

RESEARCH ARTICLE

# Diversity of Arbuscular Mycorrhizal Fungi in Trap Cultures Prepared from Abandoned Coalmine Overburden Spoils

Ksanbok Makdoh\* and Highland Kayang

Centre for advanced Studies in Botany, North-Eastern Hill University, Shillong - 793 022, Meghalaya, India.

## Abstract

Assessment of Arbuscular Mycorrhizal Fungi (AMF) diversity in fragile ecosystem and polluted environment is important since they play an important role in the establishment of vegetation and initiation of nutrient cycling for the development of self-sustaining ecosystem. In our present study we assessed the AMF diversity in coal mine abandoned overburden spoils of different age groups viz. 2 years, 10 years and 15 years abandoned site and an un-mined site, through trap culture using *Oryza sativa* L. and *Zea mays* L. as host plants. Spore density increases with the increase in age of overburden spoils. Five genera of AMF were isolated v.i.z. *Acaulospora*, *Claroideoglossum*, *Funneliformis*, *Glomus* and *Rhizophagus*, where, *Acaulospora* and *Glomus* were dominant. In both the trap plants un-mined site derived inoculum showed highest Shannon-Wiener Diversity index and Pielou's Evenness Index value whereas 2 years abandoned site derived inoculum showed lowest diversity index. In case of Simpson's dominance index, 10 years abandoned site derived inoculum has the highest value in *Z. mays* and 2 years abandoned site derived inoculum has the highest value in *O. sativa* trap culture respectively. Comparing the two trap plants, *O. sativa* showed higher colonization percentage, spore density and diversity index indicating that it has a capability to develop maximum interaction with study site derived inoculum. The dominant AMF species can further be utilized in studies for reclamation of abandoned coal mine overburden spoils.

**Keywords:** Mycorrhiza, species composition, inoculum, dominance.

\*Correspondence: ksanbok@gmail.com; +91-9863074005

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## INTRODUCTION

The term "mycorrhiza" was coined by A. B. Frank<sup>1</sup> which literally means 'fungus-root' and describes the mutualistic association existing between a group of soil fungi and higher plants. Arbuscular Mycorrhizal Fungi (AMF) form mutualistic symbioses with roots of over 80% of known plant species<sup>2</sup> including bryophyte, pteridophyte, gymnosperm and angiosperm. AMF enhance the uptake and transfer of nutrients with low mobility to plants such as, phosphorus (P), Zinc (Zn), Nitrogen (N) and Iron (Fe)<sup>3,4</sup>. In addition to absorption of mineral nutrients, AMF stabilize soil structure and enable plants to survive under stress conditions such as drought, saline and toxic soil<sup>5-7</sup>. AMF also influence soil processes, plant biodiversity and sustainability of terrestrial ecosystems<sup>8,9</sup>.

The coalmine overburden (OB) spoils vary widely in their physical, chemical and biological characteristics than natural soil, which affect plant establishment, survival and growth. AMF play an important role in the establishment of vegetation and initiation of nutrient cycling for the development of self-sustaining ecosystem in overburden spoils and other mined out areas<sup>10,11</sup>. Fresh OB spoils are usually dry, polluted with heavy metals and lack viable mycorrhizal population which affect the development of vegetation. Under these conditions, AMF can enhance the mineral absorption and draught tolerance of plants for their growth and survival. A thorough and systematic study on the occurrence of AMF species, spore density, species diversity and composition in abandoned coal mine overburden spoils will, therefore, be of great help in implementing successful reclamation programmes.

One of the most widely used methods for AMF identification is through morphological characteristics of spores collected either directly from the field<sup>12</sup> or from trap cultures<sup>13</sup>. Field collected spores, in some circumstances, lack informative taxonomic characteristics impairing a more accurate identification. Moreover, in the field conditions, AMF spores may be less in number or some species may be absent at the time of sampling even though they may be present within roots<sup>14</sup>. Alternatively, establishment of trap cultures produce clear and healthy spores which greatly improves the assessment of

species composition in an ecosystem<sup>15</sup>. However, all AMF species present in the field cannot be assessed through trap culture since, on one hand sporulation of some species is affected by host plant chosen for trapping<sup>13</sup> whereas on the other hand it promotes the sporulation of cryptic AMF species which were not sporulating at the sampling time or field conditions<sup>16</sup>. Despite of that, trap cultures have been widely used to assess AMF diversity and isolate indigenous fungi<sup>17</sup>.

Since AMF can be utilized for successful establishment of plants in highly disturbed habitats, more information on their ecology is required. It is essential to know the AMF species present in a particular soil environment before they can be studied for reclamation process. For the present study soil samples and root fragments from abandoned coal mine overburden spoils of different age groups (2 years, 10 years and 15 years abandoned sites and an un-mined site) were collected from East Jaintia Hills District, Meghalaya, India.

## MATERIALS AND METHODS

### Propagation of AMF spores through trap culture

The methods of AMF trap culture were followed from INVAM (<http://invam.caf.wvu.edu>). Field soil and root fragments from each study site that served as inocula for AMF trap cultures were collected in separate sterilized plastic bags and transported to the laboratory. Roots were cut into small fragments and mixed thoroughly with the associated soil. This chopped blend was mixed with autoclaved coarse sand (1:1 v/v). *Oryza sativa* L. (upland variety) and *Zea mays* L. were used as trap plants. These species were chosen as they are well-known AMF host plants frequently used for trap cultures. Seeds were surface sterilized with sodium hypochlorite (10% v/v) for 10 min and thoroughly rinsed with sterilized water. Approximately 15-20 seeds of *Oryza sativa* L. and 6-7 seeds of *Zea mays* L. were evenly sown on 25cm diameter plastic pots containing 1.5kg of inoculum and autoclaved coarse sand (1:1 v/v) and monitored in greenhouse condition. They were watered whenever required.

### AMF colonization assessment

After 4 months, all trap plant species were removed from the plastic pots and roots were separated for evaluation of AMF colonization. For each species, root samples were washed

thoroughly in tap water, then processed and stained with 0.05% Trypan blue<sup>18</sup>. Root tissues were quantified for colonization by magnified intersections method<sup>19</sup> and converted to percentage.

#### AMF spore analysis

AMF spores were extracted from 100g soil sample by wet sieving and decanting method<sup>20</sup>. AMF spores were picked up with needle in polyvinyl alcohol-lactoglycerol with Meltzer's reagent on a glass slide. The complete and broken spores were examined using a compound microscope, Olympus. Spore identification was done based on morphological descriptions published by INVAM (<http://invam.caf.wvu.edu>) and AMF phylogeny ([www.amf-phylogeny.com](http://www.amf-phylogeny.com)).

#### Data analysis

Means and standard errors were calculated. Spore density and species richness were expressed as number of AMF spores and number of AMF species in 100g soil samples. Relative abundance, isolation frequency, Shannon-Wiener diversity index, Simpson's dominance index and Pielou's Evenness index were calculated.

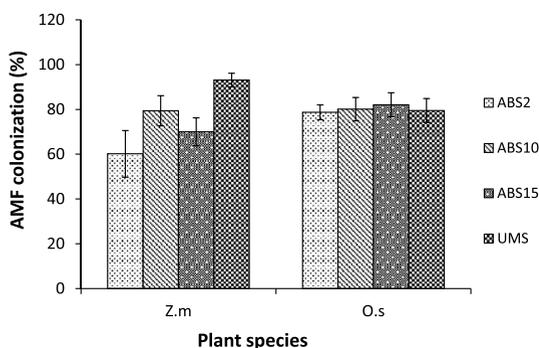
## RESULTS

*Zea mays* L. showed variation in AMF colonization in the roots whereas colonization in the roots of *Oryza sativa* L. did not show much variation. In *Z. mays* AMF colonization ranged from 60.15% (2 years abandoned site inoculum) to 93.09% (un-mined site inoculum). In *O. sativa* AMF colonization ranged from 78.71% (2 years abandoned site inoculum) to 82.07% (15 years

abandoned site inoculum) (Fig. 1). In both the trap plants AMF spore density is least in 2 years abandoned site derived inoculum and highest in un-mined site derived inoculum (82 spores/100g soil and 155 spores/100g soil respectively in *Z. mays* and 115 spores/100g soil and 204 spores/100g soil respectively in *O. sativa*) (Fig. 2).

With 2 years abandoned site derived inoculum, 10 AMF species were isolated from *Z. mays* trap culture and 13 AMF species from *O. sativa* trap culture. *Funnelformis geosporus* has the highest relative abundance in *Z. mays* (18.29%) and *Acaulospora morrowae* has the highest relative abundance in *O. sativa* (20.87%) (Table 1). With 10 years abandoned site derived inoculum, 12 AMF species were isolated from *Z. mays* trap culture where *Acaulospora mellea* and *Acaulospora morrowiae* has the highest relative abundance (11.32%) and 16 AMF species were isolated from *O. sativa* trap culture where *F. geosporus* (18.12%) has the highest relative abundance (Table 2). With 15 years abandoned site derived inoculum 14 AMF species were isolated from *Z. mays* trap culture and 18 AMF species from *O. sativa* trap culture and with un-mined site derived inoculum, 17 AMF species were isolated from *Z. mays* trap culture and 22 AMF species from *O. sativa* trap culture. *F. geosporus* has the highest relative abundance in both the trap plants set up from 15 years abandoned site inoculum and un-mined site inoculum (Table 3 and 4).

Un-mined site derived inoculum showed the highest Shannon-Wiener species diversity index in both the trap cultures (2.73 in *Z. mays*



**Fig. 1.** AMF colonization (%) in *Zea mays* L. (Z.m) and *Oryza sativa* L. (O.s) used as trap plants set up with study sites derived inocula.



**Fig. 2.** AMF spore density in trap culture set up with study sites derived inocula where *Zea mays* L. (Z.m) and *Oryza sativa* L. (O.s) are used as trap plants.

**Table 1.** AMF species isolated from trap cultures (2 years abandoned site derived inoculum) with their relative abundance and isolation frequency (IF) using *Z. mays* and *O. sativa* as trap plants

S. No.	AMF species	Relative abundance (%)		IF %
		<i>Z. mays</i>	<i>O. sativa</i>	
1	<i>Acaulospora capsicula</i> Blaszk.	10.98	6.09	100
2	<i>Acaulospora delicata</i> Walker, Pfeiffer & Bloss	10.98	2.61	100
3	<i>Acaulospora foveata</i> -Trappe & Janos	2.61	50	
4	<i>Acaulospora laevis</i> Gerd. & Trappe	9.76	6.09	100
5	<i>Acaulospora mellea</i> Spain and Schenck	13.41	13.04	100
6	<i>Acaulospora morrowiae</i> Spain and Schenck	10.98	20.87	100
7	<i>Claroideoglossum etunicatum</i> Walker & Schuessler	-	2.61	50
8	<i>Claroideoglossum luteum</i> Walker & Schuessler	8.54	10.43	100
9	<i>Funneliformis geosporus</i> Walker & Schuessler	18.29	17.39	100
10	<i>Funneliformis verruculosum</i> Walker & Schuessler	12.20	1.74	100
11	<i>Glomus multicaule</i> Gerd. & Bakshi	1.22	1.74	100
12	<i>Rhizophagus clarus</i> Walker & Schuessler	-	12.17	50
13	<i>Rhizophagus intraradices</i> Walker & Schuessler	3.66	2.61	100

and 2.94 in *O. sativa* trap culture) whereas 2 years abandoned site derived inoculum showed the least (2.17 in *Z. mays* and 2.25 in *O. sativa*) (Fig. 3). In case of Simpson's dominance index, 10 years abandoned site derived inoculum has the highest value in *Z. mays* trap culture and 2 years abandoned site derived inoculum has the highest

value in *O. sativa* trap culture (0.12 and 0.13 respectively) whereas the un-mined site derived inoculum has the least (0.07 in *Z. mays* and 0.06 in *O. sativa* trap culture) (Fig. 4). In both *Z. mays* and *O. sativa* trap cultures, un-mined site derived inoculum has the highest Pielou's Evenness Index value (0.96 and 0.95 respectively) (Fig. 5).

**Table 2.** AMF species isolated from trap cultures (10 years abandoned site derived inoculum) with their relative abundance and isolation frequency (IF) using *Z. mays* and *O. sativa* as trap plants

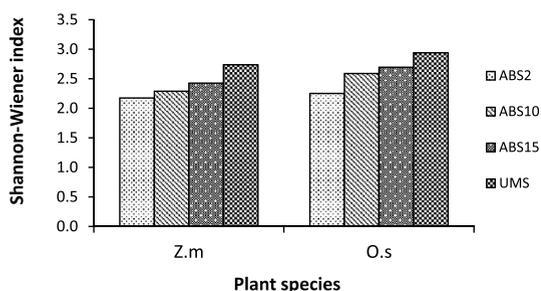
S. No.	AMF species	Relative abundance (%)		IF %
		<i>Z. mays</i>	<i>O. sativa</i>	
1	<i>Acaulospora capsicula</i> Blaszk.	8.49	5.37	100
2	<i>Acaulospora delicata</i> Walker, Pfeiffer & Bloss	-	6.04	50
3	<i>Acaulospora foveata</i> Trappe & Janos	-	4.03	50
4	<i>Acaulospora lacunosa</i> Morton	5.66	-	50
5	<i>Acaulospora mellea</i> Spain and Schenck	11.32	10.07	100
6	<i>Acaulospora morrowiae</i> Spain and Schenck	11.32	10.07	100
7	<i>Claroideoglossum etunicatum</i> Walker & Schuessler	6.60	5.37	100
8	<i>Claroideoglossum luteum</i> Walker & Schuessler	8.49	9.40	100
9	<i>Funneliformis geosporus</i> Walker & Schuessler	24.53	18.12	100
10	<i>Funneliformis verruculosum</i> Walker & Schuessler	2.83	6.04	100
11	<i>Glomus glomerulatum</i> Sieverding	-	2.68	50
12	<i>Glomus macrocarpum</i> Tul. & Tul.	-	4.03	50
13	<i>Glomus multicaule</i> Gerd. & Bakshi	2.83	2.01	100
14	<i>Glomus</i> sp.1	-	4.03	50
15	<i>Rhizophagus clarus</i> Walker & Schuessler	8.49	8.05	100
16	<i>Rhizophagus intraradices</i> Walker & Schuessler	6.60	2.01	100
17	unidentified sp.1	2.83	-	50
18	unidentified sp.2	-	2.68	50

**Table 3.** AMF species isolated from trap cultures (15 years abandoned site derived inoculum) with their relative abundance and isolation frequency (IF) using *Z. mays* and *O. sativa* as trap plants

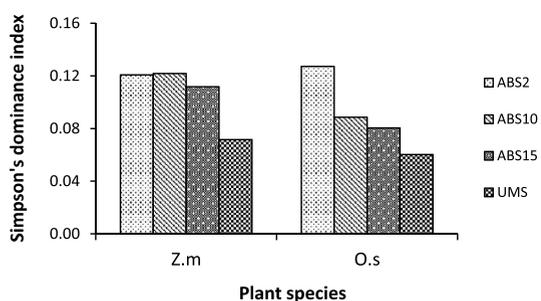
S. No.	AMF species	Relative abundance (%)		IF %
		<i>Z. mays</i>	<i>O. sativa</i>	
1	<i>Acaulospora capsicula</i> Blaszk.	-	5.92	50
2	<i>Acaulospora delicata</i> Walker, Pfeiffer & Bloss	6.72	4.61	100
3	<i>Acaulospora foveata</i> Trappe & Janos	-	4.61	50
4	<i>Acaulospora lacunosa</i> Morton	3.36	3.29	100
5	<i>Acaulospora laevis</i> Gerd. & Trappe	3.36	4.61	100
6	<i>Acaulospora mellea</i> Spain and Schenck	7.56	9.21	100
7	<i>Acaulospora morrowiae</i> Spain and Schenck	11.76	8.55	100
8	<i>Acaulospora rehmsii</i> Sieverding & Toro	-	2.63	50
9	<i>Claroideoglossum etunicatum</i> Walker & Schuessler	5.88	4.61	100
10	<i>Claroideoglossum luteum</i> Walker & Schuessler	9.24	9.87	100
11	<i>Funneliformis geosporus</i> Walker & Schuessler	24.37	17.11	100
12	<i>Funneliformis verruculosum</i> Walker & Schuessler	4.20	6.58	100
13	<i>Glomus ambisporum</i> Smith & Schenck	-	1.32	50
14	<i>Glomus macrocarpum</i> Tul. & Tul.	5.04	2.63	100
15	<i>Glomus multicaule</i> Gerd. & Bakshi	2.52	1.97	100
16	<i>Glomus rubiforme</i> Almeida & Schenck	3.36	-	50
17	<i>Rhizophagus clarus</i> Walker & Schuessler	6.72	6.58	100
18	<i>Rhizophagus intraradices</i> Walker & Schuessler	5.88	3.95	100
19	Unidentified sp.1	-	1.97	50

**Table 4.** AMF species isolated from trap cultures (un-mined site derived inoculum) with their relative abundance and isolation frequency (IF) using *Z. mays* and *O. sativa* as trap plants

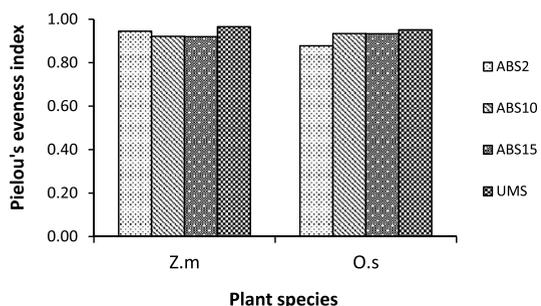
S. No.	AMF species	Relative abundance (%)		IF %
		<i>Z. mays</i>	<i>O. sativa</i>	
1	<i>Acaulospora capsicula</i> Blaszk.	6.45	6.37	100
2	<i>Acaulospora delicata</i> Walker, Pfeiffer & Bloss	4.52	4.90	100
3	<i>Acaulospora foveata</i> Trappe & Janos	3.87	5.39	100
4	<i>Acaulospora lacunosa</i> Morton	5.81	2.94	100
5	<i>Acaulospora laevis</i> Gerd. & Trappe	3.87	2.94	100
6	<i>Acaulospora mellea</i> Spain and Schenck	7.74	5.88	100
7	<i>Acaulospora morrowiae</i> Spain and Schenck	6.45	6.86	100
8	<i>Acaulospora rehmsii</i> Sieverding & Toro	-	2.94	50
9	<i>Claroideoglossum etunicatum</i> Walker & Schuessler	6.45	4.41	100
10	<i>Claroideoglossum luteum</i> Walker & Schuessler	8.39	8.82	100
11	<i>Funneliformis caledonium</i> Walker & Schuessler	-	1.96	50
12	<i>Funneliformis geosporus</i> Walker & Schuessler	14.19	11.76	100
13	<i>Funneliformis verruculosum</i> Walker & Schuessler	3.87	5.88	100
14	<i>Glomus ambisporum</i> Smith & Schenck	-	1.96	50
15	<i>Glomus glomerulatum</i> Sieverding	3.23	2.45	100
16	<i>Glomus macrocarpum</i> Tul. & Tul.	6.45	2.94	100
17	<i>Glomus microcarpum</i> Tul. & Tul.	-	1.47	50
18	<i>Glomus mosseae</i> Gerd. & Trappe	3.23	-	50
19	<i>Glomus multicaule</i> Gerd. & Bakshi	2.58	1.96	100
20	<i>Glomus rubiforme</i> Almeida & Schenck	4.52	3.92	100
21	<i>Rhizophagus clarus</i> Walker & Schuessler	8.39	8.33	100
22	<i>Rhizophagus intraradices</i> Walker & Schuessler	-	3.43	50
23	Unidentified sp.2	-	2.45	50



**Fig. 3.** Shannon-Wiener diversity index in trap culture set up with study sites derived inocula where *Zea mays* L. (Z.m) and *Oryza sativa* L. (O.s) are used as trap plants.



**Fig. 4.** Simpson dominance index in trap culture set up with study sites derived inocula where *Zea mays* L. (Z.m) and *Oryza sativa* L. (O.s) are used as trap plants.



**Fig. 5.** Pielou's evenness index of AMF in trap culture set up with study sites derived inocula where *Zea mays* L. (Z.m) and *Oryza sativa* L. (O.s) are used as trap plants. Note: ABS2 = 2 years abandoned site, ABS10 = 10 years abandoned site, ABS15 = 15 years abandoned site, UMS = un-mined site.

## DISCUSSION

In *Zea mays* trap culture AMF colonization was least with 2 years abandoned site derived inoculum and highest with un-mined site derived inoculum whereas, in *Oryza sativa* trap culture AMF colonization was lowest with 2 years abandoned site derived inoculum and highest

with 15 years abandoned site derived inoculum. However, spore density was least with 2 years abandoned site derived inoculum and highest with un-mined site derived inoculum in both the trap cultures. This indicates that AMF colonization level is not always directly related to spore density and is often poorly related to sporulation capacity in the soil<sup>21</sup>. Further, spore production cannot be explained by Mycorrhizal colonization level<sup>22</sup>.

Spore density showed an increasing trend with the increase in age of overburden spoils as reported earlier<sup>23-25</sup>. The number of AMF species isolated was found to be higher than those reported by Husin *et al.*<sup>26</sup> and increases with age of overburden spoils which is due to vegetation development and soil physical and chemical properties<sup>27, 28</sup>.

In our present investigation, five genera of AMF were isolated (*Acaulospora*, *Claroideoglossum*, *Funnelformis*, *Glomus* and *Rhizophagus*) where, *Acaulospora* and *Glomus* were dominant which is in consistent with the findings of Choudhury *et al.*<sup>29</sup> and Singh and Jamaluddin<sup>24</sup>, where they found these two genera to be dominant on spoil of all age groups. *Glomus* and *Acaulospora* species have short sporulation time and high competitive interaction and adaptability as compared to that of *Gigaspora* and *Scutellospora* species in the same environment, allowing them to establish better than the others<sup>30, 31</sup>. *Acaulospora* species are often associated with acidic soil<sup>32</sup> and a wide range of host species<sup>33</sup>. 22 AMF species belonging to five genera and one unidentified species were isolated from the trap culture with un-mined site derived inoculum which is comparable to the findings of Songachan and Kayang<sup>34</sup> from pine forest of Meghalaya.

The AMF species with 100% isolation frequency indicate that they are more tolerant to soil disturbances. Shannon-Wiener Diversity index was found to be highest in trap culture with un-mined site derived inoculum and lowest in trap culture with 2 years abandoned site derived inoculum. The diversity index is influenced by AMF species composition and relative abundance<sup>35</sup>. 2 years abandoned site derived inoculum showed the highest Simpson's dominance index (D), indicating dominance by a few species of AMF while the lower dominance index in un-mined site derived inoculum indicates shared dominance of

many AMF species.

The specific trap plants may also play an important role in sporulation and abundance of AMF spores. In our study, comparing the two trap plants, *Oryza sativa* showed higher colonization percentage, spore density and diversity index indicating that it has a capability to develop maximum interaction with study site derived inoculum. The host plant species has an influence on the fungal development in trap cultures<sup>36, 37</sup>. Spore density of AMF communities in trap culture depends on the host plants used<sup>38</sup> and their number grown in each pot. The availability of roots for colonization influences sporulation by different fungal species<sup>39</sup>. AMF host preference has been observed by various authors in different plant species<sup>40, 41</sup>. Such host preferences have obvious implications for establishing efficient and comprehensive AMF trap cultures, especially for studying AMF communities in ecosystems<sup>9</sup>.

## CONCLUSION

The number of AMF species isolated from the trap cultures increases with age of the coal mine overburden spoils. This indicates that with time the abandoned overburden spoils harbour a large number of AMF species. The trap cultures produced healthy spores which can be used to establish monospecific cultures for inoculum conservation. *Funneliformis geosporus*, *Acaulospora morrowae* and *Acaulospora mellea* were the species with highest relative abundance and can be utilized for further studies in soil reclamation.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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