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Chemistry of Neem (*Azadirachta indica*) Extracts and Their Environmental Role in Sustainable Management of *Spodoptera litura*

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ABSTRACT

The chemistry of plant-derived bioactive compounds offers promising alternatives to synthetic pesticides in sustainable agriculture. This study investigated the insecticidal and anti-feedant potential of neem (*Azadirachta indica*) leaf extracts against the tobacco caterpillar, *Spodoptera litura* (Fabricius), a polyphagous pest responsible for up to 100% crop loss in severe outbreaks. Ethanolic and aqueous neem leaf extracts were prepared and tested under laboratory and field conditions in Guntur Rural, India. Results revealed that ethanolic extracts exhibited superior efficacy, achieving 97.22 ± 2.78% mortality at 4.3% w/v and 100% mortality at 4.7% w/v, with an LC_{50} of 1.88% after 2 days. In contrast, aqueous extracts recorded only 26.39 ± 5.92% mortality at 4.3% w/v and 27.78 ± 5.56% at 5.6% w/v, with an LC_{50} of 8.52% after 4 days. Aqueous extracts, however, showed strong anti-feedant properties with complete feeding inhibition (100%) at ≥3% w/v concentrations. Field surveys confirmed that *S. litura* completes up to four generations annually under local climatic conditions, emphasizing the urgent need for effective eco-friendly management. These findings highlight the chemistry of neem phytochemicals and their environmental significance in reducing pesticide dependence, protecting biodiversity, and ensuring sustainable crop protection.

Keywords: Neem extracts; *Spodoptera litura*; bioinsecticide; ethanolic formulation; anti-feedant activity; sustainable pest management.

Introduction

The use of chemical insecticides has long been a major tool in pest control, but their indiscriminate application has resulted in serious consequences such as poisoning of humans and animals, contamination of soil, water, and air, accumulation of residues in food, persistence in the environment, resistance in pests, and destruction of beneficial insects. This has encouraged the search for safer alternatives. Plant-based insecticides have emerged as eco-friendly substitutes to synthetic chemicals, offering effective pest management without harming the ecosystem.

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These natural insecticides are derived from plants that produce secondary metabolites such as alkaloids, terpenoids, flavonoids, essential oils, tannins, and fatty acids, which serve as defense mechanisms against insects. They often act as repellents, growth inhibitors, anti-feedants, or oviposition deterrents. Unlike synthetic chemicals, they degrade rapidly, are safer for humans and other organisms, and reduce the risk of resistance in pests.

For an ideal insecticidal plant, certain qualities are desirable: it should be perennial, abundantly available or easily cultivable, use renewable parts like leaves or fruits, require minimal inputs, have additional medicinal or economic uses, and be effective at low doses. Botanical insecticides also work well in integrated pest management programs alongside other eco-friendly methods such as pheromones, beneficial microbes, and predators.

Spodoptera litura (Figure 1), a polyphagous noctuid pest, poses a major challenge to global agriculture due to its extensive host range and resistance development. It infests more than 150 plant species, including cotton, tomato, soybean, potato, and capsicum, causing yield losses of up to 100% in severe cases (Thakur et al., 2024). The pest is widely distributed across Asia-Pacific, with outbreaks influenced by climatic factors such as rainfall, temperature, and humidity (Selvaraj et al., 2010; Fand et al., 2015). Overreliance on chemical insecticides like organophosphates, pyrethroids, and carbamates has accelerated resistance, reducing control efficacy (Saleem et al., 2008; Kranthi et al., 2002). Resistance mechanisms include metabolic, target-site, and behavioral adaptations (Wang et al., 2021). This necessitates sustainable alternatives, where biopesticides, entomopathogenic nematodes, and microbial formulations have shown promise (Thakur et al., 2022; Thakur et al., 2023). Molecular identification techniques such as DNA barcoding are increasingly recommended for accurate species detection and resistance monitoring (Kress & Erickson, 2008). Integrated pest management (IPM) combining chemical rotation, biological control, and ecological strategies is therefore essential to mitigate resistance and safeguard crop productivity (Srivastava et al., 2018).

In India, *Azadirachta indica* (Neem) is one of the most widely studied and used botanical insecticidal plants. Neem trees are abundant in regions like Guntur Rural, where farmers have traditionally used neem extracts for pest management. Almost every part of the tree—seeds, leaves, bark, and oil—has bioactive compounds such as azadirachtin, nimbin, and salannin. These compounds exhibit insecticidal, repellent, growth-disrupting, and anti-feedant properties against a wide range of agricultural and household pests.

Neem has additional medicinal and cultural value in India, being used in traditional healthcare for its antibacterial, antifungal, and antiviral properties. In agriculture, neem-based products have been effective against pests of cotton, pulses, rice, and vegetables. Farmers in Guntur Rural often prepare neem seed kernel extracts and apply them directly to crops as a cost-effective and sustainable pest management practice.

Given the increasing pest challenges in agricultural regions, the potential of neem-based insecticides offers a safe, affordable, and ecologically balanced solution. Their integration into farming practices ensures crop protection while safeguarding human health, livestock, and the natural environment.

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Figure 1. Adult individuals of *S. litura* and damage to leaves

2.2. Method

2.2.1. Collection and Breeding of Insects

Larvae from the late instar stages were collected from infested plants and transferred in aerated containers to the Entomology Laboratory of the College of Agricultural Sciences, Guntur Rural. The insects were maintained in Petri dishes lined with moist filter paper and fed with fresh host plant leaves until pupation. After pupation, moisture was maintained until adult emergence. Newly emerged adults were fed fresh leaves and later used in the bioassays.

2.2.2. Collection of Leaves

Approximately 1 kg of neem leaves was collected from randomly selected trees to minimize bias from any single tree.

2.2.3. Preparation of Extracts

The preparation of neem (*Azadirachta indica*) leaf extracts was carried out in the Chemistry Laboratory. Fresh neem leaves collected locally were washed, shade-dried, and then placed in a hot-air oven (Remi® model) (Figure 2A) at 60 °C until constant weight was achieved (initial moisture content: ~59.3%). The dried leaves were ground into a fine powder using an electric grinder (Philips® HL7756/00) (Figure 2B) and stored in airtight, labeled glass jars (Figure 3B) to avoid moisture absorption.

For extraction, the powdered neem leaves were mixed separately with distilled water and ethanol to obtain solutions of maximum possible concentration. Each mixture was stirred for 18 h on a magnetic stirrer (Remi® 2MLH) (Figure 2C), with mild heating at 37 °C during the first hour to enhance solubilization. The extracts were then filtered through Whatman No. 1 filter paper and centrifuged at 5000 rpm for 15 min using a laboratory centrifuge (REMI® R-8C, India) (Figure 2D). The supernatant

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was again filtered to yield the base extract (Figure 3C), which was subsequently used in bioassays against *Spodoptera litura*.

Figure 2. Laboratory equipment. A) Forced air stove; B) Electric grain mill; C) Magnetic stirrer; D) Centrifuge.

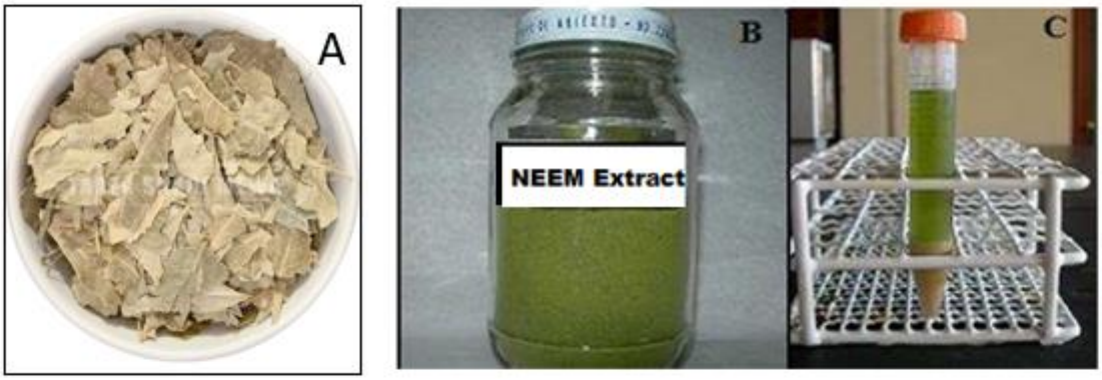

Figure 3. Neem leaf states. A) Dry leaves; B) Dust from crushed leaves; C) Base liquid extract.

2.2.4. Evaluation of Insecticidal Efficacy

The bioassays were conducted in Petri dishes (10 cm diameter) containing three adult insects, fresh host leaves, and moist filter paper. Leaves were dipped for one minute in the prepared neem extracts, air-dried, and offered to insects. Controls consisted of leaves dipped only in water or ethanol.

Mortality was recorded daily, and survival percentages were calculated. Data were analyzed to determine lethal concentration (LC_{50}) values using appropriate statistical methods.

2.2.5. Evaluation of Anti-Feedant Activity

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To assess anti-feedant properties, paired leaf disc choice tests were conducted. One disc was treated with neem extract and the other with solvent (control). Each Petri dish contained a single insect with both discs. After 24 hours, leaf area consumed was measured using image analysis software, and percentage repellency was calculated.

2.2.6. Study of Insect Life Cycle

Field observations were made on naturally infested plants in Guntur Rural to document the developmental stages of the insect. Weekly monitoring was carried out for one year to record egg, larval, pupal, and adult stages. Based on these observations, the duration of each life stage and the complete life cycle was established.

3. RESULTS AND DISCUSSION

3.1. Evaluation of the Efficacy of Neem Leaf Extracts on Adults of *Spodoptera litura*

The mean mortalities obtained from adults of *S. litura* with ethanolic extracts of neem leaves were considerably higher than those obtained with aqueous extracts, for concentrations ranging from 2.0 to 5.6% w/v. Statistical analyses revealed significant differences between treatments and their respective controls, both for ethanolic and aqueous extracts, confirming that the observed mortality was due to the insecticidal activity of neem extracts (Tables 1 and 2).

Table 1. Average mortality (% ± standard error) of *Spodoptera litura* due to the effect of ethanolic extracts of neem (*Azadirachta indica*) leaves at different concentrations.

Ethanolic Extracts (% w/v)	Mortality (% ± SD)
Control (0.0)	8.33 ± 6.88[a]
2.0	73.61 ± 5.92[b]
2.5	80.56 ± 4.58[b]
3.5	87.50 ± 4.42[bc]
4.3	97.22 ± 2.78[c]
4.7	100.00 ± 0.00[c]

* Different letters in the same column indicate significant differences between concentrations (Tukey's test, $p \leq 0.05$).

Ethanolic extracts recorded the highest mortality rates, exceeding 95% at concentrations of 4.3% and 4.7% w/v. In contrast, aqueous extracts at 4.3% w/v produced only about 25% mortality, which increased slightly to nearly 28% at 5.6% w/v, the maximum concentration tested. The lowest mortality rates for both extracts were observed in the lowest concentration treatments.

These findings suggest that neem extracts, particularly ethanolic formulations, are highly effective against adult *S. litura*, even at relatively low concentrations. Mortality rates above 80% were consistently obtained with ethanolic extracts in concentrations between 2.5 and 4.7%, demonstrating strong pesticidal potential.

The superior efficacy of neem is attributed to its diverse bioactive compounds, including azadirachtin, salannin, and nimbin, which interfere with insect feeding, growth, and reproduction.

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Neem extracts are known to act as antifeedants, growth regulators, and oviposition deterrents. The higher mortality in ethanolic extracts compared to aqueous extracts can be explained by the enhanced solubility of these bioactive compounds in ethanol, which results in stronger pesticidal action.

Table 2. Average mortality (% ± standard error) of *Spodoptera litura* due to the effect of aqueous extracts of neem (*Azadirachta indica*) leaves at different concentrations.

Aqueous Extracts (% w/v)	Mortality (% ± SE)
Control (0.0)	0.00 ± 0.00 a
2.5	15.28 ± 5.53 b
3.0	16.67 ± 5.56 b
4.3	26.39 ± 5.92 b
5.6	27.78 ± 5.56 b

* Different letters in the same column indicate significant differences between concentrations (Tukey's test, $p \leq 0.05$).

Daily survival analysis showed that ethanolic extracts significantly reduced insect survival within the first 24 hours of exposure, with mortality exceeding 50% in most treatments soon after feeding on treated leaves. In contrast, aqueous extracts displayed a delayed effect, with mortality exceeding 20% only from the fourth day of the bioassay (Figure 4).

These results demonstrate that neem leaf extracts, particularly ethanolic formulations, are effective botanical insecticides for controlling *S. litura* in Guntur Rural conditions. Their integration into pest management strategies could reduce reliance on synthetic insecticides, promote sustainable farming, and safeguard environmental and human health.

Figure 5 corroborates the effectiveness of neem (*Azadirachta indica*) leaf extracts prepared with ethanol at all concentrations, compared to aqueous extracts, and demonstrates the direct relationship between increasing concentrations and the rise in *S. litura* mortality with both solvents. Furthermore, the LC_{50} value was lower for ethanolic extracts than for aqueous extracts, confirming the higher potency of the ethanolic extract.

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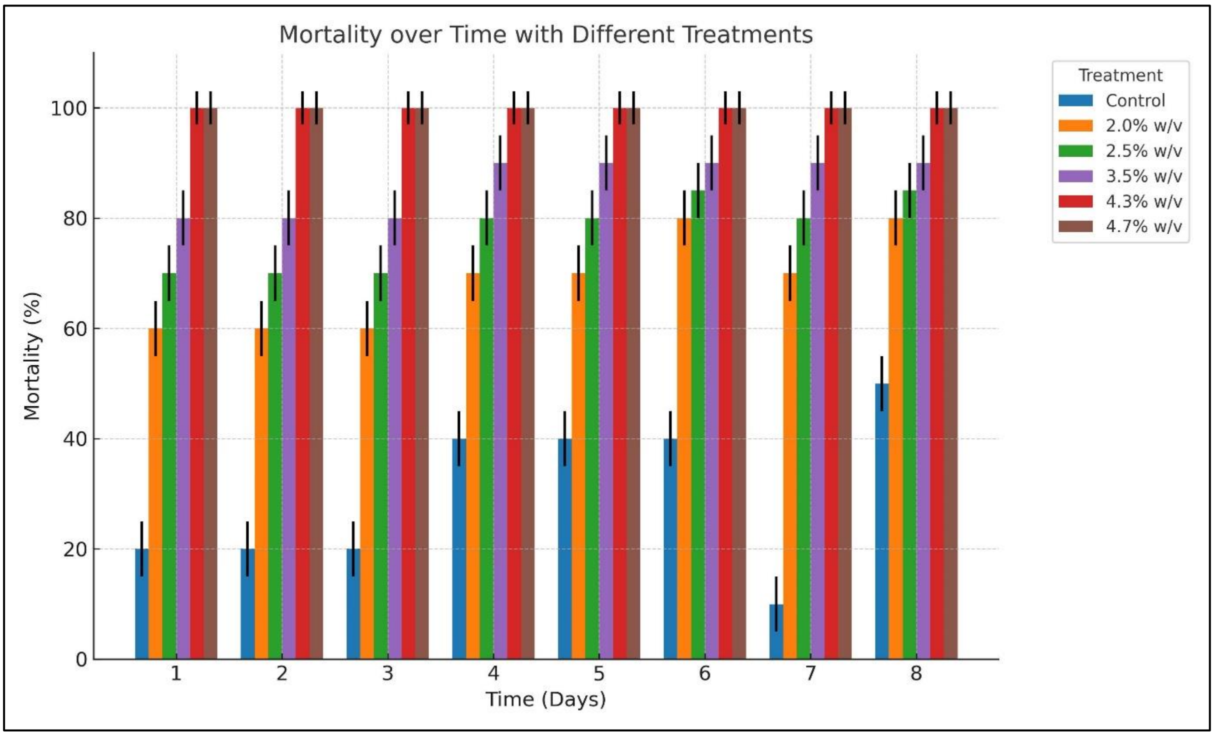

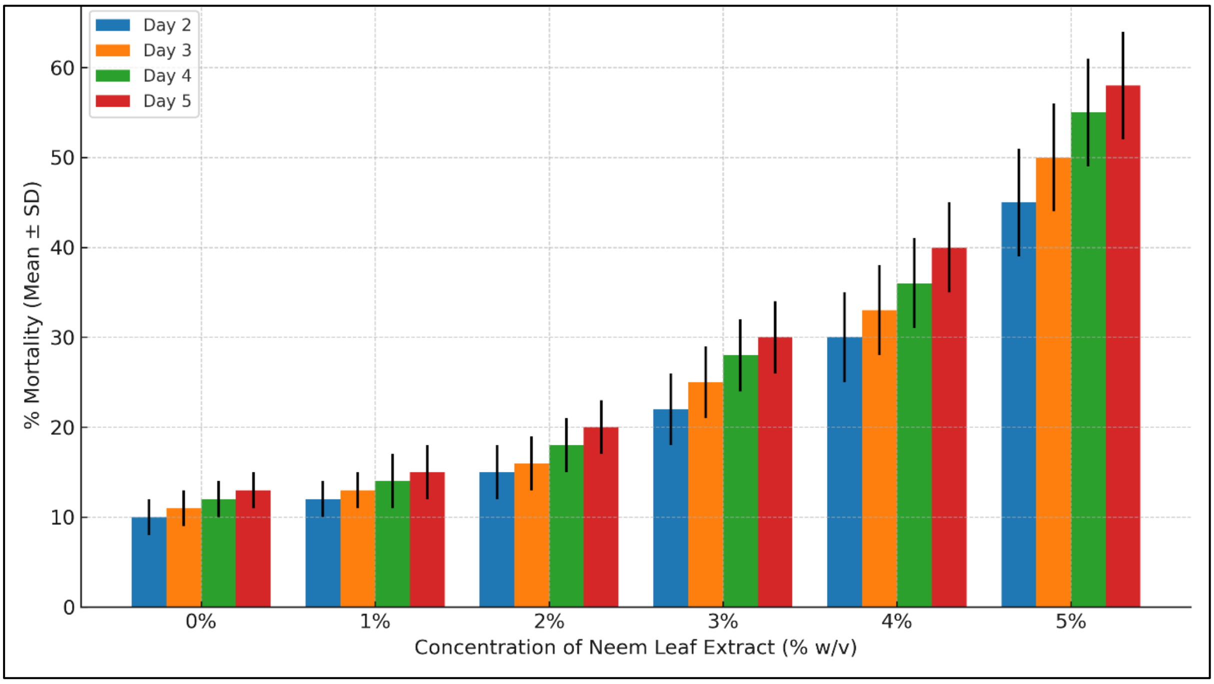

Figure 4. Average mortality (% ± standard error) of *S. litura* adults treated with neem leaf extracts at different concentrations across evaluation days. A) Ethanolic extracts (Above); B) Aqueous extracts (below).

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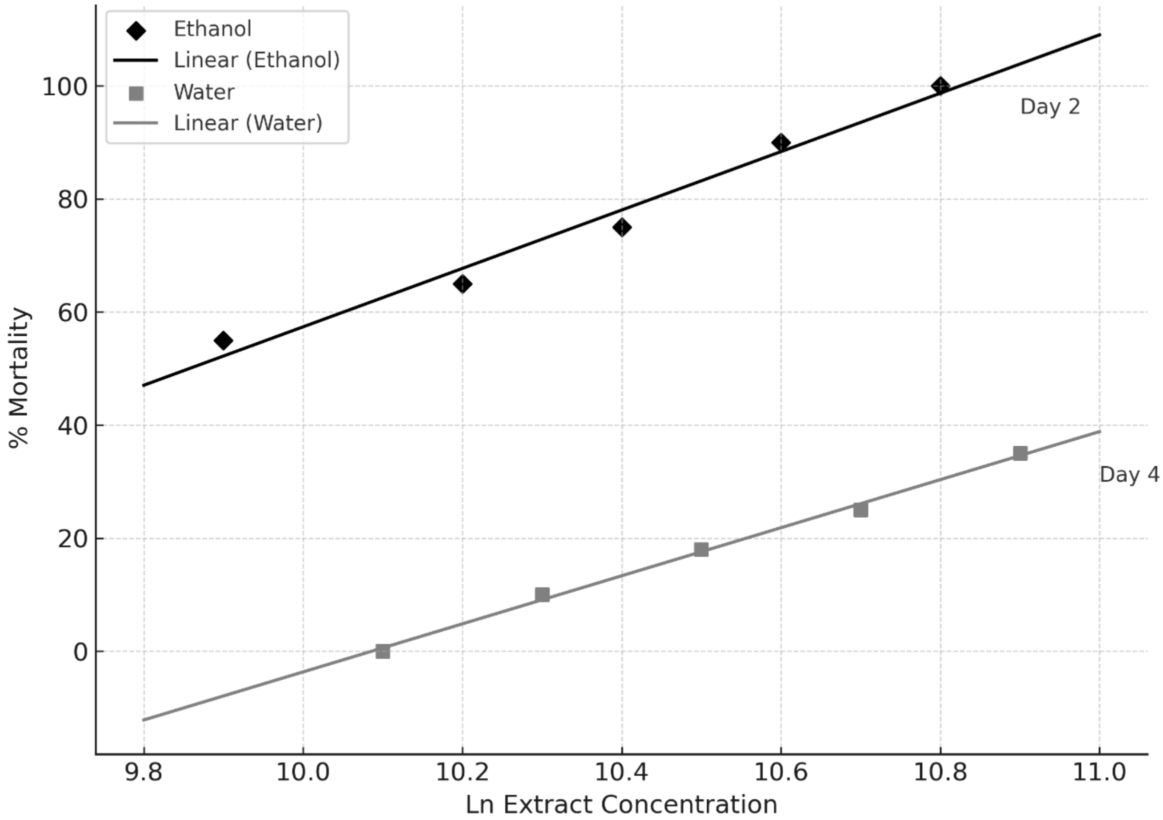

Figure 5. Graphical representation of the Probit analysis of *S. litura* mortality caused by ethanolic and aqueous extracts of neem (*Azadirachta indica*) leaves at different concentrations on the 2nd and 4th day, respectively.

3.2. Probit Analysis of Neem Extracts

The results of the Probit test indicated that ethanolic extracts of neem leaves produced an early mortality response in *S. litura* adults as early as 2 days after treatment, with clear differences observed between concentrations. This linear dose–mortality response was maintained until the end of the bioassay. In contrast, aqueous extracts produced a delayed response, with noticeable mortality only after 4 days, but with a consistent linear relationship between concentration and mortality thereafter (Table 3).

According to the Probit analysis, the LC_{50} value for ethanolic extracts was 1.88% at 2 days, while aqueous extracts required a higher concentration of 8.52% at 4 days to achieve comparable results. This indicates that ethanolic extracts of neem were more effective in achieving mortality at lower concentrations and within a shorter period. Figure 6 corroborates these findings, demonstrating a strong and direct relationship between concentration and mortality, with ethanolic extracts consistently outperforming aqueous extracts.

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Table 3. Effect of aqueous and ethanolic extracts of neem (*Azadirachta indica*) leaves on *Spodoptera litura* mortality.

Extract	Time (days)	Mortality (%) ± SE	LC_{50} (%)	χ^{2*}
Ethanol	2	55.2 ± 2.94	1.88	2.39
	8	15.9 ± 1.92	0.19	0.14
Water	4	39.5 ± 2.22	8.52	3.33
	8	27.7 ± 6.19	4.06	0.65

* The calculated χ^2 values were lower than the tabulated values (Ethanol: df = 4, $p \leq 0.05$ = 9.49; Water: df = 3, $p \leq 0.05$ = 7.81), indicating that the Probit model adequately fit the experimental results.

3.3. Anti-Feedant Activity of Neem Extracts

In addition to mortality effects, feeding deterrence was also observed. Ethanolic extracts of neem leaves exhibited strong toxic action, resulting in high adult mortality of *S. litura*, and the surviving individuals showed minimal feeding activity on both treated and control leaves, indicating a toxic rather than a deterrent effect.

On the other hand, aqueous extracts exhibited pronounced anti-feedant activity. In all treatments, adults clearly avoided the treated leaves and fed only on control leaves, indicating complete inhibition of feeding (Table 4). This suggests that while ethanolic extracts act primarily as a toxicant, aqueous extracts function more as an antifeedant against *S. litura*.

Table 4. Effect of aqueous extracts of *neem* leaves on the diet of adults of *Spodoptera litura* in an open-choice trial.

Treatment (% w/v)	Control Consumption (%)	Treated Leaf Consumption (%)	Feeding Inhibition (%)
Control	2.50	0.04	98.32
3.0	0.00	6.67*	100.00
4.3	0.00	3.50*	100.00
5.6	0.00	2.87*	100.00

* Significant differences between treated and control leaves (ANOVA, $p \leq 0.05$).

The larva has three stages, and goes through two molts during its development, initially they are dark in color and gradually acquire more yellowish tones, with two lateral longitudinal stripes of black dots; The larval period lasted approximately 3 to 4 weeks. From the second half of November until mid-December, pre-pupae and pupae were found, mainly at the base of the trunks of elms, covered by leaf litter. At the beginning of December, the first adults emerged, thus initiating the cycle of a second generation (Figure 6). The eggs and later larvae that developed between December and January give way to the second generation of adults at the end of January.

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Thus, neem extracts exert a dual mode of action: ethanolic extracts are rapidly insecticidal, while aqueous extracts act as strong feeding deterrents, preventing crop damage even when insect survival is not immediately reduced.

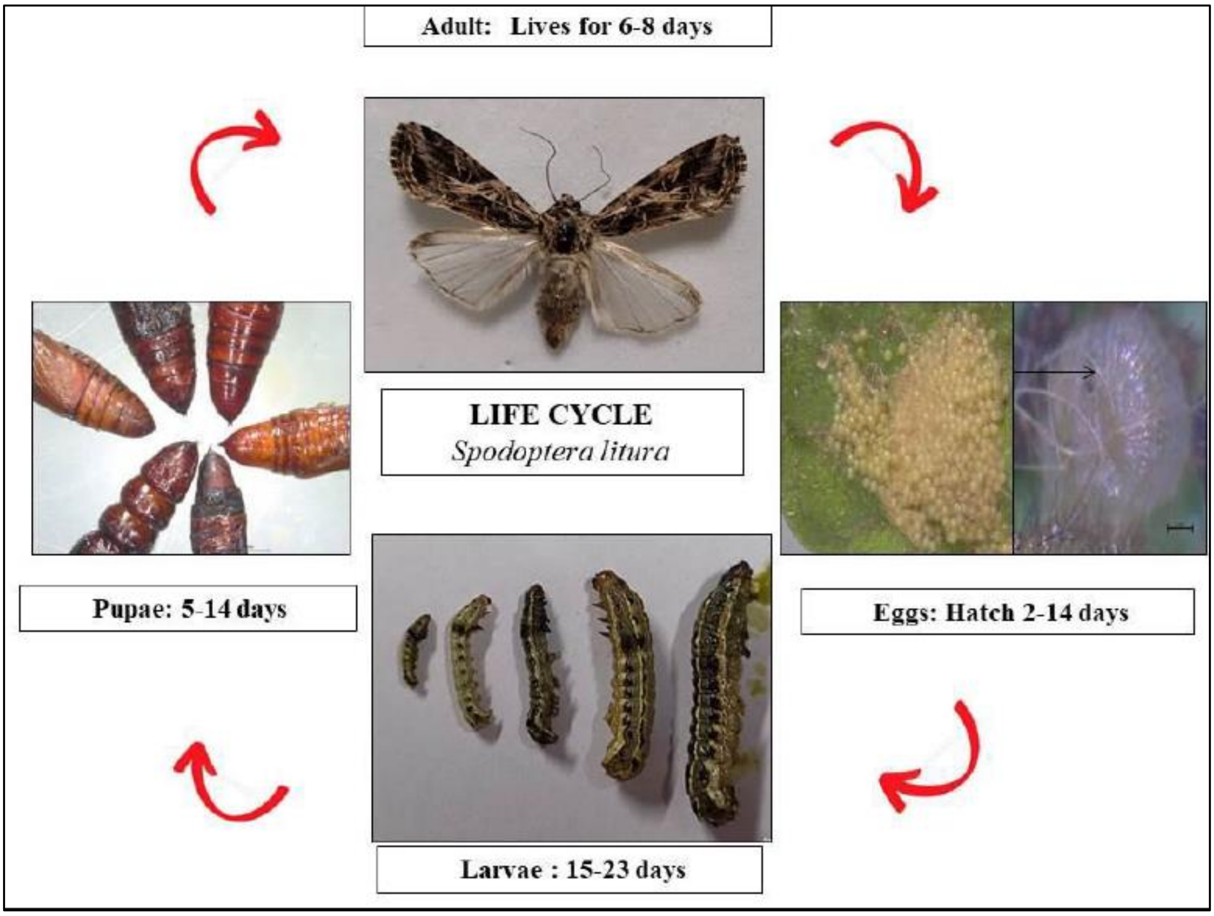

Figure 6. Developmental stages and biological cycle of *S. litura.*

3.4. Life Cycle of *Spodoptera litura* in Guntur Rural

Field observations in Guntur Rural revealed that *S. litura* undergoes multiple overlapping generations throughout the crop season. The first appearance of adults was observed in early October, when post-monsoon climatic conditions became favorable. Egg laying commenced in the second week of October, and the first larvae emerged about one week later.

The larval stage consisted of three instars, lasting approximately 3–4 weeks. Larvae were initially dark in color and gradually developed greenish-brown shades with characteristic markings. Pupation occurred in the soil or crop residues, and adults of the next generation emerged by early December. This marked the beginning of the second generation.

Eggs and larvae from December to January gave rise to the second and third generations of adults by late January and February. A fourth generation was observed in March, with adults surviving until April, after which population levels declined with the onset of higher summer temperatures.

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The observations confirmed that under Guntur conditions, *S. litura* can complete up to four generations per year. Each generation lasted approximately one to two months, depending on prevailing temperature and rainfall. Such multivoltine behavior explains the persistent and severe infestations of *S. litura* in the region, making its management a critical priority for farmers.

CONCLUSIONS

- Neem (*Azadirachta indica*) leaf extracts were highly effective as bioinsecticides against adults of *Spodoptera litura*, reaching mortalities close to 100% when obtained with ethanol and applied at concentrations of 4.3 and 4.7% w/v.
- Ethanolic extracts caused significantly higher mortality than aqueous extracts at similar concentrations.
- On *S. litura* adults, ethanolic extracts exhibited primarily toxic effects, whereas aqueous extracts displayed strong antifeedant activity.
- The lowest LC_{50} value was obtained with ethanolic extracts (1.88%), indicating their superior potency compared to aqueous extracts.
- Field observations in Guntur Rural indicated that *S. litura* completes multiple generations annually, with each generation lasting about 1–2 months under favorable climatic conditions.
- These findings highlight the potential of neem-based extracts as sustainable bioinsecticides for integrated pest management programs, reducing dependence on synthetic chemical pesticides and supporting eco-friendly farming practices.

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