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# Arbuscular Mycorrhizal Species Composition During Rainy Season From Polluted Sites And Their Role As Biofertilizer Mrs. Ashwini Deshpande<sup>1</sup>, Dr. Mahavir Gosavi<sup>2</sup>

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# **Abstract:**

Arbuscular mycorrhizae (AM) exhibit the most positive obligate symbiotic relationship, with roots of majority of plants. They show higher ecological amplitude under adverse environmental conditions. The current study deals with the study of diversity of AM fungi during rainy season with respect to rhizosphere soil analysis for AM species composition and their subsequent role as bio-fertilizer. Spores of *Glomus, Sclerocystis* and *Scutellospora* were observed. The observed species were also detected in root colonization analysis, using standard staining methods. The spore density was highest for *Glomus*. It proved to be a dominant species. The application of bio-fertilizer product prepared with these dominant species was proved to be a potent bio-fertilizer in the field trials with chili crop.

**Keywords:** -ArbuscularMycorrhiza (AM), *Glomus, Sclerocystis, Scutellospora* **Introduction:** 

The mutually beneficial relationship between the feeder roots of plants and fungi is called mycorrhiza (Frank, 1885)'*Mycos*' meaning fungus and '*rhiza*' meaning root (Trappe,2005).Arbuscular Mycorrhizal(AM) fungi show obligatory symbiosis and form natural partnership with Bryophytes, Pteridophytes, Gymnosperms and Angiosperms. They are even found in nutrient deficient soils. AM fungi play an essential role in plant growth, plant protection (from drought, temperature, and salinity) and soil quality. Around 80% of plants are colonized by AM fungi which belong to Glomeromycota and members of family Endogonaceae.AM fungi are employed both in agriculture and forestry (Rodrigues and Muthukumar, 2009).

The present paper deals with investigation of species composition of Arbuscular Mycorrhizae in industrially polluted MIDC area of Dombivli in Thane district of Maharashtra, with respect to their association with seasonal weeds surrounding fifteen industries of MIDC area, located at Sagarli in Dombivli (East) during rainy season. Thus, in present context, study of AM fungi for myco-remediation is an emerging significant alternative technology in the clean-up of metal contaminated soil to maintain status of environment and to use it as biofertilizer inoculums in more efficient way to serve in better way for fulfilling the growing nutritional needs of humankind.

**Statement of the problem:** The current study deals with identifying AM fungi species prevalent during rainy season that can be used as bio-fertilizers.

**Scope of research:** The seasonal AM fungi species found during rainy season can be effectively used as bio-fertilizer, with increased concentration of macronutrients.

**Need of research subject:** AM spores that are isolated during rainy season can be multiplied through trap culture and can be successfully used as bio fertilizer, throughout the year.

**Hypothesis of problem:** The pure inoculum of AM species, observed during rainy season can be used to clean up heavy metal pollution from the soils around industries, thus mycoremediation through AM fungi can be used as effective and ecofriendly technology.

**Research methodology**: The selected study sites were fifteen industries from MIDC, Sagarli of Dombivli (East). Different seasonal weed samples were collected growing near selected industries, along with rhizosphere soil and the spores were isolated, identified and recorded. The original percentage of carbon, nitrogen, phosphorus and potassium was estimated in the soil collected from paddy field and the field trials were taken with chili (*Capsicum annuum*, L.) crop in the same paddy field by using VAM bio fertilizer product with composition of same species, raised through trap culture technique.

**Research techniques:** For collection of roots and rhizosphere soil samples, a steel pipe was inclined and driven into soil up to 25cm of root zone at different depths.AM fungal spores were isolated from rhizosphere soil samples, by wet sieving and decanting method (Gerdemann and Nicolson, 1963). The isolated spores were observed by lifting them with pinhead and mounting on the slide containing lactophenol as mounting medium. The isolated spores were observed under stereomicroscope, identified, and categorized. Ink and vinegar staining (Vierheilig *et.al.*, 1998) and Trypan blue staining (Philips and Hayman, 1970) of roots were carried out for root colonization of AM fungal species. Thus, AM fungal species, inhabitants of soil polluted sites were surveyed during winter season (November 2021-February, 2022). The percent root colonization with soils of rhizosphere zone from all the weed samples was calculated. The percentage frequency with individual industry was also calculated. Root colonization was

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calculated using formula:-

# Percent AM colonization = $\frac{\text{No. of root segments with AM structures}}{\text{Total no. of root segments examined}} \times 100$

Data analysis: The percent root colonization, presence of vesicles, arbuscules, spores and coiled hyphae were noticed. The spore density was also calculated. The data of percentage of organic carbon, nitrogen, potassium and phosphorus of the field soil samples, taken before the field trials was compared with data of percentage of organic carbon, nitrogen, potassium and phosphorus of the field soil samples taken after the field trials, carried out by using VAM biofertilizer product.

Testing hypothesis: The data was analyzed and tested for significant increase in uptake of macronutrients after the treatment of VAM bio-fertilizer. Thus the hypothesis of role of VAM as bio-fertilizer was tested.

Observations: About 80% of Glomus species were recorded from genus Glomus such as G. albidium, G. badium, G.citricola, G. coronatum G. diphanum, G. fecundisporum, G. hoi G. leptotichum, G. macrocarpum, Glomus occultum, Glomus reticulatum and G. tenerum along with species of *Sclerocystis* like S. rubiformis and S. sinuosus. The species recorded from genus Scutellospora were S. calospora and S. persica respectively. There was significant increase in macronutrient contents after the treatment of bio-fertilizer product with composition of above mentioned AM fungi species.

# **Description of spores observed:**

1) Glomus albidum- Walker & Rhodes (1981)

The spores were isolated from rhizosphere soil of Commelinabenghalensis and Oxaliscorniculatacollected during growing season and Paspalam conjugatum and Cynodon dactylon during winter season. The spores are observed with one subtending hypha borne singly in the soil on coenocytic hyphae. Mature spore shows diameter about 196 x 140 µm, globose to sub globose, occasionally ovoid or irregular. The observed spores were yellowish to brownish yellow. Spore walls continuous with hyphal walls consisting of an outer hyaline wall and lightyellow inner wall. The subtending hypha was bilayered, straight with 12 µm, in thickness. Spores of Glomus albidum can be easily distinguished from others due to their brownish yellow colouration, globose to sub globose shape and size ranging from 150-200 µm (Gehlot and Singh, 2015). This species is closely related to G. gibbosum (Walker et. al., 1995) and can be distinguished based on four layered walls in the G. gibbosum as compared to two layered in G. albidum (Blaszkowski et. al., 2001).

# 2) Glomus badium- Oehl, Redecker and Sieverd (2005)

The spores were isolated from rhizosphere soil of *Phyllanthus amarus* collected during winter and summer season, Eragrostistenella (Poatenella) in summer season and Cleome rutidosperma and Commelinabenghalensis in rainy season. The observed spores were brownish, globose with diameter of 280 µm. The germ tubes initiation is observed at two positions. The spore wall consists of three layers. The subtending hypha is recurved.

The most distinguishing characters of G. badium are its small sporocarps lacking a peridium and composed of many, brownish orange to reddish brown, relatively small spores. The innermost flexible to semi-flexible and coloured layer of the three-layered spore wall also is a diagnostic property of this species (Ohel et.al., 2005; Blaszkowski, et.al., 2010 and Goto et.al., 2012).

# 3) *Glomus citricola*- Tang and Zang (1984)

The spores were isolated from rhizosphere soil of Commelinabenghalensis, Cynodon dactylon and Oxalis corniculata during rainy season and rhizosphere soil of Scoparia dulcis from summer season. The single spores were observed in sporocarps. The observed spores were globose with dimensions of about 56 x 84 µm. The subtending hypha was hyaline. The spore wall is thick.

The observed spores were sub-pyriform, the average observed dimensions are  $112 \ge 56 \mu m$ . The spore wall is thin, composed of two walls. The attachment of spore is sublateral.

This species is reported by several authors from India before (Jayaprakash& Nagarajan, 2017; Shrivastava et.al., 2012). Like other Species of Glomus it also shows globoid spores produced on soil surface (Frank et.al., 2003). Not much literature is available on the said species.

4) *Glomus coronatum*- Giovannetti(1983) and (1991)

The spores, present in sporocarps, were isolated from rhizosphere soil of *Calotropis gigantea* in summer, rhizosphere soil of Commelina benghalensis. Oxalis corniculata and Cynodon dactylon collected during rainy season. The sporocarps were surrounded by a peridium. The isolated spores were globose, brownish orange in colour with single funnel-shaped subtending hypha. The observed diameter was 252 µm. The observed spore wall was bilayered.

The distinctive characters of G. coronatum are its large and greyish orange to brownish orange spores and the wide, funnel-shaped subtending hypha. The wall of spores consists of two layers (Blaszkowski, 1994 and Cavagnaro et. al., 2001). According to Giovannetti et. al., (1991), G. coronatum produces spores in sporocarps surrounded by a peridium.

# 5) *Glomus diphanum*- Morton & Walker (1984)

The spores were isolated singly from rhizosphere soil of *Commelinabenghalensis*, *Heliotropium indicum* and *Cleome rutidosperma* during rainy season. The spores were found in isolated clusters. The observed spores were globose, hyaline, blackish, with about 56  $\mu$ m in diameter. The spore wall comprises of three layers and elongated subtending hypha was observed. The length observed was about 196  $\mu$ m and the diameter was 28  $\mu$ m.

*Glomus diaphanum* probably has a worldwide distribution. The spores of *G. diaphanum* occur singly in the soil, globoid and hyaline with flexible to semi-flexible innermost wall layer (Morton & Walker, 1984; Morton 1985; Oehl *et. al.*, 2003 and Oehl *et. al.*, 2005).

# 6) *Glomus fecundisporum*- Schenck and Smith(1982)

The spores were isolated from rhizosphere soil of *Commelina benghalensis* and *Oxalis corniculata* during rainy season. The spores were formed singly or in loose clusters, with globose, pale yellow to white in colour. The observed diameter was140  $\mu$ m. The spore wall was smooth in young spores and rough in mature spores. The subtending hyphae were hyaline, about 84  $\mu$ m in length and 28  $\mu$ m in diameter. The hyphal coils were seen in root colonization samples of *Commelina benghalensis*.

*Glomus fecundisporum* is a hyaline to dirty-white spored species with spores frequently borne in clusters. The spores consisting of inner and outer walls of approximately equal thickness (Lee *et.al.*, 1993).

It forms mycorrhizal associations with plant roots but not forming typical vesicles or arbuscules; coiling hyphae formed in outer cortical cells with hypha, walls becoming indistinct and after growth through 2 to 3 cortical cells; hypha contents frequently enlarging to fill cell lumen (Schenck & Smith, 1982).

# 7) *Glomus hoi*-- Berch and Trappe (1985)

The spores were isolated from rhizosphere soil of *Commelinabenghalensis* and *Cleome rutidosperma*, during rainy season and *Phyllanthus amarus and Heliotropium indicum* during winter season.

The spores were as single spore in the soil. Each one of them was globose, subglobose, ellipsoidal or irregular in shape, in diameter, light brown in colour. The observed diameter is 140  $\mu$ m x 112 $\mu$ m.

The spores can be easily distinguished from other species on the basis of solitary appearnce; globose, subglobose, ellipsoidal or irregular in shape and light brown in colour. The spore wall is composed of two distinct, separable layers. (Berch& Trappe, 1985; Blaszkowski, 2003 and Wilde et.al., 2009).

# 8) *Glomus leptotichum* –Schenck and Smith (1982)

The spores were isolated from *Commelina benghalensis*, *Cleome rutidosperma* and *Oxalis corniculata* during rainy season. The spores were found singly but in loose clusters. The spores were globose, hyaline, light yellow to pinkish in colour (as collected from polluted environment). The observed diameter ranges from 56  $\mu$ m to 252  $\mu$ m. The length of subtending hypha was about 112  $\mu$ m and diameter was 28  $\mu$ m. The spore wall is composed of three layers some ornamentations are also observed. The root colonization in the roots of *Cleome rutidosperma* is observed in the form of hyphal coiling.

Glomus leptotichum typically has large, white to cream-colored spores with hyaline walls bearing a faint reticulum of ridges. Spores and extrametrical vesicles are produced both terminally and in an intercalary manner (Schenck and Smith, 1982). Because of its large size and white to cream-colored spores it could possibly be confused with *G. lacteum* but *G. leptotichum* lacks the merging hyphae on the attachment associated with *G. lacteum*. *Glomus fecundisporum* which *G. leptotichum* resembles somewhat, has generally smaller spores with yellow to brown walls, gray-white contents, and lacks a reticulum (Morton *et.al.*, 1997; Johnson *et. al.*, 1991 and Murakoshi *et. al.*, (1998).

# 9) *Glomus macrocarpum-* Tulasne and Tulasne (1845)

The spores were isolated from *Cynodon dactylon* during summer season. *Cleome rutidosperma*, *Commelina benghlensis* and *Oxalis corniculata* during rainy season.

The spores were found singly in the soil. The observed spores were globose, pear shaped, orange to reddish. The diameter observed was 112  $\mu$ m. The spore wall was 14  $\mu$ m, in thickness. The thin, curved subtending hypha. The breadth of observed hypha was 14 $\mu$ m.

Literature indicates that it is a widely distributed throughout the world, although it occurs irregularly (Blaszkowski *et. al.*, 2002).

Spores usually slightly longer than wide, sub globose, avg. of  $150-250 \ \mu m$ . Spore wall composed of two distinct layers, spores tapering to the point of attachment of the single persistent hypha (Gehlot & Singh, 2015)

# 10) Glomus occultum- Walker (1982)

The spores were isolated from *Paspalam conjugatum* rhizosphere soil during winter and *Oxalis corniculata* rhizosphere soil from rainy season. The spores occur singly and are sub globose. The observed diameter is 56X86  $\mu$ m. The spore wall consists of two layers. The sub tending hypha, observed appeared as funnel- shaped 28  $\mu$ m in length and 14  $\mu$ m in diameter.

It is probably globally widespread (Walker, 1982; Miller *et al.*, 1985; Morton, 1985 and Puppi *et al.*, 1986). Although it is one of the most widespread but difficult to detect (Millner, 2001).

*Glomus occultum* spores are often colorless, and globose, ellipsoid, or irregular, possessing three thin walls, the inner two often adhering tightly (Morton, 1985).

11) Glomus reticulatum- Bhattacharjee and Mukerji (1980)

The spores were extracted from rhizosphere soil of *Commelina benghalensis* and *Portulaca oleracea* from rainy season and *Eclipta prostrata* and *Vernonia sinusoroides* collected during summer season. The spores were found singly, dark brown to reddish or black, globose with 140  $\mu$ m in diameter. The outer and inner walls of the spores are clearly visible under 100X. The outer wall was about 5  $\mu$ m in thickness. The subtending hyphae were funnel- shaped and about 10  $\mu$ m wide (Bhattacharjee & Mukerji, 1980).

Spores are borne freely and singly in soil, globose to subglobose or ellipsoidal, dark brown to brownish black, having reticulate ornamentation. Spore wall structure consists of double wall. Outer wall is laminated, and inner wall is with regular reticulate (Manoharachary*et.al.*, 2005; Kulkarni *et.al.*, 1997; Mishra *et.al.*, 2016; Wang *et.al.*, 2015 and Shekhar and Basu, 2017). **12)** *Glomus tenerum*- Tandy (1975)

The spores were isolated from rhizosphere soil of *Commelinabenghalensis* collected during rainy season, *Calotropis gigantea* and *Vernonia sinusoroides* collected during summer season and from Phyllanthus amarus in winter season. The observed spores were shiny, translucent, yellowish brown, thin walled, with some oil globules. The diameter of observed spores was about 56 µm.

The spores are yellow, orange to brown, globose rarely pyriform. Subtending hyphae are cylindrical with globose thick-walled vesicles (Tandy, 1975).

*Glomus tenerum* differs from other *Glomus* species in having two walls, larger spores, a plug cutting off spore contents and finer-subtending hyphae (McGee, 1986; McGee & Trappe, 2002 and Oehl *et.al.*, 2011).

13) Scelerocystis rubiformis - (Gerd. & Trappe, 1974) Almeida and Schenck (1990)

The spores were isolated from rhizosphere soil of *Paspalam conjugatum* collected in winter, *Cassia tora* soil collected in rainy season and rhizosphere soil of *Cleome rutidosperma* collected during summer season. The sporocarps were dark brown, with 392  $\mu$ m in diameter. The peridium was not found. The spores were found in tightly interwoven hyphae. The spores were dark brown, ellipsoidal with 112  $\mu$ m in diameter. The small, stalk like projection was observed protruding near the base of spores. The subtending hypha was thick walled.

It probably is a widely distributed fungus in the world and very common in India (Bhattacharjee *et. al.*, 1980; Ragupathy and Mahadevan 1993).

The distinctive features of *G. rubiforme* are its sporocarps with relatively small, coloured spores originated from a centrally positioned hyphal plexus (Almedia and Schenck, 1990; Blaszkowski *et. al.*, 1998; Wu, 1993).

The wall of *G. rubiforme* spores is composed of two layers: outer layer is a thin and hyaline whereas the inner layer is thicker and laminated. The subtending hypha is funnel shaped (Gerdemann and Trappe, 1974 and Almeida and Schenck, 1990).

14) Sclerocystis sinuosus- Gerdemann and Bakshi (1976)

#### =Glomus sinuosum

The spores were isolated from rhizosphere soil of *Commelina benghalensis*, *Oxalis corniculata* and *Phyllanthus* amarus from rainy season. The spores are found in compact, thick walled reddish brown sporocarps with sinuous or wavy hyphae, occurring in the soil in groups. The observed diameter of sporocarps was 280  $\mu$ m. The observed spores were orange to brownish orange or were reddish brown, sub globose, pulvinate and ellipsoidal, the dimensions observed were 28  $\mu$ m X 56  $\mu$ m, radiating and in single layer with single subtending hypha.

The spores are obovate, elliptical, fusiform-elliptical to clavate, radiating out in a single layer from a central plexus of hyphae (Ammani *et.al.*, 1986; Almeida and Schenck, 1990 and Muthukumar *et.al.*, 2000). The spore wall is brown and thick, generally thickest near spore base. The thick-walled sinuous hyphae that tightly enclose the sporocarps easily distinguish *S. sinuosa* from all other *Sclerocystis* (Gerdemann and Bakshi, 1976).

15) Scutellospora calospora- Nicolson and Gerdemann (1968) Walker and Sanders (1986)

The spores were isolated from rhizosphere soil of *Cleome rutidosperma* during winter season, *Scoparia dulcis* from summer soil sample collection and *Commelina benghaensis* in rainy season. The spores were found singly, terminally on a subtending hypha, which was bulbous, suspensor-like cell. It was ovoid, pale greenish yellow in colour, with dimensions of 196X252  $\mu$ m. The spore wall consists of two tightly attached layers. The bulbous sporogenous cells were found terminally on septate subtending hypha. The germination shield was ellipsoidal and hyaline.

The shape of spore ranges from sub globose to ellipsoid to oblong, sometimes irregular. Both the wall layers are of same thickness (Nicolson and Gerdemann, 1968).

Spores resemble those of spores of *S. pellucida* except they have a smaller size and are oblong. They are very similar to the spores of *S. dipurpurascens*, in size, shape, and color and differs in its inner wall structure (Koske and Walker, 1986; Morton, 2000)

**16)** *Scutellospora persica*- Koske and Walker (1985)

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The spores were isolated from summer collection from rhizosphere soil of Eragrostistenella (Poatenella) and rainy season rhizosphere soil collection of Commelinabenghalensis. The observed spore was found as single spore, terminally on a bulbous suspensor-like cell, apricot yellow in colour, subglobose, with dimensions as 364 µm in length and 336 µm in width. The germination shield is light yellow to brownish. The germ pore was protruding out, prominently visible as globoid structure. (Deshpande and Gosavi, 2022)

Spores are formed singly in the soil, terminally on a bulbous suspensor-like cell. They are globose to subglobose. The distinctive properties of S. persica are its large and dark yellow to apricot yellow spores ornamented with small warts (Morton, 1995)

# Tables and figures for observation: -

TABLE I Root colonization with AM species found in rhizosphere soil of rainy season weeds:

Name of the weeds	AM Spores observed from rhizosphere soil	Average percentage of root colonization
Cynodon dactylon	Acaulospora mellea, Glomus citricola, Glomus coronatum,	40
Commelina benghalensis	Acaulospora mellea, Glomus albidum, Glomus ambisporum, Glomus aurantium, Glomus badium, Glomus caledonium, Glomus citricola, Glomus claroideum, Glomus convolutum, Glomus coronatum, Glomus diphanum, Glomus etunicatum, Glomus fecundisporum, Glomus glomerulatum, Glomus hoi, Glomus leptotichum, Glomus macrocarpum, Glomus pubescens, Glomus reticulatum, Gloms tenerum, Glomus trimurales, Sclerocystissinuosa, , Scutellosporacalospora, Scutellosporapersica	80
Oxalis corniculata	Acaulospora mellea, Glomus albidum, Glomus ambisporum, Glomus citricola, Glomus claroideum, Glomus convolutum, Glomus coronatum, Glomus fecundisporum, Glomus fulvum, Glomus glomerulatum, Glomus leptotichum, Glomus macrocarpum, Glomus occultum, Glomus pubescens, Glomus pulvinatum, Sclerocystissinuosa	70
Cleome rutidosperma	Gigasoracalospora, Glomus badium, Glomus diphanum, Glomus hoi, Glomus leptotichum, Glomus macrocarpum, Glomus monosporum	30
Portulaca oleracea	Glomus pubescens, Glomus reticulatum	50
Heliotropium indicum	Glomus diphanum	40
Phyllanthus amarus	Sclerocystissinuosa	10
Cassia tora	Sclerocystisrubiformis	10

# TABLE II AM Fungi spore diversity (Source-Primary)

Glomus albidum	Glomus badium	Glomus citricola	Glomus coronatum
CALL!	<ul><li>A</li></ul>		
Glomus diphanum	Glomus	Glomus hoi	Glomus
	fecundisporum		leptotichum

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			B
Glomus	Glomus occultum	Glomus	Glomus tenerum
macrocarpum		reticulatum	
-	-		
Sclerocystis	Sclerocystis	Scutellospora	Scutellospora
rubiformis	sinuosus	calospora	persica

# TABLE III-Percentage of organic carbon, nitrogen and phosphorus in a field before and after the application of VAM bio-fertilizer product

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Field condition	% carbon(C)	%nitrogen	% phosphous	% potassium
	in the field	(N) in the	(P) in the field	(K) in the
	soil	field soil	soil	field soil
Before the application	1.14	0.13	0.14	0.20
of VAM bio-fertilizer				
product				
After the application of	1.75	0.20	0.24	0.26
VAM- biofertilizer				
product				

**Discussion:** Based on current survey, seasonal weeds like *Commelina benghalensis* and *Oxalis corniculata* show more diversity by harboring different species of AM fungi in their rhizosphere soil. Root colonization studies also support this observation. The most common and potent AM fungi species have adapted for polluted habitats but their colour and size has been deviated from spores growing symbiotically with weeds of normal habitat. Plant receives support from AM fungi, with the help of its symbiotic association, in the aspect of uptake of Phosphorus and other nutrients (Hart and Forsythe, 2012), enhancement of growth hormones, increase of protein content, increase of lipid, sugars, amino acid levels, increase of tolerance to heavy metals, increase of salinity tolerance and resistance to root-borne pathogens (Upadhyaya *et al.*, 2010; Orwin *et al.*, 2011 and Jacott *et al.*, 2017).

**Conclusions:** Thus, it may be concluded that these rainy season species of AM fungi especially genus *Glomus* from Endogonaceae can play a significant role as biofertilizer. Thus, *Glomus* as dominant genera, along with other genera like *Sclerocystis* and *Scutellospora* have played a great role as potent bio-fertilizers that are not only eco-friendly, but have shown remarkable increase in uptake of all the macronutrients, responsible for increasing soil fertility and thereby supporting the boosted plant growth.

**Suggestions and recommendations:** It is further suggested that the potential role of AM fungi in mycoremediation of soil polluted with heavy metals can be enhanced by inoculating hyper-accumulator plants with mycorrhizal fungi, most appropriate for the polluted site. However, there is a need to develop new methods and to optimize the conditions to grow in enormous quantities and characterize, develop, and screen considerable number of AM fungi for tolerance to metals (Miransari, 2011). The dominant species of AM fungi observed during rainy season can be used to degrade heavy metals in the soil and thus can be used as a great tool for safe environmental cleanup strategy. Thus, mycoremediation through local dominant AM fungal species can be used as cost effective, highly specific, easy, eco-friendly model for such industrial belts all over the world.

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