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Antifeedant and Growth Inhibitory Activities of *Syzygium lineare* Wall (Myrtaceae) Against *Spodoptera litura* Fab (Lepidoptera: Noctuidae)

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Abstract: Antifeedant and growth inhibitory activities of hexane, diethyl ether, dicholoromethane and ethyl acetate crude extracts of *Syzygium lineare* leaves were tested against fourth instar larvae of *Spodoptera litura*. All the crude extracts showed antifeedant activity in a dose dependent manner. Significant antifeedant activity was found only in ethyl acetate extract. Bioctive ethyl acetate extract was subjected to fractionation using silica gel column chromatography. Seven fractions were obtained. Highest antifeedant activity (91.58%) and larval deformity were observed in sixth fraction. Third fraction showed high pupal, adult deformities and decreased adult emergence (30.2%). This plant has the potential to serve as an alternate biopesticide in the management of Lepidopteron pests.

Key words: Antifeedant, myrtaceae, *Spodoptera litura*, *Syzygium lineare*

INTRODUCTION

In recent years, the use of synthetic organic insecticides in crop insect pest control programme around the world has resulted in damage to the environment, pest resurgence, pest resistance to insecticides and lethal effects on non-target organisms. These negative impacts of chemical insecticides have forced scientists to search of alternate techniques for the management of insect pests (Abudulai *et al*., 2001; Isman 1995).

There is an increasing scientific interest in the role of secondary plant metabolites in insect-plant interaction, particularly in host acceptance and rejection (Jacobson, 1989). While plant chemicals may produce toxic effects when ingested by insects, antifeeding activity may determine the extent of insect herbivory. Several papers have been published on the entomotoxic properties of crude extracts from different plant species (Sadek, 1997, 2003; Rodriguez-Saona and Trumble, 1999; Ciccia *et al*., 2000; Tapondiou *et al*., 2005; Ulrichs *et al*., 2008; Baskar *et al*., 2009). Plant derivatives are highly toxic to many insect species without phytotoxic properties (Schmutterer, 1992). Binder *et al*. (1991) reported that a lot of physiologically active substances isolated from plants affect behaviour, development and reproduction of insects.

Syzygium lineare Wall. (Myrtaceae) is a small tree with white flowers distributed in riverbeds of Tirunelveli hills of Western Ghats, Tamil Nadu, India (Manickam *et al*., 2004). The leaf powder is used for body cooling and the paste of the fruit is used for increasing stamina. *Spodoptera litura* is an economically important polyphagous pest in India, China and Japan, causing considerable economic loss to many vegetable and field crops. Crop loss due to insect pests varies between 10 to 30 % for major crops (Ferry *et al*., 2004). The objective of the present study was to evaluate antifeedant and growth inhibitory activities of *S. lineare* against *S. litura.*

MATERIALS AND METHODS

Plant materials: This experiment was conducted at Entomology Research Institute, Loyola College, Chennai, Tamil Nadu, India during the year 2004-2005. The leaves of *Syzygium lineare* were collected from Kalakad Mundanthurai Tiger Reserve Forest, Western Ghats of Tamil Nadu, India. Plant specimen was identified by Dr. D. Narasimhan, a plant taxonomist, Department of Botany, Madras Christian College, Chennai, India. The voucher specimen (MPH No. 170) was prepared and deposited at Entomology Research Institute, Loyola College, Chennai, India.

Extraction and fractionation: Dried leaf powder (2 kg) of *S. lineare* was extracted with hexane for about 48 hours at room temperature (Saxena and Yadav, 1983). The extract was filtered through a Buchner Funnel with Whatman number 1 filter paper. The filtrate was

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evaporated to dryness under reduced pressure using rotary evaporator. The remaining of the plant materials was extracted with Diethyl ether, Dicholoromethane and ethyl acetate, sequentially in a similar manner (Baskar *et al.*, 2009). Crude ethyl acetate extract (30 g) was separated by silica gel (500 g-acme's 100-200 mesh) column chromatography and eluted with hexane 100% followed by the combination of hexane: ethyl acetate and ethyl acetate: methanol ranging from 90:10 to 0: 100 and 50:50 to 0: 100, respectively. A total of 59 fractions were collected in 200 ml conical flask and pooled into seven fractions based on similar RF values using thin layer chromatography.

Rearing of *Spodoptera litura*: Egg masses of Spodoptera litura were collected from the insectory at Entomology Research Institute, Loyola College, Chennai. The eggs were surface sterilized with 0.02% sodium hypochlorite solution and allowed to hatch. The larvae were reared on normal diet (castor leaf) in a controlled environmental chamber (27ºC, 75% RH.). Second generation of larvae was used for subsequent experiments.

Antifeedant activity: Antifeedant activity of the crude extracts was studied using leaf disc no choice method (Isman *et al.*, 1990). Fresh castor leaf (*Ricinus communis* L.) discs of 4 cm in diameter were punched using cork borer and were dipped in 0.5, 1.0, 2.5 and 5% concentrations of crude extracts and 125, 250, 500 and 1000 ppm concentrations of fractions individually. The leaf discs treated with acetone and water were used as negative control and with Azadirachtin (40.86% purity, obtained from EID- Parry, India Ltd., Chennai) was used as positive control. In each petri dish (1.5x9 cm^2) wet filter paper was placed to avoid early drying of the leaf discs and single fourth instar larva was introduced into each petri dish. Progressive consumption of leaf area by the treated and control larvae after 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 replications were maintained for each treatment. The antifeedant activity was calculated by the formula of Isman *et al.* (1990).

Growth inhibitory activity: Growth regulation activities of fractions were studied at 500 ppm concentration against IV instar larvae of *S. litura.* Ten larvae were introduced in plastic tray having castor leaves treated with 500 ppm concentration of selected fractions. Acetone and water treated leaves were considered as negative control and the leaves treated with azadirachtin were considered as positive control. After 24 h feeding the larvae were transferred to normal castor leaves for studying the developmental periods. For each concentration five replicates were maintained. During the developmental period-deformed larvae, pupae, adults and successful adults and the mortality of larvae, pupae and adults were recorded (Sahayaraj, 1998).

RESULTS AND DISCUSSION

Statistical analysis: Antifeedant and growth regulation activities were analyzed using one-way ANOVA. Significant differences between treatments were determined using LSD test ($p<0.05$).

Antifeedant effect of crude extracts and fractions on *Spodoptera litura*: Antifeedant activity of solvent crude extracts and fractions was assessed based on antifeedant index. Higher antifeedant index normally indicated decreased rate of feeding. In the present study irrespective of concentration and solvents used for extraction the antifeedant activity varied significantly. Data pertaining to the experiment clearly revealed that maximum antifeedant activity was recorded in ethyl acetate extract (79.4%) at 5% concentration compared to control (Table 1). The effective ethyl acetate crude extract was subjected to column chromatography.

Antifeedant activity of fractions against fourth instar larvae of *S. litura* is presented in table II. Maximum antifeedant activities of 53.98, 60.54 and 91.58% were found in fraction 1, 3 and 6 respectively at 1000-ppm concentration. Least feeding deterrencies of 7.15 and 10.6% were observed at 125 ppm concentration in fraction 2 and 7 respectively (Table 2). The results revealed that the sixth fraction showed statistically significant feeding deterrent activity when compared to other fractions. The positive control (Azadirachtin) showed 92.18% antifeedant activity. Antifeedant activity was dose dependent.

Percentage of deformities due to the treatment of promising selected fractions at 500ppm concentration is presented in table III. Maximum larval (9.4%) deformity was found in sixth fraction. Highest pupal (11.8%) and adult (15.5%) deformities and also significant decreased adult emergence were observed in fraction 3 (Table 3). Percentage of mortality of larvae, pupae and adults was maximum in fraction 3 (i.e. total mortality 53.5%) compared to control. Highest mortality was found in positive control (Azadirachtin) (Table 4).

The ethyl acetate extract of the plant reduced the feeding rate of *S. litura.* This indicated that the active principles present in the plants inhibit larval feeding behaviour or make the food unpalatable or the substances directly act on the chemosensilla of the larva resulting in feeding deterrence. Antifeedant chemicals play a major role in the unsuitability of non-host plants as food for insects. Isolation and structure elucidation of these chemicals is important not only for understanding the

Table 1: Antifeedant activity of crude extracts of *S. lineare* against fourth instars larvae of *S. litura*

	Concentrations (%)			
Crude extracts	0.625	1.25	2.5	5
Hexane	18.65±5.48[ab]	26.58±5.11[b]	35.61±3.44[b]	41.41±4.88[b]
Diethyl ether	12.75±3.41[a]	26.45±2.46[b]	35.91±4.83[b]	38.88±6.32[b]
Dichloromethane	10.44±2.98[a]	22.54±3.78[b]	40.05±4.50[bc]	43.33±3.97[b]
Ethyl acetate	20.64±3.41[ab]	31.26±3.10[bc]	50.99±3.87[d]	79.4±7.61[c]

Percentages within the column followed by the same letters are not significantly difference ($p<0.05$ based on LSD test).

Table 2: Antifeedant activity of fractions of *S. lineare* against fourth instars larvae of *S. litura*

	Concentrations (ppm)			
Fractions	125	250	500	1000
Fraction 1	12.64±3.65[a]	12.68±3.54[a]	29.23±6.77[c]	53.98±15.64[c]
Fraction 2	7.15±15.64[a]	11.14±12.52[a]	18.69±15.80[b]	28.71±12.88[b]
Fraction 3	15.28±2.85[ab]	18.27±9.33[b]	31.75±8.41[c]	60.54±12.10[c]
Fraction 4	16.76±5.82[b]	27.75±9.31[b]	30.46±9.48b[c]	32.25±10.40[b]
Fraction 5	18.18±6.93[b]	29.17±7.82[bc]	24.89±11.25[b]	30.68±8.73[b]
Fraction 6	37.4±8.46[c]	48.39±4.89[d]	55.94±10.64[d]	91.58±18.31[d]
Fraction 7	10.6±2.64[a]	15.59±6.32[ab]	32.02±6.74[c]	27.81±14.71[b]
Azadirachtin	42.13±3.78[d]	48.14±3.98[d]	61.33±2.70[d]	92.18±4.18[d]

Percentages within the column followed by the same letters are not significantly difference ($p<0.05$ based on LSD test).

Table 3: Effect of fractions of ethyl acetate extract of *S. lineare* on *S. litura* deformed stages

	Deformity (%) (500 ppm concentration)			Successful adult emergence
Fractions	Larvae	Pupae	Adult	
Fraction 1	7.3 [a]	10.2[a]	9.0 [a]	53.5[c]
Fraction 3	2.5 [a]	11.8 [a]	10.5 [a]	30.2[b]
Fraction 6	9.4[a]	11.4[a]	12.5[a]	66.7[d]
Azadirachtin	27.5[bc]	26.8[b]	21.8[b]	23.9[a]

Percentages within the column followed by the same letters are not significantly difference ($p<0.05$ based on LSD test).

Table 4: Percentage of mortality of *S. litura* treated with fractions of ethyl acetate extract of *S. lineare*

	Mortality (%) (500ppm concentration)			Total mortality
Fractions	Larvae	Pupae	Adult	
Fraction 1	2.0 [a]	2.5 [a]	20.5[b]	25.0
Fraction 3	9.5 [b]	16.5[b]	27.5 [bc]	53.5
Fraction 6	0.0[a]	8.0[ab]	12.5[a]	20.5
Azadirachtin	20.5[c]	16.5[b]	21.0[b]	58.0

Percentages within the column followed by the same letters are not significantly difference ($p<0.05$ based on LSD test).

ecological aspects of insect pest's relationship, but also for their potential in insect pests control (Yasui *et al.*,1998). In the present investigation seven fractions were isolated from promising ethyl acetate extract using the combination of n-hexane, ethyl acetate and methanol. Several workers have suggested similar solvent system for isolation of many natural products (Harborne, 1998; Supratman *et al.*, 2000, 2001; Baskar *et al.*, 2009). Among the seven fractions, fraction 6 showed highest antifeedant activity at 1000ppm concentration. Preliminary phytochemical investigations of the fraction 6 revealed the presence of coumarin, quinone, alkaloids and terpinoids. Several authors have reported that plant extracts and fractions possess similar type of antifeedant activity against *S. litura* (Caasi Lit and Rejesus 1990; Sahayaraj, 1998; Morimoto *et al.*, 1999, 2002; Raja *et al.*, 2005; Ignacimuthu *et al.*, 2006; Ulrichs *et al.*, 2008). Most potent insect antifeedants are quinoline, indole alkaloids, sesquiterpene lactones, diterpinoids, and triterpinoids (Schoonhoven, 1982).

Insect growth inhibitory activity of fraction of *S. lineare* against *S. litura*: Insect growth regulation properties of plant extracts are very interesting and unique in nature, since insect growth regulator works on juvenile hormone. The enzyme ecdysone plays a major role in shedding of old skin and the phenomenon is called ecdysis or moulting. When the active plant compounds enter into the body of the larvae, the activity of ecdysone is suppressed and the larva fails to moult, remaining in the larval stage and ultimately dying (Koul and Isman, 1991). In the present study deformed development of larvae, pupae and adults were noted. Among the fractions, fraction 3 produced maximum percentage of deformed larvae, pupae and adults. The morphological deformities at larval, pupal and adult stages are due to the toxic effects of fractions on growth and development processes. Since morphogenetic hormones regulate these processes, it can be suggested that these fractions interfere with the hormones of the insect. These results are consistent with the earlier reports on various lepidopteran species

(Fagoonee, 1984; Barnby and Klocke, 1987; Koul and Isman, 1991; Suryakala, 1997; Sahayaraj, 1998; Akhtar and Isman, 2004). Several plants have growth inhibitory activities against *S. litura* (Caasi Lit and Rejesus, 1990; Alvarez *et al.*, 2007; Malarvannan *et al.*, 2008) and on *Helicoverpa armigera* (Baskar *et al.*, 2009).

This is first report on the antifeedant and growth inhibitory activities. This plant could be used for the development of new botanical pesticidal formulation for the control of this serious lepidopteran pest.

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