Effect of of VA-Mycorrhizal Fungi Inocula Produced In Vitro on Some Maize Fungal Diseases And Growth Parameters Under Greenhouse Conditions

BY

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ABSTRACT

Percentages of survived maize seedlings, shoot and root length, stem diameter, number of leaves/plant, fresh and dry weight/plant and total chlorophyll were significantly decreased by the pathogenic fungi tested (*C. maydis*, *R. solani* and *F. monilliforme*) compared to their respective control treatment. All these parameters were significantly increased in presence of tested 4 VAM isolates particularly VAM-O and VAM-S compared to their respective controls. The interaction between a known pathogen and a particular VAM isolate had no significant effect on survival of maize plants and stem diameter, but all interactions improved both parameters when compared to any pathogen alone. However, all other determined parameters were significantly improved by the VAM/pathogen interaction compared to the pathogen alone. The VAM/pathogen interaction involved VAM-B or VAM-S showed the best responses for most if not all determined parameters.

The soil pH was increased by the pathogenic fungi (8.02-8.45) as well as by the VAM isolates (8.54-8.74) when each organism was used alone in comparison with the general control (7.86). Such trend was slightly varied when both pathogen and VAM fungi are present together. For example, soil pH value was increased by *R.. solani*/VAM combinations (8.22-8.52) compared to *R. solani* alone (8.02), but it was clearly decreased when compared to VAM isolates each alone (8.54-8.74).

Keywords: VAM fungi, greenhouse, maize, soil pH.

INTRODUCTION

According to the available literatures and until current date, the obligate microorganisms associated with plant roots, called vesicular arbuscular mycorrhizal (VAM) fungi, could never be grown *in vitro* away from roots of their host plants **(Buckner *et al.*, 1996)**. **Brundrett (2004)** reported that the Glomeromycete fungi are not capable of saprobic existence, probably because their soil hyphae cannot absorb sugars.

Rajapakse and Miller (1987*)* found that inoculation by VAM fungi increased plant height and the N and P content of the shoots but decreased root length in comparison with uninoculated controls. **Hetrick *et al.* (1988)** found that mycorrhizal inoculation significantly improved growth of big bluestem plants in pasteurized soil, and increased root length and the number and the diameter of the primary, secondary and tertiary roots. Growth in non-sterile soil was suppressed, and mycorrhizal responses were not detected since all of the plants in non-sterile soil became mycorrhizal whether or not they were inoculated. **Sainz *et al.* (1998)** studied the interaction between *Glomus deserticola* and *Phytophtoraa cactorum* on the growth of 2 apple rootstocks obtained from *in vitro* culture. Inoculation of both apple rootstocks with *Glomus deserticola* resulted in increased stem length, number of stem nods, shoot dry weight and shoot P concentration whether the plant was inoculated or not inoculated with *Phytophthora cactorum*. **Pawlowska *et al.* (1999)** recorded that the progress in understanding the biology of arbuscular mycorrhizal fungi is hampered by the limited number of species that can be successfully propagated and studied *in vitro*. They established monoxenic cultures of *Glomus etunicatum* in association with excised Ri T-DNA transformed carrot roots.

The present study was carried out to evaluate efficacy of different VAM fungal isolates (previously isolated and propagated under the *in vitro* conditions) for improving survivals of maize plants (in pathogen-infested soils) as well as some of their growth characters. Effect of these VAM isolates on soil pH was also investigated.

MATERIALS AND METHODS

Three pathogenic fungi i.e. *Cephalosporium maydis*, *Fusarium monilliforme* and *Rhizoctonia solani* (obtained from Maize diseases, Plant. Path. Res., Inst. Agric. Res. Center, Giza and Fungi and Plant Path. Branch, Agric. Bot. Dept., Fac. Agric., Moshtohor, Benha Univ.) and 4 isolates of VAM fungi (VAM-O, VAM-B, VAM-S and VAM-M) were used in the present work. Surfaced sterilized plastic pots (ϕ 20 cm) were filled with soil previously autoclaved for 2 hours at 121ºC. Inoculum for each pathogen [grown at 26°C for 2 weeks on autoclaved sand-barely medium] was added to the potted soil at rate of 3.0% by weight, mixed thoroughly with the soil then watered and left for one week to insure even distribution of the inoculum. Pathogen-free sterilized sand-barely was mixed at the same rate with the potted soil in control treatment. Seven days later, the potted soil was inoculated with the VAM-O, VAM-B, VAM-S and VAM-M each alone [grown on barely modified "BM" medium] at rate of 1, 2, 6 and 2g/kg potted soil, respectively (**El-Fiki *et al.*, 2001**). Inocula of the isolated VAM fungi were prepared under the *in vitro* conditions as described by **El-Fiki *et al.* (2001)**. **Plate 1** shows growth and sporulation of the 4 VAM isolates tested. VAM-free pots were served as control. Pots were sown with surfaced sterilized maize seeds (Local cv.) at the rate of 10 seeds/pot 7 days after soil inoculation with VAM fungi. Five pots were used for each particular treatment. Pots were irrigated as needed and percentages of survived seedlings were recorded 7 weeks after sowing.

At the end of the experiment, the maize plants were gently removed from pots, cleaned and the following growth parameters were measured (shoot and root lengths, stem diameter, number of leaves/plant and fresh and dry weights/plant) and leaf pigments (total chlorophyll). Maize root samples were taken from each treatment, prepared and examined for the presence of VAM-infections (structures) using the technique described by **Phillips and Hayman (1970)**.

Leaf pigments were extracted from fresh leaves (fourth upper leaf) by grinding in a mortar with 90% aqueous ethanol alcohol. The pigments were filtrated through funnel No.G4, then the filtrate was made up to a known volume with 90% aqueous ethanol alcohol. The optical density of filtrate was determined using Carl-Ziess spectrocolourimeter at the wavelengths of 665 and 650 nm for Chlorophyll a and b, respectively. Concentrations of leaf pigments (mg/g dry weight of leaves) were calculated by means of Mackinney's formula **(Mackinney, 1941)** as follows:

Chlorophyll (a) mg/g =((9.784*E665)-(0.99*E650))*(v/w*0.1)

Chlorophyll (b) mg/g =((21.426*E650)+(4.65*E665))* (v/w*0.1)

Total chlorophyll mg/g = Chlorophyll (a) + Chlorophyll (b)

Where E = optical density at the given wavelength.

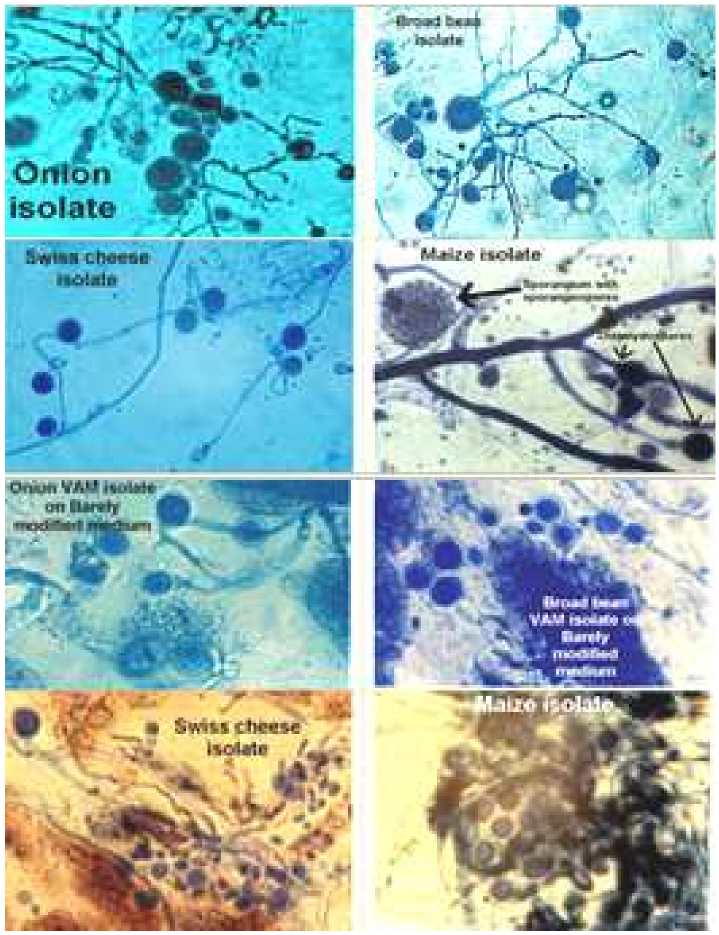

Plate 1. VAM fungi isolated from roots of onion, broad bean, Swiss cheese and maize plants grown on MS modified medium (above) and barely modified medium (below) from left to right, respectively.

After termination of experiment, the pH values were determined in the potted soil in all treatments. All data obtained, when necessary, were statistically analyzed according to the least significant difference (L.S.D.) method described by **Snedecor and Cochran** (**1982**).

RESULTS

1. Effect of VAM/pathogen interaction on survival of maize plants:

Percentage of survived maize plants affected by the VAM/pathogenic fungi interactions was investigated. Data in **Table (1)** clearly indicate that regardless VAM treatments, the pathogenic fungi *C. maydis* and *R. solani* caused the lowest percentage survived plants (77.6%) followed by *F. monilliforme* (78.0%) compared to the control (92.4%). The tested VAM isolates significantly improved % survivals compared to the control treatment. VAM-O, VAM-S and VAM-M isolates recorded the highest increase (84.0-83.5%) without significant differences in between followed by VAM-B (81.0%) compared to 75.0 in control treatment. The VAM/pathogen interaction had no significant effect on survived maize plants. However, VAM-M/*C. maydis* recorded higher survival (84.0%) compared to *C. maydis* alone (68.0%). Similarly, VAM-O and VAM-S combined with *R. solani* increased survivals (84.0 & 84.0%) compared to *R. solani* alone (68.0%). Also, the combination between *F. monilliforme* and VAM-O and VAM-M increased percentage survival compared to that of the pathogen alone.

Table (1): Effect of VAM/pathogenic fungi interactions on survival (%) of maize plants.

VAM treatments	Pathogen(s) treatments				
	Control (no pathogen)	*C. maydis*	*F. monilliforme.*	*R. solani*	Mean
Control (no VAM)	90.0	68.0	74.0	68.0	75.0
VAM-O	94.0	76.0	82.0	84.0	84.0
VAM-B	92.0	80.0	76.0	76.0	81.0
VAM-S	92.0	80.0	78.0	84.0	83.5
VAM-M	94.0	84.0	80.0	76.0	83.5
Mean	92.4	77.6	78.0	77.6	

L.S.D. at 5% for:	VAM treatments (V)	Pathogens (P)	V x P
	1.155	0.924	NS

3. Effect of VAM/pathogen interaction on some growth parameters of maize plants:

3.1. Shoot length:

Data in **Table (2)** indicate that the shoot length (cm) was affected negatively and significantly by all the tested pathogenic fungi compared to the control. In this respect, *R. solani* caused the highest decrease (72.34) followed by *F. monilliforme* (75.46) and *C. maydis* (76.09) compared to control (77.48). On the opposite side, shoot length was significantly increased by inoculation with VAM isolates in comparison with the control (**Fig. 1**). The highest increase was produced by VAM-S (85.8) and VAM-B (85.7) followed by VAM-O (83.7) and VAM-M (82.3), respectively compared to the control (73.9). Also, shoot length was affected positively and significantly by the VAM/pathogen interactions. In the absence of the pathogens, VAM-O recorded the highest increase in the shoot length (87.2) followed by VAM-S (86.3), VAM-B (86.1) and VAM-M (85.8), respectively compared to the control (76.8). While, applying VAM/pathogen combination increased shoot length to (80.3-86.5), (82.7-88.4) and (79.3-84.1) compared to each pathogen alone i.e. *C. maydis*

(74.5) *F. monilliforme* (73.9) and *R. solani* (70.2), respectively. Among all VAM/pathogen interactions, VAM-B/ *C. maydis*, VAM-B/ *F. monilliforme* and VAM-S/ *R. solani* were the best of all for improving shoot length of maize plants.

Table (2): Effect of VAM/pathogenic fungi interactions on shoot length (cm) of maize plants.

VAM treatments	Pathogen(s) treatments				Mean
	Control (no pathogen)	*C. maydis*	*F. monilliforme.*	*R. solani*	
Control (no VAM)	76.8	74.5	73.9	70.2	73.9
VAM-O	87.2	85.5	82.7	79.3	83.7
VAM-B	86.1	86.5	88.4	81.8	85.7
VAM-S	86.3	86.4	86.4	84.1	85.8
VAM-M	85.8	80.3	82.9	80.2	82.3
Mean	77.48	76.09	75.46	72.34	

L.S.D. at 5% for: VAM treatments (V) 0.222 Pathogens (P) 0.089 V x P 0.886

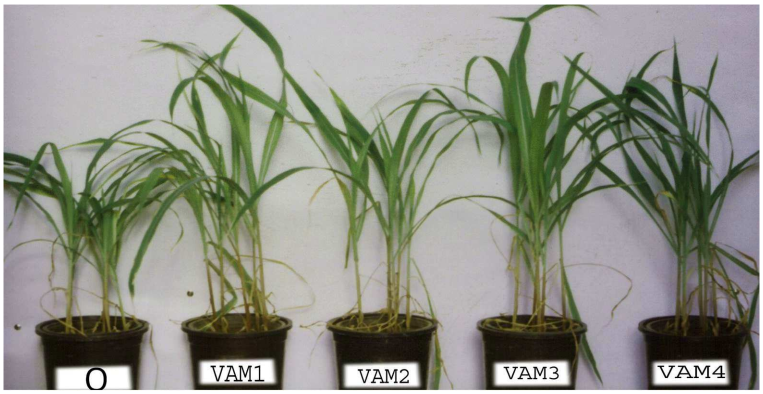

Fig.1: Shoot length of maize plants as affected by inoculation with VAM1, VAM2, VAM3 and VAM4 isolated from roots of onion, broad bean, Swiss cheese and maize plants, respectively compared to uninoculated control (O).

3.2. Root length:

Data in **Table (3)** show that all the tested pathogenic fungi decreased the root length (cm) significantly compared to the control. *R. solani* caused the highest decrease (29.98) followed by *C. maydis* (32.93) and *F. monilliforme* (33.02) compared to the control (34.64). On the contrary, it was significantly increased by all VAM isolates tested compared to the control. VAM-O, VAM-B and VAM-S induced the highest significant increase (34.05-34.10) without significant differences in between followed by VAM-M (32.38) compared to the control (28.6). The interaction between VAM isolates and pathogenic fungi significantly affected the root length. In the pathogen free soil (control) VAM-O was the best (39.0) followed by VAM-S (36.3), VAM-B (34.3) and VAM-M (33.3) compared to the control (30.3). The VAM-B/pathogen interactions, however, were the best of all for increasing root length in comparison with the pathogens each alone. All tested VAM isolates successfully colonized roots of maize plants. The microscopic examination of the colonized roots showed the different specific VAM structures **(Plate 2)**. VAM spores with germination shield were produced by VAM fungi isolated from roots of onion and maize plants whereas, the H-shaped structure characterized the infection with the VAM fungus was clearly seen in maize roots infected with the VAM fungus isolated from maize roots **(Plate 3)**.

Table (3): Effect of VAM/pathogenic fungi interactions on root length (cm) of maize plants.

VAM treatments	Pathogen(s) treatments				Mean
	Control (no pathogen)	*C. maydis*	*F. monilliforme.*	*R. solani*	
Control (no VAM)	30.3	30.3	28.2	25.7	28.60
VAM-O	39.0	33.4	33.3	30.7	34.09
VAM-B	34.3	34.4	36.0	31.8	34.10
VAM-S	36.3	34.1	35.0	30.8	34.05
VAM-M	33.3	32.7	32.6	31.0	32.38
Mean	34.64	32.93	33.02	29.98	

L.S.D. at 5% for:	VAM treatments (V)	Pathogens (P)	V x P
	0.073	0.059	0.293

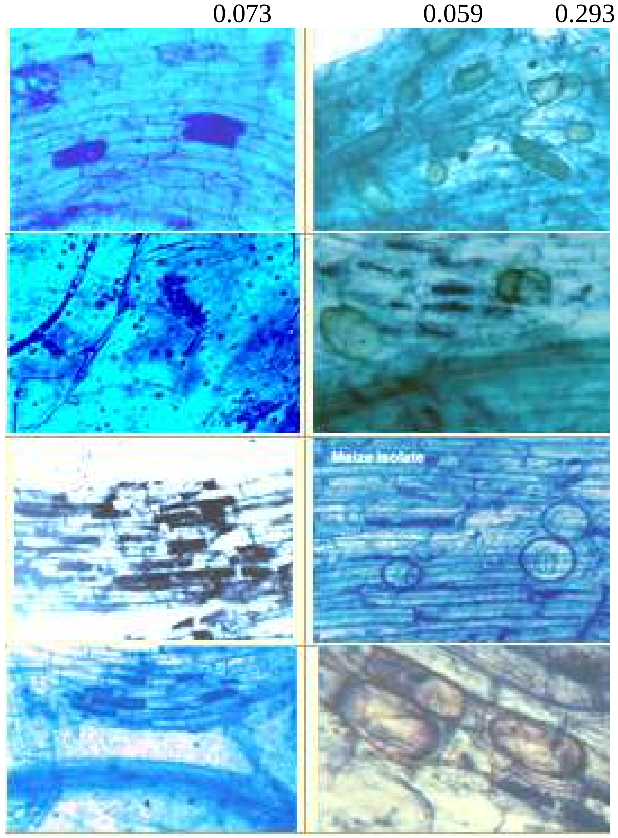

Plate. 2: Characterized VAM infection structures i.e. srbuscules (Left) and vesicles (right) formed in inoculated maize roots by VAM fungi isolated

from roots of onion, broad bean, Swiss cheese and maize plants from above to below, respectively. Note variations between VAM isolates.

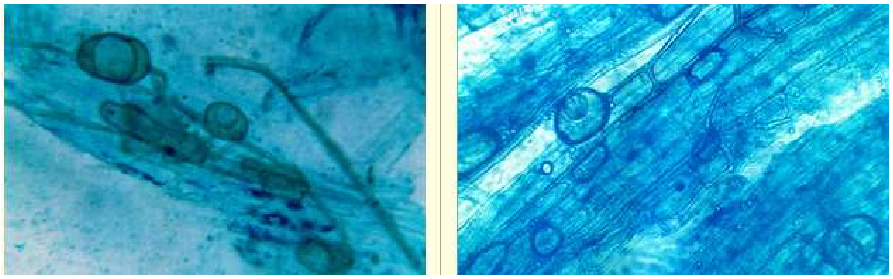

Plate 3: Spores with germination shield seen in roots of maize plants inoculated with VAM fungi isolated from onion roots (left) and maize roots (right). Note the H-shaped structures characterized infection with the VAM fungus *Glomus* (left).

3.3. Stem diameter:

Data in **Table (4)** show that the stem diameter (cm) of maize plants was reduced significantly by all the tested pathogenic fungi. *Rhizoctonia. solani* recorded the highest reduction (6.22) followed by *F. monilliforme* (6.28) and *C. maydis* (6.46) compared to the pathogen-free control (6.72). On the other hand, the stem diameter was significantly increased by using all VAM isolates tested compared to the control. In this respect, VAM-B produces the highest increase (6.6) followed by VAM-S (6.64), VAM-O (6.49) and VAM-M (6.42), respectively compared to the control (6.06). The interaction between VAM isolates and pathogenic fungi had no significant effect on the stem diameter.

Table (4): Effect of VAM/pathogenic fungi interactions on stem diameter (mm) of maize plants.

VAM treatments	Pathogen(s) treatments				Mean
	Control (no pathogen)	*C. maydis*	*F. monilliforme.*	*R. solani*	
Control (no VAM)	6.2	6.1	6.0	5.9	6.06
VAM-O	6.9	6.5	6.3	6.2	6.49
VAM-B	6.9	6.7	6.4	6.4	6.60
VAM-S	6.9	6.6	6.4	6.3	6.54
VAM-M	6.8	6.3	6.3	6.3	6.42
Mean	6.72	6.46	6.28	6.22	

L.S.D. at 5% for:	VAM treatments (V)	Pathogens (P)	V x P
	0.024	0.019	NS

3.4. Number of leaves/plant:

Data in **Table (5)** reveal that the tested pathogenic fungi significantly reduced number of leaves/plant compared to the control treatment. *Rhizoctonia. solani* recorded the highest reduction (5.76) followed by *F. monilliforme* (6.36) and *C. maydis* (6.51) compared to 6.66 leaves/plant in the control treatment. On the other hand, all VAM isolates tested significantly increased number of leaves/plant compared to the control treatments. In this regard, VAM-B recorded the highest increase in the number of leaves/plant (6.64) followed by VAM-S (6.6), VAM-O (6.54) and VAM-M

(6.26), respectively compared to 5.58 in the control. The VAM/pathogen interaction exerted significant effect in this respect. In the pathogen-free soil, VAM-O recorded the highest number of leaves/plant (7.0) followed by VAM-S (6.9) and VAM-B and VAM-M (6.8), respectively compared to the control (5.8). In soils infested with *C. maydis*, VAM-B and VAM-S recorded the highest figure (6.8) compared to *C. maydis* alone (5.8). Also, VAM-B combined with *F. monilliforme* (6.8) or *R. solani* (6.2) produces the highest number of leaves/plant compared to 5.6 and 5.1 for the two pathogens each alone, respectively.

Table (5): Effect of VAM/pathogenic fungi interactions on number of leaves/maize seedling.

VAM treatments	Pathogen(s) treatments				Mean
	Control (no pathogen)	*C. maydis*	*F. monilliforme.*	*R. solani*	
Control (no VAM)	5.8	5.8	5.6	5.1	5.58
VAM-O	7.0	6.7	6.6	5.8	6.54
VAM-B	6.8	6.8	6.8	6.2	6.64
VAM-S	6.9	6.8	6.7	6.1	6.60
VAM-M	6.8	6.5	6.2	5.6	6.26
Mean	6.66	6.51	6.36	5.76	

L.S.D. at 5% for:	VAM treatments (V)	Pathogens (P)	V x P
	0.009	0.007	0.036

3.5. Total fresh weight/plant:

Data in **Table (6)** reveal that all the tested pathogenic fungi significantly reduced the total fresh weight "g"/plant compared to the control treatment. In this regard, *R. solani* recorded the highest reduction (13.754) followed by *F. monilliforme* (15.438) and *C. maydis* (15.652) compared to the control (16.731). Inoculation with any of the VAM isolates tested, however, significantly increased total fresh weight/plant (15.067-16.572) compared to the control of both sowings (12.556). In this regard, VAM-B was the most effective (16.572) followed by VAM-S (16.522), VAM-O (16.253) then VAM-M (15.067), respectively. The total fresh weight/plant (g) in both sowings was significantly affected by the interaction between VAM isolates and pathogenic fungi tested in comparison with their controls. VAM-S or VAM-O added to the pathogen-free soil and VAM-B added to soils infested with *C. maydis*, *F. monilliforme* or *R. solani* showed the highest fresh weight/plant compared to soils infested with pathogens each alone.

3.6. Total dry weight/plant:

Data in **Table (7)** show that, all tested pathogenic fungi significantly reduced the total dry weight "g"/plant compared to the control. *Rhizoctonia. solani* recorded highest reduction (3.234) followed by *F. monilliforme* (3.463) and *C. maydis* (3.601) compared to the control (3.793). Regardless pathogenic fungi, inoculation with VAM fungi significantly increased total dry weight/plant. In this respect, VAM-S recorded the highest increase (3.803) followed by VAM-B (3.763), VAM-O (3.72) and VAM-M (3.547), respectively in comparison with the control (2.782). The total dry weight/plant was affected significantly by the VAM/pathogen interaction. In the pathogen-free soil (control), VAM-S recorded the highest increase (4.204) followed by VAM-O (4.186), VAM-M (3.83) and VAM-B (3.745), respectively compared to their control (3.002). However, VAM-B was significantly better than the other VAM isolates tested when combined with any of *C. maydis*, *F. monilliforme* or *R. solani*.

Table (6): Effect of VAM/pathogenic fungi interaction on total fresh weight (g/plant) of maize.

VAM treatments	Pathogen(s) treatments				
	Control (no pathogen)	*C. maydis*	*F. monilliforme.*	*R. solani*	Mean
Control (no VAM)	13.68	12.67	12.37	11.50	12.556
VAM-O	17.96	16.55	16.32	14.18	16.253
VAM-B	17.08	16.91	17.38	14.92	16.572
VAM-S	18.00	16.68	17.00	14.41	16.522
VAM-M	16.94	15.45	14.12	13.76	15.067
Mean	16.731	15.652	15.438	13.754	

L.S.D. at 5% for: VAM treatments (V) 0.043 Pathogens (P) 0.034 V x P 0.172

Table (7): Effect of VAM/pathogenic fungi interaction on total dry weight (g/plant) of maize plants.

VAM treatments	Pathogen(s) treatments				
	Control (no pathogen)	*C. maydis*	*F. monilliforme.*	*R. solani*	Mean
Control (no VAM)	3.002	2.932	2.804	2.390	2.782
VAM-O	4.186	3.738	3.598	3.358	3.720
VAM-B	3.742	3.888	3.878	3.542	3.763
VAM-S	4.204	3.800	3.724	3.484	3.803
VAM-M	3.830	3.648	3.310	3.398	3.547
Mean	3.793	3.601	3.463	3.234	

L.S.D. at 5% for: VAM treatments (V) 0.014 Pathogens (P) 0.011 V x P 0.054

3.7. The total chlorophyll content in maize leaves:

Data in **Table (8)** show that, the total chlorophyll (mg/g fresh weight) was decreased considerably by all tested pathogenic fungi. *Fusarium monilliforme* recorded highest reduction (6.441) followed by *R. solani* (7.628) and *C. maydis* (13.695), respectively compared to the control (14.307). All VAM isolates tested, regardless pathogenic fungi, considerably increased the total chlorophyll content in comparison with the control. VAM-S recorded the highest increase (14.474) followed by VAM-M (10.497) meanwhile VAM-O recorded the lowest increase (10.07) compared to control (7.4). The efficacy of VAM isolates tested for increasing total chlorophyll content, however, was greatly varied in the pathogen(s)-infested soils. Using VAM-S in the pathogen-free soil or in the *C. maydis*- or *R. solani*-infested soils and VAM-O in the *F. monilliforme*-infested soil resulted in the highest total chlorophyll content compared to their controls.

Table (8): Effect of VAM/pathogenic fungi interaction on the total Chlorophyll (mg/g fresh weight).

VAM treatments	Pathogen(s) treatments				Mean
	Control (no pathogen)	*C. maydis*	*F. monilliforme.*	*R. solani*	
Control (no VAM)	9.239	9.095	5.668	5.597	7.400
VAM-O	13.462	11.909	7.561	7.346	10.070
VAM-B	13.054	12.052	7.343	8.144	10.148
VAM-S	21.866	21.636	5.709	8.686	14.474
VAM-M	13.914	13.781	5.924	8.369	10.497
Mean	14.307	13.695	6.441	7.628	

5. Effect of VAM/pathogen interaction on soil pH value:

Data in **Table (9)** illustrate that soil pH was affected differently by pathogenic fungi and VAM isolates when compared to their control treatments. All pathogenic fungi caused considerable decreases in the soil pH. *Rhizoctonia solani* caused the highest decrease (8.26) followed by *F. monilliforme* (8.32) and *C. maydis* (8.46) compared to the control (8.48). On the contrary, the soil pH was increased considerably by VAM isolates tested. The highest increase in the soil pH was recorded by VAM-M (8.51) followed by VAM-B (8.43), VAM-O and VAM-S (8.4) compared to the control (8.17). The interaction between pathogens and VAM isolates proved that the soil pH was increased by the pathogenic fungi (8.02-8.45) as well as by the VAM isolates (8.54-8.74) when each was used alone in comparison with the control (7.86). Such trend was slightly varied when both pathogen and VAM fungi are present together. Th soil pH value was increased by *R.. solani*/VAM combinations (8.22-8.52) when compared to *R. solani* alone (8.02), but it was clearly decreased when compared to VAM isolates each alone (8.54-8.74). This trend was also noticed concerning combination between VAM-M and each of *C. maydis* and *F. monilliforme* as well as VAM-B and *C. maydis*. However, the interactions between *C. maydis* and VAM-S and *F. monilliforme* and each of VAM-O, VAM-B and VAM-S decreased soil pH value even compared to the pathogen or VAM isolate each alone.

Table (9): Effect of VAM/pathogenic fungi interaction on pH values in soil just after harvesting the maize plants.

VAM treatments	Pathogen(s) treatments				Mean
	Control (no pathogen)	*C. maydis*	*F. monilliforme.*	*R. solani*	
Control (no VAM)	7.86	8.35	8.45	8.02	8.17
VAM-O	8.54	8.55	7.99	8.52	8.40
VAM-B	8.62	8.51	8.37	8.22	8.43
VAM-S	8.65	8.30	8.32	8.32	8.40
VAM-M	8.74	8.60	8.49	8.22	8.51
Mean	8.48	8.46	8.32	8.26	

DISCUSSION

The plants colonized by the vesicular arbuscular mycorrhizal (VAM) fungi are better nourished and better adapted to its environment consequently its growth and health are improved and protection against environmental conditions detrimental to their survival is increased **(Sylvia and Williams, 1992)**. VAM symbiosis tends to reduce the incidence of root diseases and minimizes the harmful effect of certain pathogenic agents **(St-Arnaud *et al.*, 1996)**.

In the present work, percentages of survived maize plants, shoot and root length, stem diameter, number of leaves/plant, fresh and dry weight/plant were significantly decreased by the pathogenic fungi tested (*C. maydis*, *R. solani* and *F. monilliforme*) compared to their respective control treatment. On the contrary, all the studied parameters were significantly increased by the tested 4 VAM isolates particularly VAM-O and VAM-S compared to their respective controls. The interaction between a known pathogen and any particular VAM isolate had no significant effect on survived maize plants and stem diameter but all interactions improved both parameters when compared to any pathogen alone. However, all other determined parameters were significantly improved (to different extents) by the VAM/pathogen interaction compared to the pathogen alone. The VAM/pathogen interaction involved VAM-B or VAM-S resulted in the post responses for most if not all determined parameters. The results about improvement the growth are in harmony with several investigators who mentioned that growth of mycorrhizal plants was enhanced especially under field conditions, mainly, because improvement of nutrients uptake **(Graham *et al.*, 1976; Kucey and Paule, 1983; Hwang *et al.*, 1992; Ahmed *et al.*, 1994; Gaur *et al.*, 2000)**.

Our *in vitro* grown VAM fungi isolated from roots of onion, broad bean, Swiss cheese and maize plants were quietly varied specially in shapes of their arbuscules, spores and vesicles formed in roots of maize plants inoculated with each, reasonably the observed variation in their efficacies could be expected. **Gurumurthy *et al.* (1999)** screened symbiotic responses of VAM fungi on growth, nutrition and dry matter production of shisham (*Dalbergia sissoo*) in unsterile red sandy clay soil. Four standard VAM fungi viz. *Glomus fasciculatum, Gigaspora margarita, Acaulospora laevis, Sclerocystis dussii,* and two local isolates from Dharwad, viz., local isolate-1 and local isolate-2 were used. Shisham plants responded well to inoculation with VAM fungi. Plant height, stem diameter, leaf area, root length, shoot dry weight, root dry weight and the concentrations of P, Zn, Cu, Mn and Fe in shoot which were significantly higher in shisham plants inoculated with *G. fasciculatum* as compared to other VAM fungi. All these variables were the least in the uninoculated plants.

In fact, the VAM fungi are able to increase growth and yield of their hosts as well as their disease resistance through different ways. **Nelson and Achar (2000)** used VAM fungi (*Glomus fasciculatum, G. aggregatum, G. mosseae*) as an antagonist against *Peronospora parasitica*. The disease incidence was directly correlated to the production of phenolic compounds due to presence of VAM. Similarly deposition of lignin on host cell wall in the presence of VAM prevented further penetration and establishment of the downy mildew pathogen. The formation of structural barrier such as lignin was considered as a defense mechanism against the downy mildew pathogen. Peroxidase activity in VAM inoculated host was enhanced compared to the uninoculated ones. Increased peroxidase activity has long been related to defense mechanism in host pathogen interactions. Establishment of VAM in the xylem vessels of the host was also confirmed. VAM in the transport system of the host acted as a physical barrier against the downy mildew pathogen restricting its further penetration and establishment.

García-Garrido1 and Ocampo (2002) mentioned that the response of plants to VAM fungi involves a temporal and spatial activation of different defense mechanisms. The activation and regulation of these defenses have been proposed to play a role in the maintenance of the mutualistic status of the association, however, how these defenses affect the functioning and development of VAM remains unclear. A number of regulatory mechanisms of plant defense response have been described during the establishment of the VAM symbiosis, including elicitor degradation, modulation of second messenger concentration, nutritional and hormonal plant defense regulation, and activation of regulatory symbiotic gene expression.

Soil pH was affected differently by each of the pathogenic fungi and VAM isolates when compared to their control treatments. All pathogenic fungi caused considerable decreases in the soil pH. *Rhizoctonia solani* caused the highest decrease followed by *F. monilliforme* and *C. maydis* compared to the control. On the other hand, the soil pH was increased considerably by using the VAM isolates tested. The highest increase in the soil pH was recorded by VAM-M followed by VAM-B, VAM-O and VAM-S compared to the control.

The interaction between the tested pathogens and VAM isolates proved that, the soil pH was increased by pathogenic fungi as well as by the VAM isolates when each was used alone in comparison with the general control. Such trend was slightly varied when both pathogen and VAM fungi are present together. Th soil pH value was increased by *R.. solani*/VAM combinations when compared to *R. solani* alone but it was clearly decreased when compared to VAM isolates each alone. This trend was also noticed concerning combination between VAM-M and each of *C. maydis* and *F. monilliforme* as well as VAM-B and *C. maydis*. However, the interactions between *C. maydis* and VAM-S and *F. monilliforme* and each of VAM-O, VAM-B and VAM-S decreased soil pH value even compared to the pathogen or VAM isolate each alone. In this regard, **Sylvia, (1990)** mentioned that the flow of carbon to the soil mediated by mycorrhizae serves several important functions. The extramatrical hyphae of some mycorrhizae, produce hydrolytic enzymes, such as proteases and phosphatases, that can have an important impact on organic matter mineralization and nutrient availability. **McArthur and Knowles (1993)** stated that the low-P VAM plants had recovered 42% more of the available soil P than low-P nonVAM plants. However, the VAM fungus only partially alleviated P deficiency stress and did not completely compensate for inadequate abiotic P supply. Although the specific activities of acid phosphatases and microsomal ATPases were only marginally influenced by VAM. **Sharma and Adholeya (2000)** stated also that VAM fungi significantly change the physiology and chemical constituents of the host, the pattern of root exudation, and the microbial composition of the rhizosphere. The present results suggest, in general, that the VAM fungi might play significant role in altering the soil pH by unknown way that permits to increase solubility of phosphorus and other soil elements necessary for plant nutrition.

Conclusion

Management of VAM fungi is an important aspect in agricultural systems. The VAM fungi now be easily isolated and grown in axenic cultures and their *in vitro* inocula could be produced in large quantity and could be contributed in agriculture, in similar way as the nodule bacteria (*Rhizopium* spp.), and are gradually could be integrated with conventional agricultural practices. The appropriate management of VAM fungi in agriculture allows a substantial reduction in application of fertilizers and pesticides, reducing farm work, maintenance and costs of production, while maintaining yields at their highest levels.

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تأثير لقاح فطريات الميكورهيزا الحويصلية الشجيرية المنتج معمليا على إصابة نباتات الذرة ببعض الفطريات وصفاتها النباتية تحت ظروف الصوبة

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أجريت هذه الدراسة تحت ظروف الصوبة بهدف تقييم تأثير بعض عزلات من فطريات الميكورهيزا الحويصلية الشجيرية (VAM) على نسبة بقاء نباتات الذرة وكذلك بعض خصائص نموها وكذلك درجة حموضة التربة فى وجود بعض الفطريات الممرضة للذرة.

سببت جميع الفطريات الممرضة تحت الدراسة (سيفالوسبوريوم مايدس ، فيوزاريوم مونيليفورم ، ريزوكتونيا سولانى) نقصا معنويا فى نسبة نباتات الذرة القادرة على البقاء ، ارتفاع النبات ، طول الجذر ، قطر الساق ، عدد الأوراق/نبات ، الوزن الغض والجاف/نبات ، وكذلك المحتوى الكلى للكلوروفيل فى الأوراق مقارنة بالكنترول (غياب المسبب الممرض). وعلى العكس من ذلك أدت جميع عزلات الميكورهيزا الحويصلية الشجيرية (VAM) تحت الاختبار خاصة تلك المعزولة من البصل (VAM-O) والفول (VAM-B) إلى زيادة معنوية فى جميع تلك المقاييس مقارنة بالكنترول الغير محقون بتلك الفطريات. هذا ولم يكن للتفاعل بين المسببات المرضية وعزلات الـ VAM تأثيرا معنويا على نسب بقاء بادرات الذرة أو قطر الساق بينما تأثرت جميع الصفات الأخرى معنويا بهذا التفاعل مقارنة بوجود المسبب المرضي منفردا. وعلى أية حال ، كانت أعلى درجات التحسن فى تلك المعايير مصاحبة للتفاعلات المشتملة على عزلات الـ VAM المعزولة من الفول (VAM-B) والقشطة (VAM-S).

أوضحت النتائج أيضا أن رقم حموضة التربة قد زاد بوضوح تحت تأثير الفطريات الممرضة (8.02-8.45) وكذلك عزلات الــ VAM المختبرة (8.54-8.74) عند المقارنة بمعاملة الكنترول الخالية منهما (7.86). وقد تغير هذا الاتجاه نسبيا عند تواجد (تفاعل) كلا من السبب المرضي وفطريات الـ VAM سويا. فعلى سبيل المثال، كان للتفاعل بين عزلات الـ VAM والفطر الممرض ريزوكتونيا سولانى تأثيرا إيجابيا على رقم الحموضة بالتربة (8.22-8.52) عند المقارنة بتأثير نفس الفطر الممرض منفردا (8.02) بينما كان هذا التأثير سلبيا عند المقارنة بتأثير عزلات الـ VAM منفردة (8.54-8.74).