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Research Article

BIOEFFICACY OF *CLEOME VISCOSA* L. AND *SINAPIS ALBA* L. SEED EXTRACTS AGAINST *HELICOVERPA ARMIGERA* (HUBNER) (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT

Solvent extracts of *Cleome viscosa* L. (Cleomaceae) and *Sinapis alba* L. (Brassicaceae) seeds were incorporated in semi-synthetic diet of *Helicoverpa armigera* (Hubner) at 0.5, 1.0, 1.5 and 2.0% concentrations to evaluate their antifeedant, larvicidal, pupicidal and adult malformation activities against *H. armigera*. Methanol extract of *S. alba* recorded very high antifeedant activity (71.42%) and pupicidal activity (54%) at 2% concentration. At 2% concentration the hexane extracts of *S. alba* (33.93%) and *C. viscosa* (32.86%) produced more deformities at adult stage of the insect. The results clearly indicated that the methanol extract of *S. alba* seeds have potential antifeedant activity against *H. armigera* and can be used to prevent crop damage by *H. armigera* in the field.

Keywords: *Cleome viscosa, Sinapis alba,* Podborer, Antifeedant, Pupicidal, Adult malformation.

INTRODUCTION

Helicoverpa armigera is an agriculturally important insect. It is a polyphagous pest causing heavy yield loss in agricultural, ornamental and horticultural crops (Talekar *et al*., 2006). World-wide, synthetic pesticides are commonly used for *H. armigera* control. But application of these synthetic pesticides has resulted in many unwanted effects such as environmental pollution, pesticide resistance, non-target effect and human health hazards (Raguruman and Singh, 1999). *H. armigera* has developed resistance to almost all classes of insecticides including pyrethroids (Babu and Subrahmanyam, 2010; Dhingra *et al*., 1988).

Since the past few years, people are aware of organic pesticides and are intended towards using botanical extracts to offer alternatives to synthetic insecticides (Ladhari *et al*., 2013). Botanical pesticides have multiple modes of actions such as antifeedant activity, growth inhibiting, repellent and oviposition deterrent activities (Metcalf *et al*., 1992). Plant-derived extracts and phytochemicals have been investigated on insect pests for the past 30 years to develop alternatives to conventional insecticides (Ca´ssia Seffrin *et al*., 2010). Solvent extracts of many plants have been reported as antifeedants, larvicides and growth regulators against *H. armigera* (Paulraj and Ignacimuthu, 2009; Baskar and Ignacimuthu, 2012; Pavunraj *et al*., 2013; Sivaraman *et al*., 2014). Aqueous extracts of neem seed and leaf were found to extend the larval developmental period and reduced adult emergence, longevity, fecundity and fertility in *H. armigera* (Wondafrash *et al*., 2012).

Sinapis alba, the White mustard, is an annual plant of the family Brassicaceae and is distributed worldwide. It is grown for its seeds, mustard, as fodder crop or as a green manure. The seeds of *S. alba* have many medicinal properties including anti-colon cancer property (Yuan *et al*., 2011). The genus has a sub

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cosmopolitan distribution throughout the tropical and warm temperate regions of the world. Species of *Cleome* are commonly known as spider flowers, spider plants, spider weeds, or bee plants. The plant is used for treating various human ailments (Aparadh *et al*, 2012).

In the present study solvent extracts of *Cleome viscosa* and *Sinapis alba* seeds were screened against third instar larvae of *H. armigera* for antifeedant, larvicidal and growth inhibiting properties.

MATERIALS AND METHODS

Plant seeds

Dry seeds (500gm) of *C. viscosa* and *S. alba* were purchased from the market at Parrys in Chennai, Tamil Nadu, India. The seeds were identified by taxonomist at Entomology Research Institute and voucher specimens (*C. viscosa*: ERI-BP-GS-008; *S. alba*: ERI-BP-GS-009) were deposited at the herbarium of the Institute.

Extraction

The seeds of both plants were powdered coarsely powdered using electric blender. One part of seed powder (500 gm) was soaked sequentially in three parts (1.5L) of hexane, chloroform, ethyl acetate and methanol for a period of 48 h with intermittent shaking and filtered through a Buchner funnel using Whatman number 1 filter paper. The extracts were concentrated under reduced pressure using rotary evaporator (Medica instruments Mgf. Co. Sl. No: EV11. JF. 012) and stored at 4°C.

Phytochemical analyses

Phytochemical analysis of *C. viscosa* and *S. alba* seeds extracts were carried out following the method of Harbone (1998).

Insects

Third instar larvae of *H. armigera* were obtained from a pure stock culture maintained over ten generations on semi-synthetic diet (Vanderzant *et al*., 1962) in the Institute laboratory at 27±1^{o}C; 11±0.5 h photoperiod and 65-70% R.H.

Antifeedant activity

All the solvent extracts of the two seeds were screened at four different concentrations viz., 0.5, 1, 1.5 and 2%. Extracts were incorporated into the semi-synthetic diet as described by Nathala *et al*. (2006) and Jadhav *et al*. (2012). About 1 gm of extract-incorporated diet was weighed (AB54-S Meter Toledo Balance, Switzerland) and kept in petri-dish (13 mm × 90 mm). A single third-instar larva (3 h pre-starved) of *H. armigera* was placed on the treated diet taken in the petri-dish. Untreated diet was maintained as a control. After the treatment period of 24 h, the unfed diet in control and treatments were quantified and the larvae were reared continuously on fresh untreated semi-synthetic diet. Twenty replications were maintained for each treatment and the control. The antifeedant activity was calculated according to the formula of Bentley et al. (1984):

Antifeedant activity = C-T/C×100

Where ‘C’ is the consumption in control and ‘T’ is the consumption in treatment.

Larvicidal bioassay

The treatments were given orally through semi-synthetic diet to the larvae according to the antifeedant experiment bioassay method. After 24 h, the treated diet was removed and the larvae were continuously maintained on normal fresh semi-synthetic diet. Then larval mortality was calculated up to 96 h after the treatment. Control larvae were maintained separately on untreated diets. Ten replications were maintained for each concentration and each experiment was repeated five times.

Pupicidal activity and adult abnormalities

The survived larvae in the larvicidal experiments were continuously fed with untreated normal fresh diet pupation. Pupal mortality was calculated by subtracting the number of emerging adults from the total number of pupae. Abnormalities in adult moths were recorded and percentages of abnormal adults developed from each treatment were calculated.

Statistical analysis

Data obtained from antifeedant, pupicidal and adult malformation experiments were subjected to one way Analysis of Variance (ANOVA) and mean values were separated by Tukey's multiple range tests using SPSS software package (11.5 version).

RESULTS

Preliminary phytochemical analysis: Preliminary phytochemical analysis of seed extracts of two plants was done and the results are presented in table 1. The results clearly showed that the ethyl acetate extracts of both plant seeds contained more number of secondary metabolites. Ethyl acetate extract of *S. alba* showed positive results for five phytochemical groups namely alkaloids, coumarins, quinones, tannins and terpenoids. Ethyl acetate extract of *C. viscosa* contained four groups of secondary metabolites namely coumarins, quinones, tannins and terpenoids. The most effective treatment, i.e. the methanol extract of *S. alba* contained three types of metabolites *viz*., alkaloids, quinones and saponins.

Antifeedant activity: Table 2 shows the percentage of antifeedant activities of *C. viscosa* and *S. alba* seeds extracts against *H. armigera* third instar larvae at 0.5, 1.0, 1.5 and 2% concentrations. Methanol extracts of seeds of both plants were found to be the most effective. The significantly greater antifeedant activity (71.42%) was recorded in methanol extract of *S. alba* seeds at 2% concentration ($p \leq 0.01$). The methanol extract of *C. viscosa* presented 50.91% antifeedant activity at 2% concentration. Hexane extracts of two plants presented the lowest antifeedant activity. In all the treatments, the antifeedant activity was directly proportional to the concentration.

Pupicidal activities: None of the treatments caused larval mortality during the observation period, but caused the pupal mortality. Table 3 shows pupicidal activity of *C. viscos*a and

Table 1. Preliminary phytochemical analysis of *C. viscosa* and *S. alba* seed extracts.

Plant species	Extracts	Phytochemical groups								
		A	C	F	Ph	Q	S	St	T	Tp
C. viscosa	Hexane	-	-	-	-	-	-	-	-	+
	Chloroform	-	-	-	-	+	-	-	-	+
	Ethyl acetate	-	+	-	-	+	-	-	+	+
	Methanol	+	-	-	-	+	-	-	+	-
S. alba	Hexane	-	-	-	-	-	-	-	-	+
	Chloroform	+	-	-	-	+	-	-	-	+
	Ethyl acetate	+	+	-	-	+	-	-	+	+
	Methanol	+	-	-	-	+	+	-	-	-

A–Alkaloids; C–Coumarin; F–Flavanoids; Ph – Phenols; Q– Quinones; S–Saponins; St– Steroid; T–Tannins; Tp–Terpenoids (+ indicates presence; – indicates absence).

Table 2. Antifeedant activity of *C. viscosa* and *S. alba* seed extracts against *H. armigera* larvae.

Plant species	Extracts	Concentration (%)			
		0.5	1.0	1.5	2.0
C. viscosa	Hexane	1.78 ± 3.10^{d}	8.48 ± 4.22^{e}	11.51 ± 3.54^{f}	23.71 ± 6.05^{e}
	Chloroform	11.64 ± 5.06^{c}	17.13 ± 9.29^{de}	21.44 ± 6.48^{e}	26.88 ± 6.32^{de}
	Ethyl acetate	25.00 ± 6.72^{b}	30.85 ± 7.24^{bc}	38.37 ± 6.13^{bc}	47.81 ± 8.13^{b}
	Methanol	23.34 ± 5.62^{b}	38.09 ± 6.18^{ab}	43.26 ± 6.14^{b}	50.91 ± 6.42^{b}
S. alba	Hexane	16.62 ± 8.68^{bc}	22.02 ± 8.66^{cd}	28.35 ± 7.94^{de}	34.71 ± 9.26^{cd}
	Chloroform	12.76 ± 6.64^{c}	33.28 ± 9.47^{b}	36.31 ± 8.45^{bcd}	41.57 ± 8.33^{bc}
	Ethyl acetate	21.92 ± 8.79^{b}	27.24 ± 9.73^{bcd}	29.91 ± 8.56^{cde}	42.87 ± 9.16^{bc}
	Methanol	43.53 ± 8.93^{a}	47.69 ± 12.06^{a}	55.05 ± 12.13^{a}	71.42 ± 9.96^{a}

Within the column, mean ± SD followed by the same letter do not differ significantly (Tukey's test, $P \leq 0.05$), (n=20).

Table 3. Pupicidal activity of seed extracts of *C. viscosa* and *S. alba* against *H. armigera* (Mean±SD; n=5).

Plant species	Extracts	Concentration (%)			
		0.5	1.0	1.5	2.0
C. viscosa	Hexane	14.0±4.9^{b}	18.0±4.0^{b}	24.0±4.9^{b}	32.0±4.0^{b}
	Chloroform	4.0±4.49^{c}	6.0±4.49^{cd}	16.0±4.49^{bc}	22.0±4.0^{c}
	Ethyl acetate	0^{c}	6.0±4.9^{cd}	20.0±8.94^{bc}	26.0±4.9^{bc}
	Methanol	0^{c}	6.0±4.9^{cd}	12.0±4.0^{c}	22.0±4.0^{c}
S. alba	Hexane	6.0±4.9^{c}	10.0±0^{c}	18.0±4.0^{bc}	24.0±4.9^{bc}
	Chloroform	0^{c}	0^{d}	10.0±0^{c}	22.0±4.0^{c}
	Ethyl acetate	0^{c}	0^{d}	0^{d}	0^{d}
	Methanol	22.0±7.48^{a}	32.0±7.48^{a}	38.0±4.0^{a}	54.0±4.9^{a}

Means followed by same letter(s) in a column do not differ significantly by Tukey‘s test at P≤ 0.05.

Table 4. Percent deformed adult moths of *H. armigera* produced by *C. viscosa* and *S. alba* seed extract treatmemts (Mean±SD; n=5).

Plant species	Extracts	Concentration (%)			
		0.5	1.0	1.5	2.0
C. viscosa	Hexane	5.0±6.12^{a}	12.22±0.56^{a}	13.21±0.87^{b}	32.86±8.57^{a}
	Chloroform	0^{a}	0^{c}	0^{d}	0^{c}
	Ethyl acetate	0^{a}	0^{c}	0^{d}	0^{c}
	Methanol	0^{a}	0^{c}	0^{d}	0^{c}
S. alba	Hexane	6.67±5.44^{a}	13.33±4.44^{a}	19.44±5.76^{a}	33.93±4.37^{a}
	Chloroform	4.0±4.9^{a}	8.0±4.0^{b}	11.11±0^{b}	20.71±6.83^{b}
	Ethyl acetate	0^{a}	0^{c}	6.0±4.9^{c}	16.0±4.9^{b}
	Methanol	0^{a}	0^{c}	0^{d}	0^{c}

Means followed by same letter(s) in a column do not differ significantly by Tukey‘s test at P≤ 0.05.

S. alba seed extracts. Among the different treatments, methanol extract of *S. alba* seeds recorded the highest pupal mortality (54%) at 2% concentration. In *S. alba,* the highest pupicidal activity (32%) was recorded in hexane extract. Ethyl acetate extract of *S. alba* did not show any pupicidal activity.

Growth inhibiting properties: Growth inhibiting property of seed extracts of two plants was expressed through adult deformities. The deformed adult moths were recognized by their relatively poor body size, highly curled wings and undergrown wings. High number of deformed adult moths was produced by hexane extracts of the two plants. At 2% concentration hexane extracts of *S. alba* and *C. viscosa* seed extracts produced 33.93 and 32.86% deformed adults respectively (Table 4). Chloroform, ethyl acetate and methanol extracts of *C. viscosa* seeds did not record any malformed adults and methanol extract of *S. alba* seed did not produce any deformed adults.

DISCUSSION

Plant seed extracts were found to be possessing feeding deterrent molecules, toxicants and growth inhibitors against *H. armigera* (Sonkamble *et al*., 2000; Lingathurai *et al*., 2010; Baskar and Ignacimuthu, 2012). In the present investigation maximum antifeedant activity (71.42%) was observed in methanol extract *S. alba*.

Previously several investigators (Yasui *et al*., 1998; Koul *et al*., 2000; Rongsriyam *et al*., 2006; Kamaraj *et al*., 2008) have reported that methanol extracts of plants had higher antifeedant, insecticidal and growth inhibiting activities. Arivoli and Tennyson (2012) showed that methanol extract of *Zanthoxylum limonella* leaves showed antifeedant activity of 44.11% in 72 h at 1000 ppm concentration against *S. litura* larvae. Somboon and Pimsamarn (2006) studied the contact toxicity of methanol extracts of three *Cleome* species against *Sitophilus oryzae*. They found that *C. viscosa* was the most toxic plant, which recorded the least median lethal concentration (LC_{50}). They also reported ovipositon deterrent and ovicidal activities of *C. viscosa* against *S. oryzae*. Koul *et al.* (2000) showed that the methanol extract of *Melia dubia* had antifeedant activity and inhibited the growth of *Spodoptera litura* and *Helicoverpa armigera* larvae. Deborah *et al*. (2001) observed that the methanol extract of *Trichilia americana* exhibited antifeedant activity of 50% in a choice leaf disc bioassay with 0.18 μg cm^{-2} on *Spodoptera litura*. Methanol extracts *Ocimum canum* and *Rhinacanthus nasutus* leaves reported mortality of 100% at 1000 ppm against the larvae of *S. litura* and *Culex quinquefasciatus* (Kamaraj, *et al*., 2008). Contrary to the above findings Islam *et al*. (2014) reported that chloroform extract of *C. viscosa* caused higher mortality in *Tribolium castaneum* and *Culex* mosquito larvae.

Many plants contain phytochemicals like phenols, alkaloids, flavanoids, terpenes, quinone, coumarin etc., which play a defensive role against insect pests. These phytochemicals possess broad range of biological activities such as antifeedant, oviposition deterrent, insecticidal, ovicidal and growth regulatory activities on agricultural pests (Jeyasankar *et al*., 2012). In the present study the preliminary phytochemical analysis of the effective treatment, the methanol extract of *S. alba* seeds, revealed the presence of alkaloids, quinone and saponins. Alkaloids present in *Tylophora asthmatica* plant inhibited the feeding of *S. litura* (Verma *et al*., 1986). González-coloma *et al*. (2004) and Kathuria and Kaushik (2005) reported that alkaloids were effective feeding deterrents against lepidopteran pests.

The present study clearly showed that the seed extracts of *C. viscosa* and *S. alba* were not lethal the larval stage, but at the pupal stage. The treatments also caused abnormal growth as evidenced from the development of deformed adults. Methanol extract of *S. alba* seeds was the most lethal to the pupae and hexane extract produced higher number of abnormal adults. These findings suggested that the active principles in the plant extracts interrupted the metamorphosis process and led to the pupicidal activity and abnormalities in *H. armigera*. Similarly, Jeyasankar *et al*. (2012) have reported that ethyl acetate extract of *Solanum pseudocapsicum* seeds produced pupal mortality and adult deformities in *H. armigera*. Methanol extract of *S. alba* seeds contained major groups of phytochemicals such as quinones, alkaloids, saponins and hexane extract contained terpenoids.

CONCLUSIONS

The present study showed that *C. viscosa* and *S. alba* seed extracts possess antifeedant and chronic toxic properties against *H. armigera*. These extracts can be used for controlling *H. armigera* in ecofriendly pest management programmes.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest associated with this article.

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