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Ovicidal activity of *Atalantia monophylla* (L) Correa against *Spodoptera litura* Fab. (Lepidoptera: Noctuidae)

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PEER REVIEW

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Comments

The manuscript is well written and follows suitable methodology. Active crude extract and its fraction from the plant showed good activity. The study revealed significant results which will help the other researchers/industries to develop new pesticidal formulation.
(Details on Page)

ABSTRACT

Objective: To evaluate the efficacy of *Atalantia monophylla (A. monophylla)* leaf in different solvent crude extracts and fractions against eggs of *Spodoptera litura (S. litura)*. **Methods:** Hexane, ethyl acetate and chloroform solvent extracts of *A. monophylla* leaf and 12 fractions from hexane extract were screened at 5.0%, 2.5%, 1.0% and 0.5% for crude extracts and 1000, 500, 250 and 125 mg/kg for fractions against the eggs of *S. litura* for the ovicidal activity. LC_{50} and LC_{90} were calculated using probit analysis. **Results:** Hexane crude extract showed maximum ovicidal activity of 61.94% at 5.0% concentration with a correlation value of r^2=0.81, and least LC_{50} value of 3.06%. Hexane extract was fractionated using silica gel column chromatography and 12 fractions were obtained. Fraction 9 was active which showed maximum ovicidal activity of 75.61% at 1000 mg/kg with the LC_{50} value of 318.65 mg/kg and LC_{90} value of 1473.31 mg/kg. In linear regression analysis, significant and high correlation (r^2=0.81%) was seen between concentration and ovicidal activity of hexane crude extracts and its active fraction. **Conclusions:** As per our knowledge, this is the first report for ovicidal activity of *A. monophylla* against *S. litura*, *A. monophylla* could be used for the management of *S. litura* and other insect pests.

KEYWORDS

Atalantia monophylla, Crude extracts, Fractions, Ovicidal activity, *Spodoptera litura*

1. Introduction

Indiscriminate use of chemical pesticides to control pests has led to the development of resistance; it also pollutes the environment and causes ill effects on non target organisms. Allelochemicals are defensive substances from plants which protect them from insect pests[1]. Plant extracts are good crop protectants than synthetic pesticides owing to their low toxicity, effectiveness at low concentrations, rapid degradation and low impacts on non-target organisms[2,3]. In many parts of India, small farmers control pests using locally available plants. Natural products are used to control insect pests in various ways, *viz.*, deter feeding, produce egg, larval and pupal mortality, cause growth inhibition, produce abnormality of larvae and pupae, and increase the larval and pupal duration[4-6]. Plants extracted with organic solvents and their isolated fractions and compounds showed increased activities at low doses[7,8].

Plant extracts and their compounds have been subjected to bioassay for the past 30 years with an effort to discover a botanical insecticide to control pests. Among the plant families, Meliaceae, Rutaceae, Asteraceae, Labiateae, Piperaceae and Annonaceae are the most promising plants to control pests at various stages[9-11]. Meliaceae and Rutaceae have received much attention at least partly owing to the presence of triterpenoids called limonoids[12]. *Atalantia monophylla (A. monophylla)* from Rutaceae exhibited antifeedant, larvicidal and pupicidal activities against agricultural pests and larvicidal activity against human vector mosquito[13-16].

Field insect pests cause great harm to the crops and reduce their productivity[17]. The Asian armyworm,

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Spodoptera litura (S. litura) is a wide spread pest; it affects nearly 300 crop species. It has become serious pest of the majority of crops at the seedling stage, and also it has high resistance level of 61-to-148 fold to synthetic pesticides[18]. In India this pest caused losses of about 12 000 million rupees (300 million dollars) per year[19]; it also caused yield losses of about 69% among 180 crops[20]. *S. litura* severely damages many cultivated crops, especially ground nut, tomato, chilli, bhendi, cotton and green leaves at Thiruvallur and Kancheepuram districts of Tamil Nadu, India. A single egg mass contains 300-1 000 eggs. Freshly hatched caterpillars feed gregariously by scraping the chlorophyll; later disperse, feed voraciously at night on the foliage. If it is controlled at the egg stage, it will be a great boost to productivity. By keeping this in mind, the present study was undertaken to evaluate the bioefficacy of *A. monophylla* against eggs of *S. litura*.

2. Materials and methods

2.1. Plant material

Leaves of *A. 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], was deposited at the herbarium of Entomology Research. Institute, Loyola College, Chennai, India.

2.2. Extraction and isolation

Leaves were shade dried, powdered and 1 kg of plant powder was sequentially extracted with increasing polarity of solvents such as hexane, chloroform and ethyl acetate. Hexane extract was active and was fractionated further using silica gel column chromatography[14].

2.3. Insect culture

Egg masses of *S. litura* were collected from groundnut field at Eagattur near Thiruvallur District of Tamil Nadu. The eggs were surface sterilized with 0.02% sodium hypochlorite solution, dried and allowed to hatch. After hatching, the neonate larvae were reared on leaves of castor *Ricinus communis* till prepupal stage. Sterilized soil was provided for pupation at room temperature of (27±2) °C with 14:10 (light: dark) photoperiod and (75±5)% relative humidity in insectary. After pupation, the pupae were collected from soil and placed inside the oviposition chamber. After adult emergence, cotton soaked with 10% (w/v) sugar solution with few drops of multivitamins was provided for adult feeding to increase the fecundity. Potted groundnut plant was kept inside adult emergence cage for egg laying. After hatching the larvae were provided with tender castor leaves for feeding. The eggs laid by the laboratory reared insects were used for the present study[6].

2.4. Ovicidal activity

The ovicidal activity of the crude extract and fractions were studied by spraying on freshly laid eggs of *S. litura*. The sprayed concentrations were 0.5%, 1.0%, 2.5% and 5.0% for crude extracts and 125, 250, 500 and 1 000 mg/kg concentrations for fractions. Azadirachtin was used as positive control (purity-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 of (27±2) °C with 14:10 (light: dark) photoperiod and (75±5)% relative humidity. Percent egg mortality was calculated according to Abbott[21].

2.5. Statistical analysis

The ovicidal activity was analysed using one way ANOVA. Significant differences between treatments were determined using Tukey's multiple range HSD tests ($P \leqslant 0.05$). Analyses were performed with the original data after transformation with various approaches (the arcsin, logarithmic, and square root methods). The distribution of the fraction data did not show significant deviations from normality. Shapiro-wilk test for original crude data showed normality. Linear regression analyses were performed for all dose-response experimental data. LC_{50} and LC_{90} values were calculated using probit analysis[22].

3. Results

Table 1 shows the ovicidal activity of *A. monophylla* leaf derived hexane, chloroform and ethyl acetate crude extracts against *S. litura*. All the crude extracts showed ovicidal activity at all the concentrations, while maximum activity of 61.94% was noticed at 5% concentration of hexane extract. This was statistically significant, when compared to chloroform and ethyl acetate extracts of *A. monophylla*. Least activity was noticed in ethyl acetate extract of *A. monophylla*. Chloroform extract showed significant difference from ethyl acetate extract at 2.5% and 5.0% concentrations, while 1% concentration of ethyl acetate and chloroform extracts of *A. monophylla* showed statistically similar ovicidal activity against *S. litura*. More than 50% of ovicidal

Table 1
Ovicidal activity (%) and regression analysis of *A. monophylla* crude extracts against *S. litura*.

Crude extracts	Concentration (%)				Regression*		
	0.5	1.0	2.5	5.0	r	r^2	regression equation
Hexane	24.68±3.78^{b}	40.26±4.83^{b}	49.52±3.34^{c}	61.94±4.92^{c}	0.90	0.81	Y=27.72X+7.28
Chloroform	18.47±5.52^{ab}	28.89±3.05^{a}	42.26±4.12^{b}	52.57±4.12^{b}	0.92	0.84	Y=19.62X+7.07
Ethyl acetate	14.37±3.98^{a}	26.73±3.67^{a}	30.89±3.14^{a}	38.10±5.41^{a}	0.76	0.60	Y=19.25X+4.24

Means±SD followed by the same letter do not differ significantly using Tukey's test, $P \leq 0.05$. *Relationship between concentration and ovicidal activity of *S. litura*.

activity was noticed in hexane and chloroform extracts of *A. monophylla*. All the data of crude extracts showed normality, when applying Shapiro-wilk normality test. The relationship between concentration and ovicidal activity showed high correlation (r^2=0.81) (Table 1). The correlation was significant at 0.05% level for all the tested crude extracts.

Hexane extract of *A. monophylla* showed least LC_{50} and LC_{90} values of 3.06% and 9.87% for ovicidal activity against *S. litura* (Table 2). The LC_{50} values recorded for ethyl acetate extract and chloroform extract were 6.95% and 4.28% ovicdal activity, respectivly.

Table 2

Effective concentrations (%) of crude extract of *A. monophylla* for ovicidal activity against *S. litura*.

Crude extracts	LC_{50}	95% fiducial limit		LC_{90}	95% fiducial limit		χ^2
		Lower	Upper		Lower	Upper	
Hexane	3.06	2.61	3.62	9.87	8.29	12.49	33.79*
Chloroform	4.28	3.75	5.06	11.01	9.29	13.80	31.23*
Ethyl acetate	6.95	5.99	9.79	16.81	12.85	25.46	33.44*

*χ^2 values are significant at $P \leqslant 0.05$ level.

Twelve fractions obtained from hexane extract of *A. monophylla* by column chromatography were tested at different concentrations of 125, 250, 500 and 1 000 mg/kg for ovicidal activity against *S. litura*. Among them, 9th fraction exhibited 75.61% ovicidal activity at 1 000 mg/kg concentration (Table 3). All the concentrations of 9th fraction showed statistically significant activity. The 9th fraction exhibited the least LC_{50} and LC_{90} values of 318.65 and 1 473.31 mg/kg, respectively for ovicidal activity. *Chi*-Square value was also significant at 0.5% level (Table 4). The 6th fraction exhibited 56.57% ovicidal activity against *S. litura* at 1 000 mg/kg concentration with LC_{50} value of 741.14 mg/kg. Fractions 6 and 9 showed more than 50% ovicidal activity at 1 000 mg/kg concentration. Fractions 1, 5 and 11 showed more than 30% ovicidal activity at 1 000 mg/kg. At 125 mg/kg, fractions 3 and 12 did not show any ovicidal activity. Least ovicidal activity of 14.42% was noticed in second fraction at 1 000 mg/kg. Mean values for treated fractions were analyzed using original data with Shapiro-wilk test. Chi-Square values were significant at all the tested crude extracts and fractions. In regression analysis there was significant and high correlation of r^2=0.81 (Table 3) between concentration and ovicidal activity.

Table 3

Ovicidal activity (%) and regression analysis of *A. monophylla* fractions against *S. litura*.

Fractions	Concentration (mg/kg)				Regression*		
	125	250	500	1 000	r	r^2	regression equation
1	13.31±5.61^{bc}	16.47±4.22^{c}	24.78±4.61^{d}	34.94±5.92^{de}	0.87	0.76	Y=10.73X+0.25
2	3.05±2.78^{a}	8.21±2.69^{ab}	11.31±2.07^{ab}	14.42±4.17^{a}	0.77	0.60	Y=3.85X+0.12
3	0.00±0.00^{a}	6.21±2.41^{a}	13.47±4.45^{abc}	20.63±0.57^{abc}	0.94	0.88	Y=−0.38X+0.02
4	7.21±2.79^{ab}	10.31±5.14^{abc}	16.47±4.22^{abcd}	29.78±6.03^{bcd}	0.90	0.80	Y=3.84X+0.03
5	17.47±4.37^{c}	29.82±3.69^{d}	38.10±4.10^{e}	46.36±6.04^{ef}	0.87	0.75	Y=19.08X+0.03
6	26.78±4.17^{d}	34.94±5.92^{d}	46.42±5.29^{e}	56.57±6.79^{f}	0.88	0.78	Y=25.89X+0.03
7	5.10±3.53^{a}	8.21±2.69^{ab}	11.26±4.03^{ab}	18.47± 5.52^{ab}	0.80	0.64	Y=3.8X+0.02
8	4.10±2.29^{a}	6.21±2.41^{a}	15.47±3.74^{abc}	21.63±4.19^{abc}	0.90	0.80	Y=2.25X+0.02
9	37.15±5.90^{e}	49.42±4.98^{e}	61.89±5.41^{f}	75.61±7.88^{g}	0.90	0.81	Y=36.69X+0.04
10	4.10±2.29^{a}	8.21±2.69^{ab}	18.57±2.95^{bcd}	22.68±4.64^{abc}	0.87	0.76	Y=3.56X+0.02
11	7.21±2.79^{ab}	15.52±3.92^{bc}	21.63±4.19^{cd}	31.94± 4.15^{cd}	0.91	0.83	Y=6.79X+0.026
12	0.00±0.00^{a}	5.15±0.14^{a}	9.26±2.25^{a}	15.47±3.74^{a}	0.92	0.84	Y=−0.23X+0.02
Azadirachtin	42.26±4.12^{f}	57.73±4.12^{f}	68.10±4.15^{f}	80.42±5.57^{g}	0.91	0.82	Y=43.65X+0.36

Means±SD followed by the same letter do not differ significantly using Tukey's test, $P \leq 0.05$. *Relationship between concentration of fraction and ovicidal activity of *S. litura*.

Table 4

Effective concentrations (mg/kg) of *A. monophylla* fractions for ovicidal activity against *S. litura*.

Fractions	LC_{50}	95% fiducial limit		LC_{90}	95% fiducial limit		$\chi2$
		Lower	Upper		Lower	Upper	
1	1 443.87	1 209.57	1 865.59	3 032.95	2 440.73	4 134.28	28.87*
2	2 504.47	1 871.05	4 207.10	4 423.45	3 189.43	7 775.96	29.99*
3	1 589.70	1 338.46	2 053.79	2 622.14	2 133.64	3 549.24	45.58*
4	1 504.00	1 290.90	1 857.84	2 762.86	2 297.86	3 554.05	29.37*
5	1 030.52	872.41	1 303.92	2 606.95	2 096.57	3 572.26	34.69*
6	741.14	639.47	885.11	2 259.05	1 868.29	2 937.58	29.72*
7	2 163.12	1 697.59	3 201.85	3 859.96	2 927.01	5 969.85	29.63*
8	1 724.18	1 446.75	2 221.26	3 011.79	2 448.81	4 04041	30.23*
9	318.65	222.10	398.88	1 473.31	1 258.17	1 820.85	38.07*
10	1 702.49	1 398.70	2 295.13	3 060.37	2 424.98	4 328.40	37.36*
11	1 449.85	1 236.95	1 809.35	2 820.02	2 323.80	3 682.96	29.04*
12	1 805.74	1 473.99	2 482.25	2 990.72	2 357.59	4 308.88	42.51*
Azadirachtin	170.41	58.98	253.27	1 313.71	1 137.20	1 587.01	31.30*

*χ^2 values are significant at $P \leqslant 0.05$ level.

4. Discussion

A. monophylla from Rutaceae showed ovicidal activity against *S. litura*. Our results coincide with the earlier findings of Elumalai *et al.*[23], who noticed that *Citrus limonum* and *Citrus aurantifolia* from Rutaceae showed ovicidal activity against *S. litura*.

A. monophylla derived hexane crude extract showed ovicidal activity of 61.94% at 5.0% concentration against *S. litura*. The present findings corroborate with the findings of Pavunraj *et al.*[24], who reported that hexane, chloroform and ethyl acetate extracts of *Excoecaria agallocha* showed ovicidal activity against *S. litura*. Different organic solvent extracts from *Melochia chorcorifolia* and *Hyptis suaveolens* exhibited ovicidal activity against *H. armigera*[25]. Hexane, ethyl acetate, and methanol extracts of *Acorus calamus* leaf showed ovicidal activity against *S. litura*[26]. Similarly Raja *et al.*[27] reported that *Aegle marmelos* derived hexane, diethylether, dichloro methane, ethyl acetate and methanol extracts showed ovicidal activity against *S. litura*. Ovicidal activity of methanol extracts of 10 plants were studied by Yanar *et al.*[28] and they observed that *Eucalyptus camaldulensis* and *Xanthium strumarium* had activity of 63.26% and 59.64%, respectively against *Tetranychus urticae*. Aqueous and methanol extracts of different plants were studied and they observed notable amount of ovicidal activity against *H. armigera*[29]. Some botanical extracts exhibited ovicidal activities against disease causing mosquitoes[30].

In our study hexane leaf extract of *A. monophylla* exhibited more than 60% ovicidal activity against *S. litura*. This finding corroborates with the results of Gokce *et al.*[31], who reported that *Bifora radians* hexane extract showed more than 60% ovicidal activity against *Paralobesia viteana*. Also *Vitex negundo* leaf hexane extract exhibited 60.7% ovicidal activity against *Hyblaea puera*[32-35]. In this study, dose dependent ovicidal activity was observed for crude extracts. Our finding coincides with the earlier findings of Malarvannan *et al*[36], who observed the dose dependent ovicidal activity of *Cipadessa baccifera* against *H. armigera*.

Fractions derived from hexane extract of *A. monophylla* showed more than 75% ovicidal activity against *S. litura*. This finding coincides with the results of Baskar and Ignacimuthu[5], who reported that fractions from hexane extract of *A. monophylla* showed 72.21% ovicidal activity against *H. armigera*. Pavunraj *et al* noticed that hexane extract derived fraction showed ovicidal activity against *S. litura*. Similarly, *Hyptis suaveolens* and *Melochia chorcorifolia* derived fractions showed ovicidal activity against *H. armigera*[24,37]. Ethyl acetate extract derived fractions of *H. suaveolens* showed ovicidal activity against *H. armigera* and *S. litura*[7].

The regression analysis showed significant and high correlation (r^2=0.81) between concentration and ovicidal activity for both hexane crude extract and active fractions of *A. monophylla*. The present results coincide with the findings of Mallikarjuna *et al.*[38], who reported a value of r^2=0.82 between larval mortality and concentration of quercetin against *S. litura*. *Annona squamosa* extracts exhibited strong correlation of r^2=0.80 between growth and concentration of extracts against *Trichoplusia ni*[39].

Hexane extract and its active fractions from *A. monophylla* leaves showed significant ovicidal activity against *S. litura*. Concentration dependent ovicidal activity was recorded. This is the first report for ovicidal activity of *A. monophylla* against *S. litura*. *A. monophylla* could be used in pest management programmes.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

After the second world war, people started using more synthetic pesticides in controlling insect pests. But the repeated use of synthetic pesticides for several decades pave the way for insects to develop resistance, cause damage to the fauna and flora of the earth. Worldwide attention now focuses towards alternative methods to control the pest using plant derived substances which are non toxic, low cost, biodegradable and safer to environment. With this background, the study was carried out to find out the ovicidal properties of *A. monophylla*.

Research frontiers

This study deals with crop protection. The extracts and fractions of *A. monophylla* exhibited good ovicidal activity against *S. litura* Fabricius which is broad spectrum insect pest attacking leguminous, cruciferous, and other economically important crops. The manuscript is very systematic and presents useful findings which will help the development of bio pesticides in the field.

Related reports

The study has been conducted based on the earlier reports. They were selected based on the previous report. Example –Baskar *et al.*, 2008; Baskar *et al.*, 2009; Muthu *et al.*, 2010; Baskar and Ignacimuthu, 2012) The manuscript results and methodology have been discussed with suitable other reports.

Innovations and breakthroughs

Botanical pesticides from the plants derived secondary metabolites are important in the field of agriculture. Selected plant has significant ovicidal activity against *S. litura*. As far as I am concerned this is first report from this plant for ovicidal activity against *S. litura*.

Applications

The present study helps to develop a novel botanical pesticidal formulation to control economically important agricultural pests.

Peer review

Over all, the manuscript is well written and follows suitable methodology. Active crude extract and its fraction from the plant showed good activity. The study revealed significant results which will help the other researchers/ industries could develop new pesticidal formulation.

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