SUCCESSFUL GROWTH AND SPORULATION OF THE VESICULAR ARBUSCULAR MYCORRHIZAL FUNGI IN AXENIC CULTURES

BY

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ABSTRACT

Three VAM-fungi were isolated from roots of onion (*Allium cepa*, Liliaceae), broad bean (*Vicia faba*, Leguminosae) and Swiss cheese (*Monstera deliciose*, Araceae). Another isolate was isolated from roots of maize (*Zea mays* Graminae) plants raised from sterilized seeds sown in sterilized soil. All fungi have coenocytic coarse aseptate mycelium and reproduced asexually by sporangia and sporangiospores. Chlamydospores with different sizes were produced also by all fungi. Chlamydospores were formed apically and/or intercalary, either singly and/or in chains.

Fungal growths on barely-sand medium and suspensions of sporangiospores (obtained from cultures grown on solid medium) were used for inoculating sterilized soil (at different levels) 7 days before and after sowing, respectively. Maize (Zea maize) seedlings showed significant improvement in their growth when grown in sterilized soil inoculated by these isolated fungi. However rate of growth improvement was quite varied and depended on source of fungal isolate and its inoculum level. Onion-isolate was more effective at the lowest inoculum level and this trend was completely reversed in Swiss cheese isolate. Broad bean-isolate gave the best results at the intermediate inoculum levels. Roots of maize plants raised in soils inoculated with fungal materials showed structures characterizing mycorrhizal infections i.e. inter- and intracellularly aseptate hyphae, chlamydospores, arbuscules and vesicles.

These findings are innovative and reported herein for the first time.

INTRODUCTION

The VA mycorrhizae have a little effect on root morphology, not readily distinguishable from uninfected roots and have not been isolated from infected roots except for an *Endogone*-like fungus isolated by Barrett (1961). Hawker, (1962) stated that the endophyte in garlic roots isolated in pure culture and described as a *Pythium* considered to be the VA endophyte which produced mycorrhiza when back-inoculated into the host. Also the VA fungi could not be grown in pure culture and do not form colonies on the traditional soil dilution plates of the soil microbiologist even though they can constitute a major portion

of the hyphae present in soil (Warcup, 1957, and Jackson, 1965). The strict application of Koch's postulates to *Endogone* was not possible because of its inability to grow in pure culture The inability of the VA endophyte to grow alone in pure culture is surprising in view of its lack of specificity in the host plants it will infect. The same isolate of *Endogone,* for example, can establish VA mycorrhiza with completely unrelated plants such as onion, strawberry, violet, sweet gum, and diverse legumes and grasses.

The VA fungi were classed as phycomycetes because they lack regular septa. They have usually been assigned to the genus *Endogone,* family Endogonaceae, and order Mucorales. The nomenclature of the spore types or species of *Endogone* is somewhat confusing. Mosse and Bowen, (1968a) gave the different spore types descriptive names based on distinctive morphological features. While Gerdemann and Trappe, (1974) gave them different generic and species names, partly according to whether they are chlamydosporic, zygosporic or azygosporic. The latter scheme includes four genera, viz. *Glomus, Gigaspora, Acaulospora* and *Sclerocystis,* which contain species forming VA mycorrhiza. The name *Endogone* is restricted, in accordance with taxonomic priorities, to those species forming sporocarps that contain true zygospores (Gerdemann and Trappe, 1974) and does not embrace any species known to form VA mycorrlhiza. Ultimately it is hoped that different species will be established in pure culture and specific isolates compared and their life cycles determined to confirm species separation. At present different VA species are maintained in pot cultures with host plants grown in sterilized soil.

Various species of *Endogone* differ in the extent to which they stimulate plant growth. This specificity is more related to soil type than to the species of host plant, the most effective *Endogone* species in one soil, for example, being surpassed by four others on the same host in another soil (Mosse, 1972).

The present study concerned mainly to isolate fungi responsible for VAM-infection from roots and other organs of different plants belonging to different families and growing them in axenic cultures. Effect of soil inoculation

with the isolated fungi on some growth criteria of maize plants was also investigated.

MATERIALS and METHODS

Isolation of fungi from healthy roots:

The MS-medium (Murashige & Skoog, 1962) modified by El-Fiki et al., (1999a & b) was used in this study. Isolation technique was similar to that used for isolation of the ordinary root pathogens. Healthy root samples were taken from onion (*Allium cepa*, Liliaceae), broad bean (*Vicia faba*, Leguminosae) plants grown under field conditions as well as from aerial roots of 3-years old Swiss cheese (*Monstera deliciose*, Araceae) plants grown in pots. The root samples were washed in tap water, surface sterilized by dipping in 2% sodium hypochlorite solution for approx. 2 min., thoroughly washed with sterilized distilled water, dried between sterilized filter paper, cut into pieces and placed onto plates containing modified agar MS-medium. The plates were incubated at 25 °C for 3-5 days and observed daily. The fungal growth spreading from root pieces were purified using both hyphal tip and mono-sporic culture techniques. The resulting purified fungi were repeatedly subcultured on the above medium.

Infectivity of the isolated fungi:

Pots containing sterilized loamy clay soil (without amendment) were prepared and sown with maize grains, 5 grains per each pot. Sterilized soil was inoculated with modified barely-sand cultures (at levels of 0.1, 0.2, 0.4 and 0.6%, w/w) 7 days before sowing or with suspension of fungal sporangiospores at the rate of 0.5, 1, 2 and 3ml /pot (each containing one kg of soil) 7 days after sowing. Four pots were used for each particular treatment. Preparation of fungal inocula was as follows:

Preparation of modified barely-sand cultures and soil inoculation:

Modified barely-sand medium (referred here as modified BS medium) was prepared in 100 cc conical flasks each containing 75 g barely grains and 25 g of clearly washed sand. Flasks were supplemented with enough amount of the liquid modified MS-medium (El-Fiki et al., 1999a & b) and autoclaved as usual. Flasks

were inoculated with equal discs of 7 day-old culture (grown on modified MS-agar medium) of each fungal isolate and incubated for 2-3 weeks at 25 °C. The resulting BS-culture for each isolate was used for soil inoculation at the aforementioned rates. However, sterilized BS-medium was used at the same levels for soil inoculation in control treatment.

Preparation of suspensions of fungal sporangiospores and soil inoculation:

The isolated fungi were grown for 7 days at 25 °C on plates containing modified MS-agar medium. Each plate was flooded with 10 ml of sterilized distilled water, subjected to ultrasonic waves for 30 min. The resulting spore suspension(s) were collected (for each particular isolate), filtered through 2-layer sterilized cheesecloth. For each isolate, 0.5, 1.0, 2.0 and 3 ml of spore suspension (250.000 spores/ml) were taken, supplemented with tap water to about 50 ml and poured onto potted-soil 7 days after sowing. The former control treatment was also used herein.

Effect of the isolated fungi on root infection and growth of maize plant:

Root samples were taken weekly from each treatment and prepared for investigating VAM-infections using the technique described by Phillips and Hayman (1970). Effect of different inoculation treatments on growth of maize plants expressed as plant height, root length, stem diameter, number of leaves, average fresh and dry weight of roots and shoots per plant was recorded 4 weeks after sowing.

Isolation of VAM-like fungi from plant organs other than roots:

These studies were carried out after detecting mycorrhizal infection-structures in roots of maize seedlings raised from surface sterilized seeds sown in sterilized potted soil as well as in the leaf-sheath of Swiss cheese plant. A lot of surface sterilized seeds of maize (in 2% sodium hypochlorite for approx. 2 min.) were allowed to germinate for one week under aseptic conditions on sterilized wetted filter paper. Small portions of roots (from germinated seeds) were aseptically transferred onto Petri-dishes containing modified MS-agar medium and incubated for 3-5 days at 25 °C. Also, small portions were cut with

sharp knife from leaf petiole of 3-years old Swiss cheese plant, surface sterilized (as in case of maize seeds), thoroughly washed with sterilized distilled water, macerated into small pieces, cultured on modified agar MS-medium and incubated as mentioned before. Mycelial growth produced from these plant materials was isolated, purified as mentioned before and compared with the VAM-fungus previously isolated from roots of these plants.

RESULTS

Isolated fungi:

Four fungi were isolated from roots of onion, broad bean plants (grown under field conditions), Swiss cheese plants (grown in outdoor pots) and maize seedlings (gown in vitro under aseptic conditions). All fungi could grow and sporulate on modified MS-agar medium producing white-grayish branched mycelium. Hyphae in all fungi were aseptate, hyaline, vacuolated and showed oil-like droplets (Fig.1). The highest and lowest thickness of hyphae of different isolates was greatly varied. Thickness of hypae was ranged between 2.5-17.5, 3.2-30.0, 3.3-24.0, 2.5-35.0 µm, for isolates of Swiss cheese (Fig.1), onion (Fig.2 & 3), broad bean (Fig.4), and maize (Fig. 5-b), respectively.

All isolates (except maize-isolate) formed sporangia individually at the terminal ends of aseptate unbranched hyphae. Sporangia of *Mucor*-type measuring 25.0-37.5 µm in diameter were observed in Swiss cheese isolate (Fig. 6). While isolates of onion (Fig.3) and broad bean (not photographed) produced deciduous sporangia measured 30.0-57.5 and 40.0-45.0 µm, respectively. Sporangia in all isolates contained rounded hyaline 1-celled sporangiospores (2.5-3.0 µm in diameter). Formation of sporangia was not detected in maize-isolate. Onion-isolate only (Fig.7) produced oval shaped zygospores measuring 32.5 x 42.5 µm.

All isolates produced apical and intercalary round- and oval-shaped chlamydospores singly and/or in chains. Swiss cheese-isolate (Fig. 1) produced abundant chlamydospores (singly and in chains) mainly with round-shape (5.0-

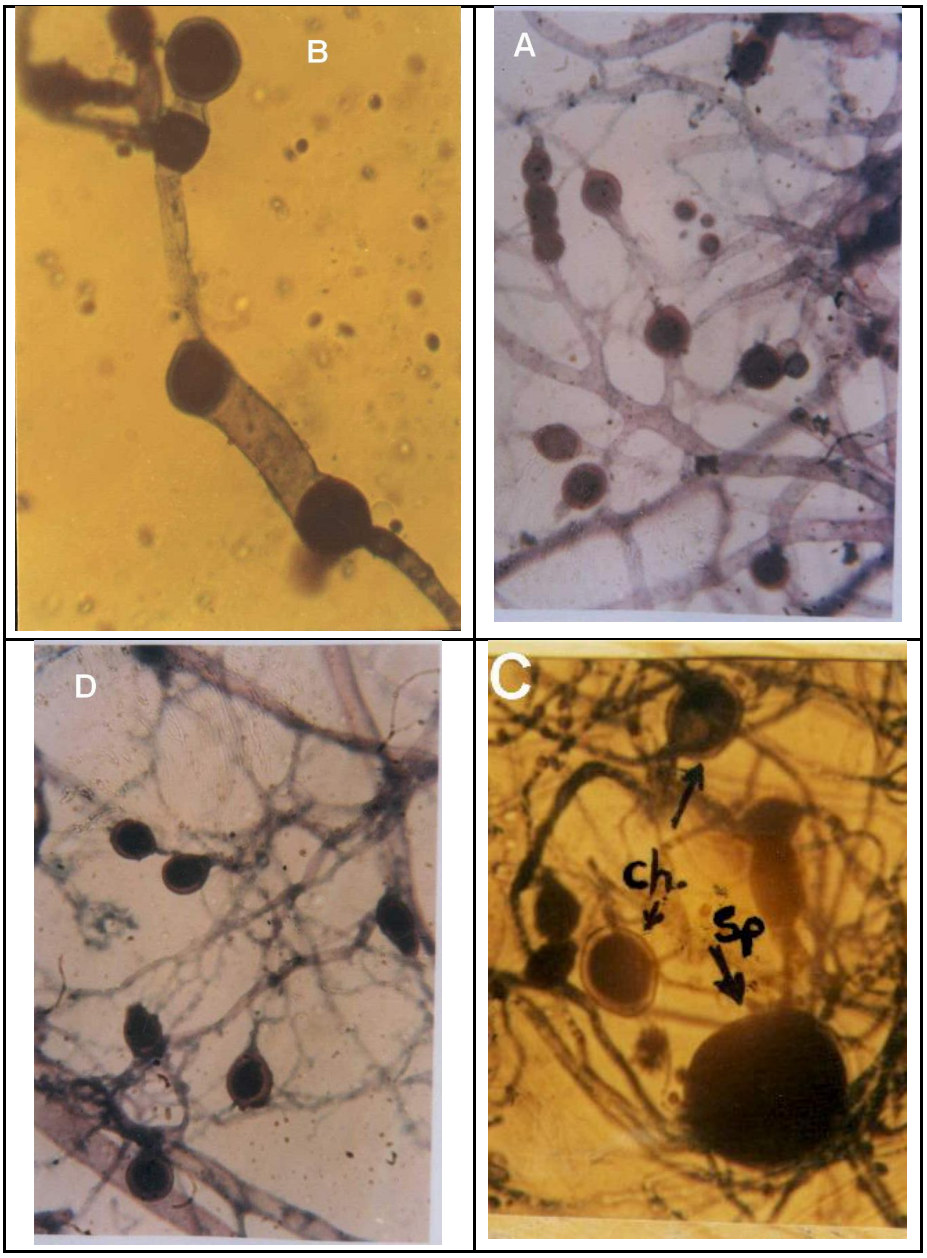

Fig.(1): a = Show Swiss cheese isolate (X=400); b = Show onion isolate (X=630); c = Show onion isolate (X=400) and d = Show broad bean isolate (X=400). Notice vacuolated hyphae with oil droplets (a), large deciduous sporangia "sp" (c), variation in chlamydospore formation and thickness of aseptate hyphae in different isolates. (All Figures were minimized by 60%).

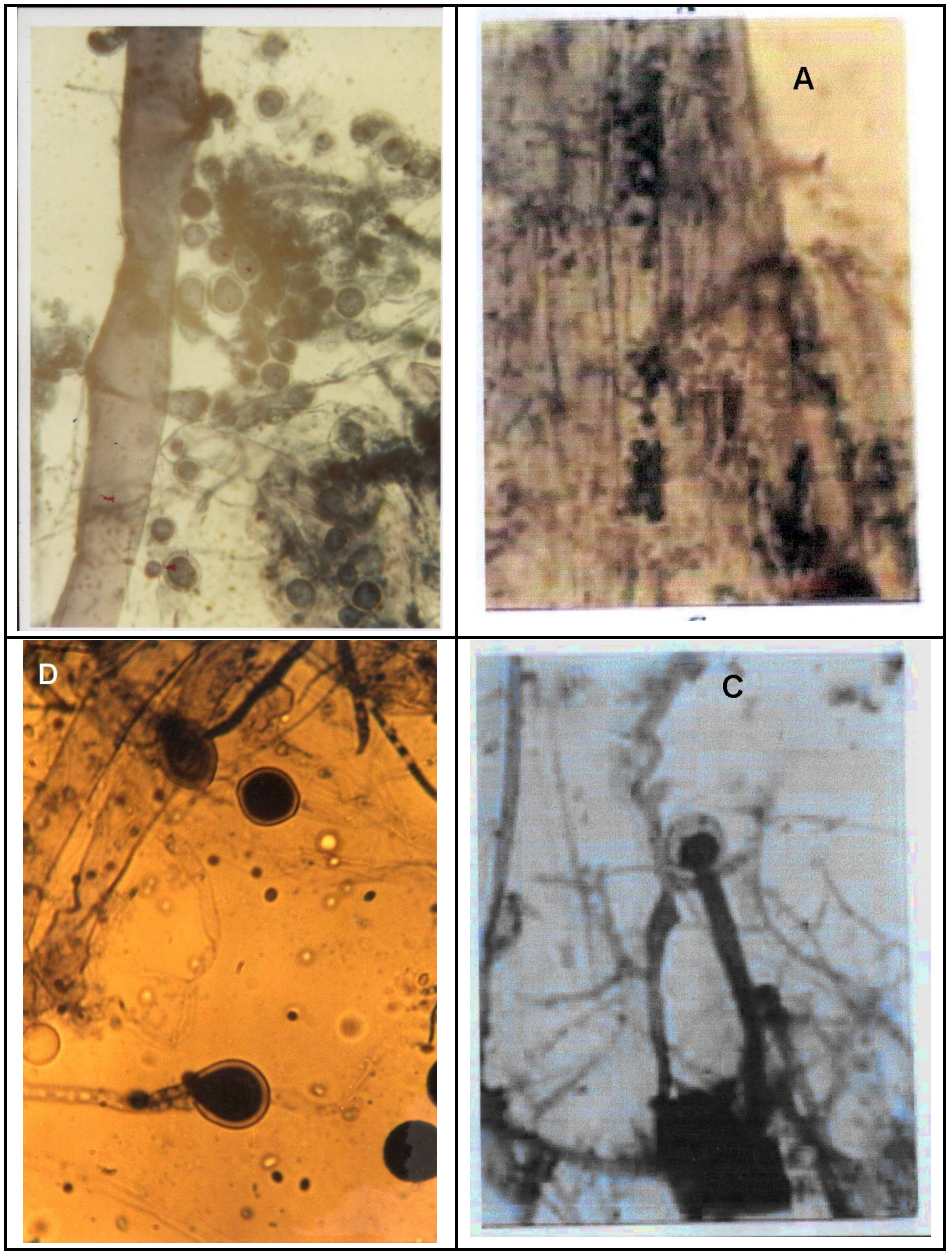

Fig. (2): a = Show mycorhizal infection in roots of aseptically grown maize seedlings (X=200); b = Show maize isolate showed thick hypha and round-shaped chlamydospores (X400); c = Show *Mucor*-type sporangia in Swiss cheese isolate (X=400) and d = Show oval-shaped zygospore (zy) in onion-isolate (X=400). (All Figures were minimized by 60%).

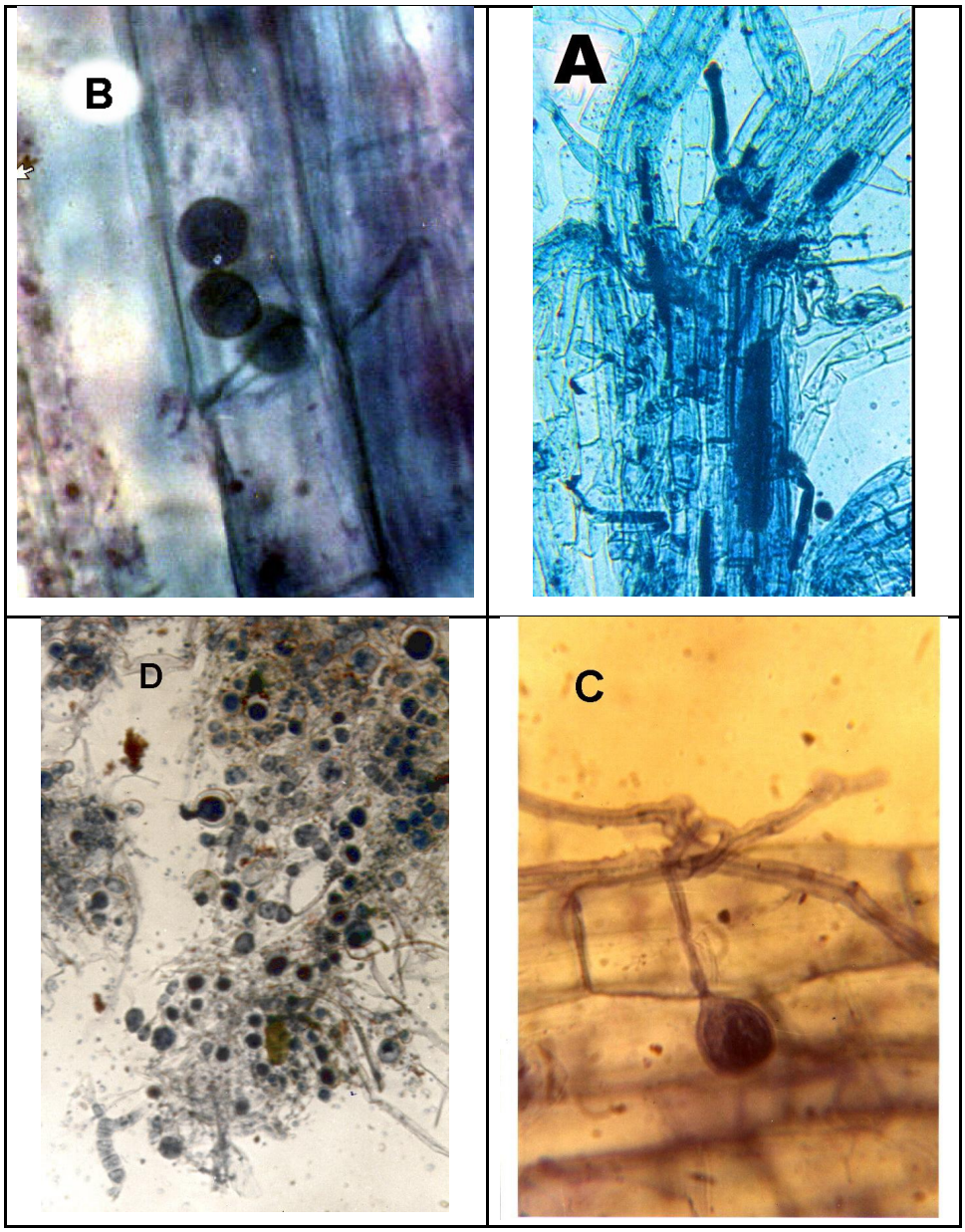

Fig. (3): a = Show arbuscules in roots of maize plants inoculated with onion-isolate (X=150); b = Show vesicles in roots of maize plants inoculated with onion-isolate (X=300): c = Show vesicles and intracellular aseptat hyphae in maize-roots inoculated with onion-isolate showed (X=480) and d = Show excessive growth and sporulation of broad bean-isolate on BS- medium (X=200). (All Figures were minimized by 60%).

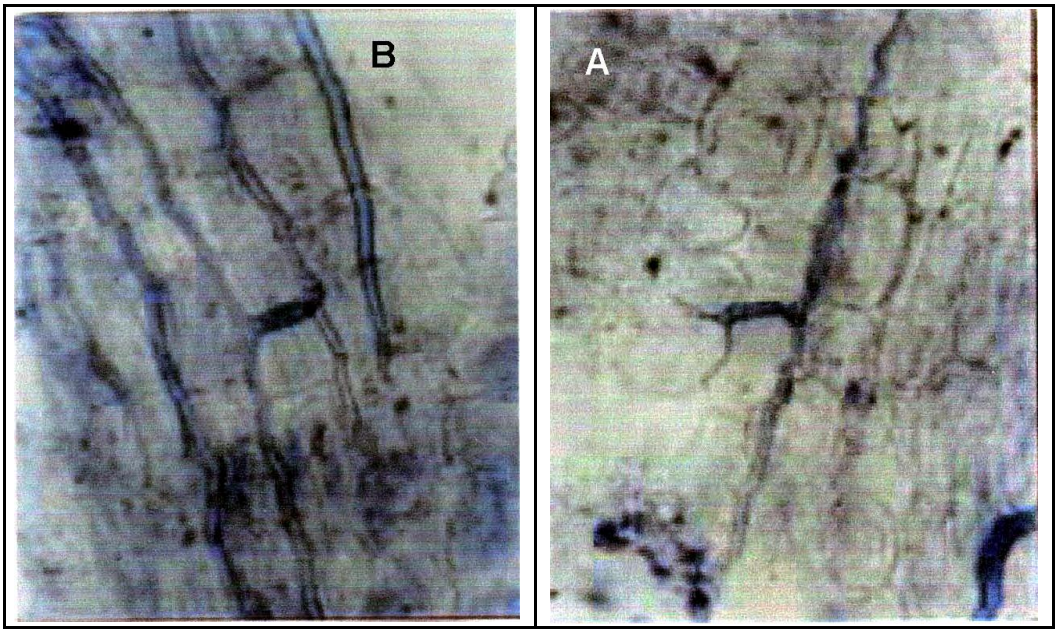

Plate 4: shows single hyphal unit with lateral perpendicular projection between two cells of Swiss cheese root (a) and two hyphal units fused together forming H-connection in Swiss cheese roots (b).

25.0 µm with an average of 15.5 µm in diameter) and few oval shaped chlamydospores (12.5 x 33 µm with an average of 17.4 x 22.2 µm). Onion-isolate (Figs.2 & 3 and 6), produced chlamydospores mainly singly and oval in shape (15.8 x 33.0 µm with an average of 20.3 x 25.2 µm) and few rounded shaped spores (12.5-30.0 µm with an average of 24.2 µm). Broad bean-isolate (Fig. 4) produced both round- shaped (20.0-30.0 µm with an average of 24.2 µm) and oval-shaped (15.8 x 33.0 µm with an average of 20.3 x 25.2 µm) chlamydospores at equal frequency. They were formed mainly singly and rarely in chains. Maize-isolate (Fig. 5-b) produced chlamydospores singly and in chains. The round-shaped chlamydospores (7.5-22.0 µm with an average of 15.5 µm) were produced more frequently than the oval-shaped one (15.0 x 25 µm with an average of 15.0 x 22.5 µm).

Roots of maize seedlings emerged from surface sterilized seeds sown in sterilized soil showed mycorrhizal infection (Fig.5-a). The fungus isolated from roots of these maize seedlings has similar characters of mycelial growth of the above isolates (Fig.5-b). Thickness of hyphae ranged from 2.5-35.0 µm. Both

sporangia and zygospores were not detected in this isolate. However, round-shaped chlamydospores (7.5-22.0 µm with an average of 15.5 µm in diameter) were formed more than the oval shaped (15.0 x 25.0 µm with an average of 15.0 x 22.5 µm in diameter).

Effect of soil inoculation with isolated fungi on growth of maize plants:

A- Effect of fungal cultures grown on modified barely-sand (BS-) medium:

All fungal isolates used in this experiment could grow and sporulated well on the modified barely-sand medium. Excessive growth and sporulation of broad bean-isolate on this medium could be seen in Fig. (11).

Data in Tables (1 & 2) showed that, the growth criteria of maize plants were significantly increased by some soil inoculation treatments. Compared with control, shoot length was increased by 11.5-36.6%, root length increased by 18.9-45.7%, stem diameter increased by 8.8-15.8%, shoot fresh weight increased by 79.5-115.1%, root fresh weight increased by 68.2-102.7%, shoot dry weight increased by 51.1-172.3%, and root dry weight was increased by 42.9-107.1%. Applying BS-culture of broad bean-isolate at level of 0.2% (w/w) produced the highest increases in shoot length (36.6%), root length (45.7%) and stem diameter (23.6%). However, BS- culture of onion-isolate (at the level of 0.1%) and Swiss cheese-isolate (at the level of 0.6%) came the next, respectively. Only broad bean-isolate (at 0.2%) and onion-isolate (at 0.1%) caused significant increase in fresh weights of both shoots and roots while broad bean-isolate (at 0.1%) and onion-isolate (at 0.2%) caused significant increase in fresh weight of shoots only. On the other hand, dry weight of shoot was significantly increased by applying the different inoculum levels of the three tested isolates except broad bean-isolate used at the level of 0.6% (w/w). The highest increases in dry weight of shoot were produced by onion-isolate (at level of 0.1%), followed by broad bean-isolate (at level of 0.2%) and onion-isolate (at level of 0.2%), respectively. Dry weight of roots showed significant increases by applying all inoculum levels of

Table (1): Effect of soil inoculation with different inoculum levels of BS-cultures of three VA-mycorrhizal fungi isolated from roots of swiss cheese, broad bean and onion plants, on some growth measurements of maize plants after 4 weeks from sowing.

Treatments		Shoot length (cm)	Root length (cm)	Stem diameter (cm)	No. of leaves /plant	Fresh weight of		Dry weight of	
VAM Isolate	Inoculum level (g/Kg soil)					Shoot system (g/plant)	Root system (g/plant)	Shoot system (g/plant)	Root system (g/plant)
Control		64.5 e	31.7 f	0.386 e	3.60 abc	2.58 d	1.10 bcd	0.47 f	0.28 ef
Swiss cheese isolate	1 g	71.9 cd	38.0 cde	0.377 e	3.40 bc	3.40 bcd	0.73 d	0.81 cd	0.23 f
	2 g	77.8 bc	38.0 cde	0.390 e	3.60 abc	3.40 bcd	1.03 cd	0.82 cd	0.30 def
	4 g	78.2 bc	38.0 cde	0.396 de	3.88 abc	3.23 bcd	0.90 d	0.82 cd	0.37 cde
	6 g	79.9 b	41.0 bc	0.437 bc	4.00 ab	3.57 bcd	1.15 bcd	0.90 bcd	0.49 ab
Broad bean isolate	1 g	82.0 ab	38.3 cd	0.433 bc	3.60 abc	4.80 ab	1.73 abc	0.71 de	0.46 bc
	2 g	88.1 a	46.2 a	0.477 a	4.30 a	5.55 a	2.23 a	1.09 ab	0.58 a
	4 g	79.6 b	38.3 cd	0.437 bc	4.00 ab	4.32 abcd	1.79 ab	0.71 de	0.47 bc
	6 g	68.1 de	33.0 ef	0.387 e	3.30 bc	2.75 d	1.02 cd	0.52 ef	0.40 bcd
Onion isolate	1 g	83.3 ab	43.3 ab	0.453 ab	4.00 ab	4.79 ab	1.85 a	1.28 a	0.45 be
	2 g	79.2 bc	37.7 cde	0.400 de	3.70 abc	4.63 abc	1.65 abc	0.97 bc	0.44 bc
	4 g	77.1 bc	34.3 def	0.420 cd	3.70 abc	3.32 bcd	1.11 bcd	0.90 bcd	0.40 bcd
	6 g	66.7 de	31.0 f	0.382 e	3.17 c	2.95 cd	1.06 cd	0.75 cd	0.30 def
L.S.D at 0.05		6.657	4.546	0.024	0.673	1.553	0.631	0.203	0.095

In a column, means followed by a common letter(s) are not significantly different at the 0.05 level by DMRT.

Table (2): Calculation of % increase (+) or decrease (-)in different growth criteria of maize seedlings due to treatments exhibited significant differences only compared with control treatment (data derived from Table 1).

Treatments		% significant increase in different growth characters over control							
VAM Isolate	Inoculum level (g/Kg soil)	Shoot length (cm)	Root length (cm)	Stem diameter (cm)	No. of leaves /plant	Fresh weight of		Dry weight of	
						Shoot system (g/plant)	Root system (g/plant)	Shoot system (g/plant)	Root system (g/plant)
	Control	*	*	*	*	*	*	*	*
Swiss cheese isolate	1 g	+ 11.5	+ 19.9	*	*	*	*	+ 72.3	*
	2 g	+ 20.6	+ 19.9	*	*	*	*	+ 74.5	*
	4 g	+ 78.2	+ 19.9	*	*	*	*	+ 74.5	*
	6 g	+ 23.9	+ 29.3	+ 13.2	*	*	*	+ 91.5	+ 75.0
Broad bean isolate	1 g	+ 27.1	+ 20.8	+ 12.2	*	+ 86.0	*	+ 51.1	+ 64.3
	2 g	+ 36.6	+ 45.7	+ 23.6	*	+ 115.1	+ 102.7	+ 131.9	+ 107.1
	4 g	+ 23.4	+ 20.8	+ 13.2	*	*	*	+ 51.1	+ 67.9
	6 g	*	*	*	*	*	*	*	+ 42.9
Onion isolate	1 g	+ 29.2	+ 36.6	+ 17.4	*	+ 85.7	+ 68.2	+ 172.3	+ 60.7
	2 g	+ 22.8	+ 18.9	*	*	+ 79.5	*	+ 106.4	+ 57.1
	4 g	+ 19.5	*	+ 8.8	*	*	*	+ 91.5	+ 42.9
	6 g	*	*	*	*		*	+ 59.6	*

@ = Significant difference (Increase or decrease) % at 5% level =

* = Treatment has no significant difference compared with control.

Table (3): Effect of inoculating soil with sporangiospore suspension (ml/kg soil) of three VA-mycorrhizal fungi, (grown on modified MS-medium), isolated from roots of Swiss cheese, broad bean and onion plants on some growth measurements of maize plants after 4 weeks from sowing.

Treatments		Shoot length (cm)	Root length (cm)	Stem diameter (cm)	No. of leaves/ plant	Fresh weight of		Dry weight of	
VAM Isolate	Spore suspension (ml/Kg soil)					Shoot system (g/plant)	Root system (g/plant)	Shoot system (g/plant)	Root system (g/plant)
Control		64.5 cd	31.7 g	0.386 fgh	3.60 cd	2.58 ef	1.10 d	0.47 de	0.28 c
Swiss cheese isolate	0.5 ml	58.0 e	35.3 efg	0.383 gh	3.53 de	2.90 def	1.45 cd	0.47 e	0.33 abc
	1.0 ml	63.9 cd	37.0 defg	0.413 cde	3.67 cd	3.37 cdef	1.47 cd	0.53 cde	0.42 abc
	2.0 ml	66.1 c	41.0 cde	0.407 cdef	3.70 cde	3.43 cdef	1.60 bcd	0.61 bcde	0.43 abc
	3.0 ml	78.1 ab	44.3 bc	0.447 ab	4.10 a	3.90 bcd	1.97 abcd	0.78 abc	0.43 abc
Broad bean isolate	0.5 ml	59.0 de	33.7 fg	0.371 h	3.13 f	3.30 cdef	2.20 abc	0.57 cde	0.41 abc
	1.0 ml	65.7 c	36.3 defg	0.396 efg	3.27 ef	3.64 bcde	2.57 ab	0.60 bcde	0.46 abc
	2.0 ml	80.1 a	47.7 ab	0.452 a	3.90 abc	5.25 a	2.80 a	0.95 a	0.51 abc
	3.0 ml	72.8 b	37.7 def	0.420 cd	3.43 def	3.90 bcd	1.70 bcd	0.73 abcd	0.32 bc
Onion isolate	0.5 ml	81.0 a	50.0 a	0.447 ab	4.03 ab	4.76 ab	2.14 abc	0.92 a	0.61 a
	1.0 ml	76.5 ab	42.0 cd	0.427 bc	3.75 bcd	4.30 abc	1.97 abcd	0.85 ab	0.59 ab
	2.0 ml	65.1 c	35.7 efg	0.400 defg	3.58 cde	3.27 cdef	1.95 abcd	0.59 bcde	0.42 abc
	3.0 ml	54.4 e	31.7 g	0.370 h	3.25 ef	2.16 f	1.12 d	0.41 e	0.24 c
L.S.D at 0.05		5.393	5.215	0.021	0.318	1.138	0.848	0.231	0.247

In a column, means followed by a common letter(s) are not significantly different at the 0.05 level by DMRT.

Table (4): Calculation of % @ increase (+) or decrease (-)in different growth criteria of maize seedlings due to treatments exhibited significant differences only compared with control treatment (data derived from Table 3).

Treatments		% significant increase in different growth characters over control							
VAM Isolate	Inoculum level ml/Kg soil)	Shoot length (cm)	Root length (cm)	Stem diameter (cm)	No. of leaves /plant	Fresh weight of		Dry weight of	
						Shoot system (g/plant)	Root system (g/plant)	Shoot system (g/plant)	Root system (g/plant)
	Control	*	*	*	*	*	*	*	*
Swiss cheese isolate	0.5 ml	- 10.1	*	*	*	*	*	*	*
	1.0 ml	*	*	+ 7.0	*	*	*	*	*
	2.0 ml	*	+ 29.3	*	*	*	*	*	*
	3.0 ml	+ 21.1	+ 39.7	+ 15.8	+ 13.9	+ 51.2	*	+ 66.0	*
Broad bean isolate	0.5 ml	*	*	*	- 13.1	*	+ 100.0	*	*
	1.0 ml	*	*	*	- 9.2	*	+ 133.6	*	*
	2.0 ml	+ 24.2	+ 50.5	+ 17.1	*	+ 103.5	+ 154.5	+ 102.1	*
	3.0 ml	+ 12.9	+ 18.9	+ 8.8	*	+ 51.2	*	*	*
Onion isolate	0.5 ml	+ 25.6	+ 57.7	+ 15.8	+ 11.9	+ 84.5	+ 94.5	+ 95.7	+ 117.9
	1.0 ml	+ 18.6	+ 32.5	+ 10.6	*	+ 66.7	*	+ 80.9	+ 110.7
	2.0 ml	*	*	*	*	*	*	*	*
	3.0 ml	- 15.7	*	*	- 9.7	*	*	*	*

@ = Significant difference (Increase or decrease) % at 5% level =

* = Treatment has no significant difference compared with control.

broad bean-isolate, onion-isolate at levels of 0.1, 0.2 and 0.4% and Swiss-cheese isolate at level of 0.6% only. The highest increases in root dry weight of roots i.e. 107.1% and 75.0% were produced by broad bean-isolate (at level of 0.2%) and Swiss cheese-isolate (at level of 0.6%), respectively. On the other hand, no significant variations due to tested treatments was detected in number of leaves per plant compared control treatment.

From these results it could concluded that, all growth criteria of maize plants have responded inversely with inoculum levels of Onion-isolate meanwhile, they were increased proportionally by increasing levels of inoculum of Swiss cheese-isolate. In this regard, broad bean-isolate seems to be slightly varied since all growth criteria showed conspicuous increase by increasing inoculum from 0.1% to 0.2% (w/w) then decreased significantly by using higher levels of inoculum (0.4% and 0.6% w/w).

B- Effect of fungal sporangiospores on growth of maize plants:

Data in Tables (3&4) show that all examined criteria of growth of maize seedlings were significantly increased when soil was inoculated with sporangiospore suspension of onion-isolate at level of 0.5-ml/kg soil. This treatment showed the following significant increases compared with control: shoot length (25.6%), root length (57.7%), stem diameter (15.8%), number of leaves per plant (11.9%), fresh weight of shoot (84.5%) and root (94.5%), dry weight of shoot (95.7) and roots (117.9%). However, number of leaves and dry weight of roots only showed no significant variations by applying sporangiospore suspension of this isolate at level of 1.0ml/kg soil when compared with control. Increasing sporangiospore suspension of onion-isolate up to 2.0ml/kg soil had no significant effects on all tested growth criteria but 3.0 ml/kg soil caused significant decreases in shoot length and number of leaves per plant only compared with control. Broad bean and Swiss cheese isolates produced significant increases in most tested growth criteria when their sporangiospore suspension was applied only at levels of 2.0 and 3.0 ml/kg soil, respectively.

The above results concluded that, among all treatments the soil inoculated with sporangiospore suspension of onion-isolate at level of 0.5 ml/kg soil (w/w) was best of all treatment for inducing the highest significant improvement in growth of maize plants.

VAM-infection structures caused by the isolated fungi:

Microscopic examination for squash's of root taken from maize seedlings grown in soils inoculated with different levels of fungal inocula (BS-cultures or suspensions of sporangiospores) showed typical VA-mycorrhizal infection structures. Squash's made in roots of maize seedlings inoculated with onion-isolate showed VA-mycorrhizal infection structures i.e. Arbuscules (Fig.8), vesicles (Fig.9) and vesicles and aseptat hyphae grow mainly between and rarely across cells in root cortex (Fig.10). Roots of inoculated seedlings showed no any detectable changes in their external morphology when compared with those of control treatment. H-shape structures were also observed in roots of Swis cheese plants (Fig. 12 &13).

Discussion

VAM-fungi with typical dichotomously branched, coenocytic mycelium and aseptate vacuolated hypae with oil-like droplets were isolated from roots of field grown onion and broad bean plants, and roots of Swiss cheese plants (in pots). Similar fungi were isolated also from leaf petioles of Swiss cheese plants (in pots) and roots and stems of maize seedlings emerging in sterilized soil from surface sterilized maize seeds. All isolated fungi were repeatedly isolated, subcultured and produced abundant growth and sporulation on modified MS-agar medium. This medium was used with great success for isolation and growing *Uromyces fabae*, the causal of broad bean rust (**El-Fiki et. al., 1998, 1999a & b**).

The mycelium in all isolates was dichotomously branched, coenocytic, coarse and aseptate. Hyphae were vacuolated with oil droplets and varied in thickness (2.5-30.0 μm). The isolated fungi reproduced asexually by hyaline

1-celled sporangiospores (2.5-3.0 µm in diameter) formed in terminal sporangia of different sizes. Swiss cheese isolate formed *Mucor*-type sporangia while deciduous sporangia were formed by onion- and broad-bean isolates. All isolates formed round- and oval-shaped chlamydospores with different size. They were borne apically and/or intercallary, singly and/or in chains. Sexual reproduction through formation of zygospores was detected only in onion-isolate. These fungal characters are similar to those of VAM-fungi, which had been described by several investigators. Nicolson, (1967) and Varma (1994) stated that, the dimorphic nature of the external loose mycelium around the VA mycorrhizae is highly characteristic. The main network is formed from coarse hyphae 20 to 30, µm in diameter which are thick-walled and often "knobbly". From the "knobs" arise fine hyphae 2 to 7, µm in diameter which are thin-walled and ephemeral. Large resting spores are produced on the coarse external hyphae. Zygospore formation in onion-isolate indicated that, this isolate is homothallic while isolates of broad bean and Swiss cheese might be heterothallic. These findings may emphasize the phycomycetous nature of the isolated fungi. Singh (1982) mentioned that, Mucorales include both homo- and heterothallic fungi. Tommerup and Sivasithamparam (1990) reported gametangial fusion in the VAM-fungus *Gigaspora decipies*.

Inoculating sterilized soil with the isolated fungi at relatively smaller inoculum levels (BS-cultures or sporangiospores) resulted in significant increases in growth of maize seedlings emerged in these soils. However, growth improvement was greatly varied and depending upon fungal isolate and kind and level of fungal inoculum. Onion-isolate induced the highest growth when used at the lowest inoculum level of both kinds of inoculum. This was in contrast with Swiss cheese-isolate did that at the highest inoculum level of both kinds of inoculum. The best effect of broad-bean isolate was attained at the moderate levels of both kinds of inoculum. These results could be explained in light of the following three aspects.

The first aspect, is the interaction between these VAM-fungal isolates (refer here as VAM-invaders) and the VAM-maize-inhabitant fungus existed naturally in maize seedlings which has been actually detected and isolated from maize seedlings grown completely under aseptic conditions. In fact, the so-called "plant-inhabitant-VAM fungi" could be detected and isolated from the aboveground parts of Swiss cheese and broad bean plants (un-published data). Both VAM-invader (when exist at certain level) and the VAM-maize-inhabitant-fungus may cooperated together for improving growth of maize seedlings. Increasing or decreasing inoculum levels of the VAM-invader may increase or decrease the promoting effect resulted from the invader-inhabitant cooperation. Reasonably, growth improvement was proportionally decreased with increasing levels of inocula in case of onion-isolate while proportionally increased with increasing levels of inocula in case of Swiss cheese-isolate.

The second aspect based on function of arbuscules. Arbuscules are structurally analogous to haustoria in the mildew and rust fungi but probably release materials to the cell in addition to absorbing nutrients within the cell. The number of arbuscules and kind and amount of the materials released or absorbed by these arbuscules might be varied in different fungal isolates consequently may change plant growth response. Additional studies for explaining variation in plant growth response against these VAM-fungi must be carried out.

The third aspect related to the unknown amounts of available phosphorous in the used soil. Several investigators stated that, the non-mycorhizal plants showed higher dry weight than the mycorrhizal plants in soils rich or containing higher amounts of available phosphorous. The opposite trend was occurred in soils poor or containing lower amounts of available P (Hayman and Mosse, 1971). Herein, the utilization of the unknown amounts of phosphorous available in soil through association between VAM-fungal isolates and maize seedlings may be affected differently by levels of inocula of different isolates. In other

word, amount of P suitable for mycorrhizal activity of an VAM-isolate at certain level of its inoculum, may become unsuitable to same isolate used at different inoculum level(s).

Results about improvement in growth of maize seedlings are in harmony with **Graham et. al., (1976)**, **Kucey and Paule (1983)**, and **Ahmed, et al., (1994a and b**). They stated that, growth of mycorrhizal plants was enhanced especially under field conditions, mainly, because improvement of nutrients uptake and may be due to providing further protection against soil-borne pathogens attacking their roots.

Typical structures of VA-mycorrhizal infection structures i.e. inter- and intracellular aseptate hyphae, arbuscules, vesicles, and chlamydospores were detected even in roots of 2-weeks old maize seedlings. These structures were restricted in the cortex of infected roots but not seen in the stele. Many workers stated that the chief diagnostic feature of vesicular-arbuscular mycorrhiza is the presence of vesicles and arbuscules in the root cortex (**Bonfante-Fasolo, 1984**). The endodermis, stele and root meristems are not invaded. Inter- and intracellular hyphae are also present in the cortex and the infection inside the root is directly linked to an external mycelium which spread and ramifies in the soil (**Nicolson, 1967**). **Smith (1994)** stated that, the same fungi do not develop arbuscules in roots of all species and use of arbuscules as key diagnostic structures may limit our knowledge of both structure and function of the full range of root infections formed by these members of the Glomales. Walker, (1994) mentioned that, although it is perfectly correct to refer to some member of the Glomales (e.g. most *Glomus* and *Acaulospora* spp.) as VAM fungi, because they form both vesicles and arbuscules, the application of such terminology to members of the Gigasporaceae is incorrect. So, indeed, the more general term AM must be used.

H-shaped connection structures were seen in roots of Swiss cheese plants. These H- connections may initiate and develop from sporangiospores liberated from sporangia inside intact tissues. On germination of these spores, the resultant

germ tubes grow into elongated infection units, which fused together through perpendicular connection formed in between. **Abbott and Robson (1979)** stated that the long infection units with parallel hyphae interconnected by perpendicular bridges "H-connections" are most frequently in *Glomus*. Fungi in *Acaulospora* and *Entrophospora* also produce smaller infection units, which often difficult to see because the intraradical hyphae stain only slightly in trypan blue. Accordingly, the Swiss cheese-isolate could be identified as a species of *Glomus*.

The present work may be very important to increase agricultural production if inoculation with these isolated fungi produced such growth improvement in other crop plants. The techniques used herein for isolation, growing and production of inoculum were very easy, inexpensive and avoiding disadvantages of other traditional and non-traditional techniques described by **Elmes and Mosse (1984)**; **Wood (1991)**; **Chabot et al. (1992)**; and **Jarstfer and Sylvia (1992)** and facilitate scientific studies dealing with ecology, biology, physiology, taxonomy and others activities of these fungi. More isolation of these VAM-fungi from different economical crops would help in advanced researches about their life-cycles to confirm species separation.

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نجاح نمو وتجرثم فطريات الميكوريزا الحويصلية الشجيرية فى المعمل بعي العوائل الحية

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الملخص العربى

أمكن خلال هذه الدراسة الحصول على ثلاث عزلات من فطريات الميكوريزا الحويصلية الشجيرية من جذور نباتات البصل والفول (النامية تحت ظروف الحقل) ونباتات القشطة (ال الأصص) وتنميتها بنجاح على بيئة الشعير والرمل المحورة وكذلك على بيئة آجار محورة تح ظروف المعمل. فى تجارب العدوى الصناعية تم حقن التربة المعقمة بلقاح الفطريات النامى بيئة الشعير والرمل المحورة بتركيزات تتراوح بين 0.1-0.6% (وزن/وزن) قبل 7 أيام من الزراعة أو بمعلق من جراثيمها الإسبورانجية المتكونة على البيئة الصناعية (بمعدل 0.5-3 ملليلتر/كجم تربة) بعد 7 أيام من الزراعة.

أظهرت النتائج توافق قدرة عزلات الفطريات الميكوريزية الثلاثة فى تحسين نمو الذرة تبعا لمصدرها وطاقتها اللقاحية المستخدمة. أمكن الحصول على أفضل قياسات لنمو نب الذرة باستخدام التركيز الأقل لكلا الصورتين من لقاح عزلة البصل والتركيز الأعلى لكل الصورتين من لقاح عزلة القشطة. أما فى عزلة الفول فقد أمكن الحصول على أفضل قياسات باستخدام التركيز الثانى من لقاحها النامى على بيئة الشعير (0.2%) أو الثالث من معلق ج الإسبورانجية (2 ملليلتر/كجم تربة). أثبتت الفحص الميكروسكوبى وجود عدوى ميكوريز واضحة فى جذور نباتات الذرة المزروعة فى التربة المعقمة تحت ظروف الصوبة.

تم أيضا خلال هذه الدراسة إكتشاف التراكيب المميزة للعدوى بفطريات الميكوريزا الحويصلية الشجيرية فى جذور بادرات الذرة الناتجة عن حبوب ذرة معقمة سطحيا و مزروعة فى تربة معقمة وأمكن عزل وتنمية تلك الفطريات من تلك الجذور.

هذه الدراسة تعتبر أول تسجيل فى العالم عن نجاح عزل وتنمية فطريات الميكوريزا الحويصلية الشجيرية على بيئة صناعية (بعيدا عن العوائل النباتية) وكذلك إثبات قدرة معمليا على تحسين النمو تحت ظروف التجارب بالصوبة.