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Detection of Arbuscular Mycorrhizal Fungi for Some Wild Plants in Diyala Governorate

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Abstract

A survey was conducted for five regions of Diyala Governorate, including Kanaan, Mandali, Bani Saad, Khalis and Baladruz from the year 2021-2022. It aims to determine the genera of the mycorrhizal fungi in the soil of some wild plants, which included the Imperata cylindrical L., Sorghum sudanense, and Millet. The results showed a difference in the rate of infection of plants with mycorrhizal fungi according to the study areas, as Millet recorded the highest value in the rate and severity of infection and the number of spores, which were 88%, 93.7%, and 72 spores.gm-1.soil, respectively. The lowest percentage of infection, its severity, and number of spores was on the plant Sorghum sudanense, which was 72%, 80.5%, and 44 spores gm-1. soil, respectively. The genera and species that included two genera: Glomus sp. and Gigaspora sp were isolated from all surveyed areas. The results also showed that Glomus is the most common in all studied areas, while Gigaspora was the least common genus.

Keywords: Glomus, Gigaspora, infection severity, diagnosis, Arbuscular mycorrhizae.

Introduction

The term Mycorrhizae was first named by the German researcher Frank in 1885. The origin of the name goes back to the Greek language and is composed of two syllables (Myco), which means fungus, and (Rhiza), which means root. Then the concept of this term developed to describe the common symbiotic relationship that occurs between the roots of vascular plants with non-pathogenic soil fungi because within this relationship there is an exchange between the fungus and the root for some compounds and elements that are used in growth and reproduction for both parties (Trappe, 1962; Keymer and Gutjahr, 2018).

Spores are regarded to be very importance in identifying mycorrhizal fungi, as all mycorrhizal classification methods are based on the phenotypic diagnosis of the spores, such as color, the outer wall and its thickness, the number of layers composing it, the shape of the fungal hyphae to the spore, the shape of the spore and its connection to other spores or its presence individually (Aminifar and Sirousmehr, 2014). A wild plant is known as a plant that grows naturally without

human intervention, as it spreads in deserts, plains, villages, and valleys. Wild plants are divided into two perennial species that live for several years and renew whenever environmental conditions improve, such as rainfall and watering the land, and an annual species that lives for a short period (Yassin *et al*., 2017).

Due to the scarcity of studies in Diyala Governorate, the current study aims to detect Arbuscular mycorrhizal fungi in some wild plants in the rhizosphere soil.

Materials and Methods

Survey study

Random samples were collected from the rhizospher zone soil of some wild plants *(Imperata cylindrical* L., *Sorghum sudanense*, and *Millet*) from five areas of Diyala Governorate, which included Khan Bani Saad, Mandali, Kanaan, Baladruz, and Khalis, for the period from February to May of 2022. These samples were stored in clean plastic bags from the polyethylene and the information for each sample was documented (the name of the sample, the place from which it was taken, and the time when the plant was taken). The samples were mixed well and a representative sample was taken, in addition to collecting a sample of the soil surrounding the roots.

Study site

Diyala Governorate is considered to be one of the eighteen governorates of Iraq. It includes six districts and thirteen towns. Its center is the city of Baqubah. Diyala Governorate constitutes 4.1% of the area of Iraq, and its area is estimated at about 17.685 km^2. Diyala is located in the central part of eastern Iraq, located between latitudes 33.3-35.6 north of the equator and between longitudes 44.22-45.56 east of the Greenwich line. Thus, it represents the international border between Iraq and Iran to the east, and is bordered by Salah al-Din and Baghdad to the west, Sulaymaniyah to the north, and Wasit to the south (Abdul Wahab, 2020). Figure (1) shows the locations where plant and rhizosphere soil samples were taken.

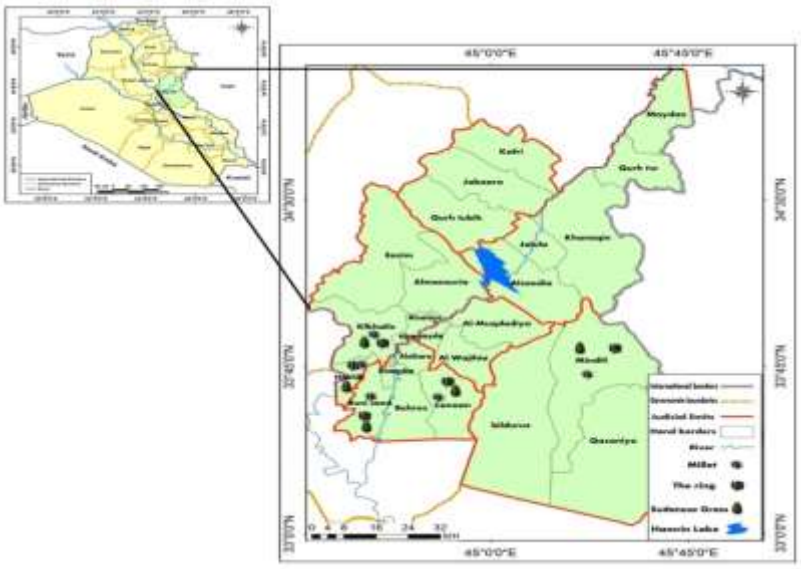


Figure (1) map showing the sites included in the study in Diyala Governorate - Iraq.

Calculating the criteria for mycorrhizal infection

3-1-Incidence rate and severity (%)

The method of dyeing the roots described by Hyman and Phillips (1970), was adopted, as the roots were cut, after washing them well with running water, into small pieces of 1 cm long and placed in 20 ml glass vials. A 10% KOH solution was added to them, then the roots were placed in a bath at 70°C for 15 minutes. Then the solution was poured and the roots were washed with distilled water three times and a 1% solution of hydrogen peroxide H_2O_2Was added to them. The roots were left for 10 minutes at room temperature. After that the solution was poured and the roots were washed well with distilled water and hydrochloric acid HCl of 1% concentration was

added to them. Then the roots were placed in the same solution for 3 minutes at room temperature, after that the acid was poured without washing the roots, and finally the roots were dyed with acid fuchsin dye, which was prepared in the laboratory according to what was reported by Korminik *et al*. (1980) and as follows: -

*63 ml glycerin

*875 ml lactic acid

*63 ml distilled water

*0.1 grams of acid fuchsin dye

After adding the dye, the roots were placed in a bath at a temperature of 70°C for 15 minutes. The roots were then transferred to a glass slide at a rate of 10 pieces per slide, which were examined under an optical microscope, and the infection rate was calculated using the equation described by Mosse and Giovanuetti (1980).

$$(\%) \text{ infection with mycorrhizal fungi} = \frac{infected\ root\ cutting\ number}{examined\ root\ number\ of\ total\ cutting} x100$$

As for the severity of infection with mycorrhizal fungi, it was calculated according to the evidence presented in Dhiab (2012), which consists of:

Grade	Ratio of infected parts to root piece
1	0% No injury
2	1 - 25% From the root is infected
3	26-50% From the root is infected
4	51-75% From the root is infected
5	76-100% From the root is infected

The severity of mycorrhizal infection was calculated according to Mckinney (1923) as in the following equation:

$$\text{Severity of infection with mycorrhizal fungi} = \frac{Total\ (number\ of\ affected\ pieces\ x\ degree\ of\ endemism)}{total\ number\ of\ pieces\ examined\ x\ highest\ degree\ of\ settlement} x100$$

3-3- Calculating the average number of mycorrhizal fungus spores in dry soil

Soil samples were taken from the area around the roots of three wild plants: *Imperata cylindrical*, *Sorghum sudanense*, and *Millet*, at a rate of 10 grams. The wet sieving and decanting method were used and the suspension containing the spores was transferred to a glass beaker, reduced the volume to 250 ml, then placed the liquid in 50 ml plastic tubes and placed in a centrifuge at 4000 rpm^{-1} for 5 minutes (without adding Sucrose solution) in order to facilitate the process of collecting spores at the surface of the suspension. Then the filtrate part of the tube was taken and the sediment was discarded. After that, the filtrate was poured into sieves and washed for 1-2 minutes with running water. Then they were transferred to a glass dish and the spores were collected using a micropipe. For the purpose of calculating the number of spores in 10 grams of soil, the equation established by Adholya and Gaur) 1994) was followed, which was: -

The number of spores in 10 grams of soil = the average number of spores calculated in 1 ml x the final volume of the suspension.

Isolation and diagnosis of Arbuscular mycorrhizal fungi

In order to isolate the mycorrhizal fungus, samples were taken from the soil surrounding the roots of some plants, namely *Imperata cylindrical*, *Sorghum sudanense*, and *Millet*, in some areas of

Diyala Governorate, which included Khan Bani Saad, Kanaan, Mandali, Al-Khalis, and Baladruz. The samples were placed in sterile polyethylene plastic bags and recorded the location and type of host. The wet sieving and decanting method described by Gerdeman and Nicolson (1963) was followed. For the purpose of isolating spores and subsequently diagnosing them, this method included weighing 250 grams of soil and placing it in a 2.5 liter glass beaker. 1 liter of running water was added and mixed well, and left for 1-2 minutes to allow the soil particles to sink to the bottom. The suspension was then passed through a set of sieves with diameters arranged in descending order of 0.025, 0.045, 0.075, 0.125, and 0.25 microns, respectively. The wash water was collected over each of the previous sieve in test tubes and centrifuged at a speed of 5000 rpm^{-1} for 5 minutes. The filtrate was poured and a 50% glycerol solution was added to the sedimented portion (Hosny *et al*., 1996). The tubes were shaken by hand and then centrifuged at a speed of 5000 rpm^{-1} for 10 minutes. The collected spores were then transferred to glass Petri dishes for examination purposes under 40x magnification to observe the spores and their number to be used as a vaccine with the aim of forming pure cultures (Al-Yahyaei *et al*. 2011).

An optical microscope was used to identify the morphological and anatomical characteristics of the spores in order to diagnose them. The genus and species of the mycorrhizal fungus were identified according to the taxonomic key mentioned by Walker *et al.* (2018). Isolates were classified to the genus and species level on the basis of the spore colour, shape and size, the nature of their attachment to the hyphae, and the number and thickness of their walls after the appearance of the fungal growths, based on the characteristics and nature of the mycelium and its composition of the fruiting body, the shape, size and colour of the chlamydial spore, the number of its covers, the method of mycelial formation and the structures it forms, depending on the taxonomic characteristics approved for the aforementioned source.

Results and Discussion

The results of Table (1) indicate that the highest average infection rate was in Millet, reaching 88.0%, while the lowest average infection rate was recorded in the Sudanese plant, amounting 72.0%. Millet was significantly superior to the Sudanese plant, with an increase rate of 22.22%. Regarding the difference between the means of the sites, Baladruz excelled, reaching 91.1%, with a significant difference from Canaan, with an increase of 32.22%.%. With respect to the interaction between plants and districts, the results showed that Imperat had the highest infection rate in Mandali, as well as Millet within Baladruz, as it reached 100%. On the other hand, the lowest infection rate was for the Sudanese knotweed within Canaan, amounting 63.3%, with an increase of 57.97%. This can be attributed to the percentage of infection which increases with an increase in pH, for the mycorrhizal fungi secrete some acids, especially oxalic. It also serves to lower the pH. This can be due to the infection rate that may depend on various factors such as humidity, temperature, soil pH, types of mycorrhizal fungi, and the host plant (Chandra and Bhardwaj, 2018).

Table (1) Percentage of infection with mycorrhizal fungi in the roots of wild plants spread in the soil of districts and towns of Diyala

Locations	Wild plants			Mean
	Imperata	Millet	Sudan grass	
Kanaan	70.0	73.3	63.3	68.9
Mandali	100.0	76.7	83.3	86.7
Bani Saad	80.0	93.3	66.7	80.0
Khalis	76.7	96.7	66.7	80.0
Baladruz	93.3	100.0	80.0	91.1
Mean	84.0	88.0	72.0	
LSD 0.05	Locations =12.12	Wild plants = 9.39	Locations*Wild plants = 21.00	

The results of Table (2) showed that the highest average infection severity was for Millet which reached 93.7%, whereas the lowest average was recorded for the Sudanese knotweed, amounting

80.5%, with an increase of 16.39%. The results showed that the highest average of injury severity was 98% in Baladruz, while the lowest average of injury severity was recorded in Kanaan, reaching 75.6%, with an increase of 29.24%. Regarding the interaction between plants and districts, the results showed that Imperat in Mandali as well as Millet in Khan Bani Saad and Al-Khalis had the highest infestation intensity (100%). By contrast, the Sudanese knotweed recorded the lowest infestation severity in Canaan, reaching 65.0%, with an increase rate of 53.84%.The difference in the intensity of settlement between the five sites can be attributed to the nature of the soil and its content of nutrients and environmental factors there, such as salinity and acidity (Liang *et al*., 2018). This can be consistent with what You *et al.* (2022) found on the common reed plant.

Table (2) Intensity of infection with mycorrhizal fungi in the roots of wild plants spread in the soil of districts and towns of Diyala

Locations	Wild plants			Mean
	Imperata	**Millet**	**Sudan grass**	
Kanaan	78.3	83.3	65.0	75.6
Mandali	100	86.7	90.	92.2
Bani Saad	88.3	100	76.7	88.3
Khalis	70	100	75.6	81.9
Baladruz	100	98.7	95.4	98.0
Mean	87.3	93.7	80.5	
LSD 0.05	Locations =12.23	Wild plants = 9.47	Locations*Wild plants = 21.19	

The results shown in Table (3) revealed that the highest average number of spores in Millet was 72 spores.gm^{-1}. soil, while the lowest average was recorded in the *Sorghum sudanense*, which amounted to 44.0 spores.gm^{-1}. soil. As a result, Millet was superior to the Sudanese knotweed. Significantly, with an increase of 63.63%. The results also showed that the highest average number of spores in Mandali was 66.7 spores.gm^{-1}. soil, whereas the lowest average was in Canaan, which amounted to 48.9 spores.gm^{-1}. soil, with an increase of 36.40%. With respect to the interaction between plants and sites, the results of Table 2 showed that there were no significant differences between the plants at the site of Mandali. In contrast, a significant superiority was found for *Millet* over the *Sorghum sudanense* at Canaan, Khan Bani Saad, Al-Khalis, and Baladruz sites, with an increase rate of 137.07%, 160.36%, 37.52%, and 49.89. % Respectively.

The most likely cause of this is the place where the samples were taken, for the differences in the number of spores may be related to seasonal patterns of reproduction, which may differ according to the types of mycorrhizal fungi or the host plant. In terms of the density of spores in a particular site, it can be due to the role of clay or sandy soil and the lowest content of organic carbon and phosphorus and nitrogen. This large number of mycorrhizal fungi spores may be related to optimal environmental conditions that can be more suitable for supporting the growth and developing the spores of mycorrhizal fungi in the root zone at the specific location. In addition, the possibility of the absence of antifungals could lead to an increase in mycorrhizal fungi compared to other sites (Shi *et al*., 2007).

It is noted from the results that the spores are present in most of the surveyed soil areas (Bani Saad, Mandali, Baladruz, Kanaan and Khalis), and their numbers varied from one region to another. The results also indicate that the dominant genus in these regions is Glomus mosseae, and this may be attributed to the competition between species (Inter Specific competition), which is considered to be one of the main factors in determining the emergence and growth of other types of mycorrhizal fungi, and this may be attributed to the adaptation of this species, G. mosseae, to the Iraqi environment in terms of soil type, type of plant families, and environmental conditions.

Table (3) Number of mycorrhizal fungi spores in the rhizosphere of wild plants spread in the districts and towns of Diyala

Locations	Wild plants			Mean
	Imperata	Millet	Sudan grass	
Kanaan	56.7	63.3	26.7	48.9
Mandali	66.7	73.3	60.0	66.7
Bani Saad	76.7	86.7	33.3	65.6
Khalis	63.3	73.3	53.3	63.3
Baladruz	70.0	63.3	46.7	60.0
Mean	66.7	72.0	44.0	
LSD 0.05	Locations = 10.74	Wild plants = 8.32	Locations*Wild plants = 18.60	

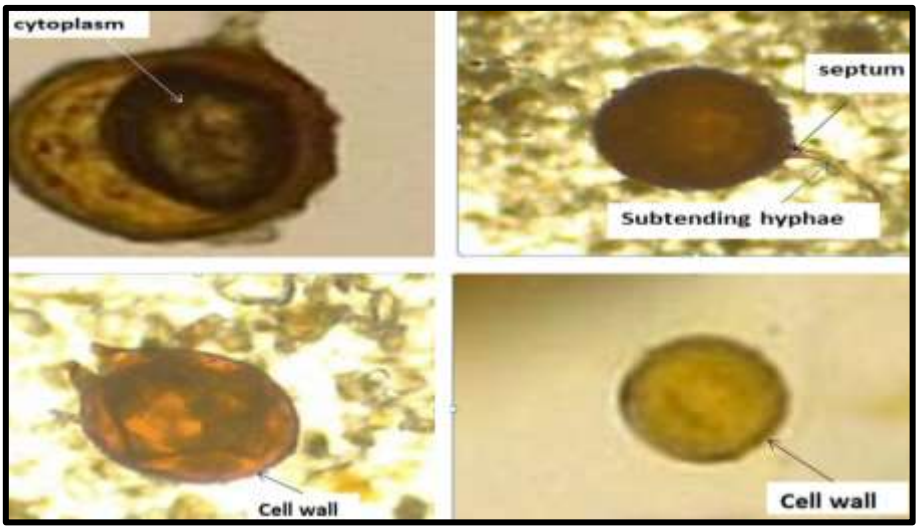
Table (3) shows the mycorrhizal compositions that were detected in the roots of, *Imperata cylindrical* L., *Sorghum sudanense*, and *Millet,* which differed from one site to another. The reason for the difference can be attributed to the difference in fungi, since not all endomycorrhizal fungi form fungal structures inside the root and thus, the difference emerged in naming these fungi. Some call them Arbuscular mycorrhizal fungi if they merely form Arbuscular structures inside the root. Some people call them vesicular mycorrhizal fungi because some mycorrhizal fungi do not form Arbuscular structures, while some have Arbuscular and vesicular structures, so they are called vesicular Arbuscular mycorrhizal fungi (Bharadwaj, 2007). Among the fungi that form vesicular structures inside the root are *Glomus mosseae*, *Glomus fasciculatum*, and *Acaulospora spinose*. As for the types; *Gigaspora* sp. and *Scutellospora* sp., they are distinguished by not forming vesicular structures inside the root (Olivera *et al.,* 2019).

Table (4) Nature of mycorrhizal structures in *Imperata cylindrical*, *Sorghum sudanense* and *Millet*

Locations	Wild plants	Hypha	Arbusclers	Vesicles
Kanaan	Imperata	+	+	+
	Millet	+	+	+
	Sudan grass	+	+	-
Mandali	Imperata	+	+	-
	Millet	+	+	+
	Sudan grass	+	+	+
Bani Saad	Imperata	+	+	-
	Millet	+	+	+
	Sudan grass	+	+	+
Khalis	Imperata	+	+	-
	Millet	+	+	+
	Sudan grass	+	+	-
Baladruz	Imperata	+	+	+
	Millet	+	+	+
	Sudan grass	+	+	+

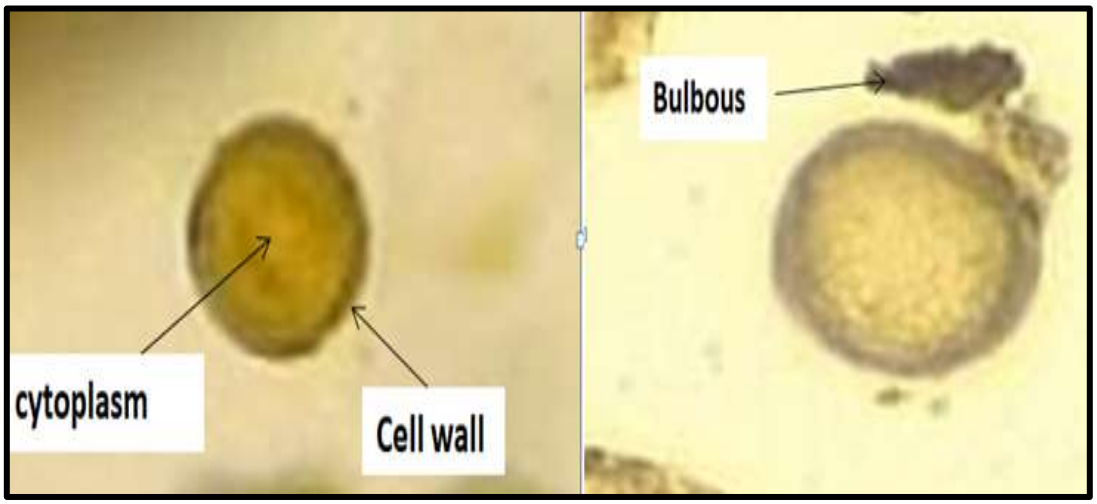
Based on the taxonomic key mentioned in Walker *et al*. (2018), the results of isolation and phenotypic diagnosis of spores of mycorrhizal fungi taken from different sites in Diyala, showed that they belong to two genera of mycorrhizal fungi. They are found to be the most frequent in soil samples, after matching their characteristics with the taxonomic key that is based on colour, the

spore, the number of spore walls, the thickness of the walls, the external shape of the spore, the contents of the spore, the area where the sporophyte meets the spore, the shape of the sporophyte, the collection of spores in the soil, and the shape of the vesicular structures inside the root. The genera are *Glomus* sp. and *Gigaspora* sp. The most frequent genus is Glomus sp in all study sites, it was isolated from the soil surrounding the *Imperata cylindrical*, *Sorghum sudanense*, and *Millet*. The infection rate of this genus was (90%), followed by the genus *Gigaspora* sp. It was isolated from the soil surrounding Millet and *Sorghum sudanense*. The reason for the discrepancy between the genera may be attributed to the difference in soil texture, salinity, acidity, organic matter, ready phosphorus in the soil, and other soil and environmental factors. These factors have been proven to be directly related to the density of each type of mycorrhizal fungus, or the reason for this may be due to the difference in the regions from which these fungi were isolated, because each region has its own environmental conditions (Yang *et al*., 2018). When *Glomus mosseae* was diagnosed, it appeared that the color of the spore was light yellow, spherical in shape with a smooth, reticulated surface. When *Glomus mosseae* was diagnosed, it appeared that the colour of the spore was light yellow, spherical in shape with a smooth, reticular surface. When examined with an optical microscope under 40x magnification, it was found that the diameter of the spore was 60 micrometers, and the diameter of the area where the spore connects to the hyphae was 10 micrometers. It is separated from the spore contents by a septum, which has one wall whose thickness is 4 micrometres, the wall thickness of the fungal hyphae in the contact area is 2 micrometres, and the spore was found individually in the soil.



Picture (1) spores of the mycorrhizal fungus genus *Glomus mosseae* under 40x magnification

It also appeared upon diagnosis that the second genus, Gigaspora sp, the colour of the spore is transparent white, spherical in shape. It is characterized by a bulge in the area where the spore connects to the bulbous sporophore. The surface of the spore is smooth, the diameter of the spore is 40 micrometers. The germination tube extends directly from the outer wall near the base of the spore. It has one thin and transparent wall. The spore is found to be sole in the soil.



Picture (2) Spores of the mycorrhizal fungus, *Gigaspora* sp. Under 40x magnification power

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