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ORIGINAL ARTICLE

Antifeedant and larvicidal activities of *Acalypha fruticosa* Forssk. (Euphorbiaceae) against *Plutella xylostella* L. (Lepidoptera: Yponomeutidae) larvae

S. Lingathurai, S. Ezhil Vendan, M. Gabriel Paulraj, S. Ignacimuthu *

Entomology Research Institute, Loyola College, Chennai 600 034, India

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Abstract Antifeedant and larvicidal activities of hexane, chloroform and ethyl acetate extracts of *Acalypha fruticosa* Forssk. Leaves were studied using leaf disc no-choice method against third instar larvae of *Plutella xylostella* L. Chloroform extract showed maximum antifeedant activity of 92.8% at 5% concentration with a LC_{50} value of 1.86%. Nine fractions were collected from the chloroform extract (30 g) by silica gel column chromatography, among which the seventh fraction (eluted by 100% ethyl acetate) recorded maximum antifeedant activity (84.3%) at 1000 ppm concentration with a LC_{50} value of 385.7 ppm against the third instar larvae of *P. xylostella*. Preliminary phytochemical analysis of this effective fraction showed the presence of terpenoids, tannins, coumarins, anthraquinones and saponins.

1. Introduction

Diamondback moth, *Plutella xylostella* L. is a serious pest of cauliflower, cabbage, broccoli, mustard, radish and turnip (Atwal, 1976; Eusebion and Rajesus, 1996; Capinera, 2001; Mohan and Gujar, 2003). Due to intensive use of insecticides, this insect has developed resistance to nearly all classes of insecticides (Shelton et al., 1993) including *Bacillus thuringiensis* (Bt) crystal (Cry) toxins (Sayyed and Wright, 2002). Outbreaks of *P. xylostella* in Southeast Asia often caused more than 90% crop loss (Verkerk and Wright, 1996), mainly because it produced numerous generations per year and was able to rapidly increase in numbers (Rowell et al., 2005). The lack of effective natural enemies and the destruction of natural enemies by chemical insecticides contributed to the rise of pest status of this insect in many regions (Waterhouse and Norris, 1987; Ooi, 1992).

It is imperative to discover a novel ecofriendly pesticide against this pest. Several investigators have screened many plant products against *P. xylostella*. Morallo-Rejesus (1986) has recorded the oviposition and repellent activity of plants belonging to Asteraceae, Fabaceae and Euphorbiaceae families. Ling et al. 2008 found that *Momordica charantia* leaf

* Corresponding author. Tel.: +91 44 2817 8348; fax: +91 44 2817 5566.
E-mail address: entolc@hotmail.com (S. Ignacimuthu).

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extract and compounds showed strong feeding deterrent activity and developmental inhibition in *P. xylostella* larvae.

The present work was undertaken to study the efficacy of hexane, chloroform and ethyl acetate extracts and fractions of *Acalypha fruticosa* Forssk. leaves, against *P. xylostella* larvae. *A. fruticosa* is a medicinal plant used in traditional medicines to cure stomachache, digestive disorders, dyspepsia, colic and diarrhoea (Sripathi and Uma, 2010). No previous reports are available on the biological activity of *A. fruticosa* against *P. xylostella*.

2. Materials and methods

2.1. Plant collection

Leaves of *A. fruticosa* Forssk. were collected from Maruthamalai hills, Coimbatore district, Tamil Nadu, India. The plant was authenticated by a plant taxonomist from the Division of Ethnopharmacology Entomology Research Institute, Loyola College, Chennai. A voucher specimen (ERIH 1351) has been deposited at the herbarium of Entomology Research Institute, Loyola College, Chennai.

2.2. Preparation of plant extract

Leaves were shade dried at room temperature and powdered by an electric blender. One kilogram of leaf powder was soaked in 3 L of *n*-hexane for a period of about 72 h and extracted through Buchner funnel with filter paper. The filtrate was evaporated to dryness under reduced pressure using rotary vacuum evaporator. The remains of the plant material were extracted with chloroform and ethyl acetate sequentially in a similar manner.

2.3. Chromatographic separation of fractions

The crude chloroform extract was subjected to column chromatography. A glass column (8 cm diameter:60 cm long) was packed with silica gel (240 g-acme's 100–200 mesh) and an admixture of crude chloroform extract (30 g) and silica gel (60–120 mesh) was loaded on the top of the silica gel column. The column was continuously eluted with *n*-hexane followed by different combinations of *n*-hexane:chloroform, chloroform: ethyl acetate and ethyl acetate:acetone ranging from 95:5 to 0:100, 5:95 to 0:100 and 50:50 to 100, respectively. Fractions were collected in 200 mL conical flasks and they were checked with thin layer chromatography (TLC). Based on the TLC patterns similar fractions were pooled together and nine major fractions were finally obtained.

2.4. Insects

P. xylostella L. larvae were obtained from a culture that was maintained on cauliflower heads at laboratory conditions (26 ± 1 °C; 11 ± 0.5 h photoperiod; 65–70% R.H.). Newly emerged third instar larvae were used in bioassay studies.

2.5. Treatment schedule

Different concentrations of crude extracts viz., 0.625%, 1.25%, 2.5% and 5% and fractions viz., 125, 250, 500 and 1000 ppm were prepared by dissolving required amount of crude or fraction in 500 µL of acetone and made up to 20 vmL with distilled water.

2.6. Antifeedant activity experiment

Antifeedant activity of crude extracts and fractions were studied following leaf disc no-choice method. Fresh and tender cauliflower leaf discs of 3 cm diameter were punched using cork borer; the leaf discs were dipped in different concentrations of crude extracts and fractions separately and air dried for 3 min. One treated leaf disc was kept in a petri plate and a single 4 h pre-starved third instar larva of *P. xylostella* was introduced into the petri plate. The leaf discs treated with acetone were used as solvent control and azadirachtin (40.86% purity, obtained from EID-Parry, India Ltd., Chennai) was used as a reference control. Each test was replicated 24 times. Progressive consumption of leaf area by the larva in 24 h period was recorded in control and treatments using leaf area meter (Delta-T Devices, Serial No. 15736 F 96, UK). Leaf area consumed in treatment was corrected from the control. Antifeedant activity was calculated by the modified formula of Bentley et al. (1984):

$$\text{Antifeedant activity} = [(C - T)/C] \times 100 \quad (1)$$

where C is the leaf area consumed in control discs and T is the leaf area consumed in treated disc.

2.7. Larval mortality bioassay

For toxicity bioassay experiment, third instar larvae were treated by oral application through cauliflower leaf discs. Concentrations were the same as in the antifeedant bioassay. After 24 h of treatment the larvae were continuously maintained on non-treated fresh cauliflower leaves. Diet was changed every 24 h. Larval mortality was recorded up to 96 h of treatment. Twenty four replicates were maintained for each treatment.

2.8. Preliminary phytochemical analysis

Phytochemical analysis of all the nine fractions was carried out following the method of Harborne (1998).

2.9. Statistical analysis

Antifeedant and larvicidal activities of the different crude extracts and fractions were statistically analyzed by one way Analysis of Variance. Significant differences between treatments were determined using Tukey's multiple range test at $P = 0.05$. Probit analysis was done to calculate median lethal concentration (LC_{50}) and LC_{90} using SPSS 11.5 version software package.

3. Results

3.1. Antifeedant activity of A. fruticosa crude extracts

Table 1 shows the per cent antifeedant activity of *A. fruticosa* crude extracts against *P. xylostella* larvae after 24 h treatment. Among the three solvent extracts chloroform extract recorded

Table 1 Antifeedant activity of crude extracts of *A. fruticosa* leaves against 3rd instar *P. xylostella* larvae ($n = 24$) after 24 h.

Plant extract	Concentration (%)			
	0.625	1.25	2.5	5
n-Hexane	12.5 ± 1.9^{c}	23.2 ± 2.0^{bc}	29.6 ± 3.5^{c}	41.4 ± 2.2^{c}
Chloroform	25.8 ± 1.4^{a}	42.7 ± 3.2^{a}	78.3 ± 3.3^{a}	92.8 ± 4.8^{a}
Ethyl acetate	19.2 ± 0.5^{b}	26.5 ± 2.5^{b}	50.6 ± 4.2^{b}	64.0 ± 5.1^{b}

Within columns, mean ± SD followed by the same letter do not differ significantly using Tukey's test, $P \leqslant 0.05$.

significantly high antifeedant activity at all four concentrations. At 0.625%, 1.25%, 2.5% and 5% concentrations of chloroform extract 25.8%, 42.8%, 78.3% and 92.8% antifeedant activities were recorded respectively. The effect of chloroform extract was statistically significant ($P \leqslant 0.05$). Ethyl acetate extract showed 64% antifeedant activity at 5% concentration. In all the treatments the antifeedant activity was directly proportional to the concentration of the extract.

3.2. Larvicidal activity and lethal concentrations of crude extracts

Among the three different solvent extracts tested chloroform extract was identified as the most toxic treatment against third instar larvae of *P. xylostella* at 5% concentration. Chloroform extract recorded 69.6% larvicidal activity at 96 h. The larval mortality was directly related to the concentration of the treatments. Ethyl acetate extract also recorded high larval mortality. The chloroform extract showed LC50 value of 1.86% and LC90 value of 6.27% (Table 2). The Chi-square values were significant at $P \leqslant 0.05$ level. The high Chi-square values in the bioassays probably indicated the heterogeneity of the test population. Different crude and fractions influenced larval mortality differently.

3.3. Chromatographic separation of chloroform extract of A. fruticosa

The crude chloroform extract of *A. fruticosa* yielded nine fractions when it was separated by column chromatography.

3.4. Antifeedant activity of fractions

Table 3 shows the per cent antifeedant activity of nine different fractions of chloroform extract of *A. fruticosa* against third instar larvae of *P. xylostella* after 24 h treatment. Among the nine different fractions, fraction seven (eluted by 100% ethyl acetate) recorded significantly high antifeedant activity at 125 (39.6%), 250 (56.4%), 500 (62.2%) and 1000 (84.3%) ppm concentrations compared to other fractions. Azadirachtin was used as reference control. The active fraction (7) was found to be significantly more effective than azadirachtin at 125 and 250 ppm concentrations. However at 500 and 1000 ppm concentrations, the antifeedant activity of azadirachtin was slightly higher than fraction 7. The antifeedant activity of all fractions was directly proportional to the concentration. Fraction 8 also showed good antifeedant activity at all concentrations.

3.5. Larvicidal activity and lethal concentration of fractions

Fraction 7 recorded the maximum larvicidal activity at all concentrations. At 125, 250, 500 and 1000 ppm concentrations fraction 7 recorded 24.7%, 42.9%, 50.5% and 66.7% larval mortality respectively. Table 4 shows that fraction 7 required the least median lethal concentration (LC50) of 385. 7 ppm for 96 h. The lower and upper limits for 95% confidence were calculated as 273.5 and 563.6 ppm respectively for fraction 7. The LC90 of fraction 7 was calculated as 1025.3 ppm which was the least concentration compared to other fractions. The Chi-square values were significant at $P \leqslant 0.05$ level.

3.6. Preliminary phytochemical analysis of fractions

The preliminary phytochemical analysis of all the fractions was done to identify the type of phytochemical groups present in them. The results indicated that more phytochemical groups were present in fractions 6 and 7 (Table 5). The effective fraction 7 contained maximum number of phytochemical groups namely terpenoids, tannins, coumarins, anthraquinones and saponins. Fractions 1, 3, 4, 5 and 9 contained only one phytochemical group.

4. Discussion

The antifeedant and insecticidal bioassays clearly indicated that chloroform extract of *A. fruticosa* leaves was more effective than hexane and ethyl acetate treatments. Feeding

Table 2 Lethal concentrations calculated for different solvent extracts of *A. fruticosa* leaves after 24 h treatment.

Plant extract	LC_{50}%	95% Confidence limit		LC_{90}%	95% Confidence limit		Chi-square
		Lower	Upper		Lower	Upper	
n-Hexane	5.20	3.96	12.65	12.90	10.50	25.30	0.528*
Chloroform	1.86	1.38	2.43	6.27	4.69	12.48	0.632*
Ethyl acetate	3.31	2.41	7.67	7.70	6.45	21.99	0.092*

LC_{50} and LC_{90} values are expressed as percentage ($n = 24$).
* χ^2 values are significant at $P \leqslant 0.05$ levels.

Table 3 Antifeedant activity of *A. fruticosa* fractions against third instar larvae of *P. xylostella* (mean ± SD) ($n = 24$).

Fractions	Concentration (ppm)			
	125	250	500	1000
1	9.2 ± 0.5^{e}	21.5 ± 3.5^{de}	30.6 ± 4.2^{g}	34.0 ± 5.0^{f}
2	21.9 ± 0.7^{c}	30.6 ± 1.9^{d}	45.5 ± 3.6^{d}	50.5 ± 1.0^{de}
3	18.1 ± 3.3^{d}	25.3 ± 0.6^{e}	29.9 ± 1.5^{g}	38.5 ± 5.0^{ef}
4	22.5 ± 1.0^{c}	31.2 ± 1.2^{d}	39.7 ± 1.4^{e}	52.3 ± 1.0^{de}
5	18.1 ± 3.7^{d}	31.7 ± 3.0^{de}	35.4 ± 2.9^{f}	57.9 ± 3.8^{d}
6	23.4 ± 1.3^{c}	37.2 ± 2.6^{c}	49.9 ± 3.6^{cd}	68.8 ± 3.0^{d}
7	39.6 ± 0.7[a]	56.3 ± 3.5[a]	62.2 ± 1.2^{b}	84.3 ± 1.5^{b}
8	27.4 ± 1.8^{b}	41.2 ± 2.9^{c}	54.9 ± 2.1^{c}	76.8 ± 6.0^{c}
9	16.1 ± 3.3^{d}	29.7 ± 3.0^{de}	32.3 ± 2.9^{f}	37.4 ± 1.3^{ef}
Azadirachtin[a]	24.5 ± 0.8^{c}	46.9 ± 3.4^{b}	68.4 ± 3.8[a]	89.5 ± 5.0[a]

Thin columns, mean ± SD followed by the same letter do not differ significantly using Tukey's test, $P \leqslant 0.05$.
[a] Reference control.

Table 4 Lethal concentrations calculated for each fraction obtained from chloroform extract of *A. fruticosa* leaves after 24 h treatment against third instar *P. xylostella* larvae.

Fractions	LC_{50} ppm	95% Confidence limit		LC_{90} ppm	95% Confidence limit		Chi-Square
		Upper	Lower		Upper	Lower	
1	3035.72	1773.40	9913.48	6932.91	4732.94	10240.06	2.642*
2	1715.57	1179.16	3377.23	1839.78	1249.94	13066.07	2.548*
3	745.72	596.72	1023.08	4955.44	2140.62	12719.43	3.208*
4	1320.12	1025.94	1968.29	5423.80	3213.18	13369.29	2.946*
5	902.86	652.94	1387.07	1845.08	1198.00	25530.27	0.868*
6	1105.92	702.75	3097.65	3746.11	1499.91	19928.75	0.611*
7	385.72	273.55	563.66	1025.37	785.93	13990.84	0.814*
8	1033.25	858.84	1298.07	2437.96	1943.43	28420.00	0.611*
9	993.52	755.76	1396.98	1759.79	1177.36	29639.16	4.466*
Azadirachtin[a]	271.48	223.97	321.84	1420.50	1038.70	2285.64	0.233*

LC_{50} and LC_{90} values are expressed as parts per million ($n = 24$).
[a] Reference control.
* χ^2 values are significant at $P \leqslant 0.05$ levels.

Table 5 Preliminary phytochemical analysis of *A. fruticosa* fractions of chloroform extract.

Phytochemical	Fractions								
	1	2	3	4	5	6	7	8	9
Steroids	−	−	−	−	−	−	−	−	−
Terpenoids	+	−	−	−	−	+	+	−	−
Phenol	−	−	−	−	−	−	−	−	−
Tannin	−	−	+	−	−	+	+	+	+
Coumarin	−	−	−	+	+	+	+	−	−
Flavonoid	−	−	−	−	−	−	−	−	−
Quinone	−	+	−	−	−	−	−	−	−
Anthraquinone	−	+	−	−	−	+	+	+	−
Alkaloids	−	−	−	−	−	−	−	−	−
Saponins	−	−	−	−	−	−	+	−	−

(−) Absent. (+) Present.

deterrent activity of plant extracts and plant products against *P. xylostella* has been reported by many investigators (Ling et al., 2008; Patil and Goud, 2003). *Azadirachta indica* A. Juss. (Meliaceae), *Acorus calamus* L. (Araceae) and *Melia azedarach* L. (Meliaceae) treatments were found to inhibit *P. xylostella* feeding after 24 h of treatment (Patil and Goud 2003). An important finding in this study was that the most effective antifeedant treatment also gave the maximum larvicidal activity. Koul et al. (2004) and Chen et al. (1996) also stated that *Aglaia elaeagnoidea* extract caused maximum antifeedant as well as larvicidal activities against *Helicoverpa armigera* and *Pieris rapae*. Ling et al. (2008) also reported that *Mimordica*

charantia ethanol extract had both feeding deterrent and larvicidal activities against *P. xylostella*. Fraction 7 was composed of five different phytochemical groups namely, terpenoids, tannins, coumarins, anthraquinones and saponins. Already terpenoids (Lago et al., 2002; Nathan et al., 2005), tannins (Lago et al., 2002), anthraquinones (Lingathurai et al., 2010) and coumarins (Baskar et al., 2010) in plants were reported to be antifeedants and insecticides against *H. armigera* and rice leaf folder larvae. Tannins are generally considered to be harmful to lepidopteran insects (Coley et al., 1985). The maximum insecticidal and antifeedant activity of fraction 7 might be due to the additive effect of these five phytocompounds. Paulraj and Ignacimuthu (2010) reported that the solvent extracts of mangrove plants *Bruguiera cylindrica* and *Rhizophora apiculata,* which contained flavonoids and quinones showed higher antifeedant and insecticidal activities against *Atractomorpha crenulata* adults and *Epilachna viginioctopunctata* grubs. Presence of more than one major phytocompound inhibited the growth of microbes and insects (Park et al., 2002; Mansour et al., 2004; Ahmad and Aqil, 2007). Saponins were reported to be insecticidal by many investigators (Marston and Hostettmann, 1985; Jeong et al., 2004; Sparg et al., 2004; McGaw et al., 2008). Tabashnik (1985) has reported that the secondary plant compounds, coumarins and rutins, were known to deter *P. xylostella* oviposition. Jackson and Peterson (2000) stated that the legume *Melilotus officinalis* (L.), containing coumarin, was toxic to *P. xylostella*. *A. fruticosa* belongs to the family Euphorbiaceae. Morallo-Rejesus (1986) reported that plants belonging to Euphorbiaceae family were highly repellent to *P. xylostella* and other lepidopteran pests.

In conclusion the plant *A. fruticosa* showed feeding deterrent and insecticidal activities against the larvae of *P. xylostella*. Since the active fraction possessed both antifeedant and larvicidal activities a single application of this phytopesticide in cruciferous crops can reduce crop damage as well as pest population.

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