International Research Journal of Biological Sciences _____ ISSN 2278-3202
Vol. **4(7),** 73-78, July **(2015)** *Int. Res. J. Biological Sci.*

Natural Mycorrhizal Colonization of Plant Species growing in a Limestone Mine Spoil: Case Study from ACC, Coimbatore, India

Logaprabha V.* **and Tamilselvi K.S.**
Department of Botany, PSGR Krishnammal College for Women, Peelamedu, Coimbatore, Tamil Nadu – 641004, INDIA

Available online at: www.isca.in, www.isca.me
Received 25th April 2015, revised 19th May 2015, accepted 30th June 2015

Abstract

In the present study we examined the Arbuscular mycorrhizal association in forty seven angiospermic plants of the limestone mine spoils of Coimbatore, India. Forty one plants were colonized by AM fungi while Achyranthes aspera, Aerva lanata, Amaranthus viridis, Alternanthera pungens, (Amarantaceae), Carex speciosa (Cyperaceae)and Argemone mexicana (Papavaraceae) did not possess mycorrhizal association. Some of the common non-mycorrhizal plants, viz., Boerhaavia diffusa, Commelina bhengalensis and Trianthema portulacastrum were found to be associated with AM fungi. The soils of the study area were calcareous, sandy loam with a slight alkaline pH and low in available nutrients. A total of ten AMF spores were identified, which included the genus Acaulospora, Gigaspora and Glomus. Glomus was the most frequent genus observed in the study area.

Keywords: Mine spoils, limestone, ACC, Madukkarai, rhizosphere.

Introduction

Mine spoils are the overburden dumps of waste rocks removed by opencast mining operations. Mine spoils are unstable, physically, chemically and biologically disturbed ecosystems. The soil profile, microbial biota and nutritional aspects are greatly altered and hence vegetation in mine spoils is almost nil. The establishment of a stable nutrient cycle from plant growth and microbial processes is the most important step required in the long term mine spoil reclamation[1-3]. Arbuscular mycorrhizal fungi had been reported to be the effective micro-organism in mine reclamation[4-6]. They are obligate symbionts, which improves the plant to access the limited soil minerals like nitrogen, phosphorus and potassium in exchange for the photosynthetic products. Mycorrhiza can promote plant growth[7], enhance the soil structure[8], and thereby maintain the stability of the ecosystem[9]. The present study reveals the mycorrhizal status of the plants found in a naturally revegetated limestone mine spoils of Coimbatore, India.

Material and Methods

Study site: The experimental site is the different aged limestone mine spoil dumps located around the Associated Cement Carriers factory, at Madukkarai, Coimbatore. The study area, limestone mine spoil dumps at Madukkarai is located 2.5 km around ACC Ltd., which lies about 10 km from Coimbatore, on NH 47 connecting Cochin and Salem.

Sampling: Sampling was done for a period of two years from September 2011 to August 2013. The study area was surveyed periodically every month, for the availability of plants in the different aged mine spoils. The rhizosphere soil was collected at a depth of 3-30 cm, where most root proliferation occurs. The soil was brought to the laboratory in clean polythene bags, shade dried and stored at 2 - 5°C until further analysis. Roots from the plants were collected by digging out the soil and carefully removing the roots without damaging them.

Soil analysis: The physico-chemical properties of the soil such as texture, pH, EC, available nitrogen (Kjeldahl method)[10], available phosphorus (Bray I method)[10] and available potassium[11] were analysed.

Mycorrhizal Colonization: The fine roots were carefully removed, washed free of soil in running tap water without damaging the external mycelium and cut into 1-2 cm long segments. The root bits were preserved in FAA (Formalin Acetic acid Alcohol – Formaldehyde, Glacial Acetic Acid and Ethanol in the ratio 1:1:18, respectively) immediately after collection. Percent root colonization of AM fungi was measured according to the root clearing and staining technique[12]. Fixed roots were thoroughly washed and cleared in 2.5% KOH[13], acidified with 5N HCl and stained with trypan blue (0.05% in lactophenol). Darkly stained roots were bleached using 3% H_2O_2. The roots were left overnight in the dye for staining and the excess stain was removed in clear lactophenol. The root segments were mounted on slides using polyvinyl lactoglycerol as a mounting medium and examined under compound microscope for the vesicles, arbuscules and hyphae. The slides were made semi-permanent by sealing the edges of the cover slip with DPX mountant.

Isolation of AM spores: The AM spores were isolated from the soil sample by following the wet-sieving and decanting

technique developed by Gerdemann and Nicolson[14]. One hundred grams of the soil sample were suspended in a litre of water. Heavier soil particles were allowed to settle down gradually and the liquid was decanted through nested sieves of 37µm to 710µm. The residues from the sieves were washed into separate beakers and collected over a filter paper. Each filter paper was spread over a glass plate and the spores were counted under an appropriate magnification (x100) of a stereo microscope. The spore population was expressed as the number of spores 100 gram^{-1} of soil. Intact spores were mounted using polyvinyl lacto glycerol and identified using Schenck and Perez manual[15] and Trappe[16]. Spores were identified based on spore morphology and sub cellular characters and compared with original descriptions[17]. Spore morphology was also compared with the culture database established by INVAM

Results and Discussion

Physico-chemical properties of the study area: The soils in the study sites were calcareous, sandy loam with a slight alkaline pH and low in available nutrients (table-1). The pH varied between 7.88 and 8.81. Electrical conductivity ranged from 0.41 to 3.07 dSm^{-1}. The amount of available N, P and K ranged from 108 to 179 kg/ha, 7 to 18 kg/ha and 102 to 1552 kg/ha respectively.

Occurrence of mycorrhiza: In the present study, 47 plants were collected, of which the major families were Amarantaceae, Asteraceae and Euphorbiaceae. Among the 47 plant species, 41 were colonized by AM fungi (Table 2). AM fungal colonization was absent in *Achyranthes aspera, Aerva lanata, Amaranthus viridis, Alternanthera pungens,* (Amarantaceae), *Carex speciosa* (Cyperaceae) and *Argemone mexicana* (Papavaraceae).

Majority of the plants under study were herbs (38), few were shrubs (7) and only 2 trees. Vesicular colonization was noticed in 37 species, *viz.,Ruellia tuberosa*, *Peristrophe bicalyculata* (Acanthaceae), *Catharanthus roseus* (Apocynaceae), *Pergularia daemia* (Asclepediaceae), *Conyza bonariensis, Eclipta prostrata, Eupatorium odoratum, Parthenium hysterophorus, Taraxacum officianale, Tridax procumbens, Vernonia cinerea* (Asteraceae), *Tecoma stans* (Bignoniaceae), *Cassis occidentalis* (Caesalpiniaceae), *Commelina bhenghalensis* (Commelinaceae), *Ipomoea indica, I. obscura* (Convolvulaceae), *Coccinia grandis* (Cucurbitaceae), *Acalypha indica, Croton bonplandianum*, *Euphorbia hirta, E. hypericifolia*, *E. prostrata, Phyllanthus niruri* (Euphorbiaceae), *Abutilon indicum* (Malvaceae), *Tinospora cordifolia* (Menispermaceae), *Prosopis juliflora* (Mimosaceae), *Muntingia calabura* (Muntingiaceae), *Boerhaavia diffusa* (Nyctaginaceae), *Passiflora foetida* (Passifloraceae), *Plumbago zeylanica* (Plumbaginaceae), *Chloris barbata, Cynodon dactylon,* (Poaceae), *Santalum album* (Santalaceae), *Scoparia dulcis* (Scrophulariaceae), *Datura metel, Solanum nigrum* (Solanaceae), *Lantana camara* (Verbenaceae).

Arbuscular colonization was observed only in 16 plant species out of the 47 studied. They were *Catharanthus roseus* (Apocynaceae), *Eupatorium odoratum, Taraxacum officianale, Tridax procumbens* (Asteraceae), *Tecoma stans* (Bignoniaceae), *Ipomoea obscura* (Convolvulaceae), *Melothriamadaraspatana* (Cucurbitaceae), *Acalypha indica, Croton bonplandianum*, *Euphorbia hirta, E. prostrata, Phyllanthus niruri* (Euphorbiaceae), *Passiflora foetida* (Passifloraceae), *Scoparia dulcis* (Scrophulariaceae), *Datura metel, Solanum nigrum* (Solanaceae).

The extent of colonization ranged from 15% (*Trianthema portulacastrum*) to 92% (*Euphorbia hirta*) in herbs, 10% (*Lantana camara*) to 64% (*Abutilon indicum*) in shrubs and 49% (*Muntingia calabura*) to 64% (*Santalum album*) in trees.

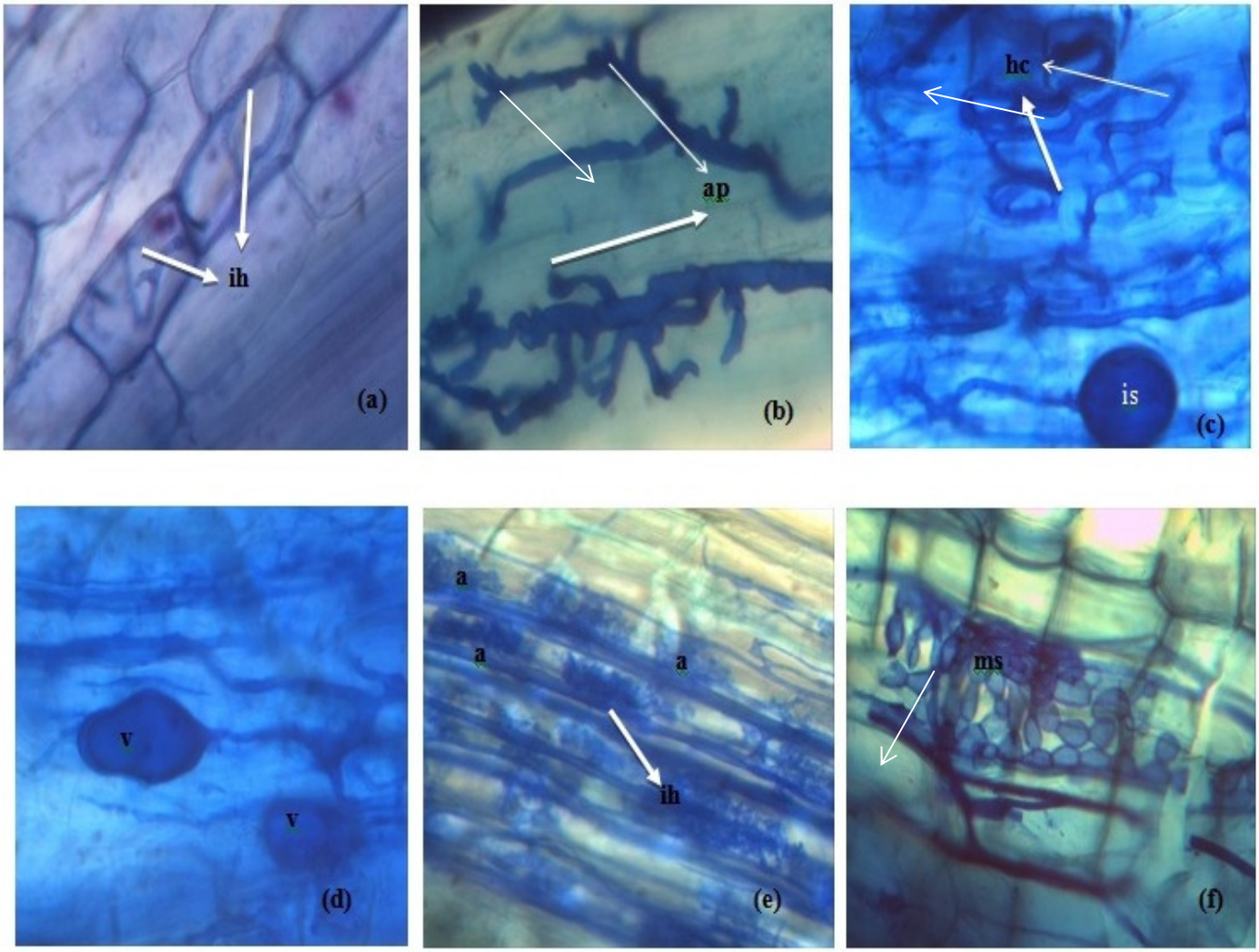
The entry of fungal hyphae into the roots was characterized by the presence of an appressorium (figure-1.b). The different AM fungal structures *viz.,* hyphal coils, intra-radical hyphae, inter or intra cellular vesicles and micro-sclerotia were observed (figure-1).

AM Fungal Diversity: A total of ten AM fungal spore morphotypes were identified based on their spore morphology. They included two species in *Acaulospora* (*Acaulospora leaves* and *A. scrobiculata*), one species in *Gigaspora* (*Gigaspora gigantea*) and seven species in *Glomus* (*Glomus aggregatum, G. diaphanum, G. etunicatum, G. fasciculatum, G. geosporum, G. intraradices* and *G. microaggregatum*) (figure-2).

Table-1
Physico-chemical characteristics of the 5 different limestone mine spoils of ACC, Madukkarai, Coimbatore

Soil No.	Lime Status	Texture	pH	EC dSm^{-1}	N kg ha^{-1}	P kg ha^{-1}	K kg ha^{-1}
1	Calcareous	Sandy loam	7.88	1.78	179	15.0	977
2	Calcareous	Sandy loam	8.81	0.41	109	17.0	102
3	Calcareous	Clay loam	7.96	0.75	132	18.0	1552
4	Non Calcareous	Sandy loam	8.30	3.07	108	7.0	1016
5	Calcareous	Sandy loam	8.09	0.50	140	13.0	1106

Samples taken in triplicate; EC- Electrical Conductivity; N- Available Nitrogen; P- Available Phosphorus; K- Available Potassium.



(a) intercellular hyphae in *Eclipta prostrata;* (b) appressorium in *Conyzabonariensis*; (c) hyphal coils and intra-radical spore in *Conyza bonariensis;* (d) vesicles in *Eupatorium odoratum*; (e) arbuscules in *Ipomoea indica*; (f) micro sclerotia in *Pergularia daemia* . ih, intercellular hyphae; ap, appressorium; hc, hyphal coils; is, intra-radical spore; v, vesicle; a, arbuscule; ms, micro sclerotia.

Figure-1
AM colonisation in the roots of the plants in the study area

Discussion: The limestone mine spoils generally supported several plant species. Majority of the primary colonizers of the mine spoils were associated with AM fungi. The level of AM infection markedly differed among various plants. Heavy root infection was recorded in *Croton bonplandianum* (92%) and *Ipomoea indica* (91%). Some of the plants which were previously reported to be non-mycorrhizal, were found to possess mycorrhizal association.

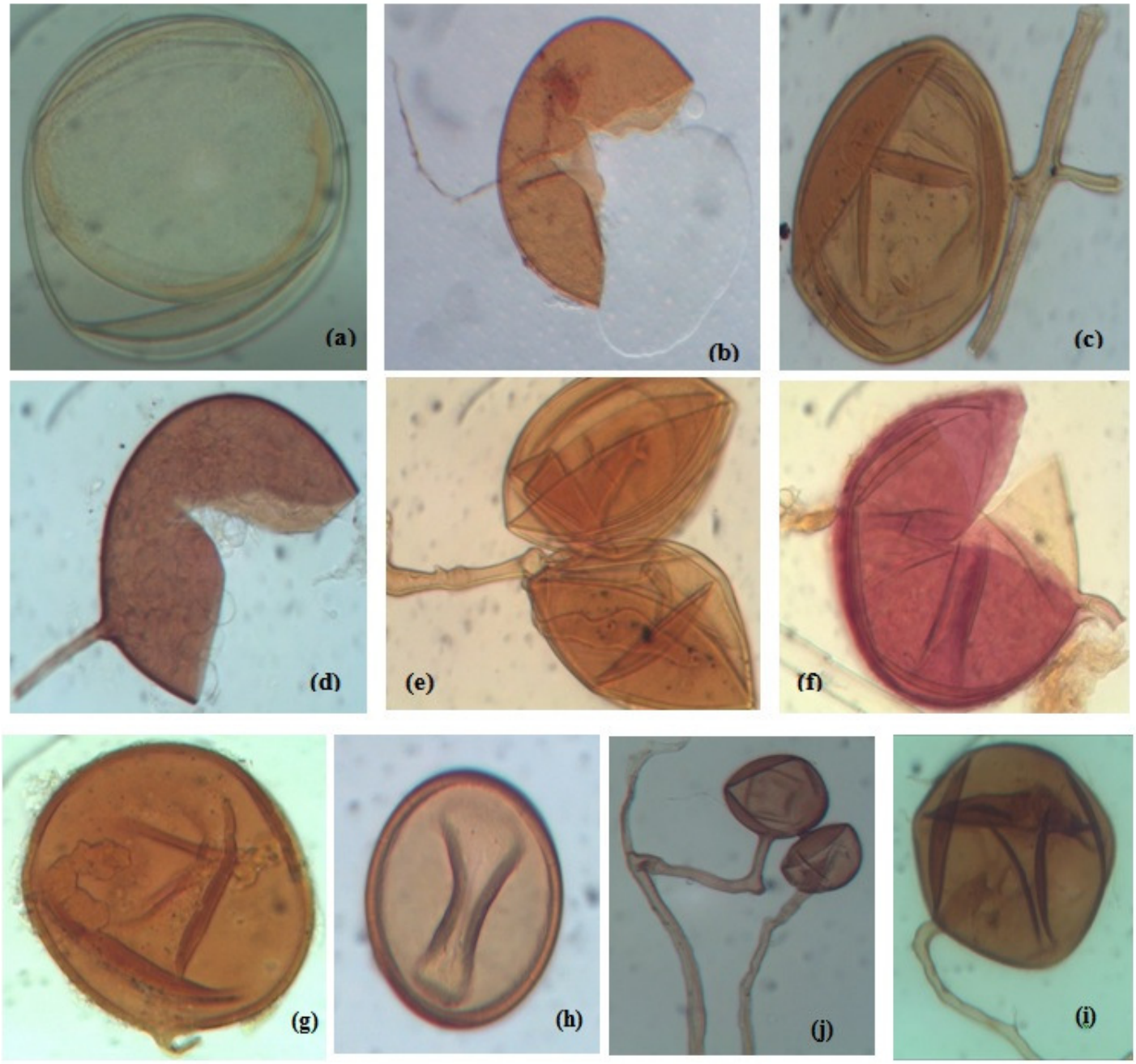
Plants belonging to the family Aizoaceae (*Trianthema portulacastrum*), Commelinaceae (*Commelina bhengalensis*) and Nyctaginacea (*Boerhaavia diffusa*), believed to be non-mycorrhizal plants were found to be associated with AM fungi. The extent of colonization was 15% in *Trianthema portulacastrum*; 26% in *Commelina bhengalensis* and 28% in *Boerhaavia diffusa.* AM colonisation has also been reported in *Boerhaavia diffusa* and *Commelina bhengalensis*[18,19]. Arbuscular colonization was not present in any of the three plant species, whereas vesicles were present in *Commelina bhengalensis* (less than 1 %) and *Boerhaavia diffusa* (3%).

Glomus was the dominant genus followed by *Acaulospora.* This is in accordance with the previous results of many researchers[20-22]. Pattern of spore population of *Glomus* might cause the dominancy of this genus[23]. Spores of *Glomus* are borne in clusters and hence they sporulate more frequently while the others like *Gigaspora* and *Scutellospora* sporulate singly[24].

Table-2
Arbuscular mycorrhizal association in the angiospermic plants of the study area

Family name	Plant name	Habit	Life cycle	AMF Colonization (%)		
				HC	VC	AC
Acanthaceae	*Ruellia tuberosa*	H	A/B	34.0±12.27	3.88±4.0	0.00
	Peristrophe bicalyculata	H	A/B	62.26±8.13	41.12±9.08	0.00
Aizoaceae	*Trianthema portulacastrum*	H	A	14.72±4.16	0.00	0.00
Amarantaceae	*Achyranthes aspera*	H	A	0.00	0.00	0.00
	Aerva lanata	H	A	0.00	0.00	0.00
	Amaranthus viridis	H	A	0.00	0.00	0.00
	Alternanthera pungens	H	A	0.00	0.00	0.00
Apocynaceae	*Vinca rosea*	H	A/B	53.89±5.45	20.83±5.47	3.06±2.51
Asclepediaceae	*Pergularia daemia*	TH	A/B	51.11±5.22	24.45±3.99	0.00
Asteraceae	*Conyza bonariensis*	H	A	41.95±9.24	6.11±7.73	0.00
	Eclipta prostrata	H	A	41.28±10.43	8.43±7.13	0.00
	Eupatorium odoratum	S	A	78.89±9.61	42.78±13.95	9.17±5.19
	Parthenium hysterophorus	H	A	57.78±6.79	20.55±7.23	0.00
	Taraxacum officianale	H	A	45.83±3.23	14.17±11.61	11.94±11.85
	Tridax procumbens	H	A	56.95±5.27	16.11±5.64	13.06±8.17
	Vernonia cinerea	H	A	49.72±9.07	7.50±6.12	0.00
Bignoniaceae	*Tecoma stans*	S	P	79.44±8.02	43.61±11.25	26.95±10.78
Boraginaceae	*Trichodesma indicum*	H	A	37.22±10.25	0.00	0.00
Caesalpiniaceae	*Cassis occidentalis*	S	P	68.33±3.62	10.28±4.37	0.00
Commelinaceae	*Commelina bhenghalensis*	H	A	25.56±10.86	0.56±0.56	0.00
Convolvulaceae	*Ipomoea indica*	TH	A	91.39±3.79	34.72±7.66	0.00
	I. obscura	TH	A	60.28±5.97	32.50±13.51	2.50±2.89
Cucurbitaceae	*Coccinia indica*	C	A	37.22±10.70	20.56±9.71	0.00
	Melothria madaraspatana	C	A	35.28±9.06	0.00	10.0±6.12
Cyperaceae	*Carex speciosa*	H	A	0.00	0.00	0.00
Euphorbiaceae	*Acalypha indica*	H	A	78.33±7.41	28.61±8.02	16.67±8.55
	Croton bonplandianum	H	A	91.94±2.88	9.44±4.82	4.20±2.17
	Euphorbia hirta	H	A	75.0±0.0	13.06±5.59	46.67±11.48
	E. hypericifolia	H	A	78.06±8.07	5.83±4.03	0.00
	E. prostrata	H	A	75.0±0	19.45±4.38	56.11±14.03
	Phyllanthus niruri	H	A	83.88±2.07	25.25±6.18	7.13±4.29
Malvaceae	*Abutilon indica*	S	A	63.88±3.24	39.50±3.06	0.00
Menispermaceae	*Tinospora cordifolia*	C	P	65.0±7.66	29.72±10.65	0.00
Mimosaceae	*Prosopis juliflora*	S	P	28.71±13.15	15.74±8.80	0.00
Muntingiaceae	*Muntingia calabura*	T	P	48.89±8.10	26.39±12.49	0.00
Nyctaginaceae	*Boerhaavia diffusa*	H	A	27.50±8.18	2.78±1.92	0.00
Papavaraceae	*Argemone mexicana*	H	A	0.00	0.00	0.00
Papilionaceae	*Crotolaria verrucosa*	S	P	25.0±0	0.00	0.00
Passifloraceae	*Passiflora foetida*	C	A	41.39±13.65	6.11±4.73	2.22±2.81
Plumbaginaceae	*Plumbago zeylanica*	H	A	16.03±4.56	1.50±3.0	0.00
Poaceae	*Chloris barbata*	H	A	68.79±7.11	5.46±2.71	0.00
	Cynodon dactylon	H	A	61.46±9.14	27.47±10.29	0.00
Santalaceae	*Santalum album*	T	P	63.61±5.63	10.0±3.69	0.00
Scrophulariaceae	*Scoparia dulcis*	H	A/B	53.89±10.89	33.89±15.86	3.89±3.01
Solanaceae	*Datura metel*	H	A	85.88±7.98	37.50±8.06	12.25±4.95
	Solanum nigrum	H	A	79.13±4.98	15.50±6.41	7.50±2.78
Verbenaceae	*Lantana camara*	S	P	10.35±20.41	8.33±4.45	0.00

Abbreviations used: H, herb; S, shrub; T, tree; TH, twining herb; C, climber; A, annual; B, biennial; A/B, annual or biennial; HC, hyphal colonisation; VC, vesicular colonisation; AC, arbuscular colonisation.



(a) Acaulospora laevis; (b) Gigaspora gigantea; *(c) Glomus fasciculatum*; *(d) G. microaggregatum; (e) G. diaphanum; (f) G. intraradices; (g) G. etunicatum; (h) A. scrobiculata*; *(i) G. aggregatum; (j) G. geosporum*

Figure-2
AM fungal spores isolated from the rhizosphere soils of the study area

Conclusion

From the study, it can be concluded that most of the plants in the limestone mine spoils survive due to the AM fungal associations. *Glomus* was the dominant genus found in the limestone mine spoils.

Acknowledgement

We thank the University Grants Commission, New Delhi, for the financial support.

References

1. Singh A.N., Raghubanshi A.S. and Singh J.S.,

Plantations as a tool for mine spoil restoration, *Curr. Sci.,* **82(12)**, 1436-1441 **(2002)**

2. Lone M.I., He Z.L., Stoffella P.J. and Yang X., Phytoremediation of heavy metal polluted soils and water: Progress and perspectives, *J. Zhejiang. Univ. Sci. B.,***9(3)**, 210-220 **(2008)**

3. Kavamura V.N. and Esposito E., Biotechnological strategies applied to the decontamination of soil polluted with heavy metals, *Biotechnology Advances.,* **28**, 61-69 **(2010)**

4. Quoreshi A.M., The use of mycorrhizal biotechnology in restoration of disturbed ecosystem, In: Mycorrhizae: *Sustainable agriculture and forestry,* Springer, Oxford, **(2008)**

5. Mukhopadhyay S. and Maiti S.K., Biofertiliser: VAM fungi-Future prospect for biological reclamation of mine degraded lands, *Ind. J. Environ. Prot.,* **29(9)**, 801-808 **(2009)**

6. Singh A.K., Jamaluddin, Status and diversity of arbuscular mycorrhizal fungi and its role in natural regeneration on limestone mined spoils, *Biodiversitas*., **12(2)**, 107-111 **(2011)**

7. Chen B., Roos P., Zhu Y. and Jakobsen I., Arbuscular mycorrhizas contribute to phytostabilization of uranium in uranium mining tailings, *J. Environ. Radioactiv.,* **99**, 801-810 **(2008)**

8. Rillig M.C. and Steinberg P.D., Glomalin production by an arbuscular mycorrhizal fungus a mechanism of habitat modification, *Soil. Biol. Biochem.,* **34**, 1371-1374 **(2002)**

9. Koide R.T. and Dickie I.A., Effects of mycorrhizal fungi on plant populations, *Plant Soil*., **244**, 307-317 **(2002)**

10. Jackson M.L., Soil chemical analysis, Prentice Hall, New Delhi, 498 **(1973)**

11. Sankaram A., A laboratory manual for agricultural chemistry, Asia Publishing House, 340 **(1966)**

12. Phillips J.M. and Hayman D.S., Improved procedure for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungus for rapid assessment of infection, *Trans. Br. Mycol. Soc.,* **55**, 158-161 **(1970)**

13. Koske R.E. and Gemma J.N., A modified procedure for staining roots to detect VA mycorrhizas, *Mycol. Res.,* **92**, 486-488 **(1989)**

14. Gerdemann J.W. and Nicolson T.H., Spores of mycorrhizal *Endogone* species extracted from soil by wet seiving and decanting, *Trans. Br. Mycol. Soc.,* **46**, 235-244 **(1963)**

15. Schenck N.C. and Perez Y., Manual for identification of VA mycorrhizal fungi. Synergistic Publications, Gainesville, Florida, USA., 1-286 **(1987)**

16. Trappe J.M., Synoptic keys to the genera and species of zygomyceteous (vesicular-arbuscular) mycorrhizal fungi, *Phytopathol.,* **72**, 1102-1108 **(1982)**

17. Schenck N.C. and Perez Y., Manual for the Identification of VA mycorrhizal fungi, Synergistic publications, Gainesville, Florida, 286 **(1990)**

18. Jain R.K., Hasan N., Singh R.K. and Pandey P.N., Vesicular arbuscular mycorrhizal (VAM) associations in some weeds of forage legumes, *Mycorrhiza News*., **5**, 9-11 **(1997)**

19. Muthukumar T. and Udaiyan K., Arbuscular mycorrhizas of plants growing in the Western Ghat region, Southern India, *Mycorrhiza.,***9**, 297-313 **(2000b)**

20. Muthukumar T. and Udaiyan K., Influence of organic manures of arbuscular mycorrhizal fungi associated with *Vigna unguiculata* (L.) Walp in relation to tissue nutrients and soluble carbohydrate in roots under field condition, *Biol. Fertil. Soils.,* **31**, 114-120 **(2000a)**

21. Klironomos J.N., Hart M.M., Gurney J.E. and Moutoglis P., Interspecific differences in the tolerance of arbuscular mycorrhizal fungi to freezing and drying, *Can. J. Bot.,***79**, 1161-1166 **(2001)**

22. Robinson-Boyer L., Grzyb I. and Jeferies P., Shifting the balance from qualitative to quantitative analysis of arbuscular mycorrhizal communities in field soils, *Fungal Ecology*, **2**, 1-9 **(2009)**

23. Dhar P.P. and Mridha M.A.U., Arbuscular mycorrhizal associations in different forest tree species of Hazarikhil forest of Chittagong, Bangladesh, *J. For. Res.,* **23(1)**, 115-122 **(2012)**

24. Dhar P.P. and Mridha M.A.U., Biodiversity of arbuscular mycorrhizal fungi in different trees of Madhupur forest, Bangladesh, *J. For. Res.,***17(3)**, 201-205 **(2006)**

25. http://invam.cag.wvu.edu, **(2015)**