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Diversity and efficacy of AM fungi on *Jatropa curcas* L., and *Panicum miliacaeum* L. in mine spoils

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The present study was undertaken in the mined areas for the survey of AM fungal diversity. Total of Sixteen AM fungal species were identified in the mined areas of Yallapur. The spores were classified into the following genera: *Glomus* (12), *Acaulospora* (3), *Gigaspora* (2) and *Scutellospora* (2). AM fungi belonging to genus *Glomus* were found to be dominant in the study site. The more value for per cent mycorrhizal colonization was observed in plants belonging to poaceae member. The pot experiments were conducted with inoculation of AM fungi and different levels of mine spoil at Department of Botany, Karnatak University, Dharwad, India. The plants treated with minimum amount of mine spoil and AM fungi increased plant growth responses and mycorrhizal colonization, but the higher concentration of mine spoil results reduced plant growth.
Key words: Mine spoil, AM fungi, Glomus, Colonization and Growth.

Introduction

Among the soil micro biota, mycorrhizal fungi play a pivotal role. The arbuscular mycorrhizal (AM) symbiosis is a mutually beneficial association between the roots of most crop plants and Glomeromycotan fungi (Schußler *et al*., 2001). It is primarily recognized for increasing the mineral status of plants via the mycorrhizosphere (i.e., combined surface area of AM roots and extraradical hyphae). It has also been suggested that the mycorrhizosphere plays a key role in the regulation of soil metal bioavailability through biosorption processes, then contributing to the alleviation of plant metal toxicity and nutrient imbalances (Christie *et al*., 2004; Meharg, 2003). Recently there has been considerable interest in the possible utilization of arbuscular mycorrhizal fungi (AMF) in the reclamation of mine waste. Much of the interest has stemmed from the experimental evidences, that AM fungi improve the survival and growth of seedlings by alleviating most of the deficiencies

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encountered by plant species during their establishment on mine wastes (Lambert and Cole, 1980; Khan, 1981; Call and Mc Kell, 1984 and Jasper *et al*., 1988; Lakshman, 2000; Akhileshkumar *et al*., 2010). In addition arbuscular mycorrhizal fungi are involved in binding loose soil and sand grains into stable aggregates through their extrametrical hyphae (Tisdall, 1991). Mycorrhizal fungi play a vital role in plant growth and productivity has been well documented (Fere-Cerrato and Villenas, 1985). Arbuscular mycorrhizal fungi provide the access to nutrients like phosphorous, Nitrogen and Potassium, which are often limited in disturbed lands (Ried and Grossnickle, 1978; Bheemareddy and Lakshman, 2011). Therefore, present study was undertaken to assess the diversity of AM fungi in mined areas and their effect on growth and yield of *Jatropa curcas* and *Panicum miliaceum* L. Because, several studies reporting the role of mycorrhizal fungi in stressed habitats (Kumar *et al*., 1991; Mehrotra, 1995; Lakshman, 1997 a).

Diversity of AM fungi in the mined areas around Yellapur in Uttara Kannada district was investigated and the efficacy of selected AM fungi on growth and P uptake of *Jatropa curcas* L., and *Panicum miliacaeum* L., with different levels of mine spoil was also conducted.

Materials and methods

Study site and mine spoil Sample collection

Fifteen kg of mine spoil was collected from the mining areas 6 km from Yallapur in Uttara Kannada District. The same mine spoil was used for experiment and for the recovery of AM fungal spores. Uttara Kannada District (Formerly North Kanara) is located in between $13^{0}55^{1}$ to $15^{0}32^{1}$ North latitude $74^{0}05^{1}$ to $75^{0}05^{1}$ East latitude long. Its geographic area is 10,291Km^{2}. The district has boundaries with Goa and Belgaum towards the north, Dharwad, Haveri and Shimoga towards the east and Udupi towards south. The Arabian Sea borders it on the west creating a long continuous, though narrow coastline of 120 Km. The soils of the district are basically derivatives of the Dharwad system- the most ancient metamorphic rocks in India- which are rich in iron and manganese (Pascal, 1988).

Root clearing and staining

One to five grams (1-5g) of *Jatropa curcas* and *Panicum miliaceum* fine roots were collected and maintained in a glycerol/ethanol/distilled water (GEE) solution (Ducousso, 1991). Roots were then cleared in 10% KOH and stained with 0.05% trypan blue in lactophenol (Phillips and Hayman, 1970) to reveal

fungal structures. Stained roots were cut into 1 cm fragments and crushed on slides in a drop of polyvinyl alcohollacto-glycerol (Philips and Hayman, 1970). 5 to 10 fragments were mounted on each slide with 10 replications. Each fragment was observed under a microscope (10 x and 40 x magnification) to estimate the extent of arbuscular mycorrhizal infection as described by Trouvelot *et al*. (1986).

Extraction and counting of AM fungal spores

The Gerdemann and Nicolson (1963) method was used to extract Glomalean spores from the soil. 100 g of soil was wet sieved on 450 to 45 µm mesh sieves and centrifuged in a water sucrose solution (50% w/v) for 10 min at 1500 rpm. Spores were counted under a stereomicroscope and grouped according to their morphological characteristics.

Spore identification

Spore size and colour were assessed in water under a stereomicroscope (Olympus SZ H10 research stereomicroscope). Spore wall structures and other specific attributes were observed under a microscope on permanent slides prepared according to Azcon- Aguilar *et al*. (2003). Identification was mainly based on morphological features, e.g. colour, size and wall structure (Morton and Benny, 1990). They were identified by using AM fungal identification manual (Schenck and Perez, 1990).

Experimental Design

The isolated spores of AM fungi were mass multiplied by using *Sorghum vulgarae* L. as a host plant in separate earthen pots. Pots measuring 30 cm diameter containing sterilized soil: sand mixture in the ratio of 3:1. A 10 g of AM fungal inoculum containing dry soil, hyphae, spores (200-250/ 50 g soil) and root bits was mixed above the surface of the potting mixture. The control plants were not inoculated with any AM fungal inoculum. The amount of AMF inoculum for each treatment was adjusted that equal quantity of soil inoculum could be added to each pot. The following treatments were maintained as follows:- $T_{1:}$ Non-Mycorrhizal, T_2: AM fungus (Glomus fasciculatum), $T_{3:}$ AM fungus with mine spoil (1:0.25%), $T_{4:}$ AM fungus with mine spoil (1:0. 50%), T_5: AM fungus with mine spoil (1:0.75%) and T_6: AM fungus with mine spoil (1:1%).

Surface sterilized with 1% mercuric chloride for three minute was done, seeds of *Jatropa curcas* and *Panicum miliaceum* were sown in each pot above the soil inoculum and pots were arranged in Randomized Completely Block

Design. Each treatment was maintained in triplicates under greenhouse conditions. Plants were uprooted periodically and per cent colonization of the roots was assessed by methods of Philips and Hayman (1970). The spores were isolated from rhizospheric soil of *Jatropa curcas* and *Panicum miliaceum* and spore count was recorded. The growth parameters such as plant height, dry weight of shoot and root, and phosphorous content in shoot in terms of µg/ mg tissue were measured (Jackson, 1973). The data was statistically analyzed.

Results and discussions

AM fungal Diversity

Sixteen AM fungal spores were identified in the mined areas of Yallapur. The spores were classified into the following genera: *Glomus* (12), *Acaulospora* (3), *Gigaspora* (2) and *Scutellospora* (2). AM fungi belonging to genus *Glomus* were found to be dominant in the study site (Table 2). The finding of *Glomus* spp., as the dominant genus was in agreement with the results found in gypsum mining impacted semiarid areas reported by Adália *et al*. (2010); and Beena, *et al*. (2000) at coastal sand dunes Mehrotra (1998). Lakshman (1990 and 1997) reported that *Glomus* was more dominant among the recovered AM fungal spores. Muthukumar and Udaiyan (1999), Lakshman *et al*. (2001) and Lakshman and Jayashankar (2004) documented that *Glomus* was more dominant in tropical soil and mined spoils than other mycorrhizal genera. Similar results were also found in study of AM fungi associated with three species of turf grass (Koske *et al*., 1997). In areas degraded by mineral extraction, the AMF and many plant species that depend on mycorrhization for survival and establishment were reduced or totally eliminated (Allen, 1991). The AMF species with low index of abundance and frequency (IAF) might be less adapted to the mining areas. Marinho *et al*. (2004) registered high IAF for *Glomus claroideum* and *G. macrocarpum* in mining degraded areas, and suggested that they should be used as inoculum in similar degraded areas since they are well established for envirionmental impacts. Conversely, AMF with medium to high IAF might be more adapted to local conditions. If these assumptions are correct, in the investigated areas *Glomus fasciculatum*, *G. macrocarpum* and *Acaulospora* sp are the most promising species for field inoculation.

AM fungal colonization

From the study area overall 29 plant species belonging to 14 families were scanned for AM fungal colonization (Table 1). In general all the plant

species showed AM fungal colonization, but the percentage of colonization was varied with each plant species. The highest percentage of root colonization was observed in plants which belongs to Poaceae family, among the Poaceae members, *Setaria intermedia* had more value than other species. Minimum value for AM colonization was recorded in plants belonging to family Cyperaceae (*Cyperus ridifolius* Steud.). The variation in the amount of AM fungal colonization is due to edaphic factors and environmental conditions.

Table 1. AM fungal colonization and spore number in stock mined spoil areas

Plant name/ Family	**PMC**	**SN**
Asclepiadaceae		
Hemidesmus indica R.Br.	27± 5.3 (31)	19 ± 4.1
Caesalpiniaceae		
Cassia tora L.	24 ± 5.2 (29)	27 ± 3.3
Asteraceae		
Ageratum conyzoides L.	58 ± 6.2 (62)	60 ± 5.2
Eupatorium odoratum Vahl.	67 ±8.0 (71)	51 ± 3.4
Tagetes minuta L.	81 ± 6.2 (87)	53 ± 4.2
Commelinaceae		
Commellina communis L.	37 ± 5.4 (43)	31 ± 8.2
Convolulaceae		
Evolvulus alsiloides L.	53 ± 6.3 (59)	3 ± 2.2
Cyperaceae		
Cyperus esculentus L.	13 ± 4.1 (36)	9 ± 1.3
Cyperus ridifolius Steud.	5 ± 3.1 (7)	10 ± 7.2
Euphorbiaceae		
Euphorbia hirta L.	32 ± 4.1 (36)	44 ± 3.3
Euphorbia sparsiflorus L.	24 ± 7.3 (31)	57 ± 2.4
Poaceae		
Chloris pycnothrix L.	71 ± 6.2 (81)	63 ± 2.2
Cynodon dactylon Pers.	77 ± 4.2 (81)	96 ± 7.1
Ergrotis tunnifolia Hochst.	83 ± 2.3 (89)	104 ± 4.5
Imparata cylindrica (L.) Raeu.	61 ± 5.1 (68)	61 ± 5.1
Setaria intermedia Roem.	87 ± 4.3 (93)	63 ± 4.2
Malvaceae		
Sida vernifolia Lam.	17 ± 6.2 (24)	51 ± 2.3
Mimosaceae		
Acacia melanoxylon R.Br.	48 ± 5.1 (52)	46 ± 6.1
Mimosa pudica L.	45 ± 8.3 (49)	53 ± 8.3
Fabaceae		
Desmodium trifoliata L.	18 ± 4.3 (24)	22 ± 5.1
Zorina didyphylla (L.) Pers.	71 ± 6.2 (78)	43 ± 3
Rhamnaceae		
Corchorus acutangulus Lam.	44 ± 4.5 (49)	11 ± 3.1
Tiliaceae		
Ziziphus trinarvia Roxb.	14 ± 6.1 (21)	71 ± 1.2
Utricaceae		
Terma orientalis (L.) Br.	21 ± 7.3 (30)	54 ± 4.4
Verbenaceae		
Lantana camara L.	23 ± 2.3 (27)	14 ± 5.4

Effect of AM fungi and mine spoil on growth and P uptake of selected plants

Effects of AM fungal inoculation on the growth and phosphorous uptake of *Jatropa curcas* and *Panicum miliaceum* are shown in Table 2 and 3. Plants inoculated with AM fungi *Glomus fasciculatum* and *Acaulospora leavis* respectively showed significantly higher values than non-mycorrhizal plants for all the parameters. The experiments were laid out with different concentrations of mine spoil along with unsterilized potting mixture. The varied plant growth responses were observed with respect to different concentrations of mine spoil. Analysis of variance revealed that increased plant height in *Jatropa curcas* and *Panicum miliaceum* grown with 0.50% and 0. 25% mine spoil respectively than other treatments (Table 2 and 3). It was found in present research that only 0.50% and 0.25% mine spoil with AM fungus contributed to P uptake in *Jatropa curcas* and *Panicum miliaceum* respectively causing higher total amounts of P per pot than any other treatment. In addition, only the plants inoculated with AM fungus found to have significantly higher biomass than uninoculated plants. Our results corresponds to findings reported by Perner *et al*. (2007) who found that P and K uptake in pelargonium (*Pelargonium peltatum*) was enhanced by AMF. They found low P and K concentrations in shoots of nonmycorrhizal plants whereas plants treated with AMF had high P concentrations and adequate K concentrations.

Table 2. Different AM fungal species recovered from the mined areas near to Yallapur in Uttara Kannada district

Sl. No	AM fungal species	Species Code*
1	*Acaulospora delicata* Wal.	ADLC
2	*Acaulospora mellea* Sp. & Smith	AMLL
3	*Acaulospora foveata* Trappe & Janos	AFVT
4	*Gigaspora albida* Sch & Smith	GABD
5	*Gigaspora decipiens* Hall & Abbott	GDCP
6	*Glomus claroides*	LCRD
7	*Glomus aggregatum* Schenck and Smith emend. Koske	LAGR
8	*Glomus clarum* Nicolson and Schenck	LCLR
9	*Glomus fasciculatum* Gerdemann & Trappe emend. Walker and Koske	LFSC
10	*Glomus geosporum* (Nicolson and Gerdemann) Walker	LGSP
11	*Glomus macrocarpum* Tulasne and Tulasne	LMCC
12	*Glomus callosum*	LCLL
13	*Glomus constrictum*	LCST
14	*Glomus microcarpum* Tulasne and Tulasne	LMRC
15	*Glomus fragile*	LFRG
16	*Glomus mosseae* (Nicolson and Gerdemann) Gerdemann and Trappe	LMSS
17	*Glomus reticulatum* Bhattacharjee & Mukerji	LRTC
18	*Scutellispora calospora* (Nicolson and Gerdemann) Walker and Sanders	CCLS
19	*Scutellispora erythropa* (Koske and Walker) Walker and Sanders	CERT

*According to Schenck and Perez manual (1990).

Table 3. Effect of AM fungus *Acaulospora leavis* and varied concentrations of mine spoil on growth of *Panicum miliaceum* L.

Treatments	SL (Cm)	RL (Cm)	FWR (g)	FWS (g)	DWR (g)	DWS (g)	PC (%)	SN / 25g soil
Non-Mycorrhizal	60.58 ±0.57d	18.00 ±0.57d	0.58 ±0.01e	3.08 ±0.03e	0.23 ±0.01e	1.21 ±0.01e	37.33 ±0.57e	65.33 ±1.52d
AM fungus (*Acaulospora leavis*)	72.19 ±0.57c	20.08 ±0.57c	0.84 ±0.00d	4.25 ±0.06d	0.26 ±0.01d	1.45 ±0.01d	48.08 ±1.15d	79.66 ±1.73c
AM fungus with mine spoil (1:0.25%).	107.82 ±0.57a	31.19 ±0.57a	3.19 ±0.01a	14.25 ±0.26a	1.36 ±0.00a	5.82 ±0.00a	95.33 ±1.15a	158.33 ±1.15a
AM fungus with mine spoil (1:0. 50%).	74.23 ±0.57b	24.66 ±0.57b	1.85 ±0.01b	8.16 ±0.00b	0.52 ±0.00b	3.83 ±0.00b	82.99 ±3.21b	140.25 ±1.52b
AM fungus with mine spoil (1:1%).	70.66 ±0.57b	20.33 ±0.57b	1.73 ±0.00c	4.74 ±0.00c	0.46 ±0.00c	2.23 ±0.05c	79.33 ±1.52c	138.33 ±1.15b

Table 4. Effect of AM fungus *Glomus fasciculatum* and varied concentrations of mine spoil on growth of *Jatropa curcas* L.

Treatments	SL (Cm)	RL (Cm)	FWR (g)	FWS (g)	DWR (g)	DWS (g)	PC (%)	SN / 25g soil
Non-Mycorrhizal	56.56 ±0.29e	20.71 ±0.36e	250.57 ±0.29	38.24 ±0.24d	28.87 ±0.47d	10.55 ±0.29e	0.00 ±0.00f	0.00 ±0.00e
AM fungus (*Glomus fasciculatum*)	74.75 ±0.24c	26.50 ±0.28b	254.26 ±0.63c	39.64 ±0.32c	31.98 ±0.56bc	12.58 ±0.32c	87.77 ±0.22c	253.66 ±0.33c
AM fungus with mine spoil (1:0.25%)	76.61 ±0.31b	29.04 ±0.04a	267.49 ±0.28b	42.28 ±0.64b	33.55 ±0.55b	13.72 ±0.28b	89.66 ±0.33b	267.33 ±0.33b
AM fungus with mine spoil (1:0. 50%)	81.87 ±0.47a	23.57 ±0.29c	270.97 ±0.55a	45.25 ±0.38a	35.63 ±0.63a	14.99 ±0.01a	92.99 ±0.01a	279.33 ±1.76a
AM fungus with mine spoil (1:0.75%)	69.99 ±0.57d	21.70 ±0.35d	251.54 ±0.29de	39.67 ±0.32c	30.72 ±0.28c	11.62 ±0.31d	75.74 ±0.37d	201.33 ±0.88d
AM fungus with mine spoil (1:1%)	65.43 ± 0.47de	20.63 ±0.33e	252.66 ±0.28de	38.46 ±0.28d	29.98 ±0.31d	11.02 ±0.01de	71.34 ±0.63e	197.43 ±0.33de

Increased plant growth due to AMF inoculation is mainly through improved in uptake of diffusion limited nutrients such as P (Krishna, and Bagyaraj, 1991; Lambert *et al.*, 1979). AM fungi improving plant biomass were

also good in increasing the P content of the host, significantly highest being in plants inoculated with AM fungus and optimum level of mine spoil. Selected efficient fungi enhancing plant biomass and P uptake has been reported in other plants by several workers (Ulfath *et al*., 2006; Vasanthakrishna *et al*., 1995). Such higher P content in AMF inoculated plants is attributed to higher influx of P into the plant system through AM fungi which explores the soil volume beyond P depletion zone (Bagyaraj and Varma, 1995; Hattingh *et al*., 1973 and, Sanders and Tinker, 1971). The enhancement in growth and nutritional status was also related to mycorrhizal root colonization and spore numbers in the root zone soil. This upholds the observations made by earlier workers on other plants (Gracy and Bagyaraj, 2005).

In terms of per cent root colonization of *Jatropa curcas* and *Panicum miliaceum*, with mine soil 0.50% and 0.25% gave highest figures. However, AMF spore number in the AM fungi inoculated plants with same concentration of mine spoil varied slightly. The colonization levels of AM fungi may indicated the degree to which biological activity has been restored in stock piled mine spoils (Zak and Parkinson, 1982; Miller *et al*., 1985). This would lead to restoration of an ecosystem and combating environmental pollution on stock piled soils by introducing mycorrhizal plants. Mycorrhizal saplings transplantation to degraded mined soils from pot experiment is practically required.

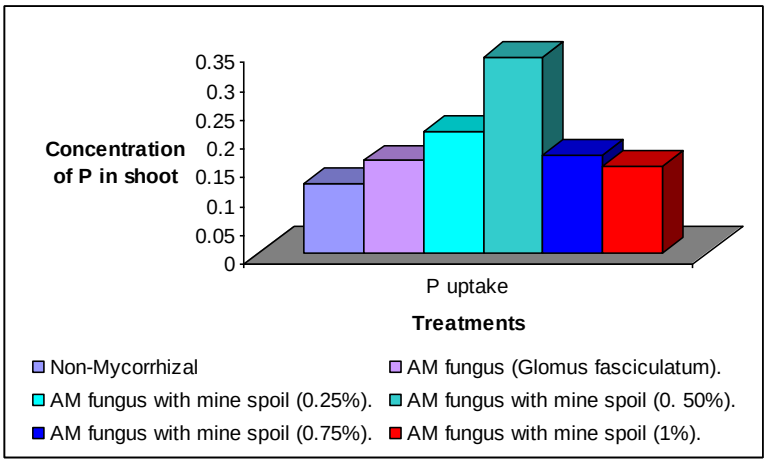

Fig. 1. Effect of AM fungus Glomus fasciculatum and different levels of mine spoil on P uptake of *Jatropa curcas* L.

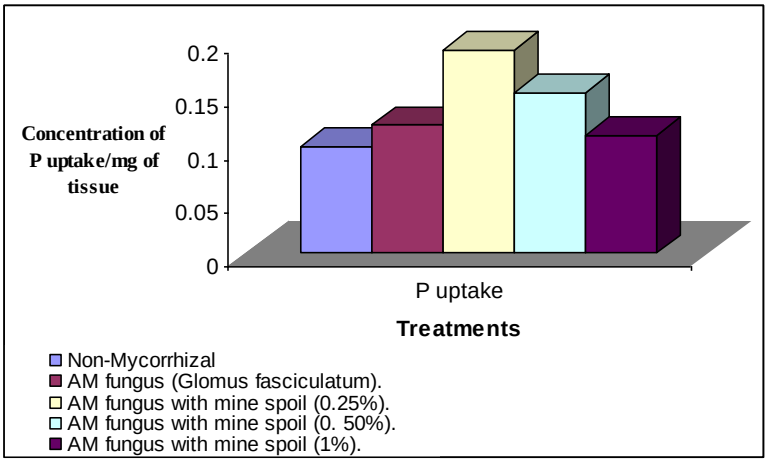

Fig. 2. Effect of AM fungus Glomus fasciculatum and different levels of mine spoil on P uptake of *Panicum miliacaeum* L.

Conclusion

The results of this study showed that 16 AM fungal species were identified in the study area, *Glomus* (12), *Acaulospora* (3), *Gigaspora* (2) and *Scutellospora* (2). *Glomus* was the dominant genus. The *Glomus fasciculatum* and *Acaulospora leavis* were found to be efficient *Jatropa curcas* and *Panicum miliaceum* growth promoters respectively from the preliminary studies. They not only increased growth but also increased P uptake. Additionally, mycorrhizal plants treated with 0.25% and 0.50% showed significant growth over the higher concentrations of mine spoil in potting mixture along with AM fungal inoculation. These findings suggested the potential of AM fungi and optimum levels of mine spoil for use as an inoculum for the increased production of biomass of *Jatropa curcas* L., and *Panicum miliaceum* L.

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