
Bioefficacy of plant extracts against Asian army worm *Spodoptera litura* Fab. (Lepidoptera: Noctuidae)

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Bioefficacy of hexane, chloroform and ethyl acetate leaf extracts of *Blumea mollis* and *Hygrophila auriculata* was studied against *Spodoptera litura* to find out their antifeedant, larvicidal, larval duration, pupal duration and pupicidal activities. There was a corresponding increase in antifeedant, larvicidal, larval duration, pupal duration and pupicidal activities when there was increased in the concentration of the test extracts. Ethyl acetate extract of *H. auriculata* at 5.0% concentration had higher antifeedant (68.48%) and pupicidal (70%) activity. The LC₅₀ value of 3.34% was observed in ethyl acetate extract of *H. auriculata* for larval mortality. Ethyl acetate extract prolonged the larval and pupal duration of *S. litura*.

Key words: *Blumea mollis*, *Hygrophila auriculata*, *Spodoptera litura*, antifeedant, pupicidal

Introduction

The indiscriminate use of chemical pesticides has given to many serious problems, including genetic resistance of pest species, toxic residues, increasing costs of application, environmental pollution and hazards (Ahmed *et al.*, 1981). This was created a world-wide interest in the development of alternative strategies, including the search for new types of insecticides, and used for traditional botanical pest control agents. Plant crude extracts often consist of complex mixtures of compounds which may act synergistically (Berenbaum, 1985). The concept of using nontoxic antifeedant to protect crops without killing the pest directly is an attractive one and has received considerable attention (Bernays, 1983). Botanical pesticides are highly effective, safe and ecologically acceptable (Senthil Nathan and Kalaivani, 2005). *Spodoptera litura* infests a wide range of cultivated food plants numbering around 112, belonging to 44 families (Sharma and Seth, 2005). Eight larvae of tobacco cut

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worm on a plant can reduce the yields up to 50% (Patel et al., 1971). In the present investigation two plants namely, *Blumea mollis* (D. Don) Merr. (Asteraceae) and *Hygrophila auriculata* (Schum.) Heine (Acanthaceae) were evaluated for their biological activities against the economically important polyphagous pest *S. litura* Fab. The essential oil from the leaves of *B. mollis* showed larvicidal activity against *Culex quinquefasciatus* (Senthil kumar *et al.*, 2008).

Materials and methods

Plant collection and Extraction

Leaves of *Blumea mollis* and *Hygrophila auriculata* were collected from Uthukottai, Thiruvallur district, Tamil Nadu, India. The plant was identified by Dr. Ayyanar, taxonomist at Entomology Research Institute, Loyola College. The voucher specimens of *B. mollis* and *H. auriculata* [ERIH: 1311-1312] were deposited at the institute herbarium. The collected plant materials were washed, shade dried under the room temperature ($27 \pm 2^\circ\text{C}$) and powdered using electric blender; 500 gm powder was macerated with 1.5 liter of hexane, chloroform and ethyl acetate sequentially for a period of 48 hrs each and filtered. The extracts were concentrated at reduced temperature on a rotary evaporator and stored at 4°C . The yield of *H. auriculata* crude extract was 4.6, 6.3 and 7.1gms and *B. mollis* 5.2, 4.3 and 5.7 gms in hexane, chloroform and ethyl acetate, respectively.

Insect culture

Egg masses of *Spodoptera litura* were collected from groundnut field at Eagattur near Thiruvallur District of Tamil Nadu. The eggs were surface sterilized with 0.02% sodium hypochloride solution, dried and allowed to hatch. After hatching, the neonate larvae were reared on leaves of castor *Ricinus communis*, till prepupal stage and sterilized soil was provided for pupation. After pupation, the pupae were collected from soil and placed inside the oviposition chambers (40 x 25 x 25 cm). 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 tender castor leaf for feeding. These laboratory reared larvae were used for bioassay, at room temperature ($27 \pm 2^\circ\text{C}$) with 14-10 light: dark photoperiod and $75 \pm 5\%$ relative humidity.

Antifeedant activity

Antifeedant bioassay was carried out using leaf disc no choice method. The crude extracts were dissolved in acetone and fresh castor leaf discs of 4-cm diameter were punched using cork borer and dipped in 0.5, 1.0, 2.5 and 5.0% concentrations. The leaf discs treated with acetone were used as negative control. In each plastic petridish (1.5cm x 9cm) wet filter paper was placed to avoid early drying of the leaf discs and single third instar larva was introduced into each petridish. Progressive consumption of leaf area by the larva after 24 hrs was recorded in control and treated discs using the leaf area meter. Leaf area eaten by the larva in plant extract treatment was corrected from the control. Five replicates were maintained for each treatment with 10 larvae per replicate (total, n=50) and the percentage of antifeedant activity was calculated using the following formula.

$$\text{Antifeedant activity} = \frac{\text{Leaf area consumed in control} - \text{Leaf area consumed in treatment}}{\text{Leaf area consumed in control}} \times 100$$

Larvicidal activity

Different concentrations of crude extracts were applied using leaf dip method. The treated leaves were exposed to the larvae. After 24 hr of treatment, the larvae were continuously maintained on the non-treated fresh castor leaves. Fresh castor leaves were provided at every 24 hr. Larval mortality was recorded after 96 hr of treatment. Five replicates were maintained for each treatment with 10 larvae per replicate. Per cent mortality was calculated (Abbott, 1925). The experiment was conducted at laboratory temperature of 27 ± 2 °C with 14:10 light photoperiod and $75 \pm 5\%$ relative humidity.

$$\text{Abbott's corrected mortality} = \frac{\% \text{ mortality in Treatment} - \% \text{ mortality in control}}{100 - \% \text{ mortality in control}} \times 100$$

Pupicidal activity

The survived larvae were continuously fed with diet (castor leaf) until they became pupae and adults. Pupicidal activity was calculated by subtracting the number of emerging adults from the total number of pupae.

Larval and pupal durations

The larval duration was calculated after treatment of larvae till pupation. Pupal duration was calculated from the day of moulting of the larvae to adult emergence.

Statistical analysis

The antifeedant and pupicidal activities and larval-pupal duration were subjected to analysis of variance (ANOVA). Significant differences between treatments were determined by Tukey's multiple range tests ($P \leq 0.05$). LC_{50} and LC_{90} values were calculated using probit analysis (Finney, 1971).

Results

The feeding deterreny of the crude extracts of *B. mollis* and *H. auriculata* at different concentrations against the third instar larvae of *S. litura* is given in Table 1. Statistically significant feeding deterrent activity (68.48%) was observed in ethyl acetate extract of *H. auriculata* at 5.0% concentration followed by hexane and chloroform extracts. The extracts from *B. mollis* showed minimum activity when compared to *H. auriculata* at 5% concentration.

The least LC_{50} and LC_{90} values were obtained at 3.34% and 8.27% concentrations, respectively in ethyl acetate extract of *H. auriculata* for larval mortality. The chisquare values are significant at $P < 0.05$ level. The high chisquare values in the bioassays indicated probably the heterogeneity of the test population. The highest LC_{50} and LC_{90} values were obtained at 8.01% and 16.20% concentrations, respectively in ethyl acetate extract of *B. mollis* (Table 2). The pupicidal activity of *B. mollis* and *H. auriculata* is presented in Table-3. Maximum pupicidal effect (70%) was observed in ethyl acetate extract of *H. auriculata* followed by chloroform and hexane extracts at 5.0% concentration. In the case of *B. mollis*, maximum pupicidal activity (46%) was noticed in hexane extract which was on par with the hexane extract of *H. auriculata*.

Among the extracts, ethyl acetate extract of *H. auriculata* at 5% concentration recorded a prolonged larval duration of 14.08 days (Table 4), where as in control, the larval duration was 8.6 days. A notable prolonged larval duration was recorded in hexane extract of *H. auriculata* (11.92 days) which was on par with the hexane extract of *B. mollis* (11.68 days) at 5% concentration. Irrespective of the concentrations, the ethyl acetate extract of *H. auriculata* showed significant prolonged larval duration when compared to other solvents of the same plant and all the extracts of *B. mollis*. From the results, it was observed that higher concentration of the test extract prolonged the larval duration.

In the present investigation, it was observed that all the extracts prolonged the pupal duration of *S. litura*. Ethyl acetate extract of *H. auriculata* recorded a maximum prolonged pupal duration (14.80 days) (Table 5) at 5% concentration. Significant prolonged pupal duration was observed in all the extracts in all the concentrations of *H. auriculata* when compared to the extracts of *B. mollis*. The pupal duration was dose dependent.

Table 1. Per cent Antifeedant activity of two plant extracts against *Spodoptura litura*.

Crude extracts	Concentrations (%)			
	0.5	1.0	2.5	5.0
<i>Blumea mollis</i>				
Hexane	29.29 ± 3.28 ^c	35.10 ± 3.10 ^c	43.32 ± 2.20 ^c	46.80 ± 3.32 ^c
Chloroform	16.97 ± 4.30 ^b	21.30 ± 3.70 ^b	27.25 ± 4.14 ^b	32.50 ± 2.24 ^b
Ethyl acetate	15.85 ± 3.94 ^b	20.09 ± 4.21 ^b	26.38 ± 3.96 ^b	31.71 ± 2.23 ^b
<i>Hygrophila aurigullata</i>				
Hexane	28.17 ± 2.91 ^c	43.58 ± 3.61 ^{de}	46.77 ± 2.37 ^{cd}	52.95 ± 1.21 ^d
Chloroform	27.02 ± 2.98 ^c	38.45 ± 3.98 ^{cd}	50.36 ± 3.70 ^{de}	54.94 ± 1.73 ^d
Ethyl acetate	31.32 ± 3.22 ^c	46.65 ± 4.09 ^e	56.52 ± 3.43 ^e	68.48 ± 3.97 ^e
Control			3.30 ± 1.87 ^a	

Within the column, mean ± SD followed by the same letter do not differ significantly using Tukey 's test, P ≤ 0.05

Table 2. Effective concentrations of LC₅₀ - LC₉₀ and χ^2 -values of plant extracts on *Spodoptura litura*.

Crude extracts	LC ₅₀	95% confident limit		LC ₉₀	95% confident limit		Chi-square
		Lower	Upper		Lower	Upper	
<i>Blumea mollis</i>							
Hexane	4.36	3.65	5.53	9.40	7.59	12.97	100.37*
Chloroform	6.30	5.34	8.00	11.15	9.12	14.96	71.80*
Ethyl acetate	8.01	6.34	11.74	16.20	12.26	25.28	47.57*
<i>Hygrophila aurigullata</i>							
Hexane	6.42	5.32	8.49	12.79	10.17	18.01	57.91*
Chloroform	5.29	4.64	6.26	11.68	9.91	14.51	30.24*
Ethyl acetate	3.34	3.00	3.75	8.27	7.30	9.65	32.48*

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

Table 3. Per cent Pupicidal activity of plant extracts against *Spodoptera litura*.

Crude extracts	Concentrations (%)			
	0.5	1.0	2.5	5.0
<i>Blumea mollis</i>				
Hexane	00 ± 00 ^a	00 ± 00 ^a	31.33 ± 11.92 ^c	46.0 ± 8.94 ^d
Chloroform	00 ± 00 ^a	00 ± 00 ^a	00.00 ^a	28.09 ± 6.81 ^c
Ethyl acetate	00 ± 00 ^a	00 ± 00 ^a	00.00 ^a	14.76 ± 1.06 ^b
<i>Hygrophila aurigullata</i>				
Hexane	00 ± 00 ^a	00 ± 00 ^a	13.69 ± 1.83 ^b	43.33 ± 9.12 ^d
Chloroform	00 ± 00 ^a	00 ± 00 ^a	23.80 ± 6.52 ^{bc}	46.00 ± 5.47 ^d
Ethyl acetate	00 ± 00 ^a	27.26 ± 8.58 ^b	50.00 ± 10.0 ^d	70.00 ± 4.56 ^c
Control	00 ± 00 ^a			

Within the column, mean ± SD followed by the same letter do not differ significantly using Tukey's test, P ≤ 0.05

Table 4. Total larval duration (days) of *Spodoptera litura* after treatment with plant extracts.

Crude extract	Concentration (%)			
	0.5%	1.0%	2.5%	5.0%
<i>Blumea mollis</i>				
Hexane	9.2 ± 0.40 ^a	9.56 ± 0.74 ^{ab}	10.52 ± 0.52 ^{bc}	11.68 ± 1.06 ^b
Chloroform	9.0 ± 0.68 ^a	9.36 ± 0.72 ^{ab}	9.6 ± 0.49 ^{ab}	9.76 ± 0.82 ^a
Ethyl acetate	9.0 ± 0.87 ^a	9.0 ± 0.64 ^a	9.28 ± 0.50 ^{ab}	9.32 ± 0.95 ^a
<i>Hygrophila auriculata</i>				
Hexane	8.8 ± 0.55 ^a	10.52 ± 0.97 ^b	11.24 ± 1.17 ^c	11.92 ± 0.90 ^b
Chloroform	9.2 ± 0.74 ^a	10.4 ± 0.21 ^b	10.7 ± 0.83 ^{bc}	11.9 ± 0.92 ^b
Ethyl acetate	11.5 ± 0.61 ^b	12.22 ± 0.75 ^c	12.82 ± 0.97 ^d	14.08 ± 0.75 ^c
Control	8.6 ± 0.53 ^a			

Within the column, mean ± SD followed by the same letter do not differ significantly using Tukey's test, P ≤ 0.05.

Table 5. Total pupal duration (days) of *S. litura* after treatment with plant extracts.

Crude extract	Concentration (%)			
	0.5%	1.0%	2.5%	5.0%
<i>Blumea mollis</i>				
Hexane	11.24 ± 0.75 ^{ab}	11.56 ± 0.45 ^{abc}	12.09 ± 0.90 ^{bc}	12.50 ± 1.34 ^b
Chloroform	10.0 ± 0.90 ^a	10.4 ± 0.64 ^{ab}	10.84 ± 0.71 ^{ab}	11.06 ± 0.95 ^{ab}
Ethyl acetate	9.94 ± 1.07 ^a	10.16 ± 0.84 ^{ab}	10.50 ± 0.91 ^{ab}	10.84 ± 0.65 ^{ab}
<i>Hygrophila auriculata</i>				
Hexane	9.84 ± 0.92 ^a	10.0 ± 0.80 ^a	10.28 ± 1.13 ^{ab}	11.21 ± 1.33 ^{ab}
Chloroform	10.64 ± 1.05 ^{ab}	11.8 ± 1.33 ^{bc}	12.2 ± 1.43 ^{bc}	12.87 ± 0.96 ^{bc}
Ethyl acetate	12.36 ± 1.02 ^b	12.74 ± 1.11 ^c	13.18 ± 1.33 ^c	14.80 ± 1.09 ^c
Control	9.8 ± 0.62 ^a			

Within the column, mean ± SD followed by the same letter do not differ significantly using Tukey's test, P ≤ .05.

Discussion

The results revealed that the antifeedant activity against *S. litura* was maximum in ethyl acetate extract of *H. auriculata*. Similar results were reported in crude extract with specific mode of action against insects is a complex mixture of compounds (Tewary *et al.*, 2005). Many researchers have reported crude extracts on *S. litura* (Raja *et al.*, 2005; Kamaraj *et al.*, 2008), on *S. frugiperda* (Rodriguez-Lopez *et al.*, 2007). Larval population was significantly reduced. The LC₅₀ value of 3.34% was observed in ethyl acetate extract of *H. auriculata* which showed significant reduced larval population. This is in accordance with the findings of Oigiangbe *et al.* (2007) who obtained LC₅₀ value at 3.5% concentration with leaf extract of *Alstonia boonei* on *Sesamai calamistis*. Pavela (2004) observed LC₅₀ value of 3.74 % in *Melissa officinalis* on *S. littoralis*. Ethyl acetate extract of *B. mollis* showed the highest LC₅₀ value of 7.15%. Similarly, Pavela (2004) reported the LC₅₀ value of 7.71% in *Salvia splendens* on *S. littoralis* and Baskar *et al.* (2010) observed ethyl acetate extract of *Couroupita guianensis* showed LC₅₀ value of 7.22% against *Helicoverpa armigera*. Pupicidal activity was high in ethyl acetate extract of *H. auriculata*. This is in accordance with the findings of Pavela (2004) on *S. littoralis*, who observed pupal mortality of 40.2 and 40.8% at 10 and 5% concentrations, respectively in the crude extract of *Origanum benedictus*. The extracts of *B. mollis* exhibited lowest pupal mortality at 5% concentration in all the extracts when compared with *H. auriculata* extracts. Similar finding was reported by Baskar *et al.* (2009) reported that the ethyl acetate extract of *Atalantia monophylla* showed 66.03% pupicidal activity against *Helicoverpa armigera*.

In the present investigation, *H. auriculata* recorded a maximum prolonged larval duration when compared to *B. mollis*. This is due to the reduction in consumption by larvae. Prolonged larval period increased the larval mortality rate on *S. exigua* (Yoshida and Toscano, 1994) on *Cnaphalocrocis medinalis* (Senthil Nathan *et al.*, 2006) and on *S. frugiperda* (Torres *et al.*, 2003). Mala and Muthalagi (2008) reported that the larval duration was increased when the fifth instar larvae were fed with 0.8% neem oil extract treated leaves. Pupal duration was increased in the plant extract treated *S. litura*. Similar observation was reported by extract of *Myrtillocactus geometrizans* on fall army worm, *S. frugiperda* Torres *et al.* (2003) and *Yucca Periculosa* (Cespedes *et al.*, 2005).

The ethyl acetate extract of *H. auriculata* at 5.0% concentration showed higher antifeedant and pupicidal activity of 68.48% and 70%, respectively. Ethyl acetate extract of *H. auriculata* also prolonged the larval and pupal duration. Hence it is inferred that the ethyl acetate extract of *H. auriculata* can

be used further for the isolation of active molecules and in the production of botanical formulations for the management of *S. litura*.

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