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CHARACTERIZATION OF *Azospirilla* AND VESICULAR ARBUSCULER MYCORRHIZAL COLONIZING ROOTS OF WILD AND ECONOMICAL PLANTS IN EGYPT.

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ABSTRACT

Enumeration, isolation and identification of the diverse species of associative N_2 - fixing *Azospirilla* and VA–mycorrhizae of selected group of wild plants from different locations in Egypt was studied. Twenty two isolates of *Azospirillum* strains were obtained from roots of different wild plants. Most of the isolates were found to belong to *A. lipoferum* and *A. brasilense*, which gave a positive biochmical characterastic, while other isolates were classified as unidentified isolates. VA mycorrhizal spores was markadly affected by cultures as well as the age of plant and sites,the highest numbers of spores were recorded with *Triticum* sp., *Hordeum* sp., *Plantago* albicanse and *Sonchus* oleraceus (12000 – 40000 spores kg^{-1} soil) .

INTRODUCTION

Asymbiotic dinitrogen fixers and VA – mycorrhizal fungi appear to play a significant role in the nutrients-economy of soil and grasses grown in different soils

Azospirillum is widely distributed in close association with roots of economically important plants, and is probably the most non-symbiotic produced plant growth promoting rhizobacteria in soil (Bashan and Levanony 1990).It is common in the rizosphere of grassas (Boddey and Dobereiner 1988, *Khammas et al; 1989*) and it is also reported to colonize the root, cortex of many plants (Baldani and Dobereiner, 1980)

The broader term endomycorrhizea, which has been replaced to a great extent the term vesicular – arbuscular mycorrhizea (VAM), are found in all climatic zones and so far in all soils (Mosse, 1975, and Redhead 1968).

The aim of this study is isolate and identify strains of *Azospirillum* and V.A mycorrhizae from wheat, Sorghum and a number of wild species.

MATERIALS AND METHODS

A – Isolation and purification of N_2 – fixing *Azospirilla*

Blocks of rhizosphere soil samples with their growing plants were collected from different localities of desert Egyptian soils for the isolation of *Azospirilla* (Table 1)

Whole plants were transferred to sterile paper bags for isolation, identification and characterization of Azospirillium spp. from root region. The enrichment culture technique was adapted by using the nitrogen deficient semi solid malate medium (NFM) recommended by Dobereiner and Day (1976), dispensed in test tube at a rate of 5 ml/tube . Root free soil was homogenized through mixing and shaking for 10 min .

Dilution with 5 gm of roots together with adhering soil were carried out by the method of (Holm and Jensen 1972, Reinhold *et al.* (1987).
Pure isolates with characteristic spining motile rods , were selected for further characterization. Sole carbon source utilization, acidfiction of glucose, requirement for biotin were carried according to Tarrand *et al.* 1978 . Catalase production was carreid out according to Neyra and Dobereiner (1977).

B- SDS- polyacrylamide gel electrophoresis :

Isolation *Azospirillum* sp.were grown on MAZ liquid medium containing 1 g NH_4Cl/l for 72 hrs, cells were harvested by centrifugation (500 rpm at 30 min) and washed twice with 0.85% (w/v) NaCl and once with distilled water. The cell pellets were lypohilized and stored at – 20 C ؛ before use .

Bacterial protine was extracted excretion by heating lyophilized cells at 100 C؛ for 10 min in 0.01 M sodium phosphate buffer, pH 7.0 containing 1% SDS and 1% mercaptoethanol (Begbie and Stewart, 1984). Protein sample excracts were identified by SDS- PAGE according to the method of Laemmli (1970) .

C- Survey, isolation and characterization of VA mycorrhizae from a number of wild plant species:

1- Extraction and counting of mycorrhizal spores:-

Samples from root region were collected from different localities. Whole plants with soil were transferred to sterile paper bags .The soil mass was gently removed from the root system of each plant and 250 gm taken and diluted to 1 litre tap water and suspended then sieved using wet-sieving and decanting technique Amer *et al.* 1997 Four sieves (400, 250, 150, 75 um mesh) were used throughout .the 250, 150 and 75 um fractions were transferred into a glass bottle and diluted with water to give between (20-50 spores / ml). The numbers of spores were estimated by spreading certain volume of mycorrhizal spore suspension onto a grided filter paper or petri dish which was divided into squares from its base .Number of spores was recorded using a binocular microscope (30-50 X)(Daft and Hogarth, 1983) .

2- Identification of extracted spores :-

Some of mycorrhizal spores representing different plants were picked up from the extracted spores. The morphological characteristic of the spore were determined according to the key by Trappe (1982) .

3- Estimation of root infection rate :-

This method was estimated according to Kormanik *et al.* (1980) .

RESULTS AND DISCUSSION

1- Azospirilla characterization: -

Twenty two isolates were taken from rhizosphere of of wild and econimecal plants taken (Table 1) from different locations in Sinai area. Identification studies of the collected wild plants revealed that they belonged to *Pulicaria crispa* (Compositae) *Bromus sp.* (Gramineae), 3 (Gramineae), 4

(Leguminosae), 5 (Gramineae), *A. hybridus* (Amaranthaceae), *Sisybrim Sp.*(Cruciferae), 10(Gramineae), *Sentria sp.* (Gramineae),*Conyza canadensis* (Composite), *Centaurea glomerate* (Composite) , *Atriplex stylosa* (Chenopodiaceae) *Poa sp.* (Composite), *Xanthium spinosis* Grramineae (Composite) *Amaranthus sp.* (Amaranthaceae), *Cicer sp.* (Leguminoseae), *Sisymbrium irio* (Cruciferae), *Chenopodium sp.*(Chenopodiaceae) and *Triticum sp.* (Gramineae) . *Azospirillum* as a nitrogen fixer which can perform on associative symbiosis with higher plants was isolated efficiently from the rhizosphere of (16) of the recorded plant samples, where we obtained *Azospirilum lipoferum* from the plant species : *Triticum sp.*(Gramineae), (3) (Gramineae), *Cicer arietinum* (Leguminoseae), *Chenopodium sp.* (Chenopodiaceae), (10) (Gramineae), *Poa sp.* (composite) (Gramineae),*Sispymbrum irio* (Cruciferae) and *Centaurea glometara* (Compositae) .We obtained *Azosprillum brasilense was obtaied* from the plant species : *Pulicaria crispa* (Compositae) , (5) (Gramineae), *Sisymbrum sp.* (Cruciferae), *Amaranthus sp.* (Amaranthaceae), *Bromus sp.* (Gramineae) (4) (Leguminosae), *Setaria sp.* (Gramineae), *Chenopodium album* (Chenopodiaceae) and *Atriplex stylosa* (Chenopodiceae) . It was were not able to detect any Azospirilla from plant species : *Cicer sp.* (Leguminosae) ,*A. hybridus* (Amaranthaceae) , *Conyza canadensis* (Compositae) .

Motility revealed that 18-24 hour old isolates were slightly motile to active (Table 2). Active isolates could be divided into (1) slow worm like active spiral (++) and very active spinnig motility (+++) . Six isolates were slightly motile while the other nine isolates were active spiral and four isolates were very active spinning.

1- Nitrogenase activity :

The acetylene reduction assay for measurement of N_2- fixation is considered as one of very important characteristics for *Azospirillum* . Data in (Table 2) indicates the activity of this enzyme of 22 isolate and found is the highest activity were recorded for the plants, (3) (Gramineae) (4) (Leguminosae), (7) *Sisymbrium sp*, (Cruciferae) and (8) *Cicer arietinum* (Leguminosae) where the recorded N_2 – ase activity was 16.8 , 16.1 , 17.4 and 19.4 nmoles C_2H_4 ml^{-1} hr^{-1} culture respectively. All other isolates recorded lower N_2 – ase activity and the isolate (8) from *Cicer arietinum* (Leguminosae) recorded the highest activity than all other isolates. The isolates of (Gramineae) , *Sisymbrium sp.* (Cruciferae) and *Cicer arietinum* (Leguminosae) were found to belong to *A. lipoferm* while isolate No. 4 (Leguminosae) only belonged to *Azospirillum brasilense* . Attention shoud be drawn to the prevalence of symbiotic N_2 – fixers in soils in general and in poor desert soils in particular , such as those investigated in this study . The beneficial effect of these organisms could be attributed to N_2- fixation which was indicated by N_2- ase activity (Vlassak, 1982) , Bashan and Levanony (1990) , Elbakry *et al* . (2001) and Mitkees *et al.* (1996)

Table (1): Checklist of plant species and their localities for the present study .

Isolate No.	Family	Species	Location
1	Compositae	Pulicaria crispa	Ismsilia
2	Gramineae	*Bromus sp.*	North Sinai
3	Gramineae	-----	" "
4	eguminosae	-----	"
5	Gramineae	-----	" "
6	naranthaceae	A.hybridus	Vadi El-Arish
7	Cruciferae	*Sisymbrum sp.*	Vadi El- Bruk
8	eguminosae	Cicer arietinum	"
9	Gramineae	*Setaria sp.*	" "
10	Gramineae	-----	" "
11	Compositae	nyz cf. canadensis	loza- Romana
12	enopodiaceae	enopodium album	Gulf El-Suaz
13	Gramineae	*Poa sp.*	Isamailia
14	Compositae	ntaurea glomerata	Gulf El-Suze
15	enopodiaceae	Atriplex stylosa	anzala Region
16	Gramineae	-----	"
17	Compositae	anthium spinosis	El- Giddi area
18	naranthaceae	*Amaranthus sp.*	Vadi El- Jerafi
19	eguminosae	*Cicer sp.*	"
20	Cruciferae	Sisymbrum irio.	ebel Katherine
21	enopodiaceae	*Chenopodium sp.*	rdawil Region
22	Gramineae	*Triticum sp.*	" "

2 – Catalase test :

Catalase enzyme which is formed in most bacteria catalyses the breakdown of H_2O_2 to realase free oxygen . A number of diazotrophs possess this enzyme (Nur *et al.* 1982). Difco Manual (1985) .Examination of the 22 isolates revealed that, most of these isolates (19) posses catalase activity but the reaction on the addition vary in strength . Weak reaction releasing few oxygen bubbles on the addition of H_2o_2 , an indication of few number of *Azospirilla* but strong and very strong bubbles indicated high number of *Azospirilla* .

Eight isolates were releasing few oxygen bubbles (+) while seven isolates were releasing bubbles of oxygen (++) and six isolates were releasing very strong bubbles of oxygen (+++) and only one isolate did not release any oxygen bubbles .

3- Nitrate reduction and denitrification :

Reduction of nitrate ions is one of important characteristics for bacteria of genus *Azospirillum* , where NO_3^- is used as final electron acceptor under conditions of low oxygen tention (Reinhold *et al.* 1987 and Doberainer 1991). Examination of 22 isolates of *Azospirilla* revealed that (19) of these isolates were able to reduce nitrate (Table 2).These isolates involved the group of *A.lipoferum A. brasilense* and an unidentified isolates.The reduction of nitrate into NO_2 or even N_2 gas differs from one

isolate to another according to the amount of gases released . Five isolates were releasing little amount of gases (+) while nine isolates were releasing moderate amount of gases and five isolates were releasing large amount of gases , but three did not releas any gases .

Table (2): Characterization of Azospirillum isolates from different wild plants

plant No. (1)	Moltolity	Shape	Glucose as sole Carbon source	Acidification of glucose		Biotin requirement	Catalase test	Nitrate reducation	Nitrogenase activity (n mole C2 H4 / h /ml culture)	Denitrification malate media
				Aerobic	anaerobic					
1	++	Spiral	-	+	+	NO	++	+	0.1992	++
2	++	Spiral	-	-	++	NO	+	+	11.621	++
3	++	spiral	+	+	+	Yes	+++	++	16.829	++
4	++	Spiral	-	-	+	Yes	+	++	16.121	+++
5	++	Slightly	-	+	+	NO	++	++	9.561	+
6	+++	Spinging	-	-	+	Yes	+	+	0.0151	-
7	+	Slightly	+	+	+	Yes	+	++	17.371	++
8	++	Spiral	+	+	+	Yes	+++	+++	19.426	+
9	+++	Spinging	-	-	+	NO	+	+	8.631	++
10	+	Slightly	+	+	+	Yes	++	+++	11.151	+++
11	+++	Spinging	+	+	+	NO	+	-	NO	-
12	+	Slightly	-	-	+	NO	++	++	NO	-
13	+++	Spinging	+	+	+	Yes	++	++	13.651	++
14	++	Spiral	-	+	+	Yes	+++	+++	11.361	+
15	+	Slightly	-	-	+	NO	-	-	NO	-
16	++	Spiral	+	-	+	Yes	++	++	8.516	++
17	+++	Spinging	-	-	+	NO	+++	+++	6.210	+++
18	+	Slightly	-	+	-	NO	+	-	NO	-
19	++	Spiral	+	-	+	Yes	+	+++	1.672	+
20	+++	Spinging	-	+	++	NO	+++	++	7.139	++
21	+	Slightly	+	+	+	Yes	++	+	4.812	+
22	++	Spiral	+	+	+	Yes	+++	++	6.250	+

KEY FOR IDENTIFICATION OF ISOLATES .
(1): Identifled isolates
(2): Motility (+) slightly motile (++) Spiral (+++) Spinging
(3): Acidification of glucose (+) Low acidity (++) Moderate acidity (+++) High acidity

4-Glucose as sole carbon source :-

Bacteria of the genus *Azospirilla* has the ability to use glucose as sole carbon source for growth in yeast malate medium (Tarrand *et al.*, 1978). Our examination of the 22 isolates revealed that the isolates 3, 8, 21, 10, 13, 7 and 17 isloated from 3 (Gramineae), 8 *(Cicer arieatinum)* (Leguminosae) , *Chenopodium sp.* (Chenopodiaceae), 10 (Gramineae) ,13 *Poa sp.* (Gramineae), 7 *Sisymbrium sp.* (Cruciferae) and 17 *Xanthimum spinosis* (Compositae) belonged to *Azopirillum lipoferum* which gave positive with the utilization of carbon source this means that *Azospirillum lipoferum* used glucose as sole carbon source in its growth medium. These results agree with Tarrand *et al* (1978). Other isolates gave negative test, these belonged to *A. brasilense*. In contrast, the isolates of 19 *Cicer sp.* (Leguminosae) and 11 *Conyza canadensis* (Compositae) g ave p ositave w ith g lucose a s s ole

carbon source but they did not belong to *A. Lipoferum* or *A.brasilense* so they were put under unidentified isolates .

5- Acidification of Glucose: -

Bacteria of the genus *Azospirilla* can grow aerobically in glucose fermentation medium (Difco, 1985) where it can acidify glucose in the medium and release gases and also produce acid which reduce the pH in the medium. Results show that isolates No.1, 3, 5, 6, 7,8 , 10, 11, 13, 14, 18, 20, 21, 22 can grow aerobically and characterized by producing gas and acid , while other isolates can grow anaerobically in peptone – based medium (Tarrand *et al.* 1978), and have the ability to acidify glucose by producing gas and acid.

Results also show all isolates except isolate No.18 can grow anaerobically.Table (3) show that the isolates from this study would be characterized into three types:, *Azospirillum lipoferum* , *Azospirillum brasilense* and unidentified isolates . *Azospirillum liopferum* isolates gave positive with biochemical reaction like biotin requirement, glucose as sole carbon source and aerobic , anaerobic acidification of glucose. *Azospirillum barsilense* isolates gave negative results with biotin and with glucose when used as the sole carbon source . Isolates which were put in special unidentified group do not belong to *A . lipoferm* or to *A. brasilense* , where isolates of this group sometimes induced positive results with some tests and

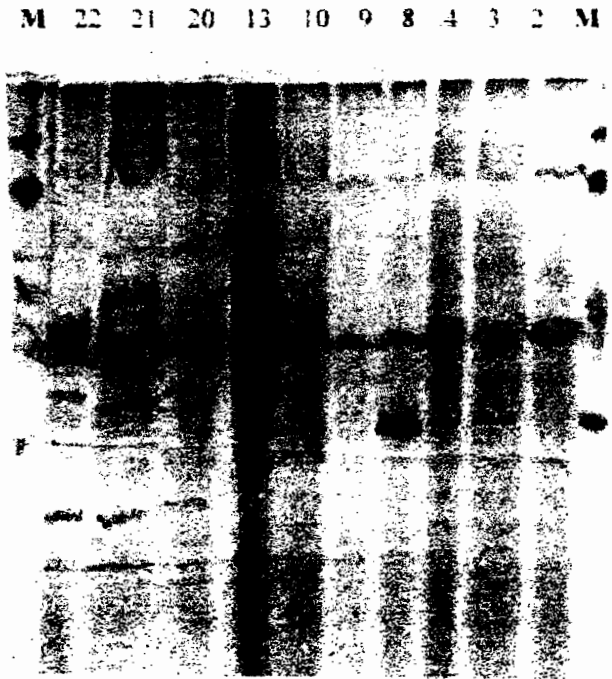

Fig. (1) : SDS- PAGE patterns of total cellular proteins of ***A.brasilense*** (2,4,9) and ***A. Lipoferum*** (3,8,10,13,20,21,22) staind coomassie blue with M. Protein marker, contained phosphorylase b 92 K.D Boviane serum Albumin 67 K.D egg albumine 45 K.D and Cassine anhydraus 29 K.D

Fig. (2a) : × 300 / Photo 9× 10 : Vesicules and Arbuscules and mycelium from infected sorghum root (45 days old)

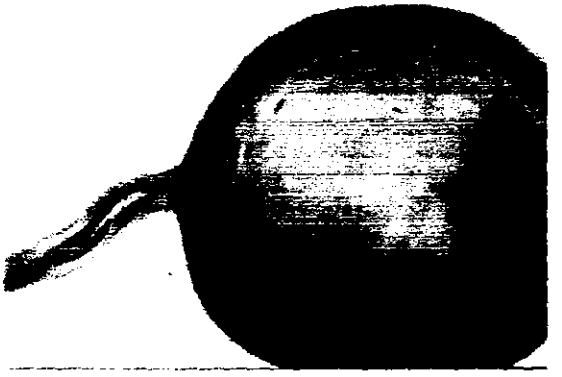

Fig. (2b) : × 500 / Photo 9× 10 : Glomus caledonius

Fig. (2c) : Photo × 200 : Glomus macrocarpus

negative with other tests . Isolates No, 6, 16 were positive with biotin requirement and anaerobically acidify glucose , while isolate No.19 can use . glucose as sole carbon source and anaerobically acidify glucose and isolate No. 11 can use glucose as sole carbon source and aerobically acidify glucose. Isolate No. 14 can use glucose as sole carbon source and aerobically anaerobically and acidify glucose erobically and anaerobically . According to Tarrand *et al.* (1978), they were put in special unidentified group .Results obtained revealed that all *Azospirillium* isolates have the ability to fix atmospheric nitrogen and nitrogenase activity of 8 isolates of *Azospirillum lipoferum* and 9 isolates of *Azospirillum brasilense* was estimated beside other biochemical tests and are recorded in table (2) . It was found that isolates No.7,8 isolated from *Sisymbrium sp.* and *Cicer arietinum* gave the best activity being 17.3 and 19.4 (n. moles) $C_2 H_4 h^{-1}$. Cultures gave comparable to isolate 22 which was isolated from Agricultural Research Center Farm which gave 6.25 n mole Our study draws attention to desert soils and wild plants, in particular of highly efficient N-fixing (Azospirilla) Tarrand *et al.* (1978) and Vlassak (1982), and their potainal application in agriculture .

Table (3) : Biochemical studies of different *Azospirillum spp.*

Identification species	Isolate Number	Biotin requirement	Glucose as sole carbon source	Acidification of glucose	
				Aerobic	anaerobic
A.lipoferum	22	+	+	+	+
A.lipoferum	3,8,21,10,13	+	+	+	+
A.brasilense	7,17	+	+	+	+
Group A	1,5	---	---	+	+
Group B	20	---	---	+	+
Group C	18	---	---	+	+
Group D	2,4,9,12,15	---	---	---	+
Undefined isolates					
Group A	19	---	+	---	+
Group B	6,16	+	---	---	+
Group C	11	---	+	+	---
Group D	14	+	---	+	---

B- SDS – PAGE patterns of total cellular proteins of *Azospirillum spp.*

In order to obtain more information about 10 isolates of Azospirilla (2, 4, 20, 9, 22, 3, 8, 21, 10 and 13) by
Protein pattern , SDS – PAGE was used to Separate total cellular protein in each isolates. Fig.(1) showed that many of the bands were common for all isolates. However, some bands appeared to be different between isolates according to intensity and molecular weight .It was observed that the isolates No. 4, 3, 8 and 13 which hight N_2–ase activity had a major characteristic common bands (ranging between 40-43 Kd) were chosen as differ on N_2–ase activity and grew and shaped a single band with appearances of common polypeptide .It is concluded that the SDS – PAGE of celluar proteins patterns for Azospirilla could be a useful tool for identification and

classification.Sundaram *et al*, (1988) , Rene and Jos 1989 and Rakhshanda *et al*, (1990) . reported that the electrophoretic patterns of total proteins on SDS – PAGE was used to compare various strains of *Azospirillum spp.* and various mutants derived from *A. barasilense* .

C – Characterization of VAM. :

Number of VAM spores :-

Results in Table 4 showed the density of vesicular arbuscular Spores extracted from 20 soil samples. The number of mycorrhizal spores was markedly affected by plant species and sites. Samples No. 2,5,13,14,19,20 which were isolated from plants: Juncus rigidus, Allium cepa , Triticum sp., Hordeum sp., Plantago albicanse and Sonchus oleraceus gave the highest number of spores compared to other plants (12000–40000) spores Kg^{-1} soil) In contrast, samples No., 6, 8, 3, 4, 9,11 which their plants are *Sonchus sp., Conyza sp. Avena fatua, Saccharum officinarum, Cauaring glauca, Zea mays* gave the lowest density of mycorrhizal spores being (3000 – 8000 spore Kg^{-1} soil). The occurrence and distribution of *V.A. Mycorrhizae* in different locations in Egypt inculud temperate , semi desert and desert have a broad ecological range, which agree with Hayman (1982) and Pacovesky (1998) .

Root infection :

With respect to root infection levels. The percentage highly varied from site to another (Table 4 and Fig. 2 (a) & (b) & (c)). Plant samples 19,20,12. *Plantago albicanse, Sonchus oleraceus* , and *Medicago sativa* samples gave the highest infection percentage being 80 , 70 and 70 respectively . These plants were collected from Sadat desert area and Giza area. Lowest infection was recorded in samples No. 6, 8, 3, 9, *Sonchus sp., Conyza sp., Avena fatua and Causarina glauca* collected from Giza and Quesna sites respectively .It could be concluded that the rhizosphere of *Plantago albicanse, Sonchus oleraceus* gave higher numbers of mycorrhizal spores as well as fungal hyphae and vesicles . Other plants show lower number of vesicles , that indicates that some plant species are considered to be efficient hosts for *vesicular arbuscular mycorrhizae*, this may be attributed to the exudates of these plants which stimulate the germination of mycorrhizal spores , increasing the infection percentage .VAM were found in most angiosperms as well as in some gymnosperms, petridophyes , and bryophytes (Hayman , 1982) .Most plant species in Leguminosae and Gramineae families are normaly mycorrhizal (Hayman , 1982). Infection percentage in Leguminosae ranged from moderate as in *Glycin max* (40%) to high in *Medicago sativa* (70,5%). In Gramineae infection ranged from low to moderate where it was in *Avena fatua* (20 %) , *Saccharum officinarum* (30%),*Zea mays* (50 %) and *Hordeum vulgare* (60 %) .These results agreed with Hayman (1983) and Zaghloul *et al*., (1996) .

3. Spore type: -

Results revealed that ; some soils have one abundant type of spores while others contain different types of spores. Description and names were based on distinctive morphological features Saleh *et al.* (1998) .

Table (4) : Survey of VA – mycorrhizae in selected Location in Egyptian soil

Sample No.	Family	Scientific name	Location	Soil Texture	Character of spores	Total Count / g soil	Infection %	Probable Identified name
9	Causarinaceae	*Causurina glauca*	Quesna	Clay	Honey – rigid	4	20 %	*Glomus spp.*
8	Compositae	*Conyza sp.*	Giza	Sandy loam	Honey rigid Honey circular	8	10 %	*Gigaspora spp.*
6	Composite	*Sonchus sp.*	Giza	Clay	Honey circular smooth	3	0 %	*Gigaspora spp.*
1	Cyperecae	*Cyperus laevigatus*	Quesna	Sandy Loam	Honey – blackish circular	10	30 %	*Gigaspora nigra*
3	Gramineae	*Avena fatua*	Giza	Sandy Loam	Honey blakish rigid wall	6	20 %	*Gigaspora spp.*
4	Gramineae	*Saccharum officinarum*	Giza	Sandy Loam	Brown rigid	10	60 %	*Entrophospora sp.*
7	Garmineae	*Hordeum vulgare*	Giza	Sandy Loam	Brown rigid	10	60 %	*Entrophospra sp.*
11	Gramineae	*Zea mays*	Giza	Sandy	Honey circular smooth	6	50 %	*Gigaspora spp. Entrophospora spp.*
2	Juncaceae	*Juncus rigidus*	Quesna	Sandy	Honey blackish rigid wall	15	25 %	*Gigaspora nigra*
10	Leguminosae	*Glycine max*	Quesna	Clay	Brown circular small and big	10	40 %	*Entrophospora sp.*
12	Leguminosae	*Medicago sativa*	Giza	Sandy clay	Honey cicular smooth	10	70,5 %	*Gigaspora spp. Entrophoshpora sp.*
5	Liliaceae	*Allium cepa*	Giza	Sandy Loam	Honey rigid / brown smooth	12	40 %	*Gigaspora spp. Entrophospora spp.*
16	Chenopodiaceae	*Brassica indica*	Sadat desert	Sandy	Honey circular Dark brown circular	12	50 %	*Gigaspora nigra, Entrophospora spp., Glomus spp.*
20	Compositae	*Sonchus oleraceus*	Sadat desert	Sandy	Small / medium Brown / Honey Grey	25	70 %	*Acaulospora, Gigaspora nigra*
13	Gramineae	*Triticum sp.*	East owinat	Sandy	Circular Honey and Brown and orange	22	10 %	*Gigaspora spp., Entrophospora spp., Gigaspora nigra*
14	Gramineae	*Hordeum sp.*	East owinat	Sandy	Circular Honey and Brown and orange	30	30 %	*Acaulospora , Gigaspora nigra*
18	Liliaceae	*Lolium rigidum*	Sadat desert	Sandy	Small medium Honey brown	12	30 %	*Acaulospora , Gigaspora nigra*
17	Plantagoinaceae	*Pintago sp.*	Sadat desert	Sandy	Large Honey small – medium brown	10	20 %	*Gigaspora nigra Entrophospora spp., glomus spp.*
19	Plantaginaceae	*Plantago albicanse*	Sadat desert	Sandy	Small , medium Honey	40	80 %	*Acaulospora , Gigaspora*
15	Solanaceae	*Lycopersicon esculentum*	Quesna	Clay	Orange and Honey circular and Rigid	10	50 %	*Acaulospora Gigaspora spp.*

Soils at Quesna had one abundant type of spores, where spores of *Gigaspora nigra* were abundant while *Cyperus laevigatus* and *Juncus rigidus* were growing. he same type of spores was also found in Giza soil on *Avena fatua*, *Saccharum officinarum*. Spores of *Enterophospora spp.* were abundant in Giza soil on *Hordeum vulgare*. In contrast, all other isolates had different types of spores.

Results also indicated that spores varied in their colour some honey blackish, orange, others were brown and grey. But according to the wall of spores, some have circular walls others have elevated wall shape. VAM have four known genera they are, *Glomus, Gigaspora* , Acaulospora and Sclerocystis Trappe (1982) . In conclusion, Our results show that we have *Gigasspora spp.* From isolated plant species Cyperus *laevigatus, Juncus rigidus, Avena fatua , Saccharum officinarum , Allium cepa Sonchus sp., Conyza sp., Zea mays, Medicago sativa , Triticum sp. Hordeum sp. Lycopersicon esculentum , Brassica indica , Plantago sp., Lolium rigidum , Plantago sp., Lolium rigidum , Plantago albicans and Sonchus oleraceus . Glomus sp.* Were isolated from plant species *Conyza sp.* and *Casuarina glaca . Entrophospora sp.* Were identified from plant species *Allium cepa , Hordeum vulgare , Glycine max, Zea mays, Medicago sativa , Triticum sp. , Hordeum sp., Brassica indica* and *Plantago sp. Acaulospora sp.* Were isolated from plant species *Lycopersicon esculetnum , Lolium rigidum, Plantago albicanse and Sonchus oleraceus* . Results also suggest that the probable identification names are: *Gigaspora nigra, Gigaspora spp., Entrophospora spp., Glomus spp.* and *Acaulospora.* From the result obtained it is clear that the density of VAM spores was markedly affected by cultivars as well as the age of plant and sites where there are some cultivars recorded high infection while others gave low infection regardeless cultivars. This ensure the symbiotic relation between the fungus and the plant.

Miller (1992) , indicated that the number of spores varied from plant to another and the highest number of spores recorded with the plants: *Triticum sp., Hordeum sp., Plantago albicans* were associated and *Sonchus oleraceus* being 12000 – 40000 spores Kg^{-1} soil .

Infection percentage varied from plant to another regardless of the number of spores where the highest infection percentage recorded was with the isolates from plants *Plantago albicanse* (80%), *Medicago Sativa* (70-5 %) and *Sonchus oleraceus* (70%)

An identification of spores was conducted according to Trappe(1982) as spore morphology , sporocarps , spore

Dimensiont , spore colour , hyphal mantle , spore Walls , spores contents and manner of spore germination .

Identifed spores given probable identified names were put under four main genera *Gigaspora , Acaulospora, Entrophospora and Glomus.*It was concluded that some plants had high numbers of spores but the root infection was low, this may be due to soil fertility and the moisture content in soil (Bagarya and Menge , 1978) Pacovsky (1989) and Gaur–Ac and Getasingh (1995) .

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عزل وتصنيف بكتريا الازوسبيرلا ونظريات الميكروهيزا المتواجدة فـى جـذور بعض النباتات البرية والمحاصيل الاقتصادية فى مصر

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ويهدف هذا البحث إلى حصر وعزل وتصنيف الأنواع المختلفة من بكتريا الازوسبيريلم الموجودة فى ريزوسفير بعض النباتات البرية والاقتصادية وتقدير النشاط الإنزيمي لهـا وعمـل اختبارات بيوكيميائية وجينية لها كذلك تصنيف أنواع مـن فطريـات الميكروهيـزا وجراثيمهـا الموجودة فى بعض المحاصيل الحقلية والنباتات البرية الصحراوية وأوضحت النتائج أن هناك ٢٢ عزلة من الاسبيريلم اختلفت فى الحركة والشكل والنشاط حسب نوع النبات والمكان كذلك اختلفت كثافة جراثيم فطريات الميكروهيزا أو نوعها حسب نوع النبات وعمره .

وأوضحت الدراسة دور الكائنات الحية الدقيقة فى مساعدة النباتات المختلفـة بامـدادها بالعناصر الغذائية اللازمة وتأثيرها على نمو وإنتاجية كثير من المحاصيل الحقلية التي تزرع فى مصر .