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Pathogenicity of Root – Knot Nematode *Meloidogyne javanica* on Cucumber Plants at Different Inoculum levels Under Greenhouse Conditions

Sulaiman Naif Ami*, Shireen Ghaeib Ali Shingaly

Department of Plant Protection, College of Agricultural Engineering Sciences, University of Duhok, Duhok, Kurdistan Region – Iraq (sulaiman.ami@uod.ac, shireenghaeeb@yahoo.com)
**Correspondence: sulaiman.ami@uod.ac*

Abstract

Root-knot nematodes are dangerous and economically very important pest of completely cultivated crops in the world, so this study was conducted to test the pathogenicity of root-knot nematode *Meloidogyne javanica* at different levels of soil infestation by nematode eggs against one of the most common cultivar (hybrid) of cucumber cultivated in Duhok province. Sterilized sandy loam soil was infested with different levels of inoculum of root – knot nematode *M. javanica* included: 0, 1000, 2000, 4000, 8000 and 16000 eggs/pot after 20 days of sowing cucumber seeds cv. Sayff F1 under greenhouses conditions at College of Agricultural Engineering Sciences / Semel, University of Duhok. Results revealed that the growth criteria of cucumber plants (length, fresh and dry weight of roots and shoots) and chlorophyll content of leaves decreased with increasing of inoculum levels of nematode eggs, whereas the infection criteria increased except with level 16000 eggs / pot at which the infection criteria decreased. At the same time nematode reproduction rate decreased with increasing of nematode inoculum levels.

Keywords: *Pathogenicity, M. javanica, Inoculum Levels, Cucumber*

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I. INTRODUCTION

Root-knot nematodes are dangerous and economically very important pest of completely cultivated crops in the world (Hussain *et al.,* 2011). Species of this nematode are mainly damaging the vegetable crops in tropical and subtropical regions (Mukhtar *et al.*, 2013).The most important species of root-knot nematodes are *M. javanica*, *M. arenaria*, *M. incognita* and *M. hapla* that cause high economic damages in different crops (Khalil, 2013). In a pot experiment Maleita *et al.*, (2012) studied the effect of egg levels (2500, 5000 and 10000 eggs/pot) of *M. javanica* and *M. hispanica* on tomato, results showed that there was a trend of decreasing in growth criteria (length and weight of roots and shoot) with increasing of initial nematode population levels. The effects of initial population levels (0, 0.125, 0.25, 0.5, 1, 2, 4, 8 and 16 eggs/gm of soil) of *M. javanica* on cucumber were conducted in greenhouse pots, and it was noted that the fresh weight and shoot length were decreased at 16 eggs of *M. javanica* /gm of soil, while the number of galls and nematode eggs increased with the increasing of inoculum levels and at the end nematode reproduction rate decreased (Charegani *et al.*, 2012). On the effect of inoculum levels (0, 500, 1000, and 2000 J2s) of root-knot nematodes on growth of tomato cv. Pectomec, results revealed that increasing the nematode inoculum levels caused increasing in nematode population density and number of galls (Kankam and Adomako, 2014). Maximum galls and egg masses/root system of green gram were recorded in 100 and 1000 J2 of *M. javanica*/kg of soil, which ultimately decreased at 10000 inoculum level as nematode population density increased in the soil with an increasing the inoculum level of nematode (Sumita, 2014). This study was designed to test the pathogenicity of root-knot nematode *M. javanica* at different levels of soil infestation by nematode eggs against one of the most common hybrid cultivars of cucumber cultivated in Duhok province.

II. MATERIALS AND METHODS

A. Pathogenicity of root-knot nematode M. javanica on cucumber plants

Plastic pots with a diameter of 19cm and depth of 18cm were filled with sterilized sandy loam soil (17.63 clay, 18.64 silt and

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63.73% sand) by 3.5 kg of soil/pot. Five seeds of hybrid cucumber cv. Sayff F1 were planted in each pot as it is the most widespread cultivated hybrids in Duhok province.10 days later thinning process were done to keep 2 plants/pot, then the pots were buried to specific level (plunged) randomly in the soil of one of the greenhouses at College of Agricultural Engineering Sciences in Semel/University of Duhok. The inner lining of the greenhouse was covered with tulle fabric carefully to avoid entry of insects. After 20 days from seed sowing, the seedlings were infested individually with different inoculum levels of root-knot nematode *M. javanica* included: 0, 1000, 2000, 4000, 8000, 16000 eggs/pot by making holes around the plant with a sterilized glass rod, and with a depth of 3cm and 2cm away from the plant. Nematode eggs inoculum was added to the soil through these holes which covered directly with sterilized soil. Pots were watered as needed and plants were uprooted carefully after 2 months of soil infestation with nematode eggs to calculate the following characters:

a. Plant growth criteria: (1)-Chlorophyll (SPAD) measured by chlorophyll meter (SPAD502/Konica Minolta Sensing, INC. made in Japan). (2) Shoot and root length (cm/plant). (3) Fresh weight of shoot and root (g/plant). (4) Dry weight of root and shoot (g/plant):

b. Calculation of reduction percentage in growth criteria and chlorophyll content of leaves according to the following equation:

$$\% \text{ Reduction} = \frac{\text{Control (non -infested soil) - Treatment}}{\text{Control (non -infested soil)}} \times 100$$

c. Infection criteria

(1) The number of root galls/root system of the plant. (2) Root gall severity: Depending on galling index (GI) measure in 0-5 scales and as follow: (0=0, 1= 1-2, 2= 3-10, 3=11-30, 4=31-100, 5= more than 100 galls /root system (Kumar *et al.*, 2014). (3) Nematode population density in the soil for each pot by the tray method as mentioned by Coyne *et al.* (2007). 4-Number of egg masses/root system by staining method as described by Stetina and Young, (2006). (5) Nematode population density/root system by staining method (Stetina and Young, 2006) as follow: Cucumber roots were washed with tap water to remove soil. Then, roots were drained on paper towels and cut to 1cm. Next, roots were stained with red food color according to the method mentioned by Thies *et al.* (2002). Egg masses and nematode inside the roots were counted using a stereomicroscope. Results were expressed as females per each gram of roots. (6) Number of eggs according to the method mentioned by Coyne *et al.* (2007). (7) Rate of nematode reproduction according to the following equation:

Final population density = Number of nematodes in soil + Number of nematodes in roots + Number of eggs root system.
Rate of nematode reproduction = Final population density of nematode / initial population density of nematode.

B. Experimental Design and Statistical analysis

This experiment consisted of 6 treatments with 4 replications. Data were analyzed using the SAS program to compare the means of studied characters depending on Duncan's multiple range test, $p \leq 0.05$ (SAS, 2001).

III. RESULTS

A. Effect of different inoculum levels of M. javanica eggs on some growth criteria and chlorophyll content of cucumber plants under greenhouse conditions

Results indicated that infested soil with different inoculum levels of nematode eggs caused a notable reduction in some growth criteria of cucumber plants included fresh, dry weight and length of root and shoot system with chlorophyll content of leaves as compared with the control treatment (Fig. 1). The values of the studied characters were decreased with increasing of inoculum levels starting with 13.25g/plant,3.5 g/plant, 17.25 cm/plant, 24.25 gm/plant, 7.5 g/plant, 56.25 cm/plant and 30.48 SPAD respectively at the minimum level (1000 eggs/pot) (Table 1), thus, it achieved a reduction by 19.69, 16.67, 27.37, 27.61, 18.92, 12.79 and 9.70 (Table 2) and ending with 6.25 gm/plant, 1.5 gm/plant, 3.75 cm/plant, 5.5 gm/plant, 1.75 gm/plant, 9cm/plant and 25.29 SPAD respectively at the maximum level (16000 eggs/pot) (Table 1) that is, it caused a reduction by 62.12, 60, 84.21, 83.58, 81.08, 86.05 and 18.21% in previous characteristics (Table 2). Results of statistical analysis also showed that the reduction in most growth criteria caused by all the inoculum levels of nematode eggs was significant compared to the control treatment (non-infested soil) except in chlorophyll content at all inoculum levels, dry weight of root system at the level 1000, 2000 and 4000 eggs / pot respectively and dry weight of shoot system at the level 1000 eggs / pot where the reduction was not significant compared to the control treatment. However, the difference was not significant between inoculum levels in their impact in some growth criteria of cucumber plants.



Figure 1. Healthy cucumber plants (1) compared to diseased plants (2,3,4,5 and 6) grown in soil infested with 1000, 2000, 4000, 8000, 16000 eggs of *M. javanica* respectively.

Table 1. Effect of different inoculum levels of *M. javanica* eggs on some growth criteria and chlorophyll content of cucumber plants cv.Saffy F1 under greenhouse conditions.

Treatments (eggs/pot)	Growth Criteria						
	Root system			Shoot system			
	F.W. (gm/plant)	D.W. (gm/plant)	R.L. (cm/plant)	F.W. (gm/plant)	D.W. (gm/plant)	S.L. (cm/plant)	Chl (SPAD)
1000	13.25 b	3.5 ab	17.25 b	24.25 b	7.5 a	56.25 b	30.48 a
2000	11.75 b	3 ab	15.5 b	21.5 b	4.75 b	34.5 c	30.13 a
4000	9.5 c	2.5 abc	10.75 c	16.75 c	4.25 bc	25.75 d	30.09 a
8000	7.75 cd	2.25 bc	7.5 d	8.25 d	2.5 cd	15.75 e	26.73 a
16000	6.25 d	1.5 c	3.75 e	5.5 d	1.75 d	9 f	25.29 a
Control (non infested soil)	16.5 a	3.75 a	23.75 a	33.5 a	9.25 a	64.5 a	30.923 a

- Means with different letter (s) are differ significantly according to Dunca,s Multiple Range test ($P \leq 0.05$) -Each number is a mean of 4 replications.
- Treatments (inoculum levels), F.W. = Fresh weight, D.W. = Dry weight, R.L. = Root length, S.L. = Shoot length, Chl. = Chlorophyll.

B. Effect of different inoculum levels of M. javanica eggs on some infection criteria in cucumber plants

Results of statistical analysis revealed that the inoculum levels of nematode eggs affected significantly on the infection criteria included, number of: root galls, egg masses, (Fig. 2), eggs and nematode stages in cucumber roots as well as nematode population density in the soil and final nematode population density in addition to nematode reproduction rate while the effect was not significant on the root gall index because it depends mainly on a range of a specific number of the root galls (Table 3). The lowest values of the infection criteria were recorded by the last level of infestation (16000 eggs/pot) except number of nematode /root system where it excelled on the first level (1000 eggs/pot) after which values of infection criteria began to increase and thus the highest values were reported by the level 8000 eggs/pot, namely: 241 galls/root system, 140 egg mass/root system, 65362.5 eggs / root system,7727 nematode stages / root system, 142362.5 J2 / pot, 215452 nematode stages / pot including soil and cucumber root system and 26.93 as a rate of nematode reproduction.

Table 2: The percentage of reduction in some growth criteria and chlorophyll content of cucumber plants as affected by different inoculum levels of root- knot nematode *M. javanica* eggs.

Treatments (eggs/pot)	Percentage of reduction in growth criteria %						
	Root system			Shoot system			
	F.W. (gm / plant)	D.W. (gm/plant)	R.L. (cm/plant)	F.W. (gm/plant)	D.W. (gm/plant)	S.L. (cm/plant)	Chl (SPAD)
1000	19.69	16.67	27.37	27.61	18.92	12.79	9.70
2000	28.79	23.33	34.74	35.82	48.65	46.51	9.59
4000	42.42	36.67	54.74	49.99	54.05	60.08	15.77
8000	53.03	40	68.42	75.37	72.97	75.58	13.55
16000	62.12	60	84.21	83.58	81.08	86.05	18.21



Figure 2. Effect of different inoculum levels of eggs on some infection criteria in cucumber plants. A. Signs of root-knot disease on roots. B. Symptoms of root-knot disease on roots

Table 3. Influence of different inoculum levels of *M. javanica* eggs on some infection criteria in cucumber plants cv.. Saffy F1.

Treatments (eggs/pot)	Infection Criteria							
	N.O.G.	R.G..	N.E.M.	N/R	N/S	N.O.E.	F.P.D.	R.O.R.
1000	112 c	4.5 a	90 c	2017 e	35262.5 d	30150 d	67429.5 d	67.4295 a
2000	140 b	5.0 a	93 c	5161 c	95200 c	31162.5 c	131523.5 c	65.7618 b
4000	224 a	5.0 a	128 b	7113.5 b	110337.5 b	64312.5 b	181763.5 b	45.4409 c
8000	241 a	5.0 a	140 a	7727 a	142362.5 a	65362.5 a	215452 a	26.9315 d
16000	97 c	4.5 a	78 d	3042.5 d	29225 e	23287.5 e	55555 e	3.4722 e
Control (without soil infestation)	0 d	0 b	0 e	0 f	0 f	0 f	0 f	0 f

- Means with different letter(s) are significantly differ depending on Duncan's Multiple Range test ($P \leq 0.05$)
- Each number is a mean of 4 replications.
-N.O.G. = Number of galls, R.G.I. = Root gall index, N.E.M. = Number of egg masses, N/R = Number of nematodes/roots system, N/S = number of nematodes/soil pot , N.O.E. = number of eggs, F.P.D.= Final population density of nematode, R.O.R.= Rate of reproduction

IV. DISCUSSION

These results showed that increasing in inoculum levels of nematode affected negatively on the growth criteria of cucumber plants which consistent with those cited by Al-Sabie and Ami, (1989); Kankam and Adomako, (2014) and Charegani *et al.* (2012) where root-knot nematodes cause reduction in growth criteria by consumption of food and energy necessary

for normal growth of the plant, as also observed by Anwar (1995) and McClure (1977). Nematodes in infected sites disrupt the vascular region of root system and retard transmission of nutrients and water to other parts of the host plant (Hajera *et al.*, 2009) in addition to that nematode injury restricted the growth of cucumber root system and reduced the absorption of water and nutrient by the roots (Anwar and Din, 1986; Karssen and Moens, 2006) leading to stunting of the plants as well as increased respiratory rate in infected tissue that led to the imbalance between the processes of catabolism and anabolism of the plant which in turn caused a decrease in plant height and this view is consistent with what have been mentioned by Paez *et al*. (1976). On the other hand root-knot nematodes destroyed root tissues, including epidermis, cortex, endodermis and pericycle during its penetration toward vascular cylinder, where females stabilized there causing formation of giant cells then malformation of vascular cylinder followed by obstruction of water and nutrient transmission to shoot system, causing a decrease in plant growth criteria, this finding is consistent with what have been indicated by Azam *et al*. (2010).

The translocation rate, photosynthesis and absorption decreased in infected plant by increasing the infestation levels of nematode and photosynthetic product move to root tissues, including giant cells, which developed by plant parasitic nematode infection and maintain the reproduction and development of nematode (Sharma and Sharma, 2015). The invasion of nematode reduces the ability of the plant root epidermis to take up water with pruning of root hairs and reducing root length and their branches. Damaged roots by nematode do not utilize water or fertilizers as effectively, causing additional damages for the farmers (Anwar and McKenry, 2012). The waste and toxic enzymes that put down by nematodes in the roots had a negative impact on plant growth and this effect increases with increasing the number of nematodes in the roots (Al-Sabie and Ami, 1989). In addition to the deterioration of root portability in the absorption process and moving elements to shoot system accompanied by morphological and physiological changes which adversely affect the growth and weight of root growth and other criteria (Golden and Van Gundy, 1975). It is obvious that the decrease of the root and shoot length led to decrease of their dry weight (Malekeberhan *et al.*, 1985).

Feeding activities of nematodes interfere with plant physiological functions including photosynthesis and leading to a decline in growth criteria (Carneiro *et al.*, 2002). The role of increasing level of nematode inoculum has been related to an increase in the formation of galls (Carneiro *et al.*, 1999; Abrao and Mazzafera, 2001). The root gall severity increase with increasing the inoculum levels of nematode which has also recorded by Maleita *et al.* (2012). Nematode population density increased in the soil by increasing the level of infestation as reported by Sarmah and Sinha, (1995). The number of root galls and egg masses increased with increasing number of nematodes that entered the roots (increase the level of infestation) where increased competition for space and food (Sumita, 2014) and reduce the efficiency of females in egg production at the highest level of infestation, also due to an increase in the number of males in the root to be another reason in decreasing the amount of food (David and Triantaphyllou, 1967). Regarding nematode rate of reproduction, which decreased with increasing of inoculum levels is attributed to competition of root-knot nematode in space, food and penetration (Ibrahim, 2002; El-Sharif et al., 2007; Joymatidevi, 2009; Kumar *et al.*, 2011 and Charegani *et al.*, 2012). The decreasing of reproduction rate may also be due to the deterioration of roots and failure of nematode to find new location of infection for generations (Hussain *et al.*, 2011).

V. Conclusion

The growth criteria of cucumber plants (length, fresh and dry weight of roots and shoots) and chlorophyll content of leaves decreased with increasing of inoculum levels of nematode eggs, whereas the infection criteria increased except with level 16000 eggs / pot at which the infection criteria decreased. At the same time nematode reproduction rate decreased with increasing of nematode inoculum levels.

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