Effect of VA-Mycorrhizal Fungi Inocula Produced *in Vitro* on Some Maize Fungal Diseases and Growth Parameters under Field Conditions Abd El-Monem I. El-Fiki, G.M.D. El-Habaa, M.A. Hafez and K.E. Eid

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> \mathbf{F} our vesicular arbuscular mycorrhizal (VAM) fungi (*Glomus* spp.) isolates namely VAM-O, VAM-B, VAM-S and VAM-M were isolated from onion, broad bean, swiss cheese and maize roots, respectively, used to produce in vitro inocula which were evaluated under field conditions. The VAM isolates were grown in vitro on barely modified (BM) cultures to produce their applicable inocula. The in vitro inocula were used at low (2.5/g ridge), medium (5.0/g ridge) and high levels (10.0/g ridge) equivalent to 10, 15 and 20 kg/feddan, respectively. Two field experiments were carried out during 2002 and 2003 growing seasons to evaluate the effect of VAM inoculation treatments compared to P-fertilizer and VAM inoculation N-fertilization compared to N-fertilizer alone on the seed + germination, percentage of smutted and stunted plants, fresh and dry weight/plant, ear weight, grain yield/plant and weight of 100-kernels. Response of maize to VAM inoculation was greatly varied depending on the VAM isolate, inoculum level and growing season. The aforementioned maize growth parameters were significantly enhanced by VAM inoculation than P-fertilization, whereas most VAM treatments + N-fertilization showed similar response when compared with the N-fertilizer alone.

Keywords: Vesicular arbuscular mycorrhizae, VAM, inoculum, *in vitro*, P-fertilization, N-fertilization, common smut, stunt, maize.

Vesicular arbuscular mycorrhizae (VAM) are Zygomycetous fungi belonging to order Glomales that form the most common symbiosis with the plant kingdom as it colonize more than 80% of vascular plants (Barek *et al.*, 2001). A symbiosis refers to an association of living organisms that benefits both partners, enabling them to survive, grow and reproduce more effectively and, above all, acquires increased resistance to environmental stresses such as drought, cold and root pathogens (Sylvia and Williams, 1992 and Mohan, 2000).

Isolation and inoculum production of VAM fungi have many difficulties. Obtaining isolates of VAM fungi is more difficult because they will not grow apart from their hosts. Spores can be sieved from soil, surface disinfested, and used to initiate "pot cultures" on a susceptible host plant in sterile soil or an artificial plantgrowth medium. Inoculum is typically produced in scaled-up pot cultures.

Alternatively, hydroponic or aeroponic systems are possible; a benefit of these systems is that plants can be grown without a supporting substratum, allowing colonized roots to be sheared into an inoculum of high propagule number (Jarstfer and Sylvia, 1992 and 1994).

El-Fiki *et al.* (2001) isolated four VAM fungi isolates namely; VAM-O, VAM-B, VAM-S and VAM-M from roots of onion (*Allium cepa*), broad bean (*Vicia faba*), swiss cheese (*Monstera deliciose*) and maize (*Zea mays*) plants. Inocula of these VAM isolates produced *in vitro* on barley modified (BM) medium were effective at different concentrations for improving growth of maize plants under greenhouse. The present work aimed to evaluate these *in vitro* VAM inocula in the field conditions as a biofertilizer on crop protection, growth and yield of maize compared to P- and N-fertilization each alone. The VAM inoculum was either used alone or combined with P- or N-fertilizers.

Materials and Methods

The symbiont-BM (barely modified) cultures for the VAM fungi namely; VAM-O, VAM-B, VAM-S and VAM-M, were prepared as described by El-Fiki *et al.* (2001) and used separately as VAM inocula.

Two field experiments were conducted at the Experimental Farm, Faculty of Agric., Moshtohor, Benha Univ. during 2002 & 2003 seasons in a randomized complete block design, with three replications (plots) each $(10.5m^2 = \frac{1}{400}$ fed.) consisted of 5 ridges 3m long and 70cm apart. Inoculum (BM-culture) of a known VAM isolate was placed, at time of sowing, in a furrow (5-10 cm deep) at the rate of 2.5, 5.0 and 10 g/ridge (equivalent to 10, 15 and 20 kg/feddan, respectively). Barley culture only (without mycrorrhizae) were used as control. Plots fertilized with calcium super phosphate (15.5% P₂O₅) at the rate of 200 kg/feddan served as controls. The P-fertilizer was added at sowing time. In parallel experiment, the same VAM inoculum levels were combined with N-fertilizer (ammonium nitrate 33.5% N) at the rate of 450 kg/fed. Plots receiving N-fertilizer only served also as control. The N-fertilizer was applied at two equal doses, *i.e.* at sowing time and before the 2nd irrigation (after thinning), respectively.

Seeds of maize (*Zea mays* cv. Local) were planted, at 22nd June in 2002 and 2003 growing seasons, in hills 30cm apart. Maize plants were thinned immediately before the 2nd irrigation to two plants per hill (approximately 75 plants/plot). The normal culture practices for growing maize were used.

Percentage of seed germination was recorded 21 days after sowing. 75 days after sowing the above ground plant parts, in taken samples, were used to determine total fresh and dry weight/plant (oven dried at 70°C for 24 hours). Concerning with symptoms only and regardless the causal agent, common smutted and stunted plants were calculated after 90 days from sowing. After harvest (110 days after sowing), averages of ear weight (g), grain yield/plant (g) and 100-kernel weight were estimated.

All data obtained, when necessary, were statistically analyzed according to the least significant difference (LSD) method described by Song and Keane (2006).

Results

1- Evaluation of different levels of VAM inocula compared to application of Pfertilizater:

Data in Table (1) reveal that inoculation with any VAM isolate at any level has significantly increased germination of maize seeds particularly in 2002 season compared to application of P-fertilizer. VAM-O was the best of all in season 2002 (80.7-81.7%) followed by VAM-S (79.3%), VAM-B (67.0-81.3%) and VAM-M (66.0-71.0%), respectively compared to application of P-fertilizer (62.3%). In the same season, seed germination was increased significantly as inoculum level was increased particularly in VAM-B and to some extent in VAM-M only. However, seed germination in 2003 season was significantly increased by all inoculum levels of VAM-O (75.8-79.2%) and the high inoculum level of VAM-B (70.0%) compared to application of P-fertilizer (64.0%). All other VAM treatments particularly VAM-M and VAM-S had no effect or significantly decreased seed germination compared to application of P-fertilizer. Inoculation with different inoculum levels of VAM-M only significantly decreased incidence of the common smut (0.4%) in season 2002 only compared to application of P-fertilizer (1.8%). All other VAM treatments in both seasons had no significant effect. However, percentage of stunted maize plants was significantly reduced by applying different VAM treatments particularly VAM-S at 5.0 and 10.0 g/ridge inoculum levels (0.0%) compared to application of Pfertilizer (4.0%).

The data in Table (1) also show that the fresh and dry weight/plant, ear weight, grain yield/plant and weight of 100-kernels were significantly increased, during 2002 and 2003 seasons, by using most of tested VAM inoculation treatments. Using VAM-O at 5 g/ridge level produced the highest increase in the fresh weight (g/plant) in season 2002 followed by the same isolate and VAM-B at 10.0 g/ridge level, respectively, whereas VAM-O and VAM-S used at the 2.5 g/ridge level produced the lowest increase. Using 2.5 g/ridge level of VAM-M significantly decreased fresh weight/plant whereas its medium level had no significant effect in 2002 season compared to P-fertilization (204.7). The same trend was observed also in season 2003. Using VAM-O at 5.0 and 10.0 g/ridge levels in both seasons and VAM-B and VAM-M at the 10.0 g/ridge level in 2002 and 2003 seasons, respectively caused the highest significant increase in dry weight (g/plant) compared to P-fertilization. However, using VAM-M at the low and medium levels and VAM-S at the 2.5 g/ridge level in both seasons in addition to VAM-O at the 2.5 g/ridge level in season 2002 and VAM-S at the low and medium levels in season 2003 had no significant effect in this respect. All VAM inoculation treatments significantly increased ear weight (g) particularly in 2002 season compared to application of P-fertilizer. Using VAM-B and VAM-S at the 10.0 g/ridge level in 2002 season and VAM-O at 5.0 and 10.0 g/ridge levels in 2003 season were the best of all treatments in this respect. However, using the 5.0 and 10.0 g/ridge levels of VAM-M and the 2.5 g/ridge level of VAM-B had no significant effect on ear weight compared to P- fertilization. Most VAM inoculation treatments increased grain yield and weight of 100-kernels in both 2002 and 2003 seasons. Using the 10.0 g/ridge level of VAM-B VAM-S in seasons 2002 & 2003, respectively produced the highest increases in grain yield/plant. Meanwhile, VAM-S at the 2.5 g/ridge level in 2002 season and VAM-M at 2.5 and

2002 and 2003 seasons									
	Parameters								
VAM isolates & inoculum level (g/ridge)	Seed germination %	Smut (%)	Stunt (%)	Fresh weight (g/plant)	Dry weight (g/plant)	Ear weight (g)	Grain yield (g/plant)	100-Kernel weight (g)	
Control (P alone *)		1.8	4.0	204.7	66.3	133.4	50.1	25.0	
	67.0	1.8	0.9	230.2	75.7	155.8	70.0	26.9	
VAM-M 2.5 g	66.0	0.4	2.2	180.6	66.8	153.6	58.3	25.1	
VAM-O 2.5 g	80.7	1.3	2.2	216.5	73.2	153.9	58.2	26.7	
VAM-S 2.5 g	79.3	1.8	1.3	217.6	72.1	152.1	54.2	26.4	
VAM-B 5.0 g	73.0	1.8	0.9	244.5	79.9	156.6	74.5	28.2	
VAM-M 5.0 g	67.3	0.4	1.8	212.1	70.4	154.2	61.2	25.4	
VAM-O 5.0 g	80.7	1.8	1.3	273.4	84.6	158.3	64.3	27.5	
VAM-S 5.0 g	79.3	1.8	0.0	230.6	81.8	151.1	73.6	26.7	
VAM-B 10.0 g	81.3	1.8	0.9	250.6	83.0	172.2	80.6	28.1	
VAM-M 10.0 g	71.0	0.4	2.7	222.8	74.6	154.2	60.4	25.3	
VAM-O 10.0 g	81.7	1.3	1.3	266.7	82.3	155.8	57.3	28.1	
VAM-S 10.0 g	79.3	1.8	0.0	221.4	78.4	164.8	75.5	28.9	
at 0.05	2.79	0.60	0.99	11.125	7.297	9.278	5.764	1.569	
· · · · · ·	64.0	1.4	1.3	163.9	51.8	99.4	35.1	25.0	
VAM-B 2.5 g	64.0	1.9	1.0	181.9	54.5	115.6	61.5	27.2	
VAM-M 2.5 g	60.8	1.9	1.0	175.2	49.5	86.1	29.6	24.4	
VAM-O 2.5 g	75.8	1.4	1.3	183.9	58.8	123.3	58.5	26.7	
VAM-S 2.5 g	64.7	1.9	1.0	176.8	47.7	128.6	76.4	28.1	
VAM-B 5.0 g	66.3	1.9	1.0	188.9	59.2	125.6	65.4	28.6	
VAM-M 5.0 g	63.7	1.9	1.0	181.1	54.5	104.5	37.9	26.4	
VAM-O 5.0 g	79.2	2.4	1.3	202.8	65.2	155.8	84.0	27.5	
VAM-S 5.0 g	68.7	1.9	1.0	184.6	51.6	128.7	79.7	28.1	
VAM-B 10.0 g	70.0	1.9	0.7	197.9	58.8	139.7	73.1	29.2	
VAM-M 10.0 g	64.2	1.9	0.7	187.8	62.1	136.1	59.8	26.7	
VAM-O 10.0 g	77.3	1.4	0.7	197.8	60.9	154.2	80.4	26.7	
VAM-S 10.0 g	68.7	1.9	1.0	193.3	53.1	136.7	87.0	28.3	
	3.04	NS	NS	8.787	5.159	20.770	15.277	0.799	
	VAM isolates & inoculum level (g/ridge) VAM-B 2.5 g VAM-B 2.5 g VAM-B 2.5 g VAM-O 2.5 g VAM-S 2.5 g VAM-S 2.5 g VAM-S 5.0 g VAM-S 5.0 g VAM-S 10.0 g VAM-S 10.0 g VAM-S 10.0 g VAM-S 10.0 g VAM-S 10.0 g VAM-S 2.5 g VAM-B 2.5 g VAM-B 2.5 g VAM-B 2.5 g VAM-B 2.5 g VAM-C 2.5 g VAM-S 5.0 g VAM-S 5.0 g VAM-S 5.0 g VAM-S 5.0 g VAM-S 5.0 g VAM-S 10.0 g VAM-S 10.0 g VAM-N 10.0 g VAM-O 10.0 g	VAM isolates & inoculum level (g/ridge) 3.0 rol (P alone *) 62.3 VAM-B 2.5 g 67.0 VAM-B 2.5 g 66.0 VAM-O 2.5 g 80.7 VAM-S 2.5 g 79.3 VAM-B 5.0 g 73.0 VAM-S 2.5 g 79.3 VAM-S 2.5 g 79.3 VAM-S 0 g 67.3 VAM-S 5.0 g 79.3 VAM-S 5.0 g 79.3 VAM-O 5.0 g 80.7 VAM-S 10.0 g 81.3 VAM-O 10.0 g 81.7 VAM-S 10.0 g 79.3 at 0.05 2.79 rol (P alone *) 64.0 VAM-S 2.5 g 64.0 VAM-M 2.5 g 60.8 VAM-M 2.5 g 60.8 VAM-S 2.5 g 64.7 VAM-B 5.0 g 63.7 VAM-S 5.0 g 63.7 VAM-S 5.0 g 68.7 VAM-S 5.0 g 68.7 VAM-S 5.0 g 68.7 VAM-S 10.0 g 77.3 VAM-S 10.0 g 68.7 VAM-S 10.0 g 68.7	VAM isolates & inoculum level $(g/ridge)$	VAM isolates & inoculum level $(g/ridge)$	VAM isolates & inoculum level (g/ridge) inoculum level or inoculum level or inoculum level or inoculum level (g/ridge) inoculum level or inoculum level or inoculum level or inoculum level or inoculum level inoculum level or inoculum level or inoculum level inoculum level or inoculum level inoculum level or inoculum level inoculum level or inoculum level inoculum lev	VAM isolates & inoculum level (g/ridge) initian formation of the second se	VAM isolates & inoculum level (g/ridge) inoculum level be in the set of the set o	VAM isolates & inoculum level (g/ridge) if b b b b b b b b b b b b b b b b b b b	

 Table 1. Effect of VAM inoculation treatments alone compared application of P-fertilizer (control) on different parameters under field conditions during 2002 and 2003 seasons

* P-fertilization with super phosphate at 200 kg/feddan was used as control.

5.0 g/ridge levels in 2003 season had no significant effect compared to P-fertilization. Concerning weight of 100-kernels, using VAM-S, VAM-B and VAM-O (at 10.0 g/ridge level) in 2002 season and VAM-B (at 5.0 and 10.0 g/ridge levels) and VAM-S (at all levels) in 2003 season were the best VAM inoculation treatments in this respect. However, VAM-M (at all levels) in 2002 season and VAM-M (at the low level) in 2003 season had no significant effect when compared with P-fertilizer application.

2- Evaluation of different levels of VAM inoculation + N-fertilization compared to the application of N-fertilization alone:

Data in Table (2) show that N-fertilization + VAM inoculation treatments (except VAM-M at all levels and VAM-S at 2.5 g/ridge level in 2003 season only) significantly increased seed germination in both seasons more than N-fertilizer alone. N-fertilization + inoculation with VAM-O particularly at its 10.0 g/ridge level produced the highest significant increase in seed germination in 2002 season (85.33%) and 2003 season (82.17%) compared to 63.33 and 73.5% for application of N-fertilizer alone in both seasons, respectively. However, seed germination in 2003 season was significantly decreased by using combination between Nfertilization and all levels of VAM-M (63.17-69.17%) and 2.5 g/ridge level only of VAM-S (70.17%) compared to N-fertilizer alone. Levels of VAM-M only were significantly varied in their effect on seed germination. VAM-M at 10.0 g/ridge level in season 2002 and medium level in season 2003 produced the highest increase compared to other its levels. Using N-fertilization + any VAM inoculation treatment significantly decreased the common smut disease incidence in 2002 season only (1.91-2.22%) compared to N-fertilizer alone (3.11%). Utilizing N+VAM-M (at different levels) were the best in this respect. Percentages of stunted maize plants in both seasons seemed not significantly affected by N+VAM treatments compared to N-fertilizer alone.

The same data in Table (2) illustrate the growth and yield parameters which differently positive responded as a result to combination of N-fertilization with VAM treatments compared with N-fertilization alone. In this respect, using N-fertilization + VAM-O at the high level (10.0 g/ridge) produced the highest significant increase in the fresh and dry weight (g/plant) in both 2002 & 2003 seasons. However, N+VAM-B at all levels and VAM-S at 10.0 g/ridge in 2002 season and VAM-M at all levels in both seasons produced the lowest increase in the fresh weight/plant.

Concerning yield components, VAM-S and VAM-B at the 2.5 g/ridge level and VAM-O at 10.0 g/ridge level in 2002 season produced the highest significant increase in ear weight, grain yield/plant and 100-kernels weight, respectively compared to N-fertilization alone. Using VAM-O at high level, VAM-O and VAM-B at medium level gave the best results for the three yield parameters in 2003 season. However, N+VAM-M particularly at low and high levels produced the lowest significant increases in these yield parameters in both seasons compared to applying N-fertilizer alone.

	seasons	Parameters							
		rarameters							
Season	VAM isolates & inoculum level (g/ridge)	Seed germination %	Smut (%)	Stunt (%)	Fresh weight (g/plant)	Dry weight (g/plant)	Ear weight (g)	Grain yield (g/plant)	100-Kernel weight(g)
Cont	rol (N alone *)	63.33	3.11	0.44	305.6	110.4	144.2	61.11	26.67
	VAM-B 2.5 g	82.33	2.22	0.00	318.3	124.5	199.4	125.59	29.36
	VAM-M 2.5 g	73.67	0.89	0.00	327.8	106.1	183.6	103.50	26.83
	VAM-O 2.5 g	83.67	2.22	0.44	323.1	113.8	191.7	102.33	29.17
	VAM-S 2.5 g	85.00	1.78	0.00	339.1	127.8	216.5	123.93	29.94
	VAM-B 5.0 g	83.00	2.22	0.00	322.2	124.9	198.1	121.19	29.92
2002	VAM-M 5.0 g	75.33	0.89	0.00	332.8	118.3	206.7	111.66	28.70
	VAM-O 5.0 g	83.33	2.22	0.89	343.9	123.3	193.4	119.83	29.72
	VAM-S 5.0 g	84.00	2.22	0.00	325.0	118.7	193.3	106.83	29.95
	VAM-B 10.0 g	84.33	2.22	0.00	318.8	122.9	186.3	97.58	29.05
	VAM-M 10.0 g	79.00	0.89	0.00	326.1	113.4	181.1	103.84	26.47
	VAM-O 10.0 g	85.33	2.22	0.89	355.8	132.2	193.7	122.49	30.00
	VAM-S 10.0 g	83.00	1.78	0.00	318.2	117.6	182.9	104.02	29.36
	at 0.05	3.61	0.727	NS	10.788	7.776	6.527	16.395	0.763
Cont	rol (N alone *)	73.50	4.29	1.33	224.5	61.3	107.0	54.71	26.67
	VAM-B 2.5 g	76.67	1.91	0.67	239.1	63.8	151.7	78.68	30.00
	VAM-M 2.5 g	65.33	2.86	0.67	241.6	64.0	141.9	80.96	27.50
	VAM-O 2.5 g	81.33	3.34	0.67	247.2	70.1	167.0	94.10	27.50
	VAM-S 2.5 g	74.50	2.38	0.67	255.7	71.6	164.4	105.27	29.17
	VAM-B 5.0 g	77.67	2.38	0.67	249.6	68.3	163.3	95.03	30.83
2003	VAM-M 5.0 g	69.17	2.86	0.67	239.4	66.9	139.7	72.67	27.78
20	VAM-O 5.0 g	81.33	2.86	0.67	250.7	73.4	176.7	109.67	29.44
	VAM-S 5.0 g	73.33	1.91	0.67	246.7	71.0	164.4	104.72	29.17
	VAM-B 10.0 g	76.67	1.91	0.67	245.7	68.8	151.4	99.78	30.28
	VAM-M 10.0 g	63.17	2.38	0.33	242.1	64.9	137.8	68.02	26.67
	VAM-O 10.0 g	82.17	1.91	0.33	261.1	77.7	177.2	106.92	29.44
	VAM-S 10.0 g	70.17	1.91	0.67	242.8	69.2	159.7	100.37	28.61
LSD	at 0.05	3.02	NS	NS	7.375	3.767	20.585	16.887	1.088

Table 2. Effect of VAM inoculation + N-fertilization compared to N-fertilization alone on different parameters under field conditions during 2002 and 2003 seasons

* N fertilization (Ammonium nitrate 33.5% N) alone at 450 kg/feddan was used as control.

Discussion

Comparing with P-fertilization, using VAM-O (particularly at the medium and high levels) caused the maximum increase in seed germination. Using VAM-M and VAM-S (both at the low and medium levels) were the best for reducing percentage of common smutted and stunted plants, respectively (particularly in 2002 season). Using VAM inoculation treatments particularly VAM-O (at the medium level) and

VAM-B (at the high level) have significantly increased the fresh and dry weight/plant in both seasons. Also, ear weight was significantly increased by all VAM inoculation treatments in 2002 season. Using VAM-B and VAM-S (at the high level) in 2002 season and VAM-O (at the medium and high levels) in 2003 season were the best in this respect. Using the 10.0 g/ridge level of VAM-B (in 2002 season) and VAM-S (in 2003 season) produced the highest increases in grain yield/plant. However, using VAM-S, VAM-B and VAM-O (at the high levels) in 2002 season and VAM-B (at the medium and high levels) and VAM-S (at all levels) in 2003 season were the best VAM inoculation treatments for increasing weight of 100-kernels.

However, seed germination in both seasons was significantly increased by applying all other N-fertilization+VAM treatments except VAM-M (at all levels) and VAM-S (at 2.5 g/ridge level) in 2003 season only. Using N+VAM-O (at high level) produced the highest significant increase in seed germination in both seasons. Using N+VAM-B (at all levels) and VAM-S (at low level) significantly decreased seed germination in 2003 season. Using VAM-M at 10.0 g/ridge level in season 2002 and medium level in season 2003 produced the highest increase compared to other levels. All N+VAM treatments particularly VAM-M (at different levels) significantly decreased the common smut disease incidence in 2002 season only. Percentages of stunted maize plants in both seasons were not significantly affected. All N+VAM inoculation had a positive significant effect on all growth and yield parameters. Using N+VAM-O (at high level) produced the highest increase in the fresh and dry weight in both 2002 & 2003 seasons. Using VAM-S and VAM-B (at low level) and VAM-O (at high level) in 2002 season produced the highest increase in ear weight, grain yield/plant and 100-kernels weight, respectively. While, VAM-O (at high level), VAM-O (at medium level) and VAM-B (at medium level) gave the best results for the three yield parameters, respectively in 2003 season.

In fact, the VAM colonized plants are better nourished and adapted to its environment consequently its growth and health is improved and protection against environmental conditions detrimental to their survival is increased (Sylvia & Williams, 1992). That symbiosis tends to reduce the incidence of root diseases and minimizes the harmful effect of certain pathogenic agents (St-Arnaud et al., 1996). However, tested VAM isolates might vary concerning their beneficial effects to the maize plants under certain circumstances. To increase the effectiveness of phosphate extraction from soil, Jakobsen et al. (1992a, 1992b) reported that, some VAM fungal species mainly explore the soil immediately adjacent to the root, while others explore it more distantly. Efficiency of VAM fungi was greatly variable. The VAM fungi seemed to be more effective under low than high levels of P-application (Trotta et al., 1991; McArthur and Knowles, 1993; Mahaveer and Adholeya, 2000). The seed yield of VAM inoculated-plants, under field conditions receiving 10.0 g/ridge level of P and N were increased by 28 and 17% in comparison with non- and only rhizobia-inoculated soybean, respectively (Vejsadova et al., 1993). Gurumurthy and Sreenivasa (1996) studied the response of Capsicum annum to different levels of Glomus macrocarpum (0, 25, 50, 75 or 100 g/10 kg unsterile soil) in 2 soil types. A matching trend was recorded in fresh fruit weight and plant dry weight, which increased with the increase in inoculum level up to 50 g/10 kg soil in the both soil types. All these parameters were significantly higher in red soil than black clay soil.

Das *et al.* (1997) inoculated seeds of *Verticillium radiata* with Rhizobium and/or VAM (*Glomus fasciculatum*) culture applied at 15 kg/ha. Shoot and root lengths, number of pods/plant and dry weight of pods were increased with dual inoculation compared with the uninoculated control. Seed yield was significantly higher with Rhizobium + VAM compared with no inoculation.

The present results indicated that the VAM fungi are not specific in terms of the partner plant they choose, which means that the same fungus can colonize a large number of plant species (Requena et al., 1999 and Buee et al., 2000). In addition, variations among VAM isolates might be more benefitial to a host plant than others under certain conditions and might be only of benefit in infertile soils. The benefit provided by mycorrhizas decreased as the degree of (inoculum level) mycorrhizal colonization of roots increased (Gange, 1999). The VAM-M (isolated from maize), however, showed the lowest improvement in the most determined parameters concerning plant growth, yield components. These results could be explained in light of the well-known detrimental effects of the monoculture crop rotation. In the present study, VAM-M was inoculated into to the same crop from which it was isolated and this resulted in similar depressions and detrimental effects associated with monoculture crop rotation. Johnson et al. (1992) suggested that, compared to other fungi, proliferating VAM fungal species might be less beneficial (or perhaps detrimental) to the crop in which they proliferate. Barnola et al. (1996) stated that the VAM fungus Glomus intraradices decreased pepper mass, whereas G. microcarpum increased mass of corn plants under greenhouse conditions (pasteurized soil) compared with the controls. However, G. intraradices had no significant effect on pepper mass under field conditions, whereas it significantly decreased mass of sweet corn. However, Dickson et al. (1999) found that the fungal isolates that perform poorly in some experiments may provide substantial benefits to plants in other trials where growing conditions are more suitable for that particular fungus.

El-Fiki *et al.* (2001) investigated the effect of adding VAM inocula (BMcultures) to the potted sterilized soil at different levels (1, 2, 4 and 6 g/pot) on different growth parameters maize plants. Degree of improvement, however, was quite variable and depended on the growth parameter tested, VAM isolate and its inoculum level. They added that, the VAM-O (isolated from onion) was more effective at the lowest inoculum level and this trend was completely reversed in VAM-S (isolated from swiss cheese) while, VAM-B (isolated from broad bean) gave the best results at the intermediate inoculum levels.

In fact, the VAM fungi could increase growth and yield of their hosts as well as their disease resistance through different ways. Sharma and Adholeya (2000) stated that, in most instances, VAM could significantly change the physiology and chemical constituents of the host, the pattern of root exudation, and the microbial composition of the rhizosphere. In this respect, the VAM effects on plant nutrition, especially P- nutrition is a mechanism of disease control. Garcia-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.

Conclusion

The VAM fungi can now could be isolated and grown in axenic cultures and their inocula could be produced in mass production *in vitro*. The *in vitro* VAM inocula could be contributed in agriculture systems, in similar way as the nodule bacteria (*Rhizobium* spp.), and gradually could be integrated with conventional agricultural practices. The appropriate management of VAM fungi in agriculture allows a substantial reduction in applications of fertilizers and pesticides, reducing farm work, maintenance and costs of production, while maintaining yields at their highest levels. The *in vitro* VAM inocula could be used, sometimes, instead of P-fertilizers and maximized beneficial responses of N-fertilizers.

References

- Barek, M.T.; Becard, G.and Philipp, F. 2001. Host-Parasite Interactions: Gene expression during pre-symbiotic development of arbuscular mycorrhizal fungi. *XXI Fungal Gen. Conf.* Asilomar, California (March 2001).
- Barnola, L.; Hamel, C.and Smith, D. 1996. Evaluation of the efficiency of different AM fungi on sweet corn (*Zea mays*) and pepper (*Capsicum frutescens*) under greenhouse and field conditions. *Internat. Conf. on Mycorrhizae [ICOM1]*, August 4-9, 1996. [Poster]
- Buee, M.; Rossignol, M.; Jauneau, A.; Ranjeva, R. and Bécard, G. 2000. The presymbiotic growth of arbuscular mycorrhizal fungi is induced by a branching factor partially purified from plant root exudates. *MPMI* 13:693-698.
- Das, P.K.; Sahoo, P.N. and Jena, M.K. 1997. Effect of VA-mycorrhiza and rhizobium inoculation on nutrient uptake, growth attributes and yield of greengram (*Vigna radiata* L.). *Environ. and Ecol.* **15**(4):830-833.
- Dickson, S.; Smith, S.E. and Smith, F.A. 1999. Characterization of two arbuscular mycorrhizal fungi in symbiosis with *Allium porrum*: inflow and flux of phosphate across the symbiotic interface. *New Phytologist* **144**:173-181.
- El-Fiki, A.I.I.; El-Habaa, G.M.D. and Eid, K.E. 2001. Successful growth and sporulation of the Vesicular Arbuscular Mycorrhizal fungi in axenic cultures. *Ann. Agric. Sci., Moshtohor* 39(2):933-952.
- Gange, A.C. 1999. On the relation between arbuscular mycorrhizal colonization and plant 'benefit'. *Oikos* 87:615-621.
- Garcia-Garrido1, J.M. and Ocampo, J.A. 2002. Regulation of the plant defence response in arbuscular mycorrhizal symbiosis. *J. Exp. Bot.* **53**(373):1377-1386.
- Gurumurthy, S.B. and Sreenivasa, M.N. 1996. Response of chilli to different inoculum levels of *Glomus macrocarpum* in two soil types of northern Karnataka. *Karnataka J. Agric. Sci.* 9(2):254-259.

- Jakobsen, I.; Abbott, L.K. and Robson, A.D. 1992a. External hyphae of vesiculararbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. 1. Spread of hyphae and phosphorus inflow into roots. *New Phytologist* **120**:371-380
- Jakobsen, I.; Abbott, L.K. and Robson, A.D. 1992b. External hyphae of vesiculararbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. 2. Hyphal transport of 32P over defined distances. *New Phytologist* **120**:509-516
- Jarstfer, A.G. and Sylvia, D.M. 1992. Inoculum production and inoculum strategies for vesicular-arbuscular mycorrhyzal fungi. In: Metting B (ed.), Soil Microbial Ecology: Application in Agriculture and Environmental Management. Marcel Dekker, New York, pp 349-377.
- Jarstfer, A.G. and Sylvia, D.M. 1994. Aeroponic culture of VAM fungi. In: Varma, A. and Hock, B (eds.), Mycorrhyzae *(structure, function, molecular biology and biotechnology)*. Springer Verlag, pp, 427-441.
- Johnson, N.C.; Copeland, P.J.; Crookston, R.K. and Pfleger, F.L. 1992. Mycorrhizae: possible explanation for yield decline with continuous corn and soybean. *Agron. J.* **84**(3):387-390.
- Mahaveer, P.S. and Adholeya, A. 2000. Enhanced growth and productivity following inoculation with indigenous AM fungi in four varieties of onion (*Allium cepa* L.) in an Alfisol. *Biol. Agric. and Hortic.* **18**:1-14.
- McArthur, D.A.J. and Knowles, N.R. 1993. Influence of vesicular-arbuscular mycorrhizal fungi on the response of potato to phosphorus deficiency. *Plant Physiol.* 101(1):147-160.
- Mohan, V. 2000. Endomycorrhizal interaction with rhizosphere and rhizoplane mycoflora of forest tree species in Indian arid zone. *Indian Forester* **126**(7):749-755.
- Requena, N.; Füller, P. and Franken, P. 1999. Molecular characterization of *GmFOX2*, an evolutionarily highly conserved gene from the mycorrhizal fungus *Glomus mosseae*, Down-regulated during interaction with Rhizobacteria. *MPMI* 12: 934-942.
- Sharma, M.P. and Adholeya, A. 2000. Sustainable management of arbuscular mycorrhizal fungi in the biocontrol of soil-borne plant diseases. *Biocontrol Potential and its Exploitation in Sustainable Agriculture (Vol. 1: Crop Diseases, Weeds and Nematodes)* pp. 117-138, eds. R. K. Upadhyaya, K. G. Mukerji, and B. P. Chamola New York: Kluwer Academic Publishers.
- Song, W. and Keane, A.J. 2006. "Parameter Screening Using Impact Factors and Surrogate- Based ANOVA Techniques", AIAA-2006-7088, 11th AIAA/ISSMO Multidisciplinary Analysis and Optimization Conference, Renaissance Portsmouth, Portsmouth, Virginia, 6 - 8 Sep 2006.
- St-Arnaud, M.; Hamel, C.; Vimard, B.; Caron, M. and Fortin, J.A. 1996. Co-culture with mycorrhizal *Tagetes patula* inhibited Fusarium wilt in the non-mycorrhizal host *Dianthus caryophyllus* as well as the pathogen in the soil. *Internat. Conf. on Mycorrhizae [ICOM1]*, August 4-9, 1996. [Abstract]
- Sylvia, D.M. and Williams S.E. 1992. Vesicular-arbuscular mycorrhizae and environmental stress. In: *Mycorrhizae in Sustainable Agriculture*. Eds. G.J.

Bethlenfalvay and R.G. Linderman. ASA Special Publication Number 54, Madison Wisconsin pp. 101-124.

- Trotta, A.; Carminati, C.; Schellenbaum, L.; Scannerini, S.; Fusconi, A. and Berta G. 1991. Correlation between root morphogenesis, VA mycorrhizal infection and phosphorus nutrition. *Develop. in Agric. and Managed-Forest Ecol.* 24:333-339.
- Vejsadova, H.; Siblikova, D.; Gryndler, M.; Simon, T. and Miksik, I. 1993. Influence of inoculation with *Bradyrhizobium japonicum* and *Glomus claroideum* on seed yield of soybean under greenhouse and field conditions. J. Plant Nutrit. 16(4):619-629.

تأثير لقاح فطريات الميكور هيزا الحويصلية الشجيرية المنتج معملياً على إصابة نباتات الذرة ببعض الفطريات وصفاتها النباتية والمحصولية تحت ظروف الحقل عبد المنعم إبراهيم إسماعيل الفقى ، جهاد محمد دسوقى الهباء ، محمد السيد حافظ ، خالد السيد عيد قسم النبات الزراعى - كلية الزراعة بمشتهر ، جامعة بنها - مصر

تم في هذه الدراسة استخدام أربعة عزلات من فطريات الميكور هيزا الحويصلية الشجيرية سبق عزلها من جذور البصل (VAM-O) ، الفول Tتك العزلات تم تنميتها تحت ظروف المعمل على بينة محورة تحتوى على الشعير – و تم استخدام هذا اللقاح بثلاثة مستويات منخفضة ومتوسطة وعالية (تقابل . . ، ٥ و ٢ كيلوجرام/فدان على التوالي) من خلال مجموعتين من تجارب الحقل تم تنفيذهما أثناء موسمى ٢٠٠٢ ، ٢٠٠٣ لتقييم تأثير استعمال لقاح تلك العزلات منفردا (مقارنة بالسماد الفوسفاتي) أو مصحوبا باستخدام السماد الآزوتي (مقارنة بالأخير منفردا) على المعايير التالية (معدل إنبات حبوب الذرة ، النسب المئوية لنباتات الذرة المتقزمة والمصابة بالتفحم العادى وكذلك الأوزان وزن الد ١٠ حبة.

أظهرت النتائج اختلاف الاستجابة لتأثير المعاملات المستخدمة تبعا لمعيار القياس ، مصدر العزلة الفطرية، تركيز اللقاح المستخدم ، موسم النمو. وبشكل عام كانت استجابة بعض المعايير لبعض معاملات تلقيح معينة (عزلات ومعدلات تلقيح) أفضل معنوياً عند مقارنتها باستخدام السماد الفوسفاتي كما كانت الاستجابة لبعض معاملات التلقيح المترافقة مع استعمال السماد الآزوتي أفضل معنويا عند مقارنتها باستعمال الأخير منفردا.