IN VITRO EVALUATION OF NEEM-BASED NEMATICIDES AGAINST *MELOIDOGYNE INCOGNITA*

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Summary. An experiment was conducted to test the effects of five neem-based pesticides on the mortality of juveniles and egg hatch of the root-knot nematode *Meloidogyne incognita*. Among the five formulations tested, Econeem and Neem seed kernel extract (NSKE) gave the greatest nematode juvenile mortality (73.3-77.2%) after 96 hours and the least juvenile emergence per egg mass (69.8-73.7 compared to 315 in the control) after 144 hours, in 1% solution. Nimbecidine, Neem Azal and Neem Gold were also effective but significantly less than the two previously mentioned formulations. The nematicidal activity increased with the increase of the concentration of the formulation and the exposure time of the nematode.

Key words: Control, egg hatch, juvenile mortality, root-knot nematode.

The use of alternatives to chemicals for the control of plant parasitic nematodes is a response to environmental and human health concerns. Several plants, belonging to different botanical families, contain principles possessing nematocidal 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. Therefore, investigations were undertaken to evaluate the efficacy of the new neem-based formulations Econeem, Nimbecidine, Neem Azal, Neem Gold and Neem seed kernel extract on the mortality of juveniles and eggs of the root-knot nematode *Meloidogyne incognita* (Kofoid *et* White) Chitw.

MATERIALS AND METHODS

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 the single egg mass were inoculated onto seedlings of brinjal (*Solanum melongena* L.) cv. Pusa Purple Long, grown in earthen pots containing 4,000 g steam sterilized growth medium (one part soil, one part sand, one part FYM). 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 all of the experiments.

Formulations tested. 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). Each of them was tested for its effects on juvenile mortality and egg hatch. Based upon the recommended dose (2-5 ml/litre of water, 0.2-0.5% concentration against insect pests), three concentrations viz., 0.25, 0.5 and 1.0% were tested.

To obtain the NSKE, dried seed kernels were powdered in an electric grinder. A sample of 100 g powder was soaked in 300 ml distilled water overnight in a beaker (Sasanelli and Di Vito, 1991). The content was filtered 3-4 times through a fine muslin cloth. The extract thus obtained was made up to 1000 ml with distilled water. This solution was designated as 10 per cent standard NSKE (aqueous solution). To remove the dense inert matter, the aqueous extract was centrifuged in a bench centrifuge at 1,200 rpm for five minutes and filtered through Whatman filter paper No. 1.

Two per cent concentrations of all of the neem-based nematicides tested and the NSKE aqueous extract were prepared on a v/v basis and designated as stock solutions. Three concentrations (0.25, 0.5 and 1.0%) were prepared from the stock solutions of each neem product by adding distilled water and stored in cool dry conditions (0 °C) until their use.

Effect on juvenile mortality. Fresh juveniles of *M. incognita* were obtained by incubating egg masses collected from the infected brinjal roots (of the pure nematode culture) in distilled water at 25 °C. One millilitre of each concentration of each neem formulation was poured into a sterilized cavity block (2-ml capacity). Cavity blocks containing only distilled water were used as a control. All treatments were replicated six times

and each replicate received 100 freshly hatched (within two days from emergence) juveniles of *M. incognita*. All cavity blocks were arranged according to a completely randomized block design in a B.O.D. incubator at 25 ± 2 °C. Observations of juvenile mortality were made after 12, 24, 48, 72 and 96 hours of exposure. For mortality confirmation, inactive juveniles were transferred to another cavity block containing distilled water to see whether or not they regained motility.

Effect on egg hatch. The methodology was similar to that of the previous experiment, except that in place of juveniles each replicate contained a single egg mass of the nematode, with an average egg content of 350. Counts of emerged juveniles were made after 24, 48, 72, 96, 120 and 144 hours of exposure.

Statistical analysis. All data were grouped according to formulation, concentration and exposure time and then subjected to analysis of variance and comparison of means by Duncan's Multiple Range Test or Least Significance Difference .

RESULTS AND DISCUSSION

Effect on juvenile mortality. The mortality of the nematode juveniles increased with the increase in concentration of the different neem-based formulations. Significantly greater juvenile mortality (34.7%) was achieved at 1.0% concentration and less at the 0.5% (21.6%) and 0.25% (8.9%) concentrations. The exposure period also played a significant role and per cent juvenile mortality increased from 8.1% at 12 hours exposure to 35.7% at 96 hours exposure (Table I).

Table I. Effect of different concentrations of neem pesticides at different exposure periods on per cent of juvenile mortality of *Meloidogyne incognita in vitro*.

Exposure period (hours)	Per cent juvenile mortality at concentration (%)			Mean
	0.25	0.5	1.0	
12	1.5 a[1] A[2]	6.8 a AB	16.1 a B	8.1
24	4.1 a A	14.2 ab AB	24.9 ab B	14.4
48	7.7 ab A	20.7 abc AB	35.1 ab B	21.2
72	13.6 bc A	28.5 bc AB	45.5 b B	29.2
96	17.5 c A	37.7 c AB	51.8 b B	35.7
Mean	8.9	21.6	34.7	

[1] Figures in the same column sharing a common lower case letter are not significantly different according to Duncan's multiple range test at $P < 0.05$.
[2] Figures on the same line sharing a capital letters are not significantly different according to LSD at $P < 0.05$.

Table II. Effects of different periods of exposure to different concentrations of neem pesticides on per cent of juvenile mortality of *M. incognita in vitro*, grouped according to exposure time and pesticide concentration.

Formulation	% juvenile mortality									
	Exposure period (hours)					Mean	Concentration (%)			
	12	24	48	72	96		0.25	0.5	1.0	
Econeem	17.2 a[1]	29.4 b	38.1 b	46.4 b	55.3 b	37.3	12.2 b	40.8 c	58.9 d	
Nimbecidine	5.3 a	11.5 ab	22.7 ab	33.5 ab	39.9 b	22.5	10.2 ab	20.5 b	37.0 bcd	
Neem Gold	4.8 a	8.9 ab	16.4 ab	22.0 ab	28.4 ab	16.1	8.2 ab	15.4 ab	24.8 b	
Neem Azal	5.4 a	11.9 ab	19.6 ab	28.7 ab	35.9 ab	20.3	9.6 ab	17.9 ab	33.4 bc	
NSKE	16.0 a	24.5 ab	29.6 ab	43.7 b	53.1 b	33.4	12.4 b	34.2 bc	53.6 cd	
Control (distilled water)	0.0 a	0.3 a	0.5 a	1.0 a	1.5 a	0.7	0.7 a	0.7 a	0.7 a	

[1] Figures in the same column sharing a common letter are not significantly different according to Duncan's multiple range test at $P < 0.05$.

Table III. Effect of different concentrations of neem pesticides at different exposure periods on per cent of juvenile mortality of *M. incognita in vitro*.

Formulation	Per cent juvenile mortality at concentration (%)											
	0.25				0.5				1.0			
	Exposure period (hours)				Exposure period (hours)				Exposure period (hours)			
	12	24	48	72	96	12	24	48	72	96	12	24	48	72	96
Econeem	0.8 ab[1]	5.3 b	8.8 bc	16.0 cd	30.2 e	14.5 c	29.7 e	42.5 d	54.7 e	62.5 d	36.3 d	53.2 e	63.0 e	68.5 e	73.3 e
Nimbecidine	2.0 bc	4.7 b	10.8 c	15.2 bc	17.7 c	4.5 b	10.0 bc	18.7 b	28.7 c	40.8 c	9.3 b	19.3 c	38.7 c	56.5 d	61.2 d
Neem Gold	1.8 bc	4.0 b	7.7 b	13.2 b	14.2 b	3.5 b	7.5 b	14.5 b	21.2 b	30.2 b	9.2 b	15.3 b	27.0 b	31.7 b	40.8 b
Neem Azal	1.7 bc	4.7 b	9.2 bc	14.8 bc	17.8 c	4.5 b	10.8 c	18.0 b	23.2 b	32.8 b	10.0 b	20.3 c	31.5 b	48.0 c	57.0 c
NSKE	2.7 c	5.5 b	9.0 bc	21.5 e	23.7 d	13.8 c	26.7 d	29.8 c	42.3 d	58.3 d	32.0 c	41.2 d	50.0 d	67.2 e	77.2 e
Control (distilled water)	0.0 a	0.3 a	0.5 a	1.0 a	1.5 a	0.0 a	0.3 a	0.5 a	1.0 a	1.5 a	0.0 a	0.3 a	0.5 a	1.0 a	1.5 a

[1]Figures in the same column sharing a common letter are not significantly different according to Duncan's multiple range test at $P < 00.5$.

Maximum juvenile mortality (37.3%, the average of replications at different exposure times) was achieved with Econeem, followed by NSKE (33.4%) (Table II). Neem Gold was the least effective (16.1%). All neem formulations showed greatest efficacy against *M. incognita* juveniles at 1.0% concentration after an exposure of 96 hours.

All of the pesticides tested (except for Econeem at 0.25%) had significant effects on juvenile mortality at the 0.25% and 0.5% concentrations after 12 hours exposure (Table III) but there was a progressive increase in mortality with increase in exposure period. The differences between the neem formulations became obvious with increase of concentration and exposure time. In general, Econeem and NSKE killed significantly more juveniles than the other formulations, with maximum and similar nematode mortalities of 73.3% and 77.2%, respectively, achieved after 96 hours of exposure to 1% concentrations. However, Econeem appeared to act more rapidly than NSKE as it killed significantly more juveniles after exposure times of 12-48 hours at the two higher concentrations. Nimbecidine at 1% showed an increase in mortality from 9.3% at 12 hour exposure to 61.2% at 96 hours, significantly less than Econeem and NSKE but more than Neem Azal (57%), which was in turn more effective than neem Gold (40.8), the least effective.

Effect on egg hatch. Mean numbers of juveniles that emerged from the egg masses decreased from 98.5 to 71.7 with increase in concentration from 0.25% to 1.0% and increased from 7.8 to 157.6 with increase in expo-

Table IV. Effect of different concentrations of neem pesticides at different exposure periods on emergence of juveniles from egg masses of *M. incognita in vitro*.

Exposure period (hours)	Number of juveniles emerged from egg masses at concentration (%)			Mean
	0.25	0.5	1.0	
24	10.1 a[1] A[2]	7.1 a A	6.2 a A	7.8
48	30.5 a A	22.6 ab A	17.3 ab A	23.5
72	92.1 b A	80.1 bc A	62.6 abc A	77.9
96	119.8 bc A	100.6 cd A	86.8 bc A	102.5
120	161.2 c A	135.9 cd A	119.2 c A	138.8
144	177.8 c A	157.0 d A	138.0 c A	157.6
Mean	98.5	83.9	71.7	

[1] Figures in the same column sharing a common lower case letter are not significantly different according to Duncan's multiple range test at $P < 0.05$.

[2] Figures on the same line sharing a capital letters are not significantly different according to LSD at $P < 0.05$.

Table V. Effects of different periods of exposure to different concentrations of neem pesticides on egg hatching of *M. incognita in vitro*, grouped according to exposure time and pesticide concentration.

Formulation	Number of juveniles emerged from egg masses									
	Exposure period (hours)						Mean	Concentration (%)		
	24	48	72	96	120	144		0.25	0.5	1.0
Econeem	2.0 a[1]	13.7 a	40.1 a	55.3 a	76.4 a	91.5 a	46.5	60.6 a	45.3 a	33.6 a
Nimbecidine	5.2 a	20.7 a	77.0 b	98.0 b	121.9 b	144.4 b	77.8	94.8 ab	77.1 a	61.5 a
Neem Gold	4.9 a	17.3 a	79.6 b	105.6 b	132.7 b	154.8 b	82.5	102.5 ab	81.2 a	63.8 a
Neem Azal	2.5 a	14.1 a	78.0 b	98.5 b	124.5 b	149.4 b	77.9	95.8 ab	78.3a	59.4 a
NSKE	2.1 a	12.3 a	41.6 a	57.0 a	77.5 a	90.8 a	46.9	60.9 a	44.6 a	35.0 a
Control (Distilled water)	30.2 b	63.3 b	153.3 c	200.0 c	299.7 c	314.8 c	176.9	176.9 b	176.9 b	176.9 b

[1] Figures in the same column sharing a common letter are not significantly different according to Duncan's multiple range test at $P < 0.05$.

Table VI. Effect of different concentrations of neem pesticides and different exposure periods on egg hatching of *M. incognita in vitro*.

Formulation	Number of juveniles emerged from egg masses at concentration (%)																	
	0.25						0.5						1.0					
	Exposure period (hours)						Exposure period (hours)						Exposure period (hours)					
	24	48	72	96	120	144	24	48	72	96	120	144	24	48	72	96	120	144
Econeem	4.3a[1]	19.7a	55.2a	75.2a	98.0a	111.5a	1.7a	16.7a	37.2a	51.8a	74.8a	89.3a	0.0a	4.7a	28.0a	39.0a	56.3a	73.7a
Nimbecidine	8.3a	30.7a	92.8b	120.0b	146.8b	169.8b	3.2a	16.5a	84.0b	96.5b	120.5bc	141.8b	4.0a	13.3a	54.3b	77.5b	98.3b	121.7b
Neem Gold	10.2a	30.3a	98.3b	127.2b	165.5c	183.5b	3.0a	14.7a	84.7b	105.7c	127.3c	151.7b	1.7a	7.0a	55.7b	84.0c	105.2c	129.2b
Neem Azal	3.3a	20.5a	95.8b	120.2b	159.3bc	175.5b	2.7a	14.2a	86.0b	96.5b	116.7b	153.7b	1.5a	7.5a	52.2b	77.7b	97.5b	119.0b
NSKE	4.2a	18.7a	57.0a	76.0a	97.8a	112.0a	2.0a	10.0a	35.5a	53.3a	76.5a	90.5a	0.0a	8.2a	32.3a	41.7a	58.2a	69.8a
Control (Distilled water)	30.2b	63.3b	153.3c	200.0c	299.9d	314.8c	30.2b	63.3b	153.3c	200.0d	299.7d	314.8c	30.2b	63.3b	153.3c	200.0d	299.7d	314.8c

[1] Figures in the same column sharing a common letter are not significantly different according to Duncan's multiple range test at $P < 0.05$.

sure time from 24 hr to 144 hr. At 0.25% an average of only 10.1 juveniles emerged from a single egg mass within 24 hours, rising to 177.9 after an exposure of 144 hours. At 1%, an average of 6.2 juveniles emerged after 24 hours, rising to 138 after an exposure of 144 hours (Table IV). However, when the overall egg hatch of all formulations after each exposure period was considered, no significant difference was observed between the different concentrations (Table IV).

Econeem and NSKE were the most effective neem pesticides as only 46.5 and 46.9 nematode juveniles (averages of all concentrations), respectively, emerged from each egg mass as compared to 176.9 juveniles in the control (Table V). However, all tested formulations significantly suppressed egg hatch at all concentrations and exposure times (Table VI), with Econeem and NSKE allowing the least emergence of nematode juveniles per egg mass of 73.7 and 69.8, respectively, after the longest exposure of 144 hr at 1% concentration. Nimbecidine, Neem Gold and Neem Azal suppressed egg hatch similarly (119-129 juveniles/egg mass *versus* 315 in the control).

Thus, NSKE and Econeem were equally effective at suppressing hatch, and Neem Azal, Nimbecidine and Neem Gold had descending degrees of efficiency. Similar results were obtained by Paruthi *et al.* (1997) with the neem formulations Achook, Neem Guard, Neemark and Margocide against *M. javanica* (Treub) Chitw. Our results with Neem Gold also agree with findings by Sharma (2000), who reported a satisfactory nematicidal activity of this neem formulation against *M. incognita*.

The effect of the different neem formulations was greater on the mortality of the second stage juveniles than on egg hatch. Several authors (Hough and Thomason, 1975; McLeod and Khair, 1975) have demonstrated that the effects of a nematicide vary with the life stage of a nematode. Greco and Thomason (1980) found that aqueous concentrations of 0.48 µg/ml of the nematicide fenamiphos had little effect on egg hatch of *M. javanica*, while 0.1 µg/ml suppressed infectivity and movement of the juveniles. However, the effects on juveniles and eggs are both important from a practical point of view.

The neem tree is indigenous to India, where most of the research on its nematicidal properties has been conducted. All parts of neem trees possess insecticidal activity but the seed kernel has the most activity. Neem products exhibit almost every conceivable type of activity against insects. The repellent and antifeedant effects of neem have been reported against a wide range of insect pests, including desert locust, *Schistocerca gregaria* Forskal, migratory locust, *Locusta migratoria* L., the ear cutting caterpillar, *Mythimna separata* Walker, etc. Our results suggest that neem-based products can be useful in the management of nematodes of important commercial crops. Also, neem-based products could be considered for combination with other organic substrates, including industrial wastes such as composts from olive residues in the Mediterranean area. This combination could improve the nematicidal effects and be of interest as a new control strategy with minimal environmental impact against *Meloidogyne* spp. (Amirante *et al.*, 2002a, b) and, possibly, other nematodes.

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