Bio Efficacy of Atalantia monophylla (L) Correa (Rutaceae) against Spodoptera litura Fabricius (Lepidoptera: Noctuidae)
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Abstract
Hexane, chloroform and ethyl acetate extracts of Atalantia monophylla were evaluated for their larvicidal and pupicidal activities against Spodoptera litura. Bioefficacy of A. monophylla leaf extracts were studied leaf disc no choice method at 0.5, 1.0, 2.5 and 5.0% concentration of crude extracts and 125, 250, 500 and 1000 ppm concentration of fractions against S. litura. The maximum larvicidal and pupicidal activities were noticed in hexane extract of A. monophylla. The hexane extract exhibited the least LC50 value of 2.01% for larval activity and regression value (R 0.98 and Coefficients 9.33 ± 17.1) for concentration dependent larvicidal activity. The hexane extract was fractionated using increasing polarity of solvent system. Twelve fractions were isolated and were evaluated at four different concentrations. Maximum antifeedant, larvicidal and pupicidal activities was noticed in fraction 9 at 1000 ppm concentration. Ninth fraction exhibited concentration antifeedant and larvicidal activities with linear regression of R 0.93, and 0.93 respectively. Least LC50 value of 340.27 ppm was observed in fraction nine for larvicidal activity. All the concentrations of 9th fraction showed good activity.

Keywords: Antifeedant; Larvicidal; Pupicidal; Solvent extracts; Fractions

Introduction
Many synthetic pesticides are used to protect the crops from pests attack around the world which lead to environmental damage and human health due residue in fruits and food [1]. Due to this reason, many research studies are being conducted to find an alternative control method for pest management. Plants produced diverse of structurally related compounds. Erythrina alkaloids from the seeds, seed pods and flowers of Erythrina latissima E. Meyer (Fabaceae) exhibited antifeedant activity against Spodoptera litura [2]. Chlormethodium inermic L. (Lamiaceae) and Lantana camara L. (Verbenaceae) extracts exhibited many activities including feeding deterrent, mortality, reduction of nymphs and adult longevity, adult emergence and fecundity against Helopeltis theivora [3]. Most of the plant compounds act as digestive enzymes inhibitor (α-amylase, protease, α- and β-glucosidases and lipase) [4]. Baskar et al. [5] stated that plant compound inhibit protein, esterase and glutathione S-transferase enzymes activities of Helicoverpa armigera and Earias vittella.

Cistus ladanifer L. (Cistaceae), Peganum harmala L. (Zygophyllaceae), Ajuga iiva L. and Rosmarinus officinalis L. (Lamiaceae) extracts reduced the larval weight, pupation and adult emergence and decreased the protein, carbohydrate, lipid of Plodia interpunctella [6]. Baskar and Ignacimuthu [7] stated that ononitol monohydrate derived from Cassia tora (L.) Roxb. (Fabaceae) exhibited antifeedant, larvicidal and growth inhibitory activities (increased larval-pupal duration and pupicidal activity) against H. armigera and S. litura. Natural compound from Pogostemon cablin (Blanco) Benth (Lamiaceae) exhibited strong antifeedant and insecticidal activities against S. litura and S. exigua [8].

Atalantia monophylla (L.) Corr. is a medicinal plant; oil from the seeds of this plant act as anti-arthritis [9] and also used for chronic rheumatism and parasitism [10]. Boiled leaves are used to cure rheumatoi disease and glandular swelling [11] and decoction from the leaves are used to cure itching and skin problems [12]. Roots are used for antispasmodic [13]. Many insecticidal properties like antifeedant, larvicidal, growth inhibitory, reduced the adult emergence against H. armigera and E. vittella [14,15] were reported.

S. litura is a major destructive pest in most cultivated crops in tropical and subtropical regions. It affects more than 90 families of cruciferous vegetables [16]. Two years host plant survey conducted by Ahmad et al. [17] on S. litura revealed that there were 27 plant species from 25 genera of 14 families are reported. Among them, Gossypium hirsutum L., (Malvaceae), Ricinus communis L., (Euphorbiaceae), Brassica oleracea var. Botrytis L., (Brassicaceae), Colocasia esculenta L., (Araceae), Trianaehma portulacastrum L. (Aizoaceae) and Sesbania sebana (Jacq.) W. Right (Fabaceae) were major host plants for S. litura. Due to its high mobility and reproduction, it affects wide variety of host plants [18]. So we called S. litura as cosmopolitan pest which means unable to manage these pests. Hence, the present study was undertaken to evaluate the antifeedant, larvicidal and pupicidal activities of A. monophylla against S. litura.

Materials and Methods
Plant material
Leaves of A. monophylla were collected from the forest areas of 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.

Crude extraction and fraction isolation
Leaves were collected and shade dried at room temperature and

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ground in a manual mill. The powder was sequentially extracted with hexane, chloroform and ethyl acetate. The powder was soaked in the respective solvents for a period of 48 h with intermittent shaken. The extract was filtered through a Buchner funnel with Whatman number 1 filter paper. The filtrate was evaporated to dryness under reduced pressure using rotary evaporator. The crude hexane extract (50 g) was subjected to column chromatography over silica gel (50 g-acme’s 100–200 mesh) and eluted with hexane followed by the combination of hexane: ethyl acetate and ethyl acetate: acetone ranging from 95:5 to 0:100 and 50:50 to 0:100, respectively. A total of 189 fractions were collected in 200 Ml conical flasks and were pooled into twelve fractions using thin layer chromatography (TLC) [14].

Insect culture

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

Antifeedant activity

Antifeedant activity of the fractions was studied using leaf disc no choice method. Fresh castor leaf discs of 4 cm diameter were punched using cork borer and were dipped in 125, 250, 500 and 1000 ppm concentrations. The leaf discs treated with acetone were used as negative control. In each plastic Petri dish (1.5 cm 6 9 cm), wet filter paper was placed to avoid early drying of the leaf discs and single third instar larva was introduced to each Petri dish. Progressive consumption of leaf by the treated and control larvae with 24 h was recorded using Leaf Area Meter (Delta-T Devices, Serial No. 15736 F 96, UK). Leaf area eaten by larvae in treatment was corrected from the negative control. Five replicates were maintained for each treatment with 10 larvae per replicate (total, n= 50). The experiment was conducted at laboratory conditions (27 ± 2°C) with 14:10 light and dark photoperiod and 75±5% relative humidity in insectary. Antifeedant activity was calculated using the following formula of Bentley et al. [19].

\[ \text{Antifeedant activity} = \frac{\text{Leaf area consumed in control} - \text{Leaf area consumed in treated}}{\text{Leaf area consumed in control}} \times 100 \]

Larvicidal activity

Larvicidal activity was studied using leaf disc no choice method. The larvae were fed on the castor leaf disc treated with different concentrations of 0.5, 1.0, 2.5 and 5.0% for crude extracts and 125, 250, 500 and 1000 ppm for fractions using leaf disc dip method. After 24 h of treatment, the larvae were continuously maintained on untreated fresh castor leaves. The diet was changed every 24 h. Larval mortality was recorded up to 96 h of treatment. Five replicates were maintained for each treatment with 10 larvae per replicate (total, n=50). Per cent mortality was calculated [20]. All the laboratory conditions were the same as in antifeedant study.

Pupicidal activity

The survived larvae were continuously fed with untreated castor leaf until they become pupae and adults. Pupal mortality was calculated by subtracting the number of emerging adults from the total number of pupae.

Statistical analysis

The antifeedal, larvicidal and pupicidal activities were subjected to one way analysis of variance (ANOVA). Significant differences between treatments were determined by DMRT F-test (p ≤ 0.05). LC50 value was calculated using Probit Analysis [21]. Linear regression analyses were performed for all dose–response experimental data.

Results

Crude extracts

Larvicidal activity: A. monophylla derived hexane, chloroform and ethyl acetate extracts showed larvicidal activity against S. litura. Table 1 shows larvicidal activity of different crude extracts of A. monophylla; hexane extract showed maximum larvicidal activity of 79.10% at 5.0% concentrations followed by chloroform and ethyl acetate extracts, which showed 43.77% and 22.88% larvicidal activity. Hexane extract at 0.5% showed 26.88% larvicidal activity. Lower concentration of chloroform and ethyl acetate extracts did not show larvicidal activity. They exhibited less than 50% larvicidal activity at all the tested concentrations. All the concentration of hexane showed statistically superior activity. Concentration dependent activity was noticed in all the three extracts.

Hexane extracts showed high correlation of R 0.98 and coefficient value of 9.33 ± 1.71 between concentration and larvicidal activity. Chloroform and ethyl acetate extract also showed high correlation of R 0.97 and 0.92 respectively. The least LC50 value of 2.01% was observed in hexane extracts. Ethyl acetate extract showed maximum LC50 value of 7.80%. Chloroform and ethyl acetate extract statistically similar at 0.5 and 1.0% concentrations.

Pupicidal activity: Hexane extract exhibited 100% pupicidal activity at 5.0% concentration against S. litura. Ethyl acetate extract showed 52.85% pupicidal activity at 5.0% concentrations. All the concentration of hexane extract statistically differed from chloroform and ethyl acetate extracts. Low concentration of chloroform and ethyl acetate extracts showed less than 10% pupicidal activity (Table 2).

| Crude extracts   | Concentrations (%) | LC50 | R   | Coefficients |
|------------------|--------------------|------|-----|--------------|
|                  | 0.5                | 1.0  | 2.5 | 5.0          |                |
| Hexane           | 26.88 ± 4.26a      | 43.99 ± 6.73a | 58.22 ± 2.43a | 79.10 ± 1.22a | 2.01 | 0.98 | y=9.33x+17.1 |
| Chloroform       | 00 ± 00a           | 14.22 ± 5.29a | 33.55 ± 6.25a | 43.77 ± 4.12a | 5.01 | 0.97 | y=14.77x+15.1 |
| Ethyl acetate    | 00 ± 00a           | 08.22 ± 4.62a | 16.44 ± 4.86a | 22.88 ± 4.12a | 7.80 | 0.92 | y=7.33x+7.7  |

Within the column, means ± SD followed by the same letter do not differ significantly by using DMRT F test, (P ≤ 0.05). Effective concentration and complete regression equations.

Table 1: Larvicidal activity (%) and effective concentration of Atalantia monophylla crude extracts against Spodoptera litura.
Fractions

Antifeedant activity: Twelve fractions derived from hexane extract of A. monophylla showed concentration dependent antifeedant activity against S. litura. Maximum antifeedant activity of 85.31% was observed in fraction nine at 1000 ppm concentration, followed by fraction 6 which exhibited 77.63%. The R value (0.92) exhibited good correlation in fraction nine at 1000 ppm concentration followed by fraction 6 of A. monophylla. Maximum larvicidal activity of 83.55% was observed in fraction 9 at 1000 ppm concentration, fractions from hexane extracts of A. monophylla also it exhibited least LC50 value of 340.27 ppm and higher regression equations.

Larvicidal activity: Table 4 shows the larvicidal activity of different fractions against S. litura. Within the column, means ± SD followed by the same letter do not differ significantly by using DMRT F test, (P ≤ 0.05).

| Fractions | Concentration (ppm) | R | Coefficients | LC50(ppm) |
|-----------|---------------------|---|--------------|-----------|
| 1         | 0.00 ± 0.00         | 0.89 | y=-6.55x+7.71 | 1476.64   |
| 2         | 0.00 ± 0.00         | 0.97 | y=-11.55x+13.4 | 1084.36   |
| 3         | 0.00 ± 0.00         | 0.94 | y=-9.33x+9.5  | 1368.21   |
| 4         | 0.00 ± 0.00         | 0.94 | y=-10.69x+11.1 | 1063.73   |
| 5         | 0.00 ± 0.00         | 0.98 | y=-3.44x+16.4 | 710.94    |
| 6         | 0.00 ± 0.00         | 0.99 | y=-7.2x+5.6   | 1609.20   |

Within column, means ± SD followed by the same letter do not differ significantly by using DMRT-F test, (P ≤ 0.05); effective concentration and complete regression equations.

Discussion

In the present study hexane extract of A. monophylla exhibited than other fractions. Fractions 2 and 7 did not show larvicidal activity at all the concentrations. Except 9th and 5th fractions, all other fractions exhibited less than 50% larvicidal activity. Minimum larvicidal activity was noticed in fraction 12 but maximum LC50 was observed in fraction 10.

Pupicidal activity: Maximum pupicidal activity of 100% was noticed in fraction 9 at 500 and 1000 ppm concentrations followed by fraction 6 which exhibited 80 and 100% pupicidal activity at 500 and 1000 ppm concentrations. Fractions 2, 7 and 10 did not show any pupicidal activity at all the tested concentrations. More than 50% pupicidal activity was noticed in fraction 3, 5, 6 and 9 at 1000 ppm concentration (Table 5).
maximum larvicidal activity against *S. litura*. The present findings coincide with the findings of Muthu et al. [15] who noticed that hexane extract of *A. monophylla* showed 85.33% larvicidal activity at 5.0% concentration against *E. vittella*. Extracts from *Sonchus oleraceus* (Asteraceae), *Raphanus sativus* L. and *Brassica nigra* L. (Brassicaceae) exhibited larvicidal activity against *S. littoralis* [22]. Abbasipour et al. [23] reported that *Peganum harmala* L. (Nitrariaceae) seed extract showed 100% insecticidal activity against diamondback moth, *Platella xylostella* at 40 mg/ml concentration.

In this study, *A. monophylla* derived hexane extracts exhibited LC₅₀ value of 2.01% for larvicidal activity against *S. litura*. The present findings support the findings of Baskar et al. [14] who reported that hexane extract of *A. monophylla* showed LC₅₀ value of 2.46% concentration for larvicidal activity against *H. armigera*. Leaf methanol extract of *Jatropha gossypifolia* L. (Euphorbiaceae) showed least LC₅₀ value of 19.75 mg/ml concentration against *S. litura* [24].

In this study, hexane extract of *A. monophylla* exhibited 100% pomicidal activity against *S. litura* at 5.0% concentration. Present findings coincide with the findings of Baskar et al. [14] who reported that hexane extract kill 100% pupae of *H. armigera* at 5.0% concentration. Muthu et al. [15] reported that hexane extract of *A. monophylla* reduced the adult emergence of *E. vittella* in a dose dependent manner. Leaves of *Ayapana trilinervis* (Vahl) R. M. King & H. Rob (Syn. *Eupatorium trilinerve*) (Asteraceae) derived aqueous extract showed larvicidal activity against *S. litura* [25]. Abbasipour et al. [23] reported that *P. harmala* seed extract completely inhibit the adult emergence of *P. xylostella* at 40 and 30 mg/ml concentrations.

In this study fractions from hexane extract of *A. monophylla* exhibited antifeedant activity against *S. litura*. Among them 9th fraction exhibited 85.31% antifeedant activity at 1000 ppm concentration. The present findings coincide with the findings of Baskar et al. [14] who reported that hexane extract derived fractions showed 87.28% of antifeedant activity against *H. armigera* at 1000 ppm concentration. Hexane and methanol fractions from leaves of *Glycosmis arborea* (Roxb.) (Rutaceae) showed antifeedant activity against *S. litura* [26]. You-Zhi et al. [27] reported that magnifol, milletocalyxin C and isoloncharpin derived from *Derris cavaleriei* Gagnepain, Notul. Syst. (Paris) (Fabaceae) showed antifeedant activity against *P. xylostella*.

In this study, *A. monophylla* derived fractions showed larvicidal activity against *S. litura*. Maximum larvicidal activity of 83.55% was observed in 9th fraction. This result corroborates with the findings of Raman et al. [28] who reported that *Catharanthus roseus* L. (G) Don. (Apocynaceae) leaves derived crude extracts from methanol, petroleum, and fractions from methanol and ethyl acetate evaluated against *H. armigera*, all extracts exhibited moderate larvicidal effects. Maximum larvicidal activity was noticed in ethyl acetate fraction. In the present study, fraction 9 showed least LC₅₀ value of 340.27 ppm for larvicidal activity. Similarly, Baskar et al. [14] reported that fraction nine from hexane extract of *A. monophylla* showed least LC₅₀ value of 384.57 ppm for larvicidal activity against *H. armigera*. Methanol extract, its fraction and isolated compound furocoumarin and quinoline alkaloid from *Ruta chalepensis* L. (Rutaceae) exhibited LC₅₀ values of 2.42, 0.89, 1.59 and 1.21 mg/ml for larvicidal activity against *S. littoralis* [29].

Hexane derived fractions from *A. monophylla* showed pupicidal activity against *S. litura*. Maximum pupicidal activity of 100% was observed in 9th fraction at 1000 ppm concentration. The results support with the earlier findings of Baskar et al. [14] who reported that fractions from hexane extract of *A. monophylla* showed pupicidal activity against *H. armigera*. Moringa oil (Moringaceae) and saponifiable oil showed pupicidal activity against *S. frugiperda* [30]. In the present study, different fractions showed pupicidal activity at concentration dependent manner. The present findings are in agreement with the findings of Huang et al. [8] who reported that *Pogostemon cablin* (Blanco) Benth. (Lamiaceae) exhibited pupicidal activity against *S. littoralis* and *S. exigua*.

**Conclusion**

Hexane extract and their fractions from *A. monophylla* showed antifeedant, larvicidal and pupicidal activities against *S. litura*. Dose depend activity was noticed in all the bioassays. Linear regression results support the dose depend activity. The hexane derived 9th fraction could be further fractionated and isolate the active principle(s) responsible for the activity. Further, hexane extract of *A. monophylla* could be used to develop a new formulation to manage the agriculturally important pests.

**Conflict of Interest Statement**

We declare that we have no conflict of interest.

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