African Journal of Agricultural Research Vol. 2(4), pp. 159-163, April, 2007
Available online at http://www.academicjournals.org/AJAR
ISSN 1991- 637X © 2007 Academic Journals

Full Length Research Paper

The role of eggs inoculum level of *Meloidogyne incognita* on their reproduction and host reaction

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Accepted 26 February, 2007

Two pot experiments were separately conducted to determine the influence of four or three inoculum levels (0, 250, 500 and 1000 or 0, 1000 and 2000 nematode eggs/ 850 g soil/pot) of *Meloidogyne incognita* on population density of the nematode and host reaction of two solanacious plants viz tomato cv. Castle rock or pepper cv. Anaheim under partly controlled greenhouse at 23 ± 4°C. Nematode reproduction and host damage were both affected by the initial inoculum levels and revealed a reduction in total the fresh weight of the plants as the inoculum level increased from 250 to 1000 eggs/ tomato plant with values of 18.6 and 43.9%. The rate of build up of *M. incognita* on tomato increased from 1.14 to 1.48, respectively. When the initial inoculum (Pi) level was increased up to 2000 eggs per pepper plant, the percentage reduction of whole plant fresh weight (73.2%) and shoot dry weight (55.3%) as well as rate of nematode build-up (1.49) also obviously increased. Galls and egg masses/root system increased as Pi was increased on both host plants. Regression analysis of Pi vs rate of nematode build-up either on tomato or pepper plants gave values of R^2 amounted to 0.6904 or 0.8149, respectively. This means the susceptibility of tomato cv. Castle Rock to *M. incognita* infection was more than did pepper cv. Anaheim under greenhouse condition.

Key words: Population density, pepper, tomato, inoculum level, *Meloidogyne incognita.*

INTRODUCTION

Tomato, *Lycopersicon esculentum* Mill, and pepper, *Capsicum annuum* L. (Fam: Solanaceae) are important vegetable crops grown in Egypt. These crops are cultivated 2-3 seasons a year, including field condition and greenhouses. Root-knot nematodes (*Meloidogyne* spp. Goldi, 1887) are widely distributed in vegetable production areas in the temperate region of the world (Sasser and Carter, 1985), causing yield losses approximately 11%, especially in the USA (Feldmesser et al., 1971). *Meloidogyne* spp. (J2) were recently recorded to be associated with tomato and pepper plantations, in the three soil types surveyed within the cultivated areas of Dakahlia province of Egypt (Salem, 2006). Damage caused by nematodes to plants is directly proportional to their population densities in soil, and their reproduction potentials on the plant (Barker and Olthof, 1976). The minimal density that causes a measurable reduction on plant growth or yield is regarded as the damage threshold density (Barker and Nusbaum, 1971), and when the cost of production and the value of a given crop are considered, the term economic threshold density (Minimal nematode density that causes economic loss) is used. The threshold density varies with nematode species, race, plant variety and environment (Barker and Olthof, 1976). Infections of non efficient or efficient hosts by low densities of *Meloidogyne* spp. may enhance growth and yield (Madamba et al., 1965 and Olthof and Potter, 1972) have no effect (Madama et al., 1965), or cause severe damage (Barker and Olthof, 1976). The present study was undertaken to quantify the effect of different initial population densities of *Meloidogyne incognita* on its population in tomato cv. Castle Rock or pepper cv. Anahim seedlings under greenhouse conditions at 23 ± 4°C.

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MATERIALS AND METHODS

Source of nematodes

Second stage juveniles (J2) of the root-knot nematodes, *M. incognita* (Kofoid and white) chitwood were obtained from a pure culture of *M. incognita* that was previously initiated by a single eggmass and propagated on coleus plants, *Coleus blumei* in the greenhouse of Nematology Research Unit, Agricultural Zoology Department, Faculty of Agriculture, Mansoura University, Egypt.

Table 1. Impact of *Meloidogyne incognita* infection at four levels of eggs on growth of tomato under greenhouse conditions.

Infection level (eggs)	Plant growth response							
	Length (cm)		Fresh weight (g)		Fresh weight of whole plant (g)	Reduction %	Shoot dry weight	Reduction %
	Shoot	Root	Shoot	Root				
250	28.67a	17.00a	2.49ab	1.70b	4.19a	18.6	1.40ab	25.5
500	27.83a	12.00a	2.41ab	1.51bc	3.92ab	23.9	1.35ab	28.2
1000	23.83a	8.00b	1.59b	1.30c	2.89b	43.9	1.21b	35.6
0.0	29.67a	20.33a	2.94a	2.21a	5.15a	---	1.88a	---

Each value is a mean of three replicates. Means in each column followed by the same letter(s) did not differ at p<0.05 according to Duncan multiple-range test

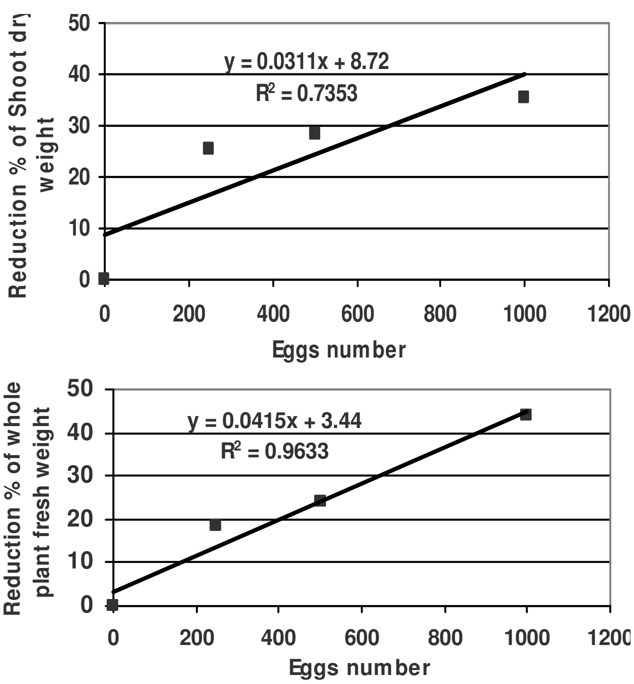

Figure 1. Impact of *Meloidogyne incognita* infection at four levels of eggs on reduction% in whole plant fresh weight and shoot dry weight of tomato plant.

Furthermore, *M. incognita* eggs as nematode inoculum was prepared by using infected root system of coleus plants with heavy eggmasses and followed the technique described by Hussey and Barker (1973).

In order to study the impact of *M. incognita* at four or three levels of eggs on growth of either tomato or pepper, respectively and nematode reproduction, twelve plastic pots 10 cm d. were filled with 850 g/ pot of steam sterilized sandy loam soil (lil) (viv), planted with one seedling for the first crop, tomato seedling (60 days old) as field susceptible plant to *M. incognita*. Then, four levels of *M. incognita* eggs viz 0, 250, 500 and 1000 were separately added to three pots with tomato seedling each, left three pots without eggs that served as control. Each treatment was replicated three times. Pots were arranged in a randomized complete block design on a bench of a partly controlled greenhouse at 23 ± 4°C and watered regularly. After 45 days from eggs of *M. incognita* inoculation, plants were harvested. Plant growth criteria i.e. shoot and root lengths and fresh weights, and shoot dry weight were also recorded.

Number of *M. incognita* J2 in 250 g/ pot soil were extracted by sieving and modified Baer-mann technique (Gocdey, 1957), counted by Hawksely counting slide under x 10 magnification microscope and recorded. Infected roots of each plant were washed with tap water, fixed in 4% formalin for 24 h and stained in 0.01 lactic acid fuchsin (Byrd et al., 1983) and then examined for recording number of galls per root system, developmental stages, females and eggmasses.

With respect to the second crop, pepper seedlings, nine plastic pots 10 cm-d. were also filled with the same capacity of soil, seed of red pepper cv. Anahein were germinated in vermiculate, transplanted when 15 days old at the rate of one seedling /pot. Seven days after transplanting, the three levels of *M. incognita* eggs viz 0, 1000 and 2000 eggs were separately monitored, three pots each. Three pots without nematode eggs were served as control. Each treatment was replicated three times. Pots were also arranged in a randomized complete block design on a bench within the same greenhouse at 23 ± 4°C, and then followed the same previous technique.

Data were subjected to analysis of variance (ANOVA) (Gomez and Gomez, 1984), followed by Duncan's multiple range tests to compare means (Duncan, 1955).

The regression analysis of the previous data was done between inoculum levels of nematode eggs and both reduction percentage of plant growth parameters and the rate of nematode build-up and recorded.

RESULTS AND DISCUSSION

Data in Table 1 and Figure 1 on host plant growth parameters of tomato revealed that the four different levels of *M. incognita* eggs reduced the plant growth as compared to the uninoculated check plants. It is interesting to observe that the marginal effect was more pronounced on roots than shoots. No significance could be detected among inoculation level treatments in shoot fresh and dry weights as well as fresh weight of whole plant. Moreover, the same trend was recorded with length of shoot or root. The lowest reduction was achieved by the inoculation levels that is., 500 and 250 on whole plant

Table 2. Rate of build-up of *Meloidogyne incognita* infecting tomato plant at four levels of eggs inoculation as well as number of galls and egg masses under greenhouse conditions.

Infection levels (eggs)	Nematode population in			Total	Rate of build-up	No. of galls	RGI*	No. of eggmasses	EGI*
	Soil	Root							
		Developmental stages	Females						
0.0	--	--	--	--	--	--	--	--	--
250	260.33c	20c	7c	287.33	1.14	7c	2.0	6c	2.0
500	538.33b	35a	15b	588.33	1.17	17b	3.0	12b	3.0
1000	1424.67a	40a	25a	1489.67	1.48	28a	3.0	23a	3.0

Each value is a mean of three replicates, Means in each column followed by the same letter(s) did not differ at p<0.05 according to Duncan multiple-rang test.; * Root gall index (RGI) or egg mass index (EGI): 0= no galling or eggmasses, 1=1-2 galls or eggmasses; 2=3-10 galls or eggmasses; 3= 11-30 galls or eggmasses; 4= 31-100 galls or eggmasses and 5= more than 100 galls or eggmasses.(Talyor and sasser,1978). ** RGI or EGI = the average of three replicates.

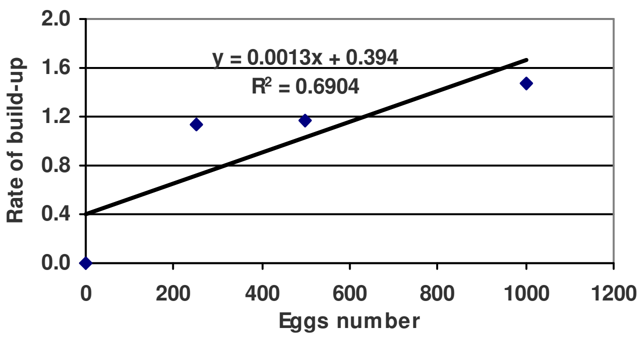

Figure 2. Rate of build-up of Meloidogyne incognita infecting tomato plant at four levels of egg inoculation under greenhouse conditions.

fresh weight and shoot dry weight since their values were amounted to 23.9, 18.6 and 28.2, 25.5% respectively. Meanwhile, the highest reduction of both above mentioned criteria was conducted at level of 1000 eggs which were 43.9 and 35.6% respectively.

The regression analysis between four levels of M. incognita eggs and reduction percentage of whole plant fresh weight and shoot dry weight of tomato plant showed R2 values of 0.9633 and 0.7353, respectively (Figure 1).

Data presented in Table 2 show the rate of build-up of *M. incognita* infecting tomato seedlings at four levels of egg inoculation, number of galls, developmental stages females and eggmasses under greenhouse conditions at 23 ± 4°C. It is evident that when the level of *M. incognita* eggs increased the number of juveniles in soil, developmental stages and females as well as final population were increased. It is also noticed that the root-knot nematode, *M. incognita* rate of build-up on tomato seedling was relatively increased. The highest rate of reproduction on tomato seedlings was recorded by the level of 1000 eggs per pot with value of 1.48. However, the lowest values for rate of reproduction resulted by 250 and 500 eggs per pot that were recorded to be 1.14 and 1.17 respectively.

It is evident that as the level of *M. incognita* eggs increased the number of nematode galls and eggmasses was significantly increased with root gall index and egg masses index values of 2 , 2 and 3 ; 3, 3 and 3, respectively (Table 2).

Moreover, regression analysis between Pi vs rate of nematode build-up on tomato plant gave R^2 value of 0.6904 (Figure 2).

Data in Table 3 and Figure 3 documented the plant growth response of pepper seedlings parameters, reduction percentage of shoot dry weight and the whole plant fresh weight as influenced by three levels of *M. incognita* eggs inoculation viz 0.0, 1000 and 2000 eggs under greenhouse conditions at 23 ± 4°C. A positive correlation has been observed between nematode inocula and reduction percentage of plant fresh weight. As the higher level of nematode inoculum was applied, the greatest reduction percentage of whole plant fresh weight was recorded. Simultaneously, this highest reduction percentage was achieved by 2000 eggs, since its value was amounted to 73.2%. Similar result was obtained for the reduction percentage of shoot dry weight for the same level of egg inoculum with value of 55.3% (Table 3). The least value of reduction percentage of the whole plant fresh weight and shoot dry weight resulted by the level 1000 egg per pot which were amounted to 40.4% and 10.5%, respectively.

Moreover, regression analysis between Pi vs reduction percentages of whole plant fresh weight and shoot dry weight of pepper plant gave values R^2 of 0.9964 and 0.8863, respectively (Figure 3).

Data in Table 4 showed the impact of three levels of *M. incognita* eggs infecting pepper seedlings on nema-tode development, population density and rate of nema-tode build-up under greenhouse conditions at 23 ± 4°C. It was an evident that as the level of *M. incognita* eggs increased, number of nematode juveniles in soil and females

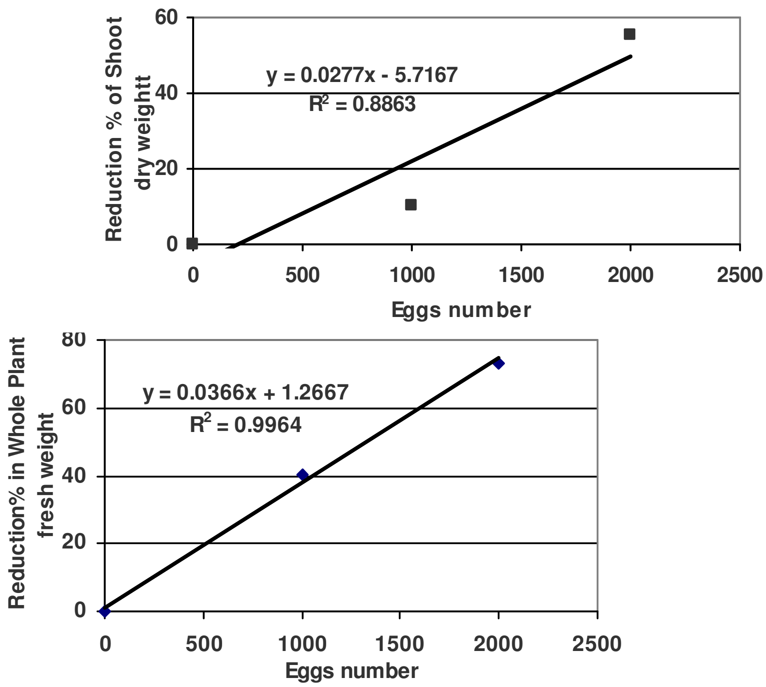

Figure 3. Impact of *Meloidogyne incognita* infection at three levels of eggs on reduction% in whole plant fresh weight and shoot dry weight of pepper plant.

Table 3. Impact of *Meloidogyne incognita* infection at three levels of eggs on growth of pepper under greenhouse conditions.

Inoculum levels (eggs)	Plant growth response							
	Length (cm)		Fresh weight (g)		Fresh weight of whole plant (g)	Reduction %	Shoot dry weight	Reduction %
	Shoot	Root	Shoot	Root				
1000	22.00a	25.50a	2.07a	1.48b	3.55a	40.4	0.34a	10.5
2000	16.33a	13.67b	0.90b	0.70c	1.60b	173.2	0.17b	55.3
Untreated plant (Ck)	23.50a	27.00a	2.24a	3.72a	5.96a	---	0.38a	---

Each value is a mean of three replicates. Means in each column followed by the same letter(s) did not differ at p<0.05 according to Duncan's multiple-range test.

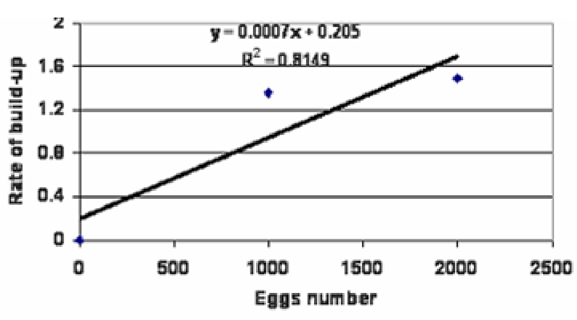

Figure 4. Rate of build-up of *Meloidogyne incognita* infecting pepper plant at three levels of egg inoculation as well under greenhouse conditions.

(final population) increased. It is also noticed that the rate of nematode build-up on pepper seedlings relatively increased from 1.36 to 1.49 as the inoculum level increased from 1000 to 2000 eggs per pot respectively. The highest rate of nematode reproduction on pepper seedling was significantly recorded by the level of 2000 eggs per pot with value of 1.49. It is also evident that as the level of *M. incognita* eggs inoculation increased from 1000 to 2000 eggs per pot, number of nematode galls and egg masses on pepper root system were significantly increased with root gall index as well as eggmasses in- dex values of 3 and 3; and 4 and 4 respectively (Table 4). Moreover, regression analysis between Pi. vs rate of nematode build-up gave R^2 value of 0.8149 (Figure 4). The present results performed a reduction percentage in plant fresh weight when the inoculum level of eggs increased on both plant hosts as was rate of build-up of *M. incognita*. Their values were 43.9% and 1.48 for the level of 1000 eggs on tomato as well as 73.2% and 1.49 for the level of 2000 eggs on pepper, respectively. These rates of nematode build up values on both host plants tested may explain the higher susceptibility of tomato cv. Castle Rock to *M. incognita* infection than in pepper cv. Anahein.

The present work also agrees with the finding of Vito et al. (1985) who studied the effect of fifteen population densities of *M. incognita* race 1 ranged from zero to 512 eggs and juveniles/ cm^3 soil on yield of sweet pepper and found that maximum reproduction rates of the nematode were 0.274 and 1.498 at the lowest initial population density.

However, the present findings are in accordance with those reported by Nadary et al (2006) with respect to different inoculum levels of *M. incognita* on greenbeans and Kheir et al. (2004) who stated that the nematode final density of *M. incognita* on banana cultivars tested, increased proportionally with the increase of initial inoculation levels and all inoculum levels suppressed the plant growth regardless of the cultivar. In conclusion, the susceptibility of tomato cv. Castle Rock to *M. incognita* infection was more than did pepper c.v Anahein. According to the values of R^2, more research is needed to ascertain the number of nematodes in soil at which the plant retained a significant loss in growth and yield; hence control programs should be maintained only when the population densities of nematode reach this level

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