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ANTIFEEDANT ACTIVITY OF SELECTED BOTANICALS AGAINST THE LARVAE OF *HELICOVERPA ARMIGERA (*Hubner)

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Abstract

The sensitivity of *Helicoverpa armigera* 4th instar larvae towards the aqueous plant extracts (*Artemisia vulgaris* and *Toddalia asiatica*) were investigated under laboratory conditions and the effect of sublethal concentrations on the feeding deterrence were evaluated on the test organism. Results revealed that the 4th instar larvae of *H.armigera* was more susceptible to *Artemisia* extracts than *Toddalia* extract as it has higher LC50 values. In addition, the results showed that the mean feeding deterrence (FDI%) of botanicals extracts was concentration-dependent. Therefore, these botanicals could be important as eco-friendly accessible pest control alternatives against *H.armigera* and other closely related species.

Key words: Antifeedant activity; *Toddalia* and *Artemisia*; Feeding deterrency; *Helicoverpa armigera*

Introduction

The cotton boll worm *Helicoverpa armigera* (Lepidoptera: Noctuidae) is one of the most destructive pests of several crops such as cotton, corn, peanut, clover, vegetables and various fruits in india as well as in Mediterranean and Middle East countries (Sundararajan and Kumuthakalavalli, 2018). The continuous and unwise use of insecticides to control agricultural pests usually lead to development of resistance, adverse effects on beneficial insects and residues in foods (Rizk*et al*. 2010 and Ehab 2012). The essential botanicals and other plant extracts, as a new class of natural products for controlling insect pests environmentally friendly have begun to play an increasing prominent role as alternatives to synthetic insecticides (El-Sinary *et al*. 2008; Tripathi *et al*. 2009 and Ragaei and Sabry 2011). The selected botanical extracts used in this study are among those compounds under investigation as potential natural pesticides.

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. 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).

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).

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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.

Preparations of aqueous botanical extracts

Healthy plants Toddalia asiatica (family: Rutaceae) and Artemesia vulgaris (family: Asteraceae) were collected in the morning hours from the study area and after separating the leaves to test their insecticidal properties against H.armigera, they were washed with distilled water and left to dry in the shade. Finally, they were transferred to an oven (70^{o}C) for 24 hour and the dried leaves were blendered to make fine powder. Fifty gram of dried powder were stirred with 1 Liter distilled water for 1 hour and incubated for 48 hour at 4^{o}C and then stirred for additional 1 h and filtered twice through whatman No.1 filter paper. The volume was made up to 500 ml and it was considered as stock solution of the extract. This stock extract was maintained in a refrigerator until being used and the diluted concentration of the extract were made up.

Bioassay study

Leaf-dip method as described by Tabashnik *et al.* 1991 was followed using tomato leaves. Fresh tomato leaves, of almost the same size, were dipped in different concentrations (0.2, 0.4, 0.6, 0.8 and 1%) in case of treatment each of *H.armigera* and 4th instars with botanical extracts treatments. The dipping lasted for ca. 5-10 seconds and left to dry in air from excess solution. The treated leaves were transferred singly in plastic cups where 10 individuals of 4th instar larvae were allowed to feed on these treated leaves. Treated leaves were offered to larvae for 48 hrs. Three replicates of each concentration were performed. The untreated tomato leaves (control) were dipped in distilled water for the same period of time as treated control. Insect mortality were recorded daily starting after 24h from treatment. The experiment was conducted at laboratory temperature of 27 ± 2 ^{o} C, 70 ± 5% R.H. with photoperiod of 16:8 (L: D) hr.

The mortality % was corrected according to Abbott's formula (Abbott, 1925) as follows:

$$\text{Corrected mortality \%} = \frac{\text{Observed mortality \% - control mortality \%}}{\text{100 - control mortality \%}} \times 100$$

Probit analysis was determined to calculate the median lethal concentration values (LC_{50}) and related parameters, according to Finney (1971).

Feeding deterrent activity (non-choice method)

Feeding deterrent activity of the botanical solutions was assayed against *H.armigera* 4th instar larvae using a leaf-dip bioassay in no-choice test method. For this purpose the concentrations (LC_{50}) of botanical extracts were prepared for each instar. The Leaf discs of (10 cm) were impregnated for 5-10 seconds in each concentrations and the control leaf discs were impregnated in distilled water for the same time. In each plastic Petri dish (2.5 cm x 10 cm) wet filter paper was placed to avoid early drying of the leaf discs and ten larvae per replicate of 4th instar were introduced. Progressive consumption of leaf weight by the larvae after 24 hrs was recorded in control and treated discs. Amount of leaf eaten by the larva in botanical extracts treatments was corrected from control. Three replicates were maintained for each treatment with 10 larvae per replicate (total, n= 30). Feeding deterrent activity was assessed by calculating the Feeding deterrence Index by the formula of Saleh *et al*. 1986:

Feeding Deterrence Index (FDI);

$$FDI = \left(1 - \frac{\text{Percentage of treated consumed leaf}}{\text{Percentage of untreated consumed leaf}}\right) \times 100$$

Statistical Analyses

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Using the computed percentage of mortalities versus corresponding concentrations, Probit analysis was adopted according to Finney (1971) using a software computer program (SAS, 2002).

This yields determination of the toxicity indices (LC_{50}) as well as the related parameters (95% confidence intervals, slope and Chi-square, χ^2) for established toxicity regression lines. Obtained data were statistically analyzed using one-way analysis of variance (ANOVA) supported by Duncan's multiple range test (Duncan, 1955).

Results and Discussion

Toxicity of tested botanicals to *H.armigera*

The toxicity activity of aqueous extract of the selected plant species is presented in Table 1. All the plants demonstrated a dose dependent increase in oral toxicity, with percentage mortality of the instar larvae of *H. armigera.* In the present study result the LC_{50} values were 2.297 and 5.016 %, respectively for *T.asiatica* compared with 2.633 and 6.527 %, respectively for *Artemisia* extract against the larvae of *H.armigera*. While the LC_{50} values were 3.456 and 6.56 %, respectively for *T.asiatica* compared with 3.818 and 8.332 %, respectively for *Artemisia* against 4^{th} instar larvae. The slope values indicated that the insect population was relatively heterogeneous in their susceptibility toward tested botanical extracts by leaf-dip method. Our results showed LC_{50} values, the range of toxicity was in the decreasing order of *T.asiatica* > *Artemisia* against *H.armigera* 4^{th} instars. The *Artemisia sp.* belonging to the important family Asteraceae (Compositae) has known to possess several important biological properties, such as insecticidal activity (Saleh 1984). Hifnawy *et al*. (2001) reported larvicidal activity of *A. vulgaris* against cotton boll worm , *H.armigera* larvae.

Among the plants found to contain insecticidal or growth regulatory effects of insects, plants from the genus *Ageratum* and *Artemisia* were reported to have insecticidal activity (Anjoo and Ajay 2008). *Artemisia herba-alba*, is rich in terpenoids such as monoterpene hydrocarbons (Behtari*et al*. 2012), oxygenated monoterpenes (Hudaib and Aburjai 2006) and sesquiterpenes (Laid *et al*. 2008 and Paolini *et al*. 2010). Sundararajan and Kumuthakalavalli (2018) and Alaguchamy and Jayakumararaj (2015) studied the effect of leaf aqueous extract of *C. roseus* and they recommended that it can potentially be used as ecofriendly bio-pesticide to control the devastating damage caused by larvae of *Helicoverpa armigera.* Kumar and Yadav (2013) showed that screened phytochemical constituents of *Catharanthus roseus*(family: Apocynaceae) possesses carbohydrates, anthraquinone glycosides, flavanoids, saponins, and alkaloids. Also, the work on the isolation of a possible insect growth regulator (IGR) from *C. roseus*is in progress (Summarwar and Pandey 2015).

Table (1): Toxicity indices (LC_{50}) of the botanical extracts against *H.armigera* (Hubner)

Plant name	LC50 (Conc.%) 95% confidence interval	Slope ± SE	χ^2
Artemisia	6.527 (4.81 - 7.96)	1.71 ± 0.24	4.83
Toddalia asiatica	5.016 (4.81 - 7.96)	1.98 ± 0.26	3.42

*LC_{50} values are significant ($p < 0.05$) whenever confidence intervals do not overlap.

Table (2): Toxicity indices (LC_{50}) of the botanical extracts against 4^{th} instar larvae of *H.armigera* (Hubner)

Plant name	LC50 (Conc.%) 95% confidence interval	Slope ± SE	χ^2
Artemisia	8.332 (6.00 - 11.17)	1.12 ± 0.24	1.52
Toddalia asiatica	6.56 (4.40 - 7.71)	1.15 ± 0.23	3.41

*LC_{50} values are significant ($p < 0.05$) whenever confidence intervals do not overlap.

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Feeding deterrence activity

Data presented in table (2) showed that the mean feeding deterrence activity (based on feeding deterrence index values) was significantly different ($P < 0.05$) between lavender and mint oil treatments on 2nd instar larvae at both LC_{25} and LC_{50} where mean feeding deterrent values at LC_{25} were higher in case of mint oil (68.369 %) than that in case of lavender oil (65.833 %) for four days after treatment. while for the same instar at LC_{50} levels, the mean feeding deterrent values were higher in case of lavender oil (79.151 %) compared to that in case of mint oil (75.272%). In connection with the 4th instar, also the mean feeding deterrent values were significantly different between lavender and mint oil treatments either at LC_{25} or LC_{50} where mean feeding deterrent values at LC_{25} were higher in case of mint oil (63.561%) than that in case of lavender oil (60.408 %) for four days after treatment. Also, at LC_{50} levels, the mean feeding deterrent values were still higher in case of mint oil (73.413 %) compared to lavender oil (70.837%) (Table 3).

Depending on the data, the mint oil exhibited relatively more feeding deterrent effect than lavender oil treatments. The higher feeding deterrence index normally indicates decreased rate of feeding.Also, the *Mentha pulegium* oil significantly inhibits the feeding of fall armyworm, *Spodoptera frugiperda* **(Zalkowet *al.* 1979)**.Any substance that reduces food consumption by an insect can be considered as antifeedant or feeding deterrent **(Isman 2002)**.

Abd El-Galeil and Nakatani (2003) indicated that the antifeedant activity was dose-dependent in some of the isolated compounds. **Elumalai*et al.* (2010)** reported that all tested essential oils are showed moderate antifeedant activity against 4th instar larvae of *S. litura*; however, the highest antifeedant activity was observed in the essential oils of *Cuminum cyminu*, *Mentha pipertia*, *Rosmarinus officinalis*, *Thymus vulgaris*.

Table (3): Percentage feeding deterrent indices (mean ± SE) of *H.armigera* 4th instars larvae treated with
LC_{50} of botanical extracts (*Artemisia vulgaris* and *Toddalia asiatica*).

Plant name	LC50 (Conc.%) 95% confidence interval	4th instar	χ^2
Artemisia	8.332 (6.00 - 11.17)	63.871± 3.920 b	1.52
Toddalia asiatica	6.56 (4.40 - 7.71)	67.750± 4.157 a	3.41

*Within the same column, means followed by the same letter are not significantly different ($P > 0.05$).

Conclusion

Our results confirmed that the tested botanicals extracts resulted in increased mortality, reduced food consumption via their feeding deterrent effect and exert a adverse impact on H.armigera growth and development. These effects were dose-dependent. The findings may be helpful and effective for studying the efficacy of such botanicals as a part of the Integrated Pest Management (IPM) against this pest and closely related ones. In the present study, the selected plant such as Artemisia vulgaris and Toddalia asiatica 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.

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