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دراسات عن الميكورايزا المرتبطة ببعض النباتات الصحراوية في المنطقة الشرقية من ليبيا والساحل الشمالي الغربي من مصر ودورها في مقاومة هذه النباتات لبعض الأمراض المرتبطة.

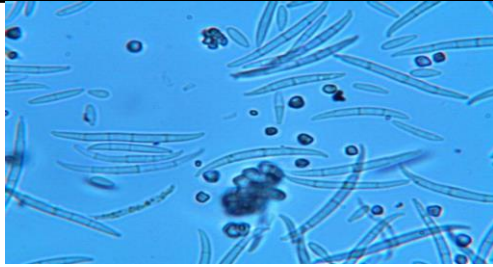
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أجريت هذه الدراسة خلال موسم (2012-2013) وذلك لتعريف وتحديد نسبة الإصابة بفطريات الميكورايزا الداخليه المرتبطة مع جذور ثلاثة نباتات صحراوية ، وهي القطف، الرمث و الشيخ وذلك في عشرة نموذجيات من المجتمعات النباتية الصحراوية شملت خمسة مناطق داخل الحدود الليبية وهي التميمي، عين الغزالة ، طبرق، كمبوت و إمسعد، و عينات مماثلة مأخوذة من خمسة مناطق داخل الأراضي المصرية شملت السلوم، سيدي براني، النجيلة، المثنى ومرسى مطروح. ودراسة دور هذه الفطريات في تثبيط العدوى بالفطريات المسببة للأمراض على النباتات المدروسة. وتبين من النتائج إرتباط الميكورايزا الداخليه مع معظم النباتات المدروسة حيث لوحظت الشجيرات والحوصلات النموذجيه للفطريات الميكورايزيه . وتبين من الدراسة ان أكثر الأجناس الفطرية إرتباطاً مع هذه النباتات هو جنس *Glomus* ، يليه جنس *Gigaspora*. و كان فطر *Glomus* أكثر الفطريات إرتباطاً بالنباتات المدروسة حيث وصلت نسبة الإصابة الي 54% في بعض الحالات، كما تبين من الدراسة أن القطف سجل أعلى معدل إصابة بفطريات الميكورايزا الداخليه في حين سجلت أقل معدلات إصابة على نبات الشيخ . كما لوحظ من نتائج هذه الدراسة أن معدلات الإصابة تختلف من منطقه الى اخرى. كما بينت نتائج هذه الدراسة ان نسبة الإصابة و أعراض الموت الرجعي المتسببه من الفطريات الممرضه وهي (*Fusarium sp* , *Pythium sp* ) (*and Rhizoctonia* ) على نباتات الدراسة تختلف من منطقه الى أخرى ولكن بشكل عام أظهرت علاقة عكسيه مع العدوى النسببيه بفطريات الميكورايزا.

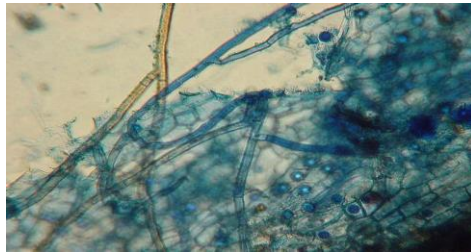
كلمات مفتاحية: ميكورايزا،نباتات صحراوية،أمراض



**Fig.(8) Spores of *Fusarium* sp.**



**Fig.(9) Spores of *Pythium* sp.**



**Fig.(10) Mycelium of *Rhizoctonia* sp.**



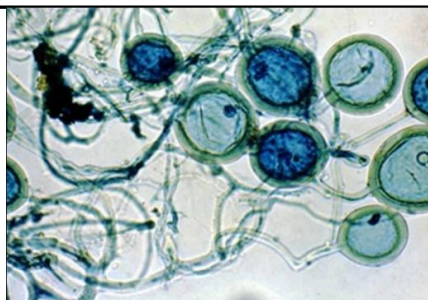
**Fig.(11) Necrosis on roots of *Atriplex halimus*.**

**Table (2).**Percentage of VAM infection, root infection with pathogenic fungi and die back symptoms on studied plants on Egyptian side.

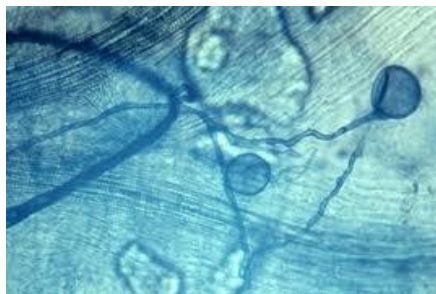
Studied Region	Plants	%VAM	Sp. or genus	%Root I	%Die back
El-Salloum	<i>Atriplex halimus</i>	34	<i>Glomus</i> sp+ Unidentified	8	11
	<i>Artemisia herba-alba</i>	2	Unidentified	33	35
	<i>Haloxylon salicornicum</i>	3	<i>Glomus</i> sp	30	28
Sidi-Brani	<i>Atriplex halimus</i>	44	<i>Glomus</i> sp+ <i>Gigaspora</i>	4	7
	<i>Artemisia herba-alba</i>	12	<i>Glomus</i> sp+ Unidentified	9	18
	<i>Haloxylon salicornicum</i>	22	<i>Glomus</i> sp	9	12
El-Ngialla	<i>Atriplex halimus</i>	41	<i>Glomus</i> sp+ Unidentified	5	8
	<i>Artemisia herba-alba</i>	13	Unidentified	8	16
	<i>Haloxylon salicornicum</i>	22	<i>Glomus</i> sp+ <i>Gigaspora</i>	8	14
El-Mathani	<i>Atriplex halimus</i>	38	<i>Glomus</i> sp	4	6
	<i>Artemisia herba-alba</i>	-	-	25	22
	<i>Haloxylon salicornicum</i>	14	Unidentified	7	8
Marsa-Matrouh	<i>Atriplex halimus</i>	39	<i>Glomus</i> sp Unidentified	4	7
	<i>Artemisia herba-alba</i>	-	-	19	18
	<i>Haloxylon salicornicum</i>	5	Unidentified	30	25

**Table (1).**Percentage of VAM infection, root infection with pathogenic fungi and die back symptoms on studied plants on Libyan side.

Studied Region	Plants	%VAM	Sp. or genus	%Root I	%Die back
Tamimi	<i>Atriplex halimus</i>	40	<i>Glomus sp+</i> Unidentified	5	10
	<i>Artemisia herba-alba</i>	8	Unidentified	20	35
	<i>Halexylon salicornicum</i>	12	<i>Glomus sp</i>	18	20
Ain Gazalla	<i>Atriplex halimus</i>	42	<i>Glomus sp+</i> <i>Gigaspora</i>	7	11
	<i>Artemisia herba-alba</i>	6	<i>Glomus sp+</i> Unidentified	13	20
	<i>Halexylon salicornicum</i>	2	<i>Glomus sp</i>	34	33
Tobruk	<i>Atriplex halimus</i>	45	<i>Glomus sp+</i> Unidentified	6	9
	<i>Artemisia herba-alba</i>	7	Unidentefy	17	20
	<i>Halexylon salicornicum</i>	12	<i>Glomus sp+</i> <i>Gigaspora</i>	10	18
Camput	<i>Atriplex halimus</i>	22	<i>Glomus sp</i>	8	8
	<i>Artemisia herba-alba</i>	-	-	30	40
	<i>Halexylon salicornicum</i>	4	Unidentified	23	32
Emsaad	<i>Atriplex halimus</i>	22	<i>Glomus sp</i>	9	12
	<i>Artemisia herba-alba</i>	-	-	25	33
	<i>Halexylon salicornicum</i>	6	Unidentified	19	18



**Fig. (6) Spores of genus *Glomes* in soil**



**Fig.(7) Spores of genus *Gigaspora* in soil**

Our studies revealed the infection of roots of studied plants with pathogenic fungi Fig.(11) identified as *Fusarium* sp , *Pythium* sp, and *Rhizoctonia*. Figs.(8,9,10) that's in agreement of the previous studies of (17) and (13). Degrees of infection of the studied plants with these fungi varied from plant to plant and from area to another but generally showed inversely proportional correlation with mycorrhizal infection, those plants show high infection with arbuscular mycorrhizal fungi, show low incidence of pathogenic fungi as well as appearance of die back symptoms there are many interpretation for these interactions . Arbuscular mycorrhizal fungi enhance P-nutrition and other essential nutrient that play a role in reducing the severity of the disease in mycorrhizal plant (9) and (16). Mycorrhizal roots have a mantle that acts as a physical barrier against the invasion of root diseases. Mycorrhizal root more lignified and secretes antibiotics that competes or antagonizes pathogens, thus aiding in disease suppression (23). Production of inhibitory phenolic cpd by arbuscular mycorrhizal fungi in their host and stimulation of chitinases, these enzymes can be inhibitory effective against certain fungal pathogen.( 25). Some genes involved in defends mechanisms are more activated during symbiotic interaction than in pathogenic infection (4). Many other arbuscular mycorrhizal fungi isolated from roots of studied plants in our studies but we can't identify them.



Fig.(3) *Halexylon salicornicum*

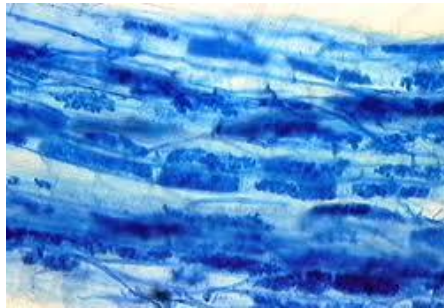


Fig.(4) Arbuscules inside root hairs

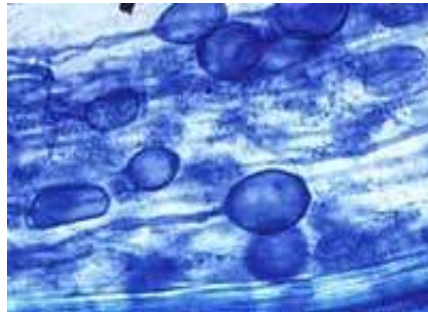


Fig.(5) Viscules and mycelium inside root hairs





Fig.(1) *Atriplex halimus*



Fig.(2) *Artemisia herba-alba*

Pure culture of each fungus was described. Each fungus was microscopically examined and spacialized key were carefully followed for identification of the isolated fungi.(1),(5),(6),(11) .

### **Spores studies in soil.**

This part of studies conducted in the laboratories of the Faculty of Agriculture (Saba Pasha) - University of Alexandria, Egypt. Spores or sporocarps were extracted from 20gm air-dried sub-samples of each soil samples, by wet sieving using sieves of different sizes, ranging from 100, 250, 400 micrometers, followed by flotation-centrifugation in 50% sucrose. (10). The spores were collected on a gride patterned filter paper, washed three times with dist. Water, then mounted on glass slides for observation and identification using current taxonomic criteria. (21 and 22) .

## **Result and Discussion**

Our results illustrated in Table (1 and 2) and Figs (4and5) indicated that there were great association of arbuscular mycorrhizal fungi(AMF) with most roots of examined plants reach 45% in some instants. These results in agreement with many previous studies (1 and 4).

*Glomus* sp Fig. (6 )was the most prevalent fungi associated with plant root as mentioned previously by (26), *Gigaspora* sp Fig. (7) was found associated with roots of *Halexylon salicornicum* in Tobruk and El-Ngialla , *Atriplex halimus* in Ain Gazalla and Sidi Brani no infection with *Gigaspora* in *Artemisia herba-alba* were notted, these results partially in agreement with those results of (2 and 19) There was variations in percent of infection with arbuscular mycorrhizal fungi between studied plants generally *Atriplex halimus* shows the higher percent of association ranging from (22 to 45) while *Artemisia herba-alba* shows the lowest association ranging from (0-13) with arbuscular mycorrhizal fungi these may be due to seasonal growth of *Artemisia herba-alba* as notted previously by (15) and (24).. Also infection with mycorrhizal fungi varied from area to another, spacemen collected from calcareous areas as camput, Emssad and Elssallum shows lower infection with arbuscular mycorrhizal fungi in other hand spacemen collected from roots of plants grown in sandy silt siols as El-Ngialla , Sidi-Brani and Tamimi showed heavy infection with arbuscular mycorrhizal fungi (27).

### Collection of soil and root samples

Soil samples were collected around the roots of five individuals of the same plant species randomly selected at each site. Care was taken during collection of individual plants that roots could be clearly identified as belonging to a particular plant. To ensure that the roots were connected to the study plants, root samples were removed while still connected to the plants. Samples were taken to the laboratory for determination of root colonization. Soil samples (ca. 250 g) included root segments and rhizosphere soil. Soil samples were air-dried prior to extraction and identification of spores, placed in nylon bags and transported to the laboratory (Central Laboratory, Faculty of Arts and Sciences Al-Gubba).

### Assessment of AM colonization

Fresh roots (ca. 0.2 g) were washed free of soil and cleared in 10% (w/v) KOH then heated at 90°C for about 1 hour in a water bath, treated segments were rinsed in water and acidified with dilute HCl. The samples were then stained by immersing for 5 minutes in 0.05% trypan blue in Lactophenol. (24). and examined at 100–400× magnification under an Olympus BX50 microscope with an automatic photomicrographic system for the presence of AM fungal structures. The percentage of root length colonized by AM fungal structures was estimated. (3).

$$\% \text{ of VAM infection} = \frac{\text{Number of segments containing VAM}}{\text{Total number of examined segments}} \times 100$$

### Determination of degree of infection with pathogenic fungi .

Degree of infection was estimated on the basis of proportion of root-hypocotyl necrosis at time of collection. A symptom severity scale based on characteristic root-hypocotyl necrosis or rotting recommended by (13) was applied throughout this study as follows:-

0 = no symptom.

1 = slight (about 10% or less of the plant part with lesions).

2 = moderate (lesions in about 10-25% of plant part).

3 = extensive (lesions in about 25-50% of plant part).

4 = severe disease (lesion on more than 50% of plant parts).

5 = dead plant.

Root pieces of each plant with or without necrosis were plated on potato dextrose agar media (10 segments/plant/petri dish) to detect the presence of fungal pathogen. The individual rating were converted to mean percent of infection using the following equation ( 17).

$$\% \text{ of diseases infection} = \frac{\text{some of individual root rating pieces value}}{5(\text{number of pieces assessed})} \times 100$$

die back index was determined as described by (12) in the same manner.

( 2 ).In many areas of the desert, the soil is sandy and poor in organic nutrients and subjected to erosion due to unexpected rainy storms that are sometimes, accompanied by high wind velocity. These environmental as well as other biotic factors are reflected on the scattered vegetation type dominating in deserts. Fungi forming mycorrhizal relationship with plants are a special group of the kingdom Fungi where this relationship is supposed to be symbiotic. This group can be classified into several types, but the arbuscular form [Arbuscular mycorrhizal fungi,(AMF)] forms 80% of this group (8). Many reports indicated the presence of fungi that form mycorrhizae in desert plants and their activities which is very important to these plant ( 22).. The role of these fungi in desert ecosystem, logically should be the same as in other ecosystems, but the difference is the mosaic and harsh environment of desert ecosystem. Now it is known that most families of desert plants form mutual association with fungi. One of the earliest observations about the presence of AMF in some grown plants such as date palm (*Phoenix dactylifera* L.) and “Nabg” plant (*Zizyphus spina-christi* Willd) in the Crescent Desert near Baghdad (Iraq) has been reported, where it seemed to contribute in the plant mineral nutrition. The report also mentioned the presence of AMF in some desert plants, with the most common plant *Peganum harmala* L. that flourish in summer months (20). Mycorrhizae are ubiquitous symbiotic associations between soil-borne fungi and plant roots. Approximate 80% of terrestrial plants establish mutualistic mycorrhizae with arbuscular mycorrhizal fungi (AMF, phylum Glomeromycota), which play a vital role in soil fertility and plant nutrition (23). Others (25), investigated that mycorrhizae enhance host plant defence against many soil-borne fungal pathogens .Mycorrhiza increased tomato resistance not only to soil borne disease caused by *Phytophthora nicotianae* var. *parasitica* . but also to foliar disease caused by necrotrophic fungus *Alternaria solani* (10). For these reasons we conducted this study to estimate the association of mycorrhizal fungi with some of desert plant in our locality and their role in defending these plants against root pathogenic fungi.

## Materials and methods

### Studied sites

Studied plants, taken from five areas within Libyan border namely, Tamimi, Ain Gazala, Tobruk, comput and Amsaad a distance of 250 km inside Libyan territory. Similar samples taken from five areas within Egyptian territory extended to distance of 250 km includes areas of El-Salloum, Sidi-Barani, Elnajailla, Al-Mthani and Marsa- Matrouh.

### Studied plants:

Three desert plants namely *Atriplex halimus* Fig(1), *Artemisia herba-alba* Fig(2) and *Halexylon salicornicum* Fig(3) were chosen to conducted this study due to their prevalent in studied area.

# Studies of Endomycorrhizal fungi associated with some desert plants in the north-eastern region of Libya and the north-west coast of Egypt in relations to associated diseases.

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## Abstract

Present investigations were conducted during the season (2011-2012) and carried out to identify, quantify the colonization by and diversity of arbuscular mycorrhizal (AM) fungi associated with three common desert shrubs, namely *Atriplex halimus*, *Artemisia herba-alba* and *Halexylon salicornicum*. were investigated in ten typical desert plant communities included five areas within Libyan border namely, Tamimi, Ain Gazala, Tobruk, Camput and Amsaad. Similar samples taken from five areas within Egyptian territory includes areas of El-Salloum, Sidi-Barani, Elnajaila, Al-Mthani and Matrouh. And to study the role of these fungi in inhibiting the infection with pathogenic fungi. Most of all three shrubs examined were found to be colonized and formed typical arbuscules or vesicles. Two genera were identified in the rhizosphere of these selected plant species belonging to the genera, *Glomus* sp and *Gigaspora*. Many other mycorrhizal fungi were isolated but couldn't identifying. *Glomus* occurred most frequently with percent of infection reach 45%. *Atriplex halimus* shows the higher percent of association, while *Artemisia herba-alba* shows the lowest association with AM fungi. Also infection with AM fungi varied from area to another. Many other genera of AMF but we couldn't identifying them. Degrees of infection and die back symptoms of the studied plants with pathogenic fungi i.e ( *Fusarium* sp , *Pythium* sp, and *Rhizoctonia* ) varied from plant to plant and from area to another but generally showed inversely proportional correlation with mycorrhizal infection.

**Key word:** Desert plants, Endomycorrhizae, Diseases.

## Introduction

Desert (semi- and arid lands) covers about 40% of land surface. This land stretches in areas of high pressure around the longitude 30° north and south of the Equator. Desert land is characterized by little precipitation (about 245 mm), hence a limited moisture, and high temperature at day time at least part of the year, and moreover high incidence of light are prevailing n manure to improve the organic matter of soil (1). The total