EFFECT OF VARIOUS INITIAL POPULATION DENSITIES OF TWO SPECIES OF *MELOIDOGYNE* ON GROWTH OF TOMATO AND CUCUMBER IN GREENHOUSE

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Summary. The effects of initial population levels of either *Meloidogyne incognita* or *M. javanica* [0, 0.25, 0.5, 1, 2 and 4 eggs and second stage juveniles (J2) per g of soil] on tomato cultivar Shaft-Falat and of *M. javanica* (0, 0.125, 0.25, 0.5, 1, 2, 4, 8 and 16 eggs and J2 per g of soil) on cucumber cultivar Super Amelia were investigated in pots in a greenhouse. Inoculation of tomato with 4 eggs and J2 of *M. incognita* or *M. javanica* per g of soil reduced shoot length and fresh dry weights to similar extents. On cucumber, the same variables were significantly reduced at 16 eggs and J2 of *M. javanica* per g of soil. The numbers of eggs and galls of the nematodes on the roots of both crop plants increased with the increase of the initial inoculum level while the reproduction rates decreased. On tomato, at 1-4 eggs and J2 per g of soil, the reproduction of *M. incognita* was more than that of *M. javanica*. *Meloidogyne javanica* reproduced more on cucumber than on tomato.

Key words: *Cucumis sativus*, root-knot nematodes, *Solanum lycopersicum*, yield loss.

Tomato (*Solanum lycopersicum* L.) and cucumber (*Cucumis sativus* L.) are among the most popular vegetables worldwide. Root-knot nematodes are known as the most damaging plant parasitic nematodes. They cause about 5% of yield reductions overall but individual crop losses can be much more severe; they are also considered one of the most important obstacles to producing sufficient food in developing countries. Root-knot nematodes, *Meloidogyne* spp., attack more than 2,000 plant species (Hussey and Janssen, 2002) and can even cause more than 50% losses to tomatoes (Natarajan *et al.*, 2006). Among the four main species of root knot nematodes, *M. javanica* (Treub) Chitw. is the most predominant in Iran followed by *M. incognita* (Kofoid *et* White) Chitw. (Akhiani *et al.*, 1984).

The extent of damage a nematode may cause to its host plant depends upon its population density in soil as well as its reproductive potential in the host plant (Seinhorst, 1965; Barker and Olthof, 1976). The nematode economic threshold, the population density of a nematode at which the value of the yield lost is equal to the cost of the treatment required to avoid that loss, depends on nematode species and/or race, plant cultivar, environmental conditions (Barker and Olthof, 1976), the value of the crop yield and the cost of the treatment (Ferris, 1978).

Therefore, the effects of different densities of root-knot nematodes on growth and yield of host plants has been the subject of many studies and review articles (Seinhorst, 1965, 1998; Schomaker and Been, 2006; Greco and Di Vito, 2009).

In microplot studies (Di Vito *et al.*, 1985, 1991), using intact egg masses as inoculum, pepper productivity was strongly affected by *Meloidogyne incognita* race 1 and tolerance limits to the nematode of 0.16 and 0.55 eggs and juveniles/cm^3 soil for pepper and tomato, respectively, were estimated. The maximum yield losses were 80% and 100%, respectively, and occurred at the largest inoculum level (512 eggs and juveniles/cm^3 soil).

In Nigeria (Chindo and Kahn, 1988), a decrease in the growth and yield of tomato was observed with the increase in nematode population of *Meloidogyne incognita* race 1. At harvest, the nematode population in the soil was found to have increased except at the largest initial population density. In Ethiopia, tomato and pepper growth were negatively correlated with population densities of *M. javanica* while root galling showed a positive correlation with the inoculum level of the nematode (Mekete *et al.*, 2003). In Egypt, El-Sherif *et al.* (2007) reported that increasing the initial inoculum density of *M. incognita* on tomato and pepper reduced tomato biomass and increased the numbers of egg masses of the nematode and galls per g of root.

Root knot nematodes are responsible for tremendous amounts of crop losses in tomato and cucumber in Iran, where they are widely cultivated in greenhouses. Hence the objective of the present study was to investigate in the greenhouse the effects of different initial densities of Iranian populations of *M. incognita* and *M. javanica* on tomato cv. Shaft-Falat and cucumber cv. Super Amelia.

MATERIALS AND METHODS

Preparation of the nematode inoculum. Tomato, cucumber, pepper, cedar and basil plants with infected roots and soil infested with *Meloidogyne* spp. were sampled from Shiraz, Marvdasht, Kavar and Abade Tashk regions, all located in Fars province in southwest Iran.

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The nematode populations were initially purified by starting from a single egg mass. In a first step, the egg masses were reproduced on tomato (cv. Shaft-Falat) and identified by morphological characteristics and perineal patterns of females. Eventually two species, *M. incognita* and *M. javanica*, were separately inoculated into 1500-cm^3 plastic pots containing transplanted tomato seedlings by making three 2.5 cm deep holes around the seedlings (Hartman and Sasser, 1985; Eisenback and Triantaphyllou, 1991). When large egg masses were present on the roots, the eggs of the two nematode species were extracted by uprooting the tomato roots and rinsing the soil off the roots with tap water. Roots were dipped in 0.5% NaOCl for 2 min (Hussey and Barker, 1973) and the nematode suspension so-obtained was poured onto a 74-µm sieve nested on a 25-µm sieve (200- and 500- mesh sieves, respectively) and washed with tap water to eliminate residues of NaOCl. Then the eggs and second stage juveniles (J2) were collected in a beaker and their numbers estimated before using them for inoculation.

Effects of initial densities of M. incognita *and* M. javanica *on tomato*. In the first experiment, initial population densities of *M. incognita* and *M. javanica* (0, 0.25, 0.5, 1, 2 and 4 eggs and J2 per g of soil) were tested. Tomato cultivar Shaft-Falat plants at the six-leaf stage were transplanted into two separate groups of pots. Each pot had been filled with 1.5 kg of sterilized sandy loam soil. The pots were maintained in a greenhouse adjusted to 30 ± 4 °C under 14:10 h (light:dark) photoperiod. The pots were laid out according to a completely randomized design with four replicates per inoculum level. Sixty days after inoculation, plants were harvested and plant growth variables (shoot length, shoot fresh and dry weights, and root fresh weight) were recorded. Furthermore, the number of nematodes per 100 g of soil was determined according to Whitehead and Hemming (1965). The number of eggs per g of root was also determined by cutting the roots into pieces 1 cm long from which 1 g was randomly selected. The eggs were extracted by agitating the root pieces in a 0.5% NaOCl solution for 2 min (Hussey and Barker, 1973), sieved as mentioned earlier and counted. The reproduction rate of the nematode and gall index were calculated as described by Sasser and Taylor (1978).

Effects of initial densities of M. javanica *on cucumber*. In the second experiment, initial population densities of 0, 0.125, 0.25, 0.5, 1, 2, 4, 8 and 16 eggs and J2 of *M. javanica* per g of soil were inoculated to cucumber cultivar Super Amelia plants at the six-leaf stage growing in 1.5 kg of sterlized sandy loam. The pots were laid out according to a completely randomized design with four replicates per inoculm level in a greenhouse adjusted to 30 ± 4 °C. Plant growth variables were recorded as described above. Also, nematode gall index (Hussey and Janssen, 2002), galls per g of root (Sasser and Taylor, 1978), eggs per g of root and reproduction rate were recorded.

Statistical analysis. Plant growth variables (shoot length, shoot fresh and dry weights, root fresh weight) of both experiments were subjected to separate analysis of variance (ANOVA) and means were compared with Duncan's multiple range tests. Statistical analysis of non-parametric data (numbers of eggs and egg masses per g of soil, gall index and reproductive factor) of both experiments was made according to Friedman's rank test followed by a Bonferroni means comparison test (Shah and Madden, 2004).

RESULTS

Effects of initial densities of M. incognita *and* M. javanica *on tomato*. When initial density of *M. incognita* increased, shoot length decreased but not significantly (Table I). However, shoot length was reduced with increasing initial density of *M. javanica*. This reduction was significant at 2 and 4 eggs and J2 per g of soil ($P < 0.05$) (Table II). A significant reduction in shoot fresh weight was observed at initial densities of 2 and 4 eggs and J2 of *M. incognita* per g of soil. Moreover, the same

Table I. Effects of different initial population densities of *Meloidogyne incognita* on growth of tomato cultivar Shaft-Falat (60 days after planting).

Treatment (eggs and juveniles/g soil)	Root fresh weight (g)	Shoot dry weight (g)	Shoot fresh weight (g)	Shoot height (cm)
0 (Control)	9.1 c	11.1 a	36.5 a	61.2 a
0.25	10.0 c	10.2 ab	35.0 ab	57.2 a
0.5	11.5 c	9.7 ab	30.1 abc	56.7 a
1	12.6 abc	9.5 b	28.2 abc	56.5 a
2	15.6 bc	9.5 b	27.2 bc	51.7 a
4	16.6 c	8.8 b	24.3 c	50.2 a

Within a column, averages sharing a letter are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test.

Table II. Effects of different initial population densities of *Meloidogyne javanica* on growth of tomato cultivar Shaft-Falat (60 days after planting).

Treatment (eggs and juveniles/g soil)	Root fresh weight (g)	Shoot dry weight (g)	Shoot fresh weight (g)	Shoot height (cm)
Control	9.1 b	11.1 a	36.5 a	61.2 a
0.25	12.6 ab	10.6 ab	34.7 a	57.5 ab
0.5	14.2 ab	10.4 abc	36.2 a	57.2 ab
1	14.8 ab	10.5 abc	34.6 a	55.0 ab
2	16.2 ab	9.1 c	33.5 a	54.5 b
4	18.9 a	8.8 c	30.9 a	53.0 b

Within a column, averages sharing a letter are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test.

trend was recorded with shoot dry weights at initial densities of 1-4 eggs and J2 per g of soil ($P < 0.05$) (Table I). No significant difference in shoot fresh weights was observed among plants inoculated with *M. javanica*. Nevertheless, the effect of various densities of *M. javanica* on shoot dry weight was similar to that of *M. incognita* on shoot fresh weight (Table II).

Effects of initial densities of M. incognita *and* M. javanica *on nematode variables in tomato*. Final population density of *M. incognita* decreased significantly at initial densities of 2 and 4 eggs and J2 per g of soil ($P < 0.01$) (Table III). Furthermore, significant reductions in final population occurred at initial densities of 1, 2 and 4 eggs and J2 of *M. javanica* per g of soil (Table IV).

Table III. Effects of different initial population densities of *M. incognita* on nematode reproduction and root galling on tomato cultivar Shaft-Falat (60 days after planting).

Treatment (eggs and juveniles/g soil)	Eggs and juveniles/g soil	Eggs and juveniles/g root	Galls/g root	Reproduction factor
0.25	3.5 a	2012 b	40 b	68.8 a
0.5	4.0 a	2137 b	45 b	41.6 b
1	3.4 a	6462 a	101 a	58.6 a
2	2.6 b	7104 a	105 a	38.9 b
4	2.7 b	7546 a	118 a	21.9 c

Within a column, averages sharing a letter are not significantly different at $P \leq 0.05$ according to Friedman's Rank Test followed by a Bonferroni Means Comparison Test.

Table IV. Effects of initial population densities of *M. javanica* on nematode reproduction and root galling on tomato cultivar Shaft-Falat (60 days after planting).

Treatment (eggs and juveniles/g soil)	Eggs and juveniles/g soil	Eggs and juveniles/g root	Galls/g root	Reproduction factor
0.25	3.6 ab	1,550 d	16 c	67.1 a
0.5	4.1 a	1,925 c	20 c	44.9 b
1	2.9 b	2,662 b	75 b	40.8 b
2	2.7 bc	2,700 b	83 b	27.3 c
4	1.9 c	2,875 a	108 a	16.4 d

Within a column, averages sharing a letter are not significantly different at $P \leq 0.05$ according to Friedman's Rank Test followed by a Bonferroni Means Comparison Test.

Table V. Effects of initial population densities of *M. javanica* on growth of cucumber cultivar Super Amelia (60 days after sowing).

Treatment (eggs and juveniles/g soil)	Root fresh weight (g)	Shoot dry weight (g)	Shoot fresh weight (g)	Shoot height (cm)
Control	5.6 e	21.0 a	49.4 a	134.5 a
0.125	5.9 de	20.9 a	45.2 a	142.2 a
0.25	7.1 d	20.8 a	43.2 ab	133.5 a
0.5	9.7 c	20.3 a	42.6 ab	117.2 abc
1	12.0 b	19.9 ab	41.0 ab	122.5 ab
2	14.5 a	19.8 ab	38.9 ab	87.2 cde
4	8.8 c	16.7 c	31.5 bc	96.7 cde
8	5.5 e	10.6 d	25.0 c	78.0 de
16	4.7 e	9.1 d	23.6 c	62.0 e

Within a column, averages sharing a letter are not significantly different at P ≤0.05 according to Duncan's Multiple Range Test.

The number of root galls increased with the increase of the initial population of both nematode species (Tables III, IV). The reproduction rate of *M. incognita* decreased from 68.8 at P_i = 0.25 to 21.9 at P_i = 4 (Table III). These figures were 67.1 at P_i = 0.25 and 16.4 at P_i = 4 for *M. javanica* (Table IV).

Effects of initial densities of M. javanica *on growth variables in cucumber*. Shoot length and shoot fresh and dry weights of cucumber plants were significantly reduced ($P < 0.01$) with the increase in the initial densities of the nematode (Table V). There was no significant difference from the control ($P < 0.01$) in shoot length of plants inoculated with 0.125-1 egg and J2 per g of soil. In comparison with the other treatments, root fresh weight was significantly increased at P_i = 2. The greatest reductions in root fresh weight was observed at P_i = 8 and 16 eggs per g of soil (Table V). Significant decreases in shoot fresh and dry weight relative to the control were recorded at P_i = 4, 8 and 16 eggs per g soil ($P < 0.01$) (Table V).

Effects of initial densities of M. javanica *on nematode variables in cucumber*. Increases in initial densities resulted in significant ($P < 0.01$) increases in the number of eggs and J2 and galls of nematode per g of roots and gall index. The number of eggs per g of root was greatest at P_i = 8 and least at P_i = 0.125 (Table VI). Interestingly, the reproduction rate of the nematode was significantly reduced as initial densities increased ($P < 0.01$) and was least at P_i = 16 (Table VI).

Table VI. Effects of different initial population densities of *M. javanica* on nematode reproduction and root galling on cucumber cultivar Super Amelia (60 days after planting).

Treatment (eggs and juveniles/g soil)	Eggs and juveniles/g root	Gall index	Galls/g root	Reproduction factor
0.125	5162 g	1.2 e	11.2 h	121 a
0.25	6875 f	1.5 de	17.5 f	98.7 ab
0.5	11125 e	2 d	23.2 g	108 a
1	11875 e	2.5 c	50.7 e	73 bc
2	28335 d	3 b	93 d	103 a
4	46500 b	3.5 b	132 c	51.2 dc
8	97750 a	5 a	234 b	34 de
16	33750 c	5 a	267 a	4.7 ef

Within a column, averages sharing a letter are not significantly different at P ≤0.05 according to Friedman's Rank Test followed by a Bonferroni Means Comparison Test

DISCUSSION

Several studies have shown that there is an inverse relationship between the nematode population in the soil and nematode reproduction on the host plants. Also, there is a positive relationship between the initial population density of the nematode and the extent of damage it causes to host plants (Seinhorst, 1965, 1970; Barker and Olthof, 1976; Schomaker and Been, 2006; Greco and Di Vito, 2009). These studies allow the estimation of the tolerance limit, defined as the nematode population density at planting up to which no measurable yield loss of a host occurs, and of the economic threshold. Estimation of these critical levels is basic to the design of nematode management programmes (Ferris, 1978; Korayem, 2006).

Although shoot height and shoot fresh and dry weights decreased with increasing initial densities of the nematodes, plants inoculated with large initial densities of the nematodes showed increases in root fresh weight. This is probably because, within the range of nematode population densities at planting used in this study, increased root weight was due to the formation of galls on the roots.

Seinhorst (1965, 1970) derived models to describe the relationship between population densities of a nematode at planting and yield of the host plant and nematode population at harvest. A much wider range of nematode population densities at planting than that used by us would have been necessary to obtain a reliable fit of Seinhorst's model to the data. However, consideration of the data suggests tolerance limits of both crops to both nematodes of roughly 0.25 eggs/g soil. This figure is close to that estimated for several vegetables under pot or microplot conditions (Greco and Di Vito, 2009).

Increasing initial densities of the nematodes increased the numbers of eggs and galls per g of root and the increase was greater in cucumber than in tomato. In general, the reproduction rate of the nematode decreased with the increase of the initial densities, thus agreeing with previous studies (Lindsey and Clayshulte, 1982; Di Vito *et al.*, 1985; Korayem, 2006; El-Sherif *et al.*, 2007). This was probably due to the reduced amount of food available per nematode at higher nematode densities and the greater competition for food and space in the roots (Seinhorst, 1970).

The number of galls per g of roots of tomato increased significantly with the increase of the initial densities of both *M. javanica* and *M. incognita*. However, the increase was smaller on tomato inoculated with *M. javanica*. At 1-4 eggs and J2 per g of soil, the numbers of eggs and galls per g of tomato root inoculated with *M. incognita* were significantly higher than those on tomato roots inoculated with *M. javanica*. This would suggest that *M. incognita* has the potential to cause more damage to tomato than *M. javanica*. Also, *M. javanica* reproduced more on cucumber than on tomato.

The greatest reproduction rate of *M. javanica* on cucumber was recorded at the smallest initial density (P_i = 0.125) of the nematode, which is consistent with Di Vito *et al.* (1985). Overall, it was found that increasing the initial densities of the two nematodes increased losses to both cucumber and tomato. However, the damage caused by these two nematodes to tomato and cucumber was evaluated under greenhouse conditions and further experiments are required under field conditions, in several growing seasons, before any recommendation to the farmers can be made.

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