PHYSIOLOGICAL STUDIES ON VESICULAR ARBUSCULAR MYCORRHYZAL FUNGI GROWN IN VITRO

BY

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ABSTRACT

Best nutritional, physiological and environmental conditions favored growth and sporiolation of VAM fungi. Bushnell's medium was the superior one for growth and production of sporangiospores and chlamydospores of VAM isolates. The organic N was better than the inorganic N sources while urea and $NaNO_2$ were not suitable for growth and sporulation. Glucose (for mycelial dry weight) and sucrose (for spore production) were the best C-sources whereas, inositol was the inferior one. The optimum temperature for growth and spore production was ranged between 28-31°C. The optimum pH for growth and sporulation was lower in VAM-B (pH 7.0) than other VAM isolates (pH 7.6-8.0). VAM growth and sporulation decreased sharply as relative humidity increased from 14 to 100%. The mycelial dry weight and sporulation of the VAM isolates was affected differently under diffused, yellow, red, green, blue lights and darkness conditions.

Key words: Vesicular arbuscular fungi, VAM fungi, axcenic cultures, nutritive media, nitrogen sources, carbon sources, temperature, relative humidity and light waves.

INTRODUCTION

The vesicular arbuscular mycorrhizas (VAM) are symbiotic associations formed between land plants and fungi of the order Glomales (Zygomycota) **(Harrier, 2001)**. The VAM fungi cannot be propagated in pure culture, since they are obligate symbionts and need to colonize roots to fulfill their life cycle **(Gadkar, *et al.*, 1997; Harrier, 2001; Diop, 2003)**. The inability of VAM fungi to grow in pure culture has spurred researchers to develop alternative forms of cultivation independent of a plant host **(Schreiner and Koide, 1993; Diop, *et al.*, 1994; Gadkar, *et al.*, 1997 and Jasper, *et al.*, 1998)**.

Asif and Khan (1996) placed surface-disinfected sweet potato roots infected with *Glomus intraradices* on a minimal medium with or without a non-infected Ri-T DNA transformed carrot root. Extensive mycelium and spores developed on both plates. When subcultured on minimal medium, without any root segment, axenic growth occurred on the plates incubated for 6 weeks in dark at 25°C. Successful vesicular infection developed in the roots of Sudan grass when inoculated with infected hairy roots or a small agar block with axenic fungal growth only as sources of inoculum. No arbuscules were observed. **Gryndler and Hrselova (1996)** studied the effects of glucose, root exudates, vitamins, mannitol, trehalose, glycin, FeNaEDTA, inositol, macrobiogenic and trace elements and selected plant growth regulators on proliferation of intraradical hyphae of VAM fungi and quantification of their growth. No significant effects of root exudates and glucose on the growth of hyphae were observed. The optimum concentrations of vitamins were determined. Complete inhibition of proliferation by riboflavin (1 mg/L) and IAA (5µM/L) was observed. They concluded that these results

might enable to design an incubation medium containing compounds ordinarily used in plant cultivation media at concentrations non-suppressive to the VAM fungi. **Karandashov, *et al.* (1998)** used Ri T-DNA transformed carrot roots to established monoaxenic culture of the AM fungus *Glomus caledonium*. The external fungal hyphae explored the whole volume of the nutrient medium and produced up to 146 spores per plate after five to seven weeks of culture. **Pawlowska, *et al.* (1999)** established monoexenic cultures of *Glomus etunicatum* in association with excised Ri T-DNA transformed carrot roots. Modifed White's medium buffered with 10 mm MES (pH 6) or MOPSO (pH 6 ± 0.5) was most optimal for the host root growth as well as for *G. etunicatum* spore germination and mycorrhiza formation. Spores appeared in dual cultures within two weeks of root inoculation. Sporulation was asynchronous and continued until root senescence.

El-FiKi, *et al.* (2001) used a modified nutritive MS-medium for isolation of different VAM isolates directly from sound healthy undamaged roots of their host plants. Four VAM fungal isolates were isolated from roots of onion (VAM-O), broad bean (VAM-B), Swiss cheese (VAM-S) and maize (VAM-M) plants. All these isolates could grew and sporulated well in axcenic pure cultures. These VAM isolates were significantly effective for improving growth of maize plants. The present work aimed to study different physiological and environmental factors that might affect production of inocula (mycelia and spores) of the VAM isolates under the *in vitro* conditions.

MATERIALS AND METHODS

Source of VAM fungal isolates:

Four VAM fungal isolates namely onion "O" isolate, broad bean "B" isolate, Swiss cheese "S" isolate and maize "M" isolate that were isolated by **El-Fiki *et al.* (2001)** from healthy root samples of a given host plant were used in this study. The obtained VAM cultures were repeatedly subcultured onto plates containing agar MS-medium **(Murashige & Skoog, 1962)** modified by **El-Fiki *et al.* (1998)** and incubated at 25 °C.

1-Effect of different media:

Different media namely Czapek's, potato dextrose, carrot dextrose, soil extract, rice extract, Bushnell's **(Bushnell and Rajendren, 1980)**, Haskin's **(Haskin, 1950)** and MS-7 **(El-Fiki, *et al.*, 2001)** were used. The solid (agar) media were used for studying rate of growth and spores production while, the liquid media were used for studying amount of growth of different VAM isolates. The warmed autoclaved agar media were poured into sterilized Petri dishes, then dishes were inoculated, each with a disc 5-mm diameter of the 7-days old mycelial growth (on MS-7 medium) of a VAM isolate and incubated at 28^{o}C. Four replicates were used for each particular treatment. Rate of growth was measured and recorded when one of the dishes of each isolate was filled with mycelial growth. Plates with VAM cultures, 7-days- old post inoculation were used for determination of spore production. A disc (10-mm diameter) was taken 1 cm apart from the inoculate place, crushed in 1ml of distilled water in a watch glass, 0.1 ml of uniform spore suspension was placed on a clean glass slide and covered with a cover slide. The numbers of sporangio-spores and chlamydio-spores per 30 microscopic field (X300) was counted for each treatment then average was calculated and recorded.

A set of four 100 cc conical flasks, each containing 20 ml of a known autoclaved liquid medium were inoculated and incubated as mentioned before. Three weeks after

incubation, the mycelial masses were collected by filtering through filter paper and washed several times with distilled water. The dry mycelia were recorded after drying for 24 hours in air-dry oven at 70°C.

The Bushnell's medium (solid and liquid) was used as basal medium for studying the *in vitro* growth and sporulation of the tested VAM isolates as affected by the following factors.

2 -Effect of temperature:

Growth and sporulation of the tested VAM isolates were estimated under different temperatures degrees (5, 10, 15, 17, 20, 23, 25, 27, 30 and 33°C) as mentioned before.

3-Effect of relative humidity:

Eight different degrees of relative humidity (R.H.%), i.e. 0, 14, 50, 74, 80, 85, 90, 95 and 100% were used. Aliquots of the specific solution for a known R.H.% **(Solomen, 1951)** were poured into plate lid of the inverted Petri dishes and four dishes were used as replicates for each particular treatment. All treatments were incubated at 30°C and observed daily. Fungal growth and spore production were measured us mentioned above.

4-Effect of different nitrogen and carbon sources:

The fungal growth and spores production of the tested VAM isolates as affected by different nitrogen and carbon sources (see results) were estimated as mentioned before. In Bushnell's medium, the N source (2g $NaNO_2$ + 1g peptone + 5g casein) or carbon source (sucrose) was replaced by a known tested N or C-source at amount equivalent to the total amount of N and C in the Bushnell's medium.

6-Effect of pH values:

Bushnell's medium was prepared in acetate or phosphate buffer at different pH values and autoclaved in the usual way. The pH of the media was checked after sterilization. For each particular pH value, a set of 4 plates and flasks containing solid and liquid media, respectively were inculcated with the tested VAM isolates and incubated at 30°C. The growth and sporulation were determined as mentioned before. Acetate and phosphate buffer stock solutions were prepared and used for obtaining different values according to **Ju–Luric (1978)**.

7-Effect of light waves on fungal growth and sporulation:

Plates and flasks with basal agar and liquid media were inoculated with known VAM isolate, enveloped separately with colored thin transparent cellophane paper i.e. hyaline, red, blue, yellow and green colors. Another group of plates and flasks were enveloped with black paper to provide dark conditions. All plates and flasks were incubated at 30°C. White florescent lamp 60 cm long continuously lighted was hanged 30 cm high above the inoculated plates and flasks. The VAM growth and spore production were determined as before mentioned.

All data obtained were statistically analyzed according to the least significant difference (L.S.D.) method described by **Snedecor and Cochran** (**1989**).

RESULTS

1. Effect of different media:

Data in **Table (1)** reveal that, the tested nutritive agar media were significantly varied concerning their effect on growth (linear and dry weight) and formation of sporangiospores (S-spores) and chlamydospores (C-spores) of VAM isolates tested.

Regardless VAM isolates, the Bushnell's medium recorded the highest values of these criteria while rice-extract and Czapek's media were the inferiors in this respect. The criteria determined for growth and spore production of VAM fungal isolates tested was significantly affected by the interaction between media and VAM isolates. **Figure (1)** showed growth of VAM-3 isolate on both agar and liquid media, respectively.

Table (1): Effect of different liquid media on the *in vitro* growth and sporulation of different VAM isolates isolated from roots onion (O), broad bean (B), Swiss cheese (S) and maize (M) plants.

		VAM fungi isolated from roots of				
	Media	Onion	Broad bean	Swiss cheese	Maize	Mean
Linear growth (mm)	MS-7 *	70.0	70.0	54.5	60.9	63.9
	PDA	70.0	67.1	70.0	66.0	68.3
	Czapek's	65.7	54.3	9.3	8.0	34.3
	CDA	70.0	70.0	70.0	56.0	66.5
	Bushnell's	70.0	70.0	70.0	70.0	70.0
	Haskin's	54.6	66.4	70.0	70.0	65.2
	Soil-extract	30.7	31.2	66.3	60.0	47.1
	Rice-extract	48.6	57.3	11.0	7.0	31.0
Weight of growth (mg)	MS-7 *	197.5	165.0	202.5	257.5	205.6
	PDA	430.0	370.0	280.0	232.5	328.1
	Czapek's	190.0	102.5	67.5	22.5	95.6
	CDA	292.5	347.5	407.5	435.0	370.6
	Bushnell's	430.0	392.5	510.0	455.0	446.9
	Haskin's	242.5	197.5	325.0	247.5	253.1
	Soil-extract	195.0	310.0	92.5	120.0	179.4
	Rice-extract	167.5	130.0	45.0	165.0	126.9
Number of sporangiospores per microscopic field (X300)	MS-7 *	21.1	9.3	47.9	29.2	26.9
	PDA	28.4	58.1	80.7	30.8	49.5
	Czapek's	11.4	2.4	7.2	2.7	6.0
	CDA	14.0	14.0	41.3	26.2	23.9
	Bushnell's	180.9	78.3	195.4	75.7	132.5
	Haskin's	14.2	16.0	67.2	36.4	33.5
	Soil-extract	44.9	11.2	52.2	13.6	30.4
	Rice-extract	7.2	3.0	8.5	22.3	10.3
Number of chlamydospores per microscopic field (X300)	MS-7 *	9.3	1.5	7.6	4.9	5.8
	PDA	4.5	4.8	9.0	5.0	5.8
	Czapek's	2.4	1.2	1.7	1.2	1.6
	CDA	2.5	2.0	3.7	3.1	2.8
	Bushnell's	10.7	7.3	10.4	6.5	8.7
	Haskin's	3.1	5.4	9.8	5.8	6.0
	Soil-extract	2.9	1.4	4.9	4.5	3.4
	Rice-extract	1.4	1.3	1.7	4.7	2.3

LSD at 5% for:	Media	Isolates	Interaction
Linear growth	0.03	0.01	0.11
Weight of growth	5.58	2.79	22.33
Sporangiospores	0.68	0.34	2.72
Chlamydospores	0.10	0.05	0.42

* MS7 is the MS-medium modified by El-Fiki et al., 1999

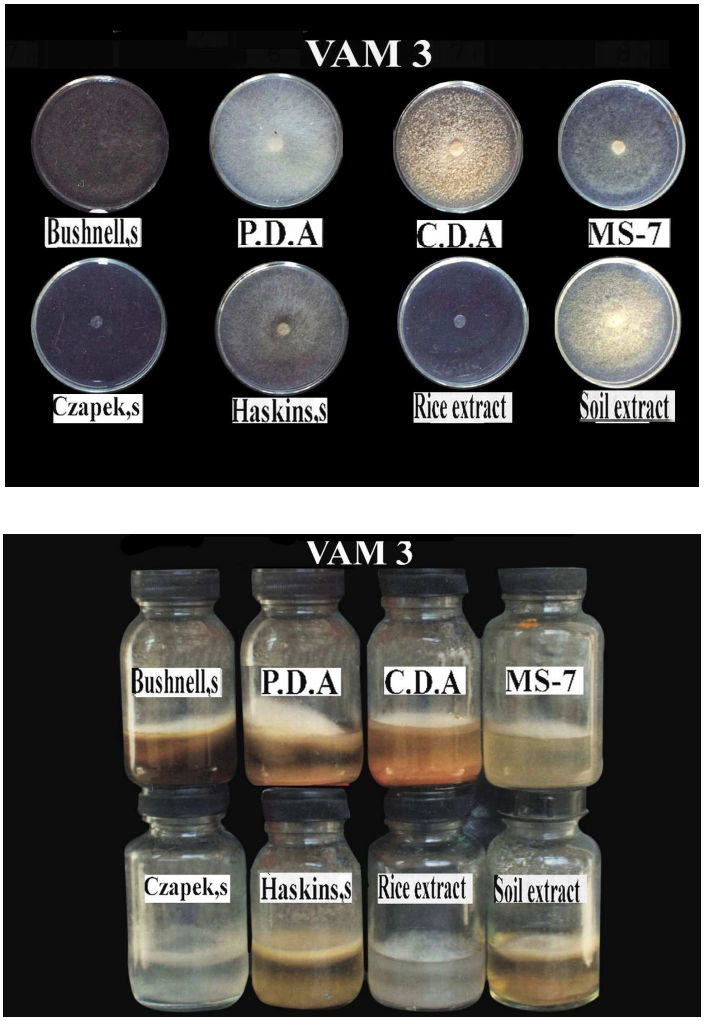

Fig. 1: Growth of VAM isolates 3 (from Swiss cheese) on different tested media on linear growth (above) and amount of growth (below).

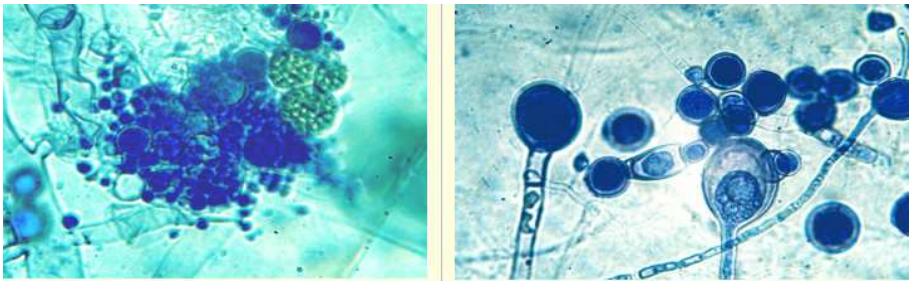

Fig. (2): In vitro produced sporangia, sporangiospores and chlamydospores of VAM fungi.

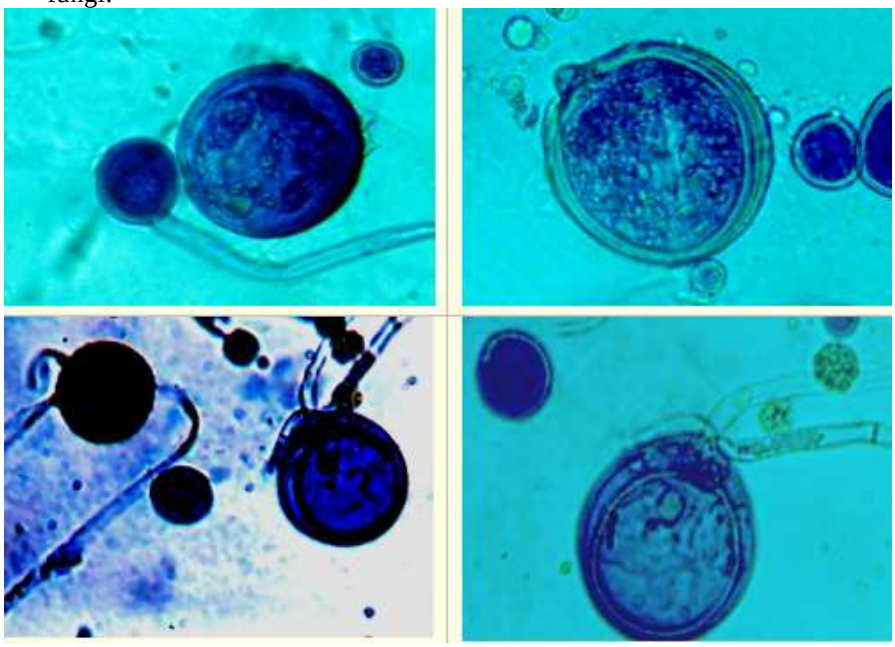

Fig. (3): Spores produced by VAM fungi *in vitro*. Note the structure of spore wall (Double and triple layered spore wall).

2. Effect of different nitrogen sources:

Data in **Table (2)** illustrate that the organic N sources (yeast-extract, beef-extract and aspragine) were the best for improving dry weight of mycelial growth and spore production (S-spores and C-spores) comparing with the control (mixture of organic and inorganic N sources). Among inorganic N-sources tested, NH_4NO_3 was the best followed by KNO_3, $NaNO_3$ and $(NH_4)_2SO_4$ particularly for spores production. All tested VAM isolates, however, could not grown or sporulate on media containing urea or $NaNO_2$ as sole sources of nitrogen. Figure 2 shows effect of different N-sources on linear growth of VAM isolates 2 & 3.

3. Effect of different carbon sources:

Data in **Table (3)** illustrated that the carbon sources tested (except inositol) were significantly equal for improving linear growth of VAM isolates. The carbon sources tested were significantly varied concerning their effect on dry weight and spore production of different VAM isolates. Glucose and sucrose were the superior C-sources for improving mycelial dry weight and spore production, respectively whereas, inositol was the inferior one. The VAM-O only could not grow or sporulate on media containing inositol as sole source of carbon.

4. Effect of different temperatures:

Data in **Table (4)** prove that the VAM growth (linear and dry weight) and their spore production (S-spores and C-spores) were significantly improved by increasing temperature from 5°C to 28-31°C then appreciably decreased by elevating it up to 33°C. VAM-B and VAM produced the highest values for linear growth and dry weight of mycelial growth, respectively. While, VAM-S and VAM-O produced the highest numbers of S-spores and C-spores, respectively. In general, the optimum temperature was obviously lower in VAM-B (28°C) than other isolates (31°C).

5. Effect of different pH values:

Linear growth and dry weight and spore production (S-spores and C-spores) of tested VAM isolates were significantly affected by the pH values of the media. All parameters were gradually and significantly increased as pH value increased from 5.0 to 7.0 (for growth parameters) and to 7.6 (for sporulation parameters) then gradually decreased by elevating pH value up to 9.0. Under stress of pH variety, VAM-S produced the highest values of dry weight and number of S-spores whereas VAM-O was best of all for C-spores production. VAM-M (**Fig. 2**), however, produced the lowest numbers of both spore types. The optimum pH value for highest growth and sporulation was lower for VAM B-isolate (pH 7.0) than other VAM isolates (pH 7.6-8.0). (**Table, 5)**.

6. Effect of relative humidity:

Data in **Table (6)** prove that, growth (linear and dry weight) and spore production (S-spores and C-spores) of tested VAM isolates were negatively affected under controlled relative humidity (14-100% R.H.) in comparison with the control (uncontrolled R.H.). See linear growth of VAM-M "VAM-4"**.** All determined criteria were decreased sharply and significantly as relative humidity increased gradually from 14 to 100%. The VAM-O produces the highest rate of growth and number of S-spores while, VAM-M and VAM-S produced the highest dry weight of mycelia and number of C-spores, respectively. Isolate M, however, produces the lowest numbers of both kinds of spores. Figure 4 shows linear growth of VAM-4 at different percentages of R.H.

7. Effect of different light waves:

Data in **Table (7)** illustrated that the linear growth for all tested VAM isolates was not affected significantly by different light waves tested (diffused, yellow, red, green, blue lights and darkness) whereas the dry weight and sporulation of different VAM isolates were significantly affected. The diffused light was best of all particularly for VAM-O followed by the green light. While, the green light followed by diffused light (VAM-B), diffused light followed by yellow and red light (VAM-S) and diffused light followed by blue and green lights (VAM-M) were the best and significantly equal in the respective VAM isolate. The darkness conditions were the inferior in this respect.

Table (2): Effect of nitrogen sources on growth the *in vitro* growth and sporulation of different VAM isolates isolated from roots onion (O), broad bean (B), Swiss cheese (S) and maize (M) plants..

	N source	VAM fungi isolated from roots of				Mean
		Onion	Broad bean	Swiss cheese	Maize	
Linear growth (mm)	Control *	70.0	70.0	70.0	70.0	70.0
	$NaNO_3$	70.0	70.0	70.0	70.0	70.0
	KNO_3	70.0	70.0	70.0	70.0	70.0
	$NH_4N\ O_3$	70.0	70.0	70.0	70.0	70.0
	$(NH_4)_2SO_4$	48.4	48.4	48.4	48.4	48.4
	Urea	5.0	5.0	5.0	5.0	5.0
	Aspragin	70.0	70.0	70.0	70.0	70.0
	Yeast extract	70.0	70.0	70.0	70.0	70.0
	Beef extract	70.0	70.0	70.0	70.0	70.0
	$NaNO_2$	5.0	5.0	5.0	5.0	5.0
Weight of growth (mg)	Control *	430.0	442.5	510.0	462.5	461.25
	$NaNO_3$	130.0	90.0	227.5	62.5	127.50
	KNO_3	275.0	42.5	440.0	115.0	218.13
	$NH_4N\ O_3$	540.0	420.0	475.0	462.5	474.38
	$(NH_4)_2SO_4$	322.5	287.5	352.5	37.5	250.00
	Urea	40.0	45.0	62.5	25.0	43.13
	Aspragine	525.0	467.5	502.5	447.5	485.63
	Yeast extract	600.0	660.0	640.0	485.0	596.25
	Beef extract	592.5	547.5	632.5	475.0	561.88
	$NaNO_2$	32.5	27.5	35.0	15.0	27.50
Number of sporangiospores per microscopic field (X300)	Control *	171.9	190.1	202.2	74.0	159.56
	$NaNO_3$	132.1	133.8	155.4	16.0	109.36
	KNO_3	150.1	142.4	165.8	33.0	122.83
	$NH_4N\ O_3$	157.1	157.9	170.0	71.8	139.18
	$(NH_4)_2SO_4$	45.9	23.3	28.4	31.6	32.28
	Urea	0.0	0.0	0.0	0.0	0.00
	Aspragin	190.1	153.1	172.0	70.4	146.41
	Yeast extract	194.2	192.4	203.6	83.8	168.51
	Beef extract	150.6	180.3	207.7	74.1	153.14
	$NaNO_2$	0.0	0.0	0.0	0.0	0.00
Number of chlamydospores per microscopic field (X300)	Control *	18.8	8.0	7.7	6.4	10.23
	$NaNO_3$	6.8	4.8	9.1	1.4	5.54
	KNO_3	6.9	5.5	11.8	2.9	6.78
	$NH_4N\ O_3$	13.2	7.0	13.6	6.2	10.00
	$(NH_4)_2SO_4$	3.6	1.2	4.2	2.7	2.94
	Urea	0.0	0.0	0.0	0.0	0.00
	Aspragin	15.9	9.0	12.6	6.1	10.90
	Yeast extract	21.1	20.0	15.9	7.3	16.06
	Beef extract	14.8	14.1	19.7	6.4	13.77
	$NaNO_2$	0.0	0.0	0.0	0.0	0.00

LSD at 5% for:	N-source	Isolates	Interaction
Linear growth	0.003	0.001	0.012
Weight of growth	9.064	3.625	36.254
Sporangiospores	2.539	1.016	10.158
Chlamydospores	0.155	0.062	0.618

* (Sodium nitrate + Peptone + Casein hydrolysate)

Table (3): Effect of carbon sources on the *in vitro* growth and sporulation of different VAM isolates isolated from roots onion (O), broad bean (B), Swiss cheese (S) and maize (M) plants.

		VAM fungi isolated from roots of				
	C-source	Onion	Broad bean	Swiss cheese	Maize	Mean
Linear growth (mm)	Sucrose	70.0	70.0	70.0	70.0	70.0
	Glucose	70.0	70.0	70.0	70.0	70.0
	Galactose	70.0	70.0	70.0	70.0	70.0
	Fructose	70.0	70.0	70.0	70.0	70.0
	Maltose	70.0	70.0	70.0	70.0	70.0
	Lactose	70.0	70.0	70.0	70.0	70.0
	Starch	70.0	70.0	70.0	70.0	70.0
	Arabinose	70.0	70.0	70.0	70.0	70.0
	Inositol	0.0	52.5	70.0	70.0	48.1
Weight of growth (mg)	Sucrose	403.8	376.3	356.3	390.0	381.6
	Glucose	450.0	412.5	412.5	446.3	430.3
	Galactose	451.3	391.3	376.3	440.0	414.7
	Fructose	415.0	430.0	405.0	407.5	414.4
	Maltose	412.5	422.5	386.3	388.8	402.5
	Lactose	362.5	370.0	356.3	383.8	368.1
	Starch	410.0	400.0	400.0	443.8	413.4
	Arabinose	372.5	407.5	390.0	400.0	392.5
	Inositol	0.0	347.5	336.3	376.3	265.0
Number of sporangiospores per microscopic field (X300)	Sucrose	260.6	177.6	256.6	128.3	205.8
	Glucose	258.7	168.0	233.7	127.5	197.0
	Galactose	241.3	184.2	252.2	117.0	198.6
	Fructose	241.3	184.2	252.2	117.0	198.6
	Maltose	236.5	180.3	241.6	110.6	192.2
	Lactose	209.2	159.0	225.6	110.3	176.0
	Starch	237.9	172.1	251.2	127.8	197.2
	Arabinose	216.3	174.5	244.9	114.6	187.6
	Inositol	0.0	149.4	210.9	107.7	117.0
Number of chlamydospores per microscopic field (X300)	Sucrose	16.2	9.0	10.2	7.9	10.8
	Glucose	16.1	8.5	9.2	7.8	10.4
	Galactose	15.0	9.3	10.0	7.2	10.4
	Fructose	15.0	9.3	10.0	7.2	10.4
	Maltose	14.7	9.1	9.6	6.8	10.0
	Lactose	13.0	8.0	9.0	6.7	9.2
	Starch	14.8	8.7	10.0	7.8	10.3
	Arabinose	13.4	8.8	9.7	7.0	9.7
	Inositol	0.0	7.5	8.4	6.6	5.6

LSD at 5% for:	C-source	Isolates	Interaction
Linear growth	0.97	0.39	3.87
Weight of growth	4.49	1.80	17.96
Sporangiospores	1.49	0.60	5.97
Chlamydospores	0.11	0.04	0.45

Table (4): Effect of temperature on the *in vitro* growth and sporulation of different VAM isolates isolated from roots onion (O), broad bean (B), Swiss cheese (S) and maize (M) plants..

		VAM fungi isolated from roots of				
	Temperature °C	Onion	Broad bean	Swiss cheese	Maize	Mean
Linear growth (mm)	5	12.1	10.7	6.9	14.2	10.96
	10	26.7	19.5	14.8	23.4	21.10
	15	39.9	40.1	37.3	27.1	36.08
	17	47.8	45.7	38.0	49.1	45.14
	20	60.5	60.0	47.5	57.3	56.31
	23	61.2	62.4	57.5	63.6	61.16
	26	62.1	62.6	58.1	63.7	61.62
	28	62.1	64.6	63.5	63.9	63.51
	31	59.0	64.2	63.7	63.9	62.69
	33	56.3	63.0	61.4	57.3	59.49
Weight of growth (mg)	5	67.5	67.5	80.0	82.5	74.38
	10	90.0	110.0	120.0	127.5	111.88
	15	105.0	115.0	157.5	142.5	130.00
	17	127.5	165.0	115.0	162.5	142.50
	20	347.5	377.5	370.0	402.5	374.38
	23	397.5	412.5	370.0	490.0	417.50
	26	422.5	417.5	437.5	455.0	433.13
	28	427.5	467.5	452.5	470.0	454.38
	31	465.0	357.5	537.5	522.5	470.63
	33	412.5	317.5	502.5	502.5	433.75
Number of sporangiospores per microscopic field (X300)	5	35.9	20.4	39.0	14.9	27.56
	10	66.2	37.6	72.8	28.5	51.27
	15	93.9	50.5	105.1	41.0	72.62
	17	99.5	56.8	94.5	39.1	72.48
	20	153.2	81.8	157.5	62.9	113.86
	23	165.5	86.4	157.5	71.2	120.15
	26	171.7	87.3	171.9	68.1	124.75
	28	174.8	93.5	178.0	69.1	128.85
	31	181.7	79.1	196.2	76.5	133.35
	33	170.2	74.4	189.6	73.7	126.98
Number of chlamydospore per microscopic field (X300)	5	2.3	1.9	2.1	1.4	1.92
	10	4.2	3.5	3.8	2.7	3.56
	15	6.0	4.7	5.6	3.9	5.02
	17	6.3	5.3	5.0	3.7	5.08
	20	9.7	7.6	8.3	6.0	7.92
	23	10.5	8.0	8.3	6.8	8.41
	26	10.9	8.1	9.1	6.5	8.65
	28	11.1	8.7	9.4	6.6	8.94
	31	11.6	7.4	11.2	7.3	9.36
	33	10.8	6.9	10.0	7.0	8.70

LSD at 5% for:	Temperature	Isolates	Interaction
Linear growth	0.044	0.018	0.178
Weight of growth	3.109	1.243	12.435
Sporangiospores	1.239	0.496	4.957
Chlamydospores	0.099	0.040	0.396

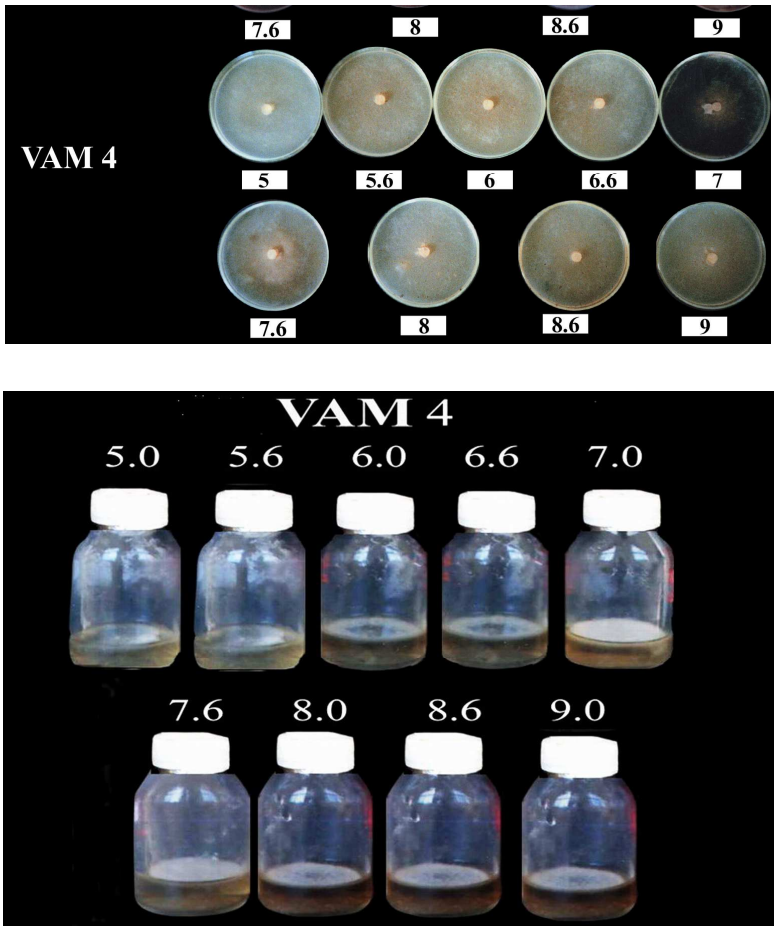

Fig. **4**: Effect of different pH value in solid growth medium on linear growth (above) and amount of growth (below) of VAM-4.

Table (5): Effect of pH values on the *in vitro* growth and sporulation of different VAM isolates isolated from roots onion (O), broad bean (B), Swiss cheese (S) and maize (M) plants.

		VAM fungi isolated from roots of				
	pH values	Onion	Broad bean	Swiss cheese	Maize	Mean
Linear growth (mm)	5.0	51.75	51.75	51.75	53.25	52.13
	5.6	52.50	52.50	52.50	54.50	53.00
	6.0	53.25	53.75	53.25	54.75	53.75
	6.6	54.25	56.25	54.25	56.25	55.25
	7.0	70.00	70.00	70.00	69.00	69.75
	7.6	70.00	66.00	70.00	70.00	69.00
	8.0	70.00	65.25	70.00	70.00	68.81
	8.6	70.00	65.25	70.00	68.00	68.31
	9.0	70.00	62.50	70.00	67.00	67.38
Weight of growth (mg)	5.0	280.0	217.5	274.5	232.5	236.88
	5.6	307.5	225.0	295.0	245.0	250.63
	6.0	315.0	237.5	317.5	247.5	259.38
	6.6	320.0	262.5	318.8	262.5	276.88
	7.0	390.0	410.0	393.0	390.0	400.00
	7.6	422.5	360.0	431.3	427.5	392.50
	8.0	417.5	352.5	428.0	412.5	383.75
	8.6	390.0	352.5	400.5	380.0	368.75
	9.0	312.5	325.0	319.0	370.0	333.13
Number of sporangiospores per microscopic field (X300)	5.0	107.10	88.13	109.69	57.20	90.53
	5.6	111.95	91.40	113.30	58.76	93.85
	6.0	115.51	94.30	116.49	60.10	96.60
	6.6	120.42	99.30	120.18	61.91	100.45
	7.0	170.36	131.15	172.07	79.32	138.22
	7.6	175.99	120.43	180.74	84.45	140.40
	8.0	173.35	118.99	178.05	82.43	138.20
	8.6	170.27	118.65	174.85	77.80	135.39
	9.0	163.74	112.90	167.75	76.45	130.21
Number of chlamydospores per microscopic field (X300)	5.0	7.78	5.73	7.72	4.69	5.72
	5.6	8.13	5.94	7.86	4.82	5.92
	6.0	8.38	6.12	8.43	4.93	6.09
	6.6	8.73	6.45	8.57	5.07	6.33
	7.0	12.36	8.52	12.00	6.50	8.47
	7.6	12.78	7.81	12.72	6.92	8.61
	8.0	12.59	7.72	12.56	6.76	8.46
	8.6	12.36	7.71	12.36	6.38	8.21
	9.0	11.89	7.33	12.07	6.27	7.94

LSD at 5% for:	pH	Isolates	Interaction
Linear growth	0.108	0.048	0.434
Weight of growth	0.659	1.483	5.932
Sporangiospores	0.829	0.368	3.315
Chlamydospores	0.065	0.029	0.260

Table (6): Effect of relative humidity on the *in vitro* growth and sporulation of different VAM isolates isolated from roots onion (O), broad bean (B), Swiss cheese (S) and maize (M) plants..

		VAM fungi isolated from roots of				Mean
	R.H %	Onion	Broad bean	Swiss cheese	Maize	
Linear growth (mm)	Control *	70.0	70.0	70.0	69.3	69.83
	14%	70.0	70.0	67.0	65.0	68.00
	50%	70.0	70.0	65.0	62.7	66.93
	70%	69.5	64.2	60.0	60.0	63.41
	74%	66.9	62.1	60.0	60.0	62.24
	80%	64.0	61.4	59.8	58.0	60.78
	85%	62.0	59.2	54.9	56.0	58.01
	90%	61.3	59.0	50.3	55.3	56.46
	95%	61.2	57.8	49.0	54.3	55.57
	100%	57.7	57.2	47.0	50.0	52.95
Number of sporangiospores per microscopic field (X300)	Control *	705.3	479.3	516.7	407.3	527.17
	14%	606.6	412.2	444.3	350.3	453.36
	50%	352.7	239.7	258.3	203.7	263.58
	70%	211.6	143.8	155.0	122.2	158.15
	74%	183.4	124.6	134.3	105.9	137.06
	80%	141.1	95.9	103.3	81.5	105.43
	85%	105.8	71.9	77.5	61.1	79.08
	90%	70.5	47.9	51.7	40.7	52.72
	95%	35.3	24.0	25.8	20.4	26.36
	100%	8.7	6.4	6.8	5.7	6.90
Number of chlamydospores per microscopic field (X300)	Control *	16.3	7.3	6.5	6.7	9.19
	14%	13.9	6.3	5.6	5.7	7.88
	50%	8.1	3.7	3.3	3.3	4.58
	70%	4.9	2.2	2.0	2.0	2.75
	74%	4.2	1.9	1.7	1.7	2.38
	80%	3.2	1.5	1.3	1.3	1.83
	85%	2.4	1.1	1.0	1.1	1.40
	90%	1.7	1.0	1.0	1.0	1.18
	95%	1.0	1.0	1.0	1.0	1.00
	100%	1.0	1.0	1.0	1.0	1.00

LSD at 5% for:	R.H.	Isolates	Interaction
Linear growth	0.063	0.025	0.254
Sporangiospores	1.298	0.519	5.192
Chlamydospores	0.069	0.027	0.275

Table (7): Effect of light wavelength on the *in vitro* growth and sporulation of different VAM isolates isolated from roots onion (O), broad bean (B), Swiss cheese (S) and maize (M) plants..

	Light wave	VAM fungi isolated from roots of				Mean
		Onion	Broad bean	Swiss cheese	Maize	
Linear growth (mm)	Diffused	70	70	70	70	70.0
	Yellow	70	70	70	70	70.0
	Red	70	70	70	70	70.0
	Green	70	70	70	70	70.0
	Blue	70	70	70	70	70.0
	Black	70	70	70	70	70.0
	Mean	70.0	70.0	70.0	70.0	
Weight of growth (mg)	Diffused	350.0	320.0	322.5	337.5	332.5
	Yellow	306.3	225.0	312.5	276.3	280.0
	Red	273.8	210.0	307.5	266.3	264.4
	Green	311.3	341.3	276.3	325.0	313.4
	Blue	260.0	278.8	257.5	330.0	281.6
	Black	243.8	240.0	247.5	311.3	260.6
	Mean	290.8	269.2	287.3	307.7	
Number of sporangiospores	Diffused	331.80	255.30	301.90	157.52	261.63
	Yellow	283.16	177.61	294.08	126.65	220.37
	Red	266.86	165.67	279.12	122.85	208.62
	Green	298.36	269.97	238.72	148.28	238.83
	Blue	240.63	222.43	220.85	152.11	209.00
	Black	231.41	191.59	255.34	143.58	205.48
	Mean	275.37	213.76	265.00	141.83	
Number of chlamydospores	Diffused	16.51	10.35	10.14	7.40	11.10
	Yellow	14.01	7.23	9.83	5.96	9.26
	Red	13.33	6.73	9.33	5.77	8.79
	Green	14.78	10.91	8.55	6.98	10.30
	Blue	11.48	8.93	7.63	7.14	8.79
	Black	11.98	7.70	7.43	6.75	8.46
	Mean	13.68	8.64	8.82	6.67	

LSD at 5% for:	Light	Isolates	Interaction
Linear growth	NS.	NS.	NS.
Weight of growth	6.369	4.246	25.477
Sporangiospores	3.876	2.584	15.504
Chlamydospores	0.231	0.154	0.924

DISCUSSION

The in vitro growth VAM fungi might require variety of nitrogen sources. In the present work, Bushnell's medium (contained peptone, casein and sodium nitrate) was the superior while, Czapek's medium (contained sodium nitrate only) was the inferior one for growth and spore formation of the isolated VAM fungi. In point, the

yeast extract, beef extract and aspragine were the superior for growth and sporulation of the isolated VAM fungi while, no growth or sporulation was detected on media containing urea or $NaNO_2$. The latter N-sources might be toxic to the VAM fungi or they caused considerable decrease in pH of the growth media to such extent not favored growth and sporulation of these fungi. The tested VAM isolates could utilized different carbon sources. Glucose was the best for producing the highest growth dry weight followed by galactose while, sucrose was the best for spore formation followed by glucose. All VAM isolates failed to grow or sporulate on media containing inositol as sole carbon source. The present results reveal that all VAM isolates tested elevate the pH values in the rizosphere region and potted soils in which maize plants were grown. The present results indicated also that, the higher pH values (pH 7.0-9.0) seemed more effective for the *in vitro* growth and sporulation of the isolated VAM fungi than the lower ones (pH 5.0-6.6).

The above-mentioned results are in harmony with several researchers. **Johnson *et al.* (1991)** reported that the diversity of the VAM fungal community was positively correlated with soil C and N. The VAM fungal density increased with increasing soil pH and H_2O soluble soil C. **Medeiros (1992)** recorded that the VAM species showed differential responses to pH, but VAM colonization generally increased as pH increased (4.0, 5.0, 6.0 or 7.0.). **Clark, *et al.* (1998)** reported that some VAM isolates were highly effective in overcoming acidic soil constraints, and tolerance of host plant to acidic soil may depend on root-VAM symbiosis. **Seema *et al.* (1998)** suggested that isolation and multiplication of alkaline-tolerant efficient strains of VAM fungi might play a role in reclamation of the barren-low vegetation soils. **Aarle, *et al.* (2002)** exposed the extraradical mycelium of *Scutellospora calospora* or *Glomus intraradices*, in association with *Plantago lanceolata*, to two different pH treatments (pH around 5 or 6), while the root substrate pH was left unchanged. Both fungi formed more extraradical mycelium at the higher pH. *Glomus intraradices* formed almost no detectable extraradical mycelium at lower pH. **Jolicoeur, et al. (1996)** observed a characteristic pH profile pattern was along hyphae of the VAM fungi. The pH profile of *G. margarita* germ tubes was higher when cultured in the presence of *Daucus carota* hairy root (non-mycorrhized). For extraradical hyphae of *G. intraradices*, the presence of root exudates also raised pH of hyphal cytosol.

VAM fungal growth and sporulation was significantly increased as temperature increased from 5°C to 28 or 31°C, depended on VAM isolate, then appreciably decreased by raising temperature up to 33°C. These results are in harmony with **Wang and Hamel (1998)** who exposed cultures of *Glomus intraradices* growing on transformed carrot roots to temperatures of 0, 5, 10, 15 and 23°C. Sporulation was largely stimulated at 23 °C as compared to all other temperatures. **Rillig, *et al.*, (2002)** reported also that the VAM soil hyphal length was increased by over 40% in the warmed plots, accompanied by a strong trend for VAM root colonization increase. They added that, the ecosystem warming might have stimulated carbon allocation to VAM fungi. Also, VAM fungal growth and sporulation was significantly decreased as constant R.H.% increased from 14% to 100% comparing with the control (normal in vitro humidity conditions). VAM fungi appeared to be highly suited to conditions of low humidity. Spore formation of isolated VAM fungi was very rare

and nearly stopped at the highest constant R.H. (100.0%). These results are in agreement with **El-Fiki, *et al.* (1999)** as they recorded that growing *Uromyces fabae* isolate A in axenic cultures under controlled relative humidity conditions (14-100%) resulted in significantly lower values of both linear growth and uredospores production compared with their corresponding obtained under uncontrolled relative humidity conditions. They concluded that fluctuation in the relative humidity might be necessary for growth and sporulation of *U. fabae* in axenic cultures.

The growth dry weight and spore formation of isolated VAM fungi but not their linear growth was significantly affected by different light waves. In this respect, the hyaline light was the best followed by the green, blue, yellow, red lights and dark, respectively. In general, the hyaline light was the best for VAM isolates O, M and S whereas green light was the best for B-isolate.

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دراسات فسيولوجية على فطريات الميكورهيزا الحويصلية الشجيرية النامية تحت ظروف المعمل

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كانت بيئة بوشنيل Bushnell أفضل البيئات المغذّية لإنتاج أعلى معدل نمو خطي ووزن جاف للميسيليوم وكذلك إنتاج الجراثيم الإسبورانجية والكلاميدية للفطريات المكونة لميكورهيزا الحويصلات الشجيرية. كما كانت مصادر النتروجين العضوية (مستخلص الخميرة، مستخلص اللحم والأسبراجين) أفضل من مصادر النيتروجين الغير عضوى (نترات الأمونيوم، نترات البوتاسيوم، نترات الصوديوم وكبريتات الأمونيوم) لتحسين النمو وإنتاج الجراثيم مقارنة بمعاملة الكنترول (بيئة بوشنيل المحتوية على خليط من النيتروجين العضوي والغير عضوي). هذا ولم تتمكن جميع عزلات ميكورهيزا الحويصلات الشجيرية المختبرة من النمو أو التجرثم على البيئة المحتوية على اليوريا أو نتريت الصوديوم كمصدر وحيد للنيتروجين. كما أظهرت النتائج أن الجلوكوز والسكروز كانا أفضل مصادر الكربون المختبرة للحصول على أعلى معدلات للنمو الجاف والجراثيم على التوالي بينما كان مركب إينوزيتول inositol الأدنى تأثيرا في هذا المجال والذى أدى إلى التوقف التام لنمو وتجرثم عزلة البصل "O--isolate".

كان نمو وتجرثم جميع عزلات ميكورهيزا الحويصلات الشجيرية المختبرة أفضل عند درجات الحرارة الأعلى منه عند الدرجات الأدنى وعموما تراوحت درجة الحرارة المثلى بين 28–31م طبقا للعزلة المختبرة. كما أدى زيادة رقم حموضة البيئة من 5 إلى 7.0 – 7.6 إلى زيادة كبيرة فى قياسات النمو (الطولى والجاف) وقياسات التجرثم على التوالى ثم نقصت تلك القياسات جميعها بزيادة رقم الحموضة حتى 9.0. وعموما كان رقم الحموضة الأمثل لنمو عزلة البصل (pH7.0) أقل منه فى باقى العزلات (pH 7.6-8.0). أيضا تناقص نمو وتجرثم جميع العزلات بشدة بزيادة الرطوبة النسبية المنضبطة من 14 إلى 100 % مقارنة بالكنترول (رطوبة نسبية غير منضبطة).

لم يتأثر النمو الخطى لجميع عزلات ميكرهيزا الحويصلات الشجيرية بتعريض مزارعها أثناء فترة التحضين لموجاتِ إضاءة مختلفة (ضوء منتشر ، ضوء أصفر ، ضوء أزرق ، ضوء أخضر ، ضوء أحمر ، إظلام تام) بينما أثر ذلك معنويا على الوزن الجاف للميسيليوم ومعدلات التجرثم لتلك العزلات. وعموما أعطى الضوء المنتشر يليه الضوء الأخضر أفضل النتائج بالنسبة لعزلة البصل بينما أعطت موجات الضوء الأخضر والضوء المنتشر أفضل النتائج بدون فروق معنوية بينها بالنسبة لعزلة الفول ، وكذلك بين موجات الضوء المنتشر والضوء الأصفر والأحمر بالنسبة لعزلة القشطة وكذلك الضوء المنتشر والضوء الأزرق والأخضر بالنسبة لعزلة الذرة.