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Interaction of VA mycorrhiza and root-knot nematode on tomato plants: effects of nematode inoculum density, soil texture and soil sterilization

E. A. E. Elsheikh and A. M. O. Mirghani

Department of Biochemistry and Soil Science, Faculty of Agriculture, University of Khartoum, Shambat, Sudan.

Abstract

The impact of VA-mycorrhizal fungi and root-knot nematodes, on tomato (*Lycopersicon esculentum*) plants, and the possibility of reducing the harmful effects of root-knot nematodes using VAM fungi, was carried out in pot experiments. The VAM fungus *Glomus* sp. significantly increased shoot and root dry weights and phosphorus (P) content of tomato plants infected or not infected with nematodes, whereas, *Meloidogyne incognita* significantly reduced them, in sterilized and unsterilized natural soil. Number of galls (nematode infection) on infected roots, was significantly increased with an increase in nematode inoculum density and significantly reduced by *Glomus* sp., whereas, mycorrhizal infection percentage was not significantly affected by *M. incognita*. Mycorrhizal infection percentage and number of galls were not affected by soil texture, however, better growth of mycorrhizal plants was observed in silt than in clay soils. The results indicated that the use of *Glomus* sp. is a promising technique for agricultural production, and could be used as a biofertilizer and a biocontrol agent.

تداخل المايكورايزا و نيماتودا العقد الجدريه علي نبات الطماطم: أثر كثافة النيماتود و قوام و تعقيم التربه

الصديق احمد المصطفي الشيخ و ايمن محمد عثمان ميرغني

قسم الكيمياء الحيوية و التربة، كلية الزراعه، جامعة الخرطوم

تمت دراسة اثر فطر المايكورايزا ونيماتودا العقد الجدرية على نبات الطماطم، ادي التلقيح بالمايكورايزا سلالة *Glomus* sp. الي زيادة معنوية في الاوزان الجافة للمجموع الخضرى و الجذرى ومحتوى الفسفورلنبات الطماطم، في وجود نيماتودا العقد الجدرية و عدمه. ادي التلقيح بنيماتودا العقد الجدرية الي نقص معنوي في الاوزان و محتوى الفسفور. ازدادت اعداد العقد الجذرية زيادة معنوية بزيادة كثافة النيماتودا المضافة، بينما نقصت معنوياً باضافة فطر المايكورايزا. لم تتأثر نسبة الاصابة في المايكورايزا عند اضافة النيماتودا, كما لم تتأثر بنوع قوام التربة. كان نمو نبات الطماطم في التربة السلتية افضل من التربة الطينية. اظهرت النتائج ان فطر *Glomus* sp. يمكن الاستفادة منه في الاسمدة البيولوجية حيث انه يعمل كسماد حيوى و يساعد في تقليل الاصابة بالامراض.

Introduction

Root-knot disease is one of the major problems facing crop production throughout the Sudan. The disease is caused by root-knot nematodes, which belong to the genus *Meloidogyne*. Among the susceptible crops the most important are tomato, egg plant, papaya and cotton. The losses due to nematode damage in tomato, egg plant, cotton and coffee were 50% or more (Sasser 1979; Yassin 1974; 1978). Indirect losses associated with root-knot disease are caused by secondary attack of other pathogens, inefficient utilization of fertilizers and water and high cost of chemical treatments. Vesicular-arbuscular mycorrhizal (VAM) fungi are beneficial soil fungi which form a symbiotic association with roots of many plants. Many reviews about the importance of VAM fungi in agriculture have been published (Harley 1989; Smith *et al.* 1992).

The subject of biological antagonism in the rhizosphere and root region is of long standing. The VAM fungi and root-knot nematodes are members of the microbial population of the root region and they can compete with each other for the same site in the rhizosphere. Hence, the beneficial VAM fungi might be expected to reduce or even eliminate the harmful effects imposed by root-knot nematodes. In plants infected with both mycorrhizal fungi and nematodes, mycorrhizal inocula-

tion substantially reduced adult nematode development (Cooper and Grandison 1986; Suresh *et al.* 1985). Therefore, the concept of using VAM fungi as a biofertilizer and a biocontrol agents is a promising perspective of these fungi. The aims of this work were to study the effects of VAM fungi and root-knot nematodes on tomato growth as influenced by nematode inoculum density, soil texture and sterilization.

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 min. and rinsed thoroughly in sterile distilled water. Two types of soils were used during this investigation, clay soil from Shambat area and silt soil from the "Gerf" of River Nile bank. Soil sterilization was carried out at 140 °C for two hours. Plastic pots were sterilized by exposure to ultra violet light for 10 min.

Source, isolation, collection and preservation of root-knot nematodes

The species used during this work was *Meloidogyne incognita*. In all experiments eggs 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 per plant except in the experiment in which different nematode inoculum densities were used. The number of eggs was adjusted with sterilized water to the required level and was poured into 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.

The 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 *et al.* (1979), in which 50 g kg^{-1} soil were placed 5 cm below surface of soil before sowing seeds.

Pot experiment (1)

Effect of nematode inoculum density on mycorrhizal tomato plants

Four weeks old mycorrhizal tomato seedlings were transplanted at the rate of one seedling per pot. Mycorrhizal tomato plants received *Meloidogyne incognita* inoculum at the rate of zero, 500, 1000 or 2000, eggs/plant. The treatments were:

1- inoculated with *Glomus* sp.;
2- inoculated with *Glomus* sp. + 500 eggs;
3- inoculated with *Glomus* sp. + 1000 eggs and
4- inoculated with *Glomus* sp. + 2000 eggs

Each treatment was replicated three times. Clay pots. (20 cm in diameter) were filled with four Kg silty soil and sterilized in oven at 140 °C for two hours. Plants were maintained in the Glass house at the Faculty of Agriculture, Shambat. Each plant received about 500 ml of tap water every two days. Pots were arranged in a randomized complete block design. Harvesting was carried out at intervals of four, six and nine 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 (Giovanetti and Mosse 1980), (c) for assaying nematode infection the number of galls per plant were counted. Also, gall rating index (R.I.) from 0-5, where 0 = 0, 1 = 1-10; 2 = 11-30, 3 = 31-100, 4 = 100-200 and 5 = more than 200 galls per plant was determined and (d) Shoot P Content which was determined by Vandomolybdate method.

Pot experiment (2)

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

Two soils were used, silt soil (Gerf) and clay soil (Shambat). The treatments were:

1- plants inoculated with *Glomus* sp.;
2- plants inoculated with *M. incognita* (1000 egg /plant) and

3- plants inoculated with *Glomus* sp. and *M. incognita* (1000 egg per plant).

The nonmycorrhizal seedlings received 10 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. Each treatment was replicated three times and raised on both types of soil. Details were otherwise as previously described for pot experiment (1).

Pot experiment (3)

Effect of Glomus *sp. on* M. incognita *infected tomato in unsterilized soil*

In this experiment, unsterilized clay (Shambat) soil was used. Treatments and details were otherwise as previously described for pot experiment (2).

Results

Effect of nematode inoculum density on mycorrhizal tomato plants

Shoot dry weights of mycorrhizal tomato plants significantly decreased with the increase in nematode inoculum densities (Table 1). However, no significant differences were observed in shoot dry weights of plants inoculated with 1000 or 2000 eggs/plant after four and six weeks from transplanting. After nine weeks from transplanting, the shoot dry weights of mycorrhizal plants were reduced by 39.5%, 53.5% and 56.4%, when 500, 1000 and 2000 nematode eggs/plant were added, respectively. Root dry weights

Table 1: Effects of nematode inoculum density on shoot and root dry weight (g per plant), mycorrhizal infection percent and phosphorus content of mycorrhizal tomato plants, nine weeks after transplanting.

	Number of eggs of *M. incognita* per plant				
	0.00	500	1000	2000	SE±
Shoot dry weight (g)	5.48	3.31	2.55	2.39	0.11
Root dry weight (g)	2.58	1.44	1.16	1.14	0.09
Mycorrhizal infection%	89.00	88.0	88.66	85.6	5.36
Phosphorus content	42.5	35.0	29.5	27.3	

(Table 1) showed similar results to that of shoot dry weights. Mycorrhizal infection percentages were not affected by the different levels of nematode inocula (Table 1). The highest P content was observed in plants not inoculated with nematodes (Table 1). Also, there was a significant reduction in P content with increasing nematode inoculum levels. However, P content of plants inoculated with 1000 eggs/plant was not significantly different from that of plants inoculated with 2000 eggs/plant.

Number of galls increased with the harvesting time and the nematode inoculum density (Table 2). No galls were developed in plants not inoculated with nematodes. The rating index (R.I.) of plants inoculated with 2000 eggs/plant was not significant compared to that of plants inoculated with 1000 eggs/plant. But, the R.I. of plants inoculated with 1000 or 2000 eggs/plant was greater than that of plants inoculated with 500 eggs/plant at four, six and nine weeks after transplanting.

Table 2: Effects of nematode inoculum density on galls number and mean gall rating index (in parentheses) of mycorrhizal tomato plants. The number of galls, after nine weeks, best fit in a polynomial equation ($Y = 0.6 + 0.15X + 0.0005X^2$; $R^2 = 0.99$, where X is the inoculum density and Y is the number of galls per plant.

Nematode inoculum density (eggs/plant)	Harvesting time (weeks after transplanting)		
	4	6	9
0	0 (0)	0 (0)	0 (0)
500	23 (2)	39 (2)	64 (3)
1000	49 (3)	66 (3)	100 (4)
2000	53 (3)	72 (4)	110 (4)

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

The VAM fungus *Glomus* sp. significantly improved shoot and root (Table 3) dry weights of nematode-infested plants in both silt and clay soils. Shoot and root dry weights of mycorrhizal plants not infested with nematodes were significantly higher in silty than in clay soil; whereas, shoot and root dry weights of nematode-infested plants were not significantly affected by soil type. On the other hand, *M. incognita* significantly reduced shoot and root dry weights of mycorrhizal plants in both silty and clay soil.

The number of galls of mycorrhizal plants inoculated with *M. incognita* was significantly less than that of nonmycorrhizal plants after four, six and nine weeks from transplanting in both silt and clay soil. After nine weeks from transplanting, the VAM fungus *Glomus* sp. reduced number of galls of nematode-infested plants by 57% in slity soil and 53%, in clay soil (Table 3).

Although mycorrhizal infection percentages increased with harvesting time in both soils (data not shown), it was neither significantly affected by *M. incognita* nor soil type (Table 3). The highest P contents were detected in plants inoculated with *Glomus* sp. in both silt and clay soil, whereas the lowest P contents were detected in plants inoculated with *M. incognita* in both silt and clay soil (Table 3).

Effect of Glomus *sp. on* M. incognita *infected tomato in unsterilized soil*

Shoot dry weight (Table 4) was significantly improved and reduced by *Glomus* sp. and *M. incognita*, respectively. Although *M. incognita* reduced the shoot dry weight of both mycorrhizal and nonmycorrhizal plants, the reduction was greater in nonmycorrhizal than in mycorrhizal plants. After nine weeks from transplanting, *Glomus* sp. improved the shoot dry weight by 79.6%, compared to uninoculated control plants, whereas *M. incognita* reduced shoot dry weight of mycorrhizal and nonmycorrhizal plants by 54.6% and 79.6%, respectively. Root dry weights also showed similar trend to that of shoot dry weights. *Glomus* sp. successfully infested tomato roots (Table 4). The mycorrhizal infection percentage in nonmycorrhizal

Table 3: Effects of soil texture on shoot and root dry weight (g per plant), mycorrhizal infection percent, phosphorus content and gall number (per plant) of mycorrhizal tomato plants, nine weeks after transplanting

	Glomus sp.	*M. inognita*	*Glomus* sp.+ *M. incognita*	SE±
Shoot dry weight (g)				
Silt	4.86	1.04	2.37	0.06
Clay	4.26	1.09	2.42	0.06
Root dry weight (g)				
Silt	2.31	0.20	1.08	0.02
Clay	2.20	0.21	1.07	0.02
Mycorrhizal infection %				
Silt	90.66	0.00	89.66	2.31
Clay	90.33	0.00	89.33	2.31
Phosphorus content				
Silt	38.10	11.80	26.00	3.16
Clay	33.50	10.20	24.60	3.16
Gall numder				
Silt	0.00	220.0 (5)	85.0 (3)	-
Clay	0.00	213.0 (5)	90.0 (3)	-

plants was very low. Also, *M. incognita* did not affect the mycorrhizal infection percentage. After nine weeks from transplanting, the number of galls of plants inoculated with both *Glomus* sp. and *M. incognita* was 55% less than that of plants inoculated with *M. incognita* alone (Table 4). No galls were developed in plants not inoculated with *M. incognita*. The gall rating index (R.I) of mycorrhizal plants was less than that of nonmycorrhizal plants.

The P content was increased in plants inoculated with *Glomus* sp. irrespective of the presence or absence of *M. incognita* (Table 4). Also, *M. incognita* reduced the P content of both mycorrhizal and nonmycorrhizal plants, but the reduction was greater in nonmycorrhizal than in mycorrhizal ones.

Table 4: Effects of *Glomus* sp. and *M. incognita* on shoot and root dry weight (g per plant), mycorrhizal infection percent, phosphorus content and gall number and gall rating index (in parentheses) of tomato plants, nine weeks after transplanting in unsterile soil.

	Control	*Glomus sp.*	*M. incognita*	*Glomus sp. + M. incognita*	SE±
Shoot dry weight (g)	1.39	2.67	0.46	1.20	0.06
Root dry weight (g)	0.44	0.88	0.12	0.39	0.02
Mycorrhizal %	1.50	83.3	2.00	80.13	3.79
Phosphorus content	24.9	37.2	0.99	23.3	3.17
Gall number	0.00	0.00	215 (5)	96 (3)	

Discussion

In all experiments, *M. incognita* formed conspicuous galls in roots of tomato, which were significantly greater in nonmycorrhizal than in mycorrhizal plants. The reduction in galls number caused by mycorrhizal inoculation was also observed by Bagyaraj *et al.* (1979). The tolerance of mycorrhizal plants to nematode infection reported here agreed with that documented in many crops such as cotton (Salih and Sikora 1984) and *Piper nigrum* (Sivaprasad *et al.*, 1992). The tolerance of mycorrhizal plants to nematode damage was attributed to changes in root physiology which in turn reduced nematode penetration and/or retarded adult nematode development (Grandison and Cooper, 1986). These physiological changes were described by Heald *et al.* (1989) as increased concentration of lignin, sugar, amino acids, phenol synthesis, and ethylene production.

The *Glomus* sp. successfully infected tomato roots forming typical VAM structures. The dry weight and P

content were significantly improved by *Glomus* sp. in both healthy and nematode-infected plants. This was obvious since the soils used were deficient in P. These results agreed with those obtained by Bagyaraj *et al.* (1979) and Heald *et al.* (1989). According to Cooper and Grandison (1986), the hyphae of VAM fungi were able to transport water and nutrients through blocked and distorted tissues caused by nematode infection.

The dry weights and P content of mycorrhizal tomato plants were significantly reduced with the increase in nematode inoculum density, whereas, the number of galls and gall rating index (R.I.) were significantly increased. This could be attributed to the increase in the nematode population infecting the root. However, no significant differences were observed in mycorrhizal infection percentage of plants inoculated with different levels of nematode inoculum (Table 1). Sivaprasad *et al.* (1992) found that the mycorrhizal infection percentage of *Piper nigrum* was not affected by root-knot nematodes. Similar results were also obtained in alfa alfa (Grandison and Cooper 1986). However, mycorrhizal infection percentage of *Cucumis melo* was reduced by *M. incognita* (Heald *et al.* 1989); whereas, that of cotton was increased (Salih and Sikora 1984). The response of tomato to VAM inoculation in unsterilized soil containing low VAM population was also observed by Smith *et al.* (1986).

In this investigation, the dry weights of mycorrhizal plants was significantly higher in silt than in clay soil. However, the mycorrhizal infection percentages were not significantly affected by soil texture (Table 3). This indicates that, the effect of VAM fungus *Glomus* sp. on dry weights of tomato plants grown in silt and clay soil was similar. But, the higher dry weight observed in plants grown in silty soil could be attributed to relatively better nutrient content of the silty soil. Mahdi and Atabani (1992) found that, the response of soybean and lablab-bean to VAM inoculation was not significantly different between plants grown in sandy and clay soil. They suggested that, it was the nutritional status of the soil that determined the dependance of the host on, and the response to VAM fungi. The results also showed that the P content was more in plants grown in silty soil. This also might be due to better P content in silty than in clay soil.

The VAM fungus *Glomus* sp. improved dry matter production, P content and reduced gall numbers of tomato plants in natural unsterilized soil. This could not be attributed only to P deficiency of the soil, but also to the low natural population of VAM fungi in the soil. The benefits of VAM inoculation in improving plant growth in natural unsterilized soil depends mainly upon natural population of VAM fungi in that soil; beside P content of the soil.

References

Bagyaraj, D., Manjunath, A., & Reddy, D., 1979. Interaction of vesicular-arbuscular mycorrhiza with root- knot nematodes in tomato. *Plant and Soil*, **51**. 397-404.

Cooper, K. M., and Grandison, G. S., 1986. Interaction of vesicular-arbuscular mycorrhizal fungi with root-knot nematodes on varieties of tomato and white clover susceptible to M. hapla. *Annals of Applied Biology*, **108**, 555-565.

Harley, J. L., 1989. The significance of mycorrhiza. *Mycological Research*, **92**, 129-139.

Heald, C. M., Burton, B. C., and Davis, R. M., 1989. Influence of *Glomus* intraradices and soil phosphorus on Meloidogyne incognita infecting Cucumis melo. *Journal of Nematology*, **21**, 69-73.

Giovanetti, M., and Mosse, B, 1980. An evaluation of techniques for measuring VAM infection in roots. *New Phytologist*, **84**, 489-500.

Grandison, G. S., and Cooper, K. M., 1986. Interaction of vesicular-arbuscular mycorrhizal fungi and cultivars of alfa alfa susceptible and resistant to Meloidogyne hapla. *Journal of Nematology*, **18**, 141-149.

Mahdi, A. A., and Atabani, E. M., 1992. Response of Bradyrhizobium-inoculated soybean and lablab bean to inoculation with vesicular-arbuscular mycorrhiza. *Experimental Agriculture*, **28**, 243-249.

Salih, H., and Sikora, R. A., 1984. Relationship between *Glomus* fasiculatus root colonization of cotton and its effect on Meloidogyne incognita. *Nematogica*, **30**, 230-237.

Sasser, J. N., 1979. Pathogenicity, host range and variability in Meloidogyne spp. In: *Root-Knot Nematodes (Meloidogyne spp) Systemic, Biology and Control*. Lamberti, F. and Tayler, C.E. (eds) Academic Press, London, UK.

Sivaprasad, R., Jacob, A., Sulochana, K., and George B., 1992. Root-knot nematode infestation and phosphorus nutrition in Piper nigrum as influenced by vesicular-arbuscular mycorrhiza. *Malaysian Plant Protection Society*, **6**, 34-37.

Smith, G. S., Rondcadori, R. W., and Hussey, R. S., 1986. Interaction of endomycorrhizal fungi, superphosphate and Meloidogyne incognita in cotton in microplot and field conditions. *Journal of Nematology*, **18**, 208-216.

Smith, S. E., Robson, A. D., and Abbott, L. K., 1992. The involvement of mycorrhiza in assessment of genetically dependent efficiency of nutrient uptake and use. *Plant and Soil*, **146**, 169-179.

Suresh, C. K., Bogyara, D. J., and Reddy, D. R., 1985. Effect of vesicular-arbuscular mycorrhiza on survival, penetration and development of root-knot nematode in tomato. *Plant and Soil*, **87**, 305-308.

Yassin, A. M., 1974. Root-knot nematode in the Sudan and their chemical control. *Nematologie Mediteranae* **2**, 102-112.

Yassin, A. M., 1978. Root-knot nematode in the Sudan. *Research and Planning conference*, Egypt. Cairo Jan-Feb 1978.