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Efficacy of Some Insecticides on Different Larval Instars of *Spodoptera litura* (Fab.) under Laboratory Condition

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Authors' contributions

This work was carried out in collaboration among all authors. Author MAT gathered data, performed analyses, and composed the original version of the paper. Authors MMR, MSH and MRA contributed to the study's conception and gave direction on analysis, writing, and editing of subsequent manuscript revisions. Author MR also assisted in data collection. Authors MSIK and MEH contributed to the development of the text. All authors read and approved the final manuscript.

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ABSTRACT

Spodoptera litura (Fab.) is a severe agricultural insect pest causing significant damage across a wide range of crops. This research aimed to determine the effect of some insecticides on different larval stages of this pest using several insecticides dosages at three distinct schedules (8h, 16h, and 24h) during the third larval stage. The mortality rate peaked after 24 hours, while the lowest rate was documented to have occurred 8 hours following insecticide treatment. After 8 hours, the highest death rate (15%) was recorded by spraying Virtako 40WG solution (thiamethoxam + chlorantraniliprole) at 0.2 g/liter of water, followed by 12.5% in Nitro 505EC solution (cypermethrin + chlorpyrifos) treated larvae at 2.0 ml/liter of water. After 24 hours of insecticide treatment, spraying with Nitro 505 EC solution resulted in the greatest larval death percentage (92.50%) with a 428.57% increase above control, followed by Virtako 40 WG solution (90.00% and 414.28%, respectively). Conclusively, Virtako 40WG and Nitro 505EC effectively controlled the immature stage of *Spodoptera litura* in lab conditions, which may be further trialed to validate this result.

***Keywords**: Spodoptera litura; larvae; thiamethoxam; chlorantraniliprole; cypermethrin; chlorpyrifos.*

1. INTRODUCTION

In traditional agriculture, insecticides are essential control options that are primary barriers against pest infestations and ensure agricultural output (Rehan & Freed, 2014; Khan et al., 2024). Traditional control methods often rely heavily on synthetic insecticides, leading to adverse effects on the environment, human health, non-targeted insects, and the development of insecticide resistance (Liu et al., 2018; Ahmad et al., 2008; Saleem et al., 2008; Ahmad et al., 2008; Sayyed et al., 2008; Ali et al., 2010). Nevertheless, t heir extensive application gives rise to significant apprehensions concerning their possible ecological consequences, specifically regarding non-target organisms (Shabir et al., 2023). *Spodoptera litura*, alternatively referred to as the tobacco cutworm, is a formidable agricultural pest that causes significant harm to a wide range of crops, including cotton, soybean, peanut, tobacco, sweet potato, taro, and cruciferous crops (El-Aswad et al., 2004; Lin et al., 2018; Ullah et al., 2020). *S. litura* exhibits a complex life cycle consisting of numerous developmental stages, wherein each stage is vulnerable to different levels of insecticide toxicity. It is crucial to comprehend the intricate ramifications of insecticides on these discrete life cycles to develop environmentally friendly pest management approaches that minimize detrimental effects (Jafir et al., 2021; Sayyid et al., 2018). *S. litura* is mainly controlled in its immature stages, especially in 3rd instar larvae, by applying chemical insecticides (Shabir et al., 2023).

Chlorpyrifos, deltamethrin, fipronil, β-cypermethrin, indoxacarb, chlorantraniliprole, thiamethoxam, imidacloprid and methomyl are common and effective insecticides for controlling *S. litura,* although the resistance of *S. litura* to these three insecticides has continued to increase (Ahmad et al., 2008; Sarfraz et al., 2010). Furthermore, considering the ever-changing characteristics of pest populations and the perpetual development of resistance mechanisms to insecticides, it is critical to conduct ongoing evaluations of the effectiveness and safety of insecticides to uphold agricultural sustainability. The objective of this research was to conduct a thorough examination of the effects of different insecticides on the distinct life phases of *S. litura* in a laboratory setting. Through a comprehensive analysis of the complex dynamics between insecticides and *S. litura* at various phases of development, this research not only enhances our knowledge of pest ecology but also provides valuable insights for the formulation of precise and ecologically sound pest management approaches.

2. MATERIALS AND METHODS

The research was carried out at the Bangladesh Sugarcrop Research Institute (BSRI) in Iswardi, Pabna. Leaves and sugar beets infested with *S. litura* caterpillars were gathered from the experimental field. In this experiment, a variety of insecticides were utilized, such as carbaryl-containing Sevin 85WP, chlorpyrifos-containing Dursban 20EC, synthetic cypermethrin, and chlorpyriphoscontaining Nitro 505EC, imidacloprid-containing Imitaf 20SL, and a combination of thiamethoxam and chlorantraniliprole, Virtako 40WG. Clusters of eggs were collected from the experimental field and subjected to a rearing cage until they matured. The population of *S. litura* collected from the experimental fields was maintained in its original, unaltered condition. Data were collected on the hatching time, larval phase, pupal period, and days of adult emergence. To evaluate the effectiveness of insecticides on different life stages of *S. litura*, a total of 440 third-instar larvae were distributed among forty-four petri dishes in the laboratory, containing 10 larvae in each petri dish. The gathered data were then used to assess the efficacy of the insecticides. Five insecticides with potential were concurrently applied at two distinct concentrations: the indicated dosage and doses exceeding 25% of the recommended amount. These insecticides will be further described below. Measurements were obtained at time intervals of eight hours, sixteen hours, and twenty-four hours. The death rates of different larval stages were determined in response to different chemical treatments. An experiment with four (4) replications was carried out utilizing a Completely Randomized Design (CRD) (Khan et al., 2020; Khan et al., 2024).

2.1 Treatments

In the laboratory, 10 different insecticide combinations were used simultaneously, which are shown in Table 1.

2.2 Data Recorded

The data were recorded on the following parameters:
Growth of larval instar, Weight of larval instar (mg), Days of adult emergence, Mortality of larvae.

According to Abbott's formula:

$$Corrected\ (\%) Mortality = \frac{X - Y}{X} \times 100$$

Where, X = percentage survival in the untreated control and
Y = percentage survival in the treated sample.

$$Mortality\ percentage\ increase\ over\ control\ = \frac{X - Y}{Y} \times 100$$

Where, X = Mortality percentage in treated petri dishes
Y = Mortality percentage in untreated (Control) petri dishes

2.3 Data Analysis

For statistical analysis, data on growth and growth-contributing characteristics were collected and tabulated according to the required format. Microsoft Excel was used to calculate mean, standard deviation (SD), and correlation as well as the necessary computation of the collected data.

Table 1. Different insecticides used in the current study, with their group name, dose L^{-1} water, and the manufacturing company name

Treatment	Trade name	Group	Dose L^{-1} Water	Manufacturing Company
T_1	Acicarb 85WP	Carbaryl	3.5 g	ACI Ltd.
T_2	Acicarb 85WP	Carbaryl	4.5 g	ACI Ltd.
T_3	Dursban 20EC	Chlorpyrifos	1.5 ml	Auto Crop Care Ltd.
T_4	Dursban 20EC	Chlorpyrifos	2 ml	Auto Crop Care Ltd.
T_5	Nitro 505EC	(Cypermethrin+ Chlorpyrifos)	1.5 ml	Auto Crop Care Ltd.
T_6	Nitro 505EC	(Cypermethrin + Chlorpyrifos)	2.0 ml	Auto Crop Care Ltd.
T_7	Imitaf 20SL	Imidacloprid	2.0 ml	Auto Crop Care Ltd.
T_8	Imitaf 20SL	Imidacloprid	2.5 ml	Auto Crop Care Ltd.
T_9	Virtako 40 WG	(Thiamethoxam+ Chlorantraniliprole)	0.15 g	Syngenta Bangladesh Ltd.
T_{10}	Virtako 40 WG	(Thiamethoxam+ Chlorantraniliprole)	0.2 g	Syngenta Bangladesh Ltd.
T_{11}	Untreated control	-	-	-

3. RESULTS

3.1 Mortality Rate at Different Time Intervals

Using three separate time intervals, viz., 8 h, 16 h, and 24 h, during the third stage of larval development, the efficacy of different insectcides doses was examined against sugar beet caterpillar *Spodoptera litura* population. Eight hours later, in a controlled environment, the T_{11} (untreated control) and T_1 treatments (Acicarb 85WP solution sprayed at a concentration of 3.5 g/liter) achieved the lowest mortality percentage of 0%. The larval mortality rate was 15% in T_{10} (Virtako 40WG solution @ 0.2 g/L water) and 12.5% in T_6 (Nitro 505EC @ 2.0 ml/L water). The larval mortality rate in T_2 was 2.5% when the Acicarb 85WP was sprayed at a concentration of 4.5 grams per liter (Fig. 1a).
Furthermore, the largest larval mortality percentage (50%) was seen after 16 h in T_6 (treated with Nitro 505EC at a rate of 2.0 ml/liter of water) and T_{10} (treated with Virtako 40WG at a rate of 0.2 g/liter of water). Fig. 1b shows that the untreated control group, T_{11}, had the lowest death rate at 7.5%. Treatment with a Nitro 505 EC solution (cypermethrin + chlorpyrifos) sprayed at 2.0 ml/lit of water for 24 h resulted in the greatest larval death percentage (92.50%) at T_6, followed by the T_{10} treatment at 90%. Virtako 40 WG solution containing 20% thiamethoxam and 20% chlorantraniliprole was sprayed at a rate of 0.2 g/lit (Fig. 1c). The T_{11} (control) treatment had the lowest larval mortality rate at 17.5%.

3.2 Percent Increase Over Control

The increase in mortality percentage compared to the control treatment ranged from 0% to more than 400% in a controlled laboratory setting. All treatments except the control one increased efficacy by 300 percent or more. The control group showed the lowest larval death rate (428.57 %), whereas T_6 (Nitro 505 EC @ 2.0 ml/lit of water) showed the highest rate (428.57 %). T_{10} consisted of spraying Virtako 40 WG solution containing 20% thiamethoxam and 20% chlorantraniliprole @ 0.2 g/lit of water, which was then applied, and the population collapsed by 414.28%. Acicarb 85 WP solution at 3.5 g/lit of water (T_1) showed the least significant increase in larval mortality percent (300%) compared to the control group (Fig. 1d). The treatment comprising Nitro 505 EC, including chlorpyrifos and cypermethrin at a rate of 2.0 ml/lit of water, yielded the best results (T_6).

3.3 Larval Weight, Larval Mortality Rate, and Larval Instar Growth Rate

The mortality rate ranged from 17.5 percent to over 90 percent when compared to the control treatment in laboratory conditions (Table 2). Following the application of treatments for 24 h, the larvae that remained alive were allowed to continue their growth. The remaining larvae successfully reached the 5th larval instar, and their weights were measured and recorded. The larvae in the controlled petri dishes (T_{11}) had the greatest weight, measuring 23.1±0.8 mg. This was followed by the group being treated with Acicarb 85WP at a concentration of 3.5 g/liter of water (T_1), which weighed 22.7±0.8 mg (Table 2). The larvae in T_6, which received a 2.0 ml/liter water solution of Nitro 505EC, had the lowest weight of 17.8±0.8 mg. The most significant impact was seen in T_6, where the application of Nitro 505EC at a concentration of 2.0 mL per liter of water resulted in the lowest weight of 5th instar larvae of *S. litura*. This treatment likely caused less stress compared to the other treatments. The remaining larvae successfully reached the 5th larval instar, and the dates of their emergence as adults were documented. The peak days of adult emergence were 27±0.7 in the control group (T_{11}) and 26±0.2 in the group treated with Sevin 85WP at a concentration of 3.5 g/liter of water (T_1) (Table 2). Nevertheless, the adult emergence was at its lowest during T_6, which included the application of Nitro 505EC at a concentration of 2.0 ml per liter of water, with an average of 19±0.5 days. The Nitro 505EC was applied to the 5th instar larvae of *S. litura* during the early stages of adult emergence, since it was hypothesized that this chemical might enhance larval development and expedite the transition to adulthood.

Death rates and adult emergence days exhibited a negative correlation (r = -0.77) under laboratory conditions (Fig. 2a). The 'r' value indicated a significant correlation. Adult emergence occurred during the shortest days when the mortality rate was greatest. The days until adulthood increased in tandem with the decline in mortality. Conversely, a positive correlation (r = 0.97; Fig. 2b) was observed between the date of adult emergence and larval weight. It is indicated by the value of 'r' that the link was substantial. The lightest weight of larvae and the earliest date of adult emergence were both recorded. The larvae experienced a progressive increase in weight as the days leading up to adult emergence passed.

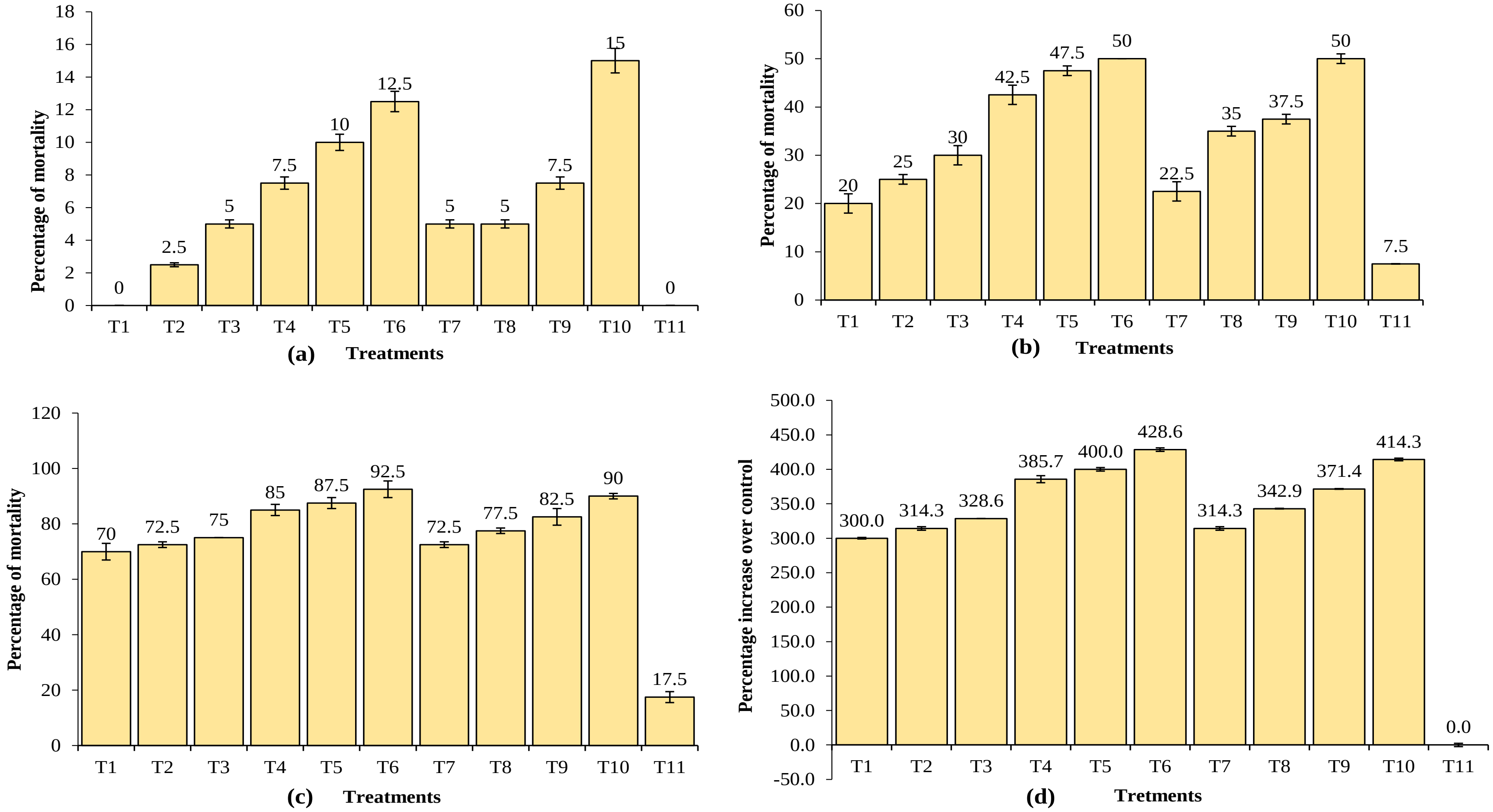

Fig. 1. Insecticidal effects on mortality percentage and their increase over control at different hours of application: (a) 8h, (b) 16h, (c) 24h, (d) mortality percentage increase over control after 24h

[T_1= Acicarb 85WP, T_2= Acicarb 85WP, T_3= Dursban 20EC, T_4= Dursban 20EC, T_5= Nitro 505EC, T_6= Nitro 505EC, T_7=Imitaf 20SL, T_8= Imitaf 20SL, T_9= Virtako 40WG, T_{10}= Virtako 40WG and T_{11}=Untreated control]

Correlation of larval weight and percentage of sugar beet caterpillar (*S. litura*) mortality with days of adult emergence

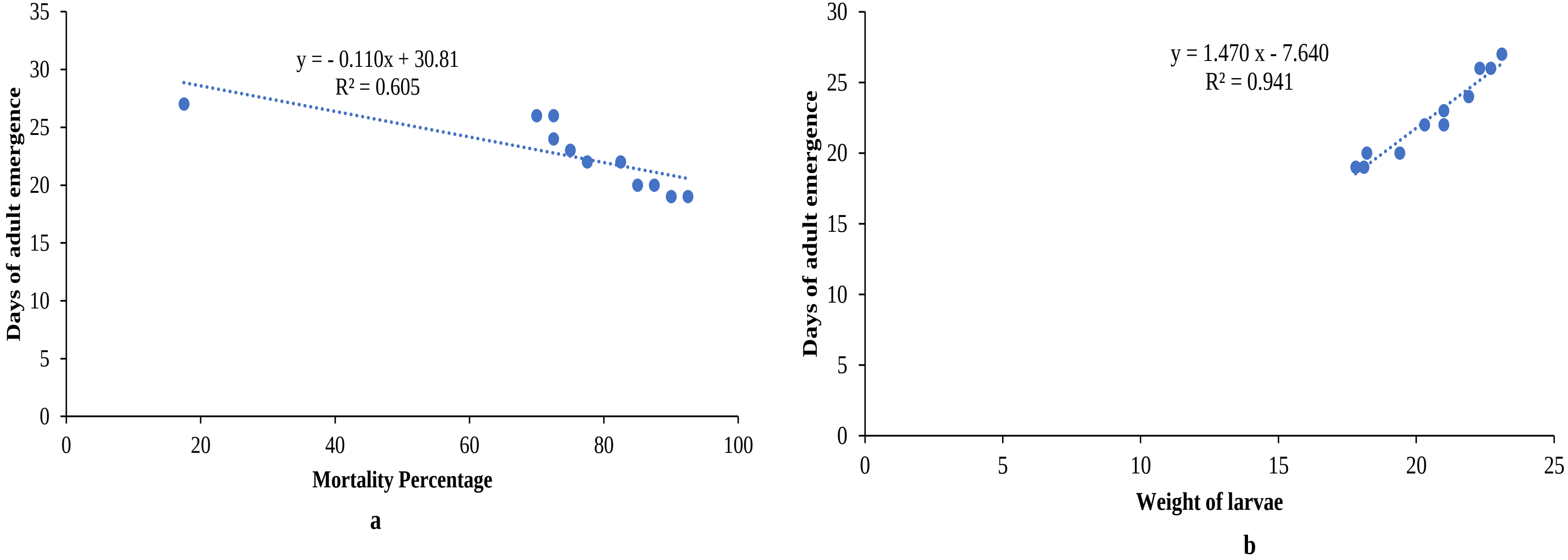

Fig. 2. Correlation between mortality percentage and days of adult emergence (a); Weight of larvae and days of adult emergence of sugar beet caterpillar *S. litura* (b)

Table 2. Impact of varying insecticides dosages on *S. litura* larval development, growth, weight, and adult emergence days

Treatment	3rd instar larval mortality (%)	Larval instar	5th instar larval weight (mg) (Mean ± SD)	Adult emergence (days) (Mean ± SD)
Acicarb 85WP	70.00	5th instar	22.7±0.8	26±0.2
Acicarb 85WP	72.50	5th instar	21.9±0.7	24±0.4
Dursban 20EC	75.00	5th instar	21±0.6	23±0.5
Dursban 20EC	85.00	5th instar	19.4±0.3	20±0.6
Nitro 505EC	87.50	5th instar	18.2±0.5	20±0.3
Nitro 505EC	92.50	5th instar	17.8±0.8	19±0.5
Imitaf 20SL	72.50	5th instar	22.3±0.6	26±0.8
Imitaf 20SL	77.50	5th instar	21±0.5	22±0.3
Virtako 40 WG	82.50	5th instar	20.3±0.4	22±0.6
Virtako 40 WG	90.00	5th instar	18.1±0.7	19±0.6
Untreated control	17.50	5th instar	23.1±0.8	27±0.7

4. DISCUSSION

Based on initial findings, the efficacy of novel insecticides that specifically target the resistant populations of this moth has been shown to be successful (Venkateswarlu et al. 2005; Khan et al., 2020). In the present study about testing the insecticides in lab conditions, we found that Nitro 505 EC (cypermethrin+ chlorpyrifos) and Virtako 40 WG (thiamethoxam + chlorantraniliprole) were the most effective insecticides against the larval stage of *S. litura* after different hours (8h, 16h, and 24h) of insecticide application. The highest mortality percentage (92.5%) was obtained after 24 h of treatment application, which is closely aligned with the result of Shabir et al. (2023), who found 98% larval mortality after 72 h of insecticide application. Our applied insecticides were more effective on the younger larvae (3rd instar) compared to older stages of larvae, which is supported by the findings of Liu et al. (2018), who found that younger larvae (3rd instar) are more susceptible to fluralaner treatment application. This scenario may be due to the greater sensitivity of juvenile stages of larvae, as they were not able to develop sufficient resistance and defense against the lethal action of insecticides. Siddiquee et al. (2017) found that Nitro 505EC at a rate of 4.5 L ha^{-1} every 15 days, when applied to sugar beet fields, revealed the greatest percentage of effectiveness in controlling larval populations. Our results are also supported by Jafir et al. (2021), who tested some synthetic insecticides with distinct modes of action, such as Coragen®, Proclaim®, Tracer®, and Talstar®, and found that early instar larvae were more susceptible to these chemicals. They also stated that the probit analysis on the 2nd, 3rd, 4th, and 5th instars of *S. litura* revealed a positive correlation between the lethal concentrations (LCs) of the investigated nanoparticles and insecticides and the age of the instars. An environment that was harmful to larval development might be responsible for the high number of larvae that died in the control environment. Anju and Srivastava (2012) also stated that synthetic pyrethroid killed eight-day-old *S. litura* larvae more effectively. Munir et al. (2005) tested three different dosages of novel chemical insecticides against *S. litura*, a leaf worm species, in its second instar larval stage. Emamectin benzoate 1.9 EC was shown to be the most effective, followed by lufenuron 5 EC, spinosad 45 SC, and indoxacarb 15 SC, according to the study. According to the investigation to determine discriminating dosages for resistance monitoring, spinosad and emamectin benzoate were recommended as more toxic towards *S. litura* showing no sign of resistance to these insecticides (Natikar & Balikai, 2015). The research conducted by Gadhiya et al. (2014) shown that the insecticides chlorantraniliprole, spinosad, and emamectin benzoate have exhibited favorable results in combating *S. litura*. According to Patil et al. (2014) and Khan et al. (2024), chlorantraniliprole had the most favorable cost-benefit ratio among the insecticides evaluated on soybeans.

The results illustrate a clear variance in the effectiveness and impact of different chemical treatments on *S. litura* larvae. While some treatments like Acicarb 85WP had a minor effect on both mortality and growth, others like Nitro 505EC were much more impactful, resulting in lower larval weights and faster adult emergence. This could indicate a dual role for Nitro 505EC, acting both as a growth inhibitor and as a potential developmental accelerator. These findings are critical for understanding the broader implications of using these chemicals in pest management. The choice of chemical treatment can have varying consequences, not just on immediate mortality but also on the growth and developmental timelines of the surviving larvae. Such information can guide more effective and targeted pest control strategies, ensuring that the most appropriate treatment is used to achieve desired outcomes in managing *S. litura* populations.

The correlation findings highlight the complex interactions between mortality, growth, and developmental timing in larvae. Treatments or environmental conditions that stress the larvae and increase mortality may also expedite development in surviving larvae, potentially as a compensatory mechanism. On the other hand, conditions that support better larval growth extend the developmental period, possibly due to the allocation of more resources towards reaching a larger size. Understanding these correlations is essential for optimizing pest management strategies, balancing the need to reduce larval populations to minimize the adverse impacts on developmental processes. Adult emergence occurred during the shortest days when the mortality rate was greatest. The days until adulthood increased in tandem with the decline in mortality. A similar correlation was also found by Punia et al. (2021), who stated that the mortality of *S. litura* larvae increases with the decrease in adult emergence days. Conversely, a positive correlation was observed between the date of adult emergence and larval weight, which is also supported by Punia et al. (2021), who discovered that the inclusion of gallic acid in the food of six-day-old *S. litura* larvae had a substantial impact on their growth, development, and survival, and the adult emergence time was considerably delayed after feeding the larvae a diet containing gallic acid, as compared to the control group.

5. CONCLUSION

The larvicidal study showed that third instar *Spodoptera litura* larvae were particularly susceptible to the chemical insecticide treatments, which resulted in over 92.50% mortality within 24 hours of application for Nitro 505EC (cypermethrin + chlorpyrifos) and 90.00% for Virtako 40WG (thiamethoxam + chlorantraniliprole). Moreover, these treatments not only caused mortality but also heavily stunted growth, accelerated the rate of adult emergence, and induced strong physiological impacts. There emerged a negative correlation between mortality rate and adult emergence time, as well as a positive correlation between larval weight and development time, indicating complex growth vs survival relationships exerted under the stress of chemicals. These results imply that *S. litura* populations can be effectively managed by early instar targeting with Nitro 505EC and Virtako 40WG. Further, these promising laboratory results should undergo field testing to evaluate environmental safety before broad use is considered.

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DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that no generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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