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Evaluation of *Lantana camara* Leaf Extract as Biopesticide for Lifecycle Disruption in *Spodoptera litura*

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ABSTRACT

Spodoptera litura is a destructive agricultural pest whose management is hindered by synthetic pesticides' ecological and health risks. Plant-based biopesticides, such as *Lantana camara* leaf extract, provide a sustainable alternative due to the presence of bioactive phytochemicals with pesticidal properties. This study aimed to evaluate the efficacy of *L. camara* extract in disrupting the lifecycle of *S. litura*. A completely randomized design was adopted, and six treatment concentrations of *L. camara* extract, namely 0%, 6.25%, 12.5%, 25%, 50%, and 100%, were applied to second-instar larvae. Observations included larval development time, leaf consumption, pupation rate, pupal weight, moth emergence, and fecundity. Data were analyzed using ANOVA, and the results showed that the extract had strong concentration-dependent effects on all variables. Interestingly, the 6.25% concentration proved to be the most efficient treatment, as it yielded statistically comparable results to higher concentrations while requiring a lower dose. Feeding activity was significantly reduced at 6.25% concentration compared to control. Larval development was delayed or halted entirely at critical thresholds. Pupation, moth development, and fecundity were inhibited by concentrations greater than 25%. In conclusion, *L. camara* extract effectively disrupted the lifecycle of *S. litura*, providing immediate suppression and long-term population control. Future studies are recommended to validate these results under field conditions and evaluate impacts on non-target species.

1. Introduction

Spodoptera litura, commonly known as the tobacco caterpillar or common cutworm, is a polyphagous pest of major economic importance. It is found in tropical and subtropical regions, including Asia, Africa, North America, and Oceania (Prajapati *et al.* 2020; Tang *et al.* 2022; Tippimath 2023). Belonging to the family Noctuidae, this pest affects a wide range of economically important crops, such as cotton, chili, tobacco, groundnut, tea, cabbage, eggplant, cauliflower, capsicum, potato, and castor. The larvae feed voraciously, causing significant defoliation and damage to leaves, fruits, and pods, which often leads to yield reductions of 10-30% (Aggarwal *et al.* 2010; Prajapati *et al.* 2020; Aliyya *et al.* 2021). Severe infestations can strip plants completely, reducing photosynthesis, stunting growth, and affecting agricultural productivity (Pushpa *et al.* 2013; Uthandi 2020).

Conventional pest control often relies on synthetic chemical pesticides, which provide rapid effects and are easily accessible. However, their widespread use raises ecological and health concerns (Hong *et al.* 2013; Zhang *et al.* 2022; Muthu *et al.* 2023). These include pesticide resistance in *S. litura*, environmental contamination, disruption of biological control, and toxic residues in crops, posing risks to human health and food safety (Shad *et al.* 2011; Sahayaraj *et al.* 2018; Nguyen *et al.* 2023). The indiscriminate use of pesticides also harms non-target organisms, such as beneficial insects and natural predators (Sahayaraj *et al.* 2018; Wang *et al.*

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2020). These issues highlight the need for sustainable pest management strategies that are both effective and environmentally safe. Biopesticides offer a promising alternative to chemical pesticides.

Recent interest in environmentally sustainable agricultural practices has led to increased exploration of plant-based biopesticides. These biopesticides are more biodegradable, environmentally friendly, and have a lesser impact on natural enemies than chemical pesticides (Shah *et al.* 2019, 2020; Acheuk *et al.* 2022). They help address challenges such as insecticide resistance and the ecological impact of synthetic pesticides (Tharamak *et al.* 2019; Zhang *et al.* 2022).

Among the explored botanicals, *Lantana camara* has drawn attention for its pest-repellent properties. This plant contains a range of phytochemicals, including flavonoids, alkaloids, saponins, terpenoids, and glycosides, which possess antimicrobial, anti-inflammatory, and insecticidal properties (Anand *et al.* 2018; Al-Snafi 2019). The extract of *L. camara* has shown toxicity to various insect species, including coleopterans, and has demonstrated larvicidal activity against stored grain pests (Aisha 2024), making it a valuable tool in pest management.

The resilience of *S. litura* further emphasizes the need for alternative pest control strategies. The pest has developed resistance to many synthetic pesticides, reducing the effectiveness of conventional control methods. Additionally, the environmental persistence of chemical pesticides poses long-term ecological risks, reinforcing the need for sustainable alternatives (Zhang *et al.* 2022). Incorporating *L. camara*-based biopesticides into integrated pest management (IPM) systems could be an effective solution. These biopesticides complement other IPM components, enhancing their efficiency while reducing reliance on harmful chemicals (Sari *et al.* 2021). Thus, *L. camara* represents a versatile, eco-friendly option for sustainable pest management.

Although previous studies have demonstrated the pesticidal potential of *L. camara*, most have concentrated on specific effects such as larval mortality or growth inhibition (Yuan & Hu 2012; Châu *et al.* 2019; Ayalew 2020; Tamboli *et al.* 2022). For example, a 15% methanolic extract and a 40% aqueous extract of *L. camara* were shown to cause over 90% larval mortality and complete growth inhibition, respectively (Deshmukhe *et al.* 2011; Singh *et al.* 2023). However, the broader impacts of these extracts on the pest's entire lifecycle remain insufficiently explored. Parameters such as pupation rates, moth emergence, and reproductive potential remain insufficiently studied. Furthermore, the critical thresholds of *L. camara* extract required to disrupt the pest lifecycle, particularly for *S. litura*, are yet to be determined. This study addresses these gaps by evaluating the efficacy of *L. camara* leaf extract in managing *S. litura* populations, focusing on developmental and reproductive parameters, including larval instar progression, leaf consumption, pupation rates, moth emergence, and fecundity. The study aims to establish *L. camara* as a viable biopesticide for immediate pest suppression and long-term population control. This study has significant implications for sustainable agriculture. Demonstrating the dual-action potential of *L. camara* extract in targeting both larval activity and reproductive capacity contributes to the development of environmentally sustainable pest management strategies.

2. Materials and Methods

2.1. Experimental Design

A Completely Randomized Design (CRD) was adopted with six treatment groups and three replicates, totaling 18 experimental units. Ten larvae per unit were sampled, resulting in 180 larvae tested in this study. Treatments included *L. camara* extract at concentrations of 0% (control), 6.25%, 12.5%, 25%, 50%, and 100%.

2.2. Rearing of *S. litura*

Larvae of *S. litura* from natural habitat were reared in 20 cm high plastic containers with a diameter of 15 cm under laboratory conditions. Each container housed five larvae, and the lids were modified with fine mesh cloth (±65 mesh) to allow ventilation. Fresh mustard leaves were provided daily as the food source, and containers were cleaned to remove residual feed and feces. Pupae were transferred to separate containers for the development of the moth.

Adult moths were fed a 10% honey solution using cotton balls (Sinyong *et al.* 2024) suspended within the containers to maintain vitality and fecundity. Male and female moth pairs were isolated in breeding chambers to obtain eggs for subsequent experiments. The eggs were hatched into larvae and reared until the second instar stage to be used as study samples.

2.3. Preparation of *L. camara* Leaves Extract

Fresh leaves of the middle part of *L. camara* were collected from the natural habitat (Figure 1) in the morning, placed in a paper envelope, and oven-dried


Figure 1. *Lantana camara* plant

at 70°C for 72 hours to achieve complete desiccation. The dried leaves were ground into a fine powder using a blender and macerated in 96% ethanol (500 g leaves per 2,000 ml ethanol) for 24 hours. The liquid extract was filtered through a fine cloth and concentrated using a rotary evaporator (Witeg 2 610 000, Germany) at 70°C with 50 rpm to yield a semi-solid crude extract. The extract was then weighed (g) according to the concentration of each treatment and then added with 100 ml of acetone solvent (except 0% concentration only using solvent as treatment).

2.4. Application of Treatments

The "leaf sandwich" (no-choice test) method was used to apply *L. camara* extract treatments. Two mustard leaves (15 × 10 cm^2) were prepared for each treatment. The lower leaf was uniformly coated (using a brush) with the appropriate concentration of *L. camara* extract. In contrast, the upper leaf was treated with adhesive paste (made from one part starch mixed with two parts hot water) and placed over the first, forming a sandwich. Second instar larvae, starved for three hours before treatment, were introduced to the containers. After 24 hours, treated leaves were replaced with fresh, untreated mustard leaves to prevent starvation, ensuring an accurate assessment of extract effects.

2.5. Data Collection

Observations were conducted daily to measure several variables, namely:

2.5.1. Larval Development Time

Time (in days) required for progression through instars II to V.

2.5.2. Leaf Consumption Rate

The extent to which larvae consumed leaf area after 24 and 48 hours of feeding was measured using the graph sheet method for both control and treated leaves. In the treatment group, the leaf area consumed was adjusted to account for the consumption of the control group. The formula used refers to Melanie *et al.* (2020) as follows:

$$\% A = \frac{(C_0 - T_0)}{(C_0 + T_0)} \times 100\%$$

Where:
A : The absolute antifeedant percentage (%)
C_0 : Leaf area consumed of control in the no-choice test (mm^2)
T_0 : Leaf area consumed of treatment in the no-choice test (mm^2)

2.5.3. Pupal Weight

This variable was measured using an analytical balance (Kern PLJ, Germany) for individual pupae.

2.5.4. Pupation and Moth Emergence Rate

Percentage of larvae successfully pupating relative to the initial larval count and percentage of moths successfully developing from pupae. The formula used refers to Siahaya & Rumthe (2018), namely % pupae or moths = (d/N) × 100%, where d = the number of larvae becoming pupae or the number of pupae becoming moths, and N = the number of larvae or pupae tested.

2.5.5. Fecundity

This study variable was conducted separately using moth samples from untreated larvae. Each treatment unit used a pair of male and female moths as experimental samples placed in a plastic container. Therefore, 18 pairs of moths were sampled in this experiment using the same experimental design as the previous stage. Treatments were given by mixing one part *L. camara* extract according to the treatment concentration (extracts + acetone solvent) and one part 10% honey

(except 0% concentration only using 10% honey as treatment). These treatments were used as food for moths following the previous rearing procedure. Each egg in the egg groups produced by female moths in three reproductive cycles was counted.

2.6. Statistical Analysis

All data were analyzed using one-way ANOVA to evaluate the significance of treatment effects across variables. The analysis continued with post hoc Honestly Significance Difference (HSD) analysis at a 95% confidence level, where significant differences were detected to determine pairwise differences among treatment groups. All stages of statistical analysis were performed using the Microsoft Excel 2021 application.

3. Results

3.1. Larval Development

The development of *S. litura* larvae showed significant variation across treatments with different concentrations of *L. camara* extract, except for instars IV and V (Table 1). Larvae in the control group (0% concentration) progressed uniformly through instars II to V (Figure 2A), with each stage requiring an average of two days. However, increasing extract concentrations led to prolonged developmental times. At 6.25% and 12.5% concentrations, instars II and III duration extended to 3.00 and 3.33 days, respectively. At concentrations of >25%, larvae failed to progress beyond instar III, showing a lethal threshold concentration (Figure 2B and C).

3.2. Feeding Inhibition

Extract significantly reduced larval leaf consumption, as shown in Table 2. In the control group, larvae consumed 25.67% and 26.67% of

Table 1. Development time of *S. litura* larvae treated with *L. camara* extract

Treatment (%)	Instar II (days)	Instar III (days)	Instar IV (days)	Instar V (days)
0	2.00±0.00^{c}	2.00±0.00^{c}	2.00±0.00	2.00±0.00
6.25	3.00±0.00^{b}	3.00±0.00^{b}	3.00±0.00	3.00±0.00
12.5	3.33±0.58^{b}	3.00±0.00^{b}	3.33±0.58	3.67±0.00
25	4.00±0.00^{a}	3.67±0.58^{b}	0.00±0.00	0.00±0.00
50	4.00±0.00^{a}	4.00±0.00^{a}	0.00±0.00	0.00±0.00
100	4.00±0.00^{a}	4.00±0.00^{a}	0.00±0.00	0.00±0.00

Different letter notations (a,b,c) in the same column are significantly different based on the HSD test at alpha 0.05

A (Control)

B (Concentration 50%)

C (Concentration 100%)

Figure 2. *Spodoptera litura* larvae at 3rd instar. (A) Control treatment, (B) treatment of *L. camara* extract at 50% concentration, (C) treatment of *L. camara* extract at 100% concentration

Table 2. Leaf consumption of *S. litura* larvae treated with *L. camara* extract after 24 and 48 hours

Treatment (%)	Leaf consumption (%) after 24 hours	Leaf consumption (%) after 48 hours
0	25.67±1.15[b]	26.67±1.53[b]
6.25	19.67±0.58[a]	18.33±0.58[a]
12.5	19.00±1.00[a]	15.00±0.00[a]
25	11.00±1.73[a]	7.33±2.08[a]
50	9.33±1.15[a]	5.33±0.58[a]
100	10.00±0.00[a]	3.67±0.58[a]

Different letter notations (a, b) in the same column are significantly different based on the HSD test at alpha 0.05

leaf material within 24 and 48 hours, respectively. Although the differences were not statistically significant, leaf consumption decreased to 19.67% and 19.00% at concentrations of 6.25% and 12.5%, respectively, after 24 hours, and further declined to 15.00% after 48 hours. At higher concentrations of >25%, leaf consumption decreased drastically, with low consumption of 3.67% at 100% concentration after 48 hours.

3.3. Pupation Rate, Pupal Weight, and Moth Emergence

The extract exhibited an inhibitory effect on pupation and subsequent life stages, as shown in Table 3. In the control group, the pupation (Figure 3A) rate was 6.67%, with an average pupal weight of 0.30 mg and a moth development (Figure 3B) rate of 6.67%. At 6.25% and 12.5%, pupation rates dropped progressively to 5.00% and 3.67%, respectively. Pupae formed at 6.25% and 12.5% concentrations exhibited abnormalities, including reduced body size and incomplete wing development (not documented). In contrast, no pupae were formed at concentrations above 25%, indicating complete suppression of moth development.

3.4. Fecundity

Because no moths were formed in the first stage of the study, specifically for fecundity observations, the study stages were repeated as described in the research procedure (fecundity variable). The results showed that fecundity declined rapidly with increasing extract concentrations (Table 4). Moths in the control group showed high fecundity, producing 78.00, 57.00, and 45.67 eggs in the first, second, and third observations, respectively (Figure 3C). However, fecundity was significantly reduced at 6.25% and 12.5%, with only 22.67 and 21.00 eggs laid during the first observation and no eggs laid afterward.

Table 3. Pupation Rate, pupal weight, and moth emergence of *S. litura* treated with *L. camara* extract

Treatment (%)	Pupation rate (%)	Pupal weight (mg)	Moth emergence (%)
0	6.67±0.58	0.30±0.00	6.67±0.58
6.25	5.00±0.00	0.17±0.00	5.00±0.00
12.5	3.67±1.15	0.20±0.00	3.33±0.58
25	0.00±0.00	0.00±0.00	0.00±0.00
50	0.00±0.00	0.00±0.00	0.00±0.00
100	0.00±0.00	0.00±0.00	0.00±0.00

A (Pupae)

B (Moth)

C (Egg group)

Figure 3. Pupation of *S. litura* and subsequent life stages. (A) Formed pupae, (B) moth emergence, (C) sample group of eggs produced after a pair of moths were treated

4. Discussion

The results of this study provide compelling evidence for the efficacy of *L. camara* leaf extract as a biopesticide against *S. litura*. *Lantana camara* extract can disrupt *S. litura's* lifecycle by inhibiting larval development, reducing feeding activity, preventing pupation and

Table 4. Fecundity of *S. litura* female moths treated with *L. camara* extract

Treatment (%)	Fecundity (eggs)-1st cycle	Fecundity (eggs)-2nd cycle	Fecundity (eggs)-3rd cycle
0	78.00±8.54^{c}	57.00±6.00^{b}	45.67±6.03^{b}
6.25	22.67±2.52^{b}	0.00±0.00^{a}	0.00±0.00^{a}
12.5	21.00±1.73^{b}	0.00±0.00^{a}	0.00±0.00^{a}
25	20.33±1.53^{b}	0.00±0.00^{a}	0.00±0.00^{a}
50	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
100	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

Different letter notations (a, b, c) in the same column are significantly different based on the HSD test at alpha 0.05

moth development, and eliminating fecundity at higher concentrations. These results are consistent with the initial hypothesis and show the potential of the extract as a sustainable alternative to synthetic pesticides.

The inhibitory effects observed in this study are consistent with previous results on plant-based biopesticides. Previous studies have shown the larvicidal and antifeedant properties of *L. camara* against coleopteran pest (Ayalew 2020; Aisha 2024) and *S. litura* (Châu *et al.* 2019), attributing these effects to the rich phytochemical profile, such as flavonoids (Aboshi *et al.* 2018; Tridiptasari *et al.* 2019), alkaloids (Ge *et al.* 2015), and saponins (Yu *et al.* 2022). This study extended these findings by comprehensively evaluating the extract's effect across multiple life stages of *S. litura*, including reproductive suppression. At higher concentrations, fecundity was completely inhibited across all observations. These results showed the ability of the extract to reduce reproductive potential, contributing to effective long-term pest population management.

The results corroborate previous studies showing dose-dependent effects of plant extracts, where higher concentrations exhibit stronger pesticidal efficacy. Although a 6.25% concentration was found to be statistically comparable to higher doses, the use of concentrations above 25% showed complete disruption of *S. litura's* lifecycle, including halted pupation and moth development. This suggests that 25% is a critical concentration beyond which larval development is effectively halted. This is important information in studies focusing solely on larval mortality or feeding inhibition. When applied at higher concentrations, these results showed the strong potential of the extract to disrupt the pest lifecycle.

The observed effects are driven by the bioactive compounds in *L. camara*, which interfere with essential physiological processes in insects. Flavonoids, saponins, and alkaloids exhibit antifeedant and growth-regulating properties through distinct biochemical and physiological mechanisms, disrupting critical pathways in insects. Flavonoids are known to act as phytoestrogens, mimicking or modulating the action of estrogen in insects. By altering reproductive hormone levels, flavonoids can disrupt critical pathways in growth and development. This includes interference with hormonal balances necessary for reproduction and molting processes, thereby affecting the progression of the insect lifecycle (Dasofunjo *et al.* 2020).

Saponins directly influence reproductive hormones, such as luteinizing hormone (LH) and follicle-stimulating hormone (FSH). The binding ability of this phytochemical to hormone receptors or enzymes in hormone synthesis can enhance or inhibit hormone production, resulting in significant disturbances in the estrous cycle and reproductive functionality. These disruptions compromise insect fecundity and population dynamics, showing the broader ecological impact of saponins (Rabadia *et al.* 2022). The dual mechanism contributes to the effectiveness of growth regulators and the ability to deter feeding.

Alkaloids exhibit neurophysiological effects through the modulation of neuropeptides, which are important in regulating key physiological processes, including feeding behavior and growth. By influencing the expression and activity of these neuropeptides, alkaloids can cause alterations in feeding patterns and developmental rates, often culminating in paralysis or death. This mechanism shows the potent role of alkaloids in targeting the neurophysiological systems of pests (Yang *et al.* 2024). The combined effect of flavonoids, saponins, and alkaloids showed diverse yet complementary mechanisms for disrupting hormonal and neurophysiological processes in insects, inhibiting larval development, and preventing pupation.

The dual-action potential of *L. camara*, such as immediate suppression of larval activity and long-term control through reproductive inhibition, makes extract an excellent candidate for IPM strategies. The biodegradability and low environmental persistence reduce the risks associated with synthetic pesticides, such as soil contamination and pest resistance development. By integrating *L. camara* extract with other biocontrol agents, such as entomopathogenic fungi or parasitoids, pest management programs could achieve synergistic effects, further enhancing control efficacy. The high efficacy at concentrations of >25% suggests the practical utility in high-infestation scenarios where rapid population suppression is critical. However, applying these concentrations should be balanced with considerations of cost-effectiveness and scalability, especially since

lower concentrations, such as 6.25%, also demonstrated statistically comparable effectiveness.

The potential impact of *L. camara* extract on non-target organisms requires careful evaluation despite showing promise as a sustainable pest control solution. The broad-spectrum activity of phytochemicals could inadvertently affect beneficial insects, such as pollinators and natural predators, thereby altering ecosystem dynamics. Future studies should focus on assessing the selectivity of *L. camara* extract and its compatibility with existing agroecosystems. Moreover, toxicity studies should thoroughly validate the safety of human health and the environment. The biodegradability and localized application suggest minimal long-term environmental risks, but further evidence should support these claims. The primary limitation of this study is the confinement to laboratory conditions. Controlled experiments provide valuable insights but do not account for real-world environmental variables like temperature fluctuations, humidity, and interactions with other biota. Therefore, field trials are essential to validate the results and assess the efficacy under diverse agricultural settings. While the study shows concentration-dependent efficacy, the long-term viability of *L. camara* extract as a pest control agent needs to be evaluated. Repeated use could lead to resistance in target pests, even though plant-based pesticides generally act through diverse mechanisms that reduce resistance risks. Investigating the potential of extract for integration with other biocontrol agents and the impacts on pest population dynamics over multiple generations further strengthens the utility of IPM programs.

In conclusion, this study provided comprehensive evidence for the efficacy of *L. camara* leaf extract as a biopesticide against *S. litura*. The results showed that extract significantly disrupted pest lifecycle through a combination of larval development inhibition, feeding activity reduction, and reproductive suppression. The extract completely halted pupation, moth development, and fecundity at higher concentrations, confirming its effectiveness for population control. The results showed the dual-action potential of *L. camara* extract, targeting immediate pest suppression while preventing subsequent generations through reproductive inhibition. This potential positioned *L. camara* extract as a sustainable alternative to synthetic pesticides, consistent with the principles of IPM and eco-friendly agriculture. Moreover, the natural origin, biodegradability, and high efficacy of *L. camara* contributed to its potential as a practical and environmentally safe pest control solution.

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