

Bio-efficacy of *Solanum torvum* (Sw.) against agricultural pest *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae)

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Research Article

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Abstract

The antifeedant, larvicidal and histological effects of *Solanum torvum* leaf extracts were investigated against *Spodoptera litura*. The study found that the ethyl acetate leaf extract showed a significant antifeedant effect against *S. litura* of (86.16%) at 5%, followed by SNP (*Solanum torvum* based silver nanoparticles synthesis) showed the a good antifeedant activity of 61.33% at 600 ppm. Ethyl acetate extract showed a larvicidal activity against *S. litura* of 88.21% and the LC50 value was 2.05%. Exposure of larvae to ethyl acetate leaf extract resulted in significant histological damage, particularly affecting epithelial, goblet and digestive cells. The results suggest that the inclusion of these plant extracts in integrated pest management approaches can promote sustainable and environmentally friendly pest control methods in agriculture.

1. Introduction

The devastating effects of *Spodoptera litura*, commonly known as tobacco worm, on agricultural and vegetable crops have been observed in numerous countries such as India, China and Japan. This voracious pest poses a significant threat to crop yields and food security and requires extensive research efforts to understand its biology, behaviour and management strategies. The researchers found that a total of 181 plant species from 39 different families were affected [1]. The use of various categories of chemical insecticides has emerged as the predominant approach to pest management and control. The indiscriminate use of synthetic compounds can lead to a number of dangerous consequences, including environmental pollution, insect species toxicity and genetic resistance, among others.

Plant extracts and secondary metabolites have emerged as promising alternatives to synthetic pesticides, offering potential solutions for sustainable pest management strategies. In recent years, there has been growing interest in the potential of essential oils as a viable alternative to synthetic pesticides [2]. The utilization of plant pesticides offers several notable benefits, primarily attributed to their swift degradation and minimal persistence and bioaccumulation within the ecosystem [3]. The field of nanotechnology has succeeded in achieving the goals of economically feasible and ecologically sustainable crop protection and sustainable crop production, which has led to a dramatic change in agricultural research. Silver nanoparticles (AgNPs) have attracted the attention of entomologists due to their potential use in crop protection. Furthermore, experts believe that nanotechnology will completely transform agriculture, including pest control, in the near future. The production of AgNO₃ in a number of plants has been successfully investigated.

Solanum torvum from the solanaceae family was used for this research. They are widespread in India, Malaysia, Pakistan, tropical America, etc., [4]. Furthermore, an extensive literature search revealed no scientific publications on pest management or control, although locals have long used this plant as food and medicine to combat intestinal parasite larvae and dental problems [5]. In addition, it is used to treat contact and repellent activity against *Callosobruchus maculatus* [6] and larvicidal and adulticidal activity

against *Aedes aegypti* [7–8]. In the present work *S. torvum* leaf extracts we on report the antifeedant, larvicidal and histological examination on insects.

2. Materials and Methods

2.1. Plant material

In 2023, leaves of a healthy *Solanum torvum* (Sw.) plant were collected from Kovilvenni, located near Thanjavur district. Reverend Dr. Robinaud Herbarium is the Director of the Center for Molecular Systems at St. Joseph's College (Autonomous), located in Tiruchirappalli, Tamil Nadu, India. S. Its classification and validity were confirmed by John Brito, SJ. The Robinot Herbarium is where the specimens are stored, and the voucher number for them is ST 004. To prepare the leaves for experimental investigation, they were first dried, then stored in an airtight container, and finally processed.

2.2. Preparation of plant extract

The *S. torvum* leaves were collected, allowed to dry in the shade at room temperature, and then ground with a mixer grinder. For 48 hours, 3 L (1:3 w/v) of hexane, ethyl acetate, and methanol were used to extract the powder (1 kilogram). Using Whatman No. 1 filter paper and a Buchner funnel, the extract was filtered. Using a rotary evaporator (Model RE 801, Yamato, Japan) at 40°C and reduced pressure, the filtrate was dried. According to Murugesan et al. [8], the crude extracts were kept cold until needed.

2.3. Mass culture of *Spodoptera litura*

The *S. litura* culture was maintained in the research department of zoology and biotechnology, A.V.V.M. Sri Pushpam College (Autonomous), Poondi, Thanjavur. A sterile plastic container containing castor leaves and a 10% honey solution was used to maintain the emerging adults on cotton wool [9], which was inserted into a tiny glass cap for egg laying. For insects, culture was carried out at 25–27°C and a relative humidity of 60–70%.

2.4. Antifeedant activity

The antifeedant effect of the *S. torvum* leaves crude extracts was examined using the leaf disc no-choice method [10]. Different concentrations of crude extracts were prepared by dissolving in acetone and tested against *S. litura*. Fresh castor leaf discs with a diameter of 4 cm were punched out using a cork drill. They were then individually immersed in crude extract concentrations of 0.625, 1.25, 2.5, 5% and 75, 150, 300, 600 ppm of SNP (Green Synthesis Silver Nanoparticle). Since acetone was used to dissolve the crude extracts, the leaf discs dipped in the substance served as a negative control. To prevent the leaf discs from drying out too early, wet filter paper was placed in each 1.5 cm × 9 cm plastic petri dish and a single third instar larva was placed in each petri dish. The progressive leaf consumption of the treated and control larvae was recorded over 24 hours using a leaf area meter (Delta-T Devices, S. No: 15736 F 96, UK). The number of leaves eaten by the treatment larvae was adjusted based on the negative control. For

each treatment, five replicates each containing 10 larvae were kept. The Ben Jannet et al. (2000) formula was used to calculate the antifeedant activity.

$$\text{Antifeedant Index} = \frac{\text{Control}-\text{Treated}}{\text{Control}+\text{Treated}} \times 100$$

In the case of the control and treated discs, C and T denote the relative amounts of leaf consumed by the larvae.

2.5. Larvicidal Activity

Different concentrations of crude extracts and SNP was applied using the leaf dipping method. The larvae were exposed to the treated leaves. The larvae were kept alive on fresh castor bean leaves that had not been treated for a whole day. Fresh castor leaves were delivered every 24 hours. Death of the larvae was noted 96 hours after treatment. For each treatment, five replicates containing ten larvae each were maintained. The mortality percentage was calculated using [11]. The experiment was carried out with a light photoperiod of 14:10, a laboratory temperature of $27 \pm 2^\circ \text{C}$ and a relative humidity of $75 \pm 5\%$.

$$\text{Abbot corrected mortality} = \frac{\% \text{mortality in treatment} - \% \text{mortality in control}}{100 - \% \text{mortality in control}} \times 100$$

2.6. Histological analysis in midgut of *Spodoptera litura*

The digestive system of *S. litura* was used for the histological examination. The abdominal part of the larval parts was fixed in 10% neutral buffered formalin. The tissue samples were dehydrated and cleaned using a tissue processor. The samples were then fixed in paraffin blocks using a rotary microtome and an embedding station, 4 μm thick sections were cut (Leica RM2255 microtome, Buffalo Grove, IL, USA), and stained with hematoxylin and eosin stains. Finally, the stained sections of the tissue sample of *S. litura* larvae were examined using light microscopy [12].

2.7. Statistical analysis

The mortality data were subjected to one-way analysis of variance (ANOVA) with Tukey's multiple range tests to find the effective treatment at $P < 0.05$ for further binary mixture studies and to determine the significant difference between treatments. Probit analysis was used to calculate the lethal concentration for 50% mortality (LC50) for a 96-h exposure period. Probit analysis was performed using SPSS statistical software (version 16.0).

3. Results

3.1. Antifeedant and larvicidal activity

The present study deals with the antifeedant and larvicidal activities of hexane, ethyl acetate, and methanol extracts of *S. torvum* against *S. litura*. Antifeedant activity of *S. torvum* crude extract was tested against *S. litura* results are presented in **Table 1**. The extract hexane and methanol were showed moderate antifeedant activity (51.33%), (77.16%) and ethyl acetate extract showed significant activity

(86.16%) at 5% concentration. Ethyl acetate exhibited larvicidal activity of 88.21% with LC50 value of 5%. The larvae after treatment with ethyl acetate extract showed mortality in larval, and adult stages (**Table 2 and 3**)

3.3. Histological analysis in the midgut of *S. litura*

Histological analysis was observed 72 h after exposure in the midgut of 3rd instar *S. litura* larvae treated with a 5% concentration of *S. torvum* leaf extract. A significant effect was observed when the larvae were treated with a concentration of 5% of ethyl acetate extract. There was disruption of the striated border and peritrophic membrane of the epithelial cells. The maximum damage was observed in larvae exposed to ethyl acetate leaf extract. Epithelium and significant vacuolization in the goblet and digestive cells of the midgut (**Fig 1**).

4. Discussion

Many plants are reported to have antifeedant, growth inhibition, and larvicidal effects against *S. litura* such as, Eg., *Zingiber officinale*, *Pedaliium murex*, *Vitex negundo*, *Citrus sinensis* [13], *Melia dubia* [14], *Ocimum basilicum* [15]. Koul et al. [16] found that the extract of *Aglaia elaeagnoidea* had maximum antifeedant and larvicidal activity against *Helicoverpa armigera* and *Pieris rapae*. *S. torvum* plant was studied with *C. quinquefasciatus* larvae achieved maximum larvicidal activity [17]. Pavunraj et al. [18] stated that ethyl acetate leaf extract of *Pergularia daemia* has good antifeedant activity against *S. litura*. Salinas-Sanchez et al. [19] reported that *T. erecta* hexane extract at 48%, acetone extract at 60% and ethanol extract at 500 ppm had a concentration of 72% compared to *S. litura* larvae. The results of Bhatt et al. [20] were closely related to our result. It was found that *D. falcata* leaves had activity of 98.58%, *A. indica* fruit extract of 85.72% and *C. reflexa* extract of 98.58%.

The study's most important discovery was that the antifeedant treatment with the highest effectiveness also had the highest larvicidal activity. Many plants have been used for their larvicidal activity against *S. litura*, including *C. calamitosum*, *C. viscosum*, *C. multiflorum*, *C. philippinum*, *C. serratum*, *C. paniculatum* and *C. splendens* [21]. These results support the validation of Jbilou et al. [22] found that acetone and ethanol extracts of *Anticarsia gemmatilis* contained potential insecticidal agents to control *A. pubescens*. *Acorus calamus* ethyl acetate leaf extract at 5% concentration 40.24% and *Annona squamosa* at 40% larval mortality was achieved against *S. litura* [23]. The leaf extract of *Marrubium vulgare* showed 42.2% larval mortality at a concentration of 5.0%. The larvae eventually stopped feeding, resulting in a developmental arrest at various stages of the larval instar [15]. The herbal insecticides and antifeedants can be important components of an integrated pest management program.

The peritrophic membrane of *S. litura* larvae remained intact in the midgut region and the untreated larvae showed signs of midgut architecture. Regenerative cells adhere to the surface of the basement membrane. Well-developed goblet cells and longitudinal muscle layers follow the epithelial cells, which have an elongated nucleus and decondensed chromatin. The epithelial cells have a densely packed,

striated brush border and form columnar epithelial cells (Fig. 1). This condition shows that the proper functioning of the midgut cells enables the metabolic activity of the insect larvae [12]. The effect was found to be dose dependent. The current results were consistent with those reported by Fiaz et al. [24] were found in the midgut digestive cells of *Anticarsia gemmatalis* and *S. litura* larvae.

In insects, digestion and food absorption take place in the midgut area, which is of endodermal origin [25]. The midgut is a crucial part of the alimentary canal and takes up a lot of space in the hemocoel. In addition, it plays a crucial role in many physiological controls such as blood flow, immunological response, and metabolism [26]. In addition, azadirachtin has been found to cause certain histological changes in the body tissues of insects [27]. When plant pesticides were applied to *Schistocerca gregaria* and *Locusta migratoria*, Cottee [28] noted changes such as cell necrosis, vacuolization of the cytoplasm, reduction in nuclear size, and cell regeneration. Therefore, interfering with any of these processes can serve as a target or strategy for subsequent pest control measures.

5. Conclusion

In conclusion, our research highlights the diverse potential of *S. torvum* leaf extracts and their transformative effect on the management of *S. litura* larvae. These findings not only expand our understanding of natural pest control, but also provide opportunities for the development of innovative, environmentally friendly solutions in the agricultural sector. We look forward to further research and application of these findings in the continued pursuit of sustainable pest control.

Declarations

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Authors Contribution

R. Murugesan: Writing – review & editing, Writing – original draft, Software, Methodology, Funding acquisition, Formal analysis, Conceptualization, Data curation, Investigation. **K. Vasuki:** Conceptualization, Data curation. **B. Kaleeswaran:** Investigation, Formal analysis, Methodology, Supervision, Validation.

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Data availability

Conflict of interest: The authors declare that they have no conflict of interest.

Availability of data and material

All the collected articles and their data are enclosed in this article in the reference section.

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Tables

Table 1. Percent antifeedant activity of *S. torvum* leaf extracts against 3rd instar larvae of *S. litura* at different concentrations.

<i>Solanum torvum</i>		Dosage	Antifeedant
Leaf Extracts (%)	Hexane	0.625	04.52 ± 0.059 ^a
		1.250	10.70 ± 0.152 ^b
		2.500	20.83 ± 0.170 ^c
		5.000	51.33 ± 0.198 ^e
	Ethyl acetate	0.625	05.35 ± 0.093 ^a
		1.250	14.19 ± 0.654 ^b
		2.500	35.57 ± 0.400 ^c
		5.000	86.16 ± 0.133 ^e
	Methanol	0.625	05.22 ± 0.144 ^a
		1.250	13.87 ± 0.306 ^b
		2.500	31.15 ± 0.094 ^c
		5.000	77.16 ± 0.126 ^e

Each value is an mean of five replicates with standard error (Mean ± SE). Means within a column followed by the different letters are significantly different (P<0.05) as determined by Tukey's test.

Table 2. Larvicidal activity of 3rd instar larvae of *S. litura* exposed to *S. torvum* leaf extracts at different concentrations.

<i>Solanum torvum</i>		Dosage	Mortality
Leaf Extracts (%)	Hexane	0.625	04.18 ± 0.244 ^a
		1.250	12.87 ± 0.200 ^a
		2.500	24.86 ± 0.244 ^b
		5.000	52.21 ± 0.374 ^c
	Ethyl acetate	0.625	14.45 ± 0.244 ^a
		1.250	28.07 ± 0.374 ^b
		2.500	52.29 ± 0.200 ^c
		5.000	88.21 ± 0.200 ^e
	Methanol	0.625	06.28 ± 0.244 ^a
		1.250	18.11 ± 0.200 ^b
		2.500	34.38 ± 0.244 ^c
		5.000	68.11 ± 0.374 ^d

Each value is an mean of five replicates with standard error (Mean ± SE). Means within a column followed by the different letters are significantly different (P<0.05) as determined by Tukey's test.

Table 3. Probit analysis of larvicidal mortality for *S. litura* exposed to *S. torvum* leaf extracts at different concentrations.

<i>S. torvum</i> extracts	LC ₅₀ (%)	95% confidence limit (LL-UL)	LC ₈₀ (%)	95% confidence limit (LL-UL)	Slope ± SE	χ ² (df)
Hexane	5.025	4.306 - 6.162	-	-	1.99 ± 0.10	75.95 (23)
Ethyl acetate	2.05	1.83 - 2.29	4.61	3.99 - 5.53	2.39 ± 0.09	88.78 (23)
Methanol	3.31	2.98 - 3.72	-	-	2.33 ± 0.09	66.65 (23)

LC = lethal concentration; LL = lower limit; UL = upper limit; SE = standard error; χ² = chi-square; df = degree freedom.

Figures

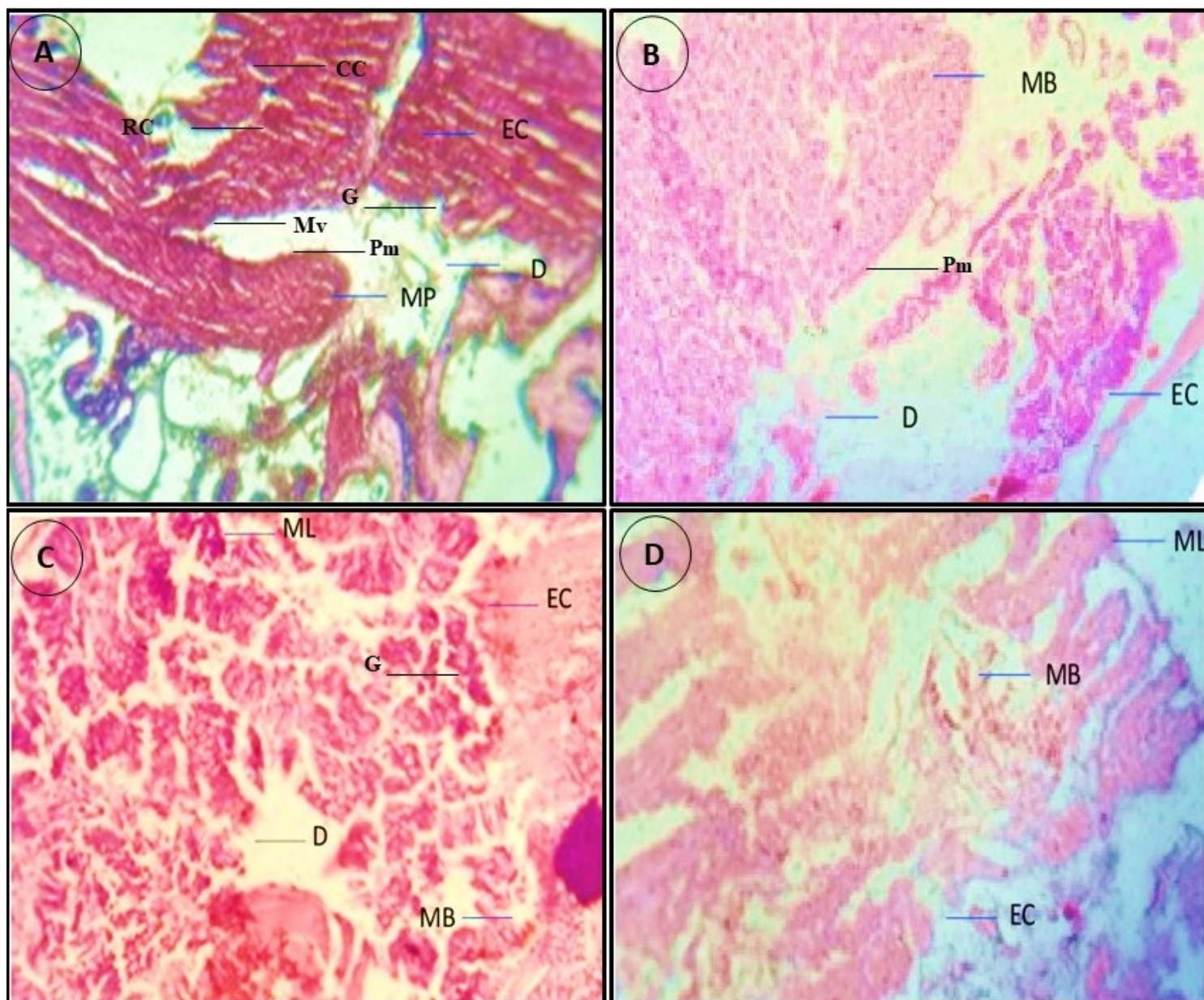


Figure 1

Histological examination of 3rd instar larvae of *S. litura* (A- control; B- hexane extract; C- ethyl acetate extract; D- methanol extract treatments). Muscular Layer (ML), Membrane Peritrophic (MP), Epithelial Cell (EC), Basal Membrane (MB) and disintegration (D), CC- Columnar cells, RC- Regenerative cells, G- Goblet cell, Mv- Microvilli, and Pm-Peritropic membrane.

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