

Survey of Indigenous Arbuscular Mycorrhizal Fungi under Ecosystem of Saudi Arabia

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Distribution and colonization of arbuscular mycorrhizal fungi were studied under plant vegetation, which was found growing naturally in the near edges of the old road between Jeddah to Mecca at western of Saudi Arabia kingdom, during the period between May to September 2013. One hundred and twenty five roots and rhizospheric soils of 12 plant species belonging to 8 families from 25 sites at different 3 locations were collected and examined. Mycorrhizal fungi were recorded in 11 taxa, 3 genera, 3 families, 2 orders, one class and one phylum from all sites. On the generic level, *Glomus* was the dominant genus in rhizospheric soil, which *Glomus sinusum* (Gerd. & Bakshi) Almeida & Schenck and *G. macrocarpum* Tulasne & Tulasne were the dominant species associated with examined plant species. The plants to family Asclepiadaceae were contained of 43.85% from total collected spores in all study sites. On the other hand the average of distribution species of mycorrhizal fungi in all examined plants between 2 and 5 species for plant family. The *G. macrocarpum* was recorded in 10 sites out of 25 sites collected from different locations.

Key words: Endo-mycorrhiza, Diversity, Distribution, Colonization, Wild plants, Saudi Arabia.

Arbuscular mycorrhizal fungi (AMF) are major component of rhizosphere microflora in terrestrial ecosystem, forming symbiotic association with the majority of plant species (Smith and Read 1997; Neelam *et al.*, 2008). Accumulating evidence indicate that AMF association plays a significant role in mineralization of plant nutrients, decomposition of soil organic matter and nutrient recycling (Tarafdar and Rao 1997; Pare *et al.*, 2000; Abdel-Fattah 2002; Almaghrabi and Abdelmoneim 2012). The population pattern of AMF and their diversity is

affected by various factors including soil, environmental condition, host plant and some agricultural treatments (Sanders 1990; McGonigle and Miller 1996; Al-khalil 2010). The geographic distribution of species of AMF influenced by edaphic factors (A permanent or nearly permanent condition of the substrate that influences the types of AMF that grow in an area), plays an important role for their distribution and predicting levels of indigenous AMF population with necessary understand fungus dynamics, quantification and identification (Neelam *et al.*, 2008; Baraka *et al.*, 2012). Taxonomy of AMF has based on morphological and anatomical characteristic of their spores and other modern techniques (Schenck and Perez 1987; Walker 1992). The present study was undertaken to isolate and identify the AMF

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associated with plant growing in study area (western of Saudi Arabia Kingdom between Jeddah and Mecca city). Also estimate the AMF status, and study the number of species and population of AMF, which coexist with different plant species growing at different sites in study area. On the other hand estimated the percentage of AMF root colonization, which influenced by some edaphic factors.

MATERIALS AND METHODS

Study area

The study area is located between Jeddah to Mecca at western of Saudi Arabia kingdom. This area lies within the subtropical dry zone of the deserts defined by Walter *et al.*, (1975). The rainfall is scanty about 71.3 mm/year at Jeddah. The air temperature is high with a mean maximum of 41.6 °C and a mean minimum of 14.5 °C. The relative humidity is high at Jeddah and decreases considerably as one proceeds in land towards Mecca.

Soil sampling

Soil samples including roots were collected from the surrounding vegetation, which were growing in the near edges of the old road between Jeddah to Mecca. Twenty five sites were separated in three locations (Table 1, Fig. 1). Five soil samples were collected from each site in sealed polythene bags. The soil sampling was done at a depth of 40±5 cm under wild plants. The soil and root samples were processed for extraction, identification of arbuscular mycorrhizal fungi (AMF) and soil analyses.

Clearing, staining and measuring of AMF root colonization

Root were separated from collected soil samples and assayed from AMF colonization after clearing and staining in 0.05% Trypan blue for 24hr at room temperature as described by Brundrett *et al.*, (1984). The root samples were de-stained at room temperature in acidic glycerol (Koske and Gemma, 1989). Randomly selected segments of fine lateral roots were mounted on microscope slides to detect the presence of vesicles arbuscules and any unusual features. A total of 10 root fragments (3cm) were examined for each site and percentage AMF colonization was measured according to Brundrett *et al.*, (1996).

Extraction of AMF from soil samples and identification

AMF spores were extracted by wet sieving and decanting technique (Gerdemann and Nicolson 1963). Semi-permanent slides were prepared by mounting the spores in polyvinyl lactophenol. The photographs were taken by using the compound light microscope (LIECA model MD502). The spore density was expressed in terms of the number of spores per 100g of soil. The spores were identified on the basis of color, size, shape, nature of spore cell wall and hyphal attachment with the help of synoptic keys of the Schenck and Perez (1987) and Walker (1992).

Soil analyses

Soil sub-samples from locations within each uniform sampling area were mixed to make a composite sample, air dried and passed manually through a 2mm sieve. Particle size distribution was carried out by sieving method (Piper, 1950). Soil classification up to the sub-great was described according to Soil Survey Manual (USAD, 1951).

Chemical analysis

Organic matter content was determined according to Wolkely and Black rapid method (Jackson 1967) and total calcium carbonates were volumetrically estimated by using Collin's Calcimeter (Wright 1939). Chemical properties including cations (Ca^{++} , Mg^{++} , Na^+ and K^+), anions (Cl , $\text{HCO}_3 + \text{CO}_3$ and SO_4), CaCO_3 , electric conductivity and pH were determined according to Page *et al.*, 1982. The pH value was estimated by using portable pH meter (model HI 8314 Hanna) and electric conductivity (EC) by using microprocessor EC/TDS meter (model HI 98360 Hanna).

RESULTS

During the study soil analysis form 25 sites in three locations, we found the total calcium carbonate (CaCO_3) value ranged between 0.28 and 11.43 meq l^{-1} . It showed a great differences among the studied samples; some showed a low content while other, especially in location 2 at site 13 (table 2). The most pH values to all sites are slightly alkaline to alkaline where soil pH values ranged between 7.5 to 9.0. The electrical conductivity (EC) in soil samples, which collected from different sites ranged between 0.12 and 0.55 dsm $^{-1}$. The mean

values of organic matter percentage ranged between 0 and 0.4%. While the cationic composition of soluble salts executed from these soils are mostly dominated by Na⁺ and Mg⁺⁺. On the other hand the anionic composition represented by Cl⁻ and HCO₃⁻. These factors are the main reasons, which lead the soil reaction towards the alkaline soils in the study area.

The total number of sampled plant species was 12 plant species belonging to 8 families distributed in 25 sites in three locations. The families Asclepiadaceae, Leguminosae and zygothyllaceae showed the highest number of arbuscular mycorrhizal fungi (AMF) species (5 species each), followed by Amaranthaceae (3 species). The remaining families Apocynaceae, Chenopodiaceae, Cruciferae and Malvaceae were

showed the same number of AMF species (2 species each). The most dominant genera of AMF in the examined rhizosphere soil of the study area were *Glomus sinusum* (Gerd. & Bakshi) Almeida & Schenck and *G. macrocarpum* Tulasne & Tulasne followed by *Glomus deserticola* Trappe, Bloss & Menge (Table 3). The AMF are assigned in 11 taxa, 3 genera (*Glomus*, *Acaulospora* and *Scutellospora*), 3 families (Glomeraceae, Acaulosporaceae and Gigasporaceae), 2 orders (Glomerales and Diversisporales), one class (Glomeromycetes) and one phylum (Glomeromycota) from all sites according to the scheme proposed by Kirk *et al.*, (2001).

The family of Glomeraceae accommodates the greatest range of species (9 species) and Acaulosporaceae and Gigasporaceae

Table 1. Global Possession System (GPS) reading of the selected sites in the study area

Location No.	Site No.	GPS reading			Plant species
		N°	E°	Elevation (m)	
1	1	21°54'10.34"	39°21'20.31"	17	<i>Calotropis procera</i> (Ait.) Ait. f.
	2	21°53'55.80"	39°21'31.27"	20	<i>Indigofera argentea</i> Burm. f.
	3	21°53'46.20"	39°21'31.77"	15	<i>Aerva javanica</i> (Burm.f.) Spreng.
	4	21°53'50.93"	39°21'45.26"	12	<i>Acacia ehrenbergiana</i> Hayne
	5	21°53'32.93"	39°22'06.28"	23	<i>Zygophyllum simplex</i> L.
	6	21°53'09.37"	39°22'53.02"	20	<i>Suaeda monoica</i> Forssk.
	7	21°53'07.38"	39°23'07.38"	16	<i>Leptadenia pyrotechnica</i> (Forssk.) Decaisne.
2	8	21°52'32.04"	39°23'54.97"	12	<i>Aerva javanica</i> (Burm.f.) Spreng.
	9	21°52'03.39"	39°24'31.79"	10	<i>Acacia ehrenbergiana</i> Hayne
	10	21°51'11.30"	39°26'15.24"	29	<i>Zygophyllum simplex</i> L.
	11	21°49'15.11"	39°27'47.84"	12	<i>Indigofera argentea</i> Burm. f.
	12	21°48'45.39"	39°28'31.00"	11	<i>Calotropis procera</i> (Ait.) Ait. f.
	13	21°47'21.08"	39°29'21.28"	9.0	<i>Aerva javanica</i> (Burm.f.) Spreng.
	14	21°46'44.82"	39°29'48.98"	23	<i>Zygophyllum simplex</i> L.
3	15	21°46'19.29"	39°30'28.44"	21	<i>Abutilon pannosum</i> (Forst. F.) Schlecht.
	16	21°46'23.46"	39°30'45.01"	22	<i>Leptadenia pyrotechnica</i> (Forssk.) Decaisne.
	17	21°46'03.31"	39°30'50.82"	20	<i>Tribulus parvispinus</i> Presl.
	18	21°45'22.47"	39°31'41.18"	16	<i>Calotropis procera</i> (Ait.) Ait. f.
	19	21°44'34.36"	39°32'15.12"	22	<i>Leptadenia pyrotechnica</i> (Forssk.) Decaisne.
	20	21°44'09.93"	39°32'42.58"	12	<i>Farsetia longisiliqua</i> Decaisne.
	21	21°42'34.63"	39°34'10.36"	31	<i>Dipterygium glaucum</i> Decaisne.
3	22	21°42'10.84"	39°34'39.66"	23	<i>Rhazya stricta</i> Decaisne.
	23	21°41'53.26"	39°34'42.50"	28	<i>Abutilon pannosum</i> (Forst. F.) Schlecht.
	24	21°41'21.22"	39°35'13.26"	21	<i>Acacia ehrenbergiana</i> Hayne
	25	21°40'51.14"	39°35'48.74"	20	<i>Calotropis procera</i> (Ait.) Ait. f.

accommodate the lowest range of species (1 of each). On the species level, genus *Glomus* came by 9 species namely: *Glomus sinusum* (Gerd. & Bakshi) Almeida & Schenck, *G. aggeratum* Schenck & Smith, *G. macrocarpum* Tulasne & Tulasne, *G. deserticola* Trappe, Bloss & Menge,

Table 2. Present status of some physical and chemical properties for the collected soil samples at the three sites

Location No.	Site No	pH	EC dsm ⁻¹	Cations meql ⁻¹				Anions meql ⁻¹				OM %	Soil texture
				Ca ⁺⁺	Mg ⁺⁺	Na ⁺	K ⁺	Cl	HCO ₃ ⁺ CO ₃	SO ₄	CaCO ₃		
1	1	8.2	0.357	1.23	2.00	1.2	1.3	2.50	1.55	0.45	0.300	0.01	Sandy
	2	9.0	0.288	1.30	0.77	0.8	0.2	1.40	1.83	2.00	0.430	0.00	Sandy
	3	8.6	0.190	1.52	0.45	1.1	0.3	1.40	1.00	2.70	8.330	0.02	Sandy
	4	8.0	0.466	1.03	0.71	0.7	0.2	1.00	1.70	0.16	10.76	0.00	Sandy
	5	8.8	0.365	1.81	0.40	0.7	0.4	1.20	2.40	1.97	8.210	0.01	Sandy
	6	8.5	0.551	1.62	0.62	1.3	1.2	1.00	2.03	0.55	8.330	0.00	Sandy
	7	7.9	0.361	2.00	0.73	1.4	0.5	1.80	1.99	0.33	9.120	0.01	Sandy
	8	8.5	0.254	1.20	1.00	0.8	0.1	0.48	1.19	0.12	7.350	0.00	Sandy
2	9	8.7	0.320	1.50	0.55	1.1	0.4	1.00	2.43	0.44	8.490	0.00	Sandy
	10	8.3	0.213	0.17	1.07	0.6	0.3	2.41	2.63	0.91	4.587	0.00	Sandy
	11	8.0	0.211	0.88	1.59	1.1	0.2	0.97	2.65	0.71	3.186	0.00	Sandy
	12	8.1	0.354	1.44	1.62	0.8	1.1	0.33	0.72	0.35	10.76	0.01	Sandy
	13	7.8	0.470	2.00	0.61	0.7	0.3	1.50	2.76	0.10	11.43	0.20	Sandy
	14	7.6	0.230	1.33	0.54	0.6	0.2	1.43	1.40	0.90	0.330	0.00	Sandy
	15	8.0	0.212	1.00	1.00	0.8	0.2	3.00	0.73	1.67	9.232	0.00	Sandy
	16	7.9	0.175	1.60	0.55	0.5	0.7	1.40	0.80	1.17	2.050	0.00	Sandy
3	17	8.5	0.155	0.45	0.67	1.1	0.5	1.22	4.63	12.5	9.430	0.03	Sandy
	18	8.7	0.154	0.23	0.86	0.9	0.6	1.52	0.65	0.30	0.280	0.00	Sandy
	19	8.1	0.123	0.44	0.77	0.8	0.3	1.00	1.73	0.53	2.050	0.25	Sandy
	20	7.8	0.432	0.50	0.88	1.0	0.2	2.00	2.54	0.90	10.56	0.40	Loamy sand
	21	8.4	0.327	0.72	0.54	1.1	0.6	1.00	1.37	0.58	8.330	0.00	Sandy
	22	7.8	0.321	0.58	0.44	1.1	0.1	1.20	2.00	0.54	3.330	0.00	Sandy
	23	8.2	0.290	1.30	0.55	0.7	0.4	1.30	2.67	0.33	8.710	0.10	Sandy
	24	7.5	0.376	0.21	0.80	0.3	0.5	1.00	1.84	0.10	9.220	0.04	Sandy
	25	8.2	0.324	0.50	1.10	0.2	0.3	1.63	2.00	1.60	10.73	0.00	Sandy

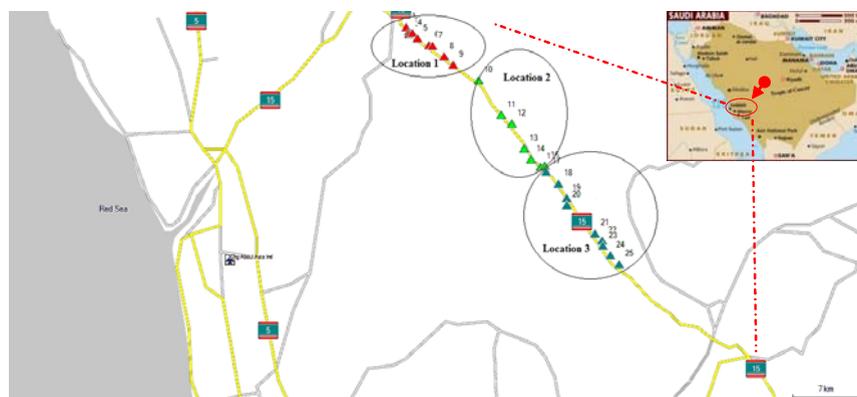


Fig. 1. Map showing the sites for sample collection

Table 3. List of arbuscular mycorrhizal fungi (AMF) associated with different plant species in the study area, the mean of number of AMF spores/100gm of soil and AMF root colonization %.

Location No.	Site No.	Dominant plant species	Common AMF species	spores/100gm soil	AMF root colonization %
1	1	<i>Calotropis procera</i> (Ait.) Ait. f.	<i>Glomus sinuosum</i>	125	31.0
			<i>Glomus aggregatum</i>		
	2	<i>Indigofera argentea</i> Burm. f.	<i>Glomus macrocarpum</i>	40.0	8.60
	3	<i>Aerva javanica</i> (Burm.f.) Spreng.	<i>Glomus deserticola</i>	54.0	17.3
			<i>Glomus mosseae</i>		
			<i>Glomus sinuosum</i>		
	4	<i>Acacia ehrenbergiana</i> Hayne	<i>Glomus sp.</i>	9.00	3.00
	5	<i>Zygophyllum simplex</i> L.	<i>Glomus mosseae</i>	6.00	2.00
	6	<i>Suaeda monoita</i> Forssk.	<i>Glomus macrocarpum</i>	27.0	8.30
			<i>Glomus deserticola</i>		
			<i>Glomus invernatum</i>	20.0	7.00
2	7	<i>Leptadenia pyrotechnica</i> (Forssk.) Decaisne.	<i>Glomus sinuosum</i>	31.0	7.60
	8	<i>Aerva javanica</i> (Burm.f.) Spreng.	<i>Glomus etunicatum</i>	22.0	8.60
	9	<i>Acacia ehrenbergiana</i> Hayne	<i>Glomus coronatum</i>	18.0	7.00
	10	<i>Zygophyllum simplex</i> L.	<i>Glomus macrocarpum</i>		
	11	<i>Indigofera argentea</i> Burm. f.	<i>Glomus macrocarpum</i>	29.0	9.00
			<i>Glomus deserticola</i>		
			<i>Glomus mosseae</i>	53.0	16.8
	12	<i>Calotropis procera</i> (Ait.) Ait. f.	<i>Glomus macrocarpum</i>		
	13	<i>Aerva javanica</i> (Burm.f.) Spreng.	<i>Glomus sinuosum</i>	11.0	2.60
	14	<i>Zygophyllum simplex</i> L.	<i>Glomus clarum</i>	26.0	19.33
			<i>Glomus macrocarpum</i>		
3	15	<i>Abutilon pannosum</i> (Forst. F.) Schlecht.	<i>Acaulospora</i> sp.	8.00	4.50
	16	<i>Leptadenia pyrotechnica</i> (Forssk.) Decaisne.	<i>Glomus deserticola</i>	5.00	1.20
	17	<i>Tribulus parvispinus</i> Presl.	<i>Glomus etunicatum</i>	7.00	2.00
	18	<i>Calotropis procera</i> (Ait.) Ait. f.	<i>Glomus aggregatum</i>	15.0	3.80
			<i>Glomus macrocarpum</i>		
	19	<i>Leptadenia pyrotechnica</i> (Forssk.) Decaisne.	<i>Glomus sinuosum</i>	3.00	0.88
	20	<i>Farsetia longisiliqua</i> Decaisna.	<i>Glomus macrocarpum</i>	3.00	1.11
			<i>Glomus sp.</i>		
	21	<i>Dipterygium glaucum</i> Decaisne.	<i>Glomus sinuosum</i>	26.0	8.50
	22	<i>Rhazya stricta</i> Decaisne.	<i>Glomus macrocarpum</i>	22.0	5.20
			<i>Scutellospora</i> sp.		
23	<i>Abutilon pannosum</i> (Forst. F.) Schlecht.	<i>Glomus multicaule</i>	15.0	9.60	
24	<i>Acacia ehrenbergiana</i> Hayne	<i>Glomus deserticola</i>	33.0	0.00	
		<i>Glomus sinuosum</i>			
25	<i>Calotropis procera</i> (Ait.) Ait. f.	<i>Glomus macrocarpum</i>	11.0	4.75	
		<i>Glomus deserticola</i>			
		<i>Glomus macrocarpum</i>			

G. mosseae (Nicol. & Gerd.) Gerd. & Trappe, *G. invermaium* Hall, *G. etunicatum* Becker & Gerdemann, *G. coronatum* Giovannetti and *G. multicaule* Gerd. & Bakshi. The association

between plant roots and AMF was not observed as the same but species *Calotropis procera* (Ait.) Ait. f. and *Acacia ehrenbergiana* Hayne were showed the highest number of AMF spores/100gm

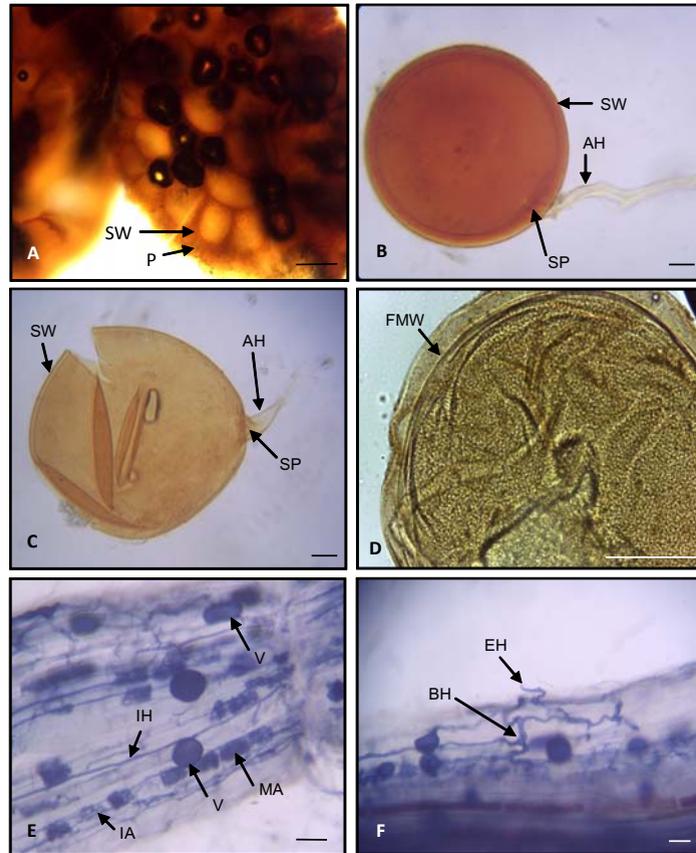


Fig. 2. Photomicrographs for some spores of arbuscular mycorrhizal fungi (AMF), which extracted from soil samples and their structures in the plant roots after clearing and staining in trypan blue at room temperature (Bar =30µm). A- Spore of *Glomus sinuosum*; B- *G. macrocarpum*; C- *G. coronatum* and their structural; D- Structural of *Acaulospora* sp.; E and F structures of AMF in plant roots. P: Peridium, AH: Attachment hyphae, SW: Spore wall, SP: Spore pore, FMW: Flexible middle wall, V: Vesicle IH: Intercellular hyphae, IA: Immature arbuscule, MA: Mature arbuscule, BH: Branched hyphae, EH: External Hyphae

Plant species	<i>Glomus sinuosum</i>	<i>G. aggregatum</i>	<i>G. macrocarpum</i>	<i>G. deserticola</i>	<i>G. mosseae</i>	<i>G. invermaium</i>	<i>G. etunicatum</i>	<i>G. coronatum</i>	<i>G. multicaule</i>	<i>Glomus</i> sp.	<i>Acaulospora</i> sp.	<i>Scutellospora</i> sp.
<i>Calotropis procera</i> (Ait.) Ait. f.	■	■	■	■								
<i>Indigofera argentea</i> Burm. f.												
<i>Aerva javanica</i> (Burm.f.) Spreng.	■			■	■					■		
<i>Acacia ehrenbergiana</i> Hayne			■				■					
<i>Zygodium simplex</i> L.	■		■					■				
<i>Suaeda monoica</i> Forssk.				■								
<i>Leptadenia pyrotechnica</i> (Forssk.) Decaisne.	■			■		■						
<i>Abutilon pannosum</i> (Forst. F.) Schlecht.				■							■	
<i>Tribulus parvispinus</i> Presl.							■					
<i>Farsetia longistylis</i> Decaisne.										■		
<i>Dipterygium glaucum</i> Decaisne.	■		■									
<i>Rhazya stricta</i> Decaisne.									■			■

Fig. 3. Distribution of AMF in different plant species collected from 25 sites from study area

soil during the study by values 125 and 54 spores/100gm soil respectively, while *Tribulus paryispinus* Presl. was recorded as lowest plant associated with AMF by value 7 spores/100gm soil (Table 3). The high percentage of AMF root colonization was found in plant family Asclepiadaceae on plant *Calotropis procera* (Ait.) Ait. f. by value 31%, and the lowest value was observed in *Farsetia longisiliqua* Decaisna belonging to family Cruciferae by value 1.11%. Also some plant families (Asclepiadaceae, Amaranthaceae and Leguminosae) were showed in high level for association with AMF comparing with other as Zygomycetaceae (Table 4 and Fig. 2).

Table 4. The mean of total number of spores per plant family

Plant family	Total No. of spores/100g soil
Amaranthaceae	96.0
Apocynaceae	22.0
Asclepiadaceae	232
Chenopodiaceae	27.0
Cruciferae	29.0
Leguminosae	93.0
Malvaceae	23.0
Zygomycetaceae	7.00

DISCUSSION

This study is a preliminary study to estimate distribution and colonization of arbuscular mycorrhizal fungi (AMF) in western site of Saudi Arabia Kingdom. The pH values of our study area were very narrow from 7.5 to 9.0. The pH values were slightly alkaline to alkaline, which may be effect on the availability of chemical nutrients in soil, which lead to make a poor fertility soil. The poor fertility condition in soil was lead to enhance AMF for spore to build up (Jasper *et al.*, 1979; Dehne 1987; Abdel-Fattah 2002; Almagrabi and Abdelmoneim 2012). The *Glomus* has been the most dominant genus of AMF in the examined rhizosphere soil of the study area.

The predominance of *Glomus* species varying edaphic conditions that may be due to the highly adaptable of this genus to varied soil and temperature conditions. That make it can survive in acidic as well as alkaline soil (Ho 1987; Neelam *et al.*, 2008; Al-Khaliel 2010; Qarawi and Alshahrani

2010). However, the rate of colonization among plant species was varied and plant species belonging to families Asclepiadaceae, Amaranthaceae and Leguminosae showed the high rate of colonization in these families has previously been reported by Yang *et al.*, (2007) and Silvani *et al.*, (2008). Our finding are against the finding of Varma (1998) who stated that there are plants, however that have been shown to be mycorrhiza free, such as Cruciferae, Zygomycetaceae and Amaranthaceae were thought to be mycorrhizae free, most of the species were found to be infected under natural stressed rangeland conditions. The number of AMF spores in rhizosphere soils differed among plant species of the same habitat. This suggests that AMF distribution does not coincide with the zonation pattern of vegetation. These differences may be related to different behavior of each AMF species, even in similar ecosystem (Kironomos *et al.*, 1993).

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