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SYMBIOTIC RELATIONSHIP OF VESICULAR ARBUSCULAR MYCORRHIZAL FUNGI WITH TOMATO PLANTS AND BIOLOGICAL CONTROL OF ROOT-KNOT NEMATODE

BY

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ABSTRACT

The effect of endomycorrhizal symbiosis on the interaction between the VA mycorrhizae, the *Meloidogyne* -susceptible tomato cv Rutgers and the root-knot nematode (*Meloidogyne incognita*) was investigated at P levels of 75 and 100% of the recommended dose (75 kg/ha), in the greenhouse. Individual plants were inoculated with 125, 250 and 500 chlamydospores of mycorrhizal fungi. The inoculum was added to seedling into the nursery. Nematode inoculum was added to plant in the pots as a 2cm deep trench around the stem-base about 350 juvenile/plant. The root colonization of the VAM fungi on the root of tomato plant and the sporulation of VAM fungi increased by increasing the VAM inoculum density either in the presence or absence of the nematode infection . VAM root colonization didn't increased by increasing the rate of Phosphorus, while the lower rate of P was more suitable for VAM root colonization either in the presence or absence of the nematode infection. The sporulation by VAM fungi decreased in the presence of nematode infection comparing with the same treatment in the absence of the nematode infection. The shoot dry weight increased by increasing the VAM inoculum density and the phosphorus levels in the presence or absence of nematode infection The numbers of J_2/150g of soil decreased by increasing the VAM inoculum density at different levels of phosphorus. The numbers of J_2/150g of soil increased at high level of phosphorus comparing with other levels of phosphorus. The root weight increased in the presence of nematode infection at different levels of phosphorus or different VAM spores concentration comparing with non infected plant. On the contrary the shoot dry weight decreased .The root weight and the shoot dry weight increased by increasing the phosphorus levels either in the absence or presence of nematode infection .The root weight decreased by increasing the VAM inoculum density in the presence or absence of nematode infection, while the shoot dry weight increased. Electrophoretic analyses of total protein on the tomato leaves of healthy and infected plants with *Meloidogyne incognita* in the presence or absence of mycorrhizal fungi at 500 chlamydospores/plant at low level of phosphorus (76.0 µg P/g of soil), was carried out by using sodium dodecyl sulfate (SDS) PAGE Two of 14 protein bands were detected only in the treatment of VAM,. However, five missing bands are specific markers for VAM+*M. incognita*. Results also indicate that eight protein patterns were missing in the leaves of infected tomato plants due to the nematode infection comparing with the

control, and four of these protein patterns, were observed again in the treatment of VAM+*M. incognita* comparing with nematode treatment . Further studies are needed to detect which proteins are responsible for that and the quantitative analysis are also needed.

INTRODUCTION

The protection of roots by mycorrhizal fungi against soilborne pathogens has received considerable attention in recent years.This attention have been developed soon after ectomycorrhizae plants were observed to be less susceptible to infection by root pathogens (Zak, 1964) . In 1973 Hussey and barker reported that the vesicular-arbuscular mycorrhizal fungi tend to make plants more tolerant to nematode attack.

Since plant-parasitic nematodes and VAMF are often intimately associated in young roots, an interaction betweent these two groups of organisms seems likely. Several investigations showed that VAMF can markedly alter plant responses to plant-parasitic nematodes Hussey and Roncadori (1982). Little and Maun, (1996) suggested that the VAMF were capable to reduce plant susceptibility to plant-parasitic nematodes.

The beneficial effect of VAMF on a nematode-susceptible plants offset damage caused by the root-knot nematode, this appear in several studies, Grandison and Cooper (1986) found that mycorrhizal inoculation improved plant growth and reduced nematode numbers (J_2) and adult development in roots in susceptible Lucerne cvs with combined inoculation . Oliveira and Zambolim (1986) recorded higher dry weight of the shoot, pod yields, nutrient uptake and fewer nematode eggs/g root than plants inoculated with only *M. javanica.* Chlamydospore production and percentage of mycorrhizal roots were not significantly affected by the nematode on *Phaseolus vulgaris* inoculated with *M. javanica* and VAM fungi. Mikhaeel *et al.,* (1997) found that the *Meloidogyne incognita* inoculation drastically reduced plant growth and nutrients uptake. Mycorrhizal colonization enhanced plant growth, nutrients uptake and decreased nematode galls, egg masses and egg as compared to nematode inoculation alone. Babu *et al.,* (1998) found that application of *Glomus fasciculatum* alone on maize reduced the population of *Pratylenchus zeae* and recorded the highest cob yield. Oliveira and Adversely, Pinochet *et al.,* (1997) found that the mycorrhizal colonization did not affect nematode (*Meloidogyne javanica*) buildup in the rootsof micropropagated 'Grand Name' banana (Musa AAA, although plants with the nematode and mycorrhiza were more galled.

Phosphorus have effect on development of nematode and mycorrhizal fungus, Thomson-Cason *et al.*, (1983) found that Walter tomato grown in high P soil had greater root weights, increased nematode penetration and egg production/plant and decreased colonization by *Gigaspora margarita* or *Glomus mosseca*, compared with plants grown in low P soil. The number of eggs/female nematode's on mycorrhizal and nonmycorrhizal plants was not influenced by P level, Oliveira and Zambolim (1986) recorded that phosphorus inhibited the nematode and development of the mycorrhizal fungus at >100 p.p.m.while

Carling *et al.*, (1989) of non-VAM and VAM plants was little affected by *M. incognita* at any P rate. Eggs production on VAM plants (eggs per root system and eggs per gram of root) was suppressed at the lowest P rate on soybean.

The aim of this work was to study the interactive effects of vesicular-arbuscular mycorrhizal (VAM)fungi and root-knot nematode (*Meloidogyne* spp.) on nematode-susceptible cultivars of tomato in soil at two levels of applied phosphate.

MATERIALS AND METHODS

(1) Collection, Extraction and Identification of the root-knot nematode

Root samples of diseased tomato plant were collected from El-Salhia region at El-Sharkia and some fields of Ismailia governorates. Single egg mass of a population of *Meloidogyne* species was used to make stock culture of this population and maintained on tomato (*Lycopersicon esculentum* Mill cv. Rutgers) in the green house at 24-28°C. Eggs and second stage juveniles (J_2) for all further studies were obtained from these green house cultures. Eggs were extracted from galled roots as described by Hussey and Barker (1973). Freshly hatched (J_2) were collected according to the procedure of Vrain (1977) by incubating egg masses on wet tissue paper supported by a plastic screen.For the root-knot identification on the basis of perineal pattern morphology (Taylor *et al.*, 1955., Taylor and Netscher 1974. and Eisenback *et al.*, 1981), live egg laying females were isolated from infected tomato plants by macerating galled roots. Posterior ends of females were cuts off in 45% lactic acid and preineal patterns sections were mounted in glycerin, and glass rod supports were used to support the cover slep.

(2) Collection, Extraction and Identification of the VAM Fungi:

A-Extraction of vesicular arbusculer mycorrhiza fungi (VAM)

Soil samples were collected from fields under different types of crops from Ismailia governorate. Each soil sample (1Kg) was first stirred thoroughly in a bucket containing 5L water and then allowed to settle for 15 sec, the contents of the bucket were then decanted through 0.5 mm sieve into a second bucket, in which the suspension was swirled vigorously and again allowed to settle for 15 sec. The supernatant was poured through a 0.036 mm sieve and the trapped material was washed into a beaker after stirring,. The material was transferred to 100 ml centrifuge tubes and centrifuged for 4 minutes at approximately 3000rpm (at sucrose solution 10%), the resulting supernatant was poured through a 0.036mm sieve and finally, the residue on the sieve was washed with water to remove the sucrose solution finally, the residue was transfer into a petri dish and examined under a binuclear and also under microscope. And collected by Pasteur pipet connected with vacuum pump Fig.(1)

B- Identification of the VAM Fungi:

The isolated fungi (VA-Mycorrhiza spores) were identified by Dr. Salah Ahmed Nasr, Assistant Professor of Microbiology, Fac. Agric., Suez Canal Univ. according to Gerdeman and Trappe (1974),

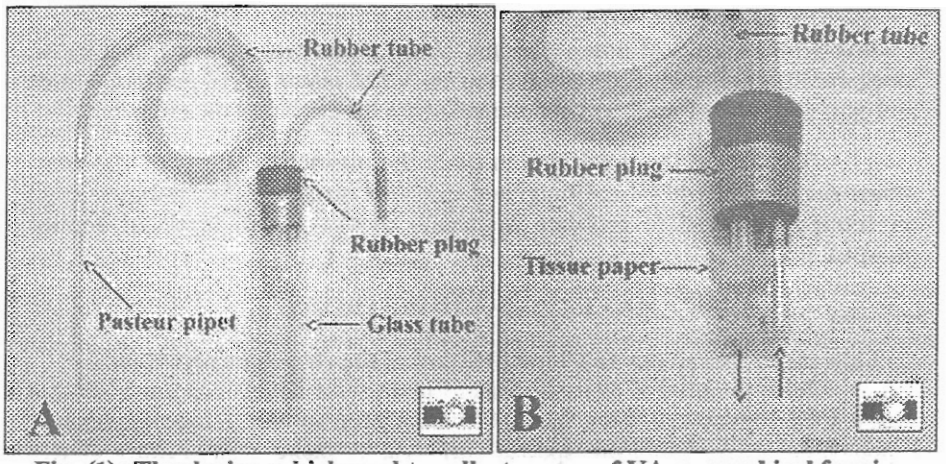


Fig. (1): The device, which used to collect spores of VA mycorrhizal fungi.

The experiment: -

Three tomato seedling (*Lycopersicon esculentum* Mill. cv. Rutgers), susceptible to *Meloidogyne* species, were planted/pot (15cm in diameter), one week later they were thinned to one seedling/pot. Twenty-four treatments were replicated five times as following:

1- Plants were inoculated by nematode (*Meloidogyne* sp.) 350 J_2/plant alone.
2- Plants were inoculated by nematode (*Meloidogyne* sp.) with different levels of phosphorus (76.0, 170.7µg P/g of soil added as rock Phosphate)
3- Plants were inoculated by VAM fungi alone as:
   A-125 chlamydospores/plant
   B-250 chlamydospores/plant
   C-500 chlamydospores/plant
4- Plants were inoculated by nematode (*Meloidogyne* sp.) and fungus (VAM) as in (A, B or C).
5- Plant were inoculated by nematode (*Meloidogyne* sp.), fungus (VAM) and different levels of phosphorus (76.0, 170.7µg P/g of soil added as rock Phosphate) as in (A, B or C).
6- Plants were left free to serve as a check.

In the end of the experiment (60 days) tomato plants were removed from the soil, shoot dry weight, VAM root colonization % and sporulation of VAM fungi were measured as well as the second stage juvenile were extracted from the soil counted.

Individual plants were inoculated with 125, 250 and 500 chlamydospores of mycorrhizal fungi. The inoculum was added to seedling into the nursery. Nematode inoculum was added to plant in the pots as a 2cm deep trench around the stem-base about 350 juvenile/plant, and was covered with soil after inoculation.

Root staining with trypan blue to detect arbusculer mycorrhiza and measuring root colonization by VAM fungi:

1- Freshly tomato Roots were stained, while the rest of the sample were preserved in 50% Ethanol.

2- The roots were rinsed thoroughly in tap water.
3- Aqueous solution of KOH (10% w/v) were placed under exhaust ventilation, either autoclave at 121°C for 3 min or heat in a water bath or on a hot-plate at 90°C for 10-30 min or leave for 24 hours at room temperature.
4- The roots were rinsed thoroughly in tap water. If necessary, bleach in alkaline H_2O_2 for up to 30 minutes
5- The root were soaked in 1%HCl overnight
6- Root were stained in acidic glycerol with 0.05% trypan blue for 24hr at room temperature. This method was described by Brundrett *et al.*, (1984)
8- Root were de-stained at room temperature in acidic glycerol according to Koske and Gemma (1989).
9- Randomly selected segments of fine lateral roots, were mounted on microscope slides to detect the presence of vesicles, arbuscules and any unusual features were noted.
10- The root colonization by VAM fungi were measured by method according to Brundrett *et al.*, (1996).

Phosphorus fertilization levels

Low P = 76.0 µg P/g of soil added as Rock Phosphate.

High Soil chemical analyses:

1- Total phosphorus : The wet ashing method by $H_2SO_2$and H_2O_2 were used for plant digestion. Total phosphorus uptake was determined colorimetrically by the chloromolybdo blue colour, while the digestion by $HClO_4$ was used to determined total phosphorus in soil according to Jackson (1973).
2- Total nitrogen, organic carbon, pH and Electrical Conductivity (EC) were determined according to Page *et al.*, (*1982*).
3 -Mechanical analysis was determined according to the method described by Piper (*1950*).
4- Cations and Anions were determined in the soil paste extract according to Richards (*1954*).

The soil was used in all experiments analysis of the raw soil gave the following results.

Chemical properties												
ECe	pH	O.M %	N µg/g	P µg/g	Cations meg/L				Anions meg/L			
					Ca^{+2}	Mg^{+2}	Na^{+}	K^{+}	CO_3^{-2}	HCO_3^{-}	Cl^{-}	SO_4^{-2}
4.51	7.80	0.448	500	90	6.83	6.53	30.38	1.36	-	2.0	25	18.10
Physical properties												
Particle size distribution							Texture grade					
Sand		Silt		Clay								
66%		14%		20%					Sand clay loam			

(Soluble cations and anions meg/L (in 1:1 extract))

P = 170.7 µg P/g of soil added as Rock Phosphate.

- The available P level, determined by double acid extraction, were approximately 20 and 50 µg P/g of soil for low P and high P.

Protein electrophoresis to determine the relative qualitative values of protein patterns of tomato leafs, the following methods were carried out.

Protein extraction: -

Tomato leafs after 60 days from planting were washed by distilled water and ground in a mortar. Total leafs proteins were extracted with extraction buffer (0.1gm/ml) which composed of 20 mM Na- borate buffer. 0.5 M NaCl, 1mM of Ethylenediamintetra-acetic acid (EDTA) at pH 8.9. After 12 hours of stirring, the extracted was centerifugated at 10.000 g for 20 minute to obtain the total protein as a supernatant (Tucci *et al.*, 1991).

Protein electrophoresis:-

The method of sodium Dodecyl Sulfate Polyacrylamid Gel Electrophoresis (SDS-PAGE) was carred out in 10% acrylamide slab gels following the system of Laemmli, *(1970)*; separating gels composed of 0.75M Tris-HCl pH 8.8, 10% SDS, 0.025% of N,N,N,N-tetramethylethylenediamine (TEMED) and 30% ammonium persulfate; stacking gels contained 0.75M Tris-HCl pH 6.8, 10% SDS, 0.025% TEMED and 30% ammonium persulfate; electrode buffer contained 0.025M Tris, 0.192M glycine, 0.1% SDS and pH 8.3. Electrophoresis was carried out with a current of 25mA and 130 volts per gel until the bromophenol blue marker reached the bottom of the gel after 3 hours. After electrophoresis, the gels were placed in the solution of coloration which was composed of 0.1% Coomassie blue G solution made of 1 gm Coomassie blue G, 23.5 ml phosphoric acid 85% and 10 gm ammonium sulphate and were dissolved in 1 liter distilled water at room temperature for 3 hours after staining, the slab gels were washed to remove the excess of in acetic acid 7% and distilled water. Then the gels were photographed. Standard proteins were used for molecular weight calibration of gels.

Statistical analysis

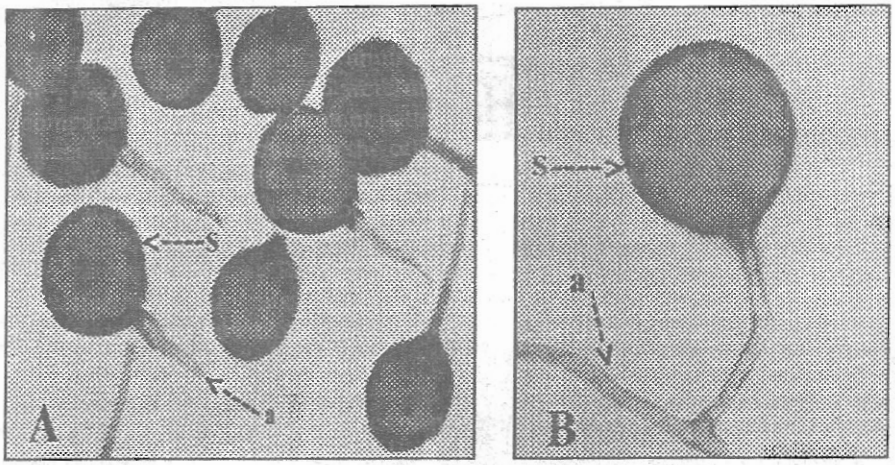
Completely randomized design was the statistical design used for the four experiments in this study. Data for each experiment was statistically analyzed using computer program (SPSS version 8) copyright by SPSS Inc. 1989-1997. Significant differences among the mean of different treatment treatments were carried out by Dancan's multiple range test ($P = 0.05$).

EXPERIMENTAL RESULTS

Nematodes isolated from infected tomato root and extracted from the soil, collected from rhizosphere, were showed that the second stage juvenile of *Meloidogyne* sp. were almost presented in the examined soil samples, while the other nematode developmental stages were detected within the infected roots.

Examination of the perineal patterns of the females which hand picked from infected tomato roots exhibit features typical for the species *Meloidogyne incognita*,which was dominant in most of the collected samples of tomato from Ismailia governorate.

VA mycorrhizal fungi were found in different soil samples collected from different locations in Ismailia governorate. The isolates were identified as *Glomus* sp., *Acalospora* sp. and *Gigaspora* sp. but most of the identified isolates belonged to the genus *Glomus sp.* Fig. (2) that was used in this experiment. The results of root staining with trypan blue to detect the structures of vesicular arbusculer mycorrhizal fungi in soil or in the root of tomato plant, which it is necessary step to measure the root colonization as a percentage were illustrated in Fig (3).



(100 X) (200 X)

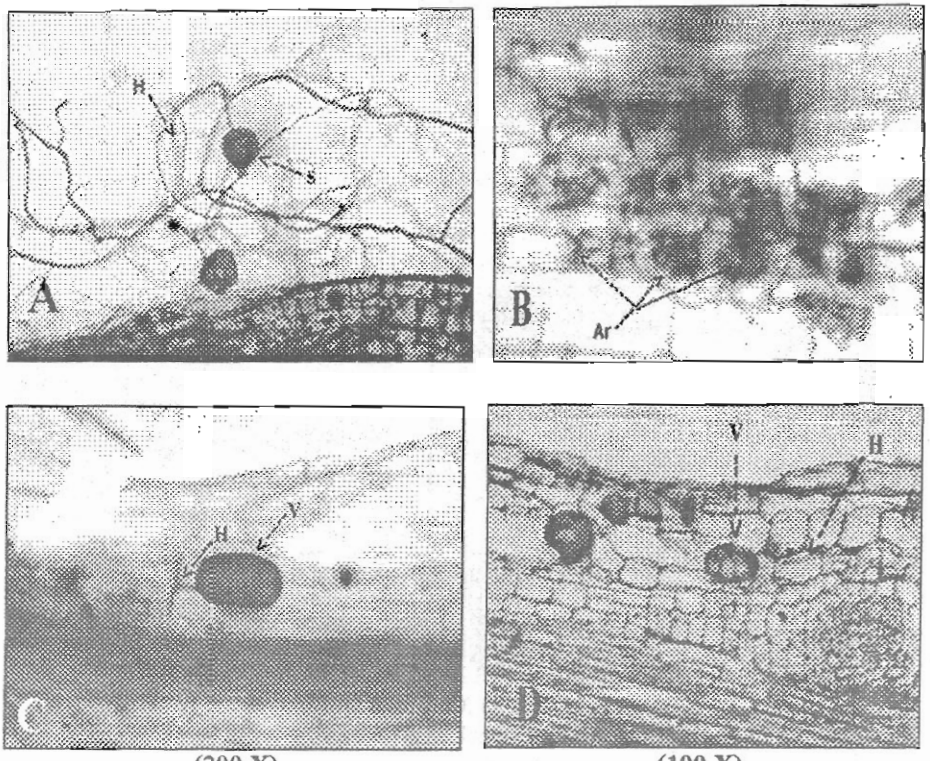
Fig. (2): A- Chlamydospores of a vesicular-arbusculer mycorrhizal fungus. s- Chlamydospore of *Glomus* sp.

a- The attachment hyphal (non septate when young and ranify within the cortex of infected plant)

B- Single spore of *Glomus* sp. at high power magnification .

Data present in Table (1) showed that in the absence of nematode infection, the lowest VAM root colonization percentage (39%) was detected at the highest level of P (170.7 µg P/g of soil) and VAM inoculum density 125 chlamydospores/plant of VAM fungi. While the highest VAM root colonization percentage (98%) was detected at P (76.0 µg P/g of soil) and VAM inoculum 500 chlamydospores/plant of VAM fungi. From the other hand, in the presence of nematode infection, the lowest root colonization percentage (33%) was observed at P (zero µg P/g of soil) and 125 Chlamydospores/plant of VAM fungi whereas the highest VAM root colonization percentage (89%) was observed at P (76.0 µg P/g of soil) and 500 chlamydospores/plant of VAM fungi.

Data presented in Table (2) show that the influence of soil phosphorus and *Meloidogyne incognita* on sporulation by VAM fungi on the tomato plant. In the absence of nematode infection the lowest VAM sporulation (300-spore/100 cm^3 of soil) was observed at zero µg P/g of soil and VAM inoculum 125 chlamydospore/plant. While the highest VAM sporulation (1400 spore/100 cm^3 of soil) was detected at P level (76.0 µg P/g of soil) and VAM inoculum density 500 chlamydospore/plant.



(200 X) (100 X)

Fig. (3): Structures of vesicular arbusculer mycorrhizal fungi in soil or in the root of tomato plant after clearing and staining.

(A): External mycelium in soil

H- A net work of hyphae forms in the soil with thicker hyphae which function as conduits and thin branched hyphae which are through to absorb nutrients.

S- Spore of *Glomus* sp. in the soil

(B), (C) and (D): Mycorrhizal structures in the root of tomato plant Ar-Arbuscules mycorrhizal infection in the inner most cortical cells . H -Hyphal of VAM in root (intercellural hyphae)

On the other hand, in the presence of nematode infection the lowest VAM sporulation (180 spore/100 cm^3 of soil) was detected at the highest level of P (170.7 µg P/g of soil) and VAM inoculum density 125 chlamydospore/plant. While the highest VAM sporulation (1350 spore/100 cm^3 of soil) was observed at P level (76.0 µg P/g of soil) and VAM inoculum density 500 chlamydospore/plant

Data presented in Table (3) show that the influence of soil phosphorus and mycorrhizal fungi on the numbers of second stage juvenile of *Meloidogyne incognita* on tomato plant. In the presence of nematode infection, the numbers of second stage juvenile of *Meloidogyne incognita* (J_2) per 150 g of soil were not

significantly differences (statistically) among at all treatments at tabulated data when comparing with a check. The lower value (274.60 J_2/150 g of soil) of population J_2/150g of soil was detected at P (76.0 µg P/g of soil) and VAM inoculum density 500 chlamydospore/plant comparing with all treatments at different rate of phosphorus with different of VAM inoculum density. On the other hand the highest value (298.8 J_2/150g of soil) was observed at P (170.7 µg P/g of soil) and VAM inoculum density zero chlamydospore/plant.

Table (1): Vesicular arbusculer mycorrhizal colonization of tomato roots growing in soil inoculated by VAM uninfested and infested with *Meloidogyne incognita* and treated with different phosphorus concentration.

Phosphorus fertilization rates (µg P/g of soil)	VAM root colonization %					
	In the absence of nematode infection			In the presence of nematode infection		
	VAM inoculum density			VAM inoculum density		
	125	250	500	125	250	500
Zero	45 [b]	76 [b]	90 [b]	33 [a]	66 [a]	79 [a]
76.0	50 [c]	83 [c]	98 [c]	43 [b]	75 [b]	89 [c]
170.7	39 [a]	70 [a]	86 [a]	41 [b]	70 [b]	82 [b]

The mean difference is significant at 0.05 level. The mean sample =3.00

Data presented in Table (4) show that the shoot dry weight of tomato plant after inoculation with different rates of VAM spores at different rates of phosphorus in the presence or absence of infection with nematode (*Meloidogyne incognita*). The reduction of the shoot dry weight reach almost 50% at zero µg P/g of soil and zero chlamydospore/plant as VAM inoculum density, comparing with non infected treatment. The lowest value of shoot dry weight (8.30 g) was observed at P level zero µg P/g of soil and zero chlamydospore/plant as VAM inoculum density. While the highest value (17.8 g) was detected at P level 76.0 µg P/g of soil and VAM inoculum density 500 chlamydospore/plant. On the other hand, in the absence of nematode infection the lowest shoot dry weight (15.47 g) was detected at P level zero µg P/g of soil and VAM inoculum density zero chlamydospore/plant. And the highest shoot dry weight (18.36 g) was observed at P level 76.0 µg P/g of soil and VAM inoculum density 500 chlamydospore/plant.

Data presented in Table (5) show the influence of the inoculation with different spore's concentration of VAM fungi and *Meloidogyne incognita* at different level of P on the root weight. In the absence of nematode infection, the lowest root weight (4.52 g) was detected at P level zero µg P/g of soil and 500 chlamydospore/plant as VAM inoculum density. While the highest value of root weight (7.86 g) was detected at P level 170.7 µg P/g of soil and VAM inoculum density zero chlamydospore/plant. On the other hand, in the presence of nematode infection the lowest root weight (6.32 g) was detected at P level zero µg P/g of soil and VAM inoculum density 500 chlamydospore/plant. And the highest root weight (9.33 g) was observed at P level 170.7 µg P/g of soil and VAM inoculum density zero chlamydospore/plant.

Table (2): Influence of soil phosphorus and infection of *Meloidogyne incognita* on sporulation by vesicular arbusculer mycorrhizal (VAM) fungi on tomato plant

Phosphorus fertilization Rates (µg P/g of soil)	Spores per 100 cm^3 of soil					
	In the absence of nematode infection			In the presence of nematode infection		
	VAM inoculum density			VAM inoculum density		
	125	250	500	125	250	500
Zero	300.0 [b]	437.0 [b]	950.0 [b]	285.0 [b]	320.0 [b]	900.0 [b]
76.0	412.0 [c]	687.0 [c]	1400 [c]	350.0 [c]	625.0 [c]	1350 [c]
170.7	240.0 [a]	367.0 [a]	855.0 [a]	180.0 [a]	280.0 [a]	832.0 [a]

The mean difference is significant at 0.05 level. The mean sample =3.00

Table (3): Influence of soil phosphorus and ycorrhizal fungi on the numbers of second stage juvenile of *Meloidogyne incognita* on tomato plant

VAM spores/plant	Phosphorus fertilization rate (µg P/g)			Means
	Zero	76	170	
	Number J_2 /150 g of soil			
Zero	290.00 [a]	282.60 [a]	298.80 [a]	290.46
125	284.20 [a]	281.52 [a]	291.60 [a]	285.70
250	283.20 [a]	278.60 [a]	284.20 [a]	282.68
500	280.40 [a]	274.60 [a]	283.00 [a]	279.33
Means	284.40	279.39	289.40	284.39

The mean difference is significant at 0.05 level. The mean sample =5.00

Table (4): Shoot dry weight of tomato plant after inoculation with different rates of VAM spores and nematoda (*Meloidogyne incognita*) at different rates of phosphorus fertilization

VAM Spores/ Plant	Shoot dry weight (g)					
	In the absence of nematode infection			In the presence of nematode infection		
	Zero µg P/g	76.0 µg P/g	170.7 µg P/g	Zero µg P/g	76.0 µg P/g	170.7 µg P/g
Zero	15.47 [a]	16.59 [a]	17.10 [b]	08.30 [a]	13.40 [a]	14.99 [a]
125	15.61 [a]	16.64 [a]	16.90 [a]	12.55 [b]	14.20 [a]	13.50 [a]
250	16.80 [ab]	17.78 [ab]	16.24 [ab]	14.33 [bc]	16.00 [b]	15.90 [ab]
500	17.45 [b]	18.36 [b]	17.57 [b]	15.27 [c]	17.80 [b]	16.30 [b]

The mean difference is significant at 0.05 level. The mean sample =5.00

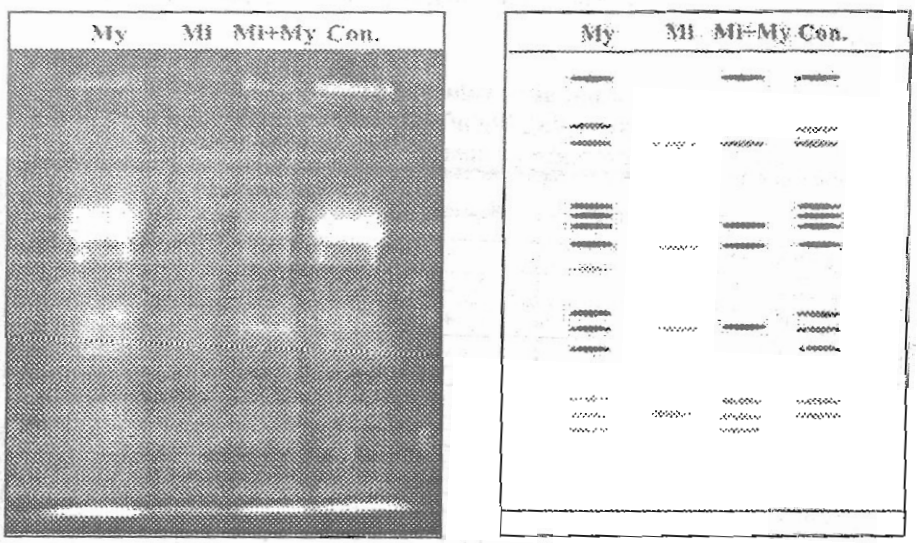
Data obtained in Table (6) show the comparative electrophoretic analyses of total protein on the tomato leaves of healthy and infected plants with *Meloidogyne incognita* in the presence or absence of mycorrhizal fungi at 500 chlamydospores/plant at low level of phosphorus (76.0 µg P/g of soil), was carried out by using sodium dodecyl sulfate (SDS) PAGE

Protein pattern of four treatments (*Meloidogyne incognita*, mycorrhizae, *Meloidogyne incognita* + mycorrhizae and the control) revealed much differences Fig. (4).

Table (5): Root weight of tomato plant after inoculation with different rates of VAM spores and nematoda (*Meloidogyne incognita*) at different rates of phosphorus fertilization

VAM Spores/ Plant	Root weight (g)					
	In the absence of nematode infection			In the presence of nematode infection		
	Zero µg P/g	76.0 µg P/g	170.7 µg P/g	Zero µg P/g	76.0 µg P/g	170.7 µg P/g
Zero	6.51 [a]	7.00 [a]	7.86 [a]	8.50 [a]	8.72 [a]	9.33 [a]
125	5.53 [a]	5.92 [ab]	6.84 [ab]	7.43 [ab]	7.92 [a]	8.82 [a]
250	5.31 [a]	5.66 [ab]	6.02 [ab]	6.51 [ab]	6.63 [a]	7.53 [ab]
500	4.52 [a]	4.81 [b]	5.20 [b]	6.32 [b]	6.50 [b]	7.00 [b]

The mean difference is significant at 0.05 level. The mean sample =5.0



(Gel protein pattern) **(Sketch of protein pattern)**

Fig. (4): The relative qualitative values of protein patterns of tomato leaves determined at 76.0µg P/g of soil and 500 chlamydospores of VAM fungi in the presence or absence of nematode Infection

Con.: Control **N:** Nematoda treatment
N/M: Nematoda and Mycorrhiza **M:** Mycorrhizal treatmen

The sketch observed in the same figure shows only 4-protein bands (light) out of 14 in treatment of nematode alone. The heavy protein bands which are not detected in *Meloidogyne incognita* pattern were found in the patterns of the control as well as in mycorrhizae and mycorrhizae + nematode (*Meloidogyne*

incognita) patterens. Dense spots which were absent in *Meloidogyne incognita* pattern, are present in sectors of *Meloidogyne* + mucorrhizas and mycorrhizas pattern. Many differences occurred in the patteren of *Meloidogyne incognita* alone and the patterns of *Meloidogyne incognita* + mycorrhizas. The comparison between *Meloidogyne incognita* and mycorrhizae patterens shows more differences than that between *Meloidogyne incognita* + mycorrhizas. Two protein bands in the control patteren are missing comparing with mycorrhizas patteren, while 9 and 6 bands were missing in the presence of *Meloidogyne incognita* and *M. incognita* + mycorrhizas patterns respectively.

The protein bands at RF (Running Factor) values 0.108, 0.154, 0.2, 0.231, 0.369, 0.477, 0.662 and 0.785 were missing in the presence of nematode infection comparing with control protein pattern. These bands consummation by nematode when infected the plant. On the other hand, the treatment of nematode + mycorrhizas the protein bands at RF values 0.108, 0.369 and 0.785 returned to observed comparing with nematode protein pattern.

The protein bands at RF values 0.415 and 0.954 were observed in mycorrhizae treatment but missing at all treatments without mycorrhizas (nematode and control) this bands formed only in the presence mycorrhizae infection.

Table (6): The relative qualitative values of protein patterns of tomato leaves determined at 76.0µg P/g of soil and 500 chlamydospores of VAM fungi in the presence or absence of nematode infection

RF	Control	Nematode	Nematode+ Mycorrhizae	Mycorrhizae
0.108	+	-	+	+
0.154	+	-	-	+
0.185	+	+	+	+
0.200	+	-	-	+
0.231	+	-	-	+
0.369	+	-	+	+
0.385	+	+	+	+
0.415	-	-	-	+*
0.477	+	-	-	+
0.631	+	+	+	+
0.662	+	-	-	+
0.785	+	-	+	+
0.815	+	+	+	+
0.954	-	-	+*	+*
Number of bands	12	4	8	14

+ = Present band (s) - = Lacking band (s) * = Specific band (s)

DISCUSSION

In the current study, the fungi (Mycorrhizas were studied as potential microbial control agents for *Meloidogyne* sp., which was found infected tomato roots, and as producers of natural compounds that could be efficacious for decreasing nematode population.

The species of root-knot nematode which isolated from the collected infected tomato root samples at Ismailia governorate, was identified as *Meloidogyne incognita*, which was reported repeatedly on tomato plants in Egypt (Oteifa, 1964 and Ibrahim *et al.*, 1986).

Mycorrhizal fungi (*Glomus* sp., *Acalospora* sp. and *Gigaspora* sp.) were found in different soil samples at Ismailia governorate and the genus *Glomus* was the most dominate, which used in this study. In the present study, the root colonization percentage of the VAM fungi on the tomato roots increased by increasing the VAM inoculum density regardless of the presence of nematode infection. These results are in agreement with those reported Saleh and Sikora (1984), they studied the interaction between *G. fasciculatum* and *M. incognita* on cotton in the greenhouse, and found that the inoculation of 30 spores/plant resulted in root colonization of only 38%, but at densities of 60,120 and 240 spores/plant, mycorrhizal root levels increased to 55-60% and at concentrations of 480 spores/plant, and root colonization levels of 87%.

Our results also revealed that the most suitable rate of P for VAM colonization on tomato roots and sporulation was the low P rate (76.0 µg P/g of soil) regardless of the presence of *M. incognita.* These results revealed that increased fertilization with P wasn't the essential role of these root symbionts and in the disease interactions with *M. incognita.* Several studies reported similar results (Thomson-Cason *et al.*, 1983 and Cooper and Grandison,1986). In addition, the sporulation of VAM fungi decreased in the presence of *M. incognita,* which may be due to the competition between both organisms or the host root become not suitable for fungal infection. These results are similar with those reported by Carling *et al.*, (1989).

The number of J_2/150 g of soil decreased by increasing VAM inoculum density at different levels of P. While this number of J_2 was increased at the high level of P (170.7µg P/g of soil) on the other hand the reduction of J_2 number was obtained at the low P level and 500 chlamydospore of VAM inoculum/plant. Our findings suggest that increased fertilization with P improved the host response, as a result of improved host nutrition, which increased the J_2 population and decreased VAM activity. Confirming this suggestion, the shoot dry weight of tomato increased by increasing P levels and increasing the VAM inoculum density, regardless of the presence of *M. incognita* (Cooper and Grandison 1986 and Carling *et al.*, 1989).

In the currant study, tomato plants grown in soil that contain 76.0 µg P/g of soil and 500 VAM chlamydospores/plant, increased tomato growth either

in the presence or absence of *Meloidogyne incognita.* Several studies reported the improve host response as a result of improved host nutrition, and competition or antagonism between the nematode and VAM, (Atilano *et al.*, (1981); Thomson-Cason *et al.*, (1983); Saleh and Sikora (1984); Heggo *et al.*, (1988); Tylaka *et al.*, (1991); Pinochet *et al.*, (1997) and Babu *et al.*, (1998)).

The root weight increased in the presence of nematode infection at the different level of P and different VAM spores concentration comparing with healthy plant. These results are expected. In addition the root weight increased by increasing P levels regardless of the presence *M. incognita.*On the contrary the root weight decreased by increasing VAM inoculum density in the presence of *M. incognita,* root knot nematode formed galls (hypertrophy and hypo-plasea) which cause increasing in the root fresh weight. Also root weight increased by increased P levels improved the plant growth this results are in harmony with Winkler *et al.*, (1999).

Results reported herein indicated that the total protein of tomato leaves could be analyzed by (SDS PAGE) to observe the specific protein which is related to the presence of VAM regardless of the presence of *M. incognita.* Results also revealed that there are some similarly in protein pattern between all treatments as well as they varied from each other. Two of 14 protein bands were detected only in the treatment of VAM, and 4 out of these 14 protein bands were found to be common in all treatments. In addition 2 bands are specific markers for VAM, however 5 are specific markers for VAM+*M. incognita.* Results also indicate that 8 protein pattern were missing in the leaves of infected tomato plants due to the nematode infection comparing with the control, and 3 of these protein pattern, were observed again in the treatment of VAM+*M. incognita* comparing with nematode treatment. These results focused on the role of VAM in improving the plant contents against the nematode infection. Further studies are needed to detect which proteins are responsible for that and the quantitative analysis are also needed.

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العلاقة التكافلية بين الفطريات الميكورزية المكونة للحويصلات والهيفات الشعيرية (VAMF) ونباتات الطماطم والمقاومة الحيوية لنيماتودا تعقد الجذور

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تم بحث تاثير التكافل الاندوميكوريزى على التفاعل بين فطريات الميكوريزا المكونة للحويصلات والهيفات الشعيرية ونباتات الطماطم القابلة للاصابة بالـــ Meloidogyne ونيماتودا تعقد الجذور *Meloidogyne incognita* عند مستويين من الفسفور ٧٥ و١٠٠ % من الكمية الموصى بها (٧٥ كم/ هكتار) فى الصوب الزجاجية. لقحت النباتات الفردية بالجراثيم الكلاميدية لفطريات الميكوريزا بمعدلات ١٢٥و٤٥٠ و٥٠٠ .فى مرحلة الشتلات فى المشتل . اضيفت النيماتودا الى النباتات فى الأصص عند عمق ٢سم حول قاعدة الساق بمعدل ٣٥٠يرقة/نبات .اوضحت النتائج ان استعمار الجذور بفطريات الميكوريزا والتجرثم زاد بزيادة كثافة القاح الميكوريزىفى حالة وجود اوعدم وجود العدوى بالنيماتودا ولم يزداد بزيادة معدل الفوسفور، بينما المعدل المنخفض من الفوسفور كان ملائم لزيادة الاستعمار لفطريات الميكوريزا فى وجود اوعدم وجود العدوى بالنيماتودا. تناقص تجرثم فطريات الميكوريزا فى وجود العدوى بالنيماتودا بالمقارنة بعدم وجود العدوى بالنيماتودا فى نفس المعاملة . زاد الوزن الجاف للمجموع الخضرى بزيادة كثافة اللقاح الميكوريزى

ومستوى الفوسفور فى حالة وجود اوعدم وجود العدوى بالنيماتودا . تناقص اعداد اليرقات لكل ١٥٠ تربة بزيادة كثافة اللقاح الميكوريزى عند كل مستويات الفوسفور. زاد وزن الجذر فى وجود العدوى النيماتودية عند مستويات الفسفور المختلفة اوتركيزات جراثيم فطريات الميكوريزا على العكس نقص وزن المجموع الخضرى. زاد وزن الجذر ووزن المجموع الخضرى بزيادة مستوى الفسفور فى حالة وجود اوعدم وجود العدوى بالنيماتودا . نقص وزن الجذر بزيادة كثافة اللقاح الميكوريزى فى وجود اوعدم وجود العدوى بالنيماتودا بينما تناقص الوزن الجاف للمجموع الخضرى. اجرى التحليل التفريدى للبروتين الكلى فى اوراق الطماطم السليمة والمصابة بالنيماتودا فى وجود اوعدم وجود فطريات الميكوريزا بمعدل ٥٠٠ جرثومة لكل نبات عند مستوى فوسفور منخفض (٧٥ ملجم فوسفور لكل جم تربة) باستخدام طريقة SDS-PAGE ظهرت حزمتين من ١٤حزمة بروتين فقط فى معاملات الميكوريزا كمعلمات نوعية. وبينما إختفت خمس حزم بروتين كمعلمات نوعية لمعاملة VAM+*M. incognita* . النتائج اوضحت ايضا ان ثمانية نماذج بروتين كانت غائبة فى اوراق نباتات الطماطم المصابة بالنيماتودا مقارنة بمعاملة الكنترول. هناك احتياج لمزيد من الدراسات لاكتشاف البروتينات المحددة للإصابة وتقديرها كميا.