

## EFFECT OF NEEM EXTRACTS ON ACTIVITY AND MORTALITY OF SECOND-STAGE JUVENILES OF *MELOIDOGYNE INCOGNITA*

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**Summary.** Three chemicals obtained from neem, viz. azadirachtin, nimbin and salannin reduced the mobility of second-stage juveniles of *Meloidogyne incognita* and caused 17.5, 16.8 and 18 per cent mortality, respectively after 48 h. They also decreased penetration of the roots of mungbean seedlings to 9, 9.8 and 0.8 per cent respectively compared to 22.8 and 22 in untreated and treated after 7 days. Salannin at 1000ppm was the most effective causing 76 and 86 per cent immobility; 9 and 18% mortality at 24 and 48 h exposure, respectively and significantly reduced the number of juveniles that penetrated mungbean seedlings (< 1 J 2 / plant). Azadirachtin and nimbin resulted in more than 50 per cent immobility at 48 h of exposure.

Neem (*Azadirachta indica* A. Juss.) with its bioactive principles has gained global attention as an alternative to chemical pesticides. Initially, crude extracts of neem fruits, seeds, seed kernels etc. were tested for their pesticidal activity. Later, isolation of neem chemicals and their testing revealed that azadirachtin is the most active constituent of neem, showing antifeeding and repellent activity and toxicity to insects. However, in the case of phytonematodes, there are only a few reports on the toxicity of chemicals obtained from neem (Mojumder, 1995). Devkumar *et al.* (1985) found that nimbin, diacetylnimbin and some other compounds extracted from neem seed kernel were nematocidal to *Meloidogyne incognita*. A new class of eneanortriterpenoides was isolated from neem cake and tested for their nematocidal properties on *M. incognita* (Kraus *et al.*, 1994). The present investigation was conducted to find out the effect of azadirachtin, nimbin and salannin on the activity (immobility and mortality) of second stage juveniles J<sub>2</sub> and their penetration in seedlings of mungbean, *Vigna radiata* L. cv. P.S. 1332.

### MATERIALS AND METHODS

The first part of the experiment used cavity blocks of 2 ml capacity to assess the effect of three pure chemical compounds from neem viz. azadirachtin, nimbin and salannin on J<sub>2</sub> of *M. incognita* (Kofoed *et al.* White) Chitw. The second part, using small plastic cups, investigated the effect of these chemicals on penetration of treated juveniles into roots of mungbean seedlings. Stock solutions of 2000 ppm of azadirachtin, nimbin and salannin were prepared. Since none of the three compounds were soluble in water, they were first dissolved in a small quantity of acetone (50 mg in 2.5 ml acetone) and distilled water was added to make 2000 ppm concentrations. Few drops of emulsifier (0.25 ml of Triton X)

were added to each solution.

The cavity blocks were maintained at room temperature (30-35 °C) after adding 1 ml suspension containing 200 freshly hatched juveniles of *M. incognita* to 1 ml double strength solution (2000 ppm) of each of the three chemicals. After stirring the solution and nematode suspension with a glass rod, the cavity blocks were covered leaving a gap for aeration. Two controls were maintained, one with nematode suspension in water and another in acetone, triton X and water mixture. All treatments including the two controls were replicated four times. After 24 and 48 h of exposure the activity of nematodes was observed immediately under a stereoscopic microscope and percent immobility was calculated. The treated and untreated nematodes were washed through 400 mesh sieve and collected in fresh water. Juveniles that did not move even after pricking the tail were considered to be dead.

Mungbean seeds were sown in sterilized sand in small plastic cups. After germination the seedlings were reduced to two plants per cup. Two hundred freshly hatched juveniles of *M. incognita* were exposed to 1000 ppm solution of azadirachtin, nimbin and salannin for 48 h in cavity blocks as described above then poured around the roots of mungbean after washing with tap water. All three treatments and two controls were replicated four times.

After seven days the plants were uprooted and the roots were washed free from the adhering sand particles. Then they were stained (Byrd *et al.*, 1983). Observations on the number of juveniles penetrated in each plant were recorded by pressing the root system in between two slides using a drop of glycerol. Average of juveniles penetrated in two plants of each cup was calculated. The data on immobility, mortality and penetration were subjected to statistical analysis.

## RESULTS AND DISCUSSION

The data on percent immobility indicated that 1000 ppm of salannin was the most effective neem chemical rendering 77.5 and 86 per cent inactivity at 24 and 48 h, respectively. Azadirachtin with 16.5 and 66.3 per cent inactivity at the respective exposure time of 24 and 48 h was the next effective chemical. Nimbin caused inactivity only at 48 h and low mortality of 51.3 per cent. The percentage mortality was not very high in any of the treatments at either exposure time (Table I). At 24 h, salannin caused 8.7 per cent mortality while azadirachtin and nimbin had no obvious effect. Even after 48 h, mortality of less than 20% was observed in all the three chemicals which was insignificant. The water control showed no mortality but acetone triton X and water control showed 3% mortality after 48 h.

The penetration studies of the treated juveniles of *M. incognita* in mungbean seedlings showed that all three neem chemicals significantly inhibited penetration as compared to controls, indicating that the chemical itself

rather than the solvent and emulsifier were responsible for poor penetration (Table I). Salannin was the most effective in inhibiting penetration (0.8 J<sub>2</sub>/plant) as compared to controls (22.8 and 22.0 J<sub>2</sub>/plant). Azadirachtin and nimbin with 9 and 9.8 J<sub>2</sub>/plant also showed a significant difference of more than 50% reduction in penetration compared to controls.

In the present investigations the mortality of the J<sub>2</sub> of *M. incognita* was similar to that found by Grandison (1992) who reported that chemicals like azadirachtin, nimbin and salannin are not directly toxic to *M. javanica*. Kraus *et al.* (1994) also reported that ten pure compounds from neem cake, including azadirachtin, nimbin and salannin, were not as nematocidal as crude extracts. However, a mixture of all the ten compounds in different proportions was as effective as the crude methanol extract. Mojumder and Mishra (1991) have earlier reported reduced penetration of root-knot juveniles using crude neem products. The present investigation also indicates that salannin is relatively more active against phytonematodes than nimbin and azadirachtin.

**Table I.** Effect of azadirachtin, nimbin and salannin on *in vitro* activity and mortality of *Meloidogyne incognita* second stage juveniles and their penetration in mungbean.

Treatments	% Immobility* after		% Mortality* after		Penetration of J <sub>2</sub> /root system**
	24 h	48 h	24 h	48 h	
Azadirachtin	16.5 (24.31)	66.3 (54.89)	0.0 (4.05)	17.5 (25.08)	9.0 (2.94)
Nimbin	0.00 (4.05)	51.3 (46.02)	0.0 (4.05)	16.8 (24.49)	9.8 (3.11)
Salannin	77.5 (62.21)	86.3 (68.88)	8.68 (17.67)	18.0 (25.44)	0.8 (0.75)
Control (water)	0.0 (4.05)	0.0 (4.05)	0.0 (4.05)	0.0 (4.05)	22.8 (4.77)
Control (acetone+Triton X+water)	0.0 (4.05)	0.0 (4.05)	0.0 (4.05)	0.0 (15.01)	22.0 (4.68)
CD (0.05 P)	(3.28)	(4.98)	(1.01)	(2.30)	(0.67)

\* Figures in parentheses represent angular transformation.

\*\* Figures in parentheses represent square root transformation.

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