Insect growth regulating and antifeedant effects of neem extracts and azadirachtin on two aphid species of ornamental plants

OPENDER KOUL

Insect Biopesticide Research Centre, 30 Parkash Nagar, Model Town, Jalandhar 144 003, India
(Fax, 91-181-274336)

Leaf disc choice test bioassay demonstrated that formulated neem seed extracts were highly deterrent and growth regulatory to rose aphid, *Microsiphum rosae* (L.) and Chrysanthemum aphid, *Macrosiphoniella sanbornii* (Gillete). Effective concentrations to produce 50% feeding deterrence was 0·80 and 0·84% respectively for 2nd instar nymphs irrespective of bioassay duration. The disruption of aphid feeding was related to the presence of azadirachtin concentration in the extract. The toxicity on contact from the leaf surface or via topical application due to azadirachtin was significantly different and topical treatment was at least 7 times more effective for both species. Thus growth regulatory effects of azadirachtin were influenced by the host plant and the stage of treatment. Field evaluation with formulated neem extracts revealed the effect to be more of growth regulatory nature thereby showing that azadirachtin is a physiological toxin for aphid species. Neem seed extracts reduced the population of aphid on respective host plants significantly, EC_{50} values being 0·88 and 0·96% for *M. rosae* and *M. sanbornii* respectively.

1. Introduction

The biological activities of neem, *Azadirachta indica* A Juss, seed extracts or its most active constituent azadirachtin (AZA) is now known for more than 400 species of insects (Schmutterer and Singh 1995). These activities include feeding deterrence, growth inhibition, antifertility, growth regulation and antioviposition properties (Schmutterer 1990; Koul *et al* 1990; Mordue and Blackwell 1993; Koul 1996a). Among them growth regulating effects are very significant, as such effects regulate the survival, longevity, moulting process and other physiological processes of insects (Koul 1996b).

Recently we demonstrated in the laboratory experiments that formulated neem seed extracts and AZA deterred feeding of cabbage aphid *Brevicoryne brassicae* (Koul *et al* 1997). Aphids are economically important pests which are difficult to control because of their mobility, parthenogenetic reproduction and resistance to many neurotoxic chemicals. In last five years number of studies have revealed the potential efficacy of neem products against these pests (Lowery and Isman 1994a). However, there are number of contradictory results regarding the deterrent activity of neem preparations. Neem seed oil, for instance, is deterrent to only half of six aphid species treated for the activity (Lowery and Isman 1993) and in some cases activity disappears within 24 h post-treatment.

Field investigations of the efficacy of neem products for the control of aphids have been few and fragmentary. Plots of lettuce, cabbage, strawberry and pepper sprayed with 1·0% neem seed oil (20 ppm AZA) have shown reduction in aphid population from approximately 40–98% relative to control plots (Lowery and Isman 1994b).

In the present study the insect growth regulatory (IGR) and antifeedant effects of purified AZA and neem seed extracts (NSE) containing known amounts of AZA have been investigated against the aphids of ornamental plants, which have been least evaluated so far, to demonstrate interspecific susceptibility among aphids.

Keywords. Azadirachtin; *Azadirachta indica*; neem seed extracts; insect growth regulatory; antifeedant; Aphididae; insect control

2. Materials and methods

Individuals of chrysanthemum aphid, *Macrosiphoniella sanbornii* (Gillete) and rose aphid, *Macrosiphum rosae* (L.) identified by taxonomical comparisons, were taken for the study. *M. sanbornii* were taken directly from the potted plants of *Chrysanthemum* (25 cm pots) and *M. rosae* from *Rosa indica* plants grown in flower beds (3 m × 0·5 m size).

Treatment was given initially in petri dishes (50 × 9 mm) with tight fitted lids according to method of Lowery and Sears (1986). For treatment leaf discs, rose buds, sepals or twigs from respective plants were immersed in treatment solutions and gently agitated for 3 to 4 s until both surfaces were wet. They were then placed on a hard plastic surface and allowed to dry before use.

Test material consisted of 3 neem seed extracts with known concentration of AZA (1400, 3000, 10000 ppm). Pure AZA (> 95% purity) was kindly provided by Prof. M B Isman, University of British Columbia, Canada. Solutions were emulsified with 0·1% Triton-X-100 in distilled water. The AZA content of extracts was determined using reverse phase high performance liquid chromatography (HPLC) (Govindachari *et al* 1990) and AZA detected at 215 nm. AZA (> 95% purity) was used as a comparative standard.

The effect of NSE on the survival of second nymphal instars and adults of *M. sanbornii* and *M. rosae* was compared with that of pure AZA. Treatments contained extract with 10000 ppm AZA at various concentrations of 0·15, 0·3 and 0·6% (i.e., 15, 30, and 60 ppm), or emulsifier only as a control. Survival of aphids was recorded daily for 3 days on treated substrate and a further 4 days on untreated substrate. The number of cast exuviae was also recorded for nymphs. For each species and stage, aphids were reared 8/dish with 5 dishes per treatment. Experiments were repeated 3 times.

Lethal concentration of AZA applied to the substrate resulting in 50% mortality (LC_{50}) was determined for adults of both species. Mortality was checked after 24 h. For each species 8 aphids were reared as per dish with 5 dishes for each concentration range (10 to 100 ppm AZA level). Contact toxicity was evaluated by application of pure AZA to dorsal abdomen of adults by the use of microapplicator with 5 µl syringe (7105 series syringe, Hamilton Co., Reno, USA) at a level of 0·03 µl of solution. AZA treatment level was up to 100 ppm.

Antifeedant activity using choice bioassay was similar to the one used for *Brevicoryne brassicae* (Koul *et al* 1997). NSE were used at a concentration of 0 to 2·0% and AZA at 10–200 ppm level for a period up to 24 h treatment.

Due to very few and fragmentary reports concerning field evaluation of neem products against aphids, NSE sprays were used against both candidate species. In case of rose aphid small rose beds of 3 × 0·5 m were used containing 5 plants in a row spaced 0·4 m apart and infested with aphids heavily. Five such beds were used for the study. In case of chrysanthemum aphids potted plants (25 cm diameter) which were heavily infested with aphids were taken for the study. Treatment was given to 20 pots with 5 pots kept in a row about 0·2 m apart. The pots were kept in the field at ambient temperature. Trials were randomized complete-block design with 4 replicates/treatment. Spray procedure was after Lowery *et al* (1993). Treatment consisted of formulated NSE at 0·5, 1·0 and 2·0% (calculated AZA in the extract = 1400 ppm) thus each formulation contained 7·0, 14·0 and 28·0 ppm of AZA at the final dilution. Foliar sprays were applied twice at weekly intervals using simple garden sprayers till the drain off situation was observed. One week after the second spray, aphid numbers/plant were recorded in both treated and control plants.

Mortality, moulting and deterrence parameters were compared using ANOVA followed by Tukey's multiple range test to determine differences between means of treatment (Wilkinson 1990). Linear regression analysis and inverse predictions (Neter *et al* 1985) were used to determine the lethal concentrations (LC_{50}).

3. Results and discussion

While 70% of the control aphids survived one week after treatment, none of the 2nd instars exposed to various concentrations of NSE or pure AZA survived for the period (table 1) except at 0·1% level where 10% survival rate was recorded. Even the number of moults were significantly lower for both *M. sanbornii* and *M. rosae* treated with NSE or AZA at all rates compared with controls. There was no significant variation between the pure AZA treatments (30 ppm) compared to 0·6% NSE for both species. Table 1 suggests that AZA accounted for the major bioactivity of neem in the two species. This is obviously true for *Myzus persicae* and *Nasonovia ribis-nigri* (Lowery and Isman 1994a) and *Brevicoryne brassicae* as well (Koul *et al* 1997). Both NSE and AZA were toxic to adult aphids at similar levels of treatment. However, their survival rate was higher when compared to nymphal treatment. There was decrease in survival with the increase in concentration (figure 1) though for reduction in survival, AZA level required was as high as 70 to 90 ppm. This is contradictory to what has been observed in case of *N. ribis-nigri* and *Chaetosiphon fragaefolii* where per cent survival and days of survival did not differ between treated and control insects (Lowery and Isman 1994b). As the mortality is related to duration, it is obvious that toxicity is primarily due to IGR activity. However, the assumption of Lowery and Isman (1994b) that activity is of IGR

type and therefore that adults could be less susceptible is not correct. There are many growth regulatory processes which occur in adult insects and could be disrupted by NSE or AZA treatment as shown in many heteropteran, lepidopteran and coleopteran insects (Koul 1996b). This conclusion gets a further support from the present data which shows that adults which survived through the treatment, apparently passed on the IGR effects to the offsprings, because considerable mortality was observed, thereby decreasing the first filial generation significantly (table 2). Obviously there is significant effect on fecundity and fertility of these insects as has been demonstrated in other aphids and many other insect species (Schmutterer 1990; Mordue and Blackwell 1993; Koul 1996b). Our studies and other related studies which demonstrate that NSE and AZA effectively inhibits aphid reproduction clearly implies that neem could directly contribute to the control of aphids in the field. This has a significant implication in a tritrophic situation as well because reduction of aphid population might allow natural enemies to effectively maintain aphid numbers below economic injury level, though there are some indications that neem is not without effects on natural enemies (Lowery and Isman 1995).

Lethal concentration of AZA resulting in 50% mortality of adult aphids placed on treated leaf discs or treated topically revealed the significance of mode of application. In topical treatment the requirement was nearly 80% lower than the contact treatment (table 3) for both candidate species. This clearly indicates that there is difference in the penetration and transport depending upon the mode of application. In our previous studies with lepidopteran species it has clearly been demonstrated that mode of application has an important role to play in AZA effects against these species (Koul *et al* 1987; Koul and Isman 1991), notwithstanding the role of the host plant (Lowery and Isman 1994a).

Initial observation after the release of both aphids on to the leaf discs was their continuous crawling on the treated surface at least for half an hour. This showed that NSE or AZA were not repellent to these aphid species as has been in the case of *Bemisia tabacci*, a sweet potato whitefly (Coudriet *et al* 1985). Deterrence of NSE to 2nd instar larvae showed the response of aphids to surface treatment was rapid and occurred within 1 h of treatment for *M. rosae*. However, for *M. sanbornii* the effect was more gradual (table 4). Rapid effect on the behaviour of aphids has been observed against *S. avenae* and *R. padii* earlier (West and Mordue 1992). There was not much decrease in EC_{50} values until 24 h as the slopes did not differ significantly ($P > 0.05$) for any of the bioassays. Similar observation has been made for strawberry aphid *C. fragaefolii* as well (Lowery and Isman 1993).

Table 1. Per cent survival after 1 week and average number of moults for 2nd instar rose aphid *M. rosae* (Mr) and chrysanthemum aphid, *M. sanbornii* (Ms) exposed to NSE and AZA applied to leaf discs, rose buds, sepals or twigs.

	Survival (%)		Av. No. of moults	
Treatment	Mr	Ms	Mr	Ms
Control	70·0[a]	70·0[a]	6	5
NSE 0·1%	10·0[b]	10·0[b]	4	3
NSE 0·3%	0·0[b]	0·0[b]	1	1
NSE 0·6%	0·0[b]	0·0[b]	< 1	< 1
AZA 30 ppm	0·0[b]	0·0[b]	< 1	< 1
AZA 60 ppm	0·0[b]	0·0[b]	< 1	< 1

Means within a column followed by same letter are not significantly different ($P > 0.05$) based on Tukey's multiple range test.

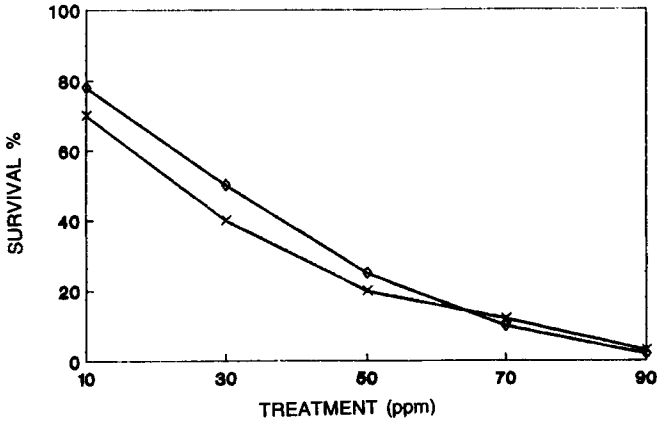

Figure 1. Per cent survival of adults of *M. sanbornii* (–X–X–) and *M. rosae* (–◊–◊–) 1 week after treatment with neem extract (= 3000 ppm AZA content) at various concentrations having AZA content of 15, 30, 50, 70, and 90 ppm respectively.

Table 2. Per cent survival of first generation aphids produced by adults exposed for 48 h to NSE or AZA.

	Survival (%)	
Treatment	*M. sanbornii*	*M. rosae*
Control	88·0[a] (95)	72·0[a] (88)
NSE 0·1%	32·5[b] (80)	38·0[b] (75)
NSE 0·3%	16·8[b,c] (32)	22·0[a,b] (42)
NSE 0·6%	8·8[c] (12)	15·2[b,c] (22)
AZA 30 ppm	0·0[c] (28)	0·0[c] (32)
AZA 60 ppm	0·0[c] (18)	0·0[c] (20)

Means within a column followed by the same letter are not significantly different ($P > 0.05$). Numbers in parentheses is No. of offsprings/treatment.
NSE (= 10000 ppm AZA).

The deterrent activity of neem to aphids have produced contradictory results. RD-9 repelin treatment, for instance, to pea aphid, *Aphis pisum* at 1 to 10% induces repellent effect (Hunter and Ullman 1992) and AZA inhibits probing and feeding of same species at 2·5% level (Wilkins *et al* 1990). In contrast NSE do not effect the probing and feeding of *Myzus persicae* (Griffiths *et al* 1989). A slight reduction due to Margosan-O-Concentrate has been observed (Lowery and Isman 1993). Although these results show that there are differences in behavioural response among the aphid species, it is quite evident from our earlier studies with *B. brassicae* (Koul *et al* 1997) and the present findings that variable level of treatment are relative to the age of the insect and the specific species evaluated.

The present results also show that deterrency of NSE to adult aphids was related to the concentration of AZA. EC_{50} values for extracts with AZA content ranging from 0·14% to 1·0% were significantly different (table 5). This shows that which the deterrency effect was AZA mediated, the requirement of AZA to produce such behaviour was different for two species (*M. rosae* = 11·0 ppm and *M. sanbornii* = 12·2 ppm on an average). That deterrence due to AZA is concentration dependent and it is demonstrated from the results obtained after pure AZA treatment to adults of both aphid species (table 6). The absolute deterrence was recorded at beyond 100 ppm, 6 h post-treatment. This level is of course on higher side as compared to lepidopteran pests (0·2–50 ppm) (Mordue and Blackwell 1993). Obviously, the treatment level around 100 ppm appears to be highly significant for required aphid control. This seems to be a generalized level required for many species like *B. brassicae*, etc. (Koul *et al* 1997). However, *M. persicae*, where AZA did not deter feeding up to 100 ppm level and EC_{50} = 119·5 ppm was recorded for the deterrence in case of *C. fragaefolii* on strawberry; it has been demonstrated that AZA is partially responsible for deterrency. This is not true in case of *M. sanbornii*, *M. rosae* and number of other aphid species (Koul *et al* 1997) wherein the effects are relative to the AZA level. Lowery and Isman (1993) explain that other components

Table 3. Contact LC_{50} values of AZA for adult aphids.

	LC_{50}^{a} (ppm AZA)			
Aphids	Contact	R^2	Topical	R^2
M. sanbornii	87·5 (82·4–90·6)	0·827	11·5 (10·2–14·6)	0·912
M. rosae	90·6 (80·5–99·5)	0·910	12·4 (10·5–15·0)	0·870

[a] Linear regression (numbers in parentheses denote 95% confidance interval). $n = 30$, mortality in 1 week after treatment.

Table 4. EC_{50} values of NSE for feeding deterrence to second instar aphids.

	M. sanbornii			*M. rosae*		
Assay time (h)	EC_{50} (% Ext.)	Slope ± SE	R^2	EC_{50} (% Ext.)	Slope ± SE	R^2
1·0	0·58	–0·176 (0·04)	0·86	0·78	–0·167 (0·04)	0·76
6·0	0·88	–0·190 (0·06)	0·87	0·80	–0·186 (0·06)	0·86
24·0	0·95	–0·195 (0·06)	0·90	0·95	–0·188 (0·08)	0·88

Slopes of regression for proportions of aphids on NSE treated discs are not significantly different ($P > 0·05$). NSE = neem seed extract with 0·14% AZA.

Table 5. Feeding deterrency of various concentrations of NSE containing variable amount of AZA to adult *M. sanbornii* and *M. rosae* aphids.

	M. sanbornii			*M. rosae*		
Extracts with AZA level (%)	EC_{50}	Calculated AZA	R^2	EC_{50}	Calculated AZA	R^2
0·14	0·86	12·1	0·90	0·78	10·9	0·96
0·30	0·42	12·6	0·92	0·37	11·1	0·88
1·00	0·12	12·0	0·88	0·11	11·0	0·92

R^2 from linear regression.

of neem are likely to make greater contribution to the deterrent activity, but this cannot be a generalization on account of two basic facts (i) that many species have shown a correlation between the AZA content and AZA effects and (ii) repellent organosulphur compounds (Balandrin *et al* 1988) did not account for the activity in their studies (Lowery and Isman 1993) and neither have salannin or other limonoids demonstrated the combined effects in any study so far. Several limonoids present in neem (Koul 1992) could produce variable behaviour responses in different magnitudes. However, unless these compounds are evaluated individually and in combinations against various aphid species such a conclusion remains only a speculation. In our recent studies with salannin it has been observed that this limonoid does not inhibit the feeding of *Brevicoryne brassicae* (unpublished data).

Application of NSE reduced the population of both *M. rosae* and *M. sanbornii* on rose and chrysanthemum plants. Average EC_{50} value for *M. rosae* in the field situation was 0·88% (table 7), similar to that established in the laboratory. A 1·0% NSE treatment reduced *M. rosae* number in the field by 65·6%. However, *M. sanbornii* reduction in potted plants at this concentration was less (53·4%) as compared to *M. rosae*. EC_{50} of 0·96% was observed for this species. The requirement of AZA to produce 50% reduction in population was 12·3 and 13·4 ppm respectively which was quite comparable to the laboratory experiments (table 5).

NSE treatment did not cause any phytotoxic effects to rose or chrysanthemum, though there are reports that neem oils are phytotoxic to tomato, potato, onion and white cabbage crops (Schmutterer 1990), which may be due to high oil content in such formulations.

In some previous studies 1·0% neem oil applied to cowpea have shown 96·7 and 43·3% mortality of third instar and adult cowpea aphids, *Aphis craccivora* after 24 h exposure (Patel and Srivastava 1989). Similarly neem seed extracts kill *Acyrthosiphon pisum* and *Aphis fabae* when applied to broad bean plants (Schauer 1984). In our studies, it is found that reduction of aphid population is due to inhibition of developmental physiology. Many aphids were seen in moribund state lying on the ground or soil of potted plants for two to three days and ultimately died apparently due to starvation and inhibition of growth regulatory effects. This shows that aphids are not killed by any neurotoxic effect of the NSE or AZA.

Thus it is clear from above discussion that neem based formulations, with standardized AZA content, are efficacious against *M. rosae* and *M. sanbornii* and many other aphid species both in the laboratory and field. Although the level of control may vary according to the level of active ingredient in the formulation, yet it can be generalized that treatment levels required for aphid control would be higher as compared to lepidopteran species, but still quite safe for a botanical pesticide. If it could be generalized that neem preparations can induce

Table 6. Deterrence of pure AZA to adult *M. sanbornii* and *M. rosae* aphids in choice bioassay.

AZA (ppm)	Aphids on treated surface (%)							
	M. sanbornii				*M. rosae*			
	After 3 h	6 h	12 h	24 h	3 h	6 h	12 h	24 h
10·0	$42·5^{a}$	$40·0^{a}$	$40·0^{a}$	$37·5^{a}$	$47·5^{a}$	$45·0^{a}$	$42·5^{a}$	$40·0^{a}$
50·0	$37·5^{a,b}$	$30·0^{a,b}$	$32·5^{a,b}$	$30·0^{a,b}$	$45·0^{a}$	$45·0^{a}$	$32·5^{a,b}$	$27·5^{b}$
100·0	$27·5^{b}$	$25·0^{b}$	$20·0^{b}$	$0·0^{c}$	$30·0^{a,b}$	$10·0^{b,c}$	$0·0^{c}$	$0·0^{c}$
200·0	$12·5^{b,c}$	$0·0^{c}$	$0·0^{c}$	$0·0^{c}$	$15·0^{b}$	$2·5^{c}$	$0·0^{c}$	$0·0^{c}$

For each time interval, means followed by the same letter are not significantly different ($P > 0·05$). Fishers LSD test $n = 40$.

Table 7. Control of aphids on plants following application of NSE.

Plant	Aphid species	Aphid reduction per plant (%)				
		0·5% (7·0)	1·0% (14·0)	2·0% (28·0)	EC_{50} (%)	AZA level (ppm)
Rose	*M. rosae*	$39·5^{a}$	$65·6^{b}$	$75·8^{c}$	0·88	12·3
Chrysanthemum	*M. sanbornii*	$28·8^{a}$	$53·4^{b}$	$87·5^{c}$	0·96	13·4

For each row means followed by the same letter are not significantly different ($P > 0·05$). Tukey's multiple range test (NSE = 1400 ppm AZA).

antifertility and antifecundity effects as demonstrated in *A. pisum, M. persicae, Nasonovia ribisnigri, Chaetosiphon fragaefolii* and *B. brassicae* (Lowery and Isman 1994a, b, 1996; Koul 1998), it will become easier and safer to control the aphids in the field.

Acknowledgements

Author is thankful to Prof. T R Govindachari, SPIC Research Foundation, Chennai for help in AZA analysis and Prof. M B Isman, University of British Columbia, Canada for authentic sample of AZA. Thanks are also due to Dr J S Shankar for technical help in the initial part of this work.

References

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

Coudriet D L, Prabhaker N and Meyerdirk D E 1985 Sweet potato whitefly (Homoptera: Aleyrodidae). Effects of neem seed extract on oviposition and immature stages; *Environ. Entomol.* **14** 776–779

Govindachari T R, Sandhya G and Ganeshraj S P 1990 Simple method for isolation of azadirachtin by preparative high-performance liquid chromatography; *J. Chromatogr.* **513** 389–391

Griffiths D C, Pickett J A, Smart L E and Woodcock C M 1989 Use of insect antifeedants against aphid vectors of plant virus disease; *Pestic. Sci.* **27** 269–276

Hunter W B and Ullman D E 1992 Effect of the neem product RD-9 repellin, on settling behaviour and transmission of zucchini yellow mosaic virus by the aphid *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae); *Ann. Appl. Biol.* **120** 9–15

Koul O 1992 Neem allelochemicals and insect control; in *Allelopathy: basic and applied aspects* (eds) S J H Rizvi and V Rizvi (London: Chapman and Hall) pp 389–413

Koul O 1996a Neem research and development: present and future scenario; in *Supplement to cultivation and utilization of medicinal plants* (eds) S S Handa and M K Koul (New Delhi: PID CSIR) pp 583–611

Koul O 1996b Mode of azadirachtin action; in *Neem* (eds) N S Randhawa and B S Parmar (New Delhi: New Age International) pp 160–170

Koul O 1998 Effect of neem extracts and azadirachtin on fertility and fecundity of cabbage aphid *Brevicoryne brassicae* (L.); *Pestic. Res. J.* **10** 258–261

Koul O and Isman M B 1991 Effect of azadirachtin on the dietary utilization and development of the variegated cutworm *Peridroma saucia*; *J. Insect Physiol.* **37** 591–598

Koul O, Amanai K and Ohtaki T 1987 Effect of azadirachtin on the endocrine events of *Bombyx mori*; *J. Insect Physiol.* **33** 103–108

Koul O, Isman M B and Ketkar C M 1990 Properties and uses of neem, *Azadirachta indica* A Juss; *Can. J. Bot.* **68** 1–11

Koul O, Isman M B and Arnason J T 1994 Toxicokinetics of [22,23-$^3H_2$] dihydroazadirachtin in the variegated cutworm, *Peridroma saucia*; *Arch. Insect Biochem. Physiol.* **25** 95–106

Koul O, Shankar J S and Mehta N 1997 Antifeedant activity of neem seed extracts and azadirachtin to cabbage aphid *Brevicoryne brassicae* (L.); *Indian J. Exp. Biol.* **35** 994–997

Lowery D T and Sears M K 1986 Effect of exposure to the insecticide azinophosmethyl on reproduction of green peach aphid (Homoptera: Aphididae); *J. Econ. Entomol.* **79** 1534–1538

Lowery D T and Isman M B 1993 Antifeedant activity of extracts from neem *Azadirachta indica* to strawberry aphid *Chaetosiphon fragaefolii*; *J. Chem. Ecol.* **19** 1761–1773

Lowery D T and Isman M B 1994a Insect growth regulating effects of neem extracts and azadirachtin on aphids; *Entomol. Exp. Appl.* **72** 77–84

Lowery D T and Isman M B 1994b Effect of neem and azadirachtin on aphids and their natural enemies; in *Bioregulation of crop protection and pest control* (ed.) P A Hedin, (Washington DC: American Chemical Society) ACS Symp. Ser. 557, pp 78–91

Lowery D T and Isman M B 1995 Toxicity of neem to natural enemies of aphids; *Phytoparasitica* **23** 297–306

Lowery D T and Isman M B 1996 Inhibition of aphid (Homoptera: Aphididae) reproduction by neem seed oil and azadirachtin; *J. Econ. Entomol.* **89** 602–607

Lowery D T, Isman M B and Brard N L 1993 Laboratory and field evaluation of neem for the control of aphids; *J. Econ. Entomol.* **86** 864–870

Mordue (Luntz) A J M and Blackwell A 1993 Azadirachtin: an update; *J. Insect Physiol.* **39** 903–924

Neter J, Wasserman W and Kutner M H 1985 *Applied linear statistical models. Regression analysis of variance and experimental designs*, 2nd edition (Homewood: Irwin) pp 172–174

Patel M B and Srivastava K P 1989 Evaluation of neem oil formulation against cowpea aphid (*Aphis craccivora* Koch); *Neem Newsl.* **6** 41–44

Schauer M 1984 Effects of various formulated neem seed extracts on *Acyrthosiphon pisum* and *Aphis fabae*; in *Natural pesticides from neem tree* (*Azadirachta indica* A Juss) *and other tropical plants* (eds) H Schmutterer and K R S Ascher (Rauischholzhausen: GTZ) Proc. 2nd Int. Neem Conf., pp 141–150

Schmutterer H 1990 Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*; *Annu. Rev. Entomol.* **35** 271–297

Schmutterer H and Singh R P 1995 List of insect pests susceptible to neem products; in *The neem tree, Azadirachta indica* A. Juss *and other Meliaceae plants* (ed.) H Schmutterer (New York: VCH Publisher) pp 326–365

West A J and Mordue A J 1992 The influence of azadirachtin on the feeding behaviour of cereal aphids and slugs; *Entomol. Exp. Appl.* **62** 75–79

Wilkins R M, Okello-Ekochu E, Jeevasatnam K and Wechakit D 1990 Effects of some feeding inhibitors on two homopteran insect pests *Nilaparvata lugens* and *Acyrthosiphon pisum*; in *Pesticides and alternatives: innovative chemical and biological approaches to pest control* (ed.) J E Casida (New York: Elsevier) pp 329–341

Wilkinson L 1990 *Systat: The system for statistics* (Evanston: Systat)

MS received 7 August 1998; accepted 11 December 1998

Corresponding editor: DEEPAK PENTAL