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Bioefficacy of *Couroupita guianensis* (Aubl) against *Helicoverpa armigera* (Hub.) (Lepidoptera: Noctuidae) larvae

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Abstract

With a view to develop an ecofriendly botanical pesticide, we evaluated the bioefficacy of crude extracts of *Couroupita guianensis* against third instar larvae of *Helicoverpa armigera.* Maximum feeding deterrency (81.67%) and least LC_{50} (2.72%) for larval mortality were seen in hexane extract. Hexane extract was subjected to column chromatography using different ratio of hexane-ethyl acetate solvent system. Totally eight fractions were collected. The fractions were screened at 125, 250, 500 and 1,000 mg kg^{-1} concentrations against *H. armigera* using no-choice leaf disc method. Fraction eight showed maximum antifeedant (86.24%) and larvicidal (80.88%) activities at 1,000 mg kg^{-1} concentration. *C. guianensis* could be utilized in pest control programme.

Additional key words: antifeedant, hexane, larvicidal, phytochemicals.

Resumen

Eficacia de *Couroupita guianensis* (Aubl) frente a larvas de *Helicoverpa armiguera* (Hub.) (Lepidoptera: Noctuidae)

Con el fin de desarrollar un plaguicida ecológico de origen vegetal, se evaluó la eficacia de extractos crudos de *Couroupita guianensis* frente a larvas de tercer estadio de *Helicoverpa armígera*. En el extracto de hexano se observó la máxima actividad disuasoria de la alimentación (81,67%) y la menor LC_{50} (2,72%) respecto a la mortalidad de las larvas. Se sometió el extracto de hexano a cromatografía en columna, utilizando diferentes proporciones del sistema disolvente hexano-acetato de etilo para eluir ocho fracciones. Las fracciones se evaluaron en concentraciones de 125, 250, 500 y 1.000 mg kg^{-1} frente a *H. armígera,* mediante ensayos de no preferencia con discos foliares. La fracción octava mostró la máxima actividad antialimentaria (86,24%) y larvicida (80,88%) a 1.000 mg kg^{-1}. *C. guianensis* se podría utilizar por tanto en programas de control de plagas.

Palabras clave adicionales: actividad antialimentaria, actividad larvicida, fitoquímicos, hexano.

Introduction

In the last five decades, many countries including India have concentrated on non-polluting and economic entomological technologies to increase the productivity of vegetable crops and economically important trees. Insect pests play a major role in damaging the crops and hence there is a need to use efficacious control agents. Crop loss due to insect pests varies between 10 to 30% for major crops (Ferry *et al*., 2004). In the last few decades, the repeated use of synthetic chemicals to manage these pests has lead to their resurgence and outbreak, resistance to insecticides, elimination of existing natural enemies and pollution of soil, water, air and food (Patel *et al*., 1992). Hence, search for viable and sustainable alternatives to synthetic pesticides is given priority (George and Seenivasagan 1998). More than 2,000 species of plants are known to possess insecticidal properties (Klocke, 1989). Prakash and Rao (1997) observed that plant pesticides do not contribute to resistance development or pest resurgence; nor do they cause negative effects on non-target organisms. They do not affect plant growth, seed viability and food quality but possess insecticidal, repellent, antifeedant and/or growth regulatory activities. Botanical pesticides tend to have broad-spectrum activity, are relatively specific in their mode of action, easy to process, produce, use and safe for higher animals and the environment (Talukder and Howse, 1994). Plants

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are endowed with a potential to produce a range of secondary metabolites like alkaloids, terpenoids, flavonoids, phenols, glycosides, sitosterols and tannins. These phytochemicals are known to protect the plants from the attack of insect-pests (Ahmad, 2007). The phytochemicals produced in response to insect-pest attack, affect feeding and oviposition of insects on the plants. Phytochemicals have shown different kinds of actions that can modify specific physiological processes in insects (Liu *et al*., 2007). Koul *et al*. (2003) noticed that plant chemicals' defense against insect herbivory almost never depends on a single compound, but instead several compounds interact with pests, individually or in unison. Application of biopesticides has been reported to have positive impacts on bollworm population management (Ge and Ding, 1996). Most of the tested botanical extracts proved to be strong growth inhibitors, acutely toxic and active feeding deterrents against lepidopteran species (Akhtar *et al*., 2008). The American bollworm, *Helicoverpa armigera* is a polyphagous noctuid feeding on 181 plant species of 39 families. *Couroupita guianensis* (Aubl.) belonging to the family Lecythidaceae possesses insecticidal properties. Methanol extract derived fractions of *C. guianensis* inhibited the growth of microorganisms (Khan *et al*., 2003). Petroleum ether and chloroform extracts of this plant exhibited larvicidal activity against vectors (Desal *et al*., 2003). The present study was undertaken to assess the antifeedant and larvicidal activities of *C. guianensis* against the notorious agricultural pest, *H. armigera*.

Material and methods

Plant collection and extract preparation

Leaves of *C. guianensis* were collected from Loyola College Campus, Chennai, Tamil Nadu, India, on 25th October 2005. The plant was identified by Dr. Ayyanar, taxonomist, at Entomology Research Institute, Loyola College. The voucher specimen (ERIH: 1310) was deposited at the institute herbarium. The plant material was shade dried at room temperature and powdered coarsely. The powder (2.0 kg) was soaked in hexane, chloroform and ethyl acetate for a period of 48 h sequentially with intermittent shaking and filtered. The extracts were concentrated under reduced pressure using rotary evaporator and stored at 4°C. The yield of hexane extract was 23.4 g, chloroform extract was 20.9 g and ethyl acetate extract was 22.1 g.

Column chromatography

Hexane extract of leaves of *C. guianensis* was fractionated using column chromatography on silica gel column. The other solvent extracts were discarded due to less activity. Hexane extract (22.2 g) was mixed with 60 g of silica gel (60-120 mesh) and the admixture was packed with 250 g-acme's of silica gel (100-200 mesh) in the column and successively eluted following a stepwise gradient ratio of hexane and ethyl acetate: 100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 25:75 and 0:100.

Phytochemical screening

Phytochemical analysis of the crude extracts was carried out following the method of Harbone (1998).

Insect culture

Larvae of *H. armigera* were collected from the field in Salamangalam, Kancheepuram district, Tamil Nadu. The collected larvae were reared individually in a plastic container (vials) and fed regularly with bhendi, *Abelmoschus esculentus* L. (Malvaceae) till the larvae became pupae under the laboratory conditions (27 ± 2°C and 75 ± 5% relative humidity). Sterilized soil was provided for pupation. After pupation, the pupae were collected from soil and placed inside the cage for emergence of adults. Cotton soaked with 10% honey solution mixed with a few drops of multivitamins was provided for adult feeding to increase the fecundity. Potted cowpea plant was kept inside adult emergence cage for egg laying. After hatching the larvae were collected from the cage and fed with standard artificial diet (Koul *et al*., 1997). The laboratory reared third instar larvae were used for the present investigation.

Antifeedant activity

Antifeedant activity of the crude extracts was studied using leaf disc no-choice method. Fresh cotton 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 and 125, 250, 500 and 1,000 mg kg^{-1} concentrations of fractions individually. The leaf discs dipped in acetone were used as negative control since acetone was used to dissolve the crude

extracts and fractions. Azadirachtin (40.86% purity, obtained from EID-parry, India Ltd., Chennai) was used as positive control. In each plastic Petri dish (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 Petri dish. 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 experiment was conducted at laboratory condition (27 ± 2°C) with 14:10 light and dark photoperiod and 75 ± 5% relative humidity**.** Antifeedant activity was calculated according to the formula of Bentley *et al*. (1984):

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

Larvicidal activity

Larvicidal activity was studied using leaf disc no-choice method**.** The cotton leaf discs were dipped in different concentrations of crude extracts and fractions. They were placed in petri dishes and the larvae were introduced as in the antifeedant experiment. After 24 h treatment the larvae were continuously maintained on the nontreated fresh cotton leaves. Diet was changed every 24 h. Larval mortality was recorded after 96 h of treatment. Five replicates were maintained for each treatment with 10 larvae per replicate (total n = 50). The laboratory conditions were the same as in the antifeedant experiment. Percent mortality was calculated according to Abbott (1925):

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

Statistical analysis

The data related to antifeedant and larvicidal activities were analysed using one way ANOVA. Significant differences between treatments were determined using Tukey's multiple range test ($P \leq 0.05$). LC_{50} and LC_{90} values were calculated using probit analysis (Finney, 1971).

Results

Phytochemical analysis

Phytochemical investigation of the crude extracts revealed the presence of alkaloids, coumarin and quinone in the crude extracts of *C. guianensis* (Table 1).

Antifeedant activity

The present study revealed that maximum antifeedant activity of 81.67% was recorded in hexane leaf extract of *C. guianensis* at 5.0% concentration followed by chloroform (73.68%) and ethyl acetate (69.70%) extracts (Table 2). The effective hexane crude extract was fractionated using silica gel column chromatography with different solvent system; finally eight fractions were isolated and they were screened for antifeedant activity. Fraction 8 showed highest antifeedant activity at all concentrations tested. Maximum antifeedant activity of 86.24% was noticed in fraction 8 at 1,000 mg kg^{-1} concentration (Table 3). Third and seventh fractions recorded more than 70% of feeding deterrency at 1,000 mg kg^{-1} concentration. Minimum antifeedant activity was recorded in fourth fraction.

Table 1. Phytochemical analysis of crude extracts of *C. guianensis*

Phytochemicals	Hexane	Chloroform	Ethyl acetate
Steroid	–	–	–
Terpinoids	–	–	+
Phenols	–	–	–
Tanin	–	–	–
Coumarin	+	+	–
Flavenoid	–	–	–
Quinone	+	+	+
Anthroquinone	–	–	–
Alkaloid	+	–	–
Saponin	–	–	–

+: indicates presence. –: indicates absence.

Table 2. Per cent antifeedant activity of crude extracts of *C. guianensis* against *H. armigera*

Crude extract	Concentration (%)			
	0.5	1.0	2.5	5.0
Hexane	45.28 ± 3.94^{d}	54.97 ± 2.69^{c}	72.83 ± 3.60^{c}	81.67 ± 2.02^{c}
Chloroform	36.69 ± 5.84^{c}	47.64 ± 5.76^{c}	63.22 ± 3.21^{b}	73.68 ± 2.75^{b}
Ethyl acetate	24.38 ± 4.24^{b}	39.64 ± 4.87^{b}	66.68 ± 4.37^{bc}	69.70 ± 2.92^{b}
Control	6.40 ± 2.35^{a}			

Within the column, means ± SD followed by the same letter does not differ significantly (Tukey's test, $P \leq 0.05$).

Table 3. Antifeedant activity (%) of *C. guianensis fractions* against *H. armigera*

Fractions	Concentration (mg kg^{-1})			
	125	250	500	1,000
1	44.41 ± 4.55^{de}	52.79 ± 5.22^{d}	56.60 ± 5.24^{de}	61.65 ± 3.88^{d}
2	46.76 ± 2.38^{ef}	55.49 ± 3.11^{d}	61.91 ± 4.82^{def}	65.44 ± 3.86^{de}
3	48.13 ± 1.89^{ef}	57.75 ± 3.96^{d}	65.89 ± 3.98^{ef}	71.70 ± 4.00^{ef}
4	18.04 ± 6.68^{b}	25.49 ± 5.92^{b}	34.28 ± 5.86^{b}	41.55 ± 5.20^{b}
5	30.97 ± 5.13^{c}	39.07 ± 3.94^{c}	43.54 ± 5.57^{bc}	49.15 ± 4.13^{bc}
6	35.45 ± 7.12^{cd}	43.60 ± 6.84^{c}	52.58 ± 5.96^{cd}	57.04 ± 7.78^{cd}
7	50.41 ± 3.50^{ef}	61.53 ± 1.99^{de}	70.03 ± 5.14^{fg}	76.09 ± 2.69^{f}
8	54.36 ± 4.52^{f}	67.05 ± 4.64^{e}	75.86 ± 4.19^{g}	86.24 ± 2.68^{g}
Azadirachtin	75.11 ± 3.52^{g}	81.03 ± 2.23^{f}	86.85 ± 2.03^{h}	93.42 ± 1.81^{g}
Control	5.19 ± 1.98^{a}			

Within the column, means ± SD followed by the same letter does not differ significantly (Tukey's test, $P \leq 0.05$).

Larval mortality

Toxic effect was observed in all the extracts. Maximum effect was seen in hexane extract at 5.0% concentration with the LC_{50} value of 2.72% followed by chloroform and ethyl acetate extracts with LC_{50} values of 5.21 and 7.22% respectively (Table 4). The Chi-square values were significant at $P < 0.05$ level. The high Chi-square values in the bioassays probably indicated the heterogeneity of the test population. Different fractions influenced larval mortality differently. Maximum larval mortality of 80.88% was observed in eighth fraction at 1,000 mg kg^{-1} and the LC_{50} and LC_{90} values were 413.30 and 1,181.68 mg kg^{-1} respectively (Table 5). Fifth fraction showed the LC_{50} and LC_{90} values at 1,456.98 and 2,233.87 mg kg^{-1} concentrations respectively. Larval mortality was nil in fourth fraction. Third, seventh and eighth fractions showed larvicidal activity at all concentrations. Less than 50% of larval mortality was observed in fifth and sixth fractions. Statistically significant activity was seen in fraction 8 when compared with other fractions. Low polar hexane extract and fractions of hexane extract produced high larval mortality comparable to that of Azadirachtin.

Table 4. Effective concentrations of LC_{50}-LC_{90} and χ^2- values of *C. guianensis* against *H. armigera*

Crude	LC_{50}	95% fiducial limit		LC_{90}	95% fiducial limit		Chi-square
		Lower	Upper		Lower	Upper	
Hexane	2.72	2.32	3.19	7.57	6.49	9.29	55.06*
Chloroform	5.21	4.48	6.42	9.40	7.82	12.28	95.33*
Ethyl acetate	7.22	5.86	10.15	12.59	9.78	18.91	84.49*

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

Table 5. Larvicidal activity (%) LC_{50}-LC_{90} and χ^2 values of *C. guianensis* fractions against *H. armigera*

Fractions	Concentrations (mg kg^{-1})				LC_{50} (95% FL) (lower-upper)	LC_{90} (95% FL) (lower-upper)	χ^2
	125	250	500	1,000			
1	0^{a}	23.55^{b}	40.44^{d}	61.77^{d}	766.72 666.25-906.68	1,435.08 1,221.25-1,802.32	129.72*
2	21.33^{cd}	23.55^{b}	59.55^{ef}	63.99^{d}	624.00 524.35-751.32	1,551.91 1,291.40-2,020.53	88.12*
3	17.11^{bc}	29.77^{bc}	40.4^{d}	61.77^{d}	737.07 666.51-825.28	1,723.38 1,521.40-2,014.20	30.78*
4	0^{a}	0^{a}	0^{a}	0^{a}	—	—	—
5	0^{a}	0^{a}	12.66^{b}	19.33^{b}	1,456.98 1,221.41-1,949.29	2,233.87 1,797.19-3,189.22	85.75*
6	0^{a}	0^{a}	21.33^{c}	33.99^{c}	1,138.69 996.36-1,379.53	1,779.37 1,503.28-2,290.31	108.82*
7	12.88^{b}	35.99^{cd}	51.08^{e}	65.99^{d}	628.86 539.84-739.99	1,510.62 1,281.27-1,895.81	76.07*
8	25.33^{d}	42.66^{d}	61.77^{f}	80.88^{e}	413.30 359.15-466.04	1,181.68 1,064.44-1,341.75	35.70*
Aza	44.88^{e}	63.99^{e}	97.77^{g}	100^{f}	163.13 134.73-186.86	396.97 361.55-446.18	43.76*

Within the column, means followed by the same letter do not differ significantly (Tukey's test, $P \leq 0.05$). * χ^2 values are significant at $P < 0.05$ levels. FL: fiducial limit.

Discussion

In the present study maximum antifeedant activity was observed in hexane extract. The antifeedant activity was high in the low polar hexane extract. Tewary *et al*. (2005) reported that low polar solvent extracts had higher activity than high polar solvent extracts. Morimoto *et al*. (2002) reported that hexane extract of *Galium aparine* had higher phagodeterrent activity against *Spodoptera litura*. Similarly hexane extracts from *Emblica officinalis* and *Ocimum sanctum*, showed good antifeedant activity against *S. litura* (Sharma and Bisht, 2008). Also, hexane portion from methanol extract of *Cryptomeria japonica* showed higher antifeedant activity against *Locusta migratoria* (Wu *et al*., 2008). Eighth fraction showed higher antifeedant activity against *H. armigera* in our study. Raja *et al*. (2005) reported that second fraction obtained from ethyl acetate extract of *Hyptis suaveolens* eluted with hexane showed potent antifeedant activity against *H. armigera*.

Phytochemical analysis revealed the presence of alkaloids, coumarin and quinone in the hexane extract of *C. guianensis* which probably contributed to the feeding deterrency in the present study. Verma *et al*. (1986) reported that alkaloids present in plants inhibited the feeding of *S. litura*. Feeding behavioral experiments showed that alkaloids acted as potent feeding deterrents in lepidopteran larvae (González-Coloma *et al*., 2004; Kathuria and Kaushik, 2005). Quinone was known to possess antifeedant activity against a variety of agricultural pests (Krishnakumari *et al*., 2001). Coumarin and quinone acted as feeding deterrents against *S. litura* (Morimoto *et al*., 1999). In the present study higher mortality was recorded due to the presence of alkaloids, coumarin and quinone**.** The results of the present investigation corroborate with the findings of Baskar *et al*. (2009) who reported that presence of alkaloids, coumarin and quinone present in the hexane extract of *Atalantia monophylla* showed higher larval mortality in *H. armigera*. Akhtar *et al*. (2008) reported that alkaloids acted as neuro-muscular toxin, contact and stomach poison. Coumarin acted as insecticide against four different insect pests (Moreira *et al*., 2007). Georges *et al*. (2008) reported that anthroquinone exhibited insecticidal activity.

Fresh cotton leaf treated with hexane extract and its fractions recorded minimum consumption which lead to death of *H. armigera* larvae. This finding coincides

with the observations of Kamaraj *et al*. (2008) who studied the extract of *Ocimum canum*, *O. sanctum* and *Rhinacanthus nasutus* treated leaf discs. They noticed low consumption with high mortality in the larvae of *H. armigera*. Similar findings were observed in low polar solvent system in different plant extracts against *H. armigera*. Janarthan *et al.* (1999) reported that petroleum ether extracts of *Parthenium histerophorus* at a concentration of 0.2 and 0.5% caused 100% mortality in the larvae of *H. armigera*. Aravinda *et al*. (2009) found that different solvent (petroleum ether, hexane) extracts of *Jatropha curcas* showed more than 70% insecticidal activity against larvae of *H. armigera*. Hexane extract of *Dodonaea angustifolia* reduced the fecundity, hatchability and larval mortality of *H. armigera* (Subashini *et al*., 2004). Malarvannan *et al*. (2009) observed that hexane extract from *Clausena dentata* showed 100% ovicidal activity against *H. armigera*. Khalequzzaman and Sultana (2006) reported that petroleum extract of *Annona squamosa* seeds recorded higher larval mortality than ethyl acetate and acetone extracts. Petroleum ether extract of *Synedrella nodiflora* caused 80% larval mortality in *S. litura* (Martin Rathi and Gopalakrishnan, 2006).

The present study will be useful in promoting the development of new botanical insecticide to manage herbivores.

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