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ORIGINAL ARTICLE

Bioefficacy of *Aristolochia tagala* Cham. against *Spodoptera litura* Fab. (Lepidoptera: Noctuidae)

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Abstract Bioefficacy of leaf and root extracts of *Aristolochia tagala* Cham. at different concentrations was evaluated at room temperature against *Spodoptera litura* Fab. Effects on feeding, larvicidal and pupicidal activities and larval–pupal duration were studied. Higher antifeedant activity (56.06%), lethal concentration for feeding inhibition (3.69%), larvicidal (40.66%), pupicidal (28%), total mortality (68.66%) and prolonged larval–pupal duration (12.04–13.08 days) were observed in ethyl acetate leaf extract at 5.0% concentration. Dose dependant effect of test extracts was observed. This plant could be used to isolate active principles and to develop a new botanical formulation in pest management programmes.

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1. Introduction

The insect pests have developed resistance to a variety of insecticides due to the indiscriminate use of chemical pesticides. Insecticides affect the non-target organisms and human beings, directly or indirectly. Plant materials are effective against a variety of agricultural insect pests; they are easily degradable. This is beneficial for both the environment and agriculture product consumers (Pascual-Villalobos and Robledo, 1999). Plant extracts or pure compounds manifest their effect on insects in several ways including toxicity, mortality, antifeedancy, growth inhibition, suppression of reproductive behavior and reduction of fecundity and fertility (Jbilou et al., 2006). Feeding deterrency is caused due to the action of botanicals (Schmutterer, 1985) that prevent the motility of the gut (Leuschner, 1972). Some compounds, either separately or synergistically, make up a chemical defense barrier in the plant against certain pests (Vendan et al., 2008). Many workers have highlighted the importance of developing botanical insecticides from plants. It has been suggested that tremendous interest has been generated in recent years about the use of pesticidal plants, particularly those that can be harvested, formulated and used easily.

Asian armyworm *Spodoptera litura* Fab. is a polyphagous insect present in high numbers in tropical countries even during rainy season (Talukder and Howse, 1994). This pest attacks more than 112 species of cultivated crops and causes severe losses. The present study was undertaken to assess the

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bioefficacy of *Aristolochia tagala* Cham. against the notorious agricultural pest *S. litura* under laboratory condition.

2. Materials and methods

2.1. Plant collection

The plant *A. tagala* Cham was collected from Kolli hills of Namakkal District, Tamil Nadu, India. The plant was identified by Dr. Ayyanar, taxonomist at Entomology Research Institute, Loyola College. The voucher specimen [ERIH: 417] was deposited in the herbarium at Entomology Research Institute, Loyola College, Chennai.

2.2. Extraction

The leaves and roots of *A. tagala* were shade dried at room temperature and powdered coarsely. Each 100 g of powder was soaked in 0.3 l of hexane and ethyl acetate solvents sequentially, for a period of 48 h. The extract was filtered through a Buchner funnel using Whatman number 1 filter paper. The filtrate was evaporated to dryness under reduced pressure using rotary evaporator. The residue (crude extract) was collected and stored at 4 °C.

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 (27 ± 2 °C) with 14–10 light: dark photoperiod and 75 ± 5% relative humidity in insectary and allowed to multiply. 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 leaf for feeding. The laboratory reared larvae were used for bioassay.

2.4. Antifeedant activity

Antifeedant activity of the crude extracts was studied using leaf disc no choice method. Fresh castor leaf discs of 4 cm diameter were punched using cork borer; they were dipped in 0.5%, 1.0%, 2.5%, and 5.0% concentrations of crude extracts individually. The leaf discs dipped in acetone were used as negative control since acetone was used to dissolve the crude extracts. In each plastic petridish (1.5 cm × 9 cm) 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 by the treated and control larvae in 24 h was recorded using Leaf Area Meter (Delta-T Devices, Serial No. 15736 F 96, U.K). 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 antifeedant activity was calculated using the formula of Isman et al. (1990):

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

2.5. Larvicidal activity

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

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

2.6. Larval and pupal durations

The survived larvae were continuously fed with castor leaf. The larval duration was calculated after treated larvae became pupae. Pupal duration was calculated from pupation to the day of emergence of adults.

2.7. Pupicidal activity

Pupicidal activity was calculated by counting dead pupae from the total larvae.

2.8. Statistical analysis

The antifeedant, larvicidal 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 ($P \leqslant 0.05$). LC_{50} and LC_{90} values were calculated using probit analysis (Finney, 1971).

3. Results

Preliminary phytochmical analysis showed the presence of steroids, phenols, flavonoids and alkaloids. The present investigation revealed that maximum antifeedant activities of 56.06% and 49.86% were observed in ethyl acetate and hexane leaf extracts of *A. tagala,* respectively, at 5.0% concentration (Table 1). Least lethal concentration (3.69%) for feeding inhibition was observed in ethyl acetate leaf extract and high square values were significant (Table 2). Minimum antifeedant activity of 31.71% was recorded in root ethyl acetate extract at the same concentration. All the extracts exhibited feeding deterrent activity to some extent and the leaf extract was more toxic than the root extract.

Table 1 Percent antifeedant activity of *A. tagala* extract against *S. litura*.

Crude	Concentration (%)			
	0.5	1.0	2.5	5.0
Leaf extract				
Hexane	20.95 ± 2.23[c]	29.05 ± 3.39[bc]	41.37 ± 3.13[d]	49.86 ± 3.32[d]
Ethyl acetate	26.48 ± 2.82[d]	33.94 ± 4.29[c]	47.48 ± 2.23[e]	56.06 ± 3.83[e]
Root extract				
Hexane	23.11 ± 2.62[cd]	27.84 ± 3.93[bc]	35.53 ± 2.71[c]	42.02 ± 2.32[c]
Ethyl acetate	16.33 ± 2.36[b]	26.56 ± 3.49[b]	28.45 ± 3.34[b]	31.71 ± 3.24[b]
Control	1.88 ± 0.65[a]			

Within the columns, means ± SD followed by the same letter do not differ significantly using Tukey 's test, $P \leqslant 0.05$.
[a] No significance compared to control.
[b] Significant compared to control.
[c] Highly significant compared to control.
[d] Very highly significant compared to control.

Table 2 Effective concentration causing LC_{50}–LC_{90} and χ^2 values of *A. tagala* against *S. litura* for feeding deterrency.

Treatments	LC_{50}	95% fiducial limit		LC_{90}	95% fiducial limit		χ^2
		Lower	Upper		Lower	Upper	
Leaf extract							
Hexane	4.64	4.11	5.39	12.49	10.67	15.29	18.3*
Ethyl acetate	3.69	3.26	4.24	11.53	9.88	14.03	18.1*
Root extract							
Hexane	6.51	5.46	8.34	18.07	14.48	24.62	10.20
Ethyl acetate	10.34	7.61	18.21	26.13	18.24	49.31	22.22*

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

Table 3 Percent larvicidal and pupicial activities and larval–pupal duration observed after treatment of *A. tagala* against *S. litura*.

Plant extract	Concentration (%)	Larvicidal activity (%)	Pucidal activity (%)	Total mortality (%)	Larval duration (days)	Pupal duration (days)
Leaf extract						
Hexane	0.5	0[a]	0[a]	0	8.84 ± 0.91[a]	10.24 ± 1.1[a]
	1.0	0[a]	0[a]	0	9.24 ± 0.95[a]	10.96 ± 0.36[ab]
	2.5	10.44[b]	0[a]	10.44	9.56 ± 0.94[a]	11.16 ± 1.07[ab]
	5.0	20.44[c]	20[bc]	40.44	9.88 ± 0.64[a]	11.48 ± 1.28[abc]
Ethyl acetate	0.5	0[a]	0[a]	0	8.88 ± 0.75[a]	10.64 ± 0.77[ab]
	1.0	8.22[b]	0[a]	0	9.72 ± 1.14[a]	10.88 ± 0.94[ab]
	2.5	22.44[c]	20[bc]	42.44	10.36 ± 1.11[ab]	12.2 ± 1.13[bc]
	5.0	40.66[d]	28[c]	68.66	12.04 ± 0.46[b]	13.08 ± 0.87[c]
Root extract						
Hexane	0.5	0[a]	0[a]	0	8.72 ± 0.70[a]	10.2 ± 0.82[ab]
	1.0	0[a]	0[a]	0	8.8 ± 0.83[a]	10.32 ± 0.54[a]
	2.5	8.22[b]	0[a]	8.22	9.56 ± 1.08[a]	10.88 ± 0.72[ab]
	5.0	12.44[b]	14[b]	26.44	9.76 ± 0.65[a]	11.68 ± 0.54[abc]
Ethyl acetate	0.5	0[a]	0[a]	0	8.76 ± 0.77[a]	10.2 ± 0.53[a]
	1.0	0[a]	0[a]	0	8.92 ± 0.99[a]	10.4 ± 0.80[ab]
	2.5	0[a]	0[a]	0	9.08 ± 0.59[a]	10.44 ± 0.52[ab]
	5.0	0[a]	0[a]	0	9.12 ± 0.52[a]	10.72 ± 0.98[ab]
Control	–	–	0[a]	0	8.64 ± 0.62[a]	10.04 ± 0.26[a]

Within the columns, means ± SD followed by the same letter do not differ significantly using Tukey 's test, $P \leqslant 0.05$.
[a] No significance compared to control.
[b] Significant compared to control.
[c] Highly significant compared to control.
[d] Very highly significant compared to control.

Ethyl acetate leaf extract of *A. tagala* at 5.0% showed higher larvicidal activity of 40.66% than the root extract which exhibited no activity at all the concentrations. Minimum larvicidal activity of 12.44% was noticed in hexane root extract of *A. tagala* (Table 3). Pupicidal activity of 28% in ethyl acetate leaf extract was recorded at 5% concentration. Pupicidal activity was observed in 5% concentration in both leaf and root extracts of all the extracts except ethyl acetate root extract. Ethyl acetate leaf extract exhibited pupicidal activity at 2.5% concentration. A total mortality of 68.06% was observed in ethyl acetate leaf extract at 5% concentration (Table 3).

Maximum larval duration of 12.04 days was found when the larvae were fed on castor leaves treated with ethyl acetate leaf extract of *A. tagala* (Table 3). In control the larval duration was 8.64 days. When the larva was treated with ethyl acetate leaf extract of *A. tagala*, there was a prolongation of pupation by 2.6 days than the control. In the present evaluation, there was a dose dependent effect on larval duration.

Maximum pupal period of 13.08 days was observed in the leaf extract of *A. tagala* against *S. litura* (Table 3). In control, pupal duration was 10.04 days. When the larva was treated with ethyl acetate leaf extract of *A. tagala*, there was a prolongation of pupal period by 3.04 days than the control. Pupal period was increased corresponding with the increased concentration of the test extracts.

4. Discussion

The present investigation revealed that maximum antifeedant activities of 56.06 and 49.86% were observed in ethyl acetate and hexane leaf extracts of *A. tagala,* respectively at 5.0% concentration. Our result coincides with the earlier report of Caasi (1983) who observed that water extract of *A. tagala* showed antifeedant activity against *S. litura*. Also methanol extract of *A. ringens* showed effects of antifeedancy, food poisoning, contact poisoning and repellency against *Sitophilus zeamais* (Arannilewa et al., 2006). Lajide et al. (1993) observed antifeedency in methanol extract of *A. albida* against *S. litura*. Baskar et al. (2010) reported that the ethyl acetate extract of *Couroupita guianensis* exhibited 69.7% against *Helicoverpa armigera* at 5% concentration. The antifeedant activity was due to the presence of steroids, phenols, flavonoids and alkaloids in the ethyl acetate extract of leaf. This observation is corroborated with some earlier findings of Baskar et al. (2009, 2010) for alkaloids who reported antifeedant activity against *H. armigera*; anti-insect and pharmacological activities have been reported for flavonoids and phenolic compounds (Yao et al., 2004); steroids present in *Ajuga reptans* inhibited the feeding (Camps and Coll, 1993).

Ethyl acetate leaf extract of *A. tagala* at 5.0% showed higher larval mortality of 40.66%. These results support the earlier findings of Jbilou et al. (2006) who observed potential insecticidal agents for the control of the larvae of *Anticarsia gemmatalis* in acetone and ethanol extracts of *A. pubescens*. *A. albida* plant had acidic metabolites like aristolic acid, aristolochic acid, aristoloctam and aristolone which exhibited larval mortality against *S. zeamais* (Arannilewa et al., 2006). Elumalai et al. (2004) reported that ethyl acetate leaf extract of *Acorus calamus* at 5.0% exhibited maximum larvicidal activity of 40.24% against *S. litura*. Ethyl acetate extract of *Artemesia nilagrica* had 40.24% of larval mortality against *S. litura* (Raja et al., 2003). Similarly Pavela (2004) reported that leaf extract of *Marrubium vulgare* at 5.0% concentration exhibited 42.2% of larval mortality. The extracts might have arrested the various metabolic activities of the larvae. Ultimately the larvae failed to feed and the development was arrested in various instar stages.

Maximum larval duration of 12.04 days was found when the larvae were fed on castor leaves treated with ethyl acetate extract of *A. tagala*. *A. ringens* extract suppressed the growth and development of *S. zeamais* (Arannilewa et al., 2006). Nascimento et al. (2004) observed that *A. pubescens* inhibited larval growth of *A. gemmatalis*. Vendan et al. (2008) observed that the ethyl acetate extract of *Mundulea sericea* increased the larval duration of *H. armigera*. Neem limnonoids increased the larval duration of *Cnaphalocrocis medinalis* (Senthil Nathan et al., 2006). The present study revealed that there was a dose dependent effect on larval duration.

Maximum pupal period of 13.08 days was observed in the leaf extract of *A. tagala* against *S. litura*. Similar results were also noticed by Sedak (2003) in *Adhatoda vasica* extracts against *S. littoralis* and Senthil Nathan and Sehoon (2006) in *Melia azedarach* extracts against *Hyblaea puera*. Pupal period was increased corresponding with increasing concentration of the test extracts.

At 5.0% ethyl acetate leaf extract of *A. tagala*, pupicidal activity was 28%. This corroborated with the findings of Baskar et al. (2009) who observed pupicidal activity in different crude extract of *Atalantia monophylla* against *H. armigera*. Malarvannan et al. (2008) observed that *Argemone mexicana* extracts reduced adult emergence and increased pupal mortality of *S. litura*.

In the present study, phytochemicals namely, steroids, alkaloids, phenols and flavonoids were found in *A. tagala*. These phytochemical compounds do not cause any harmful effects on human or environment since these phytochemicals have shown effective antioxidant and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging potential (Thirugnanasampandan et al., 2008), antifertility activity (Balaji et al., 2004), and anticancer properties (Venkata Krishnaiah et al., 2008).

5. Conclusion

The ethyl acetate extract of *A. tagala* at 5.0% concentration showed higher antifeedant, larvicidal and pupicidal activities, prolonged the larval and pupal duration. Hence it is inferred that the ethyl acetate extract of *A. tagala* can be used further for the solation of active molecules and to develop a new botanical formulation for the management of *S. litura*.

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