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Distribution of the Arbuscular Mycorrhizal Fungi in AlJabal Alakhdar Area, East Libya

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ABSTRACT

In order to investigate occurrence and distribution of Arbscular Mycorrhizal Fungi (AMF) in Aljabal Alakhthar area, east Libya. Roots and rhizosphere soil of 49 cultivated plants belonging to 7 families from 8 locations were collected. The percentage of root colonized by AMF was estimated. Spores extracted from soil samples were counted and morphologically identified to genus. The results indicate that all examined plants were colonized by AMF. Colonization rate and spore abundance differed according to location and plant. Colonization rate range from 97% in Petro selinum at Alqubbah location to 29 % in Vicia faba at Almarj. While spore abundance range from 992 spores / 100gm soil accompanied with Vicia faba at West alawilia location to 121 spores / 100gm accompanied with Avena sativa at Alqubbbah. As average of all locations, the plants belonging to Apiaceae had the highest colonization percentage where the plants belonging to Cucurbitaceae had the highest spore abundance. Lumloda location records the highest colonization rate. There was no correlation detected between root colonization rate and spore abundance. However, soil available phosphorus and clay percentage negatively correlated with degree of AM root colonization. Positive correlation was found between silt percentage and spore abundance. Glomus was the most abundant genus in all studied locations followed by Acaulosora, Gigaspora and Scutellospora

Keywords: *Arbuscular mycorrhiza, Aljabal Alakhdar, fungi*

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توزيع فطريات المايكورايزا الشجيريه في منطقة الجبل الاخضر، شرق ليبيا

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الملخص

لدراسة تواجد وتوزيع فطريات المايكرايزا الشجيريه في منطقة الجبل الاخضر ، شرق ليبيا اخذت عينات جذور وتربه من منطقة الجذور ل٤٩ نبات زراعي والتابعه لثمانية عائلات نباتيه من ٨ مواقع. قدرت نسبة اصابة الجذور بفطريات المايكورايزا وعدد السبورات في التربه. اوضحت النتائج ان جميع النباتات المفحوصه كانت مصابه بفطريات المايكورايزا الشجيريه. معدل الاصابه وعدد السبورات اختلفت باختلاف الموقع والعائله النباتيه. معدل الاصابه تراوح من ٩٧% في نبات *Petro selinum* في موقع القبه الى ٢٩% في نبات *Vicia faba* في موقع المرج. بينما عدد السبورات تراوح من٩٩٢ سبور / ١٠٠غم تربه مرافقه لنبات *Vicia faba* في موقع العويله الغربيه الى ١٢١/ ١٠٠غم تربه مرافقه لنبات *Avena sativa* في موقع القبه . كمعدل عام لجميع المواقع ،النباتات التابعه للعائله النجيليه اعطت اعلى نسبة اصابه في حين اعلى عدد للسبورات كانت مرافقه لنباتات العائله القرعيه. كما اعطت النباتات الناميه في موقع لملوده اعلى نسبة اصابه. لم يلاحظ اي علاقه معنويه بين نسبة الاصابه وعدد السبورات بينما لوحظ علاقه معنويه سالبه بين تركيز الفسفور الجاهز ونسبة الطين من جهه ونسبة الاصابه من جهه اخرى وكما لوحظ علاقه معنويه موجبه بين النسبه المئويه للغرين وعدد السبورات. كان جنس *Glomus* الاعلى وفره في جميع المواقع تبعه الجنس *Acaulospora* ثم *Gigaspora* ثم الجنس *Scutellospora* .

الكلمات الدالة: المايكورايزا الشجيريه ، الجبل الاخضر، فطريات

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1. INTODUCTION

Al Jabal Al Akhdar (Green Mountain) is located in approximately between latitudes 32° and 33° North and 20° and 23° East in the northeastern region of Libya and south Mediterranean Sea. It is 881m above sea level. It extends on the coast belt to about 300 km. The area receives total annual rainfall 250-600mm [1, 2]. Although Al Jabal Al Akhdar covering only 1% of Libyan area. It consider the biggest and most Important Plant Area (IPA) in Libya as it contains 75 – 80% of the Libyan flora and considered the most important area for agriculture in Libya [3]. 35% of the Al Jabal Al Akhdar area converted to crop areas mainly for grain , forage and vegetable production like wheat, barley and oats and 65% still the actual area of natural forest [4], [5]. Arbuscular mycorrhizal (AM) symbioses are formed between plant roots and fungi belonging of Glomeromycota [6]. Arbuscular Mycorrhizal Fungi (AMF) interactions predominate, which are found in about 80-90% of terrestrial plant families in natural and agricultural ecosystems [7]. AMF can colonize 300000 plant species belonging to 1000 genera followed 200 plant families [8]. Arbuscular mycorrhizal fungi are considered as obligate symbiotic biotopes, as they cannot grow without a host plant supplying them with carbohydrates [9], [10], [11]. In this symbiotic association, the fungus occurs inside cortex cell and also extend out in to surrounding soil as a hyphal network [9], [12]. In AM symbiosis, both organisms benefit as the fungus received photosynthesis products from host plant.The host plant receives a variety of benefits have already reported in earlier studies including:

1- Enhanced nutrient uptake over non - mycorrhizal controls [13]
2- improved water relations (increase drought resistance) [14]
3- Increase pest and disease resistance [15], [16].
4- Modification of root morphology [17].
5- Increase Efficiency of phytoremediation of contaminated water and soil [18].
6- Increase efficiency of nitrogen fixation by Rhizobium [19]
7- Enhancement of soil aggregation and stability [20]

Because of the public concerns about the side effects of pesticide and chemical fertilizer, major attention has been given to research areas concerning arbuscular mycorriza as biofertilizer and biocontrol agent.Despite the importance of arbuscular mycorrhiza in agriculture and forestry,

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there is no work has been done regarding their presence, distribution and diversity in the Aljabal Alakhdar area. The objective of this study is to evaluate the occurrence and distribution of FAM accompanying agricultural crops in AL Jabal Al Akhthar area.

2. Methods and Materials

2.1. Root and soil samples collection

Roots and rhizosphere soil samples were collected from the most common 49 cultivated plant (3 replicate), from 8 locations in Al-Jabal Al-Akhdar. These sampling locations were chosen due to their high agricultural density Fig. (1), Table (1). The roots were fixed in formalin-acetic acid-alcohol (FAA; 10:35:10:5Formalin-water-ethanol-acetic acid) as soon as possible and were kept with soil samples at 5°C for further analysis

2.2. Soil Analysis

Soil chemical and textural characteristics were determined as following. Soil texture by hydrometer method, Soil Reaction (pH) by pH meter, CEC by the sodium acetate-method. The total nitrogen by kjeldahl method, Organic Content by rapid titration method [21], available phosphorus by Olsen's method [22], Ca and Mg by titration with EDTA (23), Potassium and sodium by flame photometer [24]. The main characteristics of the soil are recorded in Table 2.

Table (1): Geographic coordinates of the sampling locations

	Longitude	Latitude
West alawilia	**E 20°45'10.64"**	**N 32°29'37.90"**
Almarj	**E 20°49'59.63"**	**N 32°28'55.64"**
Alawilia	**E 20°58'25.21"**	**N 32°33'8.30"**
Albalenge	**E 21°40'40.78"**	**N 32°45'48.36"**
Alwasita	**E 21°43'49.56"**	**N 32°48'31.33"**
Alhaboon	**E 21°57'22.22"**	**N 32°49'2.65"**
Lumloda	**E 22° 8'23.34"**	**N 32°46'54.94"**
Alqubbah	**E 22°15'0.06"**	**N 32°46'2.44"**

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Fig. (1): Sampling Locations

2.3. Assessment of AMF colonization

Root fixed in FAA and washed with water 3 times. The percentage of mycorrhizal colonization of roots were estimated by cutting the roots into 1cm pieces and clarified with 10%(w/v) KOH at 90ºC in water bath for 20-30 minutes and acidification by HCl (5%) for one minute and staining with trypan blue at 90ºC for ten minutes [25]. The stained roots placed on the glass slides for microscopic observations under 200×magnifications . AMF colonization was estimated by examination one hundred pieces of roots for each sample. Percent root colonization was counted according

$$\text{Percent Root Colonization} = \frac{\text{Number of Root Segments Colonized}}{\text{Number of Root Segments Observed}} \text{X100}$$

2.4. Recovery and counts of AM fungal spores

AMF spores were extracted from 100 g Rhizosphere soil by wet sieving and decanting [26] followed by sucrose gradient centrifugation and the Spore density (number of spores /100g soil) was counted. The spores were distinguished into morphotypes and identification up to genus level based on spore size, color, shape, hyphal attachment and spore ornamentation under a stereomicroscope using the descriptions provided by [27] ,[28]. The Relative abundance (RA) was counted according

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$$RA = \frac{\text{Number of spore of a genus}}{\text{Total number of spores observed}} \times 100 \quad [29]$$

2.5. Statistical analysis

ANOVA and correlation analyses were carried out. The means were compared using Least Significant Difference (LSD) at $p < 0.05$ after, ANOVA. The relationships between AMF parameters and soil properties were determined using Pearson's correlation analysis.

Table (2): Chemical and Textural Characteristics of soils in studied locations

	West alawailia	Almarj	Alawailia	Albalenge	AlWasita	Alhabon	Lumlluda	Alqubbah
pH	7.86	8.15	7.86	7.9	7.85	8.11	7.74	8.0
Ca (meq/L)	3.8	2.2	4.2	2.1	2.3	2.15	1.16	1.66
Mg(meq/L)	0.10	0.18	0.20	0.12	0.16	0.18	0.12	0.18
K (meq/L)	0.20	0.22	0.26	0.23	0.30	0.28	0.19	0.34
Na(meq/L)	0.38	0.36	0.8	0.58	0.28	0.31	0.22	0.12
Available P (ppm)	10.9	9.4	7.8	8.11	5.1	8.15	4.08	5.4
CEC (meq/ 100 g soil)	16.2	12.11	15	55	48	45	22	19.70
Organic Mater %	1.5	1.4	1.2	2.73	2.22	2.16	4.4	2.7
Clay %	19.6	7.6	15.6	38.03	40.30	35.20	42	30
Silt %	68	52.4	68.8	28.16	23.13	26.15	30	30
Sand %	12.4	40	15.6	33.81	36.57	38.65	28	40
Texture	Silt loam	Silt loam	Silt loam	Clay loam	Clay	Clay loam	Clay	Clay loam

3. Results and Discussion

Table (3) shows all examined plants were colonized with AMF. Root colonization and the spore abundance differed from location to location, from plant family to plant family and from plant to plant within the same family. The highest value of colonization was 97% in *Petro*

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selinum at Alqubbah location, while the lowest value was 29 % in *vicia faba* at Almarj location. The highest spore abundance was 992 spores / 100gm soil accompanied with *Vicia faba* at west alawilia location while the lowest value was 121 spores / 100gm accompanied with *Avena sativa* at Alqubbbah location.

Table (3): Means of AMF colonized root (%) and spores number in soil in various studied locations

Location	Host plant	Family	Colonization %	Spore number / 100 gm soil
West alawilia	*Hordeum vulgare*	Poaceae	37	392
West alawilia	*Triticum aestivum*	Poaceae	36	275
West alawilia	*Vicia faba*	Fabaceae	92	992
West alawilia	*Capsicum annum*	Solanaceae	46	611
Average			**52.75**	**567.5**
Almarj	Vicia faba	Fabaceae	29	337
Almarj	*Pisum sativum*	Fabaceae	58	480
Almarj	*Lycopersion esculenta*	Solanaceae	58	920
Almarj	*Capsicum anmum*	Solanaceae	61	610
Almarj	*Capsicum spp*	Solanaceae	54	425
Almarj	*Triticum aestivum*	Poaceae	77	503
Almarj	*Cucurbita pepo*	Cucurbitaceae	77	967
Average			**59.14**	**606**
Alawilia	*Hordeum vulgare*	Poaceae	71	271

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Alawilia	*Pisum sativum*	Fabaceae	65	672
Alawilia	*Vicia faba*	Fabaceae	62	693
Alawilia	*Cucurbita pepo*	Cucurbitaceae	71	920
Alawilia	*Solanum melongena*	Solanaceae	69	848
Alawilia	*Capsicum spp*	Solanaceae	55	547
Average			**65.5**	**658.5**
Albalenge	*Triticum aestivum*	Poaceae	86.5	559
Albalenge	*Hordeum vulgare*	Poaceae	77.5	572
Albalenge	*Zea mays*	Poaceae	75.5	494
Albalenge	Vicia faba	Fabaceae	80	624
Albalenge	*Pisum sativum*	Fabaceae	74	442
Albalenge	*Capsicum spp*	Solanaceae	59	351
Albalenge	*Cucumis sativus*	Cucurbitaceae	49	403
Albalenge	*Lycopersion esculentum*	Solanaceae	75.5	429
Average			**72.13**	**484.25**
Alwasita	*Hordeum vulgare*	Poaceae	79.5	572
Alwasita	*Zea mays*	Poaceae	78	832
Alwasita	*Vicia faba*	Fabaceae	81.5	910
Alwasita	*Pisum sativum*	Fabaceae	65	481
Alwasita	*Lycopersion esculentum*	Solanaceae	71	559
Alwasita	*Capsicum spp.*	Solanaceae	69	351
Alwasita	*Allium Porrum*	Amaryllidaceae	73.5	819
Average			**73.92**	**646.28**
Alhaboon	*Hordeum vulgare*	Poaceae	74	286
Alhaboon	*Vicia faba*	Fabaceae	75.5	403
Alhaboon	*Allium cepa*	Amaryllidaceae	68	312
Alhaboon	*Lactuca sativa*	Asteraceae	53	130

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Alhaboon	*Capsicum spp.*	Solanaceae	48.5	143
Alhaboon	*Allium sativum*	Amaryllidaceae	54	273
Average			**62.16**	**257.83**
Lumloda	*Vicia faba*	Fabaceae	94	140
Lumloda	*Triticum aestivum*	Poaceae	81	133
Lumloda	*Lycopersion esculentum*	Solanaceae	96	227
Lumloda	*Allium porrum*	Amaryllidaceae	74	169
Average			**86.25**	**167.25**
Alqubbah	*Sacharum officienalis*	Poaceae	76	292
Alqubbah	*Vicia faba*	Fabaceae	83	174
Alqubbah	*Coriandrum staivum*	Apiaceae	90	152
Alqubbah	*Avena sativa*	Poaceae	81	121
Alqubbah	*Medicago sativa*	Fabaceae	90	224
Alqubbah	*Lactuce pepo*	Asteraceae	91	287
Alqubbah	*Cucurbita pepo*	Cucurbitaceae	74	189
Alqubbah	*Petro selinum*	Apiaceae	97	171
Alqubbah	*Mentha arrensis*	Labiatae	86	133
Average			**85.33**	**193.66**

As average of all plants in location Fig.(2) shows the Lumloda location had the highest colonization percentage with 86.25 followed by Alqubbah 85.33%, Alwasita 73.9%, Albalenge 72.13 %, Alawilia65.5%, Alhaboon 62.16%, Almarj 59.14 and West alawilia 52.75. Where the highest spore abundance was at Alawailia location with 658.5 spore/ 100 gm soil followed by Alwasita 646.28, Almarj 606, West alawilia 567.5, Albalenge 484.25, Alhaboon 257.83, Alqubbah 193.66 and Lumloda 167.25 Fig. (3).

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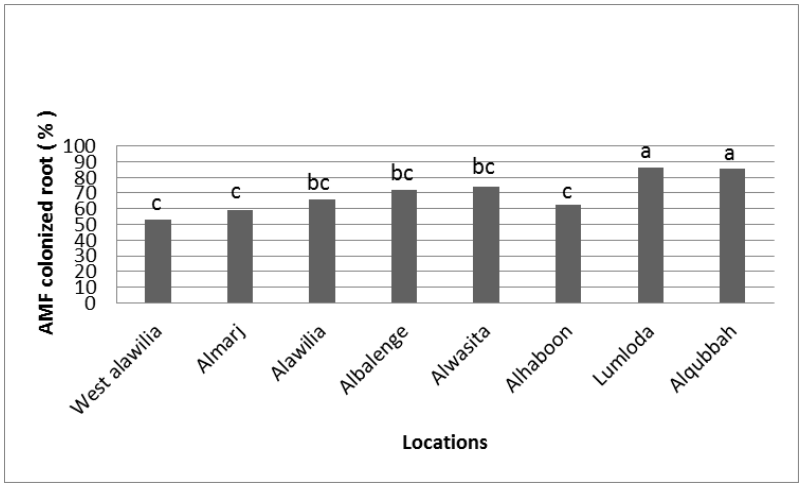

Fig. (2): AMF colonized root (%) according to location

*Different letters above the bars show significant differences (P<0.05)

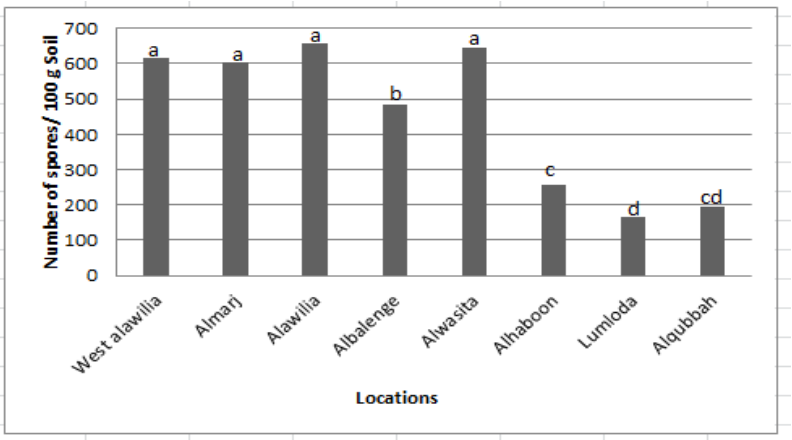

Fig. (3): AMF Spore abundance according to location

*Different letters above the bars show significant differences (P<0.05)

There was no correlation between root colonization degree and spore abundance and these results are consistent with several studies [30], [31], [32]. Also, no correlation was found between soil available phosphorus and spore abundance. However, negative correlation was found between soil available phosphorus and degree of root colonization (r= -0.6186). In earlier studies [33]، [34]، [35] obtained the same results. This negative correlation could be due to changes in permeability of root cell membrane. In earlier study [36] the researchers have concluded that the phospholipid levels in root cell wall were correlated with the percentage p content of root tissue

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and amount of p added to soil. Root exudation, soluble amino acids and reducing sugars from the roots depend upon the phospholipid level in roots and associated changes in permeability properties of root membranes. When the phosphorus increase in soil and plant tissue, lead to increase of phospholipids in root cell membrane and decrease of permeability and root exudation and decrease colonization rate. Also, negative correlation was found between clay percentage and colonization degree (r= -0.71) while positive correlation was found between silt percentage and number of spores and (r=0.74). As average of all locations, Fig.(4) shows that the plants belonging to Apiaceae had the highest colonization percentage with 93.5% followed by Labiatae 86%, Fabaceae 73%, Asteraceae 72%, Amayllidceae 71.83%, Poaceae 71.53%, Cucurbitaceae 67.75% and Solanaceae 63.5%. Fig.(5) also shows that the plants belonging to Poaceae had the highest colonization rate in Albalenge, Alwasita, Almarj, and Alawilia with 79.83%, 78.75%, 77% and 71% respectively. However, the plants belonging to Fabaceae had the highest colonization rate in West awailia and Alhaboon locations with 92% and 75.5% respectively. In Lumluda and Alqubbah locations, the plants belonging to Solanaceae and Apiaceae had the highest colonization rate with 96% and 93.5% respectively.

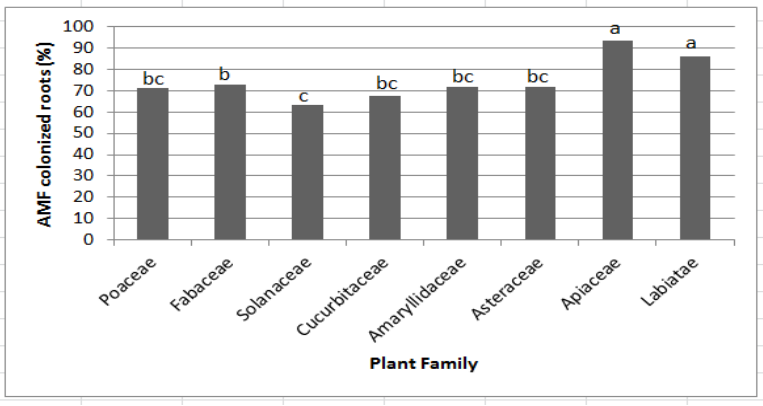

Fig. (4): AMF colonized root (%) according to plant families

* Different letters above the bars show significant differences (P<0.05)

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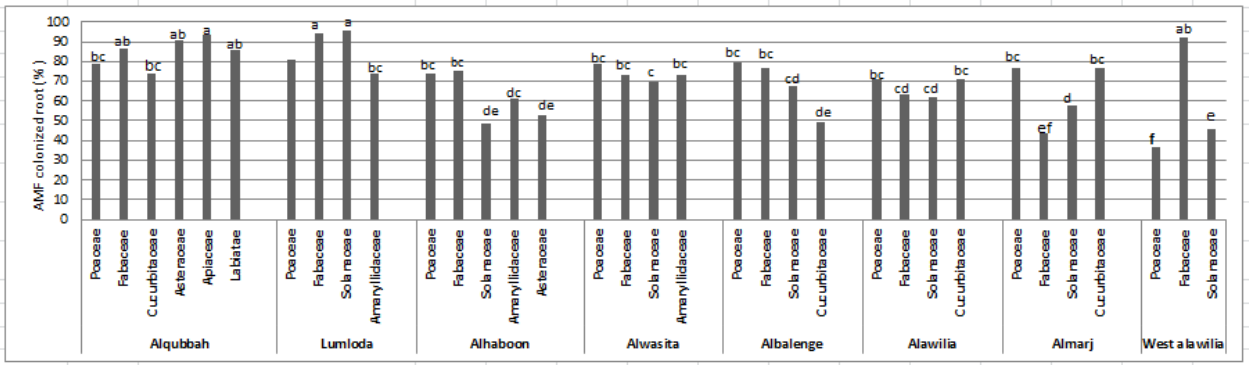

Fig. (5): AMF colonized root (%) according to plant family and location

*Different letters above the bars show significant differences (P<0.05)

The highest number of spores was accompanied with the plants belonging to Cucurbitaceae 619.75 spores/ 100gm soil followed by Fabaceae 505.5, Solanaceae 501.75, Amayllidceae 4333.3, Poaceae 407.80, Asteraceae 208.5 and Apiaceae 161.5 and Labiatae133 Fig.(6). As shown in Fig. (7) , the highest number of spores in west awailia and Alhaboon were found accompanied with the plants belonging to Fabaceae with 992 and 403 Spores/ 100gm respectively and the highest number of spores in Almarj and alawailia were found accompanied with the plants belonging to Cucurbitaceae with 967 and 920 Spores/ 100gm respectively. The highest number of spores in Albalenge, Alwasita and, Alqubbah locations were found accompanied with the plants belonging to Poaceae, Amayllidcea and Asteraceae with 541.66, 819 and 287 Spores/ 100gm respectively.

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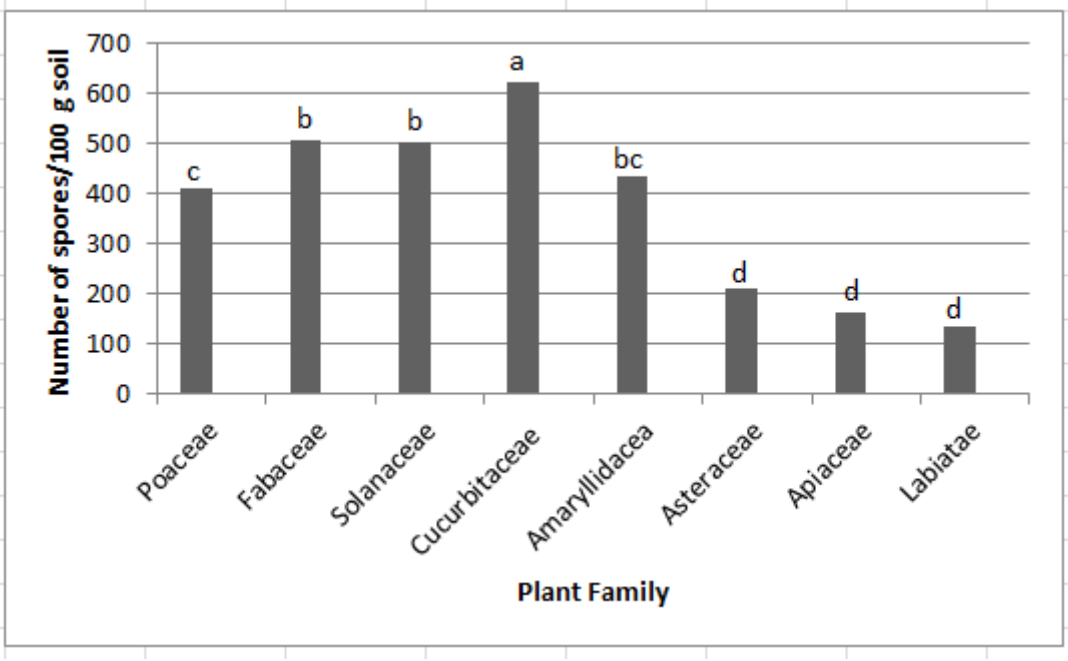

Fig. (6): Spore abundance accompanied various plant families

***Different letters above the bars show significant differences (P<0.05)**

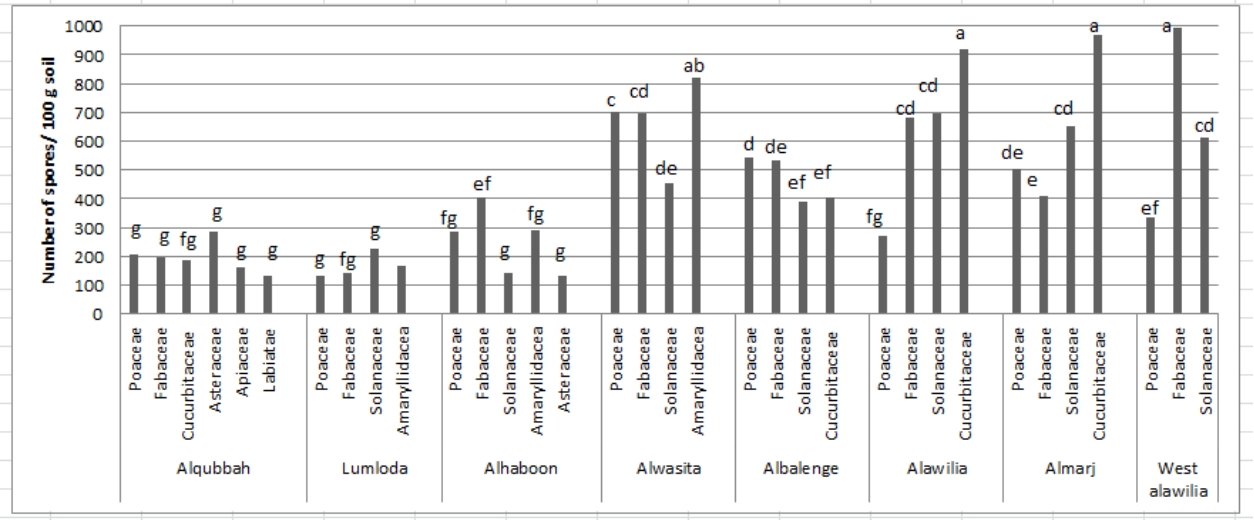

Fig. (7): Spore abundance accompanied various plant families in studied locations

*** Different letters above the bars show significant differences (P<0.05)**

Our results show that there was variation in AMF colonization and spore abundance between studied locations and plant families which could be due to one or more of multiple factors that have been reported already in several studies including:

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1. Soil Characteristics, such as moisture content [37], texture [38], fertility [39], and disturbance [40].

2. Climatic factors such as temperature [41], precipitation, Evaporation [42], level of light [43].

3. AMF factors. The differential sporulation ability of AM and the dormancy [44]

4. Local ecological factors such as plant cover and host diversity, soil cultivation and disturbance and seasonality [45], age of the host plants, host dependence of AMF species [46], host-specificity between fungi and plants [47].

5. Agricultural management such as intensive agricultural [13]. Tillage, high levels of nutrients (particularly phosphorus) [48], crop rotation [49] altering management practices, for example mediating fertilization regime [13] and introducing organic management schemes [50], land use [37]. Fig. (8) shows the relative abundance of individual AMF genera as average in all locations. *Glomus* genus had the highest spore proportion of 53.13% followed by *Acaulosora* with 26.87%, *Gigaspora* 11% and *Scutellospora* 9%.

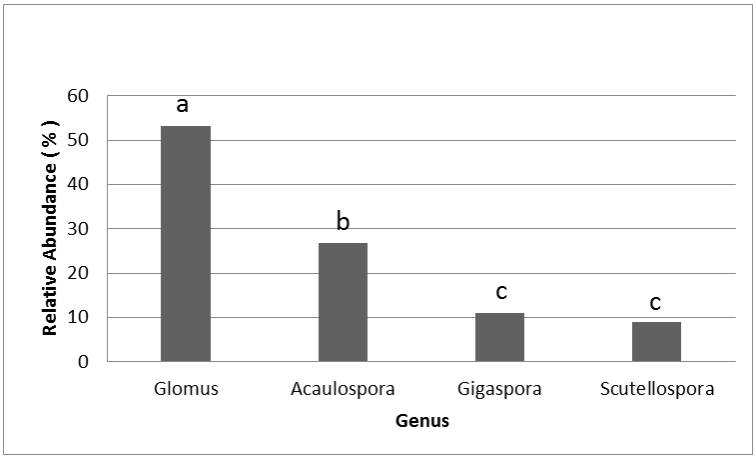

Fig. (8): Relative Abundance of AMF genera

*Different letters above the bars show significant differences (P<0.05)

Fig.(9) shows the distribution of AMF genera on studied locations. The results indicate that *Glomus* was dominate in all locations and the relative abundance of *Glomus* range from 62% at west awailia to 42% at Alhabbon location, where the relative abundance of *Acaulospora* range from 34% at Almarij to 24% at Alwasita . The relative abundance of genera *Gigaspora* and *Scutellospora* range from 19% at Albalenge and 16% at Algubbah respectively to 12% at Almarij and 5% at Albalenge respectively.

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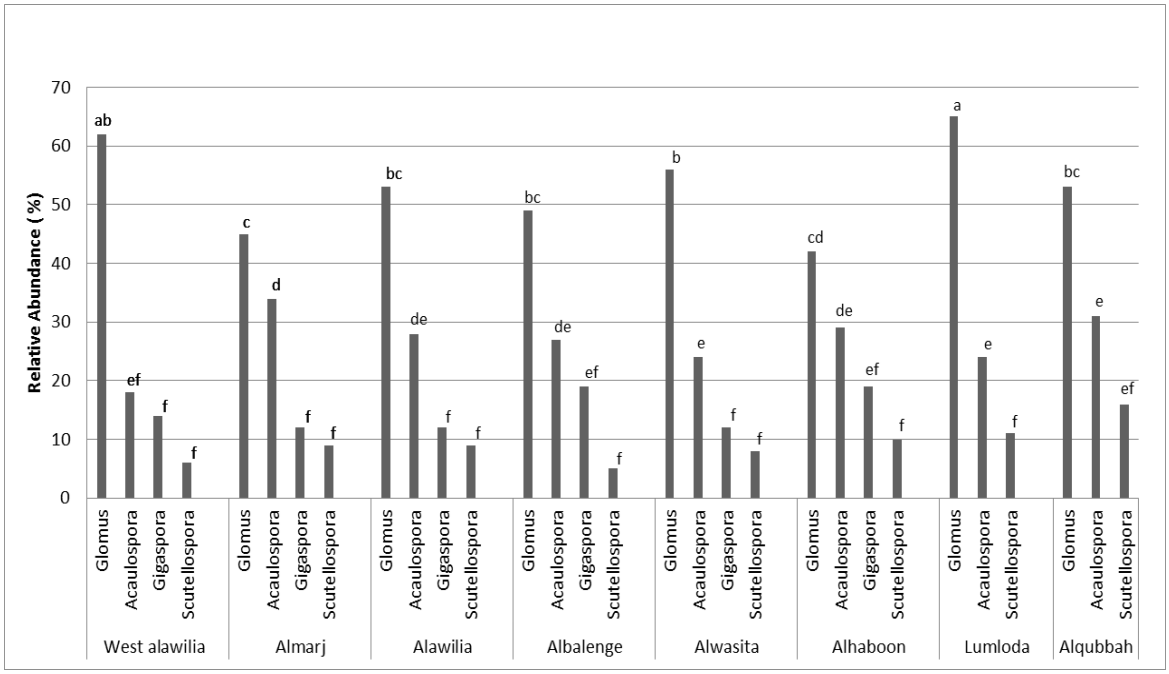

Fig. (9): Relative abundance (%) of AMF according location

*Different letters above the bars show significant differences (P<0.05

Fig.(10) shows the distribution of AMF genera in plant families. The results indicate that *Glomus* was dominant in all plant families and the relative abundance range from 55% accompanied with the plants belonging to Poaceae to 48% accompanied with the plants belonging to fabaceae where relative abundance of Acaulospora range from 38% accompanied with the plants belonging to Cucurbitaceae to 26 % accompanied with the plants belonging to Fabaceae. The relative abundance of Gigaspora range from 25% accompanied with the plants belonging to Solanaceae to 6% accompanied with the plants belonging to Apiaceae where the relative abundance of *Scutellospora* range from 20% accompanied with the plants belonging to Solanaceae to 9% accompanied with the plants belonging to Apiaceae. These results show that *Glomus* was the most abundant genus and had the highest spore proportion in all studied locations and plant families, similar results were observed in other places by [31], [46], [51]. Several studies have suggested that *Glomus* present anywhere due to the high adaptability of *Glomus* to different soil conditions, host and environment. [51], [52], [53].

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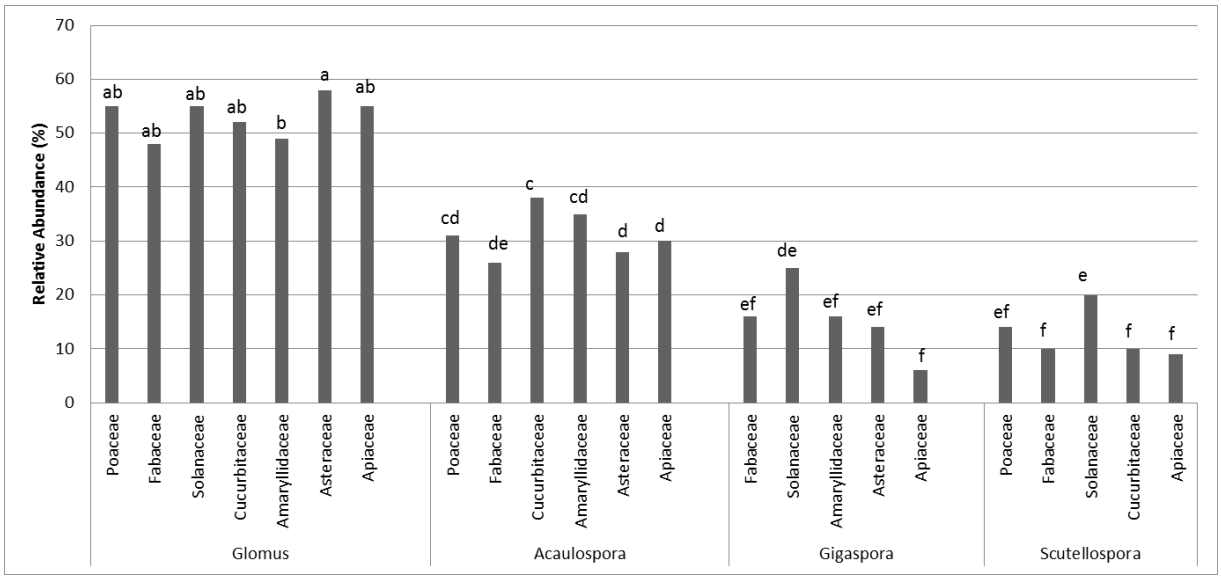

Fig. (10): Relative abundance (%) of AMF genera accompanied different plant families

*Different letters above the bars show significant differences (P<0.05

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