
Ovicidal activity of *Atalantia monophylla* (L) Correa against *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae)

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Hexane, chloroform and ethyl acetate crude extracts of *Atalantia monophylla* leaf were studied for ovicidal activity against *Helicoverpa armigera* at 0.5, 1.0, 2.5 and 5.0% concentrations. The ovicidal activity of 67.10% was statistically significant noticed in hexane extract at 5.0% concentration. The least LC₅₀ value of 2.60% was observed in hexane extract with significant Chi-square value at $P < 0.05$ level. The chloroform and ethyl acetate extracts manifested ovicidal activity of 47.49 and 43.36%, respectively at 5.0% concentration. Active crude hexane extract was fractionated using silica gel column chromatography. Twelve fractions were collected and evaluated for their ovicidal activity at 125, 250, 500 and 1000 ppm concentrations. Among them, fraction 9 showed maximum ovicidal activity of 72.21% at 1000 ppm concentration with least LC₅₀ value of 435.92 ppm.

Key words: *Atalantia monophylla*, *Helicoverpa armigera*, Ovicidal activity, Fractions

Introduction

Insect control has been a challenge to human race since the beginning of organized agriculture. With the advent of pesticides especially the chlorinated hydrocarbons, it was thought that the pest problem was solved but soon it was realized that the insects developed resistance to these chemicals and consequently the ecosystem got polluted by the insecticides (Saxena, 1994). Constant use of synthetic pesticides caused severe environmental problems as well as development of resistance in insect pests. More than 650 species of insects and mites have developed resistance to one insecticide or another (Jayaraj, 2005). Chemical pesticides like methomyl, triazophos, monocrotophos and quinalphos are used against *H. armigera* as ovicides (Ahmad *et al.* 1990).

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Antifeedants and insecticidal toxicants can play a significant role as part of integrated pest management (Isman, 2002). Usually antifeedant, insecticidal, ovicidal, oviposition deterrent and growth inhibitory activities are ascertained by screening and analyzing the bioefficacy of plant extracts or compounds. In the agro field condition the eggs of pests are mostly laid on the crop's surfaces. By foliar application of pesticide the eggs or egg masses are also targeted. Plant extracts interfere with the normal embryonic development of the eggs by suppressing hormonal and biochemical process (Fagoonee and Lauge, 1981).

Further Enslee and Riddiford (1997) suggested that the failure of egg to hatch could be attributed to incomplete blastokinesis and abnormal breakage of extra embryonic membranes in the embryo. Schmutterer (1990) pointed out that egg hatchability was reduced by neem substances. Several hundred plants have been reported as insect repellents, antifeedants, attractants, insecticides, ovicides and oviposition deterrents (Ewete *et al.* 1996). More than 400 insect species, including many key pests of agriculture, are susceptible due to various behavioral and physiological effects (repellent, antifeedant, oviposition deterrence, insect growth regulating, ovicidal and sterilant) of neem tree (Schmutterer and Singh, 1995). Plant products control the pests at various stages, which kill or extend the life stages (larvae, pupae, eggs) of *Spodoptera litura*, *Helicoverpa armigera* and protect the crops (Raja *et al.*, 2004; 2005; Ignacimuthu *et al.*, 2006; Baskar *et al.*, 2010, 2011ab, 2012). Ovicides or oval mortalities by botanicals are useful in plant protection. Previously some authors have reported the use of *Atalantia monophylla* is used to control *Spodoptera litura*, *Helicoverpa armigera*, *Earias vittella* (Baskar *et al.*, 2008; Baskar *et al.*, 2009; Muthu *et al.* 2010), *Culex quinquefasciatus*, *Anopheles stephensi*, and *Aedes aegypti* (Sivagnaname and Kalyanasundaram, 2004). But there has been no report on its ovicidal activity against *H. armigera*. Hence the present work was undertaken to assess the ovicidal activity of *A. monophylla* against *H. armigera*.

Materials and methods

Plant material

Leaves of *Atalantia monophylla* were collected from Kancheepuram district of Tamil Nadu, India. The plant was authenticated by a plant taxonomist from the Department of Plant Biology and Biotechnology, Loyola College, Chennai. A voucher specimen [ERIH - 1309], has been deposited at the herbarium of Entomology Research. Institute, Loyola College, Chennai.

Extraction and isolation

Plant material was sequentially extracted with increasing polarity of solvents such as hexane, chloroform and ethyl acetate. Hexane extract showed promising activity and was fractionated using silica gel column chromatography (Baskar *et al.* 2009).

Rearing of *Helicoverpa armigera*

Larvae of *H. armigera* were collected from the field in Salamangalam, Kancheepuram district, Tamil Nadu. The collected larvae were reared individually in a plastic container (vials) and fed regularly with bhendi, *Abelmoschus esculentus* L. (Malvaceae) till the larvae became pupae under the laboratory conditions ($27\pm 2^{\circ}\text{C}$ and $75\pm 5\%$ relative humidity). Sterilized soil was provided for pupation. Pupae were collected from soil after pupation and placed inside the cage for emergence. Cotton soaked with 10% honey solution mixed with a few drops of multivitamins was provided for adult feeding to increase the fecundity. Potted cowpea plant was kept inside the adult emergence cage for egg laying. After hatching the larvae were collected from the cage and fed with standard artificial diet as recommended by Koul *et al.* (1997).

Ovicidal activity

The ovicidal activities of the crude and fractions were studied by spraying the prepared concentration of solution on freshly laid eggs of *H. armigera* from the insectary. The sprayed concentrations were 0.5, 1.0, 2.5 and 5.0% of crude extracts and 125, 250, 500 and 1000 ppm concentrations of fractions. Azadirachtin was used as positive control (40.86%). Number of eggs hatched in control and treatments were recorded. Five replicates were maintained for each treatment with 20 eggs per replicate (total $n = 100$). The experiment was conducted at laboratory condition in room temperature at $27 \pm 2^{\circ}\text{C}$ with 14:10 light and dark photoperiod and $75 \pm 5\%$ relative humidity. Percent mortality was calculated according to Abbott (1925).

Statistical analysis

The ovicidal activity was subjected to analysis of variance (ANOVA). Significant differences between treatments were determined by Tukey's

multiple range test ($P \leq 0.05$). LC_{50} and LC_{90} values were calculated using Probit Analysis (Finney, 1971).

Results

The present investigation revealed that different solvent crude extracts of *A. monophylla* leaf exhibited ovicidal activity at all the tested concentrations against *H. armigera*. Statistically significant ovicidal activity was noticed in hexane extract (67.10%) at 5.0% concentration (Table 1). Hexane extract exhibited least LC_{50} value of 2.60% followed by chloroform and ethyl acetate extract at 5.0% concentration (Table 3). Ovicidal activity of chloroform extract was superior to that of ethyl acetate extract. The active crude extract of hexane was fractionated; the fractions were evaluated for their ovicidal activity against *H. armigera*. All the fractions exhibited ovicidal activity at 1000 ppm concentration. Among them, fraction 9 exhibited maximum ovicidal activity of 72.21% followed by fractions 6 and 5 which showed 55.78% and 51.78% ovicidal activity respectively at 1000 ppm concentration against *H. armigera*. Fractions 2,3,4,7,8,10 and 12 did not show any ovicidal activity at 125 ppm concentration (Tables 2). The Chi-square values were significant at $P < 0.05$ level. The high Chi-square values in the bioassays probably indicated the heterogeneity of the test population (Table 3-4).

Table 1. Ovicidal activity of *Atalantia monophylla* leaf crude extract against *H. armigera*

Fraction	Concentration (%)			
	0.5	1.0	2.5	5.0
Hexane	27.89 ± 6.26 ^b	37.10 ± 1.80 ^b	52.63 ± 3.22 ^b	67.10 ± 5.04 ^b
Chloroform	17.57 ± 3.18 ^a	25.78 ± 5.18 ^a	37.10 ± 3.29 ^a	47.49 ± 4.85 ^a
Ethyl acetate	15.47 ± 3.74 ^a	23.63 ± 3.94 ^a	34.05 ± 3.12 ^a	43.36 ± 6.46 ^a

Within column, means ± SD followed by the same letter do not differ significantly using Tukey's test, $P \leq 0.05$.

Table 2. Ovicidal activity (%) of *Atalantia monophylla* leaf hexane extract fractions against *H. armigera*

Fractions	Concentration (ppm)			
	125	250	500	1000
1	14.42 ± 7.40 ^b	21.63 ± 1.93 ^d	27.89 ± 3.40 ^c	34.00 ± 4.39 ^d
2	00 ± 00 ^a	00 ± 00 ^a	4.10 ± 2.29 ^a	7.15 ± 2.59 ^a
3	00 ± 00 ^a	7.15 ± 2.59 ^{bc}	11.26 ± 6.62 ^{bcd}	18.52 ± 2.53 ^{bc}
4	00 ± 00 ^a	00 ± 00 ^a	7.26 ± 2.98 ^{abc}	11.36 ± 2.48 ^{ab}
5	22.73 ± 3.29 ^{bc}	35.05 ± 2.14 ^c	44.31 ± 2.23 ^f	51.57 ± 6.60 ^e

6	25.78 ± 6.48 ^c	36.05 ± 3.08 ^c	46.42 ± 3.94 ^f	55.78 ± 6.71 ^c
7	00 ± 00 ^a	00 ± 00 ^a	6.15 ± 2.15 ^{ab}	11.26 ± 4.03 ^{ab}
8	00 ± 00 ^a	00 ± 00 ^a	8.21 ± 2.69 ^{abcd}	13.42 ± 2.90 ^{ab}
9	35.05 ± 7.14 ^d	43.21 ± 4.80 ^f	59.78 ± 2.15 ^g	72.21 ± 7.35 ^f
10	00 ± 00 ^a	4.10 ± 2.29 ^{ab}	9.26 ± 2.25 ^{abcd}	12.31 ± 2.45 ^{ab}
11	3.05 ± 2.78 ^a	10.26 ± 3.45 ^c	14.42 ± 2.21 ^d	26.73 ± 3.67 ^{cd}
12	00 ± 00 ^a	8.26 ± 2.86 ^{bc}	13.36 ± 2.61 ^{cd}	19.57 ± 2.18 ^{bc}
Azadirachtin	49.36 ± 5.57 ^d	59.73 ± 3.08 ^g	68.10 ± 3.55 ^h	80.26 ± 7.16 ^f

Within column, means ± SD followed by the same letter do not differ significantly using Tukey's test, $P \leq 0.05$.

Table 3. Effective ovicidal concentration (LC₅₀–LC₉₀ and Chi-square values) of crude extracts of *A. monophylla* leaf against *H. armigera*

Crude extracts	LC ₅₀	95% fiducial limit		LC ₉₀	95% fiducial limit		Chi-square
		Upper	Lower		Upper	Lower	
Hexane	2.60	2.24	2.98	8.46	7.35	10.11	29.21*
Chloroform	5.04	4.35	6.12	12.40	10.28	15.98	30.26*
Ethyl acetate	5.62	4.81	6.94	13.05	10.73	17.10	31.60*

* χ^2 values are significant at $P < 0.05$ levels

Table 4. Effective ovicidal concentration (LC₅₀–LC₉₀ and Chi-square values) of fractions of *A. monophylla* leaf hexane extracts against *H. armigera*

Fractions	LC ₅₀	95% fiducial limit		LC ₉₀	95% fiducial limit		Chi-square
		Upper	Lower		Upper	Lower	
1	1569.50	1254.31	2249.63	3566.53	2712.14	5470.15	30.09*
2	2009.55	1620.67	2923.51	2938.92	2285.07	4501.47	35.64*
3	1732.52	1384.07	2535.03	2869.68	2201.33	4451.20	62.60*
4	1763.84	1448.43	2461.42	2641.42	2087.44	3897.12	51.70*
5	857.57	733.51	1053.40	2459.57	1999.56	3300.30	31.21*
6	752.34	645.74	907.00	2302.96	1890.18	3037.47	30.90*
7	1741.78	1460.52	2310.79	2578.96	2087.10	3597.16	42.05*
8	1660.37	1389.39	2215.84	2494.37	2011.09	3513.09	52.63*
9	435.92	360.33	509.49	1497.80	1296.72	1807.87	37.56*
10	2045.93	1621.99	3011.15	3298.54	2524.91	5088.18	39.43*
11	15.22	1314.82	1862.70	2672.52	2239.39	3396.97	30.11*
12	1707.83	1389.79	2372.27	2882.66	2258.98	4218.68	50.37*
Azadirachtin	163.15	19.82	262.32	1316.01	1109.39	1668.47	45.12*

* χ^2 values are significant at $P < 0.05$ levels

Discussion

In this investigation, hexane extract of *A. monophylla* exhibited maximum ovicidal activity. These findings coincide with the findings of Malarvanan *et*

al. (2009) who reported that *Cipadessa baccifera*, *Melia dubia*, *Clausena dentata* and *Dodonaea angustifolia* of petroleum ether, hexane, chloroform, acetone and water extracts exhibited ovicidal activity against *H. armigera* and maximum activity was observed in hexane extract of *Clausena dentate*.

A. monophylla derived extracts exhibited concentration dependent activity. Similarly, Mehta *et al.* (1984) reported that neem seed kernel extract, margoside and repelin exhibited ovicidal activity of 13.33, 43.33 and 56.67% at 5, 0.1 and 1% concentrations, respectively against *H. armigera*. Neem oil exhibited ovicidal activity against *S. litura* and *Pericalia ricini* (Venkateswarlu *et al.* 1988). Verkerk and Wright (1993) tested azadirachtin for ovicidal activity and observed 48% activity against *Plutella xylostella*. Different neem products exhibited ovicidal activity against *Leucinodes orbonalis* (Srinivasan and Sundarababu, 1999). Nair and Thomas (2000) reported that methanol extract from *Acorus calamus* exhibited ovicidal activity of 90 and 96.67% at 0.06 and 0.08% concentrations against *Bactrocera cucurbitae*.

Different solvent extracts of *A. monophylla* leaf showed ovicidal activity against *H. armigera*. The present work is similar to the findings of Elumalai *et al.* (2003) who reported that hexane, diethyl ether, dichloromethane, ethyl acetate, methanol and water extracts of *Melochia chorcorifolia* and *Hyptis suaveolens* exhibited ovicidal activity against *H. armigera*.

Fractions derived from hexane extract of *A. monophylla* showed ovicidal activity against *H. armigera*. High ovicidal activity of 72.21% was noticed in 9th fraction at 1000 ppm concentration against *H. armigera*. The results of the present work coincided with the findings of Pavunraj *et al.* (2006) who observed that fractions from hexane extract of *Excoecaria agallocha* had ovicidal activity of 77.30% in 7th fraction at 1000 ppm concentration. Elumalai *et al.* (2005) reported that 3rd fraction isolated from the ethyl acetate extract of *Hyptis suaveolens* and *Melochia chorcorifolia* exhibited maximum ovicidal activity of 42.53 and 37.55%, respectively against *H. armigera*. Fractions isolated from *Hyptis suaveolens* ethyl acetate extract were evaluated for ovicidal activity against *S. litura* which reported by Raja *et al.* (2005). They observed ovicidal activity of 65.2% in fraction 2 at 1000 ppm concentration. *A. monophylla* fractions were more effective than crude extract at least concentrations. Previously many authors have reported that the activity increased in the isolated fractions or compounds than in crude extracts (Ignacimuth *et al.*, 2006; Baskar and Ignacimuthu, 2012). This plant could be recommended to apply in integrated pest management programmes.

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