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TOXICITY OF NEEM *(AZADIRACHTA INDICA)* SEED KERNEL EXTRACTS PREPARED WITH DIFFERENT SOLVENTS, ON THE SPIDER *CHIRACANTHIUM MILDEI*

F.A. MANSOUR*, K.R.S. ASCHER** and N. OMARI*

The toxicity of neem seed kernel extracts prepared with different solvents against the predatory spider, *Chiracanthium mildei* L. Koch, was investigated. The order of toxicity of the 4% extracts was pentane > acetone > ethanol >> methanol = water (nontoxic). All extracts were nontoxic at 2.5%.

KEY WORDS: Neem seed kernel extracts; *Azadirachta indica* A. Juss; predatory spiders; *Chiracanthium mildei.*

The neem tree, *Azadirachta indica* A. Juss, has long been known to be resistant to the attack of many insects. Steets (15), after reviewing early Indian literature, reported that extracts of the seed deter feeding by larvae of 16 species and by adults of 12 species native to that country. Neem is reported to contain different components with both insect antifeeding and growth-regulating properties (4,5,13,16).

Spiders have been observed by investigators in several countries to be promising predators of insect pests of different cultivated crops [reviewed in (10)]. Biocontrol experiments which were carried out recently in Israel in apple orchards (8), citrus groves and cotton fields (9) indicated that spiders play an important role in the suppression of serious insect pests in these agroecosystems. Surveys of the spider population throughout the year showed that *Chiracanthium mildei* L. Koch was the most dominant species and most important predator in these habitats (8,9).

Various authors have pointed out that insecticides used in different crops are detrimental to the spider populations (10). Very few attempts have been made to assess accurately the toxicity of conventional insecticides to predatory spiders associated with crops. Only some of the commonly used pesticides were selective for *C. mildei* and other spiders occurring in apple orchards in Israel (7). Since beneficial species must be conserved in the field to promote a more stabilized pest and natural enemy balance, it was felt necessary to establish the level of toxicity to spiders of compounds intended for integrated pest control, such as neem products. No work has been published on the effect of neem extracts from solvents on

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*Dept. of Entomology, ARO, Newe Ya'ar Regional Experiment Station, P.O. Haifa, Israel.
**Dept. of Toxicology, ARO, The Volcani Center, Bet Dagan, Israel.

spiders. There are very few reports on the effect of neem on natural enemies of pest insects, mainly on parasites (3,14), and two papers on the effect of neem oil on the spider *Lycosa pseudoannulata* (Boesberger & Strand) (1,11).

The present study was designed to examine the susceptibility of *C. mildei* spiders originating in citrus groves, to extracts of neem seed prepared with various solvents. Neem extracts were prepared with the extracting solvents pentane, ethanol, acetone, methanol and water as described previously (6).

The spiders used in this study were collected several times throughout the year from a citrus grove at Kibbutz Afeq near Haifa. They were of different ages (from third instar to adults) at capture and were reared in the laboratory under constant conditions of 25° ± 1°C and 55-60% RH. To avoid cannibalism, they were kept singly in 30-cm^3 clear plastic containers, with a perforated top – covered with cloth – to provide ventilation. The spiders were fed with *Drosophila melanogaster* Meig. adults. When more than half of the spiders had turned into adults, they were divided into groups consisting of the same number of adult spiders and of earlier instars. Each group served as a replicate in the toxicity tests.

Both sides of grapefruit leaves from unsprayed groves were sprayed for 5 sec from a distance of 25 cm with different concentrations of the extracts, each one in the solvent which had been employed for its extraction, with a Desaga spray-gun (Desaga GmbH, Heidelberg, FRG). The amount of liquid applied was 15-17 $\mu l/cm^2$ on each side of the leaf. In parallel, comparable control leaves were sprayed with acetone only. After the leaves had dried for 1 h at room temperature, they were cut into circles and strips and placed in 30-cm^3 plastic containers as padding for the bottom and sides of the containers. One spider was then confined to each container; the containers with the spiders were held at 25° ± 1°C and 55-60% RH. After 48 h forced contact with the extract residues, the spiders were returned to the rearing containers. To obtain dosage-mortality relationships, six spiders were used in each of the tests, which were replicated on four different days; thus, 24 spiders were assessed at each treatment. One type of test was conducted at a single concentration (4%), with mortality being recorded 2, 5 and 10 days after leaf treatment. The second test was run at different concentrations and mortality was recorded 10 days after leaf treatment.

When *C. mildei* spiders were exposed to leaves which had been sprayed 1 h previously with the different neem extracts at the 4% concentration (Table 1), the order of the toxicity of the extracts 2, 5 and 10 days after treatment was: pentane > acetone > ethanol >> methanol = water (nontoxic). Two days after treatment only the pentane extract showed a slight toxicity (8% mortality). The methanol extract was nontoxic throughout the test and thus equal to the aqueous extract and the solvent control. When *C. mildei* spiders were exposed to different concentrations of the extracts (Table 2), the order of toxicity was as above at all concentrations tested (all extracts were nontoxic at 2.5%). Activity could be correlated with the dielectric constant of the extracting solvent: pentane, 1.8; acetone,

TABLE 1

MORTALITY OF *CHIRACANTHIUM MILDEI* EXPOSED FOR 48 h TO RESIDUES OF 4% NEEM EXTRACTS ON GRAPEFRUIT LEAVES

(Six spiders/replicate; four replicates/test; 1 h after treatment)

Dried neem extract prepared with	*% Mortality ± SD after*		
	*2 days**	*5 days**	*10 days**
Pentane	8±1	54±10	71±9
Acetone	0	12±8	54±9
Ethanol	0	8±2	33±5
Methanol	0	0	4±1
Water	0	0	4±1
Control (acetone only)	0	0	4±1

*Includes 48 h of exposure time.

TABLE 2

MORTALITY AFTER 10 DAYS* OF *CHIRACANTHIUM MILDEI* EXPOSED FOR 48 h TO RESIDUES OF NEEM EXTRACTS AT DIFFERENT CONCENTRATIONS ON GRAPEFRUIT LEAVES
(Six spiders/replicate; four replicates/test; 1 h after treatment)

Dried neem extract prepared with	*% Mortality ± SD on concentration (%)*			
	2.5	4	6	8
Pentane	0	66±9	78±8	89±6
Acetone	0	50±8	60±7	80±8
Ethanol	0	30±5	45±4	55±4
Methanol	0	4±1	22±2	44±5
Water	0	4±1	4±1	4±1
Control (acetone only)	4±1	4±1	4±1	4±1

*Includes 48 h of exposure time.

20.7; ethanol, 24.3; methanol, 32.6; water, 78.5. This means that, as in spider mites (6), nonpolar solvents are more effective than polar ones in extracting substances toxic for spiders from neem kernels. If was found in all theses experiments that there was no difference in the effect of neem extracts on adult and immature spider stages. At the 2.5% concentration, as just stated, none of the extracts showed any toxicity to spiders (Table 2). It is highly encouraging that the low concentrations active against spider mites (6), *viz.*, 0.2-0.5% of the different extracts, were nontoxic against one of their predators, the spider *C. mildei.*

Lycosa pseudoannulata, a spider which is an important predator of rice pests (2,12), was not affected by neem oil even at the highest dose applied topically, 50 μg/spider (12). Recently Chiu (1) showed that a dosage of 250 μg/spider of the seed oil of *Melia azedarach* and 100 μg/spider of neem oil applied topically had no effect on *L. pseudoannulata.*

Saxena *et al.* (12) demonstrated that neem oil did not cause obvious harm to natural enemies of *Cnaphalocrocis medinalis* (Guenée). Surprisingly, the parasites were even favored by neem oil application, which prevented the larvae of the pest from folding rice leaves properly. These findings seem to support the results of Schmutterer *et al.* (14), who reported that various endoparasitic Hymenoptera pupated and emerged normally from parasitized 4th or 5th instar *C. medinalis* larvae that were fed rice leaves treated with neem fractions or extracts. This can be attributed to the lack of a contact effect of growth-regulating compounds in neem seed extracts. It is known that chitin biosynthesis inhibitors such as diflubenzuron, which act mainly through ingestion, do not harm parasites. Hellpap (3) found no detrimental effect of neem on predators of *Spodoptera frugiperda* (J.E. Smith). Joshi *et al.* (4) reported that neem seed kernel suspension in water sprayed on *Spodoptera litura* (F.) eggs before or after parasitization did not adversely affect the emergence of the egg parasite *Telenomus remus* Nixon. Because the impact of beneficial insects on pest insects is well known, the lack of adverse effects of neem extracts on predators and parasites must be considered of greatest importance when their field application is contemplated.

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