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Antifeedant, larvicidal and growth inhibitory effects of ononitol monohydrate isolated from *Cassia tora* L. against *Helicoverpa armigera* (Hub.) and *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae)

Kathirvelu Baskar [a,b], Savarimuthu Ignacimuthu [a,*]

[a] Entomology Research Institute, Loyola College, Chennai 600 034, India
[b] PG and Research Department of Advanced Zoology and Biotechnology, Vivekananda College, Thiruvedakam West, Madurai 625 217, India

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ABSTRACT

Ononitol monohydrate isolated from the ethyl acetate extract of *Cassia tora* L. using column chromatography was evaluated for its antifeedant, larvicidal and growth inhibitory activities against *Helicoverpa armigera* and *Spodoptera litura* at different concentrations of 125, 250, 500 and 1000 ppm. Leaf disc no-choice method was used for the bioassay. The compound showed significant antifeedant, larvicidal and pupicidal activities against *H. armigera* and *S. litura*. The compound also prolonged the larval–pupal duration of the insect at all the tested concentrations. The activities were concentration dependent for both the insects. Ononitol could be used as an agent to prepare botanical new pesticidal formulations.

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1. Introduction

Insect pests play a major role in damaging the crops and the crop loss varies between 10% and 30% for major crops (Ferry et al., 2004). In India, *Helicoverpa armigera* caused annual loss about Rs. 2000 crores (Ignacimuthu and Jayaraj, 2003). The repeated use of synthetic chemicals to manage this pest has resulted in resurgence and outbreak, resistance to insecticides, elimination of existing natural enemies in addition to polluting soil, water, air and food (Patel et al., 1992). *Spodoptera litura* is a polyphagous pest. It occurs worldwide because of its migration, higher reproductive rate and wide distribution. It feeds on 181 plant species belonging to 39 families; maize, sorghum, chick pea, pigeon pea, cotton, tobacco, okra, sunflower and groundnut are important economic crops attacked by this pest (Manjunath et al., 1989; Kumari and Singh, 2009; Reddy et al., 2009). Crop losses of 75–100% in chickpea (Lal, 1996), 30–50% in soyabean (Anonymous, 2007), estimated monetary loss of Rs. 20.12 million on different crops in Tamil Nadu (Jayaraj, 1990), and 300 crore in soybean alone at Kota region of Rajasthan (Dhaliwal et al., 2010) have been reported. This pest is active throughout the year on one or other crop. *S. litura* destroys a large host range of 120 host plants (Ramana et al., 1988). Bhushan et al. (2009) reported that *S. litura* has emerged as major pest of potato, attacking the crop during the vegetative phase and tubers causing heavy yield loss. Also it affects sunflower and causes more than 90% defoliation of sunflower cultivar germplasm (Sujatha and Lakshminarayana, 2007). Hence, search for viable and sustainable alternatives to synthetic pesticides is vital (Talekar et al., 1999).

Botanical pesticides tend to have broad-spectrum activity, are relatively specific in their mode of action and safe to living organisms and environment; they could be easily produced by farmers and small-scale industries (Talukder and Howse, 1994). Many medicinal plants are used for insecticidal activity (Berenbaum, 1989). Plants have chemical defense mechanisms against insects and other organisms; these defense mechanisms do not generally produce immediate death but do affect common biochemical and physiological functions (Prakash and Rao, 1997). The secondary metabolites play an important role in insecticidal, hormonal and antifeedant activities against insects (Camps, 1988). Antifeedant and insecticidal activities of many plant extracts and their bioactive compounds against several insect pests have been demonstrated (Raja et al., 2005; Ignacimuthu et al., 2006; Baskar et al., 2009, 2010; Muthu et al., 2010).

The present study was aimed at assessing the antifeedant, larvicidal, pupicidal and growth inhibitory effect of ononitol monohydrate isolated from *Cassia tora* against *H. armigera* and *S. litura*.

* Corresponding author. Tel.: +91 44 2817 8348; fax: +91 44 2817 4644.
E-mail address: entolc@hotmail.com (S. Ignacimuthu).

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2. Materials and methods

2.1. Plant material

C. tora L. leaves were collected from Padappai in Kancheepuram district near Chennai. The plant was identified by Dr. M. Ayyanar, taxonomist, Entomology Research Institute, Loyola College, Chennai. The voucher specimen (ERI H96-2006) was deposited at the herbarium of Entomology Research Institute.

2.2. Ononitol monohydrate

Isolation of ononitol monohydrate and its identification have already been described in our earlier paper (Dhanasekaran et al., 2009).

2.3. Insect culture

Larvae of *H. armigera* and *S. litura* were collected from the farmers' field in Salamangalam, Kancheepuram district, Tamil Nadu. The collected *H. armigera* larvae were reared individually in a plastic container (vials) and fed regularly with bhendi, *Abelmoschus esculentus* L. (Malvaceae) and *S. litura* larvae were reared on castor leaves and were kept 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 the 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 plants were kept for *H. armigera* and groundnut plants were provided for *S. litura* separately inside the adult emergence cages for egg laying. After hatching, the larvae were collected from the cage and fed with standard artificial diet as recommended by Koul et al. (1997) for *H. armigera.* Castor leaf was provided for *S. litura.*

2.4. Antifeedant activity

Antifeedant activity of ononitol monohydrate was studied using leaf disc no-choice method. Fresh cotton (*H. arigera*) and castor (*S. litura*) leaf discs of 4 cm diameter were punched using cork borer. They were dipped in 125, 250, 500 and 1000 ppm individually. The leaf discs dipped in acetone were used as negative control since acetone was used to dissolve the compound. Azadirachtin (40.86% purity, obtained from EID-Parry India Ltd., Chennai) was used as positive control. In each plastic petridish (1.5 cm × 9 cm) wet filter paper was placed to avoid early drying of the leaf discs. Single third instar larva of the respective insects was introduced into each petridish. Progressive consumption of treated and control leaves by the larvae after 24 h was assessed using Leaf Area Meter (Delta-T Devices, Serial No. 15736 F 96, UK). 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 conditions (27 ± 2 °C) with 14:10 photoperiod and 75 ± 5% relative humidity. Antifeedant activity was calculated according to the formula of Bentley et al. (1984);

2.5. Larvicidal activity

Larvicidal activity was studied using leaf disc no-choice method. The cotton and castor leaf discs were dipped in different concentrations of the compound and assayed as mentioned in antifeedant experiment. After 24 h the larvae were continuously maintained on the untreated fresh cotton and castor leaves for *H. armigera* and *S. litura,* respectively. 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):

2.6. Pupicidal activity

The larvae which survived were continuously fed with normal diet as specified in larvicidal activity until they became pupae and adults. Pupicidal activity was calculated by subtracting the number of emerging adults from the total number of pupae.

2.7. Larval and pupal durations

The larvae which survived were continuously fed with leaves. The larval duration was calculated after treated larvae became pupae. Pupal duration was calculated from the day of the emergence of adults from pupae.

2.8. Statistical analysis

The data related to antifeedant, larvicidal and pupicidal activities and larval–pupal durations were analysed using one way ANOVA. Significant differences between treatments were determined using Tukey's multiple range tests ($P \leqslant 0.05$). Analyses were performed with the original data, since even after transformation with various approaches (the arcsin, logarithmic, and square root methods), the distribution of the data did not show significant deviations from normality. Since data in days was normally distributed, log transformation was not done. Analyses were performed with the original data. LC_{50} and LC_{90} values were calculated using probit analysis (Finney, 1971).

3. Results

In the present study, ononitol monohydrate showed a strong antifeedant activity of 74.57% and 69.05% against *H. armigera* and *S. litura,* respectively at 1000 ppm concentration and the activity was statistically significant over control (Table 1).

Ononitol monohydrate showed 63.11% and 58.22% larvicidal activities against *H. armigera* and *S. litura*, respectively at 1000 ppm and the activity was statistically significant over control (Table 2). Ononitol monohydrate showed pronounced toxicity effect on the larvae of *H. armigera* and *S. litura*. The larvae which had consumed less amount of treated diet showed higher amount

Table 1
Antifeedant activity of ononitol monohydrate against *H. armigera* and *S. litura*.

Concentration (ppm)	Antifeedant activity (%)	
	H. armigera	*S. litura*
Ononitol monohydrate		
125	38.94 ± 3.31^{b}	34.93 ± 4.44^{b}
250	50.67 ± 4.84^{c}	45.10 ± 3.69^{c}
500	62.26 ± 2.85^{d}	57.55 ± 2.80^{d}
1000	$74.57 \pm 3.70^{e,f}$	$69.05 \pm 2.66^{e,f}$
Azadirachtin		
125	61.22 ± 2.65^{d}	66.47 ± 2.52^{e}
250	$68.37 \pm 4.16^{d,e}$	74.11 ± 4.34^{f}
500	77.51 ± 3.95^{f}	81.73 ± 2.53^{g}
1000	87.84 ± 4.65^{g}	88.61 ± 1.88^{h}
Control	4.33 ± 2.07^{a}	2.54 ± 1.74^{a}

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

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