Neem *(Azadirachta indica)* Seed Kernel Extracts and Azadirachtin as Oviposition Deterrents against the Melon Fly (*Bactrocera cucurbitae*) and the Oriental Fruit Fly (*Bactrocera dorsalis*)

Shivendra Singh[1] and R.P. Singh[2]

Neem *(Azadirachta indica* A. Juss.) seed kernel (NSK) extracts, *viz.*, NSK aqueous suspension (NSKS), ethanolic extract of NSK (EtOH. NSK), hexane extract of NSK (neem oil), ethanolic extract of the hexane extract (EtOH. oil) and acetone extract of deoiled NSK powder (Acet. DNSKP) at 1.25–20% concentrations, and pure azadirachtin at 1.25–10 ppm, were evaluated as oviposition deterrents to *Bactrocera cucurbitae* (Coq.) and *B. dorsalis* Hendel. NSKS at $\geq$5% under choice test conditions, and at all concentrations ($\geq$1.25%) in no-choice tests significantly deterred oviposition in both species. Similarly, EtOH. NSK was significantly active at all the concentrations tested for both species in choice and no-choice tests. However, with neem oil and EtOH. oil sensitivities of the two species differed considerably. Both extracts deterred oviposition by *B. cucurbitae* at all the concentrations tested under both choice and no-choice test conditions. On the other hand, with *B. dorsalis*, neem oil was significantly deterrent only at 20% in both test regimes and at 5% and 20% for EtOH. oil under choice and no-choice test conditions, respectively. Acet. DNSKP significantly deterred oviposition by both species at all concentrations tested. Azadirachtin failed to deter oviposition in either species.

KEY WORDS: Neem seed kernel extracts; azadirachtin; oviposition deterrent effect; *Bactrocera cucurbitae; Bactrocera dorsalis.*

INTRODUCTION

The melon fly (*Bactrocera cucurbitae*) and the oriental fruit fly (*Bactrocera dorsalis*) are of serious concern to fruit and vegetable growers in the Indian sub-continent. Damage caused by these species is enormous, with nearly 50% of cucurbits being partially or completely damaged by *B. cucurbitae* every year in India (1), whereas the damage caused by *B. dorsalis* is yet to be estimated.

In the past attempts have been made to control fruit flies by luring them to baits or by contact poisons (5,11,15,17). Contact poisons suffer from limitations and pose serious health hazards, especially when fruits and some vegetables are consumed raw, often unwashed. Baiting has proved to be only slightly effective, and, in addition, has harmful effects on beneficial insects, including parasitoids of *Bactrocera* spp. While the sterile

Received Oct. 31, 1997; received in final form March 22, 1998; http://www.phytoparasitica.org posting June 9, 1998.

[1]Hindustan Insecticides Ltd., R&D Centre, Udyog Vihar, Gurgaon 122 016 (Haryana), India [Fax: +91-124-341319].

[2]Div. of Entomology, Indian Agricultural Research Institute (IARI), New Delhi 110 012, India.

insect technique has been suggested (24,29), it has its own limitations and is impracticable in continental areas. In addition, these measures do not achieve satisfactory control because of high pest population densities throughout the year, due to the polyphagous and polyandrous nature and long distance migratory abilities of the flies (6).

We definitely need a lower risk alternative for agricultural workers and fruit protection, particularly when the commercial value of the fruit is reduced drastically even by slight damage.

The search for alternative, ecofriendly insecticides has resulted in the rediscovery of botanicals for plant protection. Of the numerous plants investigated, neem *(Azadirachta indica* A. Juss.) has shown the most promising results. More than 400 insect species, including many key pests of agriculture, are susceptible to the various behavioral and physiological effects of neem (22): oviposition and feeding deterrence, insect-growth-regulating, ovicidal, sterilant, repellent activity, etc.

Neem seed kernel extracts varying from crude to enriched extracts have been reported as being oviposition deterrents against many Diptera (2,7,19,31). However, in the majority of these reports and those on other insects (9,13,14,21,23,30), comparison with pure compounds was not included nor was any specific compound and/or volatile constitutent clearly indicated as responsible for oviposition deterrence.

In the present investigations neem seed kernel extracts prepared with four different solvents, *viz.*, water, hexane, ethanol and acetone, were compared with pure azadirachtin as to their effect on oviposition of *B. cucrbitae* and *B. dorsalis*.

MATERIALS AND METHODS

Extraction

Five different neem seed kernel (NSK) extracts, *viz.*, aqueous, hexane and ethanolic extracts, an ethanolic extract of the hexane extract, and an acetone extract of deoiled NSK powder and azadirachtin, were assayed for their effect on oviposition.

An aqueous suspension of NSK (NSKS) was prepared by stirring 20 g of powdered NSK in a beaker containing 100 ml of distilled water for 3–4 h, followed by filtering through a fine muslin cloth. The marc remaining on the cloth was squeezed into the beaker. The freshly prepared suspension thus obtained was taken as 20% stock solution, which was diluted to lower concentrations with distilled water.

Ethanolic (EtOH. NSK) and hexane (neem oil) extracts of NSK were prepared by stirring 100 g ground NSK in ethanol or hexane for 3 h, filtering and restirring the marc five times with a 100-ml portion of each solvent. The combined filtrate was freed of the respective solvent under reduced pressure at 50°C in a flash evaporator. The extracts were dried to constant weight leaving no trace of solvent. The marc remaining from the hexane extract was dried at room temperature and collected as deoiled neem seed kernel powder (DNSKP). This powder was similarly extracted with acetone to obtain an acetone extract of DNSKP (Acet. DNSKP).

The hexane extract (oil) was partitioned with ethanol to get ethanolic extract of oil (Et.OH. oil) as described by Singh *et al.* (25).

All the extracts were formulated as emulsions using 0.5% Triton X-100 as the emulsifier, after dissolving the extracts at 10% in their respective solvents (except NSKS and neem oil, which were emulsified directly in water) and completing the volume (100 ml) with distilled water. 1.0 mg azadirachtin was dissolved in ethanol and then emulsified in water.

Insect cultures

Adults of *B. cucurbitae* and *B. dorsalis* obtained from the Division of Entomology, IARI, were reared in 30 cm × 30 cm × 30 cm wire mesh cages at 27±1°C and 65 ±5% r.h. and provided with yeast hydrolysate, sugar cubes and water.

Bioassay

Fruit pieces (2.5" × 1.5" × 1.25", of pumpkin for *B. cucurbitae* and of guava for *B. dorsalis*) which had been cleaned previously and incubated for 48 h at the above temperature (to enable hatch of any eggs which may have been already oviposited by the flies, so that the eggs did not interfere with those laid in the experiment), were then dipped in the extracts for 30 sec and allowed to dry at room temperature. Ten pairs of 20-day-old flies were transferred to 20 × 15 cm (height × diameter) fine-muslin-cloth-covered glass jars and offered treated fruits for oviposition. Solvent-emulsified water- and untreated water-treated fruits served as controls. All the extracts were tested at 1.25, 2.5, 5.0, 10.0 and 20.0%, whereas azadirachtin was tested at 1.25, 2.5, 5.0 and 10.0 ppm aqueous emulsions. Three replications were done for treatments and controls. Experiments were conducted for 24 h under choice and no-choice conditions, after which the fruits were removed and dissected under a binocular microscope to count the number of eggs laid by ten female flies. The data thus obtained were subjected to ANOVA with Duncan's Multiple Range Test (DMRT) and Least Significant Difference (LSD) applied for comparing treatment means.

RESULTS AND DISCUSSION

The effect of NSK extracts on the oviposition by *B. cucurbitae* and *B. dorsalis* is presented in Tables 1 to 4. Under choice test conditions all the extracts (except NSKS) were significantly oviposition-deterrent for *B. cucurbitae* at 1.25% and above (Table 1); NSKS was active only at 5% and above. Similar effects were observed for *B. dorsalis* with extracts EtOH. NSK and Acet. DNSKP (activity as from 1.25%); NSKS and EtOH. oil were active only from 5% and neem oil only at 20% (Table 3).

TABLE 1. Effect of neem seed kernel extracts on the oviposition by *Bactrocera cucurbitae* under choice test conditions

Concn. (%)	% Increase (+) or decrease (-) in oviposition[z] *vs* water with emulsifier (control)				
	NSKS	EtOH. NSK	Neem oil	EtOH. oil	Acet. DNSKP
20.0	-100*	-100*	-100*	-100*	-100*
10.0	-31.3*	-99.6*	-66.3*	-100*	-100*
5.0	-33.5*	-63.4*	-51.4*	-93.6*	-84.3*
2.5	+1.8ns	-53.2*	-48.9*	-47.0*	-83.0*
1.25	+10.6ns	-42.0*	-26.4*	-43.9*	-53.4*
Untreated control	+5.6ns	+7.0ns	+7.8ns	-14.0ns	+17.1ns

[z] Means of three replications.

∗, Significant; ns, not significant; comparison by LSD.

In the experiments conducted under no-choice test conditions all extracts were significantly active against *B. cucurbitae* as from 1.25%, except NSKS (active as from 2.5%) (Table 2). Against *B. dorsalis* activity was as follows: Acet. DNSKP, as from 1.25%; NSKS and EtOH. NSK, as from 2.5%; neem oil and EtOH. oil active only at 20% (Table 4).

TABLE 2. Effect of neem seed kernel extracts on the oviposition by *Bactrocera cucurbitae* under no-choice test conditions

Concn. (%)	Number of eggs laid[z]/10 females				
	NSKS	EtOH. NSK	Neem oil	EtOH. oil	Acet. DNSKP
20.0	10.3^{a}	2.00^{a}	10.0^{a}	2.0	1.33^{a}
10.0	38.0^{b}	8.3^{b}	28.0^{b}	5.3^{a}	7.7^{b}
5.0	45.0^{b}	42.3^{c}	29.0^{b}	27.7^{b}	26.0^{c}
2.5	47.7^{b}	49.0^{c}	34.0^{bc}	31.3^{bc}	32.0^{c}
1.25	51.7^{bc}	53.7^{c}	46.7^{c}	48.3^{c}	36.3^{c}
Water with emulsifier	91.0^{cd}	98.7^{d}	96.7^{d}	75.3^{d}	84.0^{d}
Untreated control	92.3^{d}	99.7^{d}	101.3^{d}	85.7^{d}	87.7^{d}

[z] Means of three replications.
Within columns, means followed by a common letter do not differ significantly ($P = 0.05$) by DMRT.

We found that azadirachtin had no oviposition deterrence effect under choice and no-choice test conditions for either species.

TABLE 3. Effect of neem seed kernel extracts on the oviposition by *Bactrocera dorsalis* under choice test conditions

Concn. (%)	% Increase (+) or decrease (-) in oviposition[z] *vs* water with emulsifier (control)				
	NSKS	EtOH. NSK	Neem oil	EtOH. oil	Acet. DNSKP
20.0	-100*	-100*	-100*	-100*	-100*
10.0	-47.2*	-100*	-19.4ns	-55.1*	-100*
5.0	-34.5*	-55.2*	-8.6ns	-49.8*	-85.1*
2.5	-5.1ns	-50.8*	+15.8ns	-11.4ns	-69.6*
1.25	+6.0ns	-34.7*	+5.2ns	+1.8ns	-50.5*
Untreated control	+7.4ns	+7.2ns	+10.4ns	+1.8ns	+5.3ns

[z] Means of three replications.
$*$, Significant; ns, not significant; comparison by LSD.

There are reports on the oviposition deterrence effects of simple neem extracts on tephritids (3,6,16,27,28). Deterrence effects of methyl-*tert*.-butyl ether/water extract on *B. frontalis* were reported by Dreyer (8) and of 100 ppm azadirachtin on *B. tryoni* (18). The former extract contained compounds in addition to azadirachtin, whereas the latter finding was not confirmed under field conditions, probably due to the use of artificial oviposition substrates in the laboratory trials which were not preferred as oviposition sites by the flies, rather than to the effect of azadirachtin.

TABLE 4. Effect of neem seed kernel extracts on the oviposition by *Bactrocera dorsalis* under no-choice test conditions

Concn. (%)	Number of eggs laid[z]/10 females				
	NSKS	EtOH. NSK	Neem oil	EtOH. oil	Acet. DNSKP
20.0	17.0^{a}	2.3^{a}	15.0^{a}	5.7^{a}	2.3^{a}
10.0	35.7^{b}	13.0^{b}	66.7^{b}	52.7^{a}	11.0^{b}
5.0	47.3^{bc}	46.3^{c}	79.7^{b}	62.0^{b}	31.3^{c}
2.5	50.3^{bc}	52.0^{cd}	86.0^{b}	75.0^{b}	36.7^{c}
1.25	74.3^{cd}	66.0^{de}	86.3^{b}	83.3^{b}	38.3^{c}
Water with emulsifier	89.0^{d}	75.0^{e}	85.3^{b}	84.7^{b}	80.3^{d}
Untreated control	91.0^{d}	75.7^{e}	86.0^{b}	85.0^{b}	81.7^{d}

[z] Means of three replications.
Within columns, means followed by a common letter do not differ significantly ($P = 0.05$) by DMRT.

In our studies of the oviposition deterrence of *B. cucurbitae* and *B. dorsalis* on the preferred fruits, cucumber (*Cucurbita moschata*) and guava (*Psidium guajava*), respectively, we have tried to assess the deterrence effect of four neem extracts and azadirachtin and to find out the differences in the susceptibility of these two species to neem extracts.

Differences in the susceptibility of insects to neem extracts depend on the diversity of seed preparations and on the insects studied (16,26). Results have shown that the degree of deterrence varied between the extracts and the species, *B. dorsalis* being less sensitive than *B. cucurbitae*. As the extracts were prepared from the respective stock solutions, the chances of variation due to the extracts is ruled out, and difference in deterrence must be due to the differences in the susceptibility of the two species. This confirms the findings of Singh and Srivastava (27), who reported that *B. dorsalis* required a much higher concentration of ethanolic extract of hexane extract for arrest of oviposition as compared with *B. cucurbitae*.

Azadirachtin had no oviposition deterrence effect, confirming the reports of Saxena and Rembold (21). Naumann and Isman (16) and Saxena and Basit (20) advocated the role of volatile organosulphur compounds including dipropyl disulphide which are known to be present in the neem seeds (4) as probable compounds responsible for oviposition deterrence. The volatile organosulphur compounds may be responsible for deterrence in the case of EtOH. oil. However, their absence in NSKS (water cannot dissolve an apolar volatile organosulphur compound present in the apolar part – oil – of the seed kernel), which also deterred the flies, is suggestive of the fact that there may be other compound(s) responsible for oviposition deterrence, possibly a common compound, present in both oil and the deoiled part of the seed. The chances of this being a non-volatile compound are further strengthened, as the flies settled on the treated fruits and appeared quite comfortable, probed, but did not lay eggs. This is in conformity with the report of Chen *et al.* (6), who suggested the role of non-volatile neem components detected by the ovipositor as a signal to reduce egg laying.

There are several compounds present in the oil and deoiled part of the seed kernel and since both fractions have deterred oviposition, we presume that the compound(s) is/are common to the two parts. Salannin, a common compound present in oil (12,25) and the deoiled part (10) of neem seeds, may be the compound responsible for this effect. Nevertheless, the role of other compounds and the continuous input of olfactory stimuli after landing on neem-treated fruits, cannot be ruled out.

As already mentioned, the current methods used to control fruit flies have their own limitations. The incorporation of oviposition deterence in pest management programs may be an effective alternative for controlling the flies.

ACKNOWLEDGMENTS

This research was supported by the Department of Biotechnology, Ministry of Science and Technology, Govt. of India. The authors are grateful to Prof. S.S. Sehgal, Professor of Entomology, Dept. of Zoology, University of Delhi, for his keen interest in the research.

REFERENCES

1. Agarwal, M.L., Sharma, D.D. and Rahman, O. (1987) Melon fly and its control. *Indian Hortic.* 33(2):10-12.
2. Anon. (1992) Azatin. Tech. Bull. AgriDyne Technologies Inc., Salt Lake City, UT, USA.
3. Areekul, S., Sinchausri, P. and Tigvattanont, S. (1988) Effect of Thai plant extracts on the oriental fruit fly. II Repellency test. *Kasetsart J. Nat. Sci.* 22:56-61.
4. Balandrin, M.F., Lee, S.M. and Klocke, J.A. (1988) Biologically active volatile organosulphur compounds from seeds of the neem tree, *Azadirachta indica* (Meliaceae). *J. Agric. Food Chem.* 36:1048-1054.
5. Bhutani, D.K. (1975) Insect pests of fruit crop and their control – Mango. *Pesticides* 7(3):36-42.
6. Chen, C.C., Dong, Y.J., Cheng, L.L. and Hou, R.F. (1996) Deterrence effect of neem seed kernel extracts on oviposition of the oriental fruit fly (Diptera: Tephritidae) on guava. *J. Econ. Entomol.* 89:462-466.
7. Chiu, S.F. (1985) Recent research findings on Meliaceae and other promising botanical insecticides in China. *Z. Pflkrankh. Pflanzenschutz* 92:320-329.
8. Dreyer, M. (1986) Investigation of the effect of aqueous extracts and other products from neem seeds against pests of vegetables and field crops in Togo. Ph.D. thesis, University of Giessen, Germany.
9. Fagoonee, I. (1981) Behavioral response of *Crocidolomia binotalis* to neem. *Proc. 1st Int. Neem Conf.* (Rottach-Egern, Germany, 1980), pp. 109-120.
10. Feuerhake, K.J. (1984) Effectiveness and selectivity of technical solvents for the extraction of neem seed components with insecticidal activity. *Proc. 2nd Int. Neem Conf.* (Rauischholzhausen, Germany, 1983), pp. 103-114.
11. Gupta, J.N. and Verma, A.N. (1979) Relative efficacy of insecticides as contact poisons to the adults of melon fruit fly, *Dacus cucurbitae* (Coq.). *Indian J. Entomol.* 41:117-120.
12. Henderson, R., McCrindle, R., Melera, A. and Overton, K.H. (1968) Tetranortriterpenoides. IX. The constitution and sterochemistry of salannin. *Tetrahedron* 24:1525-1528.
13. Jacobson, M., Reed, D.K., Crystal, M.M., Moreno, D.S. and Soderstrom, F.I. (1978) Chemistry and biological activity of insect feeding deterrents from certain weed and crop plants. *Entomol. Exp. Appl.* 24:448-457.
14. Joshi, B.G. and Sitaramaiah, S. (1979) Neem kernel as an oviposition repellent for *Spodoptera litura* (F.) moths. *Phytoparasitica* 7:199-202.
15. Lee, W.Y. (1988) The control programme of the oriental fruit fly in Taiwan. Spec. Publ. No. 2, Entomol. Soc. Rep. China. pp. 51-60.

16. Naumann, K. and Isman, M.B. (1995) Evaluation of neem (*Azadirachta indica* A. Juss.) seed extracts and oils as oviposition deterrents to noctuid moths. *Entomol. Exp. Appl.* 76:115-120.
17. Perdomo, A.J., Nation, J.L. and Baranowski, R.M. (1976) Attraction of female and male fruit flies to food-baited and male-baited traps under field conditions. *Environ. Entomol.* 15:1208-1210.
18. Rice, M.J. (1993) Development of neem research and industry in Australia. *Proc. World Neem Conf.* (Bangalore, India), pp. 8-24.
19. Rice, M.J., Saxton, S. and Esmail, A.M. (1985) Antifeedant phytochemical blocks oviposition by sheep blow fly. *J. Aust. Entomol. Soc.* 24:16.
20. Saxena, K.N. and Basit, A. (1982) Inhibition of oviposition by volatiles of certain plants and chemicals in the leaf hopper, *Amrasca devetans* (Distant). *J. Chem. Ecol.* 8:329-338.
21. Saxena, K.N. and Rembold, H. (1984) Orientation and ovipositional responses of *Heliothis armigera* to certain neem constituents. *Proc. 2nd Int. Neem Conf.* (Rauischholzhausen, Germany, 1983), pp. 199-210.
22. Schmutterer, H. and Singh, R.P. (1995) List of insect pests susceptible to neem products. *in:* Schmutterer, H. [Ed.] The Neem Tree *Azadirachta indica* A. Juss. and Other Meliaceous Plants. VCH Publications, Weinheim, Germany. pp. 326-365.
23. Shelke, S.S., Jakhav, L.D. and Saulaunkhe, G.N. (1985) Ovipositional and adult repellent action of some vegetable oils/extracts against potato tuber moth. *J. Maharashtra Agric. Univ.* 10:284-286.
24. Sheo, S.T., Vargas, R.I., Gilmore, J.E., Kurashima, R.S. and Fujimoto, M.S. (1990) Sperm transfer in normal and gamma-irradiated, laboratory-reared Mediterranean fruit flies (Diptera: Tephritidae). *J. Econ. Entomol.* 83:1949-1953.
25. Singh, R.P., Devakumar, C. and Dhingra, S. (1988) Activity of neem (*Azadirachta indica* A. Juss.) seed kernel extracts against the mustard aphid, *Lipaphis erysimi*. *Phytoparasitica* 16:225-230.
26. Singh, R.P. and Raheja, A.K. (1996) Strategies in the management of insect pests with neem (*Azadirachta indica* A. Juss.). *Proc. World Neem Conf.* (Bangalore, India, 1993), pp. 103-120.
27. Singh, R.P. and Srivastava, B.G. (1983) Alcohol extract of neem *(Azadirachta indica* A. Juss.) seed oil as oviposition deterrent for *Dacus cucurbitae* (Coq.). *Indian J. Entomol.* 45:497-498.
28. Sombatsiri, K. and Tigvattanont, S. (1984) Effects of neem extracts on some insect pests of economic importance in Thailand. *Proc. 2nd Int. Neem Conf.* (Rauischholzhausen, Germany, 1983), pp. 95-100.
29. Wong, T.T.Y., Kobayashi, R.M., Whitehand, L.C., Henry, G., Zadig, D.A. and Denny, C.L. (1984) Mediterranean fruit fly (Diptera: Tephritidae): mating choices of irradiated laboratory-reared and untreated wild flies of California in cages. *J. Econ. Entomol.* 77:58-62.
30. Yadav, T.D. (1985) Antiovipositional and ovicidal toxicity of neem oil against three species of *Callosobruchus. Neem Newsl.* 2:25-29.
31. Zebitz, C.P.W. (1987) Potential of neem seed kernel extracts in mosquito control. *Proc. 3rd Int. Neem Conf.* (Nairobi, Kenya, 1986), pp. 555-573.