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Damage of the root-knot nematode *Meloidogyne javanica* to bell pepper, *Capsicum annuum*

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Abstract

Root-knot nematodes are the most economically important nematodes on a worldwide basis. Prediction of the crop loss that a certain population density of nematode may cause is of importance to decide whether to cultivate a plant or not. It is also of use for choosing the most suitable managing strategies. A pot experiment was devised to determine the damage potential of an Iranian population of *Meloidogyne javanica* on pepper (*cv*. California Wonder). Two leaves seedlings were planted in 3 kg pots contain different inoculum level of 0, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256 and 512 eggs and juveniles g^{-1} sandy loam soil. Shoot weight, yield and nematode reproduction rate were negatively correlated with initial inoculum level of nematode while the root response was variable. Seinhorst's model fitted the data of yield and shoot weight and a tolerance limit of 0.5 and 0.28 eggs and juveniles g^{-1} soil was respectively estimated. The minimum relative yield and shoot weight was 0.16 and 0.3, respectively. The damage threshold was estimated of 1.8 eggs and juveniles g^{-1} soil by Seinhorst's model. The maximum multiplication rate and the equilibrium density of *M. Javanica* were estimated as 708.31 and 159.65 (eggs and juveniles g^{-1} soil), respectively.

Key words: Crop loss, damage threshold, final population, initial population, population dynamics, Seinhorst model

Introduction

Plant-parasitic nematodes are of considerable importance and their harmful impacts on crops have great economic and social effects (Nicol et al. 2011). *Meloidogyne* spp. are the most serious plant-parasitic nematodes on a worldwide basis. Their annual losses are estimated to be about 5% of total plant products (Agrios 2005).

Bell pepper (*Capsicum annuum*) is widely cultivated in Iran, both in greenhouse and on farm. Root-knot nematodes (RKNs) attack this crop and often impose a considerable loss (Ghaderi et al. 2012). Reduction of plant yield directly depends on the initial population density of nematode at planting (Greco & Di Vito 2009). Estimation of the crop loss that a nematode may cause is of importance for deciding to cultivate a plant/cultivar or not. Prediction of the damage extent is also of use for picking out the most suitable managing strategies (Schomaker & Been 2006). The extent of damage to a plant by a nematode is influenced by plant cultivar and/or nematode strains. Difference in resistance of plant cultivars as well as in aggressiveness of different populations of nematode is considered as a cause for this variation (Hussey & Janssen 2002). Thus, it is rational to examine the response of major plant cultivars of each area to indigenous populations of nematodes to obtain the most precise correlation.

There are very limited data on the impact of *M. javanica* on the yield reduction of bell pepper; therefore, the present study was devised under greenhouse condition to ascertain the damage potential of increasing population densities of *M. javanica* on the growth and yield of bell pepper (*cv*. California Wonder).

Materials and methods

Preparation of bell pepper seedling

Seeds of bell pepper (*cv*. California Wonder) were washed and disinfected for 5 min in a 5% sodium hypochlorite solution. Thereafter they have been planted in seed germination trays and kept at 29 ± 1°C.

Preparation of *M. javanica* inoculum

The inoculum was adequately prepared on tomato plants (*cv*. 'Early-Urbana') starting from a single nematode egg mass previously identified as *M. javanica* (Moosavi et al. 2010, Moosavi 2012). The nematode infected roots were cut into 0.5–1 cm pieces and shaken up for 2–3 min in 0.5% sodium hypochlorite solution followed by rinsing over 60 and 20 μm sieves (Hussey & Barker 1973). Collected eggs and second stage juveniles (J_2s) were transferred to a 250 ml beaker with 100 ml of sterilized distilled water. The population densities of eggs and J_2s were estimated by means of three counts and adjusted to 100 eggs and J_2s per ml. The ability of the nematode to infect bell pepper (*cv*. California Wonder) was assessed in a pre-test.

Pot preparation with different population densities of *M. javanica*

Pots (17 cm diameter, 11 351 cm^3) were filled with 3 kg of unsterile virgin sandy loam soil composed of sand 67.3%, clay 12.1%, silt 20.6%, organic matter 3.5% with pH 7.5. The soil was tested prior to use to ensure that RKNs were absent. A geometric series of *M. javanica* inoculum (0, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256 and 512 eggs and $J_2 s g^{-1}$soil) was selected (Seinhorst 1965, 1986a) and distributed uniformly while filling the pots with soil. The pots were placed in a saucer and their top surface was covered with a 2-cm layer of polythene granules to prevent water loss (Ehwaeti et al. 1998). After three days, the pots were planted with a bell pepper seedling which had grown their second set of true leaves. Routine cultural practices were followed after transplanting and fertilizer was provided as needed.

Effect of different population densities of *M. javanica* on bell pepper growth and yield

The pots were arranged in a completely randomized design with five replications and were kept in a greenhouse for three months. Bell pepper fruits from each treatment were hand-harvested and their weights were recorded during growth period. At end of the experiment, the plants were harvested and the fresh tops and roots weighed. Nematode final population (on roots and in soil) and reproduction factor were determined at all initial inoculum levels (Greco & Di Vito 2009).

The relationship between nematode initial population and shoot weight or yield (gram fresh bell pepper) of each treatment was determined by using the Seinhorst function (equation 1; Seinhorst 1965, 1972, 1986b, 1998).

Equation 1

$$Y = m + (1 - m) \times 0.95^{\frac{(P_i - T)}{T}} \quad \text{at } P_i > T$$

$$Y = 1 \text{ at } P_i \leq T$$

In this equation Y stands for the relative yield or relative shoot weight; m is the minimum relative yield or relative shoot weight; P_i is the initial number of eggs and J_2s per g of soil; and T is the tolerance limit of the crop to the nematode. Damage threshold is defined as the number of nematodes required to cause a 10% yield loss (Stirling 2000, Mueller et al. 2012). The tolerance limit and Damage threshold were estimated by fitting the data in the Seinhorst damage function using SigmaPlot software version 11.0 (Jandel Scientific, Corte Madera, CA). Regression analysis was also performed to relate the root weight to nematode initial population using SigmaPlot software.

Population dynamics

The population dynamics of *M. javanica* on bell pepper was evaluated by population model proposed by Seinhorst (1967a, b, 1986a). The last two data points (256 and 512 eggs and J_2s per g of soil) were excluded from the predictor variable (P_i) to improve fitting data.

Equation 2

$$P_f = \frac{a \times E \times P_i}{(a - 1) \times P_i \times E}$$

a – the maximum multiplication rate

E – equilibrium density (where $P_i = P_f$)

$$M = \frac{a \times E}{(a - 1)} \text{ the maximum population density}$$

The relationship between P_i and P_f were estimated by fitting the data in the model using SigmaPlot software version 11.0 (Jandel Scientific, Corte Madera, CA).

Results

Effect of different population densities of *M. javanica* on bell pepper growth and yield

Increase in population densities of *M. javanica* up to 4 eggs and J_2s g^{-1}soil was accompanied with increase in root fresh weight (g). When P_i exceeded 4, the root fresh weight decreased (Fig. 1). Seinhorst model could not properly correlate the root fresh weight to the nematode initial population. The polynomial regression analysis was conducted up to order 5. The best equation for prediction of root fresh weight according to nematode initial population was equation 3 ($R^2 = 0.41$).

Equation 3

$$\text{Root weight} = 23.376 - (0.218 - P_i) + (0.000935 \times P_i^2) - (0.00000111 \times P_i^3)$$

The shoot weight of the plant was negatively affected by increase in *M. javanica* initial population. The aerial growth was highly suppressed when the initial population densities of nematode were equal or more than 8 eggs and J_2s per g soil (Fig. 2).

The shoot fresh weight data fitted Seinhorst model properly (equation 4). According to this equation, the minimum shoot weight (m) of bell pepper (*cv*. California Wonder) was

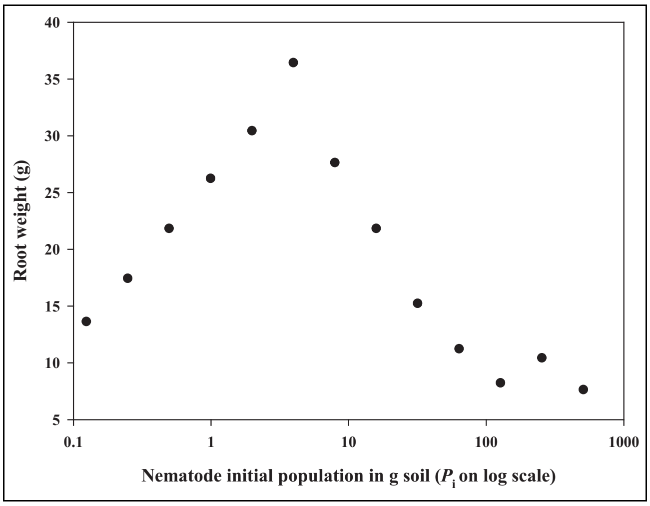

Fig. 1: Root fresh weight of bell pepper (*cv*. California Wonder) at different initial inoculum level of *Meloidogyne javanica*. The pots were kept in greenhouse for 90 days. Each treatment had five replications.

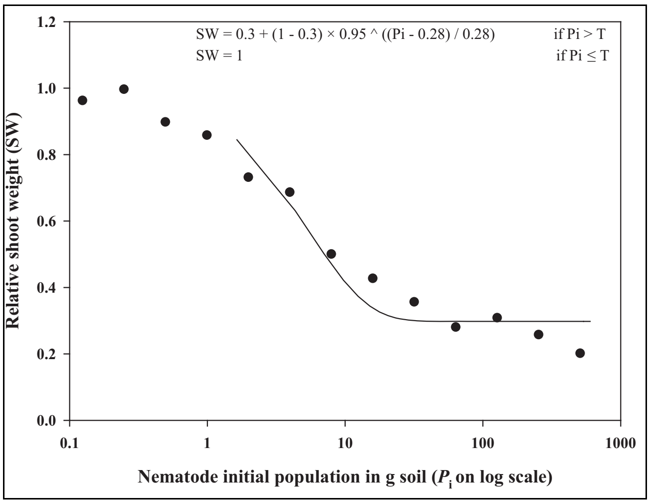

Fig. 2: Seinhorst model for the shoot weight (SW) reduction caused by *Meloidogyne javanica* on bell pepper (*cv*. California Wonder) 90 days after inoculation with different initial population densities (P_i) of the nematode. Each treatment had five replications.

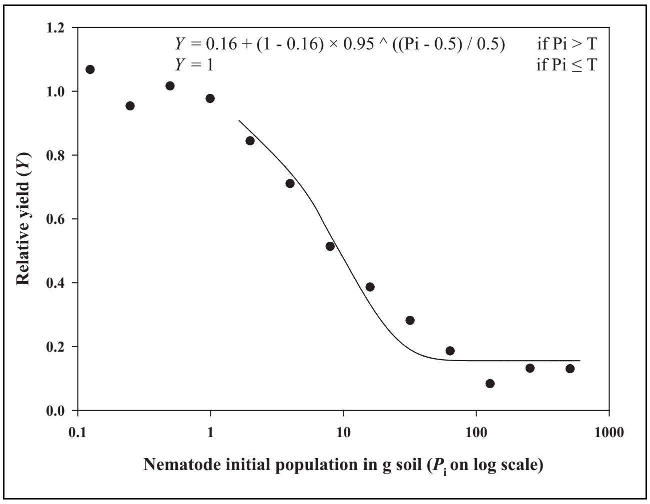

Fig. 3: Seinhorst model for the yield (Y) reduction caused by *Meloidogyne javanica* on bell pepper (*cv*. California Wonder) at different initial population densities (P_i). The pots were kept in green house for 90 days.

0.3 of unaffected plants. The tolerance limit (T) of this plant to nematode was 0.28 eggs and J_2s g^{-1} soil (standard error (se) of m = 0.02; se T = 0.03; coefficient of variation (CV) of m = 5.5; CV T = 9.6; df = 68; R^2 = 0.92).

Equation 4

$$Y = 0.3 + (1 - 0.3) \times 0.95^{\frac{(P_i - 0.28)}{0.28}} \text{ at } P_i > T$$

$$Y = 1 \text{ at } P_i \leq T$$

Infection of bell pepper with *M. javanica* caused a significant decrease in plant's yield (gram fresh bell pepper). Based on the Seinhorst model, the tolerance limit (T) of bell pepper (*cv*. California Wonder) to Iranian population of *M. javanica* was of 0.5 eggs and J_2s g^{-1} soil with an estimated minimum yield (m) of 0.16 at P_i more than 64 (equation 5; Fig. 3; se m = 0.01; se T = 0.03; CV m = 9.1; CV T = 6.5; df = 68; R^2 = 0.97).

Equation 5

$$Y = 0.16 + (1 - 0.16) \times 0.95^{\frac{(P_i - 0.5)}{0.5}} \text{ at } P_i > T$$

$$Y = 1 \text{ at } P_i \leq T$$

According to Seinhorst model, damage threshold (yield reduction of 10%) was detected when initial nematode populations exceeded 1.8 eggs and J_2s g^{-1}soil.

Population dynamics

The final population densities of nematodes on roots and in the soil increased with increasing P_i up to 16 eggs and J_2s g^{-1} soil. The reproduction factor was inversely related to initial population densities of *M. javanica*. The maximum reproduction factor of 496 was measured at the lowest P_i (0.125 eggs and J_2s g^{-1}soil). Though the reproduction factor was 1.2 for P_i at 128 eggs and J_2s g^{-1}soil, it was less than one for P_i more than 128 eggs and J_2s g^{-1} soil. The reproduction factor for the highest P_i was 0.08 and the final population density heavily decreased at the end of the experiment as compared to population density at planting.

To relate the final population of *M. javanica* to its initial population, the population dynamic model was fitted to the data. The maximum multiplication rate (a) and the equilibrium density (E) of *M. javanica* on bell pepper were estimated to be 708.31and 159.65 (eggs and J_2s g^{-1} soil), respectively. The maximum population density (M) of the nematode was predicted as 159.9 eggs and J_2s g^{-1} soil (equation 6; se a = 49.2; se E = 1.84; CV a = 6.95; CV E = 1.55; df = 53; R^2 = 0.91).

Equation 6

$$P_f = \frac{708.31 \times 159.65 \times P_i}{(708.31 - 1) \times P_i + 159.65}$$

Discussion

There are contradictory reports on susceptibility of the bell pepper cultivar California Wonder to *M. javanica* and *M. incognita*. It seems that different populations of nematodes have dissimilar abilities in parasitizing this cultivar. Cultivar California Wonder was susceptible to all four races of Indian population of *M. incognita*, while it was resistant to *M. javanica* (Khan & Khan 1991). However it is reported that Greek *M. incognita* populations virulent on the *Mi* resistant gene of tomato had a zero or decreased reproduction on California

Wonder (Tzortzakakis & Blok 2007). 30% of *M. javanica* isolates that were collected from nine districts of Uttar Pradesh (India) were infective on California Wonder (Khan et al. 2003). Though six populations of Nigerian *M. javanica* out of ten reproduced on cultivar California wonder (Ogbuji 1981), only one collected Brazilian population out of six was pathogenic on California Wonder (Carneiro et al. 1998). The susceptibility of *cv*. California Wonder to this Iranian isolate of *M. javanica* was confirmed in the current experiment; however, a pre-experiment had been arranged to test the pathogenicity of the nematode.

Unfortunately, there is no or limited information on the relationship between initial population densities of nematode and yield of host crop, even for the major RKN – crop combinations in most countries (Greco & Di Vito 2009). In this experiment the highly inoculated bell peppers flowered poorly and produced smaller fruits, but no significant effect was observed on the required time for flowering and fruiting. 50% of shoot growth and yield was reduced when the inoculum densities were 7 and 9.3 eggs and J_2s g^{-1} soil, respectively (Equation 4 and 5). At the highest P_i (512 eggs and J_2s g^{-1} soil), the shoot growth and yield were suppressed by 81 and 88%, respectively. The extent of yield as well as shoot growth decreased with the increase of inoculum level of *M. javanica* on pepper plants (Fig. 2, Fig. 3). Similar correlation was observed between *Meloidogyne* spp. and their host plants (Seinhorst 1965, 1970; Barker & Olthof 1976, Schomaker & Been 2006, Russo et al. 2007, Greco and Di Vito 2009, Hussain et al. 2011, Gharabadiyan et al. 2013, Moosavi 2014, Wesemael et al. 2014).

Increase in P_i up to 4 eggs and J_2s g^{-1} soil caused an increase in the root weight; however, the root weight decreased when the P_i exceeded 4 eggs and J_2s g^{-1} soil (Fig. 1). Root fresh weight did not significantly relate to P_i either in Seinhorst model or polynomial regression analysis. Increase in root volume in response to infection as well as gall formation raises the root weight. However, when the population level of nematode surpasses a limit, the imposed damage is too high to be tolerated by plant and the root growth and weight decreases (Karssen & Moens 2006).

At low population densities (P_i = 0.125 and 0.5) of *M. javanica*, yield showed a little increase (Fig. 3). It has been suggested that nematode-infected plants produce auxin-like compounds (Abad et al. 2009) which can keep the yield unaffected or even can cause little increase at low population density of nematode (Greco & Di Vito 2009).

Bell pepper (*cv*. California Wonder) could tolerate 0.28 egg and J_2s of *M. javanica* g^{-1} soil without any significant weight reduction in its aboveground parts. The relative minimum shoot weight was estimated as 0.3. In an experiment with the Ethiopian population of *M. javanica*, the shoot weight tolerance limit (*T*) of bell pepper (*cv*. Marekofana) was of 0.36 J_2 cm^{-3} soil. The tested population densities were 0, 1, 2, 4, 8 and 16 J_2 cm^{-3} soil and Seinhorst's model estimated the minimum shoot weight (*m*) of 0.4 at 32 J_2 cm^{-3} soil for that research (Mekete et al. 2003).

The tolerance limit (*T*) of bell pepper to *M. javanica* was of 0.5 egg and J_2s g^{-1} soil with an estimated relative minimum yield (*m*) of 0.16 (Fig. 3). Di Vito et al.(1985) defined a tolerance limit of pepper to *M. javanica* of 2.2 and 0.165 eggs cm^{-3} soil when inoculum type were dissolved egg masses and finely chopped infested roots, respectively. Tolerance limit of bell pepper (*cv*. Yolo Wonder) to *M. incognita* was estimated of 0.74 eggs and juveniles cm^{-3} soil (Di Vito 1979) while it was assessed as 0.3 eggs and juveniles of *M. incognita* per cm^3 soil for both susceptible (*cv*. Yolo Wonder) and resistance (*cv*. Line 89422) genotypes of bell pepper. However, the minimum relative yield of susceptible and resistance genotypes were 0.5 and 0.16, respectively (Di Vito et al. 1992). Ferries et al. (1986) reported tolerance limit of bell pepper (*cv*. Yolo Wonder) to *M. incognita* of 0.065 eggs and juveniles per g loamy sand soil. Difference in tolerance limits and minimum yields of various studies can be a consequence of variation in plant species and cultivar, nematode species and population, soil type (Hussey & Janssen 2002, Greco & Di Vito 2009, Ravichandra 2014), the way of collecting and using the inoculum (Hussey & Barker 1973, Vrain 1977, Di Vito et al. 1986), and environmental conditions (Mekete et al. 2003).

The term "damage threshold" is used by nematologists to define the population density that will cause about 10% reduction in yield (Thomas et al. 1995, Stirling 2000, Mueller et al. 2012). The damage threshold of *M. javanica* on bell pepper was calculated as 1.8 eggs and J_2s g^{-1}soil by Seinhorst's model. Di Vito et al. (1992) estimated the damage threshold of *M. incognita* on two cultivars of bell pepper. In their experiment, a 10% yield reduction occurred on susceptible (*cv*. Yolo Wonder) and resistant (*cv*. Line 89422) bell pepper when initial inoculum level was 1.078 and 1.669 eggs and juveniles cm^{-3} soil, respectively. In another microplot experiment on chile pepper in New Mexico, the yield of 3 cultivars, New Mexico 6–4, Sandia and Jalapeno, were respectively reduced by 10% at pre-plant inoculum level of 12, 33 and 95 eggs and juveniles of *M. incognita* per 500 ml soil (Thomas et al. 1995).

A negative correlation was observed between *M. javanica* initial population densities and reproduction factor. The growth of highly infected plants was severely reduced at both aerial and underground parts; therefore, they could not supply enough food for high populations of nematode. Consequently the final population density of nematodes seriously decreased at higher P_i. Negative correlation was repeatedly reported for nematode initial inoculum levels and reproduction rate (Benson et al. 1976, Barker & Benson 1977, Lindsey & Clayshulte 1982, Di Vito et al. 1992, Charegani et al. 2012, Moosavi 2014).

Since the density of the population in soil at planting time is a good predictor for plant damage, analysis of nematode population dynamics is an imperative discipline in nematology (Schomaker & Been 2006). Here, the population dynamics of *M. javanica* on bell pepper was described by a mathematical model which represented changes in final population in terms of the initial population densities. The last two data points was excluded from P_i as the corresponding P_f values had been influenced by growth reduction, caused by the nematodes.

The susceptibility of bell pepper (*cv*. California Wonder) to Iranian isolate of *M. javanica* is approved by this experiment. The cultivar's tolerance limit (without yield reduction) and damage threshold (10% yield reduction) was respectively estimated of 0.5 and 1.8 eggs and J_2s g^{-1}soil by Seinhorst's model.

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