International Scholars Journals

African Journal of Virology Research ISSN 3421-7347 Vol. 4 (4), pp. 001-004, April, 2010. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Effects of the Pathogen in *Meloidogyne incognita* eggs on growth of nematode and sugarbeet reproduction under conservatory conditions

Muhab Abdallah and Ullah Abdula

Plant Pathology Department, Faculty of Agriculture, Helwan University, P.O. Box: 77, Cairo, Egypt.

Accepted 14 March, 2008

The impact of three levels of *Meloidogyne incognita* eggs, that is, 1000, 2000 and 3000 eggs/ plant on plant growth parameters of sugarbeet cv. Nejma and its development and reproduction under greenhouse conditions at 17 ± 5°C was studied. Results indicated that reduction percentage of sugarbeet growth parameters were greatly affected, where the marginal effect was more pronounced on roots than shoots. Moreover, as the inoculation level increased from 1000 up to 3000 eggs of *M. incognita* /plant, the percentage reduction of plant growth parameters increased, where the highest values were recorded to be 48.97 and 24.18% by 3000 eggs/ plant for shoot dry and whole plant fresh weights, whereas, their lowest values of 30.93 and 4.05% resulted by inoculation level of 1000 eggs/ plant for the same plant growth criteria, respectively. Moreover, the highest rate of nematode reproduction on sugarbeet plants cv. Nejma was recorded by the level of 2000 eggs per plant with value of 1.05 whereas the lowest ones of the same criterion resulted by the levels of 3000 or 1000 eggs per plant that were 0.85 or 0.84, respectively. *M. incognita* infection to sugar beet plants cv. Nejma at the three levels of egg inoculation obviously reduced N, P, K and total chlorophyll content on leaves of sugarbeet with values of 9.9% (N), 18.5% (P), 8.8% (K), 1.58% (chlorophyll) and 20.0% (N), 27.4% (P), 18.4% (K) and 5.9% (chlorophyll) by 1000 and 3000 eggs/ plant, respectively compared to the uninoculated plant.

Key words: *Meloidogyne incognita*, sugarbeet, inoculum levels.

INTRODUCTION

Sugarbeet (*Beta vulgaris* L.) is an important arable crop, traditionally used for sugar extraction all over the world. Its total cultivated area reached 227.856 thousands feddans with an average 17.94 tons /Feddan (Feddan = 4200 m^2) of sugar beet tubers in the season of 2008/ 2009 (Annual technical report of the council of sugar crops, Ministry of Agriculture, 2005-2009) in Egypt, where it is grown in all types of soil especially, in newly reclaimed sand areas such as El- Hamoal Barrary, West Nubaruia, and Al- Bostan regions. Sugarbeet plants are subjected to be attacked by several plant parasitic nematodes in many countries. In recent years, sugar beet is becoming an important crop in Egypt for supporting the expansion of Egyptian sugar industry, therefore efforts to protect the crop from the most destructive pests, that is, nematode and diseases are crucial. In 1976, Jatala and Jensen (1976) described that the interrelationships of *Meloidogyne hapla* and *Heterodera schachtii* on sugarbeet in combinations of several inoculum levels given at different times were studied. The numbers of *M.*

*Corresponding author. E-mail: E-mail: Dr_muhab@yahoo.fr

hapla galls were fewer in any treatment in which *H. schachtii* was inoculated 10 days before *M. hapla* than when *M. hapla* was inoculated first or alone. Non-inoculated controls generally had higher concentrations of K, P, Mg, and B than inoculated plants. Pathak and Keshari *(*2000) showed the effect of inoculum levels of *M. incognita* on seed germination, seedling emergence, and growth of beet root (*Beta vulgaris var. crassa cv. Crimson Globe)* in Bihar, India. They stated a significant reduction in seed germination was obtained at 500 nematodes/kg soil, with the reduction being higher in unsterilized soil. Seedling emergence was significantly reduced by at least 1000 nematodes/kg soil in steam- sterilized soil, while the reduction decreased with increasing levels of nematode population in unsterilized soil. Growth significantly decreased with 50 nematodes/kg soil, and continued to decrease with increasing levels of the nematode in the soil. Therefore, the aim of the present work was to deal with the impact of *M. incognita* at three levels of eggs on growth of sugarbeet and nematode reproduction under greenhouse conditions at 17 ± 5°C.

MATERIALS AND METHODS

Nematode source (Inoculum)

To collect and determine the inocula of *Meloidogyne incognita* eggs, *M. incognita* was identified according to Taylor et al. (1955). Infected root systems with heavy egg masses of *M. incognita* various growing coleus plants grown in 25 cm plastic pots filled with sterilized loamy sandy soil at the Nematology Research Unit, Agriculyure, Zoology Department, Faculty of Agriculture, Mansoura University, Egypt, were well-washed and cleaned by running tap water, then placed in a plastic container with enough solution of 1.0% NaOCl for 90 s, shacked vigorously (manually) then, quickly NaOCl solution was passed through 60-mesh sieve, nested over a 400-mesh sieve to collect free eggs. After that, the 400-mesh sieve with eggs was quickly placed under a stream of tap water for several minutes to remove residual NaOCL. Eventually, the number of eggs per unit volume of water was counted and then the seedlings were inoculated directly with eggs levels (Hussey and Barker, 1973).

Pathogenicity test

Sixteen plastic bags were filled with 3 kg/bag of steam-sterilized clay-sand soil (1:1) (V:V) (Table 2). Three seeds of sugarbeet (*Beta vulgaris* cv. Nejma) were sown planted in each bag. Thirty days from seeds germination and thinning to one seedling / bag, the tested three levels of *M. incognita* eggs, that is, 1000, 2000 and 3000 eggs were separately monitored. Each inoculated treatment was replicated four times and another four bags left without nematodes served as control. All bags were arranged in a randomized complete block design under the greenhouse conditions at 17 ± 5°C. Plants were watered and regularly receiving conventional pesticides to control mites and insects as needed. After 60 days from the inoculation time, plants were harvested. Plant growth criteria, that is,. shoot and root lengths and fresh weights, as well as shoot dry weight were recorded.

Number of *M. incognita* second stage juveniles (J_2) in 250 g soil/bag was extracted by sieving and modified Baermann-technique (Goodey, 1957), then calculated for the soil of each bag counted by Hawksely counting slide under 10×4 magnification and recorded. Infected roots of each plant were washed with tap water, then fixed in 4% formalin for 24 h and stained with lactic acid-fuchsin 1% (Byrd et al., 1983) and then examined for the number of galls, developmental stages, females and egg-masses.

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

RESULTS AND DISCUSSION

Effect of inoculum levels of *M. incognita* eggs on sugarbeet growth

Data in Table 1 on growth parameters of sugarbeet cv. Nejma revealed that the tested inoculums levels of *M. incognita* eggs reduced the plant growth as compared to the un-inoculated plants. It is interesting to note that the marginal effect was more pronounced on roots than shoots. Significant differences could be detected among the inoculation levels and the control in total plant fresh weight except that of 1000 eggs level. The same trend was noticed with total plant length. The highest reduction percentage of shoot dry and whole plant fresh weights was achieved by 3000 eggs/bag (plant) since their values amounted to 48.97 and 24.18%, respectively, however their least values resulted by inoculation level of 1000 eggs per plant which amounted to 30.93 and 4.05%, respectively (Table 1). These findings are in accordance with those reported by Gohar and Maareg (2009) who mentioned that gall index was found to be the most sensitive method for assessing resistance of such sugarbeet varieties to *M. incognita* infection, especially at the low inoculum levels (500 and 1000 eggs) per plant.

Effect of different inoculum levels of *M. incognita* eggs on its development and reproduction

Table 2 showed that the rate of nematode build-up, number of galls, developmental stages, females and egg-masses were positively affected by the tested levels of eggs. The highest rate of nematode reproduction on sugarbeet plants was recorded at using the level of 2000 eggs per bag with value of 1.05. However, the lowest values for rate of nematode reproduction resulted by either 3000 or 1000 eggs per bag that were recorded to be 0.82 and 0.84, respectively. It was also evident that as the level of *M. incognita* eggs increased, number of nematode galls and egg-masses increased with root gall and egg-masses indices values of 3.0 and 3.0, 5.0 and 5.0, and 5.0 and 5.0 for 1000, 2000 and 3000 eggs inoculum levels, respectively. Regression analysis of nematode build-up reached the maximum value of R2 that was amounted to 0.5827 (Figure 1). These findings of the present investigation are in accordance with those reported by Osman and Kheir (1978) who reported that growth of sugarbeet cv. Detroit decreased and root-knot

Table 1. Plant growth of sugarbeet cv. Nejma as affected by three inoculum levels of *M. incognita* eggs infection under greenhouse conditions at 17± 5ºC.

Inoculum levels (eggs/ plant)	Plant growth response*								
	Length (cm)		Total plant length (cm)	Fresh weight (g)		Total plant F. wt (g)	R%	Shoot dry weight (g)	R%
	Shoot	Root		Shoot	Root				
Non-infected plant	22^{a}	14^{a}	36.0^{a}	22.05^{a}	10^{a}	32.05^{a}	-	6.37^{a}	-
1000	20.5^{ab}	10^{a}	30.5^{b}	22.00^{a}	8.75^{b}	30.75^{a}	4.05	4.4^{b}	30.93
2000	21.5^{ab}	10^{b}	31.5^{b}	20.00^{b}	7.75^{b}	27.75^{b}	13.42	3.72^{c}	41.60
3000	19.00^{b}	8^{b}	27.0^{c}	17.8^{c}	6.5^{c}	24.3^{c}	24.18	3.25^{d}	48.97

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

$$R\% = \text{Reduction } \% = \frac{\text{Non- infected plant} - \text{infected plant}}{\text{Non - infected plant}} \times 100$$

Table 2. Development and reproduction of *M. incognita* infecting sugarbeet cv. Nejma as influenced by different inoculums levels of eggs under greenhouse conditions at 17 ± 5°C.

Inoculum levels	Nematode populations			Final population	Rate of build up RF =Pf/Pi	Galls	*RGI	Egg-masses	*EI
	Soil (Larvae)	Root/ plant							
		Females	Developmental stages						
1000 eggs	661.5^{c}	25.0^{c}	155.75^{c}	842.25	0.84	28.0^{c}	3.0	20.0^{c}	3.0
2000 eggs	1154.5^{b}	167^{b}	775.5^{b}	2097.0	1.05	176.25^{b}	5.0	156.5^{b}	5.0
3000 eggs	1255^{a}	293^{a}	906^{a}	2454	0.82	387.5^{a}	5.0	288.25^{a}	5.0

RF = Reproduction factor = Rate of build-up = Final population P_f / initial population P_i *Root gall index (RGI) or egg-masses index (EI): O = No galls or egg-masses, 1= 1-2 galls or egg-masses, 2 = 3-10 galls or egg-masses, 3=11-30 galls or egg-masses, 4= 31-100 galls or egg-masses and 5 = More than 100 galls or egg-masses (Taylor and Sasser, 1978).

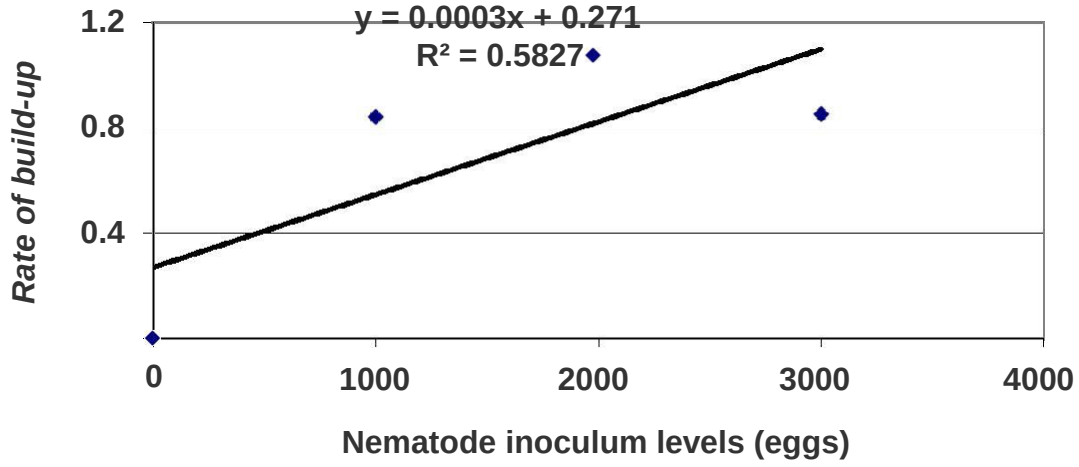

Figure 1. Regression (R^{2}) value of *Meloidogyne incognita* nematode build-up rate as affected by the three levels of eggs infecting sugarbeet cv. Nejma under greenhouse conditions at 17±5°C.

index increased with increasing inoculum levels from 1000 up to 8000 j_2 where maximum reduction in plant growth occurring with 4000 j_2 of *M. incognita* per plant, and that of Pathak and Keshari (2000) in respect to growth of sugarbeet var. crassa cv.crimson which was decreased significantly with 50 nematodes of *M. incognita* per kg soil and continued to decreased with increasing volume of nematodes in the soil.

Each value is a mean of four replicates. Mean values in each column followed by the same latter(s) did not differ

Table 3. Influence of three inoculum levels of *Meloidogyne incognita* eggs infecting sugarbeet cv. Nejma on the leaves content of N, P, K, and chlorophyll A, B under greenhouse conditions at 17± 5ºC.

Treatments	%			Chlorophyll content (mg/g)			*Decrease
(eggs/plant)	N	P	K	A	B	Total	%
Control (ck)	4.93	0.394	4.88	1.175	0.866	2.041	-
1000	4.44	0.321	4.45	1.132	0.850	1.982	1.58
2000	4.19	0.307	4.14	1.112	0.835	1.947	3.33
3000	3.94	0.286	3.98	1.082	0.813	1.895	5.91

$$*\text{Decrease \%} = \frac{\text{Control} - N}{\text{Control}} \times 100$$

Each value is a mean of four replicates.

at P< 0.05 according to Duncan's multiple- range test.

Also, data presented in Table 3 show the impact of *M. incognita* infection to sugarbeet plants cv. Nejma at the tested levels of eggs on the content of N, P, K and total chlorophyll in leaves. It was evident that nitrogen, phosphorus, potassium and total chlorophyll content of sugar beet plants cv. Nejama were obviously reduced by nematode infection at the three levels of eggs. Moreover, it was also noticed that as the eggs inoculum level increased from 1000 eggs up to 3000 eggs / plant as these tested elements gradually decreased, since their values were 9.9 (N), 18.5 (P), 8.8 (K), 1.58% (chlorophyll), and 20.0 (N), 27.4 (P), 18.4 (K), 5.9 (chlorophyll) for 1000 and 3000 eggs as nematode inoculation per plant, comparing to the uninoculated plant, respectively (Table 3). Undoubtedly, more work is needed in this respect under field condition before recommendations can be made for management programmes of root-knot nematodes.

REFERENCES

Byrd DW, Kirkpatrick T, Barker K (1983). An improved technique for clearing and staining plant tissues for detection of nematodes. J. Nematol. 15(3):142-143.

Duncan DB (1955). Multiple range and multiple F. test. Biometrics 11:1-42.

Gohar IMA, Maareg MF (2009). Effect of inoculum level, type, plant age and assessment date on evaluating sugarbeet resistance methods for root- knot nematode*, Meloidogyne incognita.* J. Agric. Sci. Mans. Univ. 34(5):5401-5419.

Gomez KA, Gomez AA (1984). Statistical procedures for Agricultural Research.2nd Ed., John Wiley & Sons: Inc., New York.

Goodey JB (1957). Laboratory methods for work with plant and soil nematodes. Tech. Bull. No.2 Min. Agric. Fish Ed. London, P. 47.

Hussey RS, Barker KR (1973). A comparison of methods of collecting inocula of *Meloidogyne* spp. including a new technique. Plant Dis. Rep. 57:1925-1928.

Jatala P, Jensen HJ (1976). Parasitism of *Beta vulgaris* by *Meloidogyne hapla* and *Heterodera schachtii* alone and in combination. J. Nematol. 8(3):200-205.

Osman AA, Kheir AM (1978). Host parasite relations of *Meloidogyne incognita* on sugarbeet, *Beta vulgaris*. Proceedings of the 4th Conference of pest control. pp. 889-894.

Pathak KN, Keshari N (2000). Effect of inoculum levels of *Meloidogyne incognita* (Kofoid and White, 1949) Chitwood 1919, on seed germination, seedling emergence and plant growth of red beet (*Beta vulgaris* var. crassa). Pest Manag.Horticult. Ecosys. 6(2):118-123.

Taylor AL, Dropkin VH, Martin GC (1955). Perineal patterns of root-knot nematode. Phytopathology 45:26-34.

Taylor and Sasser (1978).Biology,Identification and Control of Root-knot Nematodes,Meloidogyne spp. North Carolina State University Graphics, Raleigh P. 111.