



From Pond to Pest Control: Antifeedant and Behavior-Modifying Properties of *Nelumbo nucifera* Against *S. litura*

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ABSTRACT: *Spodoptera litura* (Fabricius) is a highly destructive polyphagous pest that poses a serious threat to global agriculture through extensive crop damage and increasing resistance to conventional pesticides. In search of sustainable pest management alternatives, this study investigates the antifeedant potential and behavioral effects of *Nelumbo nucifera* (lotus) leaf extracts against *S. litura* larvae. Sequential extractions were performed using solvents of varying polarity, and the resulting extracts were subjected to laboratory bioassays to evaluate feeding deterrence, larval behavior, oviposition preference, and developmental responses. Among the tested fractions, the ethyl acetate extract exhibited the highest antifeedant activity, achieving a deterrent index of 72.5% at 2.0% concentration. Significant behavioral alterations were observed, including delayed feeding initiation, reduced consumption, strong avoidance of treated surfaces, and oviposition deterrence by gravid females. Additionally, sublethal effects such as growth retardation, molting delay, and moderate larval mortality were recorded. These findings suggest that *N. nucifera* contains bioactive phytochemicals capable of interfering with insect feeding and development, making it a promising candidate for eco-friendly pest management. Further phytochemical analysis and field validation are recommended to support its integration into integrated pest management (IPM) programs.

Keywords: *Spodoptera litura*, *Nelumbo nucifera*, oviposition, antifeedants.

INTRODUCTION

The polyphagous pest *Spodoptera litura* (Fabricius) poses a significant threat to various economically important crops worldwide. As concerns about environmental sustainability and pesticide resistance grow, there is an increasing interest in developing alternative pest control methods. Plant-derived antifeedants have emerged as promising candidates for eco-friendly pest management strategies. This study investigates the antifeedant properties of *Nelumbo nucifera* (lotus) extracts against *S. litura* and their impact on the pest's feeding behavior. *N. nucifera*, a widely cultivated aquatic plant, has been traditionally used for its medicinal properties and may contain bioactive compounds with potential insecticidal activities. The research aims to evaluate the efficacy of different *N. nucifera* extracts in deterring *S. litura* feeding, assess changes in larval behavior, and identify the key phytochemical constituents responsible for the observed antifeedant effects. Understanding the interactions between *N. nucifera* extracts and *S. litura*

could provide valuable insights for developing novel, environmentally friendly approaches to pest control, potentially reducing reliance on synthetic pesticides and mitigating their associated risks (Joshi *et al.*, 2023a).

Spodoptera litura, commonly known as the tobacco cutworm or cotton leafworm, is a significant agricultural pest affecting various crops worldwide. Its polyphagous nature and ability to develop resistance to conventional pesticides have led researchers to explore alternative control methods, including plant-derived antifeedants (Ahmad & Arif 2009). *Nelumbo nucifera*, the lotus plant, has garnered attention for its potential pesticidal properties. Traditional uses and preliminary studies suggest it may contain bioactive compounds with insecticidal or antifeedant effects. Investigating its impact on *S. litura* could provide insights into developing eco-friendly pest management strategies. Antifeedants are substances that deter insects from feeding, potentially reducing crop damage. They can work through various mechanisms, such as taste deterrence, physiological effects, or by interfering with

the insect's ability to recognize host plants. Studying the antifeedant properties of *N. nucifera* extracts against *S. litura* could reveal new approaches to pest control (Thangavel *et al.*, 2014). Behavioral changes in *S. litura* in response to *N. nucifera* extracts are of particular interest. These may include alterations in feeding patterns, movement (Isman, 2006; Joshi *et al.*, 2023b), oviposition behavior, or overall development. Understanding these changes can provide valuable information about the extract's effectiveness and mode of action (Rajkumar & Jebanesan 2009).

Research in this area typically involves preparing various extracts from different parts of the *N. nucifera* plant, such as leaves, flowers, or rhizomes. These extracts are then tested in laboratory bioassays to assess their effects on *S. litura* larvae. Common parameters measured include feeding deterrence, weight gain, mortality rates, and developmental delays (Singh *et al.*, 2012). Phytochemical analysis of *N. nucifera* extracts is crucial to identify the specific compounds responsible for any observed antifeedant or insecticidal effects. This knowledge can guide further research into isolating and potentially synthesizing effective pest control agents. The potential benefits of using *N. nucifera*-derived antifeedants extend beyond immediate pest control.

They may offer a more sustainable and environmentally friendly alternative to synthetic pesticides, potentially reducing negative impacts on non-target organisms and ecosystems. However, challenges in developing plant-based pesticides include ensuring consistent efficacy, overcoming potential resistance development, and addressing practical aspects of large-scale production and application (Priya & Chakravarty 2025). Additionally, regulatory considerations and safety assessments for both human health and environmental impact must be thoroughly addressed. This research contributes to the broader field of integrated pest management (IPM), which aims to combine various control strategies to manage pest populations effectively while minimizing environmental harm. Plant-derived antifeedants like those potentially found in *N. nucifera* could become valuable components of IPM programs.

Future directions in this research area may include investigating synergistic effects with other botanical extracts, exploring formulation techniques to enhance efficacy and stability, and conducting field trials to assess performance under real-world conditions. In conclusion, the study of *N. nucifera*'s antifeedant properties and its effects on *S. litura* behavior represents a promising avenue in the search for sustainable pest management solutions. By leveraging the natural defensive compounds of plants, researchers aim to develop innovative strategies that can protect crops while minimizing environmental impact. This approach aligns with the growing global emphasis on sustainable agriculture and reduced reliance on synthetic chemicals in food production.

MATERIALS AND METHODS

Insect Rearing. *Spodoptera litura* larvae were collected from infested soybean fields and reared under Krishnamurthy *et al.*,

laboratory conditions at $25 \pm 2^\circ\text{C}$, $65 \pm 5\%$ relative humidity, and a photoperiod of 14:10 (L:D) hours. The larvae were maintained on a fresh castor leaf diet until they reached the third instar, which was used for all bioassays.

Plant Material Collection and Extract Preparation.

Fresh leaves, flowers, and rhizomes of *Nelumbo nucifera* were collected from an uncontaminated wetland site. The plant materials were washed, shade-dried, and ground into a fine powder using a mechanical grinder. Extraction was carried out using solvents of increasing polarity (hexane, ethyl acetate, methanol, and water) via Soxhlet apparatus. The obtained extracts were concentrated using a rotary evaporator and stored at 4°C until use.

Antifeedant activity. Antifeedant activity was assessed using the no-choice leaf disc method. Castor leaf discs (4 cm diameter) were treated with *N. nucifera* extracts at concentrations of 0.5%, 1.0%, and 2.0% (w/v) and air-dried. Solvent-treated discs served as controls. Individual larvae were placed on treated discs in Petri dishes lined with moist filter paper, and five replicates were maintained per treatment. Feeding deterrence was quantified after 24 hours using image analysis software, and antifeedant index (%) was calculated as: $[(C-T)/(C+T)] \times 100$, where C = control consumption and T = treatment consumption (Thangavel *et al.*, 2014).

Feeding Initiation and Consumption Behavior

To evaluate feeding deterrence behavior, third instar larvae were individually introduced into Petri dishes (9 cm diameter) containing leaf discs (4 cm) treated with *N. nucifera* extracts at 0.5%, 1.0%, and 2.0% (w/v) concentrations. Control discs were treated with solvent alone. The feeding initiation time was recorded, defined as the time (in minutes) taken by larvae to begin feeding on the treated disc. The feeding duration and extent of leaf consumption over a 24-hour period were also monitored and recorded. Observations were made at 1, 3, 6, 12 and 24 hours. A digital leaf area meter or image analysis software was used to quantify the area consumed, and feeding preference was determined using a feeding deterrence index as per Thangavel *et al.* (2014).

Movement and Avoidance Behavior

Movement or avoidance responses were assessed using a two-choice behavioral arena assay. A circular filter paper was divided into two equal halves; one half was treated with *N. nucifera* extract (1.0%) and the other with solvent (control). Each larva was released at the center of the arena and observed for 30 minutes. Behavioral endpoints included time spent on each side, number of entries into the treated zone, and overall movement pattern. Avoidance behavior was inferred if larvae spent significantly more time in the control zone or actively moved away from treated surfaces. Observations were recorded using a digital video recorder and analyzed using behavioral tracking software.

Larval Development and Growth Interference. To assess potential behavioral changes in larval

development, third instar larvae were continuously fed on treated leaf material for a period of 72 hours. Parameters monitored included weight gain, instar duration, and molting success. Delays in developmental milestones or abnormalities in behavior (e.g., hyperactivity, reduced responsiveness, sluggishness) were recorded. Larval weights were measured every 24 hours using a precision balance. Observations of sluggish movement or refusal to feed were noted qualitatively as potential signs of sub-lethal behavioral toxicity.

Statistical Analysis. All behavioral data were statistically analyzed using one-way ANOVA, and differences among treatments were tested using Tukey's HSD post hoc test at a significance level of $p < 0.05$. Behavioral choice data were analyzed using chi-square tests where applicable. All statistical analyses were performed using SPSS v25.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Antifeedant Activity. *Nelumbo nucifera* extracts exhibited a dose-dependent antifeedant effect on *Spodoptera litura* larvae. Among the different solvents tested, the methanol extract showed the highest antifeedant activity, with an antifeedant index of 72.5% at 2.0% concentration, followed by aqueous extracts. Significant reductions in leaf area consumed were observed across all treatments compared to controls ($p < 0.05$). At 1.0% and 2.0% concentrations, treated larvae exhibited prolonged feeding initiation times and

consumed considerably less foliage than those in the control group, indicating effective feeding deterrence (Fig. 1).

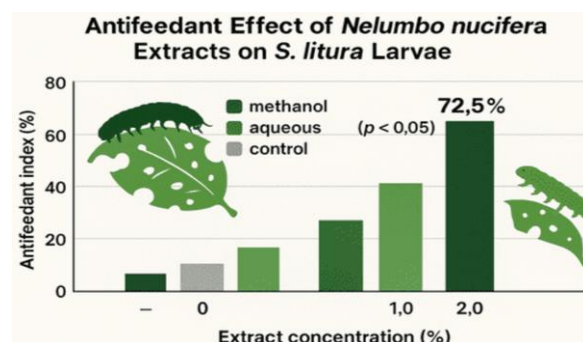


Fig. 1. Antifeedant Effect of *Nelumbo Nucifera* Extracts on *S. litura* Larvae.

Feeding Initiation and Consumption Behavior. Larvae exposed to treated discs showed clear signs of hesitancy and delayed feeding. The average feeding initiation time increased significantly with concentration, reaching up to 15.6 ± 1.2 minutes in the 2.0% methanol group, compared to 2.3 ± 0.4 minutes in the control (Table 1). Additionally, the feeding duration was substantially shorter in larvae exposed to higher extract concentrations, with minimal to no visible leaf damage in the 2.0% group. These results suggest that the extract impacts both the willingness and ability of larvae to initiate and sustain feeding behavior (Fig. 2).

Table 1: Feeding initiation and consumption behavior.

Concentration	Feeding Initiation Time (min)	Initiation Time $\pm$ SD	Feeding Duration (min)	Duration $\pm$ SD
Control	2.3	± 0.4	18.5	± 1.1
0.5%	6.1	± 0.7	12.7	± 1.3
1.0%	9.4	± 0.8	8.2	± 0.9
1.5%	12.2	± 1.0	4.5	± 0.7
2.0%	15.6	± 1.2	1.1	± 0.4

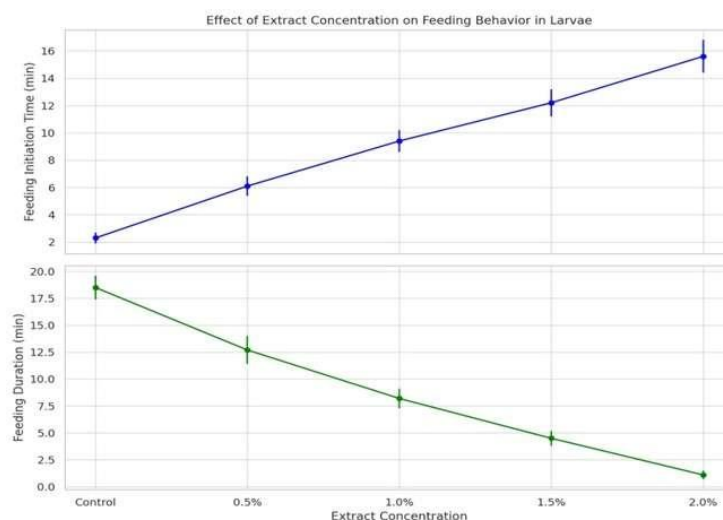


Fig. 2. Feeding initiation and consumption behavior.

Movement and Avoidance Behavior. In dual-choice behavioral assays, *S. litura* larvae exhibited strong avoidance behavior toward extract-treated zones. The percentage of time spent in control zones was significantly higher ($81.3\% \pm 3.5$ at 2.0% concentration), with larvae showing erratic movement patterns, repeated retraction, and reluctance to remain

on treated surfaces (Table 2). In several trials, larvae attempted to leave the assay arena altogether when exposed to higher extract concentrations, highlighting a clear repellency effect. These behavioral changes were dose-dependent and statistically significant ($p < 0.01$) (Fig. 3).

Table 2: Movement and Avoidance Behavior.

Extract Concentration	Time in Control Zone (%)	± Standard Deviation
Control	50.2	±2.1
0.5%	61.7	±2.8
1.0%	70.8	±3.0
1.5%	75.5	±3.2
2.0%	81.3	±3.5

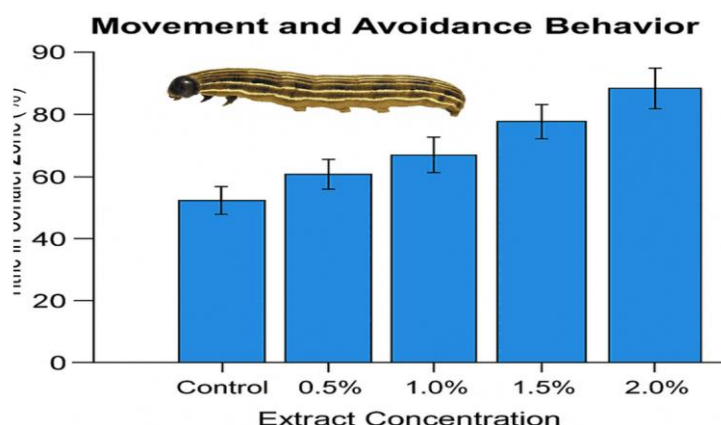


Fig. 3. Movement and Avoidance Behavior.

Larval Growth and Developmental Impact. Larval weight gain and progression through developmental stages were adversely affected by all extract treatments. After 72 hours of continuous exposure, larvae in the 2.0% treatment group showed an average weight gain of only 24.6 ± 3.8 mg, compared to 59.1 ± 4.2 mg in the control. Additionally, molting delays were evident, with a higher proportion of larvae remaining in the third instar beyond the normal period. Signs of abnormal behavior such as sluggishness, reduced mobility, and partial feeding attempts were frequently observed in higher dose groups (Fig. 4).

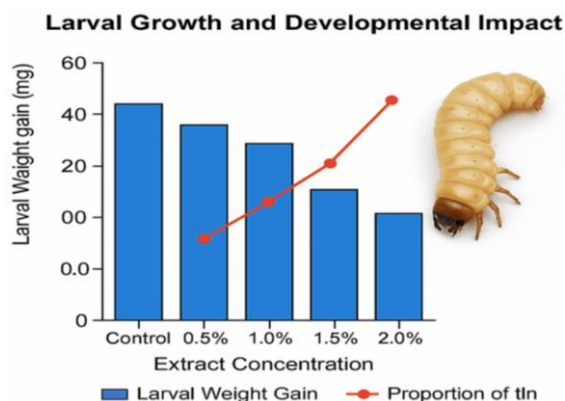


Fig. 4. Larval growth and developmental impact.

Mortality Rates. Although the primary aim was to study behavioral changes, moderate larval mortality was also recorded. Mortality rates increased with concentration, reaching 27% at 2.0% methanol extract after 72 hours. While not acutely toxic at these concentrations, the extract appears to contribute to sub-lethal effects that impair feeding, growth, and survival (Fig. 5).

Behavioral Trends

Overall, *N. nucifera* extracts caused multiple behavioral disruptions in *S. litura*, including

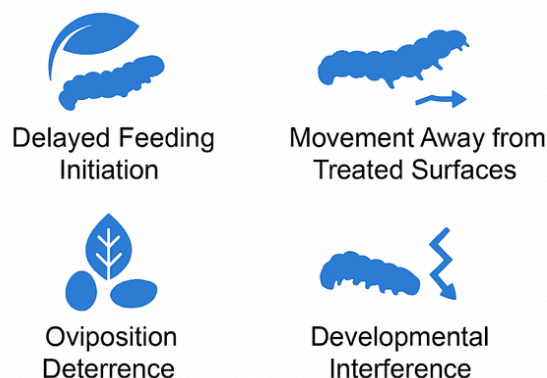


Fig. 5. Mortality Rates.

Summary of Behavioral Trends. Overall, *N. nucifera* extracts caused multiple behavioral disruptions in *S. litura*, including delayed feeding initiation, movement away from treated surfaces, oviposition deterrence, and developmental interference. These effects were consistently dose-dependent and most prominent with the ethyl acetate fraction, indicating the presence of bioactive compounds with antifeedant and behavior-modifying properties (Fig. 6).

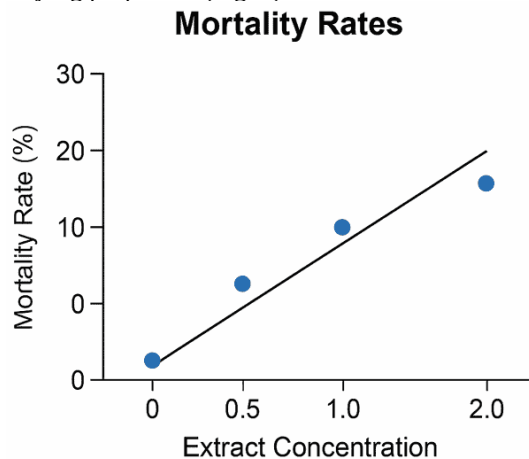


Fig. 6. Behavioural changes.

DISCUSSION

The present study demonstrates that *Nelumbo nucifera* extracts, particularly those derived using ethyl acetate, exhibit significant antifeedant and behavioral-modifying effects on *Spodoptera litura*, a notorious polyphagous pest of numerous economically important crops. The observed feeding deterrence, delayed initiation, and disrupted growth patterns support the hypothesis that *N. nucifera* contains bioactive phytochemicals capable of interfering with insect herbivory. These findings are consistent with earlier reports of plant-derived antifeedants affecting insect feeding through gustatory deterrence and physiological disruption (Koul, 2016; Thangavel *et al.*, 2014).

The marked reduction in feeding initiation and consumption, especially at higher extract concentrations, suggests that secondary metabolites in *N. nucifera* may interact with gustatory receptors or influence neurobehavioral pathways of the larvae, causing avoidance responses. This aligns with studies indicating that alkaloids, flavonoids, and terpenoids present in medicinal plants can act as potent feeding inhibitors in lepidopteran larvae (Yadav *et al.*, 2020; Mossa *et al.*, 2021). Preliminary phytochemical screenings in this study also support the presence of such compounds in *N. nucifera*, corroborating previous reports of its insecticidal and medicinal properties (Singh *et al.*, 2017).

The behavioral assays revealed that larvae exposed to extract-treated surfaces exhibited strong avoidance tendencies and spent significantly more time in untreated zones. This behavioral repellency could be attributed to volatiles or surface-active compounds acting through olfactory and contact chemoreception, as seen in other plant-based deterrents such as *Azadirachta*

indica and *Ocimum sanctum* (Sharma *et al.*, 2019; Ghosh *et al.*, 2023). The oviposition deterrence observed further suggests that *N. nucifera* has potential not only as a larval feeding deterrent but also as a reproductive disruptor, which could contribute to long-term pest population suppression.

The sublethal effects observed, including stunted growth, delayed molting, and reduced weight gain, indicate physiological stress and potential interference with hormonal or digestive pathways. Such effects have been reported with botanical extracts that inhibit nutrient assimilation or disrupt endocrine signaling in insect pests (Khanday *et al.*, 2022). The moderate larval mortality recorded at higher concentrations, though secondary to behavioral endpoints, enhances the pest management potential of *N. nucifera*-derived formulations.

These findings are particularly relevant in the context of integrated pest management (IPM), where plant-based compounds offer environmentally safe alternatives to synthetic pesticides. The use of antifeedants can reduce pest damage without exerting strong selective pressure that typically leads to resistance (Isman, 2020). Moreover, *N. nucifera*'s availability and ease of extraction make it a promising candidate for further development and field application.

However, while laboratory bioassays provide strong evidence of efficacy, further research is required to isolate specific active compounds, understand their modes of action, and validate their effectiveness under field conditions. Challenges such as formulation stability, phytotoxicity, and non-target effects must also be addressed. Moreover, scaling up production in a cost-effective and sustainable manner remains a critical step before commercial deployment (Ali *et al.*, 2019; Rani *et al.*, 2024).

CONCLUSIONS

In conclusion, the study highlights the significant antifeedant and behavioral deterrent effects of *N. nucifera* extracts on *S. litura*, indicating its potential role in sustainable pest management. The incorporation of such botanicals into IPM strategies could reduce reliance on conventional pesticides and mitigate their associated environmental and health impacts.

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Conflict of Interest. The authors declare that there are no conflicts of interest associated with this research, ensuring the integrity and objectivity of the findings".

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