Compatibility of *Heterorhabditis indica* with neem seed and kernel granules for suppressing *Meloidogyne incognita* infecting tomato

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Abstract

A glass house experiment was conducted to study the effect of neem seed granules (NSG), neem seed kernel granular (NSKG) formulation and *H. indica* alone as well as their combinations on *Meloidogyne incognita* infecting tomato. Among the different treatments, combined application of *H. indica* @ 160 J_3/g soil + NSKG formulation @ 1 g/kg soil followed by *H. indica* @ 160 J_3/g soil + NSG formulation @ 1 g/kg soil were found high to all other treatments in suppressing the *M. incognita* population (267 and 289 J_2/g soil) and thus, significantly ($P \geq 0.05$) less gall formation (237 and 361) respectively, as compared to untreated inoculated control (1368 galls per plant). Also the treatment of *H. indica* @ 160 J_3/g soil alone, reduced the number of galls (433) significantly in comparison with inoculated control (only *M. incognita* J_2/g soil). All the treatments resulted in improved plant growth characters such as root length, shoot length, root fresh weight and shoot fresh weight at $P \geq 0.05$ significance level in comparison with the treatments only *M. incognita* inoculum.

Keywords: Root-knot nematode, *Meloidogyne incognita*, tomato, neem, *Heterorhabditis indica.*

Chemical nematicides had adverse effects on the soil and also led to ground water contamination (Thomason, 1987). Hence, emphasis today on non-chemical management, including cultural practices, bio-control agents, plant resistance, non-conventional nematicides and compounds obtained from plants. Neem *Azadirachta indica* have a potential for nematode control (Vijayalakshmi *et al.*, 1985; Dasgupta & Gaur, 1986; Alam, 1993; Mojumder, 1995; 2002). Root-knot nematode species (*Meloidogyne* spp.) are the most serious pests which reported to cause serious crop losses in a wide variety of crops in open fields and protected cultivation systems (Barker *et al*., 1976; Dasgupta & Gaur, 1986; Gill & Jain, 1995). Solanaceous and cucurbitaceous vegetables were amongst the most susceptible hosts of root-knot nematodes (Khan & Khan, 1990).

Entomopathogenic nematodes (EPN) normally kill insects, but some reports have indicated suppression of plant parasitic nematodes in their presence. It is plausible that neem and EPN used for suppression plant parasitic nematodes simultaneously may inhibit one of the others. Entomopathogenic nematode *Heterorhabditis indica* used for control of root-knot nematode in some vegetable crops (Grewal *et al*., 1997; 1999; Channappa *et al*., 2004; Mojumder, 2002). Also Grossman (1997) showed that the application of *Steinernema riobravis* provide root-knot nematode control comparable to that achieved with chemical nematicides. Neem products have some effects on entomopathogenic nematodes (Hussaini *et al*., 2001; Shamseldean *et al*., 2004).

Keeping in view the above aspects the present investigations were planned to study the effect of combination of two neem products made from seed and kernel with *Heterorhabditis indica* for the management of root-knot nematode.

Materials and Methods

Preparation of soil mixture: The sandy loam soil was collected and sieved through 20 mesh sieve. Sand, soil and well rotten dried farm yard manure (FYM) were mixed thoroughly in the ratio 5:2:1 and steam sterilized at 120 ºC at 6.75 kg/cm^2 pressure for 30 minutes. This sterilized soil, sand and FYM mixture was used in pot experiments.

Raising and maintenance of nematode inoculums: Initial inoculum of *Meloidogyne incognita* Race-1 was obtained from the culture of nematodes maintained on brinjal crop. Examination of perineal pattern of adult females characteristically showed high, squarish dorsal arch with lateral ridges absent in the lateral field, marked by breaks and forks in striae; striae were coarse, smooth to wavy and the tail terminus had a distinct whorl. These findings were in conformity with those mentioned by Eisenback (1985) for *M. incognita*. Hence, species was confirmed as *M. incognita*. About 4000 juveniles were inoculated around one week old seedlings of tomato (*Solanum lycopersicum*) cultivar, Pusa Hybrid-1 in 20 cm diameter earthen pots filled with sterilized soil. The population was periodically sub-cultured as mentioned above to have a regular supply of nematodes throughout the experimental period.

Extraction of nematodes: The nematode infected plants were removed from the culture pots after 60 days and the adhering soil washed under a slow stream of water. Large numbers of adhering egg-masses were hand-picked. The egg-masses were rinsed with fresh water and placed over a double layer of tissue paper supported on a coarse aluminum gauge in a Petri dish containing fresh water in contact with tissue paper. Several such sets were kept at 29+1 ºC for 24 hrs and thus, freshly hatched juveniles were collected for inoculation.

Counting and inoculation: The population density of *M. incognita* juveniles in the suspension was determined by counting one ml aliquot separately three times, mean assumed to give a correct estimate. After bubbling vigorously, the measured amount of suspension was drawn; containing the required number of nematode juveniles and the suspension poured into 3-4 cm deep holes made by a glass rod around the established seedlings.

Preparation of different concentrations of neem seed and kernel powder: The kernels were separated from neem seeds and grinded in an electrical blender for preparation of seed kernel powder and only seeds grinded in electrical blender for preparation of seed powder. This seed powder (8 g) was placed in a muslin cloth and kept in 100 ml water for 12 hrs for making a suspension of 8% w/v from which dilutions of 1%, 2% and 4% were prepared separately.

Rearing of *Galleria mellonella*: *G. mellonella* was obtained from a culture being maintained in division of Nematology, Indian Agricultural Research Institute (IARI), New Delhi and reared in laboratory on standard artificial diet. The moths were collected in plastic jars and honey was provided as natural food. They were allowed to lay eggs on a tissue paper lining. The eggs were allowed to hatch in a separate plastic jar covered with muslin cloth. The larvae were fed with artificial diet containing maize flour 200 g, wheat flour l00 g, rice bran 100 g, milk powder 75 g, yeast powder 30 g, honey 125 ml and glycerol 125 ml. The 4th instar *G. mellonella* larvae were used for experimental purposes. A few larvae were allowed to pupate for running the culture.

Culturing of *Heterorhabditis indica*: The infective juveniles (J_3) of *H. indica* were obtained from pure culture maintained in the division of Nematology, IARI, New Delhi. The J_3 were routinely cultured on healthy 4th instar larvae of *G. mellonella* and harvested by using White's trap (White, 1927). Seven or eight 4th instar larvae of *G. mellonella* were kept on a filter paper in a Petri dish. *H. indica* J_3 (15-20) were inoculated on the larvae and incubated in a

BOD incubator at 28 ± 1 °C. The inoculated *G. mellonella* died in about 24 hrs. Petri dishes were maintained moist up to one week. Thereafter, the (cadavers) were washed with tap water and kept on a White trap to harvest the emerging J_3. The harvested J_3 were stored in sterilized culture bottles with loose cap at 15 ± 1 °C.

Tomato seedlings (cv., Pusa Hybrid-1) transplanted in 20 cm diam., earthen pots. Freshly hatched J_2 of *M. incognita* were inoculated @ 2 J_2/g of soil, *H. indica* @ 160 J_3/g of soil and neem seed and kernel formulations @ 0.1%.w/w. Treatments as i) *M. incognita* + *H. indica* + neem seed granular formulation; ii) *M. incognita* + *H. indica* + seed kernel granular formulation; iii) *M. incognita* + *H. indica*; iv) *M. incognita* alone; v) *H. indica* alone and vi) healthy control. The six treatments were replicated three times. Observations on the number of galls, nematode population in soil, root, shoot length, fresh shoot, root weight and dry shoot weight in tomato were recorded 45 days after inoculation.

Statistical analysis of data: The replicated data were subjected to single or factorial analysis. The data in percentages were subjected to angular transformation before analysis. The data on nematode population densities were transformed to square roots before subjecting to analysis. The differences among the mean of main and interactive effects were tested for significance at 5% probability level.

Results

Effects of *Heterorhabditis indica* alone and with neem seed and kernel granular formulations on *Meloidogyne incognita*: Among the different treatments, combined application of *H. indica* @ 160 J_3/g soil + NSKG) @ 1 g/kg soil followed by *H. indica* @ 160 J_3/g soil + NSG @ 1 g/kg soil, were found high to all other treatments in suppressing the *M. incognita* population (267 and 289 J_2/g soil) and thus, significantly ($P \geq 0.05$) less gall formation (237 and 361), respectively as compared to untreated inoculated control (1368 galls per plant). Also the treatment of *H. indica* @ 160 J_3/g soil and *M. incognita* 2J_2/g, reduced the number of galls (433) significantly in comparison with inoculated control (1368 only *M. incognita* 2 J_2/g soil). All the treatments were found significantly high over control in reducing the number of galls and J_2 population. The minimum root-knot index was recorded in treatment of *H. indica* @ 160 J_3/g soil + NSKG @ 1 g/kg soil followed by *H. indica* @ 160 J_3/g soil + NSG @ 1 g/kg soil, which were 2 and 3 respectively (Table1, Fig.1 and 2). Maximum root-knot index of 4.8 was observed in control (only *M. incognita* 2 J_2/g soil).

The average number of *M. incognita* (J_2) per 100 g soil were also significantly ($P \geq 0.05$) reduced. Compared to1559 in control (only *M. incognita* 2 J_2/g soil), it was 267 in NSKG @ 1 g/kg soil + *H. indica* @ 160 J_3/g soil, 289 in NSG @ 1 g/kg soil + *H. indica* @ 160 J_3/g soil and 37 in treatment of *H. indica* @ 160 J_3/g soil + *M. incognita* @ 2 J_2/g soil.

Effects of *Heterorhabditis indica* alone and with neem seed and kernel granular formulations on plant growth: All the treatments resulted improved plant growth characters such as root, shoot length, root and shoot fresh weight at $P \geq 0.05$ significance level in comparison with the treatment having only *M. incognita* inoculation. In case of overall plant growth, all the treatments were significantly high over *M. incognita* alone (76.7 cm). Treatment of only *H. indica* @ 160 J_3/g soil gave the highest plant length (92.3 cm) followed by treatment of *H. indica* @ 160 J_3/g soil + NSKG (91.3 cm), *H. indica* @ 160 J_3/g soil + NSG (89 cm) and healthy tomato (91.7 cm). The highest root growth was observed in treatment healthy tomato (13 cm) followed by only *H. indica* @ 160 J_3/g soil (12.8), *H. indica* @ 160 J_3/g soil + NSKG (12.3 cm) and *H. indica* @

160 J_3/g soil + NSG (12 cm). Maximum root growth retardation was found in control of only *M. incognita* (7.7) which significantly higher in comparison to other treatments. This showed that there was significant reduction in root growth retardation as well as population density of *M. incognita* in soil with application of neem seed formulation (Table 1 and Fig. 3).

Highest shoot growth of 82 cm was observed in healthy plant and with only *H. indica* @ 160 J_3/g soil (79.5 cm) followed by *H. indica* @ 160 J_3/g soil + NSKG (79 cm). Lowest shoot growth was observed in only *M. incognita* (69 cm). *H. indica* @ 160 J_3/g soil + NSG and *H. indica* + *M. incognita* at par with each other and significantly high in reducing the shoot growth retardation compared to control (only *M. incognita* J_2/g soil). Similar trend was observed in case of root fresh weight (Table 1).

Highest shoot fresh weight was observed in healthy (43.2 g) followed by *H. incica* olone (41.7). Lowest shoot weight was observed in *M. incognita* (36 g). Similar trend was observed in shoot dry weight (Table 1, Fig. 4 and 5).

Table 1. Effect of *Heterorhabditis indica* with neem seed and neem seed kernel (NSKG) granules on *Meloidogyne incognita* and plant growth characters of tomato.

Treatments	Number of galls per plant	Number of *M. incognita* (J2)/100g soil	Plant growth characters					
			Total length (root + shoot) (cm)	Root length (cm)	Shoot length (cm)	Root fresh weight (g)	Shoot fresh weight (g)	Shoot dry weight (g)
H. indica + NS @ 1 g/kg soil + *M. incognita*	361 (19)	289 (17)	89	12	77	13.1	37.8	5.9
H. indica + NSK @ 1 g/kg soil + *M. incognita*	237 (15.38)	267 (16.32)	91.3	12.3	79	13.6	40. 7	5.9
H. indica + *M. incognita*	433 (20.77)	37 (19.23)	87. 7	10.3	76. 7	11.8	37.2	5.8
M. incognita	1368 (36.95)	1559 (39.44)	76. 7	7.7	69	9.7	36	6.8
H. indica	0 (0.71)	0 (0.71)	92.3	12.8	79.5	14.3	41.7	6.3
Healthy plant (control)	0 (0.71)	0 (0.71)	91. 7	13	82	14. 7	43.2	6.4
SE	0.502	0.74	1.12	0.63	1.36	0.65	1.07	0.32
CD 0.05	(1.458)	(2.13)	3.26	1.82	3.95	1.89	3.11	0.92

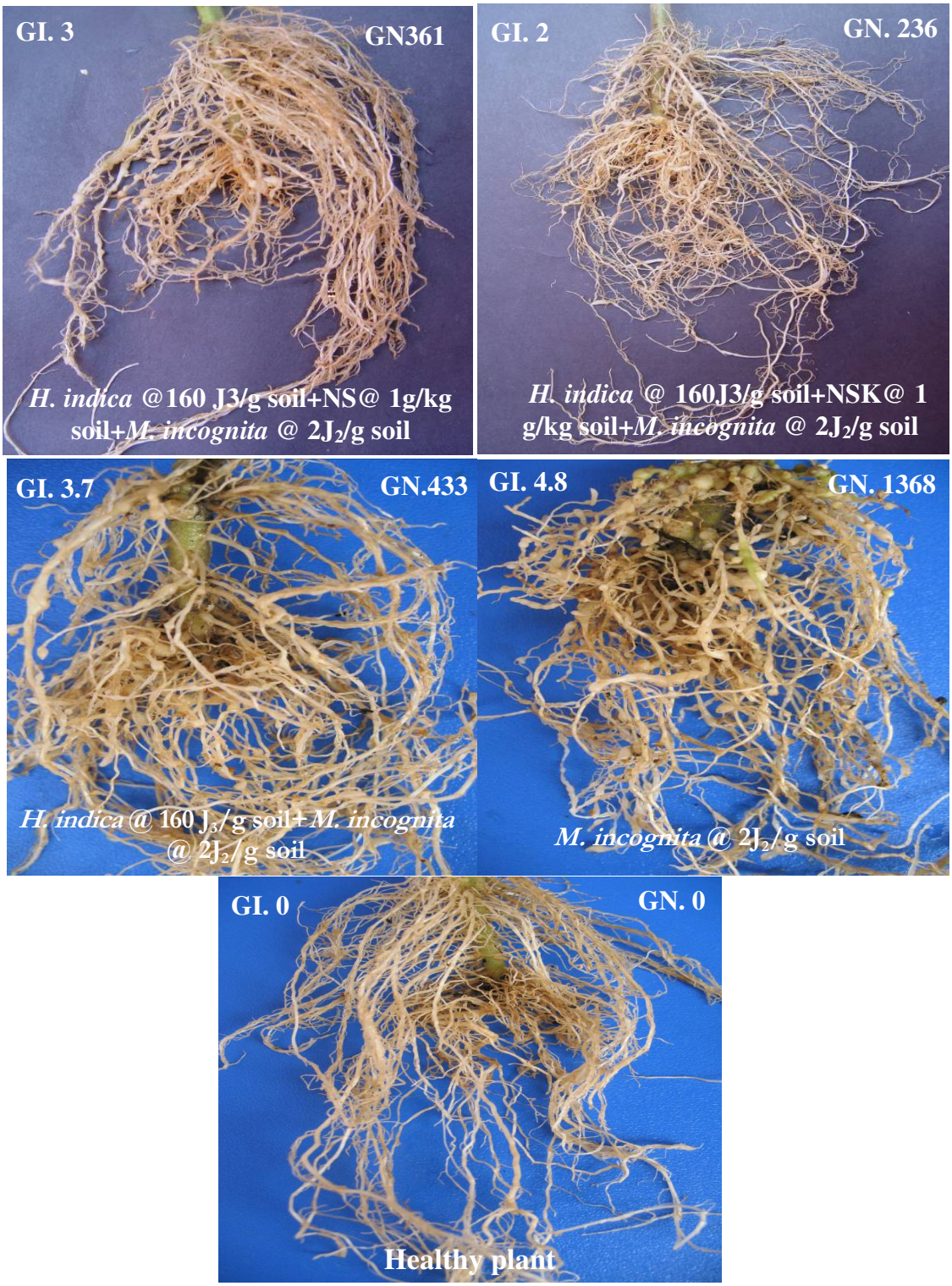

Fig. 1. Gall index (GI) and gall number (GN) on the tomato roots due to *M. incognita*, 45 days after application of the *Heterorhabditis indica* (160 J_3/g soil) and different concentrations of neem seed kernel and neem seed compared to healthy plant.

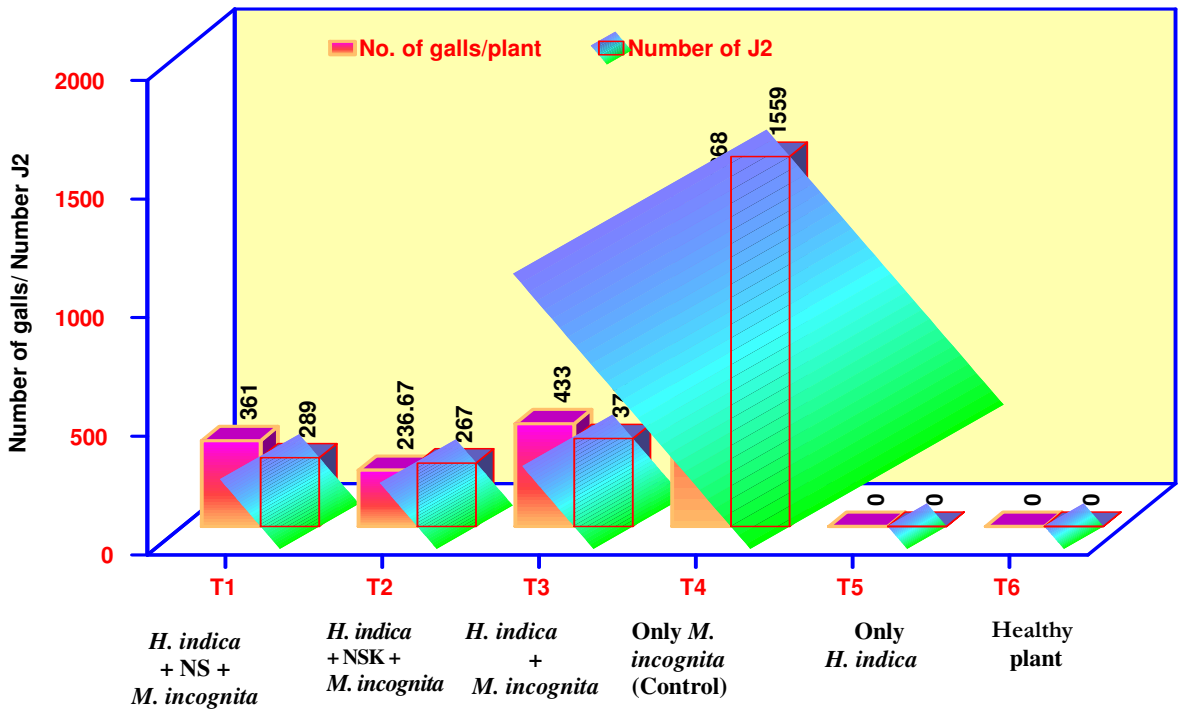

Fig. 2. Effect of *Heterorhabditis indica* alone or in combination with neem seed and kernel granules on population density of *Meloidogyne incognita* and galling.

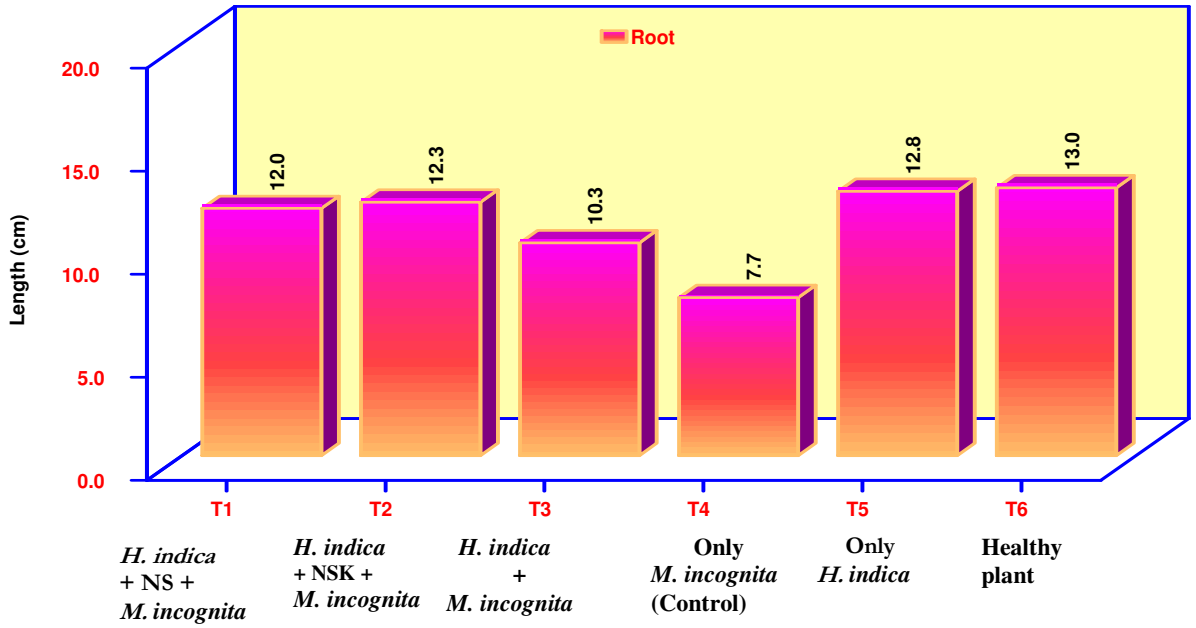

Fig. 3. Effect of *Heterorhabditis indica* alone or in combination with neem seed and kernel granules on root length of tomato, 45 days after inoculation.

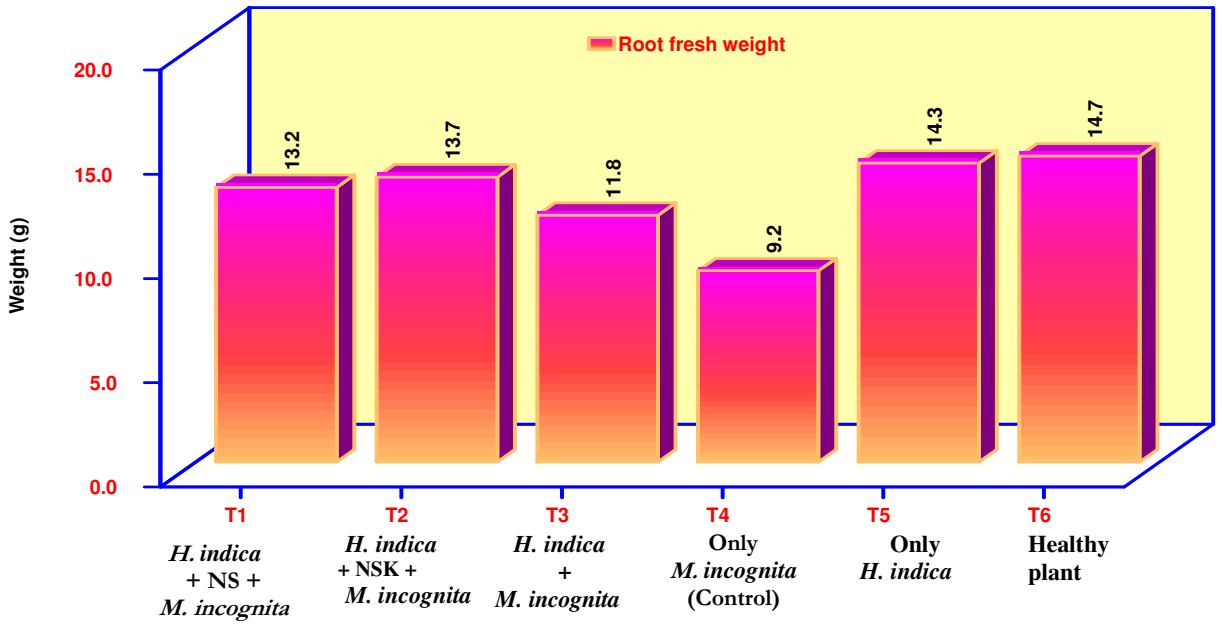

Fig. 4. Effect of *Heterorhabditis indica* alone or in combination with neem seed and kernel granules on root fresh weight of tomato, 45 days after inoculation.

Fig. 5. Effect of *Heterorhabditis indica* alone or in combination with neem seed and kernel granules on shoot fresh weight of tomato, 45 days after inoculation.

Discussion

Entomopathogenic nematodes are often used in conjunction with other pest management tactics and the lack of compatibility information is a major impediment in further expansion of their use. We evaluated the effects of NSKG and NSG in combination with different number of *H. indica* to control *M. incognita* infecting tomato in glasshouse. All combinations reduced galling and J_2 population of *M. incognita* in soil and also improved plant growth characters compared to control but *H. indica* at 160 J_3/g soil and NSK @ 1 g/kg soil showed maximum effect.

The compatibility of the nematode-bacteria complex of *Steinernema carpocapsae*, *S. abbasi* and *H. bacteriophora* with neem plant extracts (seeds and leaves) to control diamondback moth, *Plutella xylostella* (L.) on Chinese cabbage plants was investigated by Abdel-Razek & Gowen (2002) under laboratory conditions. They conclude from these investigations that the combination of entomopathogenic nematodes with neem plant extracts could be of success in controlling *P. xylostella* on Chinese cabbage plants in the field. Krishnayya & Grewal (2002) reported that neem and the fungicide azoxystrobin (Abound) could be safely tank mixed at the field recommended concentrations with the infective juveniles of *S. feltiae* for application.

Other research work also showed that Azadirachtin a constituent of neem, had minimal or no impact on non-target organisms, compatible with other biological control agents and has a good fit into classical integrated pest management programmes (Immaraju, 1998).

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