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Biopesticidal Potential of *Lippia javanica* (Burm. F) Spreng. Leaf Extracts and their Fractions against *Spodoptera litura* (Fab.)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Three organic solvent extracts of *Lippia javanica* leaves were screened for their antifeedant and larvicidal activities against the 4th instar larvae of *Spodoptera litura* at 5% concentration. The maximum antifeedant and larvicidal activities were recorded in ethyl acetate extract (76.57%) for antifeedant and (90.40%) for larvicidal activity, followed by chloroform and hexane extracts. Ten fractions were obtained from the ethyl acetate extract of *L. javanica* by using different combinations of hexane and ethyl acetate as the mobile phase through column chromatography. The fractions were screened at 1000 ppm concentrations for antifeedant and larvicidal activities. Fraction 10 (71.75%) was found to be the most effective one, followed by fraction 4 (61.32%) and fraction 7 (60.58%) for antifeedant acitivity. At a 1000 ppm concentration, fraction 3 exhibited the highest larvicidal activity (79.20%) against *S. litura*, followed by fraction 1 (76.0%), while fractions 5 and 6 have shown equal activity. Quantitative protein analysis revealed that treatment with the eighth fraction reduced the haemolymph protein drastically (1.52 mg/mL) compared to control (2.84 mg/mL) and other fractions. In the reference control azadirachtin treatment, the haemolymph protein quantity was found to be nearly the same (1.4 mg/mL) as that of the eighth fraction. Treatment with the first and second fractions resulted in increased haemolymph protein (8 and 7.6 mg/mL, respectively). The bio-efficacy of *L. javanica* in pest management is discussed.

Keywords: Antifeedant; larvicidal activity; Lippia javanica; Spodoptera litura; plant extracts; environmentally friendly bio-pesticide.

1. INTRODUCTION

"The problem associated with the use of synthetic chemicals as insecticides has led researchers to search for new, less damaging pest management tools"[1]. "Most synthetic insecticides act as acute toxic chemicals, causing the rapid elimination of pest insects. However, they cause a complete washout of beneficial insects too" [2]. So the natural balance between pests and predatory insects is disturbed, and pest resurgence occurs [3]. This ends up in the unrestrained application of the chemical insecticides. An urgent need for eco-friendly pesticides was realized in the middle of the 20th century, and more than 2000 pesticides have been tested up to the end of the 20th century [4]. Plant products are mostly target-specific and exhibit their anti-insect properties in many ways; they act as antifeedants [5], repellants [6], insecticides [7], attractants [8], ovicides and oviposition deterrents [9], and growth regulators [10]. This multifaceted action of botanicals is advantageous for the control of pest populations.

"The pesticidal plant *Lippia javanica* (Verbenaceae), commonly known as fever tea or lemon bush, is a small tree often with strong aromatic leaves. This plant is well known medicinally to many African tribes and to many avid herbalists and herbal gardeners. Different parts (the leaves, flowers, twigs, and occasionally the roots) of the plant are used for different purposes. *Spodoptera litura*, the notorious insect, is a polyphagous pest of more than 120 agricultural crops" [11]. The unlimited application of synthetic pesticides to control this pest has created pesticide resistance [12]. The present study was undertaken to assess the antifeedant and larvicidal effects of *L. Javanica* leaf extracts on *S. litura* in the laboratory.

2. MATERIALS AND METHODS

2.1 Plant Collection

The matured leaves of the plant *L. Javanica* were collected from the Coleroon River in Nathiyanur Village, Ariyalur District, Tamil Nadu, India. This is a well-known tourist destination with historical significance (Image 1 to 5). An authoritative plant taxonomist from the Department of Botany at Madras Christian College (MCC), Chennai, identified the plant specimen. The extraction and isolation of fractions were outlined in a prior study conducted by Pavunraj et al. [13].

2.2 Culture of *Spodoptera litura*

The test insect, *S. litura,* was maintained as per the method of Pavunraj et al. [13].

2.3 Antifeedant Activity Test

The antifeedant activity of crude extracts and fractions was studied using the leaf disc no-choice method [14]. The stock concentration of crude extracts (5%) and fractions (1000 ppm) was prepared by dissolving them in acetone and mixing them with dechlorinated water. Polysorbate 20 (Tween 20) at 0.05% was added as an emulsifier [15, 16]. From the stock, the required concentrations were prepared and tested against the 4th instar larvae of *S.litura.* Fresh castor leaf (*Ricinus communis*) discs of 3cm diameter were punched using a cork borer and dipped in 5.0% concentrations of crude extracts and 1000 ppm concentrations of fractions separately and air dried for 5 minutes. After air-drying, treated leaf discs were kept inside separate petri dishes (1.5 x 9 cm) for 2 hours. Pre-starved fourth-instar larvae of *S. litura* were introduced on each treated leaf disc. Leaf discs treated with acetone were considered controls. *Azadirachtin*, a company product, was tested at 50 ppm as a reference control. Ten replications were maintained for crude extract, each fraction, and control. Progressive consumption of leaf area by the larva in a 24-hour period was recorded in control and treatment groups using a leaf area meter (Delta-T Devices, Serial No. 15736 F 96, UK). The percentage of antifeedant index was calculated using the formula of Ben Jannet et al*.* [17].

Image 1.The site bound for the collection of plants is, quite rightly, a well-liked tourist destination with historical significance. Plant collection area near the banks of the Coleroon River in Nathiyanur Village, Ariyalur District, Tamil Nadu

Image 2.Vegetation of Coleroon River's banks

Image 3. Birdwatches view point

Image 4. Karaivetti Bird Sanctuary, Ariyalur District, Tamil Nadu, is nearer to the banks of the Coleroon River

Image 5. A view of the birds sitting in the sanctuary
(*Source of images: 4 & 5*);*https://www.google.com/search?q=Karaivetti+Bird+Sanctuary+ariyalur+district*

2.4 Larvicidal Activity Test

Fresh castor leaves were treated with crude extract, azadirachtin, and different fractions (as mentioned in the atifeedant activity). Castor leaf, treated with acetone, was considered a control. In each treatment, 20 pre-starved (2-h) fourth-instar larvae of S. litura were introduced and allowed to feed on the treated leaves for 24 hours. Larval mortality was recorded up to pupation. The percentage of larval mortality was calculated using Abbott's [18] formula.

2.5 Collection and Processing of Haemolymph of *S. litura*

After 3 days of treatment, the hemolymph was drawn by pricking the second proleg of the larvae with a sterilized needle. The hemolymph was collected from 5 larvae in prechilled eppendorf vials with few crystals of phenylthiocarbamide (1-phenyl-2-thiourea). The sample was centrifuged in a refrigerated centrifuge at 10,000 rpm for 10 minutes at 4 °C to get the supernatant and stored at 20 °C for protein quantification and protein gel electrophoresis studies by Koul and Wahab [19].

2.6 Quantitative and Qualitative Estimation of Haemolymph Protein

The haemolymph protein concentration per mL was estimated according to the Bradford method using bovine serum albumin as the standard. The qualitative estimation of haemolymph protein profiles was determined by one-dimensional sodium-dodecyl sulfate-polyacriylamidegel electrophoresis (SDS-PAGE) using a vertical slab gel electrophoresis unit as detailed by Laemmli [20].

2.7 Statistical Analysis

The data were subjected to one-way analysis of variance (ANOVA) to find out the significance of the treatments at the 5% level. The mean values were separated to expose the significantly effective treatments by the least significant difference (LSD) at the 5% level.

3. RESULTS

3.1Antifeedant and Larvicidal Activity

The effect of different extracts on antifeedant and larvicidal activity at 5% concentration levels is given in (Fig. 1**).** Ethyl acetate extracts exhibited promising results, followed by chloroform and hexane extracts. The maximum antifeedant activity (76.57%) and larvicidal activity (90.04%) were observed in the ethyl acetate extract treatment. Then fractions were isolated from the ethyl acetate extract of *L. javanica*. Among the fractions, fraction 10 caused the maximum antifeedant activity (71.75%), followed by fraction 4 (61.32%) and fraction 7 (60.58%)**.** However, considering the overall treatments, azadirachtin exhibited the highest antifeedant activity (80.33%) (Table 1). The highest larval mortality was recorded in fraction 3 (79.20%), followed by fraction 1 (76.0%), fraction 5 (72.8%), and fractions 2 and 6 (72.0% each). Azadirachtin caused only 26.4% of larval mortality**.** The plant products reduced the feeding duration, and food ingested by larvae led to abnormalities in insect growth and development. Deformities in the larval, pupal, and adult stages were also observed.

3.2 Pupicidal Activity

All the fractions and azadirachtin caused mortality at the pupal stage as well. This result clearly indicated that the toxins in the fractions had a prolonged effect on *S. litura* if the pest consumed the treated food. Both azadirachtin and fraction-8 killed 66.66 percent of the pupa. Fractions 10, 4, 3, and 9 gave pupal mortalities of 60.0, 57.42, 42.85, and 42.85 percent, respectively.

3.3 Qualitative and Quantitative Changes in Haemolymph Protein

According to Engelmann [21], proteins are the fundamental building blocks of all living things and change both quantitatively and qualitatively as they develop. In addition, it plays a crucial role in the synthesis and breakdown of structural materials and it is constantly changing. In the present study, the concentration of protein was reduced in the treated larvae. The eighth fraction reduced the haemolymph protein drastically (1.52 mg/mL) compared to the control (2.84 mg/mL) and other fractions. In the reference control azadirachtin treatment, the haemolymph protein quantity was found to be nearly the same (1.4 mg/mL) as that of the eighth fraction. Treatments with the first and second fractions resulted in increased haemolymph protein (8 and 7.6 mg/mL, respectively) (Fig. 2). The treatment-induced haemolymph protein changes in treated larvae may be attributed to either a higher rate of proteolysis or a toxic stress of fractions related to reduction of protein synthesis by deranging the protein machinery. Similar observations were reported by Jisheng et al [22-26].

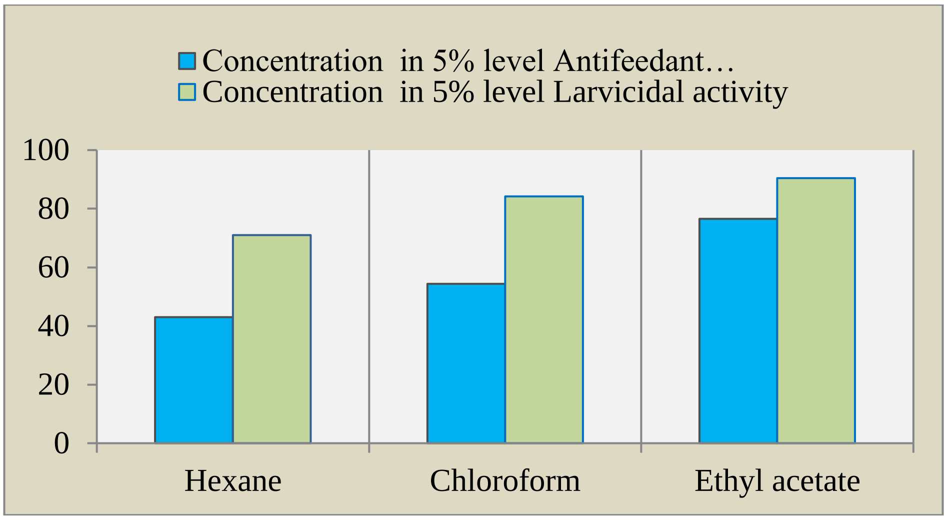

Fig. 1. Antifeedant and larvicidal activities different crude extracts of *L. javaninca* leaves against 4th instar larvae of *S. litura* (mean ±SE)

Values carrying different alphabets in a column are statistically significant at the5% level by LSD.(No=10 for antifeedant activity; No=20 for larvicidal activity)

Table 1. Antifeedant and larvicidal activities different fractions of ethyl acetate extracts of *L. javaninca* leaves against 4th instar larvae of *S. litura* (mean ±SE)

Fractions	Concentrationsin 1000ppm	
	Antifeedant activity	Larvicidal activity
Fraction=1	27.30 ± 5.92^{e}	70.00±2.82^{ab}
Fraction=1	50.94 ± 6.29^{c}	72.40±5.38^{b}
Fraction=1	41.84 ± 0.29^{d}	79.20±3.87^{a}
Fraction=1	61.32 ±3.15^{b}	42.40±4.11^{e}
Fraction=1	38.91 ± 1.40^{d}	72.80±3.20^{b}
Fraction=1	28.58 ± 2.29^{e}	72.00±2.82^{c}
Fraction=1	60.58 ± 4.13^{b}	35.20±4.27^{f}
Fraction=1	35.72 ± 2.27^{de}	66.40±3.00^{d}
Fraction=1	40.11 ± 2.82^{d}	67.20±4.63^{cd}
Fraction=1	71.75 ± 0.71^{a}	40.00±3.34^{e}
Fraction=1	80.33 ± 0.87^{a}	26.40±2.03^{g}

Values carrying different alphabets in a column are statistically significant at the 5% level by LSD. (No=10 for antifeedant activity; No=20 for larvicidal activity)

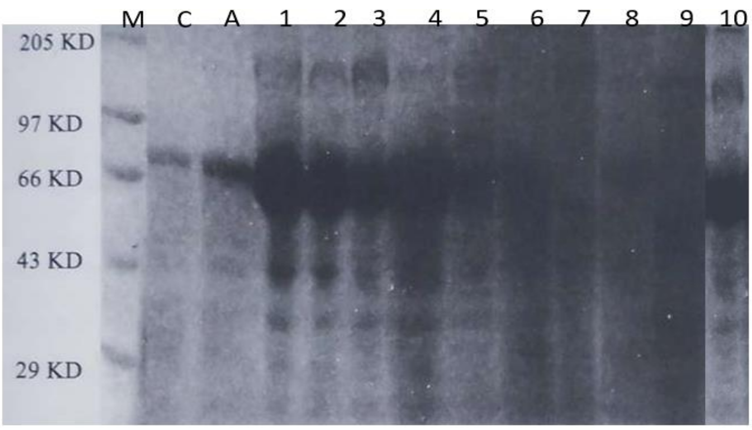

Fig. 2. SDS-PAGE analysis of haemolymph protein profile of 4th instar larvae of *S. litua* treated with *L. javanica* leaf extracts

Note: M = Marker; C = Control; A = Azadirachtin (reference control); 1–10 = fractions

In the present study, qualitative changes in the haemolymph protein were observed through electrophoretic separation of the haemolymph. Haemolymph volume changes under stress, resulting in fluctuations in the protein pattern. The appearance of protein bands in the haemolymph in the treatment indicated developmental changes due to toxic stress on fractions. The SDS-PAGE separation of haemolymph protein revealed that the polypeptide of molecular weight 66 KDa showed variations among the different treatments. This polypeptide was concentrated (quantitatively high) at fractions 1, 2, 3, and 4. Treatments with fractions 5 and 6 and control showed a qualitatively lower amount of polypeptide of 66 KDa. This suggested that treatments with fractions from 1 to 4 increased the polypeptide to 66 KDa. Further analysis of the protein banding pattern showed that a polypeptide of nearly 200 KDa molecular weight was found in treatments 2, 3, and 4 but was disintegrated in treatments 5 and 6. The variations in protein band might be due to the susceptibility of the larvae to fractions. This is in accordance with the reports of Shaurub [27-31].

The present study indicated that the ethyl acetate extract of *L. javanica* is promising in reducing the feeding rate and increasing larval mortality. The qualitative and quantitative changes in haemolymph protein and abnormal moulting in the larval stage due to the botanical treatment added points to support the fact that this plant product is interfering with the physiological activities of the pest. Similar findings have been reported by many researchers in many other plants [32-34]. This is the first report for the plant *L. javanica* having antifeedant and larvicidal activities against *S.litura*.

4. DISCUSSION

In this present investigation, different solvent crude extracts and fractions*L. javanica* leaves exhibited antifeedant and larvicidal activities against *S. litura* depending on the concentrations. This finding coincides with finding of Pavunraj [35] who noticed that “plant characteristics, such as chemicals, color, trichomes, and architecture, in concert with the insect’s internal milieu, form the basis for discrimination between acceptable and unacceptable Plants for feeding or oviposition by various species of phytophagous insects”.

“The results revealed that the antifeedant activity against *S. litura*was maximum in ethyl acetate extract of *L. javanica*. Similar results were reported in crude extract with specific mode of action against insects is a complex mixture of compounds” [36-38]. Many researchers have reported crude extracts on *S. litura* [39-41] on *S. littoralis*. Larval population was significantly reduced. The maximum larval mortality was observed in ethyl acetate extract of *L. javanica* which showed significant reduced larval population. This is consistent with the results of Pavunraj et al.[42], who found that at a 5% concentration, larval mortality was seen in DCM extract *Spilanthes acmella* against *S. litura* (44.88%).According to Paul and Choudhury [43] *H. armigera* showed significant oral toxicity to the crude extract of *L. cubeba*leaves.Pavunraj and collaborators (2024) reported that the 6th fraction obtained from the dichloromethane leaf extract of *Aristolochia bracteolata* had strong larvicidal activity (81.77%) against *E. vittella* at a 1000 ppm concentration.

As shown in the proposed investigation, the test insect's haemolymph protein underwent both qualitative and quantitative changes that were influenced by the ethyl acetate extract of *L. javanica*.The results of Huang et al.[44] who found that camptothecin (CPT), a quinoline alkaloid exposed larvae, exhibited strong insecticidal molecular target against *S. frugiperda*, were corroborated by the current findings.Furthermore, Liu et al.[45] investigated how carvacrol controlled the growth and development of *Spodoptera frugiperda* larvae by influencing the process of food digestion and applying its toxicity to the larvae through interaction with a range of insecticidal targets. This resulted in the inhibition of larval growth and the induction of mortality.

5. CONCLUSION

The present study indicated that the ethyl acetate extract of *L. javanica* is promising in reducing feeding rates and increasing larval mortality. The qualitative and quantitative changes in the haemolymph protein and abnormal moulting in the larval stage due to the botanical treatment added points to support the fact that this plant product is interfering with the physiological activities of the pests.

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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