

INTERACTION OF THE ENTOMOPATHOGENIC NEMATODE *STEINERNEMA MASOODI* AND THE ROOT-KNOT NEMATODE *MELOIDOGYNE INCOGNITA* ON TOMATO

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Summary. The effect of different population levels (100, 1000 and 10,000) of infective juveniles (IJ) of *Steinernema masoodi* was tested on the egg hatching and juvenile mortality of *Meloidogyne incognita* in cavity blocks containing distilled water. A population of 1000 or more IJs of *S. masoodi* caused significant inhibition of the hatching of eggs and induced mortality to juveniles of *M. incognita*. The effect of the entomopathogenic nematode (EPN) on root penetration by root-knot nematode (RKN) juveniles was studied using concomitant inoculation with 500 J₂ or 15 egg masses of RKN and 500, 1000, 5000 or 10,000 IJs of *S. masoodi*. The tested population levels of the EPN resulted in significant and progressive decrease in the penetration of RKN J₂ into tomato roots. The effect of the EPN on galling, reproduction and soil population of RKN and on the plant growth and yield of tomato was investigated using pre, post and concomitant inoculation with 2000 J₂ of *M. incognita* and 1000, 5000 or 10,000 IJs of *S. masoodi*/plant in earthenware pots. The pre-inoculation with 10,000 EPN IJs caused 11-39% reduction in galling, egg mass production and soil population of RKN and 19-22% increase in plant dry weight and yield of tomato. *Steinernema masoodi* at 5000 IJs inoculum level markedly suppressed nematode pathogenesis irrespective of inoculation mode, but the effect was significantly less than that obtained with 10,000 IJs, whereas 1000 IJs did not produce significant effects ($P \leq 0.05$) on nematode pathogenesis.

Keywords: Galling, hatching, plant growth, reproduction, *Solanum lycopersicum*, yield.

The biological control of plant parasitic nematodes (PPNs) mostly relies on the use of fungal and bacterial antagonists, but some researchers have shown that entomopathogenic nematodes (EPNs) also have potential to suppress PPNs (Jagdale *et al.*, 2002; Lewis, 2006). EPNs are basically pathogenic to insects (Gaugler, 1993; Grewal *et al.*, 2005) and kill their larvae with the help of symbiotic bacteria present in the gut that causes septicemia to the prey within 24-72 hours (Kaya and Gaugler, 1993) and finally the death of the insect. EPNs are known to suppress the activity of plant parasitic nematodes (Grewal *et al.*, 1999). The suppression of PPNs by EPNs may largely occur due to overcrowding, physical competition for space in the root zone (Bird and Bird, 1986; Gouge *et al.*, 1994) and/or due to an allelopathic effect of the associated symbiont bacteria (*Xenorhabdus* and *Photorhabdus*) on plant nematodes (Hu *et al.*, 1995, 1999; Grewal *et al.*, 1997). The symbiont bacteria produce a potent toxin that may kill PPNs (Akhurst and Boemare, 1990). A few studies have been conducted to determine the interaction between these two types of nematodes (Lewis *et al.*, 2001; Lewis and Grewal, 2005; Crow *et al.*, 2006).

The present study aimed to explore and ascertain the nature of the interaction that may occur between the entomopathogenic nematode, *Steinernema masoodi* Ali, Pervez, Hussain *et al.* Ahmad, and the plant parasitic nematode, *Meloidogyne incognita* (Kofoid *et al.* White)

Chitw., and the interactive effects on the plant growth and yield of tomato under artificial treatment conditions. Therefore, three experiments were conducted. The first experiment was conducted *in vitro* to investigate the effect of different population levels of EPNs on hatching of eggs and mortality of juveniles of *M. incognita*. The second experiment was done to determine the effect of EPNs on the penetration of second stage juveniles of *M. incognita* into tomato roots in cups. In the third experiment, the effects of the EPNs were studied on root-knot disease development, reproduction of root-knot nematode, and plant growth and yield of tomato in pots.

MATERIALS AND METHODS

EPN culture. *Steinernema masoodi* was isolated from a field of the Agriculture Faculty, Aligarh Muslim University, using the insect trap method developed by Bedding and Akhurst (1975). A soil sample (500 g) was collected from a depth of 2-4 cm after removing 1 cm of top layer soil from the experimental field. Two hundred and fifty ml of the moist soil was put in plastic jars of 500 ml capacity, onto which 5-6 *Galleria mellonella* L. larvae were placed as bait for EPNs. The jars were closed with a lid having perforations for exchange of air. The insect larvae were examined for mortality every 24 hrs. Dead larvae were placed on White traps (White, 1927) and incubated at 25 ± 2 °C in a Biological Oxygen Demand (BOD) incubator. The Whatman filter pa-

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per was kept moist by adding 5-10 drops of sterilized distilled water. The EPNs emerging from the cadaver were collected in culture flasks and Koch's postulates were tested to ensure the entomopathogenic behaviour of the nematodes. The nematode was identified as *S. masoodi* and a mass culture of *S. masoodi* was prepared on *G. mellonella*. The process involved application of 100-200 infective juveniles of the nematode to a water soaked filter paper lining a Petri plate and placing 5-6 *G. mellonella* larvae on it. The dish was covered with the lid and incubated at 25 ± 2 °C. After 2-5 days of incubation the dead larvae of the insect were transferred to White traps and incubated at 25 ± 2 °C for 2 weeks. The EPN juveniles that emerged from the cadavers were collected from the Petri plates by adding distilled water and stored with water in culture flasks at 25 °C.

Root-knot nematode culture. Eggplants showing knots were collected from the field. A single egg mass was picked from the infected roots and the female under the egg mass was excised and its perineal pattern was prepared to confirm that the egg mass was of *M. incognita*. The egg mass was surface sterilized in 1:500 aqueous solution of sodium hypochlorite for five minutes and was then thoroughly washed before transfer to a small coarse sieve lined with tissue paper, which was placed in a Petri plate containing sufficient water to just cover the tissue paper. The Petri plate was incubated at 27 ± 5 °C for five days (den Ouden, 1958). The second stage juveniles that emerged from the single egg mass were used to inoculate eggplant seedlings raised in autoclaved soil. This culture of *M. incognita* provided egg masses, which were collected to prepare inoculum of the nematode for use in the experiments.

Experiment 1. The effects of different population levels of *S. masoodi* (0, 100, 1000 and 10,000 IJs/5 ml) on hatching of eggs and mortality of juveniles of *M. incognita* were studied in distilled water in cavity blocks containing 10 egg masses, or 1000 J_2 of *M. incognita* separately. Three cavity blocks (replicates) were maintained for each treatment. The cavity blocks were incubated in a BOD incubator at 25 ± 2 °C for 5 days and the numbers of J_2 that emerged from the egg masses and the numbers of dead PPNs and/or EPNs were recorded every 24 hours. Then, per cent egg hatching and mortality of PPNs and/or EPNs were calculated. Nematode juveniles were considered dead if they were straight and immobile.

Experiment 2. The effect of different concentrations of IJs of *S. masoodi* on root penetration of *M. incognita* juveniles was studied in soil. Styrofoam cups (150 ml capacity, 6.5×8.0 cm, diameter \times height) were filled with 100 g steam sterilized sandy loam soil (sand 70%, silt 22%, clay 8%, pH 7.5) to which 500 J_2 or fifteen egg masses having 240 eggs/egg mass of *M. incognita* along with 500, 1000, 5000 or 10,000 IJs of *S. masoodi* were

added separately. A day later, a 3-week-old seedling of tomato, *Solanum lycopersicum* L. cv. Pusa Ruby was planted in each cup. Nine replicates were maintained for each treatment and observations were made 7, 10 and 15 days after inoculation on three of them on each occasion. Tomato seedlings inoculated with either J_2 or egg masses of *M. incognita* alone and receiving distilled water rather than a suspension of the EPNs served as controls. On the designated observation days, seedlings from three cups were uprooted; roots were washed and cut into small pieces 3-5 cm in length. The pieces were plunged into boiling 0.1% acid fuchsin for 3 minutes in a beaker. Then the roots were washed in running water to remove excess stain and placed in Petri plates with clear lactophenol for 2-3 days to differentiate nematodes in the root tissue. Small amounts of roots were gently pressed between two glass slides (Khan, 2008), examined under a stereoscopic microscope and the numbers of juveniles and adults of *M. incognita* present in the roots were counted.

The populations of *S. masoodi* and *M. incognita* that remained in the soil were extracted using Cobb's decanting and sieving method followed by Baermann funnels. The specimens of *M. incognita* and *S. masoodi* extracted were counted in a multi-chambered counting dish under a stereoscopic microscope.

Experiment 3. A pot experiment was conducted to investigate the effect of *S. masoodi* on the root-knot disease, reproduction of *M. incognita*, plant growth and yield of tomato cv. Pusa Ruby. Three-week-old tomato seedlings, raised in sterilized soil, were transplanted into earthenware pots (15 cm \times 15 cm) containing 1 kg of a mixture of autoclaved (121 °C for 20 minutes) soil and farm yard manure in the ratio 3:1. The pots were inoculated with 1000, 5000 or 10,000 infective juveniles of *S. masoodi*, 7 days before (pre-inoculation), 7 days after (post-inoculation) and simultaneously (concomitant inoculation) with 2000 freshly hatched J_2 of *M. incognita*. Uninoculated plants served as a control. For each treatment five pots (replicates) were maintained and arranged in a completely randomized design. The plants were grown for four months during which they were uniformly watered and regularly observed for any symptoms. At harvest, pots were flooded with water to facilitate maximum root recovery. The plants were gently uprooted and roots were rinsed with water. The roots were stained with phloxin B for ease of counting of egg masses. Then the roots were immersed in 50 ml of 0.5% NaOCl solution in a 100 ml conical flask and stirred for 5 minutes to dissolve the gelatinous matrix of the egg masses and separate eggs. The egg suspension rinsed from roots was centrifuged at 1600 rpm for 10 minutes in a sugar solution (specific gravity 1.18). The centrifugation separated eggs from other soil and crop debris for ease in counting the eggs and thereby determining the fecundity of female *M. incognita* (number of eggs/egg mass). The soil population of *S. masoodi* and

M. incognita was estimated in the soil collected from the pots, after uprooting the plants, using Cobb's decanting and sieving method.

The yield was determined by weighing tomato fruits collected during the growth period (4 months). At the last harvest, plant dry weight (roots and shoots) was recorded after drying the plants in an oven at 60 °C for 3-4 days.

Statistical analysis. The data on hatching, mortality, penetration and soil population were subjected to one way analysis of variance (ANOVA) and the least significant difference (LSD) was calculated at three probability levels viz., $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$. Data on the effect of EPN on galling, egg mass production, plant growth etc., were analyzed by two way ANOVA considering EPN inoculation as factor one and RKN inoculation as factor two. The LSD was calculated at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$ probability levels. Duncan's Multiple Range Test was used to identify significant differences caused by the two treatments on hatching and mortality data (Fig. 1). In all figures, significance is denoted at $P \leq 0.05$, but in the Results section smaller alpha levels are also reported.

RESULTS

Experiment 1

Hatching of eggs of *M. incognita* was inhibited by 11.6% in the cavity blocks having 100 IJs of *S. masoodi* while at 1000 and 10,000 IJs, the hatching decreased by 23 and 24.8%, respectively (Fig. 1). Mortality of the hatched juveniles of *M. incognita* increased to 13.9% with 100 IJs and was 14.6 and 26% with 1000 and 10,000 IJs of *S. masoodi*, respectively, while it was 2.9%

in the control (data not shown). On incubation of 1000 J₂ of *M. incognita* with 100 IJs of *S. masoodi*, 12% mortality in *M. incognita* juveniles over the control was recorded. This mortality increased to 29 and 45% with 1000 and 10,000 IJs of *S. masoodi*, respectively (Fig. 1).

Experiment 2

Inoculation with 500 juveniles. The penetration of *M. incognita* into the roots of tomato was invariably affected by the presence of *S. masoodi* in the root zone and was also population dependent (Fig. 2). Root penetration of *M. incognita* decreased by 10-18% during the period of 7-15 days after inoculation with 500 EPN. The highest inoculum level, i.e. 10,000 IJs, resulted in 58-68% decrease of the nematode penetration into the roots.

The greatest numbers of *M. incognita* and *S. masoodi* were recovered from soil on the 7th day after inoculation but a marked decline in the root-knot nematode population was recorded on the 10th and 15th day after inoculation in all treatments, including the control (Fig. 3). The nematode specimens extracted from the soil showed considerable variation in the proportions of live and dead juveniles. In the control, the mortality of *M. incognita* was 6% while it increased to 16% and 32% in pots inoculated with 500 ($P \leq 0.01$) and 10,000 IJs ($P \leq 0.001$) of *S. masoodi*, respectively (data not shown). However, in the presence of *M. incognita*, *S. masoodi* exhibited 11, 15, 22 and 24% juvenile mortality at the 500, 1000, 5000 and 10,000 population levels in comparison to the control (4.3% mortality without *M. incognita*, data not shown).

Inoculation with fifteen egg masses. The number of specimens of *M. incognita* that penetrated the roots of tomato inoculated with fifteen egg masses was less than

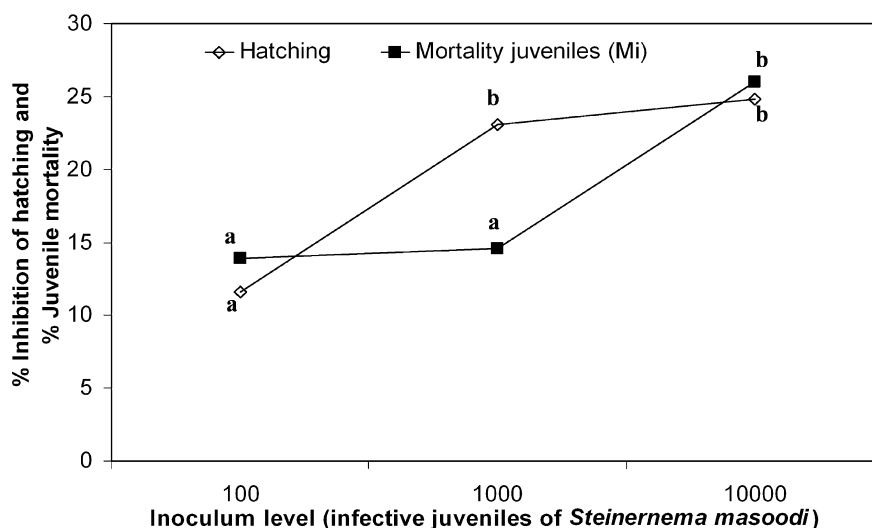


Fig. 1. Effect of *Steinerema masoodi* on hatching and mortality of *Meloidogyne incognita*. Within a line, points followed by different letters are significantly different from each other at $P \leq 0.05$ according to Duncan's Multiple Range Test.

that observed when tomato seedlings were inoculated with juveniles (Fig. 2). Root penetration 15 days after inoculation was reduced by 25, 48, 53 and 68% when the soil was inoculated with 500, 1000, 5000 and 10,000 IJs of *S. masoodi*, respectively (Fig. 2). More than 1200 juveniles of *M. incognita* were recovered from the control 15 days after inoculation (Fig. 3). However, the population was progressively lower (851, 531, 413, and 397) in the presence of increasing numbers of *S. masoodi*. The mortality in *M. incognita* juveniles ranged from 13 to 29% at the different inoculum levels of *S. masoodi* (significant at $P \leq 0.01$ and 0.001, data not shown).

Experiment 3

The experiment on the effect of pre, post and concomitant inoculation of *S. masoodi* and *M. incognita* on root galling, egg mass production, soil population of *M. incognita* and plant growth and yield of tomato revealed a significant decline in the root and shoot dry weights and yield on plants inoculated with 2000 J₂ of *M. incognita* compared to the control (Table I). An average of 87 galls and 72 egg masses were formed on tomato roots in the control. Gall formation and egg mass production by *M. incognita* decreased significantly ($P \leq 0.05$) on application of 5000 or 10,000 IJs of *S. masoodi* being greatest with the latest application (Table I). However, the fe-

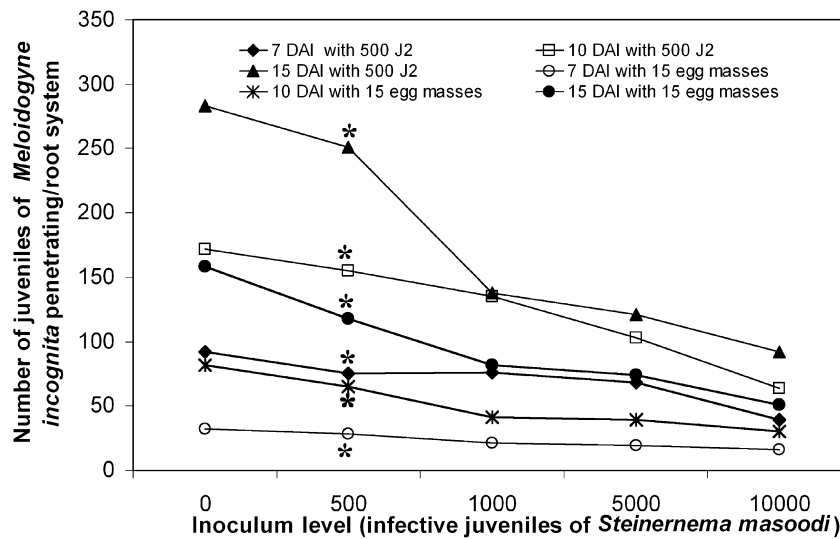


Fig. 2. Effect of *S. masoodi* on the penetration of *M. incognita* in the roots of tomato seedlings. *All treatments with 500 IJ of *S. masoodi* are significantly ($P \leq 0.05$) different from the respective controls (0 IJ of *S. masoodi*).

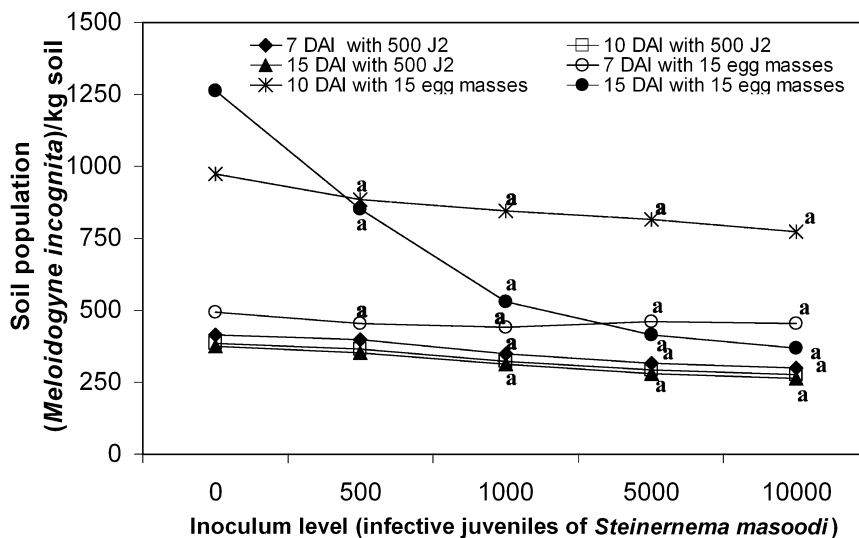


Fig. 3. Effect of inoculation with *S. masoodi* on the soil population of *M. incognita*. Within a line, points followed by letter a are significantly different from the respective controls (0 IJ of *S. masoodi*) at $P \leq 0.05$.

Table I. Effect of pre, post and concomitant inoculation with *Steinernema masoodi* and *Meloidogyne incognita* on the plant growth of tomato, and on the root-knot disease and reproduction of the nematode.

Treatment	Dry weight		Yield (g)	No. of galls/plant	No. of egg masses/plant	No. of eggs/egg mass	Soil population	
	Roots	Shoots					<i>M. incognita</i>	<i>S. masoodi</i>
Uninoculated control								
2000J ₂ Mi	5.7	14.4	388	-	-	-	-	-
1000J ₃ Sm	4.8 ^a	10.3 ^c	305 ^b	87	72	243	2904	-
5000J ₃ Sm	5.7	14.5	387	-	-	-	-	850
10000J ₃ Sm	5.6	14.4	386	-	-	-	-	3990
10000J ₃ Sm	5.8	14.3	388	-	-	-	-	8000
1000J ₃ Sm (pre-inoculation) + 2000J ₂ Mi	5.0	10.8	319	83	67	242	2468 ^a	735 ^a
5000J ₃ Sm (pre-inoculation) + 2000J ₂ Mi	5.2 ^a	11.2 ^a	340 ^a	80 ^a	65 ^a	234	2126 ^c	3690
10000J ₃ Sm (pre-inoculation) + 2000J ₂ Mi	5.7 ^b	12.6 ^b	366 ^b	77 ^a	63 ^a	249	1771 ^c	6760 ^a
1000J ₃ Sm (post-inoculation) + 200J ₂ Mi	4.9	10.8	326	84	68	254	2556 ^a	725 ^a
5000J ₃ Sm (post-inoculation) + 2000J ₂ Mi	5.2 ^a	11.0	338 ^a	81	66 ^a	260	2220 ^b	3652 ^a
10000J ₃ Sm (post-inoculation) + 2000J ₂ Sm	5.3 ^a	11.4 ^a	343 ^a	79 ^a	64 ^a	254	2059 ^c	6980 ^a
1000J ₃ Sm + 2000J ₂ Mi (concomitant inoculation)	4.9	10.8	323	84	67	239	2585 ^a	731 ^a
5000J ₃ Sm + 2000J ₂ Mi (concomitant inoculation)	5.0	10.8	331 ^a	83	66 ^a	241	2468 ^a	3659 ^a
10000J ₃ Sm + 2000J ₂ Mi (concomitant inoculation)	5.6 ^b	11.7 ^a	342 ^a	80 ^a	63 ^a	236	1960 ^c	6850 ^a

Each value is the mean of three replicates. ^{a,b,c} indicate significant difference compared with controls at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, respectively. J₂Mi, second stage juveniles of *Meloidogyne incognita* and J₃Sm, third stage juveniles of *Steinernema masoodi*.

cundity (number of eggs/egg mass) remained uninfluenced at any population level of the EPN. Soil populations of both RKNs and EPNs decreased in cohabitation but the decrease was more pronounced for *M. incognita* (Table I). A significant decrease in the soil population of *M. incognita* was observed with application of 10,000 IJs ($P \leq 0.001$), 5000 IJs ($P \leq 0.01$) and 1000 IJs ($P \leq 0.05$) relative to the control. The soil population of *S. masoodi* also decreased significantly ($P \leq 0.05$), the decrease varying from 7.5 to 15.5% at the highest inoculum level. The greatest decrease in root galling and increase in growth and yield of tomato plants were recorded in the pots pre-inoculated with 10,000 IJs of *S. masoodi*, while post-inoculation with *S. masoodi* was the least effective in suppressing nematode pathogenesis and improving plant growth and yield of tomato.

No significant effect of the inoculation with just *S. masoodi* was observed on any of the growth components considered. However, a significant increase in the growth variables was recorded in *M. incognita* infested and *S. masoodi* treated plants at the 5000 and 10,000 inoculum levels (Table I). The increase in root and shoot dry weight varied from 4.1 to 22% in plants receiving *S. masoodi* prior to root-knot nematode inoculation. The greatest increases in the plant growth variables compared with RKN controls were recorded with pre-inoculation of 10,000 IJs ($P \leq 0.01$) followed by concomitant or post-inoculation (Table I). The increase in tomato yield was greatest (20%) when 10,000 IJs of *S. masoodi* were applied prior to inoculation of *M. incognita* and just 4.5% at the 1000 inoculum level of *S. masoodi*.

DISCUSSION

In the present study, *S. masoodi* was found to suppress egg hatching and induce considerable mortality to root-knot nematode juveniles at all inoculum levels tested. Grewal *et al.* (1997) reported EPN antagonism to *M. incognita* and suggested that the allelochemicals released by the bacterial symbiont were responsible for the nematocidal effects. In the present study also allelopathy was apparently involved in the suppression of the root-knot nematode (Grewal *et al.*, 1999; Hu *et al.*, 1999).

The study on the effect of EPNs on root penetration by *M. incognita* revealed a significant decline in root penetration during the period of 7-15 days at all inoculum levels of the EPN. The decrease was greatest at the highest inoculum level of 10,000 IJs of *S. masoodi*. The presence of EPNs in soil induced mortality to juveniles and thus reduced the soil population of *M. incognita*. The suppressive effect was more pronounced when the EPN was inoculated along with the egg masses of the root-knot nematode. Perez and Lewis (2002) reported that inoculation of 150 eggs of *M. incognita* and 25 IJs of *S. feltiae* (Filipjev) Wouts, Mráček, Gerdin *et Bedding* or *S. riobrave* Cabanillas, Poinar *et Raulston* per cm³ soil significantly reduced root-knot nematode pene-

tration in tomato roots. Similar inhibitory effects of EPNs or metabolites of their bacterial symbionts on penetration by *M. incognita* into tomato roots and *Radopholus similis* Cobb into banana roots have been observed by other researchers (Aalten, 1996; Grewal *et al.*, 1999).

The study on different modes of inoculation showed that pre-inoculation of *S. masoodi* might have provided opportunity for EPN to get established in the soil, perhaps leading to release of more of the bacterial symbionts or their metabolites into the soil so that when root-knot nematodes were added the soil had already become unsuitable or suppressive to the root-knot nematodes, leading to greater juvenile mortality and/or their failure to penetrate roots in comparison to concomitant and post-inoculation treatments. This is presumably the reason for greater decrease in the galling and increase in tomato growth and yield recorded with the pre-inoculation of 10,000 IJs of *S. masoodi*. Other researchers have also demonstrated reduction in galling and egg mass production of *M. incognita* on tomato and *M. hapla* Chitw. on peanut due to application of *S. riobrave* (Lewis *et al.*, 2001; Perez and Lewis, 2002, 2004). Choi *et al.* (1988) and Ishibashi and Choi (1991) reported that the application of *S. carpocapsae* resulted in a significant decrease of root galling on tomato caused by *M. incognita*. A similar observation was recorded by Molina *et al.* (2007) on tomato infested with *M. mayaguensis* (now *M. enterelobii*) together with live and dead infective juveniles of *S. feltiae* SN strain and live IJs of *Heterorhabditis baujardi* Phan, Subbotin, Nguyen *et Moens* LPP7. Hussaini *et al.* (2009) reported a reduction in galling, egg mass production and hatching of *M. incognita* eggs with subsequent increase in root weight, shoot weight and yield of tomato due to treatments with *Steinernema* spp., whereas Fallon *et al.* (2002) recorded a reduction in biomass production in EPN-treated plants. In the present study an adverse effect of EPN treatment was not seen on tomato plants.

The present study has demonstrated the feasibility of using *S. masoodi* for the management of *M. incognita*, which causes extensive damage to many agriculturally important crops throughout the world. However, the dose of EPN required to control root-knot nematodes was found to be much higher than that required to control insects (Choi *et al.*, 1988; Hussaini *et al.*, 2009), apparently because of the higher population of the target PPN. The EPN application alone was quite effective but their integration with other management strategies could further lower the cost of management and increase the efficacy of the management strategy.

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