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Antifeedant Activity of Selected Botanicals Against the Larvae of *Helicoverpa armigera* (Hubner)

V. Arunagiri [a++] and G. Sundararajan [a#*]

[a] *Department of Botany, Govt. Arts College, Dharmapuri-5, Tamil Nadu, India.*

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The cotton boll worm Helicoverpa armigera (Hubner) (Lepidoptera: Noctuidae) is one of the most destructive pests of several crops such as cotton, corn, peanut, clover, vegetables and various fruits in India The sensitivity of Helicoverpa armigera 4th instar larvae towards the aqueous plant extracts (*Artemisia vulgaris* L. and *Zanthoxylum asiaticum* (L.) Appelhans, Groppo & J.Wen) 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 Zanthoxylum 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*. The selected botanical extracts used in this study are among those compounds under investigation as potential natural pesticides.

++ *Research Scholar;*
# *Associate professor;*
**Corresponding author: Email: drgsbotany@gmail.com;*

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Keywords: Antifeedant activity; Artemisia vulgaris and Zanthoxylum asiaticum; feeding deterrency; Helicoverpa armigera.

1. INTRODUCTION

Natural insecticides made from plants may be a preferable option than manmade chemical pesticides. Botanicals are potent insecticidal secondary metabolites derived from plants that are administered as complex combinations or as pure substances. The biochemical components found in botanicals are safer for species that are not targets and extremely target-specific, fast biodegradable [1]. Botanicals can be effective against resistant pests and decrease the development of insect resistance due to their complex chemistry and unique mechanisms of action. Because they have the potential to decrease the use of chemical pesticides and increase the sustainability, viability, and economy of pest control, botanicals are a key research priority for scientists and governments throughout the globe. Based on a cost-benefit analysis, the use of botanicals in vegetable production can be more economical for controlling major vegetable pests in Bangladesh and *Drosophila suzukii* (Matsumura) on berry crops in Italy [2].

In India as well as in the Mediterranean and Middle East, the cotton boll worm *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) is one of the most devastating pests of many crops, including cotton, corn, peanuts, clover, vegetables, and a variety of fruits [3]. According to Rizk et al. [4] and Ehab [5] persistent and careless use of pesticides to eradicate agricultural pests typically results in resistance development, negative impacts on beneficial insects, and residues in food. Essential botanicals and other plant extracts are emerging as a new class of environmentally friendly natural products that can replace synthetic insecticides in the management of insect pests [6,7], Ragaei and Sabry 2011). The chemicals being studied as possible natural insecticides include the particular plant extracts employed in this work.

2. MATERIALS AND METHODS

2.1 Collection of Plants

The Dharmapuri district of Tamil Nadu is home to the plants that were chosen for this study. The state's rural residents employ the plants for traditional purposes, and their insecticidal qualities and local abundance were taken into consideration while choosing them. The majority of the time, the samples were taken when the plants were in blossom or fruit. With the use of volumes of the Flora of the Madras Presidency [8] and the Flora of the Tamil Nadu Karnatic [9] all the chosen plant species were identified.

2.2 Test Organism

The semi-synthetic diet of chickpeas, as recommended by Singh and Rembold (1992), was used to sustain a lab culture of *H. armigera* larvae at 27±1°C, 75±1% relative humidity, and a photoperiod of 12 L: 12 D. In order to construct the colony in the laboratory, several *H. armigera* larval instars were obtained from tomato crops cultivated in tomato fields. To avoid cannibalism and contamination until pupation, the collected larvae were kept in individual containers with tomato leaves and fruits under laboratory conditions (27±1°C, 75±1% R.H., and photoperiod of 12 L: 12 D). To encourage moth emergence, pupae were moved to sterile containers containing sterilized filter paper. After emerging as adults, the male and female moths were paired off and placed into separate mating rooms, each measuring 2.5 by 1.5 feet. According to Kaushik and Kathuria (2004), the adults were given cotton strips as an oviposition medium in addition to a meal consisting of 1% sucrose solution. The laboratory colony was fed a semi-synthetic diet based on chickpeas starting with the first generation. For the bioassays, freshly molted instar larvae were utilized from the cultures.

2.3 Preparations of Aqueous Botanical Extracts

In order to test the insecticidal qualities of the plants against *H. armigera*, healthy plants *Zanthoxylum asiaticum* (L.) Appelhans, Groppo & J.Wen (Syn. *Toddalia asiatica* Lam.) (Family: Rutaceae) and *Artemisia vulgaris* L. (Family: Asteraceae) were gathered from the study area early in the morning. The plants were then cleaned with distilled water and allowed to dry in the shade. The dried leaves were then blended into a fine powder after being placed in an oven set at 70^0C for 24 hours. One liter of distilled water was used to stir fifty grams of dry powder for one hour. The mixture was then incubated for

48 hours at 4ºC, stirred again for one hour, and twice filtered using Whatman No. 1 filter paper. The extract's stock solution was created with a capacity of 500 milliliters. This stock extract was kept refrigerated until it was needed and the diluted concentration was prepared.

2.4 Bioassay Study

Tomato leaves were used, and the leaf-dip technique as reported by Tabashnik et al. [10] was applied. Nearly identical fresh tomato leaves were dipped in varying doses (0.2, 0.4, 0.6, 0.8, and 1%) to treat *H. armigera* and 4th instars with botanical extracts. After dipping for five to ten seconds, the surplus solution was allowed to dry in the air. Ten larvae in their fourth instar were allowed to feed on the treated leaves after they were placed individually in plastic containers. Larvae were given treated leaves to consume for 48 hours. For every concentration, three duplicates were used. For the same amount of time as the treated control, the untreated tomato leaves (control) were submerged in distilled water. Daily records of insect mortality were made beginning 24 hours after treatment. The experiment was carried out using a photoperiod of 16:8 (L:D) hours at a laboratory temperature of 27 ± 2ºC and 70 ± 5% R.H. The mortality percentage was adjusted using Abbott's method [11].

As follows:

Corrected mortality % = Observed mortality % - control mortality % / 100 - control mortality % x 100

Probit analysis was determined to calculate the median lethal concentration values (LC50) and related parameters, according to Finney [12].

2.5 Feeding Deterrent Activity (Non-Choice Method)

Using a leaf-dip bioassay in the no-choice test technique, the feeding deterrent efficacy of the botanical solutions was tested against larvae of the fourth instar of *H. armigera*. The concentrations of botanical extracts (LC50) for each instar were produced for this purpose. Ten-centimeter leaf discs were impregnated for five to ten seconds in each concentration, whereas the control group of leaf discs was impregnated for the same amount of time in distilled water. To prevent the leaf discs from drying out too soon, wet filter paper measuring 2.5 cm by 10 cm was put in each plastic Petri dish, and ten larvae per duplicate of the fourth instar were added. After a day, the larvae's progressive consumption of the leaf weight was noted in the treated and control discs. The amount of leaf consumed by the larvae in treatments with botanical extracts was adjusted compared to the control. For every treatment, three duplicates (totaling thirty larvae each) were kept. The Feeding Deterrence Index was computed using the method proposed by Saleh et al. [13] in order to evaluate the feeding deterrent activity.

Feeding Deterrence Index (FDI);

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

2.6 Statistical Analysis

Probit analysis was implemented in accordance with Finney [12] utilizing a software computer program, employing the calculated percentage of mortalities vs matching concentrations [14]. For known toxicity regression lines, this results in the determination of the toxicity indices (LC50) as well as the associated parameters (95% confidence intervals, slope and Chi-square, $\chi 2$). One-way analysis of variance (ANOVA) was used to statistically assess the obtained data, and Duncan's multiple range test was used as support [15].

3. RESULTS AND DISCUSSION

3.1 Toxicity of Tested Botanicals to *H. Armigera*

Plate 1, Plate 2 and Table 1 displays the cytotoxic activity of the chosen plant species' aqueous extract. The oral toxicity of all the plants showed a dose-dependent rise, accompanied by a percentage mortality of *H. armigera* instar larvae. The current study's findings showed that the LC50 values for *Zanthoxylum asiaticum* (L.) Appelhans, Groppo & J.Wen against *H. armigera* larvae were 2.297 and 5.016 percent, respectively, whereas the corresponding values for *Artemisia vulgaris* L. extract were 2.633 and 6.527 percent, respectively. In contrast, the LC50 values for *Z. asiaticum* (L.) Appelhans, Groppo & J.Wen against 4th instar larvae were 3.456 and 6.56%, respectively, whereas those for Artemisia were 3.818 and 8.332%, respectively. According to the slope values, the insect population's sensitivity to the botanical extracts that were examined using the leaf-dip method was somewhat variable. According to our findings, *Z. asiaticum* (L.) Appelhans, Groppo & J.Wen was less harmful than *A. vulgaris* L. against *H. armigera* 4th instars in terms of LC50 values. It is recognized that the *Artemisia vulgaris* Lam. a member of the significant Asteraceae (Compositae) family, possesses a number of significant biological traits, including insecticidal action [16]. According to Hifnawy et al. (2001), *A. vulgaris* Lam. has larvicidal efficacy against the larvae of *H. armigera*, the cotton boll worm.

Plants of the genera *Ageratum* and *Artemisia* were identified to exhibit insecticidal action among those revealed to possess insecticidal or growth-regulating impacts of insects [17]. Terpenoids include monoterpene hydrocarbons [18] oxygenated monoterpenes [19] and sesquiterpenes [20,21] are abundant in *Artemisia herba-alba* Asso. After studying the effects of *Catharanthus roseus* (L.) G.Don leaf aqueous extract, Sundararajan and Kumuthakalavalli [3] and Alaguchamy and Jayakumararaj [22] suggested that it may be utilized as an environmentally friendly bio-pesticide to reduce the severe damage caused by *Helicoverpa armigera* larvae. *Catharanthus roseus* (L.) G.Don (Family: Apocynaceae) has screened phytochemical contents that include alkaloids, flavanoids, saponins, anthraquinone glycosides, and carbohydrates, as demonstrated by Kumar and Yadav [23]. Additionally, research is ongoing to isolate a potential insect growth regulator (IGR) from *C. roseus* (L.) G.Don [24].

Plate 1. Zanthoxylum asiaticum

Plate 2. Artemesia vulgaris

Plate 3. Helicoverpa armigera

Table 1. Toxicity indices (LC50) of the botanical extracts against *H. armigera* (Hubner)

Plant name	LC50 (Conc.%) 95% confidence interval	Slope ± SE	χ2
Artemisia vulgaris Lam.	6.527 (4.81 - 7.96)	1.71 ± 0.24	4.83
Zanthoxylum asiaticum (L.) Appelhans, Groppo & J.Wen	5.016 (4.81 - 7.96)	1.98 ± 0.26	3.42

**LC50 values are significant (p < 0.05) whenever confidence intervals do not overlap.*

Table 2. Toxicity indices (LC50) of the botanical extracts against 4th instar larvae of *H. armigera* (Hubner)

Plant name	LC50 (Conc.%) 95% confidence interval	Slope ± SE	χ2
Artemisia vulgaris Lam.	8.332 (6.00 - 11.17)	1.12 ± 0.24	1.52
Zanthoxylum asiaticum (L.) Appelhans, Groppo & J.Wen	6.56 (4.40 - 7.71)	1.15 ± 0.23	3.41

**LC50 values are significant (p < 0.05) whenever confidence intervals do not overlap.*

Table 3. Percentage feeding deterrent indices (mean ± SE) of *H. armigera* 4th instars larvae treated with LC50 of botanical extracts (*Artemisia vulgaris* Lam. and *Zanthoxylum asiaticum* (L.) Appelhans, Groppo & J.Wen)

Plant name	LC50 (Conc.%) 95% confidence interval	4th instar Percentage feeding deterrent indices (mean ± SE)	χ2
Artemisia vulgaris Lam.	8.332 (6.00 - 11.17)	68.369± 3.920 b	1.52
Zanthoxylum asiaticum (L.) Appelhans, Groppo & J.Wen	6.56 (4.40 - 7.71)	65.833± 4.157 a	3.41

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

3.2 Feeding Deterrence Activity

Table 2 displays data indicating a significant difference (P < 0.05) in the mean feeding deterrence activity (based on feeding deterrence index values) between *Zanthoxylum asiaticum (L.) Appelhans, Groppo & J.Wen* and *Artemisia vulgaris* Lam. treatments on fourth instar larvae at LC50. For four days following treatment, *Artemisia vulgaris* Lam. had higher mean feeding deterrent values at LC50 (68.369%) than *Zanthoxylum asiaticum (L.) Appelhans, Groppo & J.Wen* (65.833%). The *Artemisia vulgaris* Lam. treatments showed a comparatively greater feeding deterrent effect than the *Zanthoxylum asiaticum (L.) Appelhans, Groppo & J.Wen* treatments, based on the data. Generally speaking, a greater feeding deterrent index means a lower feeding rate. Additionally, *Mentha pulegium* L. considerably reduces Spodoptera frugiperda's ability to eat [25]. Any material that decreases an insect's appetite is referred to as an antifeedant or feeding deterrent [26].

Some of the extracted compounds showed dose-dependent antifeedant effect, according to Abd El-Galeil and Nakatani [27]. According to Elumalai et al. [28] all investigated essential oils exhibited a moderate level of antifeedant activity against *S. litura* larvae in their fourth instar; however, *Cuminum cyminum* L., *Mentha piperita* L., *Salvia rosmarinus* Spenn [29-31]. (Syn. *Rosmarinus officinalis* L.), and *Thymus vulgaris* L. exhibited the strongest antifeedant activity [32-35].

4. CONCLUSION

Our findings demonstrated that the botanical extracts under test had a negative effect on *H. armigera* growth and development, increased mortality, and decreased food consumption due to their feeding deterrent effect. The effects varied with the dosage. The results might be useful in researching how well these botanicals work against this insect and others that are closely related as part of Integrated insect Management (IPM). The chosen plants for this study, including *Zanthoxylum asiaticum (L.) Appelhans, Groppo & J.Wen* and *Artemisia vulgaris* Lam., have shown encouraging insecticidal action against *H. armigera* larvae. Farmers in states like Tamil Nadu, where organic farming is being promoted by the Central and State governments, will benefit from the development of an affordable, environmentally friendly crop protection formulation that results from further research on the bioactivity of these widely found plants.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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