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Interaction of VA Mycorrhizal Fungi and Root-Knot Nematode on Tomato Plants: Effects of Nemacur, Phosphorus and Infection Time

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Abstract

Pot investigations were designed to study the effect of inoculation time, phosphorus (P) and nemacur on the interaction between VAM fungi, *Glomus* sp, and root-knot nematode, *Meloidogyne incognita*, on tomato (*Lycopersicon esculentum*) growth. The results indicated that addition of P or *Glomus* sp significantly increased the shoot and root dry weight and P content. The addition of P, nemacur or *Glomus* sp significantly decreased the gall number in plants infected with *M. incognita*. Addition of P significantly decreased the mycorrhizal infection percentage, whereas, the addition of nemacur did not affect it. Shoot and root dry weight and P content were significantly increased, whereas, the gall number significantly decreased, when the mycorrhizal infection preceded the nematode infection. The results indicated that tomato growth could be improved by the addition of mycorrhiza, which could also be used as a control for root-knot nematodes.

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Introduction

Root-knot nematode is one of the major problems facing tomato production throughout the Sudan especially in localities of light soil (Zeidan ,15). The first detailed investigation of the problem was undertaken by Yassin (14), who referred to 3 species of the root-knot nematodes namely *Meloidogyne javanica*, *M. incognita* and *M. arenaria*. He stated that, *M. javanica* caused up to 70% loss of tomato and tobacco in central and western Sudan. Among the infected plants tomato, egg plant and papaya were found to be more susceptible than others.

VAM fungi in the Sudan have not received much attention. In 1988, Atabani (2) used 4 introduced species namely *Glomus mossae*, *Gigaspora margarita*, *Gigaspora calospora* and *Acaulospora sp* and studies their effects on hyacinth bean (*Lablab purpureus*) and soybean (*Glycine max*). Mahdi (10) reviewed the use of VAM fungi as a biofertilizer in Sudan, and suggested that VAM fungi has a great potentiality for using as a biofertilizer in this country whose soil was found to be very poor in available phosphorus.

Because VAM fungi and root fungal or nematode pathogens commonly occur together in root and rhizosphere of the same plant, the potential role of mycorrhizae as a biocontrol agents recently received attention (5). In general severity of diseases was decreased in VA mycorrhizal plants, and particularly VAM were found to limit nematode development, to reduce disease symptoms and improve growth of nematode-infested plants (8 & 9). The aims of this work were to study the effects of VAM fungi and root-knot nematodes on

tomato growth as influenced by addition of nemacur, Phosphorus and infection time of nematode or *Glomus* sp.

Materials and Methods

Seeds and Soils

Seeds of tomato variety Beto-86 were maintained in polyethylene bags and stored at 4°C. They were sterilized in 1.25% sodium hypochoride (chlorox) for 20 minutes and rinsed thoroughly in sterile distilled water. The soils was silt soil from the "Gerf" of River Nile bank; with the following characteristics: Sand%, 22; Silt%, 68; Clay%, 10; pH, 7.6; EC, (dS/m), 1.4; total P%, 0.016; total N%, 0.340.. Soil sterilization was carried out at 140°C for 2 hours. Plastic pots were sterilized by exposure to Ultra Violet Light for ten min.

Root-knot Nematodes: Source, Isolation, Collection and Preservation

In all experiments eggs of *Meloidogyne incognita* were used as inoculum, which were extracted from infected tomato roots. Nematode inoculum was maintained and multiplied in tomato plants grown in sterilized silt: sand (1:1 w/w) in pots. The number of eggs used in inoculation was 1000 eggs/plant. The number of eggs was adjusted with sterilized water to the required level and was poured in holes made at the base of the plant, one week after transplanting. The uninoculated plants received 10 ml. of sterilized water not

containing the eggs.

VAM fungi: isolation, collection and preservation

The strain used during this investigation was *Glomus* sp. which was isolated from onion plants grown in Shambat soil. The VAM strain was multiplied and maintained in open pot cultures of Sudan grass [Garawya] (Sorghum *bicolor* var. *sudanense*) grown in sterilized sand:silt (1:1 w/w). Inoculation of tomato seedling with *Glomus* sp was carried out according to Bagyaraj (3), in which 50 g/kg soil were placed 5 cm below surface of soil before sowing seeds.

Pot experiment (1)

Effect of inoculation time of *Glomus* sp and *M. incognita* on tomato plant

Glomus sp inoculum and/or *M. incognita* inoculum were either added at transplanting or 2 weeks later. Five treatments, each with 3 replicates were used. The treatments were:-

1. Plants inoculated with *M. incognita* at transplanting and *Glomus* sp 2 weeks later.
2. Plants inoculated with *Glomus* sp at transplanting and *M. incognita* 2 weeks later.
3. Both *M. incognita* and *Glomus* sp were added at transplanting.
4. Both *M. incognita* and *Glomus* sp were added 2 weeks after transplanting.
5. Uninoculated control plants.

Each treatment was replicated 3 times. Clay pots, 20-cm in diameter were filled with 4 Kg silty soil and sterilized in oven at 140°C for 2 hours. The nonmycorrhizal seedlings received ten ml of soil filtrate from Sudan grass cultures per plant passed through filter paper. This filtrate contained all the contaminating microorganisms except the VAM fungi. Plants were maintained in the Glass house at the Faculty of Agriculture in Shambat. Each plant received about 500 ml of tap water every 2 days. Pots were arranged in a randomized complete block design. Plants were harvested, 4, 6 or 9 weeks after transplanting. Roots were thoroughly washed from surrounding soil and the following parameters were determined: (a) Shoot and root dry weights, (b) The infection percentage was determined by root-slide technique (6), (c) for assaying nematode infection, the number of galls/plant were counted. Also, gall rating index (R.I.) from 0-5, where 0=0, =1-10; 2=11-30, 3=31-100, 4=100-200 and 5=more than 200 galls/ plant was determined (d) Shoot P Content which was determined by Vandomolybdate method (Chapman and Pratt, 1961) (4).

Pot experiment (2)

Effect of nemacur and *Glomus* sp on *M. incognita* infected tomato plants

The systemic nematicide Nemacur (commonly known as phenamiphos) was used in this experiment. It was applied in granular form containing 10% active ingredient (a i). One week before nematode inoculation, each plant received 5 grams Nemacur (0.5 g a i). One

of the following treatments were used:-

1. Plants inoculated with *Glomus* sp.
2. Plants inoculated with *M. incognita.*
3. Plants inoculated with *Glomus* sp and *M. incognita.*

Each treatment was replicated 4 times and either received or not received Nemacur. Details were otherwise as previously described for pot experiment (1).

Pot experiment (3)

Effect of phosphorus and *Glomus* sp on *M. incognita* infected tomato plants

Three replicates for each of the following treatments were used:-

1. Plants inoculated with *Glomus* sp.
2. Plants inoculated with *M. incognita.*
3. Plants inoculated with *Glomus sp and M. incognita.*
4. Un inoculated control plants.

Each of the 4 treatments received either Zero, 0.5 or 6.0 g P in form of KH_2PO_4/kg soil just before planting. Details were otherwise as previously described for pot experiment (1).

Results

Effect of inoculation time of *Glomus* sp and *M. incognita* on tomato plant

Shoot dry weights (Fig. 1) of plants inoculated with *Glomus* sp before *M. incognita* were significantly higher compared to that of plants inoculated with *M. incognita* before or even with *Glomus* sp. The figure, indicates that, inoculation of tomato plants with *Glomus* sp before *M. incognita* significantly reduced nematode damage compared to inoculation with *M. incognita* before or with *Glomus* sp. Effects of inoculation time with *Glomus* sp and *M. incognita* on root dry weight were similar to that of shoot dry weight (Fig. 1).

The number of galls increased with time of harvest (Table 1). Gall rating index (R.I.) of plants inoculated with *Glomus* sp before *M. incognita* was less than that of plants inoculated with *M. incognita* before *Glomus* sp, after 6 and 9 weeks from transplanting. The highest number of galls and rating index were recorded when *M. incognita* inoculum was added before *Glomus* sp, whereas, the lowest number was recorded when *Glomus* sp inoculum was added before *M. incognita* (Table 1). Also, simultaneous inoculation of *Glomus* sp and *M. incognita* after 2 weeks from transplanting, significantly ($P < 0.01$ reduced galls number compared to simultaneous inoculation at transplanting, only after 9 weeks from transplanting.

Table (1). Effects of inoculation time on mycorrhizal infection percentage (%), galls and rating index and (in parentheses) shoot phosphorus of mycorrhizal tomato plants infested with *M. incognita*.

Inoculation (weeks from transplanting)		Harvesting (weeks from transplanting)		
0	2	4	6	9
Mycorrhizal infection (%)				
NT*	NT	0.00	0.00	0.00
M. incognita	*Glomus* sp	28.67	48.00	53.67
Glomus sp	*M. incognita*	60.67	69.33	69.67
Glomus sp + *M. incognita*	NT	49.00	60.00	63.00
NT	*Glomus* sp + *M. incognita*	27.33	51.33	56.67
SE = ±		4.23	5.99	4.07
Galls and rating index				
NT	NT	0.0	0.0	0.0
M. incognita	*Glomus* sp	83 (3)	107 (4)	160 (4)
Glomus sp	*M. incognita*	33 (3)	47 (3)	72 (3)
Glomus sp + *M. incognita*	NT	70 (3)	80 (3)	105 (4)
NT	*Glomus* sp + *M. incognita*	51 (3)	67 (3)	86 (3)
Shoot phosphorus content (mg PO^{-3}_4 /g dry weight)				
NT	NT	10.63	12.51	15.01
M. incognita	*Glomus* sp	9.38	12.51	14.39
Glomus sp	*M. incognita*	16.26	18.77	25.02
Glomus sp + *M. incognita*	NT	12.51	14.39	18.77
NT	*Glomus* sp + *M. incognita*	10.01	10.88	14.39
SE = ±		2.48	2.71	4.09

* NT = No treatment.

Mycorrhizal infection percentages were increased with the time of harvest (Table 1). highest infection percentage was observed when *Glomus* sp was added before *M. incognita*, whereas, the lowest infection percentage was observed when *Glomus* sp was added 2 weeks after transplanting. The infection percentage of plants inoculated with both microorganisms after 2 weeks from transplanting was not significantly ($P < 0.01$) different from that of plants inoculated with nematode alone before mycorrhizal (Table 1). However, there was a significant difference between infection percentages of inoculated with both microorganisms at transplanting and that of plants inoculated with *Glomus* sp alone before *M. incognita*. The highest shoot phosphorus content was detected in plants received mycorrhiza before nematode, followed by plants received both microorganisms at transplanting (Table 1).

Effect of nemacur and *Glomus* sp on *M. incognita* infected tomato plants

Number of galls and gall rating index increased with harvesting time. No galls were developed in plants not inoculated with *M. incognita*. After 9 weeks from transplanting, the number of galls of plants inoculated with *M. incognita* were reduced by 78.8%, 54.7% and 89.9% when plants treated with nemacur, *Glomus* sp or with both *Glomus* sp and Nemacur, respectively. The mycorrhizal infection percentage was neither affected by the presence of Nemacur nor by *M. incognita*. No infection was observed in plants not inoculated with *Glomus* sp.

Table 2 Effect of Nemacur and *Glomus* sp on mycorrhizal infection percentage (%), galls and rating index (in parentheses) and shoot phosphorus of *M. incognita* infested tomato plants, nine weeks after transplanting.

Treatments	*Glomus sp*	*M. incognita*	*Glomus sp* + *M. incognita*
Mycorrhizal infection (%)			
SE = ±			
Without Nemacur	78.75	0.00	78.50
With Nemacur	78.00	0.00	77.50
Galls and rating index			
Without Nemacur	0 (0)	137 (4)	62 (3)
With Nemacur	0 (0)	29 (2)	18 (2)
Shoot phosphorus			
SE = ±			
Without Nemacur	32.50	8.80	22.50
With Nemacur	33.00	15.90	27.50

The phosphorus content of plants inoculated with *M. incognita* was significantly ($P < 0.01$) improved by *Glomus* sp, however, nemacur did not significantly affect the phosphorus content of mycorrhizal plants infested or not infested with *M. incognita*. The effect of nemacur on shoot and root dry weights (data not shown) showed a similar pattern to that of shoot phosphorus.

Effect of phosphorus and *Glomus* sp on *M. incognita* infested tomato plants

Addition of phosphorus significantly ($P < 0.001$) improved the dry weight of shoots and roots (Table 3) of tomato plants regardless of the presence or absence of *Glomus* sp and/or *M. incognita*. Shoot and root dry weights of mycorrhizal plants fertilized with 6 g P/kg soil was not significantly improved, compared with that of non-mycorrhizal plants. Although *M. incognita* reduced shoot and root dry weights of mycorrhizal and nonmycorrhizal plants at all levels of applied phosphorus, the magnitude of reduction was less in mycorrhizal than in nonmycorrhizal plants.

No galls were developed in plants not inoculated with *M. incognita* (Table 4). The number of galls of mycorrhizal plants infested with *M. incognita* was less than that of nonmycorrhizal plants infested with *M. incognita* at zero or 0.5 g P/kg soil. However, in plants received 6 g P/kg soil, the gall R. of mycorrhizal and nonmycorrhizal plants were similar. No infection was observed in nonmycorrhizal plants (Table 4). The highest

Table 3 Effect of phosphorus and *Glomus* sp on dry weights of shoots and roots (g/plant) of *M. incognita* infested tomato plants, six weeks after transplanting.

Treatments	P level (g/kg soil)		
		0.5	6.0
Shoot dry weight (g) SE ± 0.75			
Control	.12	1.66	6.41
Glomus sp	1.92	3.02	6.42
M. incognita	0.42	0.76	3.69
Glomus sp + M. incognita	1.03	.67	3.64
Root dry weight (g) S.E. ± 0.18			
Control	0.42	0.62	2.64
Glomus sp	0.68	1.27	2.61
M. incognita	0.15	0.23	1.40
Glomus sp + M. incognita	0.35	0.57	1.43

infection percentage was observed in plants fertilized with 0.5 g P/kg soil. Mycorrhizal infection percentage significantly ($P < 0.001$) reduced in plants fertilized with 6 g P/kg soil (Table 4). No significant differences were observed between the infection percentage of plants inoculated with *Glomus* sp alone and of plants inoculated with both *Glomus* sp and *M. incognita* at all phosphorus levels (Table 4).

In general, there was an increase in phosphorus content with increase in level of applied phosphorus (Fig. 2). Plants inoculated with *Glomus* sp showed the highest phosphorus content. However, at the highest level of applied phosphorus (6.0 g P/kg soil), the phosphorus content of control plants was not significantly different from mycorrhizal plants. Nematode infection significantly reduced the phosphorus content of tomato plants.

Discussion

In all experiments, root-knot nematode Meloidogyne incognita formed conspicuous galls in roots of tomato plants and reduced the shoot and root dry weights and phosphorus content of shoots. Price *et al.* (13) reported similar results for cotton. The improvement of dry weight and phosphorus content by *Glomus* sp in phosphorus deficient soil is not a new concept and was documented for different crops (McArthur & Knowles, 12). The VAM fungus *Glomus* sp also significantly improved the dry weights and phosphorus content of nematode-infected plants. These results are compatible to those obtained by Bagyaraj *et al.*(3) and Heald *et al.*(9).

Table 4 Effect of phosphorus and *Glomus* sp on mycorrhizal infection percentage and galls and gall rating index (in parenthesis) of *M. incognita* infested tomato plants, six weeks after transplanting.

Treatments	P level (g/kg soil)		
		0.5	6.0
mycorrhizal infection SE= ± 4.08			
Control	0.00	0.00	0.00
Glomus sp	73.67	86.67	18.00
M. incognita	0.00	0.00	0.00
Glomus sp+M. incognita	72.33	88.00	17.67
Galls and rating index			
Control	0.0 (0)	0.0 (0)	0.0 (0)
Glomus sp	*0.0 (0)*	*0.0 (0)*	*0.0 (0)*
M. incognita	68 (3)	113 (4)	305 (5)
Glomus sp + M. incognita	32 (3)	45 (3)	313 (5)

The benefits of *Glomus* sp in improving tomato growth and reducing nematode damage were more pronounced when *Glomus* sp was added before *M. incognita*. Also, the root-knot nematode *M. incognita* didn't affect mycorrhizal infection percentage. However, when nematode was added before or at the same time with mycorrhiza, mycorrhizal infection percentage was reduced. This could be attributed to the competition for space between root-knot nematode and VAM fungi (5). The results reported in this investigation, indicate that, the nematicide, nemacur, successfully reduced *M. incognita* infestation and damage, and did not affect the VAM fungus *Glomus* sp. Similar results were reported by Marks *et al.* (11).

Application of phosphorus significantly increased the dry weights of shoots and roots and phosphorus content of tomato plants irrespective of the presence or absence of mycorrhiza and/or nematodes. The response of tomato growth to *Glomus* sp inoculation was reduced by application of high concentration of phosphorus fertilizers (5). The detrimental effect of phosphorus on reducing VAM benefits was also observed by Heald *et al.* (9) and Price *et al.* (13). Reduction in dry weights imposed by *M. incognita* was partially eliminated by phosphorus application. This may be due to the improved phosphorus content of the plant and not to reduction in nematode infestation, because number of galls were increased by phosphorus addition. According to Heald *et al.* (9), the improvement of growth of muskmelon infected with *M. incognita* was attributed to improved nutrition status and not to reduction in nematode infection. The role of phosphorus in reducing the impact of root-

knot nematodes on plant growth was also suggested by Fayad and Sweelam (6). The reduction of gall numbers caused by *Glomus* sp was affected by addition of phosphorus. When 6 g, P/kg soil had been added, *Glomus* sp failed to reduce gall numbers. This could be attributed to low mycorrhizal infection percentages associated with high phosphorus level (Table 4). Reduction in mycorrhizal infection percentage as a result of application of phosphorus fertilizers was clearly documented (Amijee *et al.*, 1). The results indicated that tomato growth could be improved by the addition of mycorrhiza, which could also be used as a control for root-knot nematodes.

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