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Research paper

Efficacy and Difference Growth, Development of Azadirachtin Against Spodoptera Litura (Lepidoptera: Noctuidae)

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Abstract

A crop pest is any harmful organism that competes for essential resources such as nutrients, water, and sunlight. Azadirachtin, a tetranortriterpenoid derived from Azadirachta indica, has been widely used for its effectiveness in integrated pest management (IPM). This study assessed Azadirachtin's impact on the mortality, growth inhibition, feeding behavior, and molting disruption of Spodoptera litura (S. litura). Conducted at the Biological Control Laboratory at Can Tho University, Vietnam, the study used artificial diet treatments on larvae collected from a cabbage field. Mortality rates increased rapidly with higher Azadirachtin dosages, reaching 90.7% by day 6 at 1463.10 ppm and 100% by day 12. At lower concentrations, mortality increased more slowly, with 48.3% at 731.55 ppm by day 6 and 35.0% at 365.78 ppm. Larval growth was also impacted, with the highest mortality showing minimal food consumption. Growth was better at lower doses, with larvae at 365.78 ppm growing from 8.35 mm to 14.38 mm. At concentrations of 365.78 ppm and 243.85 ppm, larvae exhibited moder-ate molting, but few reached the 5th instar or successfully pupated. Azadirachtin proves to be a sustainable, environmentally friendly alterna-tive to synthetic pesticides, reducing ecological impact while managing pest populations effectively.

Keywords: *Azadirachtin; Insect Control; Spodoptera Litura; Neem; Vietnam; Agricultural Sciencesipm.*

1. Introduction

This Agricultural pests control like controls of Spodoptera litura (Lepidoptera: Noctuidae) is a major issue of concern in sustainable crop production. Riyaz, et al. [1] Stated that the use of conventional chemical pesticides has been very effective, but it is prone to negative impacts on the environment and development of resistance in the pests as well as damage to non target pests, therefore, there is a need to seek environment-friendly pesticides. Azadirachtin, a botanical pesticide derived from the neem tree, has various actions such as antifeed-ant, insect growth regulation, and insecticidal. [2], [3]. An antifeedant is a substance that prevents or reduces the feeding behavior of insects. In the context of pest control, antifeedants like Azadirachtin interfere with the insects' ability to eat, leading to growth inhibition and eventually their death. The study evaluates Azadirachtin insecticidal and physiological effects on S. litura larvae, a pest in cropping systems, using experimental methods to measure larval mortality, feeding bioactivity, and molting processes. [3], [4].

While Azadirachtin has shown promising results in controlled laboratory conditions, several environmental factors could influence its effectiveness in real-world field settings. Variables such as temperature, humidity, and rainfall can affect the stability and efficacy of Azadirachtin, potentially reducing its effectiveness if the compound breaks down more quickly than expected. [5], [6]. Furthermore, in field conditions, pest population dynamics including pest migration, the presence of other pest species, and varying pest resistance levels could alter how Azadirachtin performs. [2]. For instance, pests in the field may be more or less susceptible depending on their previous exposure to Azadirachtin or other pesticides, which could affect the observed mortality rates and pest control outcomes. [7]. These factors highlight the importance of conducting further field trials to evaluate Azadirachtin's true potential and optimize its use in diverse agricul-tural systems.

However, such parameters are important in determining the efficacy of Azadirachtin not only as a toxicant, but also, as a growth inhibitor, and a behavior modulator. It is well known that Azadirachtin severely affects major physiological processes of various insects such as synthesis of hormones and Chitin formation which is crucial in their development. [2], [8]. Nonetheless, additional empirical information is needed to determine its effect on S. litura under different concentrations and under different periods of exposure.

This paper fills a gap in understanding Azadirachtin potential as a biopesticide by using robust statistical analyses of dose-response and growth inhibition mechanisms. It also discusses its implications for integrated pest management programs, preventing synthetic pesticide dependency and controlling insect resistance evolution. Any organism living on crops that feeds and competes against crops for vital life resources including nutrients and sunlight and water is known as a crop pest [9]. Different pests inflict substantial damage on crop produc-tion through their actions of plant ingestion and disease transmissions and stored food contamination which leads to post-harvest losses

[10], [11]. Pests cause two major problems in agriculture by decreasing farming output and resulting in financial losses that affect farmer profits and possibly creating challenges to food availability. Consequently, farmers employ chemical pesticides, biological control techniques, and cultural measures including crop rotation and intercropping to manage pests [12], [13]. Integrated Pest Management (IPM) combines various tactics to provide sustainable and effective pest control while reducing environmental harm [14]. However, conventional pesticides may cause pest resistance [15]. Pesticide residues can have a severe influence on the environment and human health, thus there is an urgent need for long-term, effective pest control options [16]. Botanical pesticides are considered a substitute instrument for integrated pest management (IPM). This is due to their quick environmental disintegration, low toxicity to mammals, and little likelihood of resistance development in target pests [17].

A polyphagous pest, tobacco cutworm, S. litura (Fabricius) Lepidoptera: Noctuidae is widely dispersed throughout tropical and subtropical regions and feeds on over 150 different host plants, including field crops and vegetables such as cotton, soybeans, and cabbage.[18, 19]. The S. litura, is primarily a leaf feeder and acts as cutworm, the most destructive insect and a generalist defoliator feeding on over a hundred plant species. [20], [21]. Due to its importance, it is a significant economic pest due to rapid reproduction, a potential threat for damage, and high capacity to consume different plants. [20]. Pests threaten agriculture, but long-term exposure to chemical pesticides leads to genetic resistance, loss of natural biocontrol agents, environmental pollution, and toxic residues, harming human health and disrupting ecosystems.

Most bioactive chemicals used in neem-based pesticide formulations come from the seeds, where numerous biologically active compounds have also been isolated from different parts. [22]. A botanical insecticide, Azadirachtin, a tetranortriterpenoid derived from Azadirachta indica (Juss), has been widely used for decades due to its promising and effectiveness for integrated pest management. [2]. Along with direct toxicity, it affects many physiological events in insects, including growth regulation, protein synthesis, reproduction, diapause, and behavior. [23]. Furthermore, azadirachtin interferes with chemo-receptors and directly affects many insect tissues such as muscles, fat body, and gut epithelial cells. [24].

It has been shown to have a substantial antifeedant impact on many insect pests, such as Drosophila melanogaster, Plutella xylostella, and Galleria mellonella [17]. It influences three elements of insect biology: eating behavior, growth, and development. [2]. Azadirachtin slows down or stops insect growth and development. It has been shown that Azadirachtin delayed the development of D. melanogaster by preventing the production of ecdysteroids and juvenile hormones. [25]. It also reduces the release of prothoracicotropic hormone (PTTH) by neuroendocrine cells, which slows the growth and molting of Trypanosoma cruzi [7], [26], [27].

Furthermore, Azadirachtin interfered with the proper working of the endocrine and neuroendocrine systems in Labidura riparia [28]. Application of neem plants, however, does not always have the same results because it depends in part on the insect species, application method, time, and concentration. [29], [30]. The efficiency of neem extracts also varies and can be affected by the provenance of the neem tree, technique of extraction, temperature, and humidity. [31]. This study aims to evaluate the efficacy of Azadirachtin as a bio insecticide against S. litura through bioassay experiments. This included assessing its impact on larval mortality, growth inhibition, feeding behavior, and molting disruption. The study also explored its potential application in integrated pest management (IPM) as an environmentally friendly alternative to synthetic pesticides.

2. Material and Methods

This study was conducted at the Biological Control Laboratory of the Plant Protection Department, College of Agricultural, Can Tho University, Vietnam.

2.1. Preparation of neem seed extract

The neem seed was prepared with the extraction process of neem leaves by cold maceration [32].

2.2. Preparing insect s. litura

S. litura eggs and the first instar larvae were collected from a cabbage field in Can Tho city. The larvae were subsequently treated with an artificial diet placed in a glass petri dish (23 cm in diameter and 3 cm in depth). Artificial diet was replaced daily as needed until larvae reached the pupa stage. Pupae were given cabbage leaves in a petri dish to keep humidity inside the dish. As adults emerged, they were treated in a cage made from an iron frame covered with white gauze (26 cm in diameter and 50 cm high) for two days before ensuring complete mating. Adults were fed with 10% honey solution through the cotton layer and were hung on the top of the cage. The adults were transferred into a nesting box (30 cm in diameter and 20 cm high) and fed with 10% honey solution through a cotton layer in a small plastic dish (7 cm in diameter and 1 cm in depth). The entire box surface was covered with white paper and an additional folded paper to facilitate the female laid egg. Egg masses produced by females were collected daily, and the first instar larva from this generation was used to test insects.

The study determines the effectiveness of Azadirachtin against Spodoptera litura based on various biological responses. It offers a complete toxicity screens, behavior interfering evidence, growth regulatory impact, and molting blocking evidence. The study also shows how it can help in curbing crop destruction, delaying the gaining of weight and body length improvement, disturbing molting and pupation of the insects. The results can be used to justify the use of Azadirachtin in an integrated pest management control programs since its environmental toxicity is low and its ability to develop resistance is low as well compared to conventional insecticides.

2.3. Bioassay

2.3.1. Immature development

This research was adopted from the study of Xue et al., and conducted in the Laboratory of Biological Control Laboratory, Can Tho University [33], [34]. In the current method, an artificial diet prepared based on the formulation (Kusano et al. 2025). And were placed separately into plastic cup (5 cm in diameter and 2.5 cm in length). Newly hatched larvae obtained from the culture were transferred and maintained individually in these plastic cups until the fifth instar. Each replication had 30 larvae. The larvae were observed daily to record development time and mortality. Leaves were replaced the first three days after infestation (DAI) and continued daily until larvae completed their stage. Larva that successfully developed into a pupa was maintained individually in a paper bag (20 cm x 16c m). The development

stages were recorded daily until all individuals reached adult. The researcher observed the length and weight of larva at 3 and 8 DAI; weight of pre-pupa at 13 DAI; length, width, and weight of pupa, sex ratio of adult, and survival rate. Reproduction and adult longevity Newly emerged females obtained from the first experiment were used to assess reproduction and longevity. Females were maintained together with male in cage for two days to allow mated. After that, adult females were transferred individually into a nest (15 cm in diameter and 15 cm in depth), fed with 10% honey solution, and were maintained with same method. The researcher observed the first day of oviposition to determine preoviposition period.

2.3.2. LC_{50} and LT_{50} determination

LC_{50} stands for Lethal Concentration 50%, which is the concentration of a substance (like Azadirachtin) that is required to cause the death of 50% of a group of test organisms. It is commonly used to measure the toxicity of a substance. The concentrations used for the toxicity assay were 1463.10 ppm; 731.55 ppm; 487.78 ppm; 365.55 ppm.

Data Analysis

Mortality data were corrected using Abbott's formula Abbott, 1987 [35]. The data were converted to corrected mortality using the Abbot Formula. [35].

Corrected mortality = (% treatment− % control/% control) *100%

All the generated data were subjected to statistical analysis [36] Using one-way ANOVA in SPSS Statistical Software (version 22), and LSD compare the difference among the treatment means at $p<0.05$. Tukey's HSD test was used to find significant differences.

3. Results

S. litura larvae death rates increased with both concentration and exposure time. Mortality increased quickly at the maximum dosage (1463.10 ppm), reaching 90.7% by day 6 and 100% by day 12. However, Lower concentrations caused the mortality rate to rise more slowly. At 731.55 ppm, mortality reached 48.3% by day 6 but increased to 100% by day 15. At 365.78 ppm, mortality followed a slower trend, reaching 35.0% on day 6 and 97.9% by day 15. The lowest tested concentration (243.85 ppm) showed the least effectiveness, with mortality at only 27.5% by day 6 and 96.9% by day 15. As shown in Table 1, the statistical analysis showed significant differences in mortality with different concentrations, particularly in the early days post-treatment. However, the highest concentration showed the fastest and most effective larval mortality, indicating a dose-dependent effect of Azadirachtin on S. litura.

Table 1: The Days of Larval Mortality (Corrected Percentage) After the Treatment

Concentration (ppm)	Corrected mortality percentage (%)						
	2 DAT	4 DAT	6 DAT	8 DAT	10 DAT	12 DAT	15 DAT
1463.10 ppm	34.7a	56.7a	90.7a	97.9a	98.9a	100.0a	100.0
731.55 ppm	3.0b	13.4b	48.3b	83.5b	95.8a	98.9a	100.0
365.78 ppm	3.0b	12.3b	35.0bc	63.8c	81.2b	91.6b	97.9
243.85 ppm	5.0b	11.1b	27.5c	57.6c	69.7b	87.4b	96.9

DAT: Day after treatments.

3.1. LC_{50} of azadirachtin on second instar s. litura larvae

Initially, the LC_{50} was 17,187.8 ppm (confidence interval: 9,501.24 – 84,564.29), indicating a high tolerance in early exposure stages. When the exposure was increased, the LC_{50} values progressively decreased, reaching 937.8 ppm (confidence interval: 139.26 – 1,640.81) at the lowest effective concentration. The narrowing confidence intervals at lower concentrations suggest increased precision in estimating toxicity at these levels. Azadirachtin's median lethal concentration (LC_{50}) values decreased with exposure, indicating a strong dose-response effect. At higher LC_{50} values, a greater concentration of Azadirachtin was required to achieve 50% mortality. The slope values suggest a gradual increase in mortality with concentration, with slight variations in response among the tested larvae, as shown in Table 2.

The chi-square (X^2) test values for each treatment indicate a good fit of the mortality data to the dose-response model, validating the reliability of the results. The decreasing LC_{50} values with time indicate that prolonged exposure enhances the toxicity of Azadirachtin against S. litura larvae.

Further, the results showed that azadirachtin also exhibited strong antifeedant properties, leading to anorexia in S. litura larvae. As the concentrations increased, the larvae's eating activity significantly decreased, which led to limited growth and developmental delays. Azadirachtin's effectiveness as an insect growth regulator is supported by a decrease in LC_{50} with time, which impairs feeding behavior and leads to higher mortality. Therefore, this data highlights the potential of Azadirachtin as a biopesticide for controlling S. litura, demonstrating both direct toxicity and behavioral disruption.

Table 2: LC_{50} of Azadirachtin on Second Instar Larvae

No. larvae	Slope ± SE*	LC_{50} (ppm)	Fiducial limited	X^2	df
100	1.049 ± 0.143	17187.8	9501.24 – 84564.29*	19.559	8
100	0.870 ± 0.135	8533.8	5558.77 – 24119.69 *	15.386	8
100	0.722 ± 0.134	3220.1	2075.49 – 5013.16*	12.738	8
100	0.631 ± 0.135	1522.1	356.06 – 2424.26*	13.166	8
100	0.625 ± 0.138	937.8	139.26 – 1640.81**	16.986	8

*: Slope of probit line. SE: standard error.

3.2. Effect of Azadirachtin on larval feeding behavior

Larvae devoured the least amount of food at the highest dosage (1463.10 ppm), consuming just 127.4 mg per larva at 3 DAT, which increased marginally to 279.3 mg by 9 DAT. Likewise, consumption remained modest at 731.55 ppm, with 131.0 mg at 3 DAT and 248.2 mg at 9 DAT. The pattern continued at 365.78 ppm and 243.85 ppm, with feeding rates still considerably lower than the control. Larvae in the control group, on the other hand, consumed significantly more feed; their values were 222.6 mg at 3 DAT, 863.1 mg at 6 DAT, and 1638.7 mg at 9 DAT. Azadirachtin significantly reduced the amount of food consumed by S. litura larvae, demonstrating a severe antifeedant effect, as evidenced by the significant difference between treated and untreated larvae. Compared to the control group, all treated

larvae showed a marked decrease in feed consumption across all time points (3, 6, and 9 days after treatment), as shown in Table 3. However, the observation is further supported by statistical analysis, which shows that all treated groups (designated with "b") consumed significantly less feed than the control group (designated with "a") ($p < 0.05$). The lower feeding rates directly contribute to reduced larval growth and survival, highlighting Azadirachtin's effectiveness in pest management through growth inhibition and starvation. This information demonstrates that azadirachtin significantly impacts feeding suppression, which weakens larvae over time and interferes with their growth, in addition to its effect on larval mortality.

Table 3: Effect of Azadirachtin on Larval Feeding Behavior

Concentration (ppm)	Larvae feed weight consumed (mg/larvae)		
	3 DAT	6 DAT	9 DAT
1463.10 ppm	127.4b	226.6b	279.3b
731.55 ppm	131.0b	203.3b	248.2b
365.78 ppm	128.3b	215.1b	255.2b
243.85 ppm	139.1b	227.8b	347.5b
Control	222.6a	863.1a	1638.7a

DATA: day after treatments.

3.3. Effect of azadirachtin on larval weight gain

The larvae exhibited slightly better growth as the concentration decreased but remained significantly smaller than the control group. At 365.78 ppm and 243.85 ppm, larvae showed moderate weight gain over time, with an increase of 4.95 mg and 4.86 mg, respectively, between 6 and 9 DAT. However, the difference was still stark compared to the control group, which exhibited rapid and substantial growth. The control larvae had an average weight of 118.61 mg at 3 DAT, which increased dramatically to 331.86 mg at 6 DAT and 756.11 mg at 9 DAT, reflecting a massive weight gain of 424.26 mg in the final three days, as shown in Table 4.

Larval weight increase is greatly decreased by azadirachtin in a dose-dependent manner. At 1463.1 ppm, larvae grew slowly (4.99 mg at 3 DAT to 7.61 mg at 9 DAT, increasing only 0.26 mg between 6 and 9 DAT). Likewise, weight rose from 8.48 mg to 14.15 mg at 731.55 ppm, while only 1.01 mg was acquired in the previous three days. These findings highlight the strong inhibitory effects of Azadirachtin on S. litura larvae. Higher concentrations resulted in near-total suppression of weight gain, whereas lower concentrations allowed for some growth but still significantly less than untreated larvae. This suggests Azadirachtin functions as an effective insect growth regulator, interfering with normal development and reducing the pest population.

Table 4: Effect of Azadirachtin on Larval Weight Gain

Concentration (ppm)	Average Weight (mg/larva)				
	3 DAT	6 DAT	Increase	9 DAT	Increase
1463.1 ppm	4.99 ± 0.50	7.34 ± 0.75	2.35	7.61 ± 1.22	0.26
731.55 ppm	8.48 ± 1.91	13.14 ± 1.37	4.66	14.15 ± 1.35	1.01
365.78 ppm	10.10 ± 1.65	15.04 ± 2.82	4.94	19.99 ± 1.67	4.95
243.85 ppm	12.78 ± 2.30	16.86 ± 1.02	4.08	21.72 ± 2.27	4.86
Control	118.61 ± 37.26	331.86 ±59.21	213.25	756.11 ± 83.43	424.26

DAT: day after treatments.

3.4. Effect of Azadirachtin on larval medium length

As concentrations decreased, larval growth showed a gradual improvement. At 365.78 ppm, the average length increased from 8.35 mm at 3 DAT to 14.38 mm at 9 DAT, with a broader variability range (6.5–15.5 mm). At 243.85 ppm, larvae showed slightly better growth, with an average length of 8.45 mm at 3 DAT, reaching 16.78 mm at 9 DAT, suggesting that lower concentrations of Azadirachtin allow for some developmental progress, though still limited compared to the control group, as shown in Table 5 and Figure 1.

The results indicate that Azadirachtin significantly inhibits larval growth, as reflected in the reduced body length of S. litura over time. At the highest concentration (1463.1 ppm), larvae exhibited minimal growth, with an average length of 5.35 mm at 3 DAT, increasing slightly to 6.13 mm at 6 DAT and reaching only 7.00 mm at 9 DAT. The limited range of variability (3.5–8.5 mm) suggests that high doses of Azadirachtin consistently suppress growth across individuals. Similarly, at 731.55 ppm, larvae showed a moderate increase in length, from 7.75 mm at 3 DAT to 9.42 mm at 9 DAT, but still significantly less than untreated larvae.

On the other hand, the control larvae showed noticeably higher growth, with a much larger variability range (14.5–36.0 mm) and an average length of 21.05 mm at 3 DAT that increased to 33.25 mm at 9 DAT. This reveals that untreated larvae develop properly, whereas Azadirachtin drastically retards their growth. Azadirachtin's efficacy as an insect growth regulator is confirmed by its distinct dose-dependent influence on larval length, which lowers the survivability of larvae by preventing them from developing to their maximum potential.

Table 5: Effect of Azadirachtin on Larval Medium Length

Concentration (ppm)	Medium Length (mm)					
	3 DAT	range	6 DAT	range	9 DAT	volatility
1463.1	5.35 ± 1.25	3.5 – 7	6.13 ± 1.09	4.5 – 7.5	7.00 ± 1.00	5.5 – 8.5
731.55	7.75 ± 1.40	6.5 – 11	8.94 ± 1.07	8 – 11.5	9.42 ± 0.8	8.0 – 10.0
365.78	8.35 ± 1.29	6.5 – 11	9.85 ± 1.29	8 – 12.5	14.38 ± 0.95	13.0 – 15.5
243.85	8.45 ± 0.98	7.5 – 10.5	10.25 ± 1.21	9 – 12.5	16.78 ± 1.64	14.5 – 19.5
Control	21.05 ± 5.19	14.5 – 19.5	30.20 ± 3.60	26 – 35.5	33.25 ± 1.83	31.5 – 36.0

Fig. 1: Azadirachtin on Larval Medium Length.

This figure demonstrates the negative relationship between the concentration of Azadirachtin and the growth of Spodoptera litura larvae. The regression equation (y = -0.0078x + 9.2994) reveals that as the concentration of Azadirachtin increases, the larvae's body length decreases. The R^2 value of 0.696 indicates a moderate level of correlation, meaning that about 69.6% of the variation in larval size can be attributed to the changes in Azadirachtin concentration. This suggests that higher doses of Azadirachtin effectively limit larval growth, supporting its role in inhibiting insect development.

The data analysis reveals a significant inverse relationship between the azadirachtin concentration and the body length of the 3 DAT larvae. As shown in Figure 3, the linear regression equation is y=−0.0078x+9.2994y = -0.0078x + 9.2994y=−0.0078x+9.2994, indicating that with an increase in azadirachtin concentration, the body length of the larvae decreases. The negative slope of the regression line, -0.0078, further confirms this inverse correlation.

The coefficient of determination (R2R^2R2) value of 0.696 suggests that approximately 69.6% of the variability in the larvae's body length can be explained by changes in the azadirachtin concentration. This moderate inverse correlation highlights the potential effect of azadirachtin on larval growth, supporting the hypothesis that higher concentrations of this compound reduce the growth of the larvae, as shown in Figure 2.

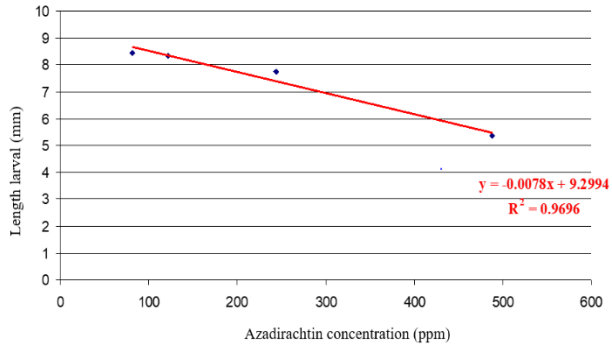

Fig. 2: Graph Showing the Inverse Correlation between Azadirachtin Concentration and Length Larval.

3.5. The ability of azadirachtin to inhibit molting in s. litura

The results showed that at 365.78 ppm and 243.85 ppm, larvae exhibited a moderate ability to molt, with 77% successfully reaching the 3rd instar and 40% and 36%, respectively, advancing to the 4th instar. However, only 3–8% of larvae at these concentrations reached the 5th instar, and none successfully pupated. The consistent failure to reach the pupal stage across all treated groups suggests that Azadirachtin disrupts hormonal regulation necessary for molting, ultimately preventing full development. The results indicate that Azadirachtin effectively inhibits molting in S. litura larvae, with higher concentrations causing the most significant disruption in development. In the control group, 97% of larvae successfully molted into the 3rd and 4th instars, with 82% reaching the 5th instar and 96% successfully pupating. This reflects normal, uninterrupted growth and metamorphosis.

In contrast, At the highest concentration of Azadirachtin (1463.10 ppm), only 56% of larvae progressed to the 3rd instar, with molting to subsequent stages nearly entirely blocked. Only 3% of larvae reached the 4th instar, and none advanced to the 5th instar or pupated, confirming that high doses of Azadirachtin severely disrupt molting. At a lower concentration of 731.55 ppm, a higher percentage of larvae (66%) molted to the 3rd instar, with 28% molting into the 4th instar. However, no larvae developed past this stage, further demonstrating the impact of Azadirachtin on molting. Figure 3 visually represents these findings, illustrating that as the Azadirachtin concentration increases, fewer larvae successfully progress through their molting stages. These results underscore Azadirachtin's role as a growth regulator, disrupting normal developmental processes and preventing larvae from reaching maturity.

These results show that Azadirachtin inhibits the progression of S. litura larvae through their life stages by acting as an efficient insect growth regulator. Higher quantities cause near-complete suppression of molting, while even lower dosages considerably delay development. This implies that by keeping S. litura from maturing into reproductive adults, azadirachtin may be a powerful tool for managing populations.

Table 6: Ability of Azadirachtin to Inhibit Molting in S. Litura

Concentration (ppm)	Percentage (%) larvae molting			
	3rd	4th	5th	pupae
146.10	56c	3c	0c	0b
731.55	66bc	28b	0c	0b
365.78	77b	40b	3c	0b
243.85	77b	36b	8b	0b
Control	97a	97a	82a	96a

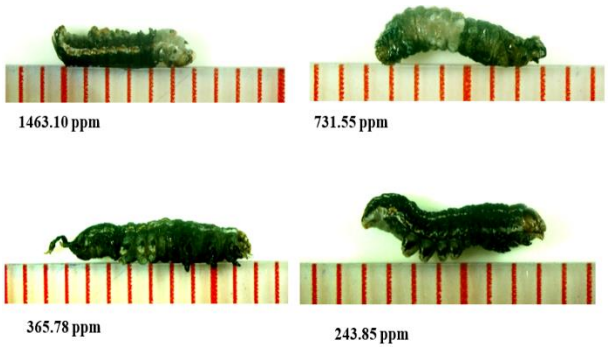

Fig. 3: Azadirachtin Inhibit Molting in S. Litura.

4. Discussion

The study results showed that Azadirachtin efficacy in significantly reducing the growth, development, and survival of S. litura. Every measure displayed changes as a result of Azadirachtin exposure with an influence felt on mortality rate and larval weight and feed consumption and body length and molting inhibition. The strength of Azadirachtin's inhibitory impact grew in direct correlation to its concentration in the solution yet reduced concentrations could generate growth delays in the life stages.

Research shows that high levels of insecticidal activity in Azadirachtin lead to substantial mortality in treated larvae when used at concentrations of 1463.10 ppm and 731.55 ppm. [2], [37]. Laboratory reports demonstrate that Azadirachtin causes three essential physiological processes to fail which ends in the death of larvae. The research conducted by Senthil-Nathan et al. revealed that Azadirachtin successfully lowered survival levels of Helicoverpa armigera and Plutella xylostella [38]

The known ability of Azadirachtin to act as an antifeedant and growth regulator is indicated by its effects on reduced weight and decreased feed consumption in treated larvae. The study results confirmed that as the A. indica concentration increased, the weight increase decreased substantially until it reached zero at the highest level of 1463.10 ppm. [39]. This supports the previously reported studies that azadirachtin inhibits the manufacture of ecdysteroid hormones, which causes lepidopteran larvae to grow more slowly and feed less. [40]. Azadirachtin dramatically decreased larval weight, delaying their growth, according to a study on Spodoptera frugiperda [23]. However, in contrast to these findings showed only minor growth suppression in Sesamia cretica, implying species-specific variability in Azadirachtin sensitivity. [41].

Azadirachtin treatment significantly reduced larval body length, resulting in smaller sizes, confirming previous research showing impaired chitin synthesis and disrupted molting cycles in Spodoptera larvae genus. [42]. Similar results were found in a study on Mythimna separata, where exposure to Azadirachtin led to reduced body size and increased mortality.[43]. Research showed that Azadirachtin acted moderately on Anopheles stephensi growth while the insecticide displayed strong effects on Lepidopteran life cycles. This indicates dipteran resistance potentially surpasses Lepidopteran resistance. [44].

Treatment with Azadirachtin resulted in impeded molting among larvae since the pupal stage was not achieved at higher concentration levels. The findings align with previous reports which demonstrate Azadirachtin disturbs Spodoptera exigua molting hormones thus preventing its development throughout the life cycle. [23]. Pupation became highly suppressed when concentrations surpassed 365.78 ppm because Azadirachtin affects juvenile hormone and ecdysone balance causing developmental problems. [45]. Researchers discovered that specific insect populations of S. litura demonstrated resistance against Azadirachtin by surviving at reduced amount concentrations. [34, 46].

Azadirachtin exerts its effects on insect development by interfering with key hormonal and molecular pathways. One of the primary mechanisms involves its disruption of ecdysone signaling, essential for molting and metamorphosis. Research on Spodoptera frugiperda demonstrated that Azadirachtin inhibits the expression of the nuclear hormone receptor HR3 in the prothoracic gland, a critical receptor involved in ecdysone action during ecdysis. [42]. This inhibition leads to a failure in molting, as the larvae are unable to shed their old cuticle, resulting in developmental arrest and mortality. In addition, studies on Spodoptera litura indicate that Azadirachtin alters protein metabolism by affecting the expression of proteins, including ecdysone receptors, which are crucial for normal larval growth and development. [47]. These findings highlight Azadirachtin's role as a potent growth regulator by disrupting hormonal regulation, thereby impairing molting and preventing proper insect development.

The present research demonstrates that Azadirachtin demonstrates wide-ranging effectiveness as a growth regulator against S. litura. The insect growth regulator properties of Azadirachtin produce limited feeding behavior along with growth stunting while raising mortality rates and halting successful metamorphosis which makes it appropriate for integrated pest management solutions. Detailed research into genetic and environmental factors influencing Azadirachtin resistance must proceed because species show different levels of vulnerability to it. Studies of biopesticide combinations with Azadirachtin for mutual benefits can boost effectiveness while protecting against resistance changes. Researchers should conduct studies on Azadirachtin applications in agricultural fields to show its effectiveness in actual farm conditions.

Azadirachtin, the primary active compound in neem, is often considered more effective than crude neem oil or other neem-based products due to its higher potency and more consistent performance. [2, 48]. While neem oil can vary in effectiveness depending on its preparation and storage conditions, Azadirachtin provides a more reliable response in pest control. [49]. Synthetic insecticides like pyrethroids and organophosphates offer quick knockdown effects and a broad spectrum of activity, but they are prone to causing resistance in pests over time. [50]. On the other hand, Azadirachtin works through multiple mechanisms, making it less likely to result in rapid resistance, which is a key advantage for sustainable pest management. [2]. This characteristic makes Azadirachtin a valuable component in Integrated Pest Management (IPM) programs, especially in regions aiming for sustainable agricultural practices.

The economic feasibility of Azadirachtin as a biopesticide for farmers, especially in low- and middle-income countries, depends on several factors, including the cost of production, application, and availability of neem-based products. While Azadirachtin can be more expensive than conventional chemical pesticides due to its extraction process and the need for large-scale neem cultivation, its long-term benefits may outweigh these costs [51]. Azadirachtin is biodegradable, has low toxicity to mammals, and poses minimal risk to non-target species, which can reduce the environmental cleanup costs associated with chemical pesticides [52]. Additionally, as global demand for organic and sustainable farming practices grows, Azadirachtin could become more economically viable through economies of scale, improved

extraction methods, and government subsidies promoting eco-friendly pest management. For smallholder farmers, the potential for reduced pesticide resistance and lower environmental impact may make Azadirachtin a cost-effective, sustainable alternative in the long run [3], [53].

Azadirachtin has shown strong potential in controlling pests like Spodoptera litura on crops such as cabbage, soybeans, and cotton, which are commonly affected by these pests. [54]. While the laboratory results are promising, it's important to test Azadirachtin's effectiveness in field conditions, as pest populations and environmental factors can vary. Furthermore, although Azadirachtin is considered less harmful to the environment compared to synthetic pesticides, its effects on non-target organisms particularly beneficial insects like pollinators and natural predators that requires careful consideration [3], [55]. When using Azadirachtin in IPM, it is crucial to ensure that its use doesn't negatively impact these important species that contribute to maintaining ecological balance.

Despite of a promising biopesticide, Azadirachtin faces challenges when scaled for field applications. One of the main obstacles is its higher production cost compared to synthetic chemicals, which could limit its widespread use, especially in regions with limited resources. The need for large-scale neem cultivation and extraction processes adds to its cost. [2]. Moreover, Azadirachtin's effectiveness can vary depending on environmental factors, such as temperature and humidity, making its field application less predictable than synthetic insecticides, which are often more consistent under various conditions. [56]. Regarding resistance development, Azadirachtin's multi-mode action affecting insect growth, feeding, and reproduction may slow the development of resistance compared to conventional chemical pesticides, which often target single pathways. However, resistance to Azadirachtin has been observed in some pest species, although it typically occurs more gradually, suggesting that its integration into pest management strategies could help mitigate the rapid resistance seen with synthetic insecticides. [2], [57].

Significance of the study for sustainable Pest Management and IPM practices

The research adds essential information to IPM science through evidence that shows Azadirachtin works well against S. litura. Pesticide resistance issues and environmental impacts from synthetic insecticides have increased concerns leading Azadirachtin to emerge as an sustainable alternative against these problems. The research findings validate the use of plant-derived insecticides in farming practices for middle-low-income nations because they offer environmentally sustainable and affordable solutions.

5. Conclusion

This research delivers important knowledge to IPM science by demonstrating that Azadirachtin provides excellent control of S. litura. The growing concerns about pesticide resistance and environmental consequences have prompted Azadirachtin to become an eco-friendly alternative among these problems. Research data confirms plant-derived pesticides should be used extensively by farming sectors in middle-low-income nations because they provide cost-effective environmental-friendly solutions.

6. Limitations of The Study

Several restrictions exist in this research although the reported outcomes appear favorable. The laboratory settings in which experiments were performed might not properly mimic actual field conditions because environmental elements including temperature and humidity together with natural predators would play a role. Lacking in this research was an investigation into the long-term effects on reproductive success and resistance development despite concentrating on larval development along with mortality. The study did not investigate the interaction effects of Azadirachtin regarding the inhibitory outcome with different biopesticides and chemical pesticides despite showing substantial blocking results.

7. Future Perspectives

Additional investigation in outdoor environments needs to take place since it will reveal Azadirachtin's functional effectiveness in real-world conditions. Research on how Azadirachtin works with other biopesticides or natural enemies will enhance effectiveness by lowering the chance of pest resistance development. Molecular studies of Azadirachtin's mechanisms on S. litura will help researchers to develop better and improved control approaches for pests by revealing resistance elements. Long-term agricultural use of Azadirachtin in agriculture requires analyzing its impacts on pollinators and beneficial insect species together with investigations of non-target effects.

Declarations

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Compliance with Ethical Standards

This study complies with all ethical guidelines and was conducted in accordance with the ethical standards established by the research institution. No live animals were involved, and all procedures were designed to minimize harm to the organisms involved.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Author Contributions

All authors contributed equally to the design, execution, and analysis of the research. Xuan Thi Trinh led the experimental work and data analysis. Hiep Pham Van assisted in the bioassays and data interpretation. Son Pham Kim contributed to the experimental setup and manuscript preparation.

Supplementary Information

Supplementary material is available upon request.

Additional Information

There is no additional information to be disclosed

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