



OVICIDAL PROPERTY OF SOME PLANT EXTRACTS AGAINST *HELICOVERPA ARMIGERA* (HUBNER)

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ABSTRACT

Ovicidal property of some plant extracts was evaluated against *Helicoverpa armigera* (Hubner) under laboratory conditions. Maximum egg mortality 59.2 and 61.4 per cent was observed with extract of *neem* (*Azadirachta indica*) seed kernel in methanol at 7.5 per cent concentration and chloroform:methanol (9:1) extract at 1.0 per cent concentration, respectively. Mortality of untreated eggs (without extract) was only 28.0 and 26.4 per cent, respectively. *Melia azedarach* drupes extract at 1.0 per cent concentration extracted in chloroform: methanol (9:1) exhibited 59.6 per cent egg mortality. Extracts from leaves of *neem* and *M. azedarach* had lower mortality of eggs as compared to fruits, but *Lantana* (*Lantana camara*), *Datura* (*Datura stramonium*) and *ak* (*Calotropis procera*) leaves extract did not exhibit significant ovicidal property as compared to control. Therefore, *neem* and *M. azedarach* could be considered as promising plants their ovicidal property against *H. armigera*.

Key words: *Azadirachta indica*, *Helicoverpa*, *Melia azedarach*, *neem*, ovicidal property

INTRODUCTION

In a bid to promote eco-friendly technologies in agriculture, biopesticides play a prominent role in sustainable crop production (Koul *et al.*, 2009). The plant kingdom affords a rich storehouse of compounds of diverse biological effects on insects. In recent years, several plants have been identified which can be used as safe renewable sources of insecticides (Jaglan *et al.*, 1997). Unlike organic chemicals, plant products comprise a large variety of molecules which reduces the chances of pest developing resistance (Saxena, 1983).

The information regarding ovicidal properties of plant based products against *H. armigera* and other species is scanty. In view of seriousness and polyphagous nature of *H. armigera*, it was considered worthwhile to study the ovicidal activity of some plant extracts under laboratory conditions.

MATERIALS AND METHODS

Collection and processing of plant materials: A selection of plants known for insecticidal properties was made from among those grown at the campus of CCS Haryana Agricultural University, Hisar (Table 1). The plant material was initially dried in the shade for a week and subse-

quently dried in an oven at 40°C for 24 h. The kernels and leaves of *Azadirachta indica*, *Melia azedarach*, *Lantana camara*, *Datura stramonium* and *Calotropis procera* were broken into small pieces manually. The drupes of *M. azedarach* were crushed to a semi-power with the help of a burr mill grinder. The plant materials thus obtained were subjected to extraction in different solvents.

Extraction of different plant parts in different solvents:

The plant materials were extracted in methanol (Meisner *et al.*, 1983) and in chloroform : methanol (9:1) and subsequently fractioned on other solvents (Singh, 1987). The plant material (500 g) was soaked separately in 1L solvent for 48h at room temperature in a stoppered round bottom flask and shaken every three hours for 5 minutes. The extracts were filtered and filtrate was evaporated under vacuum on a water bath (30–35°C) to get the crude extracts for further biological studies. For the extraction of chloroform : methanol (9:1) extract and its fractions, only four plant parts *viz.*, *A. indica* (neem) seed kernels and leaves and *M. azedarach* (*bakain*) drupes and leaves were taken for bio-efficacy as these showed good bio-efficacy in methanol extracts. For fractionation of chloroform : methanol (9:1) extract in different solvents, an amount of 200g of the crude extract was taken into 200 ml hexane and refluxed for one hour on water bath. After decantation the

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Table 1. Plants/plant part extracted and evaluated for ovicidal property against *Helicoverpa armigera*

Treatment part(s) used	Botanical name	Common name	Family	Plant
T ₁	<i>Azadirachta indica</i> A. Juss	<i>Neem</i>	Meliaceae	Kernels
T ₂	<i>A. indica</i> A. Juss	<i>Neem</i>	Meliaceae	Leaves
T ₃	<i>Melia azedarach</i> L.	<i>Bakain</i>	Meliaceae	Drupes
T ₄	<i>M. azedarach</i> L.	<i>Bakain</i>	Meliaceae	Leaves
T ₅	<i>Lantana camara</i> L.	<i>Lantana</i>	Verbenaceae	Leaves
T ₆	<i>Datura stramonium</i> L.	<i>Datura</i>	Solanaceae	Leaves
T ₇	<i>Calotropis procera</i> R. Br.	<i>Ak</i>	Asclepiadaceae	Leaves

process was repeated twice. The hexane was evaporated. The crude extract left after hexane fractionation was further fractionated with 3 × 200ml each successively with chloroform and ethylacetate by refluxing on water bath to get chloroform and ethylacetate fractions, respectively. The different extracts thus obtained were diluted in acetone (20% w/v) to prepare stock solution for further biological studies.

Biological Testing

Stock culturing of the test insect: The test insect (*H. armigera*) was reared in the laboratory on pigeonpea at 28 ± 2°C and 65–75 per cent relative humidity in an incubator under sterilized conditions. The laboratory culture of insect was initially started by collecting final stadia larvae from pigeonpea fields to obtain adults for subsequent egg laying.

Ovicidal property of different plant parts against *H. armigera*: The experiment on ovicidal property was carried out in an incubator at 28 ± 2°C and relative humidity 67–75%. The female moths were allowed to oviposit on muslin cloth and cotton swabs. The eggs were pasted on egg cards with gum. The cards containing eggs (0–24 h age) were treated with methanol extracts at different concentrations (0.5, 2.5, 5.0 and 7.5% and chloroform : methanol (9:1) extracts and their fractions at concentrations, *i.e.* 0.1, 0.5 and 1.0% using Teepol (0.2%) as emulsifier and water as diluent (Rup and Chopra, 1987). The different concentrations of methanol and chloroform: methanol (9:1) extracts and its fractions were standardized before conducting the final experiments. Emulsified water was taken as control. After treatment, air dried egg cards were put in Petricishes, which were provided with cotton swabs soaked in distilled water to provide moisture. The per cent egg mortality was calculated for determining the ovicidal property. Each treatment containing 25 eggs was replicated five times.

Statistical analysis: The data were subjected to analysis of variance (ANOVA) after necessary transformations in a

factorial completely randomized design to determine critical difference (CD) among treatments (Panse and Sukhatme, 1985).

RESULTS AND DISCUSSION

The data (Table 2) indicate that egg mortality of *H. armigera* ranged from 28.0 to 59.2 per cent in different treatments as compared to 28.0 per cent in untreated control (without extract). The extracts from *neem* seed kernels (T₁) and *M. azedarach* drupes (T₃) exhibited significantly highest average mortality of eggs, 43.0 and 42.1 per cent, respectively. The mortality of eggs progressively increased with the increase in concentration of different plant extracts. Extracts (T₁ to T₄) showed a dose dependent increase in egg mortality. The average highest egg mortality (39.5%) was observed at 7.5 per cent concentration and lowest (30.0%) at 0.5 per cent concentration.

The interactions among different treatments and concentrations (Table 2) showed that the highest mortality (59.2%) was observed in T₁ at 7.5 per cent concentration, which was at par with T₃ at the same level. Treatments T₁ to T₇ at 0.5%, T₃ to T₇ at 2.5, 5.0 and 7.5% concentrations showed parity with control statistically.

Effect of chloroform: methanol (9:1) extracts and its fractions on the egg mortality: The data (Table 3) indicate that mortality of *H. armigera* eggs, when treated with various plant parts extracted in chloroform : methanol (9:1) and its fractions, at different concentrations ranged from 34.0 to 61.4 per cent as compared to 26.4 per cent in control (untreated). Maximum egg mortality (average 59.9%) was observed in *neem* seed kernel extract (T₁) at 1.0% concentration, which was statistically at par with T₃ (*M. azedarach* drupes extract) at the same level (average 59.6%). A progressive increase in egg mortality with the increase in concentration of extracts was observed. All the treatments (T₁ to T₄) at all concentrations (0.1, 0.5, 1.0%) were superior to control. Further, the data (Table 3) indicate that all the extracts were equally effective.

Maximum mortality of eggs (average 45.0%) was observed in chloroform : methanol (9:1) extract and minimum in ethylacetate fraction (43.2%).

The different plants parts in different extracts interacted significantly and indicate that highest egg mortality (61.4%) of *H. armigera* was observed in T₁ (*neem* seed kernel extract) at 1.0% concentration in chloroform : methanol (9:1) extract, which showed parity with hexane, chloroform and ethylacetate fractions in the same treatment at the same concentration and T₃ in all extracts at 1.0%

concentration. All the treatments in different extracts at various concentrations were superior to control (Table 3).

The differences in the efficacy of methanol and chloroform : methanol (9:1) extracts were significant and could be attributed to the phenomenon of polarity. Chloroform : methanol (9:1) extracts were more effective than methanol extracts. Methanol being highly polar (less polar than water), would have also extracted much inactive polar substances such as sugars, tannins and others, thus, diluting the concentration of extract (methanol extract). In

Table 2. Efficacy of various plant extracts (methanol extract) on mortality of *Helicoverpa armigera* eggs

Treatments*		Egg mortality at different concentrations (%)				Average
		0.5	2.5	5.0	7.5	
<i>A. indica</i>	Kernels	32.0 (34.44)	37.6 (37.82)	43.2 (41.09)	59.2 (50.30)	43.0 (40.91)
<i>A. indica</i>	Leaves	31.6 (34.19)	36.8 (37.35)	40.0 (39.23)	41.4 (40.05)	37.4 (37.70)
<i>M. azedarach</i>	Drupes	32.4 (34.61)	36.8 (37.35)	42.4 (40.63)	56.8 (48.91)	42.1 (40.37)
<i>M. azedarach</i>	Leaves	31.2 (33.95)	35.6 (36.63)	37.2 (37.57)	39.6 (39.00)	35.9 (36.79)
<i>L. camara</i>	Leaves	28.2 (32.08)	28.0 (31.94)	29.6 (32.95)	31.2 (33.95)	29.2 (32.73)
<i>D. stramonium</i>	Leaves	28.4 (32.19)	28.8 (32.45)	29.6 (32.95)	30.4 (33.45)	29.3 (32.76)
<i>C. procera</i>	Leaves	28.0 (31.94)	28.0 (31.94)	29.6 (32.95)	29.6 (32.95)	28.8 (32.44)
Control (untreated)		28.0 (31.94)	28.0 (31.94)	28.0 (31.94)	28.0 (31.94)	28.0 (31.94)
Average		30.0 (33.52)	32.4 (34.68)	34.9 (36.16)	39.5 (38.82)	

CD (P = 0.05) : Treatments = (1.34); Concentrations = (1.14); Interaction = (2.68); Figures in parentheses are angular transformed valued; * Treatment details described in Table 1.

Table 3. Efficacy of various plant extracts (chloroform: methanol extract and its fractions) on the mortality of *Helicoverpa armigera* eggs

Treatment*	Concen- trations (%)	Egg mortality (%) in different extracts				Average
		Chloroform : methanol (9:1)	Hexane fraction	Chloroform fraction	Ethylacetate fraction	
T ₁	0.1	43.6 (41.32)	42.4 (40.63)	41.2 (39.93)	39.8 (39.11)	41.8 (40.25)
	0.5	52.0 (46.15)	49.6 (44.77)	54.4 (47.52)	47.2 (43.39)	50.9 (45.46)
	1.0	61.4 (51.60)	59.2 (50.30)	60.6 (51.13)	58.4 (49.84)	59.9 (50.72)
T ₂	0.1	37.2 (37.58)	36.6 (37.23)	34.0 (35.66)	34.2 (35.78)	35.5 (36.56)
	0.5	42.0 (40.40)	41.2 (39.93)	42.0 (40.40)	40.8 (39.70)	41.5 (40.11)
	1.0	49.8 (44.89)	48.8 (44.31)	49.2 (44.54)	48.4 (44.08)	49.2 (44.45)
T ₃	0.1	40.4 (39.47)	39.8 (39.11)	40.4 (39.47)	39.0 (38.65)	39.9 (39.17)
	0.5	49.6 (44.77)	47.2 (43.39)	47.2 (43.39)	48.8 (44.31)	48.2 (43.96)
	1.0	59.6 (50.54)	58.4 (49.84)	60.4 (51.01)	59.8 (50.66)	59.6 (50.51)
T ₄	0.1	36.4 (37.11)	35.8 (36.75)	36.6 (37.23)	34.0 (35.66)	35.7 (36.69)
	0.5	39.8 (39.11)	38.6 (38.41)	39.4 (38.88)	39.0 (38.65)	39.2 (38.76)
	1.0	47.2 (43.39)	46.0 (42.71)	46.4 (43.05)	46.0 (42.71)	46.5 (42.96)
Control (untreated)	–	26.4 (30.91)	26.4 (30.91)	26.4 (30.91)	26.4 (30.91)	26.4 (30.91)
Average		45.0 (42.09)	43.8 (41.41)	44.5 (41.78)	43.2 (41.03)	

CD (P = 0.05) : Treatments = (1.70); Extracts = (N.S); Interaction = (3.40); Figures in parentheses are angular transformed valued; * Treatment details described in Table 1.

chloroform : methanol (9:1) extract, the chloroform's lower polarity precluded the extract of the inactive substances, thus, increasing their effectiveness at lower concentrations. Feuerhake and Schmutterer (1982) were also of a similar view, as they expressed that by the selection of a particular solvent, the growth disrupting properties of extracts increased to 50 folds as compared to methanolic extract.

The results of the present investigations also showed that *neem* seed kernel extract had the maximum ovicidal property amongst all the plants parts, which was followed by *M. azedarach* drupes, *neem* leaves and *M. azedarach* leaves (Table 2–3). This could presumably be attributed to the presence of azadirachtin / meliacarpin and salanin the well known active compounds in *neem* and *M. azedarach* (Singh *et al.*, 1988). The slight lower toxicity of *M. azedarach* compared to *neem* may be attributed to azadirachtin content variations. Singh (1987) reported that leaves had less azadirachtin than kernels that could explain the lower toxicity of leaves than fruits. Extracts from *Lantana*, *Datura* and *calotropis* did not have significant ovicidal property due to absence of limonoids like azadirachtin/ meliacarpin or salanin that are present in the Meliaceae family (*neem* and *M. azedarach*) only.

Although information on the ovicidal property of plant products against *H. armigera* and other species is scanty, Saxena (1987) observed that the hatchability of *Mythimna separata* and *Spodoptera mauritia* was reduced when rice leaves containing eggs were dipped in *neem* oil (12.0%). Similarly, Saxena *et al.* (1981) also observed reduced hatching of *Cnaphalocrocis medinalis* eggs on rice plants sprayed with *neem* oil. Loke *et al.* (1992) recorded that *neem* oil (at more than 1.25% concentration) significantly reduced egg hatching of *Plutella xylostella* and at 2.0 per cent all eggs failed to hatch. Gajmer *et al.* (2002) also observed significant mortality of *Earias vitella* eggs when treated with methanolic extracts of *neem* and *Melia azedarach* seeds. The variations in egg mortality with respect to the reported results could be due to different solvents being used thereby altering the extraction of active compounds.

REFERENCES

- Feuerhake, K. and Schmutterer, H. 1982. Simple processes for the extraction and formulation of the *neem* seed extracts and their effect on various insect pests. *Zeitschrift für Pflanzkrankheiten und Pflanzenschutz* **89**: 737–747.
- Gajmer, T., Singh, R., Saini, R.K. and Kalidhar, S.B. 2002. Effect of methanolic extracts of *neem* (*Azadirachta indica* A. Juss) and *bakain* (*Melia azedarach* L.) seeds on oviposition and egg hatching of *Earias vitella* (Fab.). (Lepidoptera : Noituidae). *Journal of Applied Entomology*, **126**: 238–243.
- Jaglan, Maha Singh, Khokhar, K.S., Malik M.S. and Singh, R. 1997. Evaluation of *neem* (*Azadirachta indica* A. Juss) extracts against American bollworm, *Helicoverpa armigera* (Hubner). *Journal of Agriculture & Food Chemistry*, **45**: 3262–3268.
- Koul, O., Dhaliwal, G.S. and Kaul, V.K. 2009. *Sustainable Crop Protection : Biopesticide Strategies*. Kalyani Publishers, New Delhi.
- Loke, J.H., Heng, C.K., Rejab, A., Basirun, N., Mardi, H.C.A., Ooi, P.A.C., Lim, G.S. and Teng, P.S. 1992. Studies on *neem* (*Azadirachta indica* A. Juss.) in Malaysia. Proceedings of 3rd International Conference on Plant Protection in the Tropics, Genting High lands, Malaysia, 20–23 March, 1990, **2**: 103–107.
- Meisner, J., Ascher, K.R.S. and Zur, M. 1983. The residual effect of a *neem* seed kernel extract sprayed on fodder beet against larvae of *Spodoptera littoralis* *Phytoparasitica* **11**: 51–54.
- Panse, V.G. and Sukhtame, P.V. 1985. *Statistical Methods for Agricultural Workers*. 2nd Edn. ICAR, New Delhi.
- Rup, P.J. and Chopra, P.K. 1987. Ovicidal activity of diflubenzuron on *Callosobruchus maculatus* (Fab.) *Indian Journal of Agricultural Sciences*. **57**: 378–379.
- Saxena, R.C. 1987. *Neem* seed derivatives for the management of rice pests – a review of recent studies. Proceedings of 3rd International *Neem* Conference, Nairobi 1986, pp. 81–93.
- Saxena, R.C., Waldbauer, G.P., Liquido, N.J. and Puma, B.C. 1981. Effect of *neem* seed oil on the rice leaf folder, *Cnaphalocrocis medinalis*. Proceeding of 1st International *Neem* Conference, Rottach–Egern, 1981, pp. 189–204.
- Sexena, R.C. 1983. *Naturally occurring pesticides and their potential*. In: Chemistry and A World Food Supplies. The New Frontiers. Schemidt, L.W., (Eds.), Pergamon, New York, pp: 143–161.
- Singh, R.P. 1987. Comparison of antifeedant efficacy and extract yield from different parts and ecotypes of *neem* (*Azadirachta indica* A. Juss.). In: *Natural Pesticides from Neem Tree and other Tropical Plants*, N.S. Randhawa and B.S. Parmar, (Eds.), 1987, pp. 185–194, PSI, New Delhi.
- Singh, R.P., Devkumar, 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.