Bhupalpally and Mancherial location soils are acidic. However, Yellandu and Ramagundam soils are near to neutral. Water holding capacity of clay loam soils are found to be 30% (Yellandu), 25% (Ramagundam), 16% (Mancherial) silt soils and 10% (Bhupalpally) sandy loam soil. Electrical conductivities of four test soils varied between 0.11 and 0.19. The present test soils did not vary with regard to available phosphorus. It varied between 9.6 and 14.5.

Similarly, there is no significant variation in available nitrogen. The available phosphorous and nitrogen in

all the four disturbed coalmine spoils is very less when compared to normal undisturbed soils. There is much variation in cathode exchange capacities (CEC) of the soils. Least CEC was for Bhupalpally and highest for Ramagundam spoils.

Mycorrhizal status of plant species growing in four coalmine spoils

In order to assess the mycorrhizal status of four coalmine spoil sites 30 plant species commonly growing in all the four sites were selected and the mycorrhizal infection percentage, nature of infection was determined. Similarly, the population of arbuscular mycorrhizal fungal species was also estimated (Table 3-6). A critical perusal of the table-4 reveals the percentage of mycorrhizal infection in different plant species growing in Yellandu JK-5 OCP varied from species to species. The highest percentage and lowest percentage of infections were recorded in *Tephrosia purpurea* and *Digitaria r*espectively. All the plant species were associated with vesicular or arbuscular or mycorrhizal associations. Mycorrhizal spore population also varied with the plant species with maximum spore population in the rhizosphere of *Cleome viscosa* and lowest in *Phyllanthus amarus.* Mycorrhizal status of the test plants in winter is different from summer. In this season highest infection rate was recorded in *Sapindus emarginatus* and lowest in *Azadiracta indica*. The spore population in rhizosphere of different plant species ranged between 23-56 percent. Such variations were also recorded in rainy season. Vesicular structures were more frequently observed than in summer and winter.

Table 1: Geographical and climatic features of selected coal mine locations.

Study site	Latitude	Longitude	Altitude(m)	Rainfall (mm)	Humidity %			Temperature° C		
					Summer	Winter	Rainy	Summer	Winter	Rainy
Bhupalpally KtK5 incline	18.4314° N	79.8584° E	219m	977	39.6	57	68.5	40	18.2	21.5
Yellandu JK-5 OCP	17.5941° N	80.3224° E	252m	1099	32.2	49.3	59.5	41	20	21.2
Mancherial RK5	18.8891° N	79.4858° E	159m	1086	31.1	45.6	62.1	40	16.3	21.2
Ramagundam (OCP2)	18.7519°N	79.5134° E	179m	1081	29.9	47.8	66.2	42	16	18.2

Table 2: Physico- chemical characteristic of soil of selected coal mine locations.

Soil type	Sample collection area	Soil texture	pH	WHC (%)	EC (mhos /cm)	Organic mattr (%)	Available phosphorus (kg/hec)	Available Nitrogen (kg/hec)	CEC (meq /100g)
Coalmine soil	**Bhupalpally**	**Sandyloam**	4.8	10	0.11	0.10	9.6	32.1	3.94
	Yellandhu	**Clay loam**	6.1	30	0.18	0.17	14.5	38.5	6.7
	Mancherial	**Silt loam**	4.8	16	0.13	0.12	13.2	40.1	5.9
	Ramagundam	**Clay loam**	6.1	25	0.19	0.15	10.9	29.3	8.2

Mycorrhizal status Mancherial RK5 plants and soils are different from the Yellandu coalmine spoils (Table-4). Though, all the plants were observed to be mycorrhizal infection rate and spore population varied with the plant species. In summer, highest infection percentage was observed in *Ficus religiosa* and lowest in *Gompherena celosioides.* Vesicular structures were more frequently observed. Spore population varied between 34-61 percent. In general, mycorrhizal infection and spore population were less than the summer. Infection percentage in different plant species varied between 32.1-56.2 percent, and spore population between 29 and 61. In comparison to vesicles, arbuscular and mycorrhizal infections were observed to be less. Infection rates were observed to be more in rainy season than in summer and winter. Similar to summer and winter vesicular structures were found to be more. Mycorrhizal spore populations are recorded to be more in rainy season when compared to summer and winter. The highest spore population was recorded in *Parthenium hysterophorus*, however, not in proportionate with percentage of infection.

All the plant species growing in Bhupalpally coalmine sites have exhibited mycorrhizal infection and alsoin all the seasons. However, the selected values of selected parameters varied both with plant species and season. In summer percentage of infection

varied between 19.2 and 50.1 depending upon the plant species. All the plant species were with one or other characteristic features of mycorrhizal infection. Spore population varied between 16 and 51. Mycorrhizal infection percentage in winter for different plant species ranged between 21.3 and 51.2. The spore population was between 21 and 61. In general, spore population was found to be slightly more than the summer. In rainy season, mycorrhizal infection percentage was found to be more than the other two seasons. However, the minimum and maximum were less with corresponding values of summer and winter. In rainy season, more species were associated with vesicles. The spore population ranged between 16 and 56 with minimum in *Physalis minima* and maximum in *Digeria muricata.*

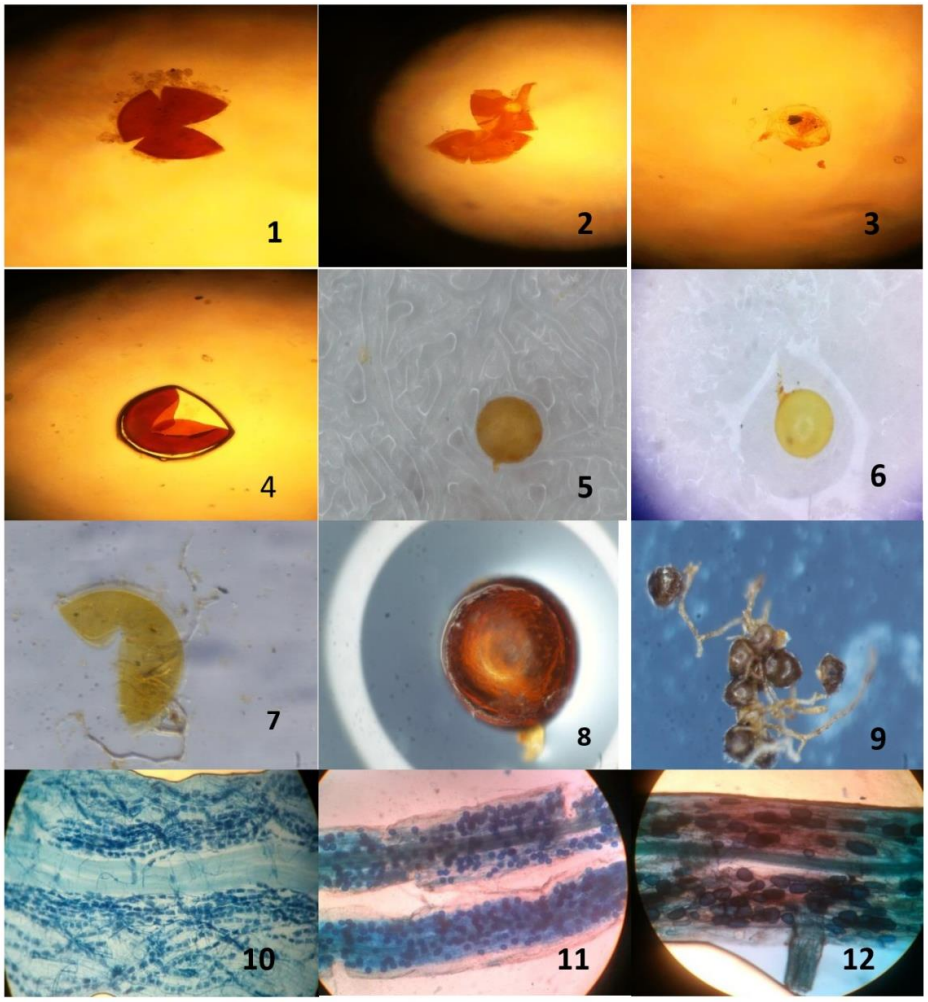

Fig:2.Some of the AM fungal isolated spores an root infection from diferent coalmine soils

1.Acaulospora laevis, 2, A. rugosa 3. Gigaspora decipiens, 4. Gigaspora margarita, 5. Entrophospora colombiana,6. Glomusclarum, 7.G. fasciculatum, 8.G. monosporum, 9. Glomus aggregatum, 10,11&12 Mycorhizal infection(Vesicles and Arbuscules) in roots.

Table 3: Arbuscular Mycorrhizal status of different plant species growing in coal mine soils of Yellandu JK-5 OCP.

Soil type: Coal mine												*Location:Yellandu JK-5 OCP*				
Sl. No.	***Plant species***	***Summer % of infection***	***V***	***A***	***M***	***No of spores***	***Winter % of infection***	***V***	***A***	***M***	***No of spores***	***Rainy % of infection***	***V***	***A***	***m***	***No of spores***
1	***Parthenium hysterophorus (Asteraceae)***	41.5	-	+	-	48	42.5	-	+	-	46	46.5	+	+	-	46
2	***Amaranthus viridis (Amaranthaceae)***	46.4	-	+	-	51	39.6	-	+	-	55	39.6	+	-	-	48
3	***Aervalanata (Amaranthaceae)***	44.9	-	+	-	48	39.8	-	+	-	55	48.5	-	+	-	47
4	***Achyranthes aspera (Amaranthaceae)***	49.1	-	+	-	45	45.6	+	-	-	56	36.5	-	-	+	44
5	***Digeramuricate (Amaranthaceae)***	39.6	-	-	+	41	44.9	+	+	-	51	41.2	-	-	+	45
6	***Gomphrena celosioides (Amaranthaceae)***	41.7	-	-	+	39	46.5	+	-	+	48	43.2	+	-	+	31
7	***Ficusreligiosa (Moraceae)***	46.4	-	-	+	56	41.8	+	-	-	41	39.5	+	-	-	36
8	***Andrographis echioides (Acanthaceae)***	49.4	+	-	-	51	49.1	-	+	+	39	38.1	+	-	-	49
9	***Calotropis gigantea (Apocynaceae)***	43.5	+	-	-	49	44.8	-	+	+	41	36.2	-	-	+	46
10	***Brassicanigra (Brassicaceae)***	44.1	+	-	-	44	49.9	+	-	-	36	39.1	-	-	+	47
11	***Tridaxprocumbens (Asteraceae)***	38.6	+	-	-	46	48.6	+	-	-	48	40.5	+	-	-	51
12	***Taraxacum sp. (Asteraceae)***	37.2	-	-	+	48	44.8	-	+	+	41	46.2	+	-	-	56
13	***Cleome viscosa (Cleomaceae)***	38.9	-	-	+	59	47.9	+	-	-	52	47.8	-	-	+	57
14	***Digitariasp.s (Poaceae)***	33.9	-	-	+	51	49.1	-	+	-	56	35.8	-	-	+	49

15	***Luffa echinate (Cucurbitaceae)***	34.9	-	-	+	56	48.5	-	-	+	48	29.4	+	-	-	41
16	***Euphorbia heterophylla (Euphorbiaceae)***	37.1	-	+	-	54	39.5	+	-	-	39	46.9	+	-	-	48
17	***Acalyphaindica (Euphorbiaceae)***	49.1	-	+	-	58	26.5	-	-	+	36	38.5	+	-	-	44
18	***Acacia nilotica (Fabaceae)***	46.1	+	-	+	47	28.9	+	-	-	23	51.2	-	-	+	45
19	***Peltophorumpterocarpum (Fabaceae)***	48.1	+	+	+	48	29.1	-	-	+	38	41.5	+	-	-	57
20	***Azadirachtaindica (Meliaceae)***	49.3	+	+	-	49	22.6	+	-	-	39	46.2	+	-	-	49
21	***Tephrosiapurpurea (Fabaceae)***	56.1	+	-	+	41	36.5	-	-	+	41	48.2	+	-	-	41
22	***Crotalaria pallida (Fabaceae)***	51.5	+	-	+	48	35.5	+	-	-	36	45.1	+	-	-	44
23	***Leonotisnepetifolia (Lamiaceae)***	48.2	+	-	-	49	34.5	+	-	-	35	47.2	+	-	+	45
24	***Leucas aspera (Lamiaceae)***	47.1	+	-	+	39	39.1	+	-	-	34	48.1	+	-	+	46
25	***Abutilon indicum (Malvaceae)***	41.2	+	-	+	36	38.2	+	-	-	41	39.5	-	-	+	51
26	***Phyllanthus amarus (Phyllanthaceae)***	48.1	-	-	+	34	46.1	+	-	-	46	39.6	+	-	-	48
27	***Phyllanthus acidus(Phyllanthaceae)***	44.1	+	-	-	44	44.7	+	-	-	56	48.5	-	-	+	47
28	***Physalis minima (Solanaceae)***	49.2	-	+	-	39	48.5	-	-	+	47	51.5	+	-	-	49
29	***Sapindusemarginatus (Sapindaceae)***	44.6	+	-	-	49	58.7	-	-	+	44	47.1	+	-	+	48
30	***Stachytarphetajamaicensis (Verbenaceae)***	48.2	-	-	+	51	48.1	+	+	+	56	47.2	+	-	+	46

a) V= Vesicles b) A= Arbuscules C) M = Myceliu

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Table4: Arbuscular Mycorrhizal status of different plant species growing in coal mine soils of Mancherial RK5.

Soil type: Coal mine											Location: Mancherial RK5				
S. No	Plant species	Summer				Winter					Rainy				
		% of infection	V	A	M	No of spores	% of infection	V	A	M	No of spores	% of infection	V	A	m	No of spores
1	*Parthenium hysterophorus* (Asteraceae)	35.3	+	-	+	42	32.3	+	-	-	48	48.6	+	+	-	72
2	*Amaranthus viridis* (Amaranthaceae)	48.6	+	-	-	38	36.8	+	+	-	46	49.7	+	-	-	71
3	*Aervalanata* (Amaranthaceae)	42.9	+	-	-	37	39.4	+	+	-	49	50.8	+	-	-	62
4	*Achyranthes aspera* (Amaranthaceae)	36.9	+	-	-	37	41.4	-	+	-	52	56.2	+	-	-	36
5	*Digeramuricata* (Amaranthaceae)	50.2	+	-	-	36	46.4	+	-	-	53	58.2	+	-	-	45
6	*Gomphrena celosioides* (Amaranthaceae)	32.1	+	+	-	39	47.4	+	-	-	34	39.4	+	+	-	49
7	*Ficusreligiosa*(Moraceae)	56.2	+	+	-	47	47.5	+	-	-	61	39.4	+	+	-	49
8	*Andrographis echioides*(Acanthaceae)	46.4	-	-	+	48	49.1	+	+	-	39	36.5	-	+	-	58
9	*Calotropis gigantea* (Apocynaceae)	48.2	-	+	-	49	48.1	+	+	-	40	39.6	-	+	-	59
10	*Brassica nigra* (Brassicaceae)	39.2	-	+	-	50	46.2	+	+	-	45	41.5	+	-	-	51
11	*Tridaxprocumbens* (Asteraceae)	36.2	+	-	-	51	47.3	-	+	-	46	37.5	+	-	-	53
12	*Taraxacum sp.(Asteraceae)*	46.5	+	-	-	42	41.3	+	-	-	48	36.5	+	-	-	56
13	*Cleome viscosa* (Cleomaceae)	47.8	+	+	-	49	46.2	+	-	-	49	32.5	+	-	-	49
14	*Digitaria sp. (Poaceae)*	48.9	+	+	-	56	47.3	-	+	-	51	46.5	+	-	-	61
15	*Luffa echinate* (Cucurbitaceae)	41.5	+	-	-	49	39.4	-	+	-	56	69.5	+	-	-	71
16	*Euphorbia heterophylla* (Euphorbiaceae)	47.5	-	-	+	49	41.5	+	-	-	51	49.5	+	-	+	46
17	*Acalyphaindica* (Euphorbiaceae)	46.5	-	-	+	48	32.8	-	+	-	61	47.5	+	-	-	49
18	*Acacia nilotica* (Fabaceae)	39.1	-	-	+	46	35.8	+	-	-	46	48.5	+	-	-	51
19	*Peltophorumpterocarpum* (Fabaceae)	46.5	-	-	+	37	41.7	+	-	-	36	49.6	+	-	-	46

20	***Azadirachtaindica (*Meliaceae)**	47.5	+	-	-	41	42.8	-	-	+	35	50.6	-	-	+	48
21	***Tephrosiapurpurea* (Fabaceae)**	48.1	+	+	-	51	39.4	+	-	-	48	58.5	+	+	-	49
22	***Crotalaria pallida* (Fabaceae)**	49.2	-	-	+	52	40.1	+	-	-	49	59.2	+	+	-	51
23	***Leonotisnepetifolia*(Lamiaceae)**	48.5	-	+	-	56	41.2	+	-	-	36	60.5	+	-	-	58
24	***Leucas aspera* (Lamiaceae)**	46.2	-	+	-	57	46.5	+	-	-	42	61.5	+	-	-	61
25	***Abutilon indicum*(Malvaceae)**	44.2	-	+	-	61	39.4	+	-	-	48	49.5	+	-	-	55
26	***Phyllanthus amarus(*Phyllanthaceae)**	36.2	+	-	-	51	42.1	-	+	-	34	21.1	+	-	+	41
27	***Phyllanthus acidus*(Phyllanthaceae*)***	39.5	+	-	-	41	56.2	-	+	-	45	42.3	-	+	-	46
28	***Physalis minima* (Solanaceae)**	45.3	+	+	-	50	32.1	+	-	+	48	43.2	-	+	+	46
29	***Sapindusemarginatus(*Sapindaceae)**	34.1	-	+	+	34	41.2	+	-	+	43	50.1	-	+	-	48
30	***Stachytarphetajamaicensis*(Verbenaceae)**	45.2	+	+	-	49	47.2	-	-	+	29	43.1	+	-	+	54

a) V= Vesicles b) A= Arbuscules C) M = Mycelium

Table5: Arbuscular Mycorrhizal status of different plant species growing in coal mine soils of Bhupalpally KTK5 Incline.

Soil type: Coal mine												Location: Bhupalpally KtK5 incline				
S.n	Plant species	Summer % of infection	V	A	M	No of spores	Winter % of infection	V	A	M	No of spores	Rainy % of infection	V	A	m	No of spores
1	*Parthenium hysterophorus* (Asteraceae)	26.5	+	-	-	46	41.5	+	-	-	46	38.9	+	-	-	45
2	*Amaranthus viridis* (Amaranthaceae)	32.4	+	-	-	44	46.2	+	-	-	51	46.9	+	-	-	46
3	*Aervalanata* (Amaranthaceae)	41.2	-	+	-	36	39.2	+	+	-	39	44.9	+	+	-	39
4	*Achyranthes aspera* (Amaranthaceae)	46.2	-	+	-	29	26.2	+	+	-	42	51.6	+	+	+	48
5	*Digeramuricata* (Amaranthaceae)	43.2	+	+	-	38	27.2	+	-	-	46	46.7	+	+	-	56
6	*Gomphrena celosioides* (Amaranthaceae)	39.5	+	-	-	32	22.4	-	+	-	45	48.9	+	+	-	51
7	*Ficusreligiosa* (Moraceae)	38.6	+	-	-	36	28.5	-	+	-	44	44.1	+	-	-	55
8	*Andrographis echioides* (Acanthaceae)	36.5	+	-	-	31	26.4	-	-	+	59	47.8	+	-	-	49
9	*Calotropis gigantea* (Apocynaceae)	32.5	+	-	-	29	36.5	+	+	-	39	40.2	-	+	-	55
10	*Brassica nigra* (Brassicaceae)	33.2	+	-	-	20	42.5	+	+	-	61	49.1	-	+	-	41
11	*Tridaxprocumbens* (Asteraceae)	41.5	-	-	+	35	46.6	+	+	+	44	50.1	+	-	-	55
12	*Taraxacum sp.* (Asteraceae)	46.5	-	+	-	40	41.2	-	-	+	45	51.5	+	-	-	51
13	*Cleome viscosa* (Cleomaceae)	49.5	-	+	+	45	47.1	+	-	-	39	56.5	+	+	-	52
14	*Digitaria sp.* (Poaceae)	49.6	-	-	+	46	39.5	+	-	-	46	49.2	+	+	+	56
15	*Luffa echinata* (Cucurbitaceae)	39.8	-	-	+	47	36.5	-	+	-	41	48.5	-	-	+	49
16	*Euphorbia heterophylla* (Euphorbiaceae)	36.8	-	-	+	48	41.4	+	-	-	42	46.1	-	-	+	48
17	*Acalyphaindica* (Euphorbiaceae)	37.7	-	-	+	49	47.4	-	-	+	43	48.2	-	-	+	49
18	*Acacia nilotica* (Fabaceae)	39.5	+	-	-	51	34.5	+	-	-	48	39.6	-	-	+	39
19	*Peltophorumpterocarpum* (Fabaceae)	40.1	+	-	-	42	36.2	-	-	+	49	34.1	+	-	-	36
20	*Azadirachtaindica* (Meliaceae)	42.5	+	-	-	41	28.4	-	-	+	51	38.7	-	+	-	38
21	*Tephrosiapurpurea* (Fabaceae)	46.3	+	-	+	46	46.4	+	-	-	56	39.5	-	-	+	41
22	*Crotalaria pallida* (Fabaceae)	47.5	+	-	-	47	41.9	-	-	+	34	40.5	+	-	-	46
23	*Leonotisnepetifolia* (Lamiaceae)	46.1	+	-	+	41	51.2	+	-	-	47	41.1	+	-	-	45

24	***Leucas aspera*** **(Lamiaceae)**	49.7	+	-	-	36	46.1	+	-	-	48	43.5	+	-	-	49
25	***Abutilon indicum*** **(Malvaceae)**	50.1	+	-	-	31	49.2	+	-	-	41	46.2	-	-	+	51
26	***Phyllanthus amarus*** ***(*****Phyllanthaceae)**	36.5	-	-	+	35	39.6	-	-	+	40	44.1	+	-	-	52
27	***Phyllanthus acidus*** **(Phyllanthaceae*)***	23.6	-	+	+	25	21.3	-	+	-	31	18.3	+	-	+	21
28	***Physalis minima*** **(Solanaceae)**	19.2	-	+	-	28	24.3	+	-	+	21	15.2	-	+	-	16
29	***Sapindusemarginatus*** ***(*****Sapindaceae)**	24.2	+	-	-	16	25.3	+	-	+	28	19.3	-	+	-	25
30	**Stachytarphetajamaicensis (Verbenaceae)**	31.2	+	-	+	25	21.5	+	-	+	24	13.5	+	-	+	25

a) ***V= Vesicles*** ***b) A= Arbuscules*** ***C) M = Mycelium***

Table6: Arbuscular Mycorrhizal status of different plant species growing in coal mine soils of Ramagundam (OCP2).

Soil type:	**Coal mine**															
		Summer					**Winter**					**Rainy**				
S. n	**Plant species**	**% of infection**	**V**	**A**	**M**	**No of spores**	**% of infection**	**V**	**A**	**M**	**No of spores**	**% of infection**	**V**	**A**	**m**	**No of spores**
1	***Parthenium hysterophorus*** **(Asteraceae)**	45.2	+	-	-	56	51.2	+	+	-	41	35.2	+	+	-	45
2	***Amaranthus viridis*** **(Amaranthaceae)**	52.1	+	-	+	47	32.1	-	+	-	48	50.2	+	-	+	61
3	***Aervalanata*** **(Amaranthaceae)**	43.1	-	+	-	41	42.1	+	-	-	42	53.2	-	+	-	45
4	***Achyranthes aspera*** **(Amaranthaceae)**	35.2	+	-	-	49	52.1	-	+	-	45	41.2	+	-	+	41
5	***Digeramuricata*** **(Amaranthaceae)**	36.2	+	-	-	41	45.2	-	+	-	41	42.1	-	+	-	52
6	***Gomphrena celosioides*** **(Amaranthaceae)**	52.4	-	+	-	54	38.9	-	+	-	45	40.2	+	-	+	49
7	***Ficusreligiosa*** **(Moraceae*)***	45.2	-	+	-	48	39.5	+	-	+	52	46.9	-	+	-	51
8	***Andrographis echioides*** **(Acanthaceae)**	56.2	+	-	+	52	50.1	-	+	-	65	46..9	-	+	-	48
9	***Calotropis gigantea*** **(Apocynaceae)**	45.2	-	+	-	48	56.2	+	-	+	45	35.5	+	-	+	56
10	***Brassicanigra*** **(Brassicaceae)**	42.1	+	-	+	35	32.1	-	+	-	35	42.1	+	-	+	36
11	***Tridaxprocumbens*** **(Asteraceae)**	52.1	+	-	+	56	45.2	+	-	-	41	45.3	+	+	+	65
12	***Taraxacum sp. (asteraceae)***	49.6	+	-	+	47	38.2	+	-	-	48	47.8	+	-	-	48
13	***Cleome viscosa*** **(Cleomaceae*)***	47.8	+	-	-	48	39.4	+	+	-	47	41.9	-	-	+	49

14	***Digitaria sp. (poaceae)***	41.9	-	-	+	49	40.1	-	+	-	48	49.1	+	-	+	44
15	***Luffa echinata*** **(Cucurbitaceae)**	50.1	+	-	-	48	39.1	-	+	-	51	39.1	-	-	+	56
16	***Euphorbia heterophylla*** **(Euphorbiaceae)**	58.5	+	-	+	61	46.2	-	+	-	56	49.1	-	-	+	34
17	***Acalyphaindica*** **(Euphorbiaceae)**	49.1	-	-	+	42	46.2	-	+	-	48	36.4	+	+	-	41
18	***Acacia nilotica*** **(Fabaceae)**	52.5	+	-	+	54	58.6	+	-	+	61	59.4	+	-	+	51
19	***Peltophorumpterocarpum*** **(Fabaceae)**	49.3	-	+	-	49	50.2	-	+	-	65	42.1	-	+	-	46
20	***Azadirachtaindica*** **(Meliaceae)**	41.2	+	-	-	54	41.2	+	-	-	52	38.2	+	-	+	42
21	***Tephrosiapurpurea*** **(Fabaceae)**	36.1	+	-	-	32	35.4	-	-	+	39	41.2	+	-	-	34
22	***Crotalaria pallida*** **(Fabaceae)**	26.1	-	+	-	41	43.2	+	-	+	51	42.3	+	-	-	39
23	***Leonotisnepetifolia*** **(Lamiaceae)**	33.5	+	-	+	25	45.1	-	+	-	46	46.3	-	+	-	54
24	***Leucas aspera*** **(Lamiaceae)**	45.2	-	+	+	61	23.1	+	-	+	36	41.2	-	+	-	58
25	***Abutilon indicum*** **(Malvaceae)**	49.3	-	+	-	36	36.4	-	-	+	24	53.1	+	-	+	26
26	***Phyllanthus amarus*** **(Phyllanthaceae)**	32.8	+	-	+	29	21.4	-	+	-	29	36.2	-	-	+	37
27	***Phyllanthus acidus*** **(Phyllanthaceae)**	26.3	+	-	-	18	36.2	+	-	+	35	46.9	+	-	-	35
28	***Physalis minima*** **(Solanaceae)**	52.3	+	-	-	24	21.4	-	+	-	34	36.4	+	-	+	41
29	***Sapindusemarginatus*** **(Sapindaceae)**	22.3	-	-	+	31	25.6	-	+	-	35	37.8	-	-	+	23
30	**Stachytarphetajamaicensis (Verbenaceae)**	32.5	-	+	-	35	31.1	+	-	+	32	28.4	+	-	+	28

a) V= Vesicles b) A= Arbuscules C) M = Mycelium

Table7: Diversity and distribution of Arbuscular mycorrhizal fungal species in four coal mine locations

AMF Species	Location			
	Bhupalpally KtK5 incline	**Yellandu JK-5 OCP**	**Mancherial RK5**	**Ramagundam (OCP2)**
Acaulospora				
A. laevis	22.1	-	16.2	-
A. delicata	-	-	-	21.3
A. rugosa	15.6	21.2	-	21.1
A. foveata	-	25.1	-	19.3
A. scrobiculatum	-	18.2	-	10.2
Gigaspora				
G.margarita	13.2	-	-	16.3
G. gigantea	-	12.1	-	11.6

G. albida	18.2	-	-	16.6
G. decipiens	-	-	-	14.2
Entrophospora				
E. colombiana	-	23.1	-	15.3
E. infrequens	13.2	-	19.1	-
Glomus				
G. clarum	21.1	19.6	-	-
G. clavispora	-	22.1	-	16.5
G. aggregatum	21.4	-	18.2	-
G. constrictum	-	14.2	-	13.2
G. caledonium	16.4	-	14.2	21.4
G. ambisporum	-	31.6	-	21.1
G. fasciculatum	24.2	-	16.5	-
G. monosporum	-	29.3	-	19.9
G. intraradix	22.3	19.6	21.1	-
G. convolutum	-	26.2	25.1	-

Mycorrhizal status of Ramagundam coalmine spoils are found to be different from the other test sites. In summer mycorrhizal infection percentage for different plant species ranged between 22.3-58.5 with former value for *Sapindus emarginatus* and latter value for *Euphorbia heterophylla.* All the plant species in all the seasons were observed to be associated with any one of the mycorrhizal structures. Spore population in the rhizosphere soil was highest in *Euphorbia hyterophylla* and lowest in *Phyllathus amarus*. In winter, variation in infection percentages and spore population were with respect to different plant species. Maximum and minimum percentages of infection were recorded in *Acacia nilotica* and *Phyllanthus amarus*. Spore population in rhizosphere for different plant species varied. It was highest for *Andrographis echioides* and lowest in *Abutilon indicum*. The values of selected parameters for rainy season differed from summer and winter. Percentage of infection ranged between 28.4-59.4 and most of the plant species infection percentages are close to maximum value. *Tridax procumbense* plant roots were found to be associated with all the three characteristic structures. Spore population varied for different plant species. The population for different plant species ranged between 23 and 65 with maximum for *Tridax procumbense.*

Diversity and distribution of different AM fungal species in four coalmine spoils was determined and the results are presented in Table 7. A critical study of the table reveals. Fourteen fungal species were recorded from Ramagundam OCP2 followed by Yellandu JK-5 OCP. The least number of fungal species (7) were recorded from Mancherial RK-5. The species distribution also varied with the location. *Glomus* species were frequently observed in all the four locations followed by *Acaulospora* and least only one species for *Entrophospora*. (In all 10 of *Glomus* species were collected from all the four sites and Yellandu soil was represented by 7 species) In all 21 AM fungal species were recorded from all the sites. However, the species distribution varied with the genus and coalmine location. *Glomus*, *Acaulospora, Gigaspora* and *Entrophospora* were represented by 10, 5, 4 and 2 species respectively. Yellandu coalmine spoil was found to harbor 7 species followed by other locations five each. Out of 5 species of *Acaulospora*, Ramagundam soil is harbored 4 species and Mancherial coalmine only one species. All the four species of *Gigaspora* were recorded from Ramagundams OCP2 and interestingly all the species of *Gigaspora* are absent from this soil. Out of 2 species of *Entrophospora* at least one species is represented in all the four soils.

Species wise distribution also varied with the test location. No single species was recorded from all the four locations. Some species like *Acaulospora delicate* was recorded from only location. On the other hand, *A.rugosa*, *G.caledorium* and *G. introradics* were distributed in there test locations. Remaining species were found to be distributed in at least two locations. The abundance of different AM species in different locations varied significantly. In general *Glomus* species were found to dominate the three other species. *Gigaspora* species were found to be less abundant. Among four different locations, Yellandu JK-5 OCP was found to have a greater number of AM fungal species. It is followed by Mancherial RK-5 coalmine spoils. Thus, there is a wide variation in the diversity, distribution and abundance of AM fungal species in four coalmine spoils.

DISCUSSION

Coal mine spoils are usually unfavorable for plant growth and have different properties compared to normal undisturbed soils. In coalmine areas the topsoil gets seriously damaged, resulting in soil degradation through loss of soil structure, accelerated soil erosion, excessive leaching, competition, reduced soil pH, accumulation of heavy metals, depletion of organic matter, reduced cation exchange capacity (13). Plant community and microbial activity in the disturbed soils is severely affected. Along with bacteria beneficial to plant growth, arbuscular mycorrhizal fungi are affected. The hyphae network established by mycorrhizal fungi breaks when soils are initially moved and stockpiled (14). Fayuan Wang (15) investigated the ecological restoration of mining sites using arbuscular mycorrhizal fungi (16). Enlighted the contribution of AM fungi to soil aggregate formation. Therefore, establishment of spontaneous natural vegetation in difficult in open mine spoil bank (17). In the revegetation of disturbed coalmine soils, apart from other strategies, application of AM fungi as inoculum is considered as a promising strategy [18]. However, it is important to know the extent of disturbance and also depletion of mycorrhizal fungi in native topsoil. Such as information is necessary to assess whether native AM fungal population can be exploited or inoculation with exotic source is necessary. Studies on the occurrence of VA mycorrhizas in plants from coalmine wastes and their potential importance in plant colonization have been relatively few (19-21). A study conducted by [http://environment.scientific-journal.com.] indicated that application of native AM fungi is prospective for tropical reforestation in open cast coal mining area. The inoculum of native fungi is important especially in sites with low AM fungi inoculums potential such as in post coalmining field. In this context, it is necessary to assess the diversity and distribution of AM fungal species in coalmine disturbed soils. Kumar *et al* 2003, (22) assessed the diversity and distribution of AM fungi of Jayan (U.P) coalmine soils by selecting 79 plant species belonging to 30 families, out of which 94% plant species showed presence of VAM hyphae. They have categorized the plant species in to 5 groups based on level of mycorrhizal association. Three coalmine tips in the Illawarra region of New South Wales were investigated for the incidence of VA mycorrhizas in plant growing on them (23). All the plant species examined except species of *Personia*a nd *Banksia* (Proteaceae) were infected by VA mycorrhizal fungi. Most of the soil samples studied contained VA mycorrhizal endophytes. The study yielded three types of spores, *Glomus macrocarpus*, *Glomus mosseae* and *Sclerocysties rubiformis*. Interestingly, they observed endogonaceous spores in the rhizosphere of plants which showed no mycorrhizal infection (24) while studying the succession of mycorrhizal fungal communities on a coalmine spoils concluded that AMF fungal species communities seem to be influenced by biotic rather than abiotic factors (25) observed that species belonging to the genus *Glomus* was found to be widely distributed among the mine spoils. Nine species of VAM fungi belonging to the 3 major genera viz, *Acaulospora*, *Glomus* and *Gigaspora* were identified from a rehabilitated coalmine spoil at Birampur (MP) (26). VAM fungal infection varied from plant to plant among the thirty-one-plant species in the gypsum mine spoils in Rajasthan. Surprisingly, the percentage of root infection was higher among the plants growing in mine spoils than those found in the normal soil (27). *Glomus* was the most dominant genus isolated among the various mine spoils and mine spoil dumps in India (28-30) investigated the species diversity of AM fungi in Dalli-Rajhara iron mine overburden dump of Chhattisgarh. They could collect 71 species of AM fungi belonging to 9 families and 10 genera and among them *Glomus* spp. were found to be the most taxonomically diverse with 18 to 29 species.

CONCLUSION

The present investigations clearly reveal the variation in geographical, physicochemical characteristics of four selected coalmine locations. The mycorrhizal status of different plant species varied with the location and also season. All the locations were found to contain mycorrhizal spores, however in different levels. We conclude that these native AMF species can be exploited for revegetation of these disturbed locations.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

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