Effects of Neemgard on Phytophagous and Predacious Mites and on Spiders

F.A. Mansour,[1] K.R.S. Ascher[2] and F. Abo-Moch[1]

The effects of Neemgard, an acaricidal and fungicidal formulation obtained from neem (*Azadirachta indica*) seed kernels, on the phytophagous mite *Tetranychus cinnabarinus*, the predacious mite *Phytoseiulus persimilis*, and the predatory spider *Chiracanthium mildei*, were investigated in laboratory experiments. Neemgard was highly toxic to *T. cinnabarinus* but had no toxic effect on *C. mildei* or *P. persimilis.* Another neem formulation – the insecticidal Neemix 45 – caused conspicuous repellency, but no mortality, in *T. cinnabarinus*.
KEY WORDS: Neem; *Azadirachta indica*; Neemgard; Neemix 45; *Tetranychus cinnabarinus; Phytoseiulus persimilis; Chiracanthium mildei.*

The neem tree – or Indian lilac – (*Azadirachta indica* A. Juss.) is widely distributed in many Asian and African countries (1) and is now being propagated energetically in Central and South America, Australia, etc. (14). Neem seed kernel extracts (NSKEs) have been shown to possess pronounced antifeeding and growth-and-development-inhibiting activity (1,6,7,14) against a wide spectrum of insect species (see ref. 15 for a detailed list). The NSKEs active against insects are obtained by extraction with polar solvents. For instance, high IGR activity against larvae of *Spodoptera littoralis* was exhibited by NSKEs from polar solvents (dielectric constant >20), namely, H_2O, MeOH, EtOH and acetone, whereas extracts from apolar solvents, such as $CHCl_3$, CCl_4 or *n*-pentane, were inactive (3). The most important active ingredient in insecticidally active NSKEs is the tetranortriterpenoid azadirachtin (AZA); this compound may prove to be one of the more promising botanical pesticides for use in insect control (1,6,14).

However, as regards extracting solvents to obtain NSKEs active against Acari, results were diametrically opposed to those in insects. We showed in 1983 (8) that the order of effectiveness of NSKEs from various solvents active against adult females of *Tetranychus cinnabarinus* (Boisd.) was pentane $>$ $CHCl_3$ $>$ *n*-butanol $>$ acetone $>$ MeOH $>$ H_2O (inactive); that is to say, activity decreased with increasing dielectric constant of the extracting solvent. These findings, that NSKEs extracted by lipophilic solvents were much more active against mites than those extracted by hydrophilic solvents, were confirmed in 1992 by Sanguanpong and Schmutterer (13); they found that the NSKE obtained by pentane extraction had the strongest effect as regards mortality of female adults of *Tetranychus urticae* Koch. They also reported that addition of large quantities of AZA to inactive MeOH extracts of neem oil did not confer miticidal efficacy; this proved that AZA contributed nothing to the toxicity of neem products against spider mites and that miticidal activity is concentrated in the apolar NSKEs. The subject has been reviewed by us recently (9).

Further work on the effect of neem formulations on phytophagous mites was conducted by Sundaram and Sloane (16). They recorded deleterious biological effects –

Received Dec. 26, 1996; received in final form May 6, 1997.
[1] Dept. of Entomology, ARO, Newe Ya'ar Research Center, Ramat Yishay 30095, Israel [Fax: +972-4-9836936; e-mail: vfnfm146@volcani.agri.gov.il].
[2] Dept. of Toxicology, ARO, The Volcani Center, Bet Dagan 50250, Israel.

mortality, reduction of number of eggs laid, reduced percentage of eggs hatched and survival of emerged mites, as well as repellence – when females of *T. urticae* were treated with different neem formulations. Activity decreased in the order: Azatin (according to their determination containing 3.1% AZA) > Neem PTI-EC4 (3.95% AZA) > Margosan-O (0.31% AZA, but containing also 14% neem oil) > RH-9999 (19.7% AZA) >AZA-A (>95% purity). The same parameters were investigated by Dimetry *et al.* (4), again with *T. urticae* for the two neem formulations Margosan-O and NeemAzal-S.

Some years ago the firm W.R. Grace & Co., Columbia, MD, USA, released a product called Neemgard (for a local data sheet, see ref. 2). Neemgard, according to the producer's specifications, contains hydrophobic (=lipophilic) extracts of neem oil. It contains none, or only traces, of AZA. According to the local data sheet cited (2), the oil extracts are clarified by a special process in order to remove phytotoxic waxes and then refined and formulated. Neemgard is active mainly as a fungicide and an acaricide.

We have shown (8) that the NSKEs prepared from various solvents affected also the behavior and fecundity of *T. cinnabarinus.* Furthermore, in previous work conducted to elucidate the activity of neem against some natural enemies, we investigated the effects of these extracts on the predacious mite *Phytoseiulus persimilis* Athias-Henriot, and the hunting spider *Chiracanthium mildei* Koch, in comparison with their effects on *T. cinnabarinus* (10,11). In continuation of these studies, the effect of three industrial formulations of NSKE: Margosan-O^{TM}, Azatin^{TM} and RD9-Repelin®, was assayed (10) on *T. cinnabarinus*, on the predacious mite *Typhlodromus athiasae* Porath and Swirski, and on the spider *C. mildei.* The present work was carried out to estimate the effect of Neemgard on these organisms.

Maintenance of the phytophagous mite stock cultures and kidney bean plants (*Phaseolus vulgaris*), and toxicity and repellency test procedures, were described by us previously (10). The *C. mildei* spiders used in this study were collected from a citrus grove at the Newe Ya'ar Research Center and reared and tested as described previously (10,11). The predacious *P. persimilis* mites were obtained from the Biological Control Industries at Kibbutz Sede Eliyyahu. The toxicological bioassay procedure was as described by us in a former study (12).

Pesticides tested: The two neem formulations employed were Neemgard, and Neemix 45 – which contains 4.5% AZA. (Both formulations formerly of W.R. Grace Sierra Crop Protection, Milpitas, CA, USA; now distributed by Thermo Trilogy, Columbia, MD, USA.) The formulations were diluted with water to the desired concentrations.

Statistical analysis: The data were analyzed by analysis of variance and Duncan's multiple range test (5).

Fresh, 1-h-old residues of the acaricide and fungicide Neemgard have strong acaricidal activity against *T. cinnabarinus* (Table 1), *e.g.* 1% killed >90% of female adults exposed for 24 h; 0.75% and 0.5% killed plus repelled at a rate of >90%. At lower concentrations (0.25% and 0.125%) the percentage of both live and repelled mites increased. The insecticide Neemix 45 exhibited conspicuous repellency under the same experimental conditions, but caused practically no mortality. These results with Neemix 45 recall our previous findings with two other insecticidal neem products, Azatin and Margosan-O (10).

Based on these results with Neemgard, we investigated the residual effect of 4- and 8-day-old 1% dilution residues of this formulation (Table 2). Following 48 h of exposure to 4-day-old residues, there was no longer any mortality, but still >90% repellency. Because of this high rate of repellency, oviposition was reduced to nearly zero. On 8-day-old residues repellency was 71%, and 24% of the mites survived on the leaf disks; the number of eggs/female/day was approximately proportional to the percentage of survivors.

Finally, it was of importance to investigate the toxicity of Neemgard to natural enemies of phytophagous mites, namely, the predacious mite *P. persimilis* and the spider *C. mildei.* After 24 h and 48 h of exposure to 1-h-old 1% Neemgard residues there was no mortality in *P. persimilis.* Similarly, no mortality was brought about by 2- or 8-day-old 1% Neemgard residues in *C. mildei* adults after a 48-h exposure.

The results showed that Neemgard had acaricidal (fresh residues) and repellent (4- and 8-day-old residues) activity against *T. cinnabarinus*, whereas even fresh residues of Neemix 45 had a repellent effect only. Neemgard was innocuous to the beneficial organisms *P. persimilis* and *C. mildei*. The use of selective pesticides is the first step in developing a mite management strategy within the framework of an integrated pest control program. Moreover, employing botanical pesticides that are relatively harmless to natural enemies could increase the effectiveness of natural predation. This may in turn allow for fewer pesticide applications, and reduce production costs and environmental pollution.

TABLE 1. Mortality and repellency of adult spider mites (*Tetranychus cinnabarinus*) by 1-h-old spray residues from various concentrations of Neemgard (six replicates) and Neemix 45 (four replicates) on kidney bean leaves (20 mites/replicate)

Concn of the aqueous dilution of the tested formulation (%)	Mean % of mites affected (±SD) after a 24-h exposure to the residues		
	Live	Dead	Repelled
Neemgard			
1	1 (±2.0) a	92 (±5.0) a	7 (±4.0) a
0.75	6 (±6.5) a	73 (±19.5) b	21 (±18.0) ab
0.50	5 (±4.0) a	67 (±13.0) b	28 (±13.5) ab
0.25	24 (±19.5) b	30 (±17.5) c	46 (±25.0) b
0.125	35 (±23.0) b	28 (±29.0) c	37 (±22.5) b
Control	97 (±6.0) c	0 (±0) d	3 (±6.0) a
Neemix 45			
0.50	22 (±11.9) a	4 (±7.5) a	74 (±8.5) ab
0.375	15 (±4.0) a	3 (±2.8) a	82 (±6.4) a
0.25	25 (±14.7) a	5 (±4.0) a	70 (±10.8) ab
0.125	35 (±17.7) a	6 (±6.2) a	59 (±13.1) b
0.062	36 (±22.5) a	5 (±4.0) a	59 (±18.8) b
Control	96 (±6.0) b	0 (±0) a	4 (±2.5) c

Within columns, and for each material, means followed by a common letter do not differ significantly at $P<0.05$ according to Duncan's multiple range test (5).

TABLE 2. Mortality, repellency and fecundity (eggs/female/day) of adult females of *Tetranychus cinnabarinus* exposed for 48 h to 4- and 8-day-old spray residues from 1% Neemgard dilutions on kidney bean leaves (25 mites/replicate; four replicates)

Age of residues (days)	Treatment	Mean % of mites affected (± SD) after a 48-h exposure to the residues			
		Live	Dead	Repelled	Eggs/female/day
4	Neemgard	4 (±4.6) a	2 (±4.0) a	94 (±7.6) a	0.3 (±0.32) a
	Control	99 (±2.0) b	0 (±0) a	1 (±2.0) b	8.5 (±0.14) b
8	Neemgard	24 (±17.2) a	5 (±2.0) a	71 (±17.0) a	0.8 (±0.39) a
	Control	100 b	0 a	0 b	5.3 (±0.14) b

Within columns, and for each material, means followed by the same letter do not differ significantly at $P<0.05$ according to Duncan's multiple range test (5).

ACKNOWLEDGMENT

Our thanks are due to Mr. U. Rosenberg, Agron, Agrochemicals Development & Marketing Ltd., Rehovot, Israel, for the samples of Neemgard and Neemix 45.

REFERENCES

1. Anon. (1992) Neem: A Tree for Solving Global Problems. National Research Council. National Academy Press, Washington, DC.
2. Anon. (1996) Neemgard – technical information. Data sheet released by Agron, Agrochemical Development & Marketing Ltd., Rehovot, Israel.
3. Ascher, K.R.S., Eliyahu, M., Nemny, N.E. and Meisner, J. (1984) Neem seed kernel extract as an inhibitor of growth and fecundity in *Spodoptera littoralis. in:* Schmutterer, H. and Ascher, K.R.S. [Eds.] Natural Pesticides from the Neem Tree and Other Tropical Plants. *Proc. 2nd Int. Neem Conf.* (Rauschholzhausen, Germany, 1983), pp. 331-344.
4. Dimetry, N.Z., Amer, S.A.A. and Reda, A.S. (1993) Biological activity of two neem seed kernel extracts against the two-spotted spider mite *Tetranychus urticae* Koch. *J. Appl. Entomol.* 116:308-312.
5. Duncan, D.B. (1955) Multiple range and multiple F tests. *Biometrics* 11:1-42.
6. Jacobson, M. [Ed.] (1989) Focus on Phytochemical Pesticides. Vol. I. The Neem Tree. CRC Press Inc., Boca Raton, FL, USA.
7. Locke, J.C. and Lawson, R.H. [Eds.] (1990) Neem's Potential in Pest Management Programs. *Proc. U.S. Dep. Agric. Neem Workshop* (Beltsville, MD, USA), ARS-86.
8. Mansour, F.A. and Ascher, K.R.S. (1983) Effects of neem (*Azadirachta indica*) seed kernel extracts from different solvents on the carmine spider mite, *Tetranychus cinnabarinus. Phytoparasitica* 11:177-185.
9. Mansour, F.A. and Ascher, K.R.S. (1995) Acarina, mites. *in:* Schmutterer, H. [Ed.] The Neem Tree – Source of Unique Natural Products for Integrated Pest Management, Medicine, Industry and Other Purposes. VCH, Weinheim, Germany. pp. 161-164.
10. Mansour, F.A., Ascher, K.R.S. and Abo-Moch, F. (1993) Effects of Margosan-O^{TM}, Azatin^{TM}, and RD9-Repelin®on spiders, and on predacious and phytophagous mites. *Phytoparasitica* 21:205-211.
11. Mansour, F.A., Ascher, K.R.S. and Omari, N. (1986) Toxicity of neem (*Azadirachta indica*) seed kernel extracts prepared with different solvents, on the spider *Chiracanthium mildei. Phytoparasitica* 14:73-76.
12. Mansour, F.A., Ascher, K.R.S. and Omari, N. (1987) Effects of neem (*Azadirachta indica*) seed kernel extracts from different solvents on the predacious mite *Phytoseiulus persimilis* and the phytophagous mite *Tetranychus cinnabarinus. Phytoparasitica* 15:125-130.
13. Sanguanpong, U. and Schmutterer, H. (1992) Laborversuche über die Wirkung von Niemoel und Niemsamenextrakten bei der gemeinen Spinnmilbe *Tetranychus urticae* Koch (Acari: Tetranychidae). *Z. Pflanzenkr. Pflanzenschutz* 99:637-646.
14. Schmutterer, H. [Ed.] (1995) The Neem Tree – Source of Unique Natural Products for Integrated Pest Management, Medicine, Industry and Other Purposes. VCH, Weinheim, Germany.
15. Schmutterer, H. and Singh, R.P. (1995) List of insect pests susceptible to neem products. *in:* Schmutterer, H. [Ed.] The Neem Tree – Source of Unique Natural Products for Integrated Pest Management, Medicine, Industry and Other Purposes. VCH, Weinheim, Germany. pp. 326-365.
16. Sundaram, K.M.S. and Sloane, L. (1995) Effects of pure and formulated azadirachtin, a neem-based biopesticide, on the phytophagous spider mite, *Tetranychus urticae. J. Environ. Sci. Health, Part B (Pestic. Food Contam. Agric. Wastes*) 30:801-814.