

Original Research Article

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Arbuscular Mycorrhizal Fungi Diversity in Two Different Regions in Saudi Arabia

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ABSTRACT

Plant root endosymbiotic arbuscular mycorrhizal fungi (AMF) are an important functional group of soil microbes in most of the terrestrial ecosystems. Since the desert land is the major covering of Saudi Arabia, the association between AMF and desert plants needs more considerable attention because of their potential importance in agriculture, horticulture, ecology and biodiversity. This study aims to investigate the colonization, composition, diversity and favorable environmental conditions of Saudi Arabian desert habituated AMF in order to explore their agricultural, ecological and biotechnological potential. The total number of collected plants from the three studied areas was 14 plants, represented by 9 unique plants. Seven different plants were collected from Riyadh and Qassim regions. However, five plants (*Rumex vesicarius*, *Echium rauwolfia*, *Anthemis deserti*, *Erucaria hispanica* and *Picris* sp.) were collected from both Riyadh and Qassim regions. The most abundant mycorrhizal genus in the collected samples was *Glomus* as it was found associated with 7 plants collected from different regions with 5 different species. Collected plants from the two different regions showed difference in mycorrhizal colonization rates. The highest hyphal colonization percentage of 75.00% was found in *Picris* sp. collected from Qassim region followed by 70.00% in *Picris* sp. collected from Riyadh region. The highest vesicles colonization percentages of vesicles were found associated with *Echium rauwolfia* (36.13%) plant collected from Riyadh region.

Keywords

Mycorrhizal diversity,
Colonization, Riyadh,
Qassim, AMF

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Introduction

Desert environment is very challenging one for plant life due to the scarce, sporadic precipitation, nutrient deficiency, intense solar radiation, and the high temperature (Apple, 2010). Hence, desert plants show unique reproductive biology, well-adapted eco-physiological, anatomical characteristics, and specialized metabolism and survival abilities. Plant-associated microorganisms are critical

components of native desert vegetation in which they are playing an active role in the survival of their plant partners. Soil drought is a widespread problem in the World, which restricts the plant growth and biomass production; particularly in arid and semi-arid regions such as in Saudi Arabia. Drought stress generally occurs when available soil water is reduced and water is continuously lost by the transpiration and/or evaporation as a result of atmospheric conditions.

Plant root endosymbiotic arbuscular mycorrhizal fungi (AMF; phylum Glomeromycota) are an important functional group of soil microbes in most of the terrestrial ecosystems. In their association with plant roots, AMF improve the plants ability to absorb water and available nutrients via increasing the absorption surface using their hyphae which increases the plant ability to adapt with extreme harsh conditions. Several studies proved the positive effects of AMF colonization on plant growth under drought stress conditions (Herrmann *et al.*, 2016). In turn, plants provide AMF with the carbon they need to survive. AMF are important players in key soil functions such as biogeochemical cycling of essential macronutrients and minerals, and maintenance of soil structure. They also facilitate plant mineral nutrient uptake from the soil and increase plant tolerance to different abiotic stresses (Smith and Read, 2008). Plants associated with AMF exhibit increased nitrogen, phosphorus, calcium, magnesium, and iron uptake, high tolerance to drought, salinity, acidity, and heavy metal stress. Moreover, mycorrhizal plants are more resistant to fungal infections and herbivorous insects comparing to other non-mycorrhizal plants. The possible mechanisms through which AMF could help plants include extensive absorption of water by external hyphae, stomatal regulation through the hormonal signals, greater osmotic adjustment in the mycorrhizal plants that promotes the turgor maintenance even though at low tissue water potentials and the indirect effects of the photosynthetic activity, proline accumulation and increasing the nutritional status in the mycorrhizal plants. Nevertheless, not all the plants showing mycorrhizal symbiosis. More than 15% of the terrestrial plants are not able to form symbiosis relation with AMF. However, Desert ecosystems mycorrhizal symbioses are of crucial importance since they are essential for native and agricultural plants survival, economically

useful desert plants and fungi successful cultivation and their impact on soil carbon dynamics (Apple, 2010).

Saudi Arabia covers roughly 80% of the Arabian Peninsula which includes the central plateau, range of deserts and mountains as well as dry and marshy coastlands. Majority of Saudi Arabia's land is characterized as arid or semi-arid regions. These areas in nature characterized by several challenging abiotic stresses including drought, salinity, acidity and nutrient deficiency which hinder plant growth and development, reduces their yield and affect their survival. During winter, few places receive occasional showers of rainfall. Depending on this little amount of water, ephemeral plants appear during winter and spring and complete their life cycle overcoming such harsh conditions. The vegetation in Saudi Arabia is very diverse and majority of the plants grow in this arid conditions play a critical role reducing the extent of desertification and soil erosion in the deserts and dry habitats. Moreover, the microorganisms present in these plants rhizosphere micro-environments plays a significant role in their survival. AMF are considered the most important microorganism via their symbiosis relation with the desert plants.

It is not well understood what is the main factors limit the abundance of mycorrhizae in nature. However, different abiotic stresses such as soil drought, salinity, pH and heavy metal concentration was shown to have significant effect on the composition of AMF communities (Maček *et al.*, 2016). Not all the desert plants have the ability to form symbiotic relationship with AMF (Collier *et al.*, 2003). Previous studies approved the presence of AMF in different sites in Saudi Arabia. A survey conducted among two sites near Riyadh, Saudi Arabia shown that roots of dominant plants like *Anisosciadium lanatum*,

Horwoodia dicksoniae, *Tripleurospermum auriculatum*, *Anthemis deserti*, *Rhazyastricta* and *Panicum turgidum*, showed AMF presence belonging to *Glomus fasciculatum* and *G. mosseeae* species. It also noted that among the two, *G. mosseeae* dominates by alkaline nature of the sand dunes, evidence for mycorrhizal species soil preference. On the other hand, 34 species belong to 20 genera of AMF were isolated from the northern east of Saudi Arabian soil where native truffles are found (Al-Whaibi, 2009). Since the desert land is the major covering of Saudi Arabia, the association between AMF and desert plants needs more considerable attention because of their potential importance in agriculture, horticulture, ecology and biodiversity (Apple, 2010). Moreover, the recognition of mycorrhizal association and distinct host specificity of the AMF are also of significant importance for the documentation of mycorrhizal status. There is a high level of functional diversity in AMF and distinct AMF taxa may have different mycorrhizal functions in an ecosystem. Therefore, the abundance and diversity of AMF is important for assessing soil quality and biological activity in arid and semi-arid areas. In general, there is a vast need for more experimental work for the description and further exploration of the observed mycorrhizal relationship among Saudi Arabian plants. Therefore, the principal aim of the current study is to investigate the colonization, composition, diversity and favorable environmental conditions of Saudi Arabian desert habituated AMF in order to explore their agricultural, ecological and biotechnological potential.

Materials and Methods

Study sites

Samples were collected from Rawdat Khuraim in Riyadh region, and Aba Alworood in Qassim region in Saudi Arabia. Seven

different plants were collected from Riyadh and Qassim regions. Rawdat Khuraim (25° 38' N, 47° 29' E, 543 m a. s. l.) is located about 100 km northeast of Riyadh in central region of Saudi Arabia. The central physiographic province is mainly dominated by a large central plateau, "Najd", which is divided into "Higher Najd" in the western and "Lower Najd" in the eastern sides. The main features of the central region are Wadi Nisah and Tuwayq mountains. It is characterized by the dissection of its landscape into an extensive system of large wadis which flow eastward, from the higher mountains in the west to the plains of Najd, responding to the general slope of the land. These wadis are not continuous, and at times covered and buried by the sand dunes of Dahna desert. Seasonal springs originate in these wadis during rainy days and often create shallow pools along the banks and depressions. Meteorological data of the central region are characterized by mean air temperature ranging from 10 °C in January to 42 °C in July and an annual rainfall from 12 to 36 mm. The mean relative humidity ranges between 10% and 50%, while the mean evaporation value is 10.35 mm/day. Aba Alworood (27° 2' N, 44° 7' E, 576 m a. s. l.) is located about 200 km northeast of Qassim. Qassim Region is located in the central part of Saudi Arabia, and at the central part of the Saharo-Arabian floristic region. The climate of the Qassim Region is characterized very hot dry summers and mild to cool winters. Mean monthly precipitation is 11.4 mm with mean monthly air temperature of 15–34°C. The relative humidity during winter ranges from 14 to 72%, whereas during summer, it ranges from 8 to 31%.

Plant sampling

Plant sampling was conducted during the flowering stage of the plants. Ten different individuals (replicates) of each plant were collected. Vegetative parts of the ten plants

were kept in plastic bags for identification. Collected plants were identified according to (Collenette, 1999; Chaudhary, 2001) at the College of Science Herbarium, King Saud University. Root systems of the ten replicates were collected with the maximum care to avoid any damage to the root systems of the different collected plants. Roots were carefully cleaned from the soil particles and put in plastic bags for transferring to the laboratory for further mycorrhizal analysis and DNA extraction. The soils loosely and tightly bound to the surface of roots were removed by clean tweezers and brush, and defined as rhizosphere soil samples for DNA extraction and spore density determination. In the laboratory, the roots were separated from the collected soils and preserved in 50% alcohol after cleaning and washing.

Root processing and assessment for mycorrhizal colonization

To evaluate mycorrhizal colonization, the root samples were washed with tap water, and then cut into about 1-cm length segments. A modified method from Phillips and Hayman (1970) was used to clean and stain root samples. The segments were first softened in 2.5% KOH at 90 °C for 1 h, bleached in alkaline hydrogen peroxide at room temperature for 30 min, acidified in 1% HCl at room temperature for 1 h, and then stained with trypan blue (0.05%) at 90 °C for 20 min. Stained root segments were mounted on glass slides. To facilitate the observation of different type of mycorrhizal structures, the slide's cover slip was gently pressed after mounting the segments. The mycorrhizal colonization was assessed by direct microscopic observation using digital computerized microscope (Model DP-72, Olympus) at 20x-40x magnification. A root segment was considered to be infected if it showed mycelium, coiled hyphae, vesicles, arbuscules, or any other combination of these

structural characteristics of AMF. Data on different structural variables, e.g. colonization rate of fungal structures (mycelium, vesicle, and arbuscules) was recorded for at least 50 root segments for each sample. The mycorrhizal colonization was estimated according to the method described by Trouvelot.

Soil sampling

Rhizosphere was defined as the soils loosely and tightly bound to the surface of roots. The remaining extracted soils were collected at the depth of 15 – 30 cm beneath the plant samples at different directions following the standard sample collection methods and homogenized and then approximately 500 – 1000 g soil was placed in plastic bags for determination of soil different physical and chemical characteristics. Ten soil samples were collected for each studied plant (one from the soil under each plant replicate) and kept in plastic bags for transferring to the laboratory for further analysis. In the laboratory, soil samples were sieved using 2 mm sieves to remove stones, coarse roots and other litter and stored at low temperature.

Soil sample processing of and spore isolation

AMF spores were isolated from 100 g of soil samples using wet sieving and sucrose density gradient centrifugation (Gerdemann and Nicolson, 1963). Distilled water was added to 100 g of soil and the solution passed through a series of ASTM-60 (250 µm), ASTM-270 (53 µm) and ASTM-400 (38 µm) sieves. The soil fraction in the last sieve was collected into plastic tubes. Spore suspensions (25 mL) were transferred to 50 mL centrifugation tubes, and another 25 mL of a 70% sucrose solution were added at the bottom of the tubes, which were then centrifuged for 2 min at 3000 rpm. Four replications of soil subsamples were used for

spore counting under the stereo-binocular microscope at 25x magnification power. The total number of spore population in each individual sample was calculated per 100g dry soil basis

Microscopic identification of mycorrhizae

The obtained spores were separated and segregated by their morphological and molecular characteristics.

The spores were observed under stereo-binocular microscope and examined using compound microscope after mounting using a mixture of polyvinyl alcohol in lactoglycerol (PVLG) and Melzer's reagent (1: 1 v: v). The identification of spores was done following standard methods of Schenck and Perez (1990), Schüßler *et al.*, (2001) and Redecker and Raab (2006). The identification process was based on spore's morphological characteristics including color, size, surface ornamentation and wall structure.

Molecular characterization of mycorrhizae

DNA extraction

Fine roots and rhizosphere soil were homogenized in liquid nitrogen using ceramic mortar and pestle. Mycorrhizal DNA was extracted from approximately 100 mg lyophilized roots using QIAamp® DNA Mini Kit (QIAGEN Korea Ltd, Korea) following the manufacturer's instructions. The purity and the concentration of the DNA were measured by NanoDrop 2000/2000c UV-Vis (ThermoFisher Scientific, USA), and the quality was checked by agarose gel electrophoresis (Invitrogen, USA) with ethidium bromide staining (Sigma, USA). The DNA samples were then diluted 1: 10 with double distilled water to be used as PCR template.

PCR amplification and DNA sequencing

Partial AMF 18S ribosomal RNA (about 550 bp) spore DNA extracts was amplified with the universal eukaryotic primer NS31 (Simon *et al.*, 1992) and primer AM1, designed to amplify AMF 18S ribosomal RNA sequences but not plant sequences (Helgason *et al.*, 1998). The PCR reactions were carried out in a final volume of 25 mL, containing 12.5 mL 2 × Taq Master Mix (Invitrogen), 0.5 mL each primer (10 mM, Invitrogen), 1 mL template and 10.5 mL RNase-Free Water with the following cycling conditions: 94 °C for 4 min, followed by 30 cycles of 94 °C for 50 s, 56 °C for 60 s, 72 °C for 70 s and extension at 72 °C for 10 min. All the PCR reactions were run in BioRad PCR cycler (USA). PCR products were examined on a 1.5% (w/v) agarose gel with ethidium bromide staining in the presence of DL2000 DNA ladder. PCR products were purified using Universal QIAquick PCR Purification Kit (QIAGEN Korea Ltd, Korea). Purified PCR products were sent for sequencing by Qacrogen, Seoul, Korea.

Phylogenetic analysis

The obtained DNA amplicons were sequenced, and the obtained sequences were searched for their phylogenetic relationships with other known sequences available in reference databases using MEGA software. The generation of phylogenetic trees was performed using 4 different phases or steps; (1) acquiring the sequences, (2) aligning the sequences, (3) estimating the tree and (4) presenting the tree.

For acquiring the sequences will be used in the tree estimation, each obtained sequence was blasted using blastn (Standard Nucleotide BLAST) against Nucleotide collection (nr/nt) database with default parameters. About 10 different sequences of different species were

chosen from the blast hits based on their similarity score and query coverage. The range of nucleotides in the subject and the query alignment status (whether query aligns with the subject sequence itself “Strand = plus/plus” or with its complement “Strand = plus/minus”) with the subject was noted and the parameters was edited according to this values before alignment step. The obtained sequences were aligned to the sequence of the mycorrhizal sample using MUCSLE algorithm with default parameters. The aligned sequences were saved to a new mega session (.mas) file and exported to a MEGA format (.meg) file to be used in tree estimation. The exported MEGA file containing the aligned sequences was used for the estimation of the Maximum Likelihood (ML) tree. To find the best model for tree estimation Find Best DNA/Protein Models (ML) command was run in the MEGA software and the results was saved to a new excel file. Then, computing of the phylogeny tree was done using the Construct/Test Maximum Likelihood Tree in the software after editing the parameters as follows:

Model/Method: was selected based on the results obtained from calculating the best model for the sequences. Rates among Sites: was selected based on the results obtained from calculating the best model for the sequences to be Gamma distributed (G) or Gamma distributed with Invariant Sites (G + I). Gaps/Missing Data Treatment: was set to partial deletion in which sites with missing data are removed only as the need arises means that option retains more information. To assess the reliability of that estimate, the bootstrap method was used with 1000 replicates. Finally, the tree was computed. The different calculated trees were represented in Unrooted Rectangular Phylogram format with bootstrap percentages on nodes, indicate the reliability of the cluster descending from that node. The higher the number, the more

reliable is the estimate of the taxa that descend from that node. In general, nodes with <70% reliability are not taken seriously.

Statistical analysis

The percentages of different mycorrhizal structures exist on the same plants collected from the different study sites was compared using T-test at significance level $P \leq 0.05$. One-way analysis of variance (ANOVA) were used to compare the difference between colonization percentages among all the collected plants ($P \leq 0.05$) and means was separated by least significant difference (LSD) method. All values were calculated from 10 different replicates and means were reported \pm standard error (SE). Correlation between percentages of different mycorrhizal structure and different soil chemical and physical characteristics was compared using the Pearson's correlation coefficient (R²). The different statistical tests were performed using the SPSS 21.0 statistical program “SPSS Inc., IBM” (Chicago, IL, USA).

Results and Discussion

Collected plants identification

The total number of collected plants from the two studied areas was 14 plants (Table 1), represented by 9 unique plants. Plant samples collected from each stand were identified according to (Collenette, 1999; Chaudhary, 2001) at the herbarium of King Saud University, Riyadh, Saudi Arabia. Seven different plants were collected from Riyadh and Qassim regions. However, were collected from both Riyadh and Qassim regions. Furthermore, no plants were repeated in the two studied regions. The repeated plants from Riyadh and Qassim were collected from comparisons between mycorrhizal infection of the same plants under different climatic conditions of the two areas. Collected plants

belong to 4 unique plant families (Asteraceae, Boraginaceae, Brassicaceae and Polygonaceae).

Mycorrhizal colonization

Mycorrhizal diversity

In total, 7 out of 14 collected plants were found to be associated with different species of mycorrhizal fungi. The most abundant mycorrhizal genus in the collected samples was *Glomus* as it was found associated with 7 plants collected from different regions with 5 different species (Table 2). In Riyadh region, two plants (out of 7 collected; P1: P7) were found to be associated with two different mycorrhizal species, namely *Glomus deserticola* and *Glomus geosporum*. Five different plants collected from Qassim region (out of 7 collected; P8: P14) were colonized by different mycorrhizal species. Mycorrhizal species found in Qassim region were *Glomus deserticola*, *Glomus aggregatum*, *Diversispora spurca*, *Glomus etunicatum* and *Glomus mosseae*

Phylogenetic analysis

Phylogenetic analysis of extracted mycorrhizal DNA was performed in order to confirm the morphological identification of mycorrhizal species. Analysis was performed on the DNA sequences of the samples showed mycorrhizal colonization as described above. The obtained results confirmed the morphological identification of mycorrhizal spores. Figure 1 shows the phylogenetic tree produced by MEGA software to identify the mycorrhizal species in each collected plant. The results show that the *Echium rauwolfia* plants collected from Riyadh region (sample 2) and from Qassim region (sample 9) were colonized by *Glomus deserticola*. *Pulicaria gvestii* plant (sample 4) collected from Riyadh region was colonized by *Hypocrea lixii*.

Anthemis deserti plant collected from Riyadh region (sample 5) was colonized by *Hypocrea nigricans*, while the same plant collected from Qassim region (sample 12) showed mycorrhizal association with *Glomus etunicatum*. *Picris* sp. plant collected from Riyadh region (sample 7) was colonized by *Glomus geosporum*; however, the same plant collected from Qassim region (sample 14) was colonized by *Glomus mosseae*. *Arnebia decumbens* plant (sample 10) collected from Qassim region was colonized by *Glomus aggregatum*. *Calendula arvensis* plant (sample 11) collected from Qassim region were colonized by *Diversispora spurca*.

Colonization rates and spore population

Collected plants from the two different studied regions showed difference in mycorrhizal colonization rates (Table 3). The hyphae colonization percentage in all collected plants ranged from 66.50 to 75.00%. The highest colonization percentage of 75.00% was found in *Picris* sp. collected from Qassim region followed by 70.00% in *Picris* sp. collected from Riyadh region. However, the lowest hyphae colonization was observed in *Calendula arvensis* (58.11%) and *Arnebia decumbens* (64.22%) plants collected from Qassim region. Vesicles colonization percentages ranged from 29.50 to 36.13%. The highest colonization percentages of vesicles were found associated with *Echium rauwolfia* (36.13%) plant collected from Riyadh region. Nevertheless, the lowest percentages of vesicles colonization were associated with *Picris* sp. (29.50%) plants collected from Qassim region. There was a significant difference in the arbuscules colonization percentages between the different collected plants. The highest and lowest arbuscules colonization rates were found in Qassim region associated with *Calendula arvensis* (60.20%) and *Echium rauwolfia* (52.40%), respectively. Mycorrhizal spores

were counted in the collected soil samples. Spore count in the rhizosphere of each collected plants were measured on the basis of 100 g of the samples soil. There was a great difference between spore counts in the rhizosphere of different collected plants (Table 3). Average spore counts were ranged from 105.2 to 141.5 spores/ 100 g of soil. The maximum spore count was found in the rhizosphere of *Arnebia decumbens* plant collected from Qassim region. However, the lowest spore abundance was mainly found in the soil sample collected from Riyadh region in the rhizosphere of *Picris* sp. (105.2 spores) and *Echium rauwolfia* (122.2 spores) plants.

The colonization percentages of different mycorrhizal structures and spore counts associated with the same plants collected from different two sites were compared using t-Test. *Echium rauwolfia* plant were collected from both Riyadh (P2) and Qassim (P9) regions (Fig. 2). It was found that the plant was colonized by the same species; *Glomus deserticola* in both sites. Furthermore, the t-Test results showed that there is no difference between colonization percentages of hyphae structures on the two plants ($P = 0.83$). However, there was a significant difference between colonization percentages of vesicles ($P \leq 0.05$) and arbuscules ($P \leq 0.01$). Spores showed a great significant difference between the two plants ($P \leq 0.01$, $t = 5.03$).

Similarly, *Picris* sp. plants were collected from both Riyadh (P7) and Qassim (P14) regions (Fig. 3). In Riyadh region, *Picris* sp. plant were colonized by *Glomus geosporum*; however, in Qassim region, it was colonized by *Glomus mosseae*. The t-Test results showed no significant differences ($P \leq 0.05$) in the colonization percentages of the different structures formed by the two fungi species. However, there was a great significant difference between the spores present in the rhizosphere the two plants ($P \leq 0.01$, $t = 4.26$).

The results obtained in this study indicated that plants grown in Riyadh and Qassim regions in Saudi Arabia have highly diverse AMF communities. In this study, we found 7 plants colonized by 5 different mycorrhizal species, dominated mainly by *Glomus* genus. The mycorrhizal colonization of the selected plants in this study was not studied before in different Saudi Arabia regions. However, some studies investigated mycorrhizal diversity in Riyadh region. Al-Qarawi *et al.*, (2012) studied the mycorrhizal colonization percentages of 10 different plants, namely *Calendula officinalis* L., *Catharanthus roseus* (L.) G. Don, *Convolvulus arvensis* L., *Cynodondactylon* (L.) Pers., *Petunia hybrida* (L.) Mill. *Gaillardia pulchella* Foug., *Ocimum sanctum* L., *Phoenix dactylifera* L., *Sesuvium portulacastrum* (L.) L., *Tagetes patula* L. They found that the highest infection was recorded in *Petunia hybrida* and *Gaillardia pulchella* (97%) followed by *Calendula officinalis* (90%) and *Cynodon dactylon* (87%); however, the lowest infection was found in *Sesuvium portulacastrum* (33%). The maximum vesicles were found in *P. hybrida* (97%) followed by *G. pulchella* (80%) and *C. officinalis* (67%), and the minimum was found in *P. dactylifera* (7%). The highest colonization percentage of arbuscules was recorded with *P. hybrida* (73%) and arbuscules were not found with *C. arvensis*, *S. portulacastrum*. Furthermore, the highest number of spores was recorded from the rhizosphere soils of *P. hybrida* and the lowest number was found with *S. portulacastrum*. Moreover, Khaleil (1989) studied the mycorrhizal colonization associated with *Anisosciadium lanatum*, *Korwoodia dicksoniae*, *Tripleurospermum auriculatum*, *Anthemis deserti*, *Rhazystricta* and *Panicum turgidum* in Riyadh region and found that two different species of AMF, namely *Glomus fasciculatum* and *G. mosseae* associated with these plants.

Table.1 The different studied plants and their families and location

No.	Plant	Family	Location
1	<i>Rumexvesicarius</i>	Polygonaceae	Riyadh
2	<i>Echiumrauwolfia</i>	Boraginaceae	Riyadh
3	<i>Zilla spinose</i>	Brassicaceae	Riyadh
4	<i>Pulicariaguestii</i>	Asteraceae	Riyadh
5	<i>Anthemis deserti</i>	Asteraceae	Riyadh
6	<i>Erucariahispanica</i>	Brassicaceae	Riyadh
7	<i>Picris sp.</i>	Asteraceae	Riyadh
8	<i>Rumexvesicarius</i>	Polygonaceae	Qassim
9	<i>Echiumrauwolfia</i>	Boraginaceae	Qassim
10	<i>Arnebiadecumbens</i>	Boraginaceae	Qassim
11	<i>Calendula arvensis</i>	Asteraceae	Qassim
12	<i>Anthemis deserti</i>	Asteraceae	Qassim
13	<i>Erucariahispanica</i>	Brassicaceae	Qassim
14	<i>Picris sp.</i>	Asteraceae	Qassim

Table.2 The diversity of mycorrhizae in studied plants

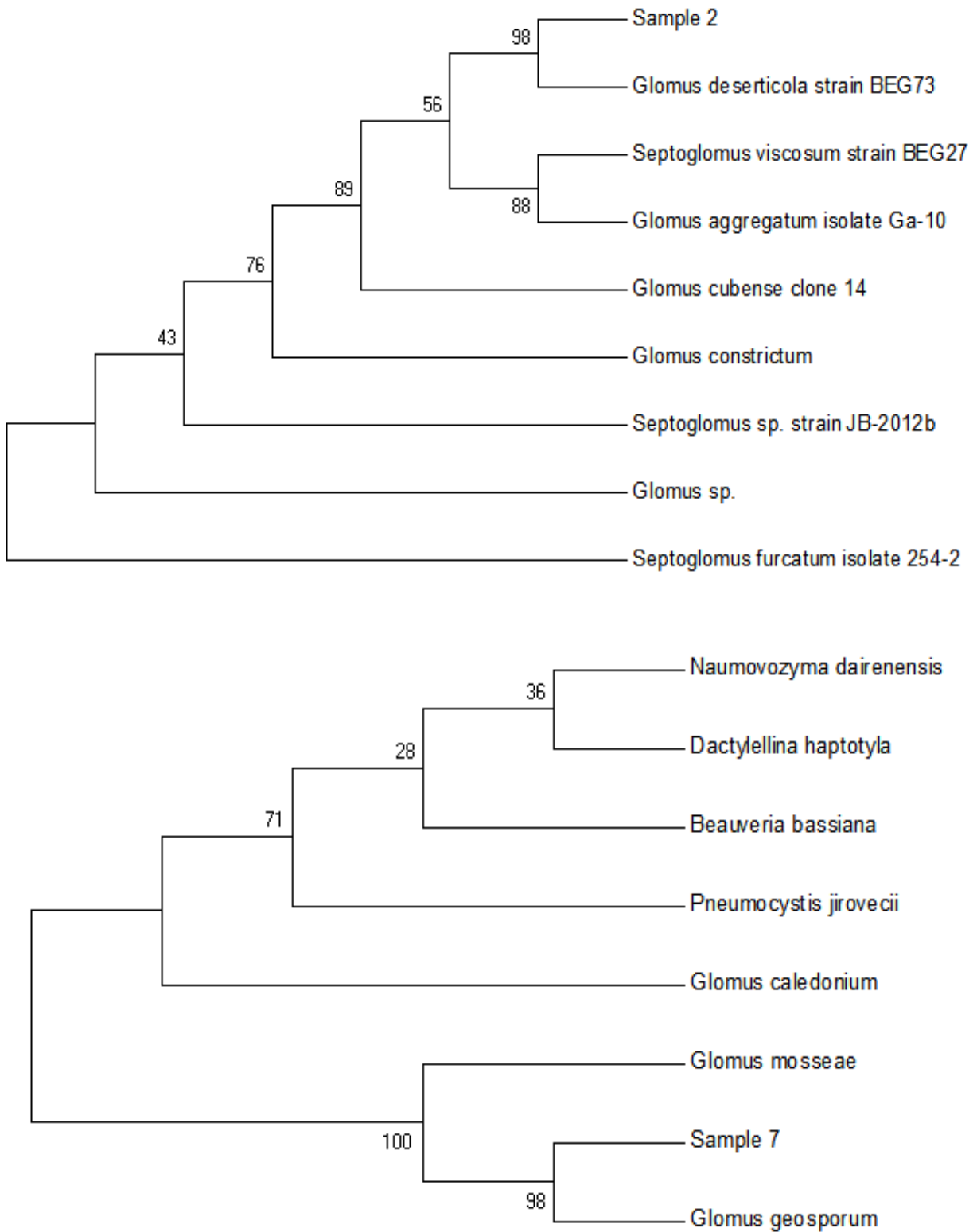
Sample No.	Mycorrhiza Species
P2	<i>Glomus deserticola</i>
P7	<i>Glomus geosporum</i>
P9	<i>Glomus deserticola</i>
P10	<i>Glomus aggregatum</i>
P11	<i>Diversisporaspurca</i>
P12	<i>Glomus etunicatum</i>
P14	<i>Glomus mosseae</i>

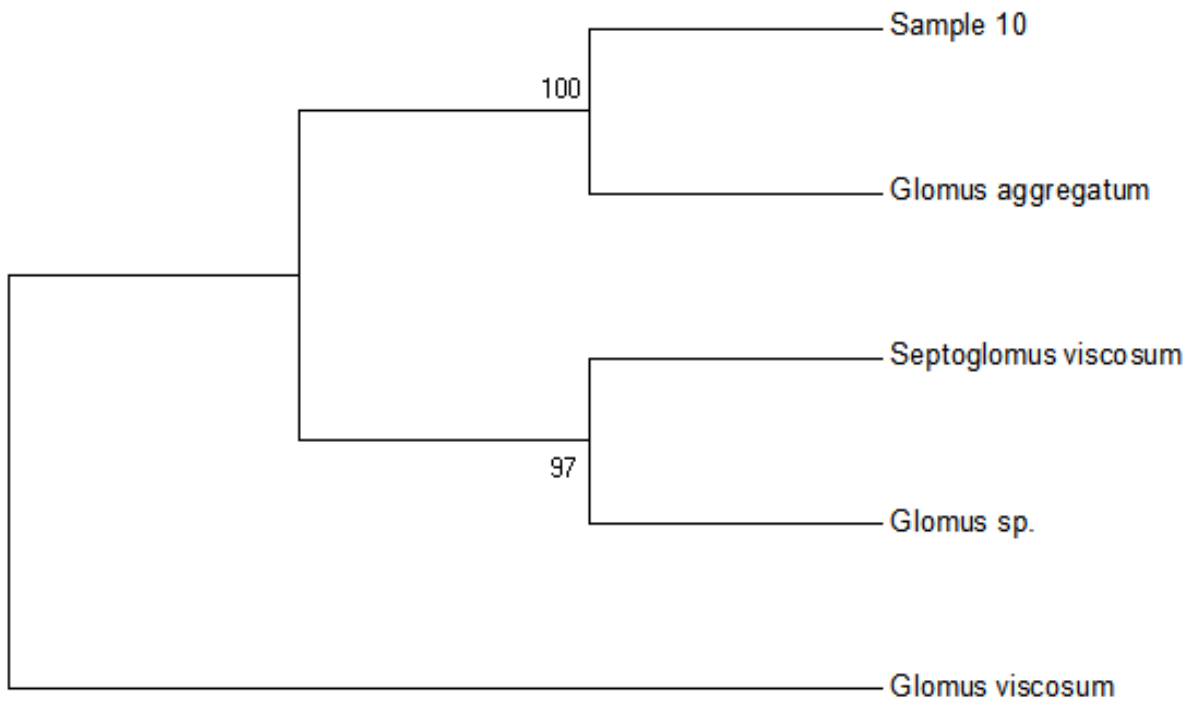
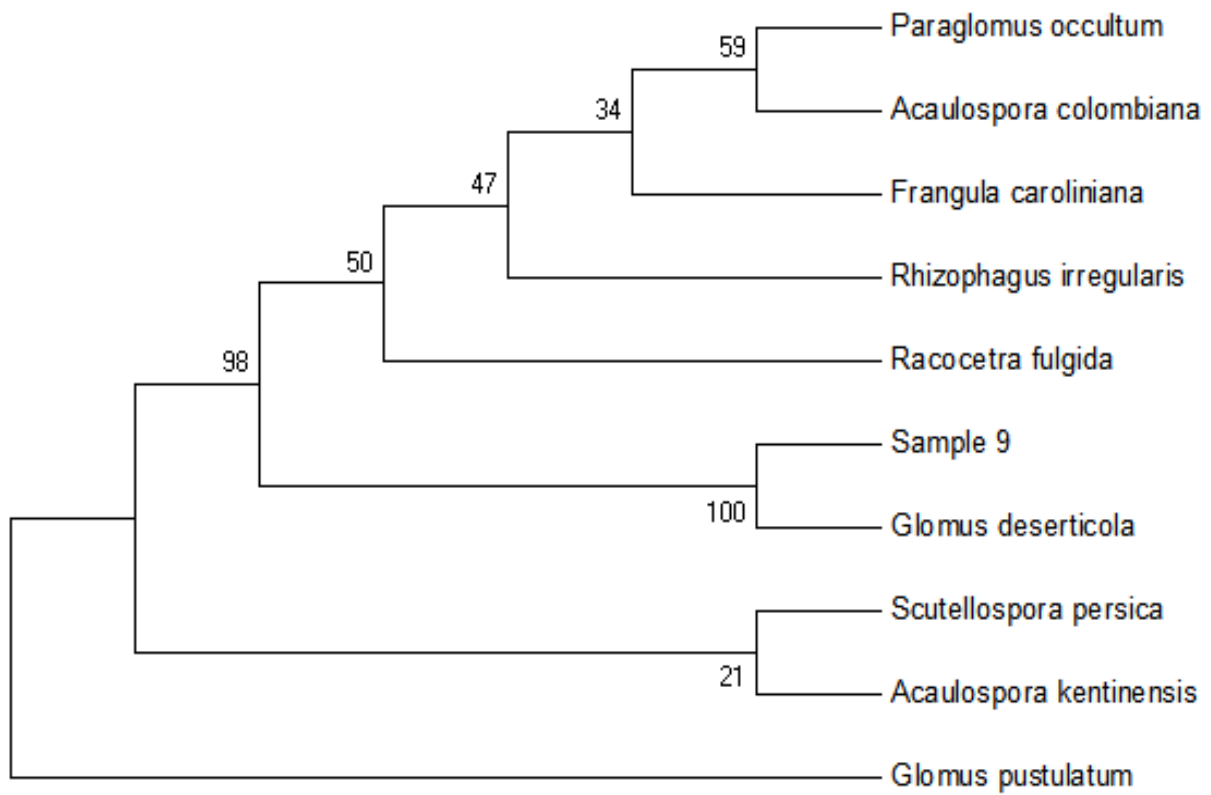
Table.3 Colonization percentage (%) and spore count (/ 100 g soil) of different mycorrhizal structures in the studied plants

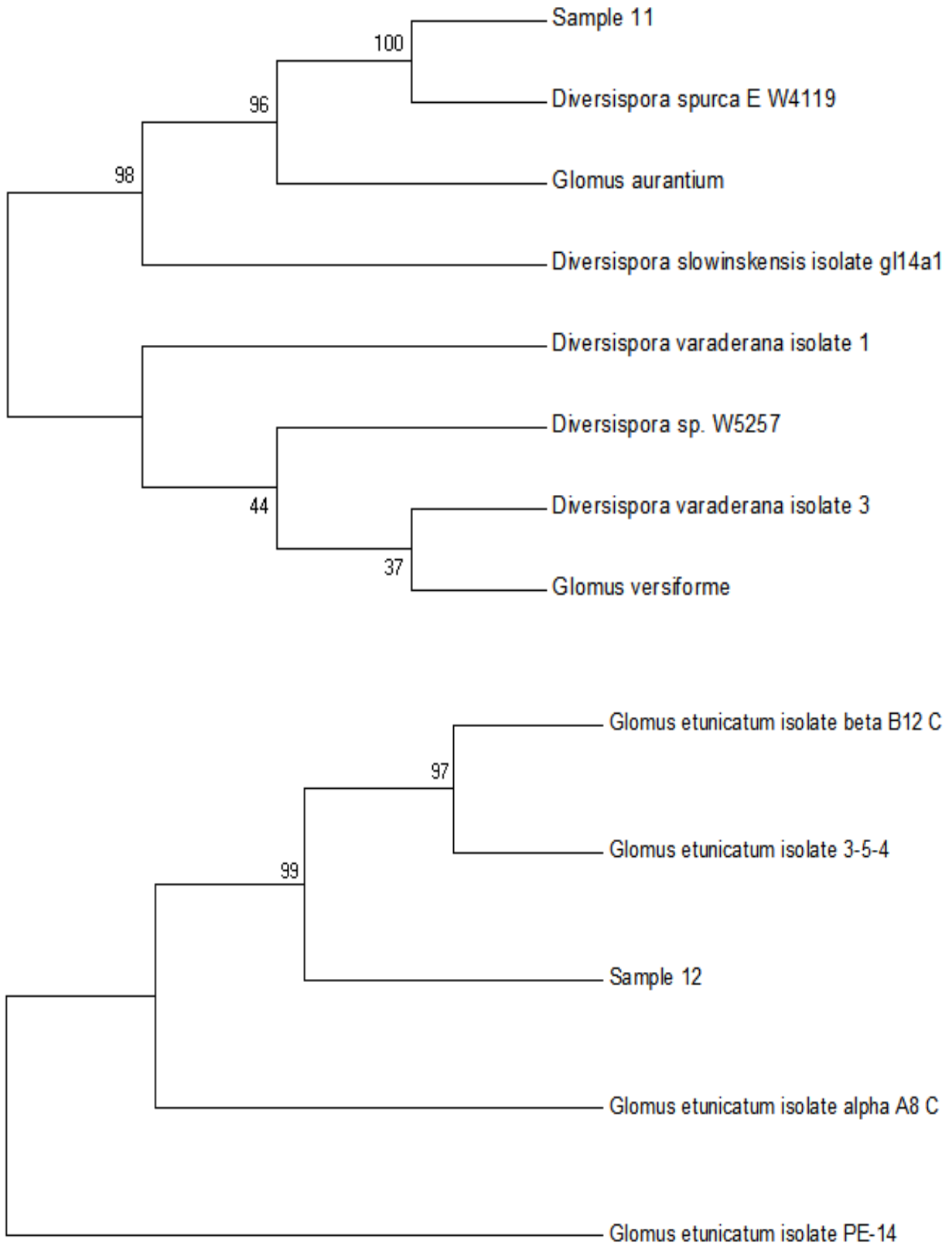
Plant	Mycorrhizal Structures			
	Hyphae	Vesicles	Arbuscules	Spores
P2	68.33 ± 2.37*	36.13 ± 1.08	57.10 ± 1.22	122.2 ± 2.26
P7	70.00 ± 1.52	33.00 ± 1.84	53.70 ± 2.12	105.2 ± 1.31
P9	67.89 ± 1.07	33.00 ± 0.85	52.40 ± 0.92	137.4 ± 2.00
P10	64.22 ± 1.41	34.38 ± 1.11	56.20 ± 0.84	141.5 ± 2.74
P11	58.11 ± 1.68	35.63 ± 1.23	60.20 ± 0.65	122.8 ± 1.60
P12	68.22 ± 3.27	34.88 ± 2.87	54.40 ± 5.38	128.7 ± 1.84
P14	75.00 ± 0.83	29.50 ± 0.70	53.10 ± 0.98	121.0 ± 3.46

*Values are expressed as mean of 10 replicates ± standard error.

Fig.1 Phylogenic analysis of the different sequenced mycorrhizal fungi







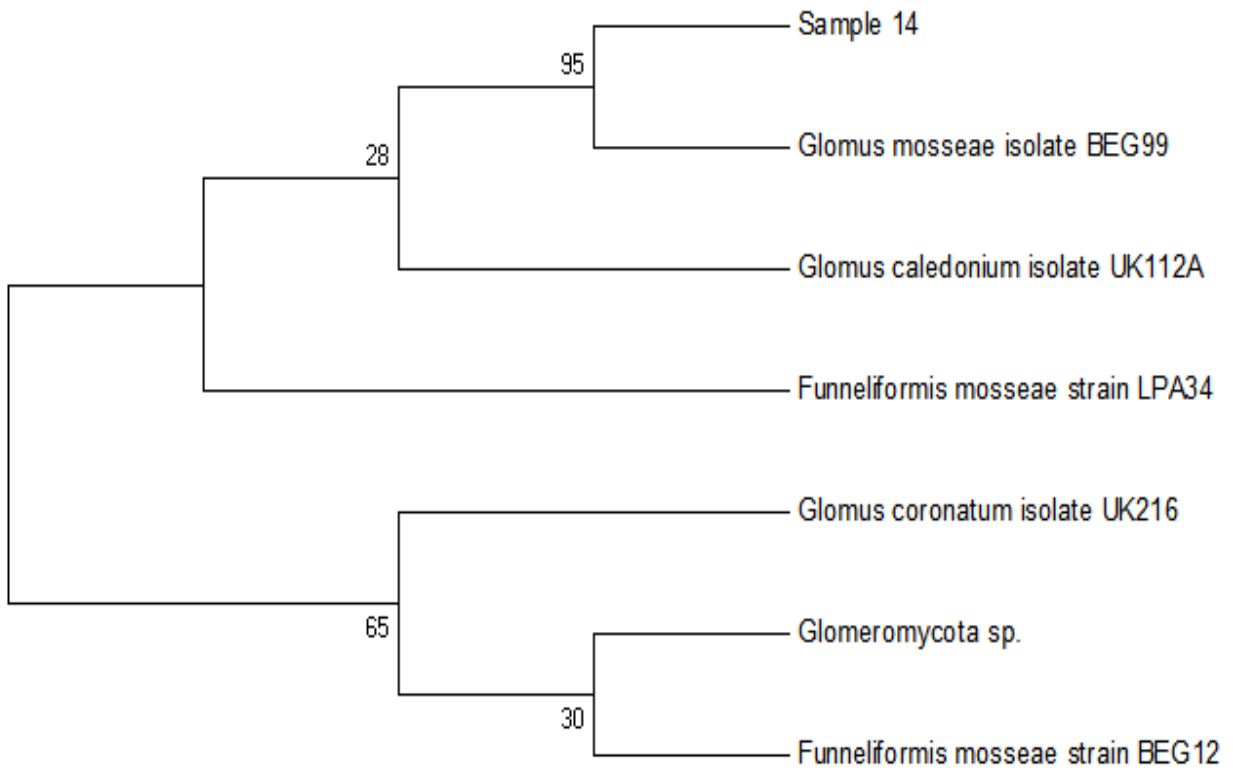


Fig.2 Difference in colonization rates (%) of hyphae, vesicles and arbuscules and spore counts (per 100 g soil) associated with *Echium rauwolfia* collected from Riyadh (P2) and Qassim (P9)

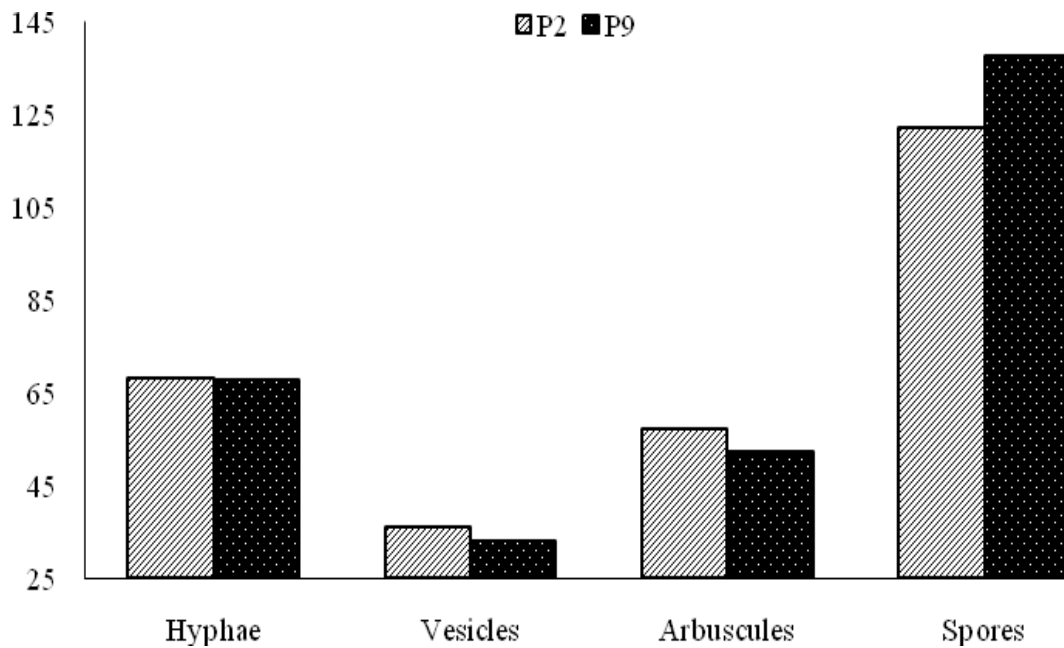
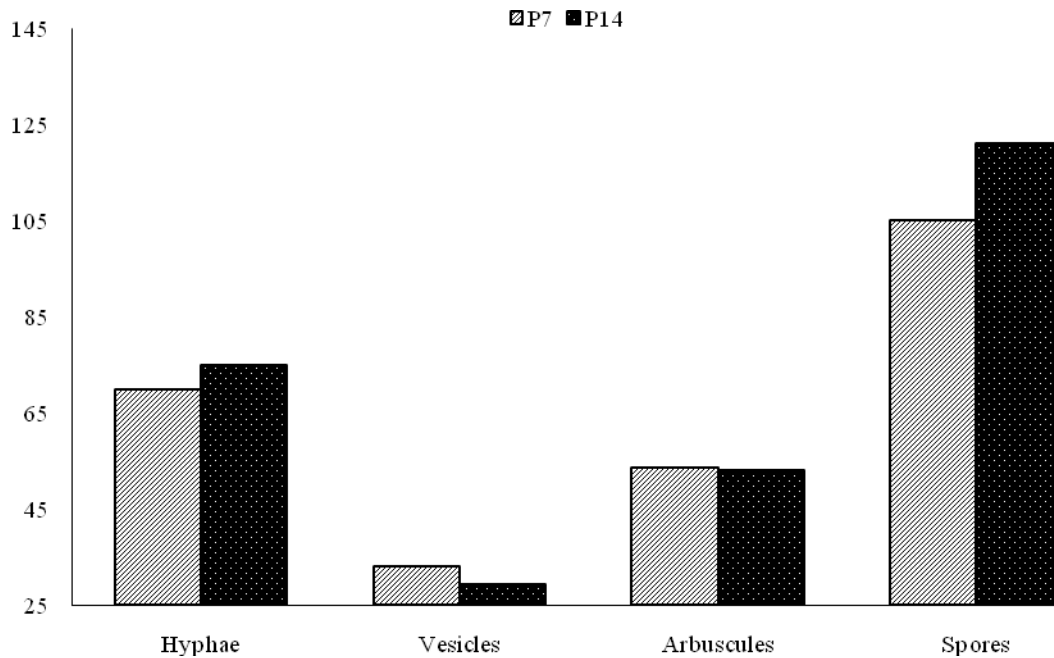


Fig.3 Difference in colonization rates (%) of hyphae, vesicles and arbuscules and spore counts (per 100 g soil) associated with *Picris* sp. collected from Riyadh (P7) and Qassim (P14)



He attributed the dominance of *G. mosseae* to the high alkalinity of the soil. In Qassim region, AMF reported to be associated with date palms (Khaleil and Abu-Heilah, 1985). Moreover, several reports indicated the presence of AMF in different regions of Saudi Arabia but without studying the colonization percentages of different mycorrhizal structures. In western region of Saudi Arabia, mycorrhizal presence was reported in Medina, Heda-Alsham and Taif (Malibari *et al.*, 1990). The different mycorrhizal species found in the studied regions include *Glomus mosseae*, *Glomus fasciculatum*, *Gigaspora* sp., *Sclerocysts* sp. However, *Glomus* species were the most dominant. The highest root infection percentage was found in the roots of alfalfa, millet and sudangrass plants. Similarly, Al-Garni and Daft (1990) studied the mycorrhizal occurrence in agricultural soils in Saudi Arabia and found six different mycorrhizal species belong to *Glomus* genus associated with maize and onion plants. Moreover, several studies examined the

presence of mycorrhizal species in different regions of Saudi Arabia (Bahabail, 1996; Al-Garni, 2001; Al-Qarawi and Al-Shahrani, 2010).

Several plants collected in this study did not show any mycorrhizal colonization. These plants include *Rumex vesicarius*, *Zilla spinosa*, *Erucaria hispanica*, *Minuartia filifolia*, *Sisymbrium orientale* and *Argemone ochroleuca*. *Rumex vesicarius* is a member of Polygonaceae plant family. Several studies indicated that Polygonaceae plants do not form any mycorrhizal symbiosis. Pendleton and Smith (1983) studied the status of mycorrhizal colonization of different plants at several disturbed sites in Utah, USA and found that Polygonaceae plants did not show any mycorrhizal symbiosis. Moreover, in British Columbia, different plants belong to this family did not show any mycorrhizal colonization (Berch *et al.*, 1988). Another study surveyed the mycorrhizal colonization of Hawaiian angiosperms and found no

colonization in plants of Polygonaceae family (Koske *et al.*, 1992). In the tropical plains of Tamil Nadu in India, mycorrhizal fungi did not colonize any of the Polygonaceae plants (Ragupathy and Mahadevan, 1993). Furthermore, plants belonging to Polygonaceae family collected from southern Poland did not show any colonization with AMF (Pawlowska *et al.*, 1997). Cripps and Leslie (2005) studied the distribution of mycorrhizal types among Alpine vascular plant families on the Beartooth plateau, Rocky mountains, USA and found that Polygonaceae plants had no mycorrhizal colonization.

Zilla spinosa, *Erucaria hispanica* and *Sisymbrium orientale* belonging to Brassicaceae families. It was well established that plants of Brassicaceae family do not form mycorrhizal symbiosis. Hirrel *et al.*, (1978) approved that Brassicaceae plants do not form any mycorrhizal symbiosis. Harley and Harley (1987) studied that mycorrhizal status of most of plants in the British flora and found that mycorrhizal colonization did not occur with Brassicaceae plants. Moreover, plants belonging to Brassicaceae family did not show any infected roots with AMF (Khan and Belik, 1995). Demars and Boerner (1996) studied the arbuscular mycorrhizal development in the Brassicaceae in relation to plant life span and found no mycorrhizal colonization with most of plants belonging to this plant family.

Minuartia filifolia is one of Caryophyllaceae plants. Several earlier reports stated that Caryophyllaceae plants do not form AMF colonization (Harley and Harley, 1987; Berch *et al.*, 1988; Pawlowska *et al.*, 1997). Alpine plants in the Northwestern Caucasus belonging to this family did not show any mycorrhizal colonization (Onipchenko and Zobel, 2000). Furthermore, in the Patagonian steppe, Argentina, Caryophyllaceae plants

had no mycorrhizal colonization (Fontenla *et al.*, 2001). Recently, Cripps and Leslie (2005) found that Caryophyllaceae plants growing in Beartooth Plateau, Rocky Mountains, USA do not form any symbiotic relationships with mycorrhizal fungi. Similarly, *Argemone ochroleuca* is one of the plants belonging to Papaveraceae family, formerly known as Fumariaceae. Plants of this family were reported not to form mycorrhizal symbiosis (Pendleton and Smith, 1983; Harley and Harley, 1987; Berch *et al.*, 1988). Brundrett and Kendrick (1988) investigated the mycorrhizal status of different plants grown in a sugar maple forest and found no colonization in plants belonging to Papaveraceae family.

In our study, fungi were identified using morphological and molecular characteristics. In 2011, a group of mycologists meet to evaluate six DNA regions (SSU, LSU, ITS, RPB1, RPB2, MCM7) and nominate the official fungal barcode, the ITS region, which was later approved by the Consortium for the Barcode of Life. Schoch *et al.*, (2012) found the ITS region to be among the markers with highest probability of correct identifications for a very broad group of sampled fungi. Additional fungal studies have provided support for the ITS region as a suitable fungal barcode (Seena *et al.*, 2010; Dentinger *et al.*, 2011; Kelly *et al.*, 2011). Moreover, most environmental surveys (metagenomic studies) of fungi are using the ITS region in their studies (e.g. O'Brien *et al.*, 2005; Bellemain *et al.*, 2010; Henrik Nilsson *et al.*, 2011; Hibbett *et al.*, 2011) to identify fungi using modern sequencing technology, such as next-generation sequencing, which can aid in rapid identification of fungi without the need to clone the amplicons. This sequencing approach has therefore created a surge in the number of ITS sequences that are available in GenBank (Seifert, 2009; Begerow *et al.*, 2010). According to Schoch *et al.*, (2012),

currently about 172,000 full-length fungal ITS sequences are deposited in GenBank, of which 56% have a Latin binomial associated, representing approximately 2500 genera and 15,500 species. The ITS region is a useful barcode marker because it usually can be sequenced from previously described fungi for which no sequence data are currently available by sequencing the type material (i.e., the specimen on which a species is originally described and deposited in a fungarium). Therefore, the practicality and broad kingdom-wide taxonomic applicability make ITS a useful tool for fungal barcoding for most (~70% of all fungi tested) (Schoch *et al.*, 2012; Yahr *et al.*, 2016) if not all lineages of fungi (Nilsson *et al.*, 2008; Seifert, 2009; Vrålstad, 2011; Kiss, 2012).

Spore population in the rhizosphere of the collected samples was calculated in this study as the number of spores present in 100 grams of soil rhizosphere. Spore population associated with some plants grown in Riyadh region was reported (Al-Qarawi *et al.*, 2012). It was found that spores count ranged from 59 to 216 spore per 100 grams of dry soil. Moreover, the highest number of spores was recorded from the rhizosphere soils of *P. hybrida* and the lowest number was found with *S. portulacastrum*. Al-Garni (2001) reported a wide variation among the samples for spore populations with the field soils and infectivity of AMF with roots from a study on Taif soils and standing crops. On the other hand, several studies reported occurrence and diversity of mycorrhizae in desert plants (Miller, 1979; Rose, 1981; Mejstřík and Cudlín, 1983; Stutz and Morton, 1996; He *et al.*, 2002; Titus *et al.*, 2002; Chaudhry *et al.*, 2006; Panwar and Tarafdar, 2006; Uhlmann *et al.*, 2006) but very few references are available with structural colonization studies and spore population in different countries with desert plants especially from Saudi Arabia.

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