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Bio-efficacy of crude leaf extracts of *Acalypha fruticosa* Forssk. against some agriculturally important insect pests

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ABSTRACT

Objective: To investigate the antifeedant and larvicidal activities of *Acalypha fruticosa* Forssk. (Euphorbiaceae) (*A. fruticosa*) leaf extracts.

Methods: Efficacy of various organic solvent extracts of dichloromethane, acetone, dimethyl sulfoxide and aqueous extracts of the leaves of *A. fruticosa* were evaluated for their feeding inhibition and larvicidal activities against third instar larvae of *Leucinodes orbonalis* (*L. orbonalis*), *Helicoverpa armigera* (*H. armigera*), *Spodoptera litura* (*S. litura*) and *Earias vittella* (*E. vittella*). Antifeedant and larvicidal activities were performed by leaf and fruit discs no-choice method at 0.625%, 1.25%, 2.5% and 5% concentrations.

Results: The results revealed that 5% concentration of dichloromethane extract had significant antifeedant activity on *L. orbonalis* (77.1%), *H. armigera* (66.2%), *S. litura* (74.8%) and *E. vittella* (67.2%) followed by acetone, dimethyl sulfoxide and aqueous extracts. The result on larvicidal activity also showed that the 5% concentration of dichloromethane extract exhibited significantly higher larval mortality for *L. orbonalis* (62.12%), *H. armigera* (62.14%), *S. litura* (55.11%) and *E. vittella* (77.15%) when compared to acetone, dimethyl sulfoxide and aqueous extracts.

Conclusions: From this study, it is concluded that the dichloromethane extracts of leaves of *A. fruticosa* could serve as a potential natural pesticide for further exploration of active compounds.

1. Introduction

The application of synthetic insecticides has been practiced as the sole method for insect pest management since decades[1]. Many synthetic insecticides act as acute toxic chemicals, causing rapid elimination of pests and complete wash out of beneficial insects as well[2]. The increasing concern on the development of resistance by insects to synthetic pesticides together with growing public concern about the use of toxic chemicals and their impacts on the environment has lead to an urgent need of more environmentally and minimal adverse effects on safer insecticides[3].

Plant products were known to have feeding deterrent and insecticidal properties on a variety of insect pests that were tested to determine whether they have potential as alternative pest control measures[4]. Triterpenoids, azadirachtin is widely used as insect antifeeding substance. It affects nearly 400 species of leaf eating insect pests. It also influences the normal development of herbivore insects[5].

The action of insects feeding on plants is supported or opposed by various natural chemical compounds present in the plants. It acts as phagodeterrent, insecticidal, growth inhibitory, ovicidal, and oviposition deterrent[5,6]. A plant molecule is know to inhibit the enzyme activities of the pests[7]. Wide-range of phytochemicals present in the plants at different levels and are bound to make a different mode of actions[8]. The majority of plants derived secondary metabolites affect the various biochemical system of lepidopteran pests, acting individually/synergistically, which results in affecting both the central and peripheral

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nervous system of pest insects^[9].

Hence, in this study an attempt has been undertaken to assess the efficacy of extracts of leaves of *Acalypha fruticosa* (Forssk.) (*A. fruticosa*) using different solvents such as dichloromethane (DCM), acetone, dimethyl sulfoxide (DMSO) and aqueous for their antifeedant and larvicidal activities against four economically important lepidopteran larvae. *A. fruticosa* is an Indian ethano-medicinal plant that was used to cure dyspepsia, stomach ache, skin diseases, wound healing and poisonous bites^[10,11]. Many ethanopharmacological investigations have shown its antidiarrhoeal^[12], antioxidant and hepatoprotective^[13], anticancer^[14], anthelmintic^[15], antimicrobial and cytotoxic properties^[14], as well as larvicidal activity against *Spodoptera litura* (*S. litura*)^[16] and *Plutella xylostella*^[17]. There are no earlier reports on the antifeedant and larvicidal activities of *A. fruticosa* leaf extracts against the larvae of *Earias vittella* (*E. vittella*) and *Leucinodes orbonalis* (*L. orbonalis*).

2. Materials and methods

2.1. Plant collection and extraction

Fresh and healthy leaves of *A. fruticosa* were collected in and around Chennai, Tamil Nadu, India. The collected plant materials were washed and shade-dried at room temperature and powdered by using electrical blender. A total of 500 g of plant powder was extracted with increasing polarity of DCM, acetone and DMSO at room temperature for 1 week with occasional shaking in an aspirator bottle. The solvent extract was filtered through Whatman No. 1 filter paper and concentrated by using vacuum rotary evaporator. Finally, the plant powder was extracted by using aqueous. The extract was concentrated with rotary evaporator below 50 °C and was stored in at 4 °C until use.

2.2. Insect culture

Helicoverpa armigera (*H. armigera*) and *E. vittella* larvae were collected from bhendi fields and *S. litura* and *L. orbonalis* larvae were collected from groundnut and brinjal fields respectively at Thandalam Village near Thiruporur, Kancheepuram District, Tamil Nadu, India. The agree-field collected larvae were maintained in the insect culture room on their natural respective food. The pupae were shifted to Petri dishes and kept inside oviposition cage. The newly emerged adults from pupae were provided with a mixture of 10% sucrose solution with multi-vitamin liquid. Brinjal (*L. orbonalis*), bhendi fruits (*E. vittella* and *H. armigera*) and groundnut seedling grown in small cups (*S. litura*) were placed in the oviposition chamber. The newly emerged third instar larvae were used for the laboratory experiments at (28 ± 2) °C, (11 ± 1) h photoperiod and (75 ± 5)% relative humidity.

2.3. Antifeedant bioassay

Different crude extracts from *A. fruticosa* were evaluated by using leaf disc no-choice method. Fresh castor leaf discs of 4 cm diameter were dipped in 0.625%, 1.25%, 2.5% and 5% concentration of crude extracts for about 5 min and shade dried. One such treated leaf disc was put inside a Petri plate and a single pre-starved (2 h) third instar larva of *S. litura* was introduced into the leaf disc. The same procedure was followed for *H. armigera* using cotton leaf discs. Respective solvents were used as negative controls. After 24 h, the discs were weighed and the difference between initial and final weights was calculated by the formula of Bentley *et al.* as follow^[18]:

$$\text{Antifeedant activity} = \frac{\text{Consumption in control} - \text{Consumption in treated}}{\text{Consumption in control}} \times 100$$

The antifeedant activity was assessed using bhendi fruit discs for *E. vittella* and brinjal fruit discs for *L. orbonalis*. Bhendi fruit discs (100 mm thick) with seeds and brinjal discs (10 mm thick) were dipped in 0.625%, 1.25%, 2.5% and 5% concentration of crude extracts for about 10 min and was shade dried and fruit discs were weighed and provided for *E. vittella* and *L. orbonalis*. A set containing 10 discs were placed separately in Petri dishes for each treatment and control for both the pests. Discs of bhendi and brinjal dipped in respective solvents were used as negative control and without larvae were also maintained to find out the weight loss in the discs due to desiccation at room temperature. After 24 h, the discs were weighed and the difference between initial and final weights was calculated. Consumption was calculated as follows:

Weight loss due to desiccation (D) = Initial weight – Final weight

Actual consumption = Initial weight – (Final weight + D)

2.4. Larvicidal bioassay

The larvicidal activity was evaluated by leaf disc and fruit disc no-choice method. Fresh castor and cotton leaves and bhendi and brinjal fruits were dipped with crude extracts (as mentioned in antifeedant activity) and acetone. In each treatment, pre-starved (2 h) third instar larvae of *L. orbonalis*, *H. armigera*, *S. litura* and *E. vittella* were obtained from laboratory culture and introduced into the respective treatments. The treated and control larval instars were maintained upto pupation with respective diets. Larval mortality was observed upto pupation. Five replicates were maintained for each treatment with 10 larvae per replicate (total n=50). Percentage of larval mortality was calculated according to Abbott's formula^[19]. The experiment was conducted at laboratory condition [(27 ± 2) °C with 14:10 h photoperiod and (75 ± 5)% relative humidity].

2.5. Statistical analysis

The data related to antifeedant and larvicidal activities were presented in graph. Concentration dependent larval mortality was calculated by linear regression analysis.

3. Results

3.1. Antifeedant activity

DCM, acetone, DMSO and aqueous extracts of *A. fruticosa* leaves were studied for their antifeedant activity against four important lepidopteran larvae that are presented in Figure 1. Maximum antifeedant activity of 77.1% was noticed in DCM extract of *A. fruticosa* at 5% concentration against *L. orbonalis*. DMSO extract was the next best treatment and recorded the antifeedant activity of 72.4% at 5% concentration followed by aqueous extract against *L. orbonalis* (Figure 1). In the case of larvae of *H. armigera*, the highest antifeedant activity was recorded in the DCM extract of *A. fruticosa* with 66.2% at 5% concentration followed by acetone, DMSO and aqueous extracts.

In the larvae of *S. litura*, the maximum antifeedant activity of 74.8% was noted in DCM extract of *A. fruticosa*, followed by acetone, DMSO and aqueous extracts at 5% concentration when compared to control. The same extracts at 2.5% concentration recorded the antifeedant activity of 66.1% against *S. litura*. In *E. vittella*, DCM extract of *A. fruticosa* showed the highest antifeedant activity of 78.1% followed by acetone extract (67.2%), DMSO extract (64.6%) and aqueous extracts (51.0%) at 5% concentration. The same extracts at 1.25% and 2.5% concentration showed antifeedant activity of 52.1% and 58.5%, respectively, compared to control. The DCM and DMSO extracts of *A. fruticosa* leaves produced various types of abnormalities in larvae, pupae and adults of the all the tested insect pests.

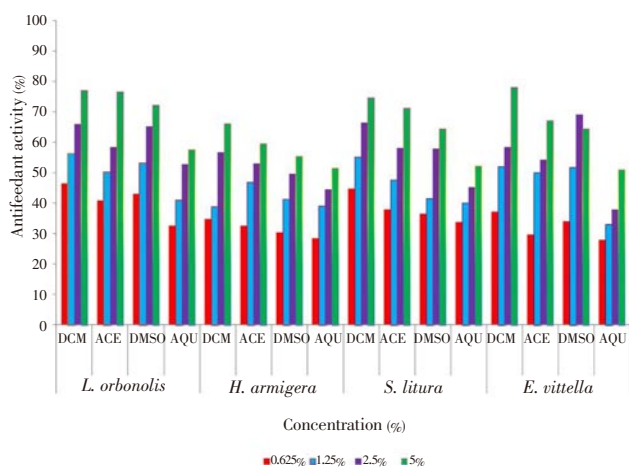


Figure 1. Antifeedant activity of different crude extracts of *A. fruticosa* against four lepidopteran insect pests. ACE: Acetone; AQU: Aqueous.

3.2. Larvicidal activity

Figure 2 shows the larvicidal activity of different solvent extracts of *A. fruticosa* against *E. vittella*. DCM extracts of *A. fruticosa* leaves at 5% concentration manifested significant larvicidal activity of 77.15% against *E. vittella* followed by acetone extract. Moderate larvicidal activity of 59.11% was observed in DMSO extract at 5% concentration followed by aqueous extract that recorded minimum larvicidal activity of 28.56% at 5% concentration.

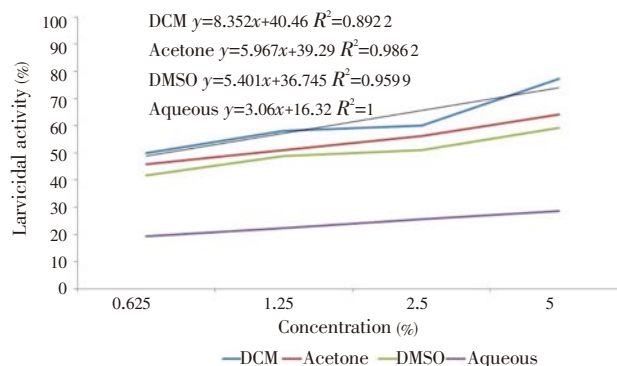


Figure 2. Larvicidal activity of different crude extracts of *A. fruticosa* against *E. vittella*.

The case of *L. orbonalis*, DCM extract of *A. fruticosa* showed larval mortality of 62.12% followed by acetone and DMSO extracts at 5% concentration. The lowest mortality was recorded in aqueous extracts (Figure 3). The DCM extract of the same plant at 2.5% concentration exhibited the maximum larvicidal activity of 53.10% against *L. orbonalis* followed by acetone, DMSO and aqueous extracts.

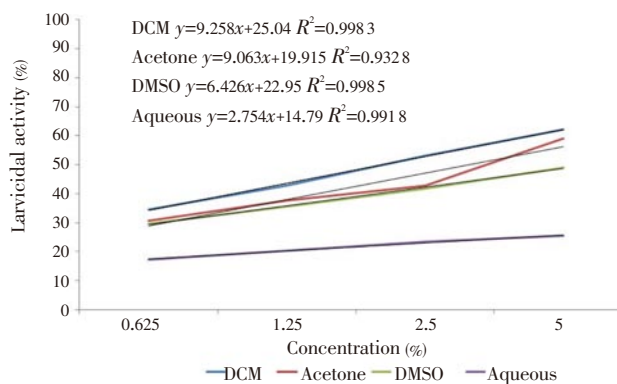


Figure 3. Larvicidal activity of different crude extracts of *A. fruticosa* against *L. orbonalis*.

Moderate larvicidal activity was observed in all the extracts in the case of *S. litura* larvae. The maximum larval mortality was seen in DCM extract with 55.10% followed by DMSO (51.12%) and acetone (48.96%) extracts at 5% concentration (Figure 4). In *E. vittella*, DCM extract of *A. fruticosa* showed larvicidal activity of 62.14% followed by DMSO and acetone.

DCM extracts exhibited 62.14% larvicidal activity against *H. armigera* larvae at 5% concentration followed by DMSO, acetone and aqueous extracts. Linear regression line for larvicidal activity (Figures 2–5) clearly showed that DCM extracts exhibited concentration dependent activity against

all the tested lepidopteran insects and aqueous extracts showed the minimum activity than all other extracts against tested insect pests.

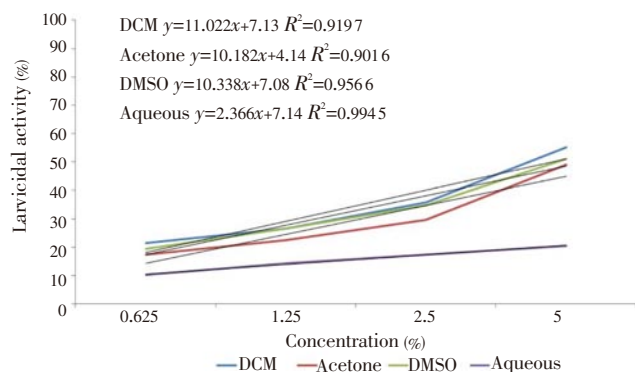


Figure 4. Larvicidal activity of different crude extracts of *A. fruticosa* against *S. litura*.

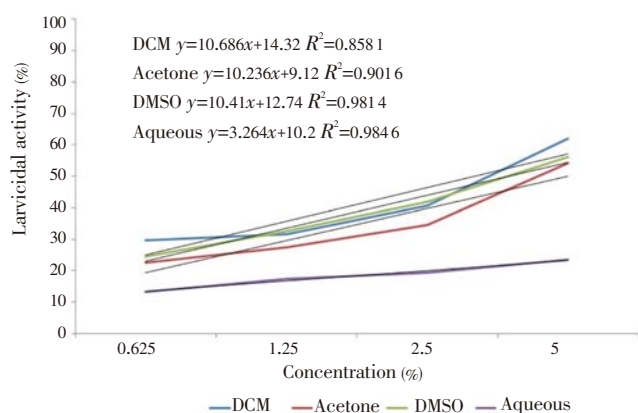


Figure 5. Larvicidal activity of different crude extracts of *A. fruticosa* against *H. armigera*.

4. Discussion

4.1. Antifeedant activity

Plants based compounds are responsible to repel insect or alter feeding behaviour, growth, development and moulting process or may disrupt mating and oviposition[20]. The results of our study show that DCM and DMSO extracts of leaves of *A. fruticosa* at 5% concentration possess potent feeding deterrence against all the tested lepidopteran larvae. This result corroborates with the reports of Arivoli and Tennyson who reported dichloromethane extract of *Strychnos nuxvomica* and *Abrus precatorious* showed antifeedant activity against *S. litura*[21]. The present results are also in agreement with the findings of Pavunraj *et al.* who reported that dichloromethane extract of *Spilanthes acmella* leaves exhibited (68.88%) good antifeedant activity against third instar larvae of *L. orbonalis*[22].

Pavunraj *et al.* reported the antifeedant activity of ethyl acetate extracts of leaves of *Melochia corchorifolia* against *H. armigera*, *S. litura*, *E. vittella* and *L. orbonalis*[23]. In the present investigation, the dichloromethane extract

also showed significant antifeedant activity against all tested larvae. Similarly, Jayasankar *et al.* reported that dichloromethane extract of *Solanum pseudocapsicum* showed antifeedant activity against *S. litura* and *H. armigera* larvae[24]. Similarly, Muthu *et al.* reported that hexane extract of *Flueggea leucopyrus* and chloroform extract of *Clerodendrum phlomidis* leaves showed antifeedant activity against third instar larvae of *E. vittella*[25].

4.2. Larvicidal activity

Most of the studies on biological activity of plants have shown killing potential against early instars[26]. The most common symptoms consequent upon feeding of the larvae on toxicants are convulsions, copious diuresis leading to dehydration, loss of body turgidity and finally cause death. The present study revealed significantly higher larval mortality in dichloromethane extract of *A. fruticosa* against *H. armigera*, *S. litura*, *E. vittella* and *L. orbonalis*. This is in accordance with the findings of Pavunraj *et al.* who observed that the larval mortality of *H. armigera* (35.33%), *S. litura* (46.66%), *E. vittella* (48.88%) and *L. orbonalis* (32.66%) at 1% concentration in the crude extract of *Hyptis suaveolens*[22]. Earlier, Muthu *et al.* reported that *Clerodendrum phlomidis* exhibited larvicidal activity against *E. vittella* at 5% concentration[25]. Phattharaphan *et al.* also reported that dichloromethane extract of *Stemona collinsae* exhibited insecticidal activity against third instar larvae of *Plutella xylostella*[27]. The larval mortality was increased significantly with the increase in the concentration of the active crude extracts. The findings of the present research work are also in confirmation with the earlier reports several workers[28–30].

This study explored the antifeedant and larvicidal activities of DCM extracts of leaves of *A. fruticosa* against selected lepidopteran pests. This plant extract could be further explored for the possible active insecticidal compounds for effective control of these pests.

Conflict of interest statement

We declare that we have no conflict of interest.

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