International Journal of Scientific Research and Management (IJSRM)
||Volume||08||Issue||05||Pages||B-2020-67-76||2020||
Website: www.ijsrm.in ISSN (e): 2321-3418
DOI: 10.18535/ijsrm/v8i05.b02

Larvicidal and Antifeedant Activity of Some Plants Extracts Against the Larvae of Helicoverpa Armigera (Hubner)

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Abstract
In the present study, selected some plants, namely, Andrographis paniculata Ness., Cardiospermum halicacabum L., Cassia tora L., Catharanthus roseus L (G) Don., Datura metal L., Eupatorium riparium and Mikania micarantha were screened for their larvicidal and antifeedant activity against the larvae of Helicoverpa armigera (Hubner) under laboratory conditions. The crude extracts of all the selected plants demonstrated a dose dependent increase in bioactivity. However the bioactivity of four plants namely, A.paniculata, Cassia tora L., C.halicacabum L., and Datura metal L. was significantly higher ($p \leq 0.05$) than the control and extracts of C.roseus, E. riparium and M.micarantha. Methanol extract of A.paniculata caused highest oral toxicity with larval mortality ranging between 29.00% and 58.22% across the test concentration (0.2%, 0.4% and 1% w/v) while extract of C.tora L., demonstrated the highest feeding deterrence with reduction in larval feeding by 59.92% and 76.61% at 0.2% and 0.4% respectively. Crude extract of C.halicacabum L., leaves demonstrated high oral toxicity and feeding deterrence while extract of D.metel showed moderate level of oral toxicity as well as feeding deterrence at the highest tested concentration. Thus it may be concluded that four out of the selected plants possess insecticidal property and can be further investigated for the development of a potent natural botanical insecticide.

Keywords: Plant extract, oral toxicity, antifeedant activity, Helicoverpa armigera.

Introduction

Helicoverpa armigera (Hubner) (Lepidoptera: Noctuidae) is a polyphagous migratory noctuid which is widespread in Asia (Lammers and Macleod, 2007). It is known to cause serious damage to hundreds of economically important crops all over the world (Setiawati *et al*., 2000; Fakrudin *et al*., 2004). In India it is reported to be feeding on 182 plant species across 47 families (Manjunath *et al*., 1985) and causes an annual loss of about Rs. 2,000 crores (Ignacimuthu and Jayaraj,2003). Fifty percent of all insecticides used in India and China are to control *H. armigera* alone (Lammers and Macleod, 2007) but the continuous and indiscriminate use of insecticides over the years has resulted in the *H. armigera* developing resistance to certain molecules belonging to different classes of insecticides in various parts of the world (Chaturvedi, 2007; Yang *et al*., 2013).Thus alternatives to the synthetic pesticides are being sought.

The search for alternatives to synthetic pesticides has focused the interest of the pest managers on natural plant derived pest control agents. Plant-based pesticides or botanicals have many advantages: firstly, they have multifarious control mechanisms against pests (Sivagnaname and Kalyanasundaram, 2004) which reduces the possibility of the development of resistance in pests (Liu *et al*., 2000); secondly, they are target-specific and hence not harmful to humans and beneficial insects; and lastly, they are not persistent in nature and hence environment friendly (Shaalan, 2005).

In the present investigation an attempt has been made to screen widely distributed plants, for their insecticidal activity against the larvae of *H. armigera*, which has been reported as a major pest of tomato and chickpea in India (Thakur *et al*., 2006). The effect of many different plants and their extracts on *H. armigera* has been studied by several authors (Sahayaraj, 1998; Sundararajan and Kumuthakalavalli 2017; Koul *et al*., 2002; Kathuria and Kaushik, 2005; Ramya *et al*., 2018; Wambua *et al*., 2011; Jeyashankar *et al*., 2012; Arivoli and Tennyson, 2013). While

extracts of certain plants such as *Ocimum basilicum, Gynandropsis gynandra, Acorus calamus, Lantana camara,* and *Toddalia asiatica* demonstrated larvicidal effect on *H. armigera* (Pandey *et al*., 1983; Sundararajan and Kumuthakalavalli, 2017), others such as neem seed kernel extract were seen to have indirect effects such as causing larval-pupal intermediaries and abnormal adults (Jotwani and Srivastava, 1984) and feeding deterrency (Hongo and Karel, 1986). Majority of the plants tested against different larval instars of *H. armiger*a have been reported to demonstrate antifeedant properties (Sahayaraj, 1998; Koul *et al*., 2002; Kathuria and Kaushik, 2005; Ramya *et al*., 2018; Wambua *et al*., 2011; Jeyashankar *et al*., 2012; Arivoli and Tennyson, 2013).

Although extensive research has been conducted on the effect of different plant extracts on *H. armigera,* there is limited literature available on the efficacy of plants such as *Andrographis paniculata Ness., Cardiospermum halicacabum L., Cassia tora L., Catharanthus roseus L (G) Don., Datura metal L., Eupatorium riparium* and *Mikania micarantha* which have a wide distribution in the study area of Tamil nadu and find application in medicinal practices of the local rural population (Neogi *et al.*, 1989; Chhetri, 2008; Hynniewta and Kumar, 2008; Kayang *et al*., 2008; Sinha *et al*., 2008; Sohkhlet, 2014). The present study is aimed at determining the oral toxicity and antifeedant activity of the above mentioned plants against the larvae of *H. armigera* (Hubner).

Materials and Methods

Collection of Plants

The selected plants for this study were collected from in and around Dharmapuri district in Tamil Nadu. The selection of the plants was based on their local abundance, insecticidal properties and uses in traditional practices by the rural people of the state (Table 1). The samples were generally collected during the flowering and fruiting stage of the plants. All the selected plant species were identified with the help of volumes of *Flora of the Madras Presidency* (Gamble 1980) and *Flora of the Tamil Nadu Karnatic* (Matthew 1983).

Table 1: Evaluation of selected plants and the plant parts used for the study.

Plant name	Common name	Plant parts used
A.paniculata (Burm.f.) Ness.,	Siriyanankai	Whole plant
C.halicacabum L.,	Mudakkaruthan	Leaves
C.tora (L.) Roxb.	Thagarai	Leaves
C.roseus (L.)G Don.	Nithyakalyani	Whole plant
D. metel L,	Oomathai	Leaves
E. riparium Regel	Snakeroots	Leaves
M.micarantha Kunth	American rope	Aerial part

Preparation of Plant Extracts

The plants were brought to the laboratory immediately after collection and washed with tap water thoroughly followed by a final rinse with dechlorinated water, following which, they were shade dried at room temperature (27±1°C) for 48-72 hours, depending on the plant. The dried plants were ground to coarse powder using an electric blender. The crude extracts were prepared using standard protocol (Harborne, 1998; Houghton and Raman, 1998; Kathuria and Kaushik, 2005; Handa *et al*.,2008; Deepa and Remadevi, 2011). For the preparation of extracts, 100 gm of each of the plant powders was extracted with 1 litre methanol using a Soxhlet apparatus for 48 hours. Prior to extraction with methanol, the plant material was defatted with petroleum ether. The extracts were taken to dryness under reduced pressure using a rotary-vacuum evaporator and stored in airtight screw capped borosil containers for future use. Prior to performance of a bioassay, a standard stock solution of 1% w/v concentration was prepared by dissolving 1 g of the extract in 10 ml acetone and volume was made up to 100 ml by adding deionized water. From the stock

solution, 0.2%, 0.4% ,0.6, 0.8% and 1% w/v concentration was prepared for ingestion toxicity test and 0.2%, 0.4% and 1.0% w/v concentration for feeding deterrence test.

Test Organism

A laboratory culture of *H. armigera* larvae was maintained on a chickpea based semi-synthetic diet as suggested by Singh and Rembold (1992) under laboratory conditions (27±1°C, 75±1% R.H., and photoperiod of 12 L: 12 D). For the initial establishment of the colony in the laboratory, different instars of *H.armigera* larvae were collected from tomato crops grown in tomato field. The collected larvae were maintained on tomato leaves and fruits under laboratory conditions (27±1°C, 75±1% R.H. and photoperiod of 12 L: 12 D) in individual containers to prevent cannibalism and contamination until pupation. Pupae were transferred to clean containers with sterilized filter paper to facilitate moth emergence. Upon adult emergence, the male and female moths were paired and two pairs were released into individual mating chambers (2.5x1.5 feet). The adults were fed on a diet of 1% honey solution and provided with cotton strips as oviposition medium (Kaushik and Kathuria, 2004). From the first generation onwards, the laboratory colony was maintained on a chickpea based semi-synthetic diet. From the cultures, newly molted instar larvae were used for the bioassays.

Bioassay Studies

The larvicidal activity of plants was studied by oral application of the extracts through leaf dip method (Sundararajan and Kumuthakalavalli, 2017; Ramya *et al*., 2018). Freshly collected tomato leaves were individually dipped in the three different concentrations (0.2%, 0.4% and 1% w/v) of each of the extracts and air dried. A single treated leaf was kept in a petri plate lined with moist filter paper and a single six hour starved instar *H. armigera* larvae was introduced into the petri plate. Leaves treated with acetone were used as control. Larval mortality was recorded after 24 hours of exposure. A total of 10 larvae were individually exposed to each treatment and each treatment was replicated thrice. The total number of subjects per treatment was 30 larvae. The mortality data were represented as corrected mortality using Abbott's formula (Abbot, 1925).

Feeding Deterrence Bioassay

The antifeedant activity of crude extracts was assayed using leaf disc method (Sundararajan and Kumuthakalavalli, 2017). Discs of size 2.5cm^2 were punched from freshly collected tomato leaves and treated on each side with 10 µl of the test solution emulsified with 0.1% Triton X-100. The extracts were tested at three different concentrations 0.2%, 0.4% and 0.1% w/v. Leaf discs treated with acetone solution and emulsifier (0.1%) were used as control. The leaf discs were air dried and arranged in a petri plate with one treated and one control leaf disc per plate. A treated instar larva of *H. armigera* was then introduced at the center of the petri plate, such that it was equidistant from the treated and the control discs. The experiment was thus conducted with one larva per petri plate with ten larvae per treatment and each treatment was replicated three times. After six hours, the leaf discs were removed and the area consumed by the larvae was measured using a graph sheet method. The feeding deterrence index was calculated by using the formula given by Bomford and Isman (1996):

$$FDI = \frac{C-T}{C+T} \times 100$$

Where, C=area of consumption in the control; T = area of consumption in the treatment.

Data Analysis

The data obtained from the two bioassays were subjected to arcsine transformation prior to statistical analysis. The transformed data were then statistically analysed by one-way ANOVA. Separation of means and comparison between the different treatments was performed by Tukey's test at $P \leq 0.05$. SPSS version 20 was used for the analysis.

Results

Toxicity Bioassay

The larvicidal activity of methanolic extract of the selected plant species is presented in Table 2. All the plants demonstrated a dose dependent increase in oral toxicity, with percentage mortality of the instar larvae of *H. armigera* being highest at test concentration of 1% w/v. When tested at the concentration of 0.2%, methanol extract of all the plants demonstrated an average mortality of 18.64% which was statistically similar ($p > 0.05$) to the mortality rate of the larvae in the control. However, at concentration of 0.4% and 1% w/v, the crude extracts of *A.paniculata, C.tora* and *C.roseus* caused significantly higher mortality ($p \leq 0.000$) than the control. The larvicidal activity demonstrated by the extract of *A.paniculata* against *H. armigera*, was the highest amongst all the selected plants with percent corrected mortality ranging from 29.77% to 78.22% across the test concentration. Its larvicidal activity was significantly higher than the control and the other plants ($p \leq 0.05$) except *C.tora* ($p = 0.672$) and *D.metel* ($p = 0.315$). The methanol extract of the leaves of *C.tora* demonstrated the second highest oral toxicity against the larvae of *H. armigera*, with corrected larval mortality ranging between 21.61% and 52.23% across the test concentration. Of the remaining plants, extract of *D.metel* caused 35.11% larval mortality at the highest concentration of 1% w/v and it was significantly higher ($p=0.014$) than the larval mortality in control, thereby making it the third best plant after *A.paniculata* and *C.tora* in terms of oral toxicity against the larvae of *H. armigera*. However, it is to be noted that the synthetic insecticide, endosulfan 10% EC, which was used as control in this bioassay, caused 100% larval mortality within 24 hours of exposure and its activity was significantly higher ($p \leq 0.000$) than the activity of the plant extracts.

Feeding Deterrence Bioassay

The antifeedant activity of crude extracts of the selected plants was studied at three different concentrations. The feeding deterrence activity of the plants was assessed on the basis of the feeding deterrence index (FDI). Higher feeding deterrence index indicates lower feeding by the test organism. All the plants demonstrated dose dependent increase in feeding deterrence but irrespective of the test concentration of the plant extracts, the antifeedance index of the control was significantly lower ($p \leq 0.0001$) in comparison to that of the plants (Table 3). Of the plants, the crude extract of *C.roseus* demonstrated the highest antifeedant activity, causing 56.20% to 72.21% reduction in feeding by the larvae of *H. armigera*, across the test concentration and thus its FDI was significantly higher ($p \leq 0.05$) than the other plants. Apart from *C.roseus*, crude extract of *C.tora*, *D.metal* and *A.paniculata* also caused high feeding deterrence, which was significantly higher than the remaining plants with $p \leq 0.05$. While the FDI on exposure to *C.tora* extract was 22.12% to 52.72% across test concentrations, *D.metel* extract reduced larval feeding by 36.07% to 49.39%; and, *A.paniculata* extract reduced larval feeding in the range of 28.66 % to 44.73% across the test concentrations.

Table 2: The larvicidal activity of the crude extracts of the selected plants against the larvae of Helicoverpa armigera.

Plant name	Concentration of Extract(% W/V)		
	0.2%	**0.4%**	**1.0%**
A. paniculata	29.77 ± 6.93^{b}	44.81 ± 5.01^{b}	58.22 ± 8.01^{b}
C.halicacabum	13.7 ± 5.48^{b}	13.70 ± 5.48^{def}	24.07 ± 5.25^{cd}
C.tora	20.37 ± 9.45^{b}	35.55 ± 3.85^{bcd}	51.48 ± 7.88^{b}
C.roseus	13.33 ± 5.77^{b}	20.00 ± 10.00^{cde}	24.07 ± 5.25^{cd}
D. metel	20.37 ± 9.45^{b}	34.44 ± 5.09^{bc}	37.77 ± 3.85^{bc}
E. riparium	7.04 ± 6.12^{b}	7.04 ± 6.12^{ef}	13.33 ± 5.77^{de}
M.micarantha	7.50 ± 6.61^{b}	7.87 ± 6.85^{ef}	18.52 ± 6.41^{d}
Control	----------	----------	----------

Mean ± SD represents mean percent corrected mortality of 3 replicates of 10 individuals each. Within columns, Means followed by the same alphabet do not differ significantly at 5% level of significance using Tukey's test.

Table 3: the antifeedant activity of the selected plants extracts against the larvae of Helicoverpa armigera.

Plant name	Concentration of Extract(% W/V)		
	0.2%	**0.4%**	**1.0%**
A. paniculata	28.66 ± 2.95^{de}	35.44 ± 6.83^{ab}	44.73 ± 8.55^{bc}
C.halicacabum	12.67 ± 1.44^{c}	12.42 ± 6.51^{cd}	17.12 ± 7.31^{d}
C.tora	22.12 ± 5.68^{c}	46.61 ± 7.16^{a}	52.72 ± 4.93^{ab}
C.roseus	56.20 ± 2.19^{a}	50.92 ± 11.21^{a}	72.21 ± 9.04^{a}
D. metel	36.07 ± 1.05^{b}	43.57 ± 6.7^{a}	49.39 ± 5.25^{b}
E. riparium	12.83 ± 0.83^{e}	15.8 ± 9.85^{bc}	17.31 ± 5.31^{d}
M. micarantha	21.57 ± 1.31^{cd}	23.99 ± 6.03^{ab}	25.26 ± 5.92^{cd}
Control	----------	----------	----------

Mean ± SD represents mean percent feeding deterrence of 3 replicates of 10 individuals each. Within columns, means followed by the same alphabet do not differ significantly at 5% level of significance using Tukey's HSD test.

Discussion

The ingestion toxicity bioassay revealed that larvicidal activity of the crude methanolic extract of the plants was much lower than that of the synthetic insecticide, Endosulfan 10% EC. However, four out of the selected plants caused significantly higher ($p \leq 0.05$) larval mortality as well as feeding deterrence in comparison to the solvent control indicating potent insecticidal activity against the notorious pest, *H. armigera*.

The results of the present study indicated that at higher concentrations, *A.paniculata* could act both as a potent oral toxicant and feeding deterrent against *H. armigera* larvae, and this result is in agreement with the findings of Prasad and Roy (2011), who had concluded from their histopathological study that extracts of *A.paniculata* could act as stomach poison in addition to some antifeedant activity against the larvae of *H. armigera.* In a study by Murugesan and co-workers (2012), it was reported that essential oil at a concentration range of 2500-10000 ppm caused 20-50% larval mortality after 24 hours exposure against third instar larvae of teak defoliator, *Hyblaea puera* while in another study, aqueous crude extract of *L. camara* leaves at a concentration of 40% caused 100% mortality of fourth instar larvae of *Spodoptera litura* (Deshmukhe *et al.,* 2011). Both these studies found that larvicidal activity of *A.paniculata* increased with increase in its concentration which corroborated with our present findings. Tennyson (2013), at a concentration of 1%, the ethyl acetate crude extract of *L. camara* showed 25-50% antifeedance against third instar larvae of *Spodoptera litura* while the hexane and dichloromethane extracts showed < 25% antifeedance. Our study indicated higher activity of methanolic extract of *A.paniculata,* causing 40.74% feeding deterrence against fourth instar *H. armigera* larvae, at a much lower concentration of 0.4% w/v. However, it may be noted that different test organisms were used in the investigations conducted by other authors and many studies have shown that even closely related insect species can show widely different susceptibilities to the same extract or compound (Isman, 1993), which could be one of the reasons for the variation between the outcome of the present study and the previous studies.

The insecticidal activity of plants is attributed to the presence of various phytochemical groups (Kabaru and Gichia, 2001) and the occurrence of more than one major class of phytocompounds is responsible for the different modes of action of plant extracts against the target pests (Park *et al.*,2002; Lingathurai *et al.*, 2011). All three groups of phytocompounds have been reported to affect herbivorous insect's growth and development either by feeding inhibition or through post-ingestive phenomena (Coley *et al.*, 1985; Barbehenn *et al.*, 2001; Hoffman-Campo *et al.*, 2001; Lago *et al.*, 2002; Treutter, 2006; Jadhav *et al.*, 2012). In addition, extracts of *L. camara* and *L. cubeba* also tested positive for terpenoids. Terpenoids in plants can act mainly as antifeedant and growth disruptor and possess considerable toxicity toward insects (Kubo and Nakanishi,1978; Khalid *et al.*, 1989). Saponins on the other hand are a class of phytochemicals which are reported to be insecticidal by many investigators (Marston and Hostettmann, 1985; Jeong *et al*., 2004; Sparg *et al.*, 2004; McGaw *et al.*, 2008). Thus, the insecticidal and antifeedant activity demonstrated by the methanol extracts of *C.roseus, A.paniculata. C.tora* and D.metel could be the result of composite effect of all these classes of phytocompounds.

However, the present study is a preliminary investigation which indicates that crude methanol extracts of the four plants possess insecticidal property. Future research has to be conducted with these plants to understand their exact mode of action/s as well as isolate and identify the bioactive compound/s responsible for the toxicity demonstrated towards the target pest.

Conclusion

From the present study it can be concluded that out of seven selected plants, four plants namely, *Andrographis paniculata Ness., Cardiospermum halicacabum L., Cassia tora L., Catharanthus roseus L (G) Don., Datura metal L., Eupatorium riparium* and *Mikania micarantha* have demonstrated promising insecticidal activity against *H. armigera* larvae. Further research on the bioactivity of these commonly found plants can lead to the development of a cost effective, eco-friendly formulation for crop protection, which will be beneficial to farmers of states such as Tamilnadu where organic farming is being encouraged by the Central and the State governments.

ACKNOWLEDGEMENT

This research did not receive any specific grant from funding agencies in the public, commercial, or not for profit sectors.

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