EFFECT OF NEEM-BASED PRODUCTS ON THE ROOT-KNOT NEMATODE, *MELOIDOGYNE INCOGNITA*, AND GROWTH OF TOMATO

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Summary. An experiment was conducted under glass-house conditions to test the effects of five neem-based nematicide products in the form of drenches on the growth of tomato and population density of the root-knot nematode, *Meloidogyne incognita*. All the tested formulations suppressed nematode multiplication and root galling severity significantly and improved plant growth. The formulations most effective at low concentrations against the root-knot nematode were neem seed kernel extracts (NSKE) and Econeem. At the highest concentration of 1.0% all the neem formulations were equally effective. NSKE drench produced the best plant growth, closely followed by Econeem. None of the formulations was phytotoxic even at the highest concentration.

Key words: Control, drench application.

Several plants, belonging to different botanical families, contain principles possessing nematicidal or nematostatic properties (Gommers, 1981; Grainge and Ahmed, 1988). Investigations on extracts from various indigenous plants and neem (*Azadirachta indica* A. Juss.) products have revealed that some of them are effective against insects and nematodes (Holyoke and Reese, 1987; Byomakesh *et al.*, 1998; Nanjegowda *et al.,* 1998; Khanna and Sharma, 1998; Sharma, 2000) and commercial formulations of them are already available. Neem-based formulations are known to have nematicidal potential, particularly against plant parasitic nematodes (Alam, 1990; Mojumder, 1995). The fresh extracts of fruits, leaf bark, root and gum of neem inhibited hatching of *Meloidogyne incognita* (Siddiqui and Alam, 1985). Therefore, investigations were undertaken to evaluate the efficacy of the new commercial neem-based formulations Econeem, Nimbecidine, Neem Azal and Neem Gold, along with a neem seed kernel extract, applied as soil drenches, on plant status, root gall index and final nematode density of the root-knot nematode *Meloidogyne incognita* (Kofoid *et* White) Chitwood in tomato.

MATERIALS AND METHODS

The studies were conducted during 2002-2003 in a glass-house at the authors' research centre.

Raising of tomato seedlings. Tomato seeds (cv. Manisha) were given pre-sowing treatment with Bavistin (0.1%) + Indofil M-45 (0.25%) to control damping off. Seedlings were raised from these treated seeds in a nursery on the departmental farm.

Rearing of Meloidogyne incognita. Egg masses of *M. incognita* were isolated from infected tomato roots and placed singly in Petri dishes containing distilled water. The second stage juveniles emerging from each single egg mass were inoculated on seedlings of brinjal (*Solanum melongena* L.) cv. Pusa Purple Long, grown in earthen pots containing 4,000 g steam sterilized soil (soil : sand : FYM in the ratio of 1 : 1 : 1). The plants were maintained in a glass-house at 25±2 °C for one year to allow the reproduction of the nematode. This nematode culture was used for the experiments.

Tested formulations. The five neem formulations were Econeem 1% (PJ Margo Private Limited), Nimbecidine 0.03% (T-Stanes and Company Ltd.), Neem Gold 0.03% (Southern Petro Chemicals, Chennai Tumkur), Neem Azal 1% (EID Pairy India Ltd., Chennai) and Neem Seed Kernel Extract (NSKE). Based upon the recommended concentrations (2-5 ml/l of water, i.e. 0.2-0.5%) for use against insect pests, three concentrations, viz., 0.25, 0.5 and 1.0%, were tested.

To obtain the NSKE, dried seed kernels were processed as described by Sasanelli and Di Vito (1991) and Khanna and Kumar (2006).

Aqueous preparations of 2% concentration of all the neem-based nematicides that we tested were prepared on a v/v basis and designated as stock solutions. Three distilled water concentrations (0.25, 0.5 and 1.0%) were prepared from stock solutions of each neem product and stored in cool, dry conditions (0 °C) until they were used.

Assay procedure. A soil mixture (soil : sand : FYM in the ratio of 1:1:1) was sterilized by autoclaving. This mixture was used to fill 1000 cm^3 plastic pots sterilized by dipping in 5% formalin for 5 minutes. One four-week-old healthy tomato seedling was transplanted into

each pot. Seedlings were grown under glass-house conditions at 25±2 °C. Fifteen days after transplanting, each pot was inoculated with 1000 freshly hatched second stage juveniles of *M. incognita*. The egg masses were isolated from infected brinjal roots and placed singly in Petri dishes containing distilled water. The second stage juveniles emerging from egg masses were collected after three days and combined for use. Sixteen days after transplanting, the soil in the pots was drenched with 100 ml of each of the concentrations, viz., 0.25, 0.5 and 1.0%, of the neem products separately. Pots receiving only 100 ml of distilled water were used as a control. Each treatment had six replications, arranged in the glass-house in a completely randomized layout. The experiment ran for 60 days after transplanting. Observations on plant status, viz., shoot length, fresh shoot weight, root length, fresh root weight and leaf count per plant, were recorded at the end of the experiment. The final nematode populations of second stage juveniles per 200 cm^3 of soil were counted after extraction by the Cobb decanting and sieving technique. Root galling index was rated on a 1-5 scale (1 = no gall, 2 = 1-10 galls, 3 = 11-30 galls, 4 = 31-99 galls, 5 = 100 galls and above). Correlations between root gall index and nematode population in soil were assessed statistically.

Statistical analysis. All data were analyzed by ANOVA and critical differences (CD) calculated.

RESULTS AND DISCUSSION

Effect on plant status. Plant height increased with the increase in concentration of all tested formulations and the increase was significant at all concentrations (Table I). Nematode inoculated plants drenched with NSKE at 1.0% concentration attained the maximum height of 74.7 cm, followed by plants treated with Econeem with a plant height of 68.5 cm at the same concentration. Shoot lengths in Nimbecidine, Neem Gold and Neem Azal drenched pots reached similar values of 66.7, 65.2 and 63.5 cm, respectively. Control plants inoculated with nematodes but not treated with any formulation remained significantly smaller, with an average height of 32.5 cm.

Mean shoot weight (Table II) increased with the increase in concentration of the formulations and the increase was significant for all formulations. NSKE treated plants had the highest mean shoot weight of 63.7 g as compared to 15.3 g in the control, followed by mean shoot weights of 58.0 and 57.8 g in Econeem and Neem Gold drenched plants, respectively.

Again, a significant increase in root length (Table III) was observed with the increase in concentration of the neem-based nematicides. Mean root length was 14.3 cm in plants treated with the 1.0% concentration of the neem products, as compared to the average root length of 11.0 cm of plants drenched with the 0.25% concentration. The formulations were not equally effective and the root lengths of the plants drenched with Nimbecidine or Neem Azal (9.8 and 9.7 cm, respectively) at the 0.25% concentration were not significantly different from the control plants (8.2 cm). However, beyond this concentration both of these formulations significantly increased root length. There were no significant differences in root size between the 0.5 and 1.0% concentrations of Econeem, Nimbecidine, Neem Gold and NSKE. Thus, the 0.5% concentration of these formulations was as good as the 1.0% concentration as far as root length is concerned. Neem Azal gave the least increase of root length (mean overall length of 11.3 cm).

In general, the root weight of tomato increased with the increase in concentration of the nematicides. The mean weights over formulations, of 11.0, 12.2 and 14.3 g at the 0.25, 0.5 and 1.0% concentrations, respectively, were significantly different from each other. The largest root weights were observed in NSKE and Econeem treated plants (15.3 g and 14.6 g, respectively), and did not differ significantly. Root weight increased significantly with the increase in concentration from 0.25 to

Table I. Effects of different concentrations of neem-based nematicides on shoot length of tomato in soil infested with *Meloidogyne incognita*.

Formulation	Shoot length (cm) at concentration (%)			Mean
	0.25	0.5	1.0	
Econeem	49.8	62.7	68.5	60.3
Nimbecidine	45.7	53.7	66.7	55.3
Neem Gold	52.5	64.7	65.2	60.8
Neem Azal	52.8	60.7	63.5	59.0
NSKE	53.3	65.0	74.7	64.3
Control (Distilled water)	32.5	32.5	32.5	32.5
Mean	47.8	56.5	61.8	
$CD_{0.05}$				
Formulation (F)	5.1			
Concentration (C)	3.6			
F × C	6.25			

0.5% of Nimbecidine but not with the same increase in concentration of Econeem, Neem Gold, Neem Azal or NSKE. However, increasing the concentration to 1% resulted in a significant increase of root weights by all formulations except Nimbecidine (Table IV).

All formulations increased the number of leaves per plants at all concentrations (Table V). The greatest numbers of leaves per plant were obtained with 1.0% NSKE (59.3) and Econeem (57.3), values that were not significantly different.

Table II. Effects of different concentrations of neem-based nematicides on shoot weight of tomato in soil infested with *M. incognita*.

Formulation	Shoot weight (g) at concentration (%)			Mean
	0.25	0.5	1.0	
Econeem	46.3	60.5	67.2	58.0
Nimbecidine	41.0	50.0	64.0	51.7
Neem Gold	49.2	60.3	64.0	57.8
Neem Azal	34.5	48.7	62.5	48.6
NSKE	50.8	65.7	74.5	63.7
Control (Distilled water)	15.3	15.3	15.3	15.3
Mean	39.5	50.1	57.9	
$CD_{0.05}$				
Formulation (F)	3.89			
Concentration (C)	2.75			
F × C	4.77			

Table III. Effects of different concentrations of neem-based nematicides on root length of tomato in soil infested with *M. incognita*.

Formulation	Root length (cm) at concentration (%)			Mean
	0.25	0.5	1.0	
Econeem	12.2	14.3	14.5	13.7
Nimbecidine	9.8	13.7	15.5	13.0
Neem Gold	12.5	14.8	16.0	14.4
Neem Azal	9.7	10.2	14.0	11.3
NSKE	13.5	15.5	17.3	15.4
Control (Distilled water)	8.2	8.2	8.2	8.2
Mean	11.0	12.8	14.3	
$CD_{0.05}$				
Formulation (F)	1.66			
Concentration (C)	1.17			
F × C	2.03			

Table IV. Effects of different concentrations of neem-based nematicides on root weight of tomato in soil infested with *M. incognita*.

Formulation	Root weight (g) at concentration (%)			Mean
	0.25	0.5	1.0	
Econeem	13.2	14.2	16.3	14.6
Nimbecidine	10.2	13.5	14.8	12.8
Neem Gold	11.8	13.2	15.7	13.6
Neem Azal	9.5	9.8	15.2	11.5
NSKE	14.0	15.2	16.8	15.3
Control (Distilled water)	7.2	7.2	7.2	7.2
Mean	11.0	12.2	14.3	
$CD_{0.05}$				
Formulation (F)	1.23			
Concentration (C)	0.87			
F × C	1.51			

Table V. Effects of different concentrations of neem-based nematicides on number of leaves of tomato in soil infested with *M. incognita*.

Formulation	Leaf count per plant at concentration (%)			Mean
	0.25	0.5	1.0	
Econeem	39.2	49.0	57.3	48.5
Nimbecidine	38.8	43.5	51.8	44.7
Neem Gold	34.8	38.3	45.7	39.6
Neem Azal	35.3	46.5	47.7	43.2
NSKE	41.8	50.0	59.3	50.4
Control (Distilled water)	24.3	24.3	24.3	24.3
Mean	35.7	41.9	47.7	
$CD_{0.05}$				
Formulation (F)	4.01			
Concentration (C)	2.83			
F × C	4.91			

Table VI. Effects of different concentrations of neem-based nematicides on final nematode populations of *M. incognita* on tomato.

Formulation	Final nematode population (per 200 cm^3 soil) at concentration (%)			Mean
	0.25	0.5	1.0	
Econeem	237.7	177.2	78.0	164.3
Nimbecidine	244.2	226.0	177.0	215.7
Neem Gold	270.2	214.0	133.2	205.8
Neem Azal	267.8	239.7	135.3	214.3
NSKE	223.7	160.8	60.83	148.4
Control (Distilled water)	576.2	576.2	576.2	576.2
Mean	303.3	265.6	193.4	
$CD_{0.05}$				
Formulation (F)	52.21			
Concentration (C)	36.91			
F × C	63.96			

Effect on population of M. incognita. All neem-based formulations greatly suppressed the soil population density of *M. incognita* (Table VI) and the suppression was greater the higher the concentration of the nematicide. The differences in the number of nematodes/200 cm^3 soil were not significant between formulations at the 0.25% concentration but some became significant at higher concentrations. The lowest nematode population was observed when treating with 1% of NSKE and Econeem.

Root gall index also appeared to be a useful parameter to evaluate the effect of the neem formulation on *M. incognita* (Table VII). Mean root gall index (1-5 scale) was reduced significantly, from 2.7 to 2.2 and 1.9 with the increase of concentration from 0.25 to 0.5 and 1.0%. Plants treated with NSKE had the lowest mean gall index of 1.6, followed by the 1.7 of Econeem treated plants. Nimbecidine (2.1), Neem Gold (2.1) and Neem Azal (1.9) treated plants showed similar degrees of root gall suppression. This indicates that, although all neem formulations reduced the infestation by *M. incognita* significantly, the degree of their efficacy varied significantly. At 0.25%, NSKE (gall index 1.8) was the most effective. At the 0.5% concentration, NSKE and Econeem (gall indices of 1.5) were significantly more effective than Nimbecidine (2.2) and Neem Gold (2) and, at the highest concentration of 1.0%, all the formulations had similar effects (gall indices of 1.3 and 1.5). The root gall index of the control plants was 4.3.

In addition, the root gall index of the tomato plants was clearly and positively correlated with the final nematode soil population density, with a correlation coefficient of 0.7796, significant at $P < 0.01$.

Our experiment revealed that the formulations tested improved plant status, exerted significant control of the nematode and reduced root galling of tomato. Plant performance was best in NSKE treated plants, closely followed by those treated with Econeem. However, at the lowest concentration of 0.25%, NSKE was the most effective treatment against *M. incognita*.

Table VII. Effects of different concentrations of neem-based nematicides on root galling of tomato in soil infested with *M. incognita*.

Formulation	Root gall index* at concentration (%)			Mean
	0.25	0.5	1.0	
Econeem	2.3 (1.5)	1.5 (1.2)	1.3 (1.1)	1.7 (1.3)
Nimbecidine	2.5 (1.6)	2.2 (1.5)	1.5 (1.2)	2.1 (1.4)
Neem Gold	2.7 (1.6)	2.0 (1.4)	1.5 (1.2)	2.1 (1.4)
Neem Azal	2.5 (1.6)	1.8 (1.3)	1.5 (1.2)	1.9 (1.4)
NSKE	1.8 (1.3)	1.5 (1.2)	1.3 (1.1)	1.6 (1.2)
Control (Distilled water)	4.3 (2.1)	4.3 (2.1)	4.3 (2.1)	4.3 (2.7)
Mean	2.7 (1.6)	2.2 (1.4)	1.9 (1.3)	
$CD_{0.05}$				
Formulation (F)	0.15			
Concentration (C)	0.11			
F × C	0.18			

Figures in the parentheses are square root transformed values.
* Root gall index on 1-5 scale: 1 = No gall, 2 = 1-10 galls, 3 = 11-30 galls, 4 = 31-100 galls, and 5 = 100 galls and above.

Naik *et al.* (1998), while working with various neem-based nematicides, also found Nimbecidine to be more effective than NSKE. Our results agree with findings of Akhatar and Mahmood, 1996; Bhatti, 1988; Hafeez *et al*., 2000).

LITERATURE CITED

Akhtar M. and Mahmood I., 1996. Evaluation of nematicidal properties of a biopesticide against root knot nematode *Meloidogyne incognita. Tests of Agrochemicals and Cultivars, 7*: 104-105.

Alam M.M., 1990. Neem in nematode control. Pp. 51-55. *In*: Nematode Biocontrol: Aspects and Prospects (Jairajpuri M.S., Alam M.M. and Ahmed J., eds). CBS Publishers and Distributors, Delhi, India.

Bhatti D.S., 1988. *Utilization of toxic plants for the control of nematode pests of economic crops. Final Technical Report (1983-1988)*. Haryana Agricultural University, Hissar, India, 231 pp.

Byomakesh D., Padhi N.N. and Dash R., 1998. Relative efficacy of neem products against root knot nematode on tomato. *Indian Journal of Nematology, 28*: 163-167.

Gommers F.J., 1981. Biochemical interactions between nematodes and plants and their relevance to control. *Helminthological Abstracts, Series B, Plant Nematology*, *50*: 9-24.

Grainge M. and Ahmed S., 1988. *Handbook of Plants with Pest-Control Properties*. J. Wiley and Sons, New York., U.S.A., 260 pp.

Hafeez U.K., Riaz Ahmad, Waqar Ahmad, Khan S.M. and Ahmad S.A., 2000. Evaluation of chemical *vs* biological control treatments against root-knot nematode (*Meloidogyne incognita*) on tomato. *Pakistan Journal of Phytopathology, 12*: 118-120.

Holyoke C.W. and Reese J.C., 1987. Acute insect toxicants from plants. Pp. 67-118. *In*: Handbook of Natural Pesticides. Vol. III Insect Growth Regulators, Part B (Morgan E.D. and Mandava N.B., eds.). CRC Press, Boca Raton, Florida, USA.

Khanna A.S. and Kumar S., 2006. *In vitro* evaluation of neem-based nematicides against *Meloidogyne incognita*. *Nematologia Mediterranea*, *34*: 49-54.

Khanna A.S. and Sharma N.K., 1998. Phytotherapeutic effect of some indigenous plants/nematicides on *Meloidogyne incognita* infesting tomato. Pp. 4-6. *In:* Proceedings of National Symposium on Rational Approaches in Nematode Management for Sustainable Agriculture, 23-25 November 1996, GAU, Anand Gujarat, India.

Mojumder V., 1995. Nematoda, nematodes. Pp. 129-150. *In:* Neem Tree and Other Meliacious Plants: Source of Unique Natural Products for Integrated Pest Management (Schmutterer H., ed.). VCH Publications, Weinheim, Germany.

Naik B.G., Nanjegowda D., Ravi K., Reddy P.P., Kumar N.K.K. and Verghose A., 1998. Effect of neem products and a nematicide for management of root knot nematode, *Meloidogyne incognita*, in tomato. Pp. 321-322. *In:* Advances in IPM for horticultural crops. Proceedings of the First National Symposium on Pest Management in Horticultural Crops: environmental implications and thrusts. 15-17 October, 1997, Bangalore, India.

Nanjegowda D., Naik B.G., Ravi K., Reddy P.P., Kumar N.K.K. and Verghese A., 1998. Efficacy of neem products and a nematicide for the management of root-knot nematode *Meloidogyne incognita* in tomato nursery. Pp. 318-320. *In*: Advances in IPM for horticultural crops. Proceedings of the First National Symposium on Pest Management in Horticultural Crops: environmental implications and thrusts. 15-17 October 1997, Bangalore, India.

Sasanelli N. and Di Vito M., 1991. The effect of *Tagetes* spp. extracts on the hatching of an Italian population of *Globodera rostochiensis*. *Nematologia Mediterranea*, *19*: 135-137.

Sharma G.C., 2000. Efficacy of neem based formulations against the root knot nematode *Meloidogyne incognita*. *Pesticide Research Journal, 12*: 183-187.

Siddiqui M.A. and Alam M.M., 1985. *IARI Neem Newsletter*, *2*: 43-47.

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