



## BEYOND MORTALITY: EVALUATING INSECTICIDE IMPACT ON *Spodoptera exigua* FEEDING AND DAMAGE

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### Abstract

The study addresses the pressing issue of effective pest control in agriculture, focusing on the impact of pesticides on *Spodoptera exigua*, a notorious pest of agricultural crops. The research aims to investigate the influence of different insecticide active ingredients and concentrations on mortality, feeding inhibition, and damage levels to develop optimal control strategies. The observed variables were mortality, feeding inhibition, and damage level. The study utilized regression and correlation analysis to determine the influence of harvested area on shallot production and relationship between harvested area and shallot production, generalized linear model (GLM) analysis to evaluate the effects of insecticide treatments and employed Duncan's multiple range test (DMRT) to identify the most effective factors. Results indicate that chlorpyrifos emerged as the most potent insecticide in terms of mortality, while chlorantraniliprole + indoxacarb and chlorpyrifos showed high feeding inhibition. Chlorpyrifos, particularly at the recommended concentration, minimized damage levels to the host plant. These findings underscore the importance of considering multiple factors when implementing pest control measures for sustainable agricultural practices.

Keywords: Shallots, Armyworm, Pesticides

## 1. Introduction

Shallots (*Allium cepa* L. var. *aggregatum*) are a vital horticultural commodity in Indonesia, including in Brebes Regency (Febrayanto & Susiyanti, 2024). Shallots hold significant economic value and serve as a primary source of income for many farmers (Febrayanto, 2023). However, shallot production in Brebes Regency is often hindered by pest infestations, particularly the *Spodoptera exigua* caterpillar (Karya & Supriyadi, 2021). *Spodoptera exigua* is a major pest that can cause substantial crop damage, leading to reduced yields and farmer income (Li et al., 2023). In Brebes Regency, the average shallot planting area from 2017 to 2023 has been approximately 30,000 hectares, with an average production of 300,000 tons (Figure 1). This highlights the significant economic importance of shallots in the region. However, despite this substantial production, shallot cultivation in Brebes Regency faces challenges due to the prevalence of *Spodoptera exigua* infestation (Aldini et al., 2020).

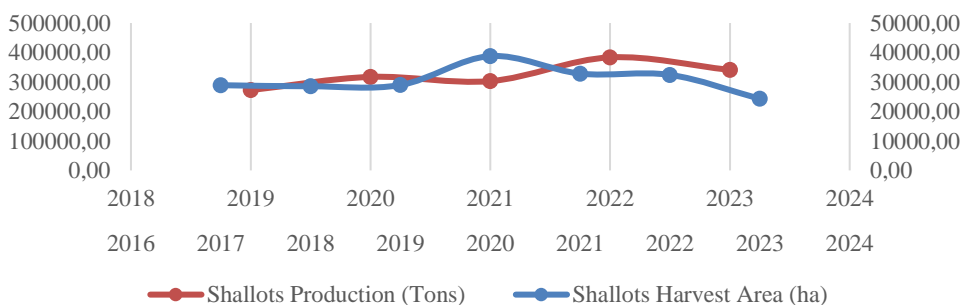


Figure 1. Shallots harvest area and production



While Brebes Regency boasts a significant shallot planting area, reaching an average of 30,000 hectares from 2017 to 2023, with an average production of 300,000 tons (BPS, 2024), this does not necessarily translate to a direct correlation between planting area and production (Table 1). Linear regression analysis revealed that planting area does not significantly influence shallot production in Brebes Regency, indicating a lack of a strong relationship between the two factors (Table 2). This suggests that other factors play a more significant role in determining the final yield. Indeed, factors such as pest and disease infestations, as well as natural phenomena like floods and prolonged droughts, have a more pronounced impact on shallot production (Utami, 2021). These external factors can significantly disrupt the growth and development of shallot crops, leading to reduced yields and impacting the overall productivity of the shallot industry in Brebes Regency (Primadita et al., 2023).

**Table 1.** Correlation of shallots harvest area and production

		Harvest Area	Production
Harvest Area	Pearson Correlation	1	-.018
	Sig. (2-tailed)		.978
	N	5	5
Production	Pearson Correlation	-.018	1
	Sig. (2-tailed)	.978	
	N	5	5

**Table 2.** Linear regression of shallots harvest area on shallots production

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	327784.968	143655.707		2.282	.107
	Harvest Area	-.137	4.515	-.018	-.030	.978

Among the various pests that threaten shallot production in Brebes Regency, the *Spodoptera exigua* caterpillar, commonly known as the beet armyworm, poses the most significant threat to farmers (Aldini et al., 2021a). This voracious insect, with its characteristic feeding habits on leaves and stems, can cause severe damage to shallot crops, leading to significant yield losses and impacting the overall profitability of shallot cultivation (Aldini et al., 2020). The *Spodoptera exigua* caterpillar's ability to rapidly multiply and its resistance to conventional control methods make it a persistent and challenging pest for farmers in the region (Che et al., 2015; Li et al., 2023).

The control of *Spodoptera exigua* in Brebes Regency has largely relied on the use of chemical pesticides. While effective in suppressing pest populations, excessive application of chemical pesticides can lead to the development of pest resistance. Pest resistance occurs when pest populations become immune to specific pesticides, rendering them less effective in controlling pests (Bolzan et al., 2019; Ishtiaq et al., 2014). Resistance of *Spodoptera exigua* to chemical pesticides has become a serious issue in various regions of Indonesia, including Brebes Regency. The active ingredients commonly used by farmers in Brebes Regency to control *Spodoptera exigua* populations are chlorfenapyr, methomyl, chlorpyrifos, spinetoram + methoxyfenozide, emamectin benzoate + chlorbenzuron, and cyantraniliprole. *S. exigua* originating from Larangan, Wanasari, Brebes, and Songgom subdistricts in 2021 showed resistance to chlorfenapyr, methomyl, and emamectin benzoate (Aldini et al., 2020, 2021a).

The emergence of *Spodoptera exigua* resistance to chemical pesticides poses significant challenges for shallot farmers in Brebes Regency. Farmers are forced to use higher doses or more frequent applications of pesticides, which can increase production costs and have negative environmental impacts. Additionally, pest resistance can lead to decreased yields and farmer income.

Therefore, this study aims to analyze the resistance of *Spodoptera exigua* to chemical pesticides in Brebes Regency. The findings of this study are expected to provide valuable information for stakeholders, including farmers, extension agents, and researchers, to develop appropriate and effective pest control strategies, ultimately enhancing shallot productivity in Brebes Regency.

## 2. Methods

This study was conducted in Brebes Regency, a major shallot producing region in Central Java, Indonesia. The study area included Bulakamba Subdistrict known for its significant shallot production. The subdistrict was selected based on its historical prevalence of *Spodoptera exigua* infestation and the widespread use of chemical pesticides for pest control. Data on *Spodoptera exigua* resistance to chemical pesticides was collected through a combination of field experiments and surveys.

The study employed a factorial experimental design with two factors: insecticide type and insecticide concentration. Three active ingredients were tested: isocycloseram, chlorpyrifos, and chlorantraniliprole + Indoxacarb. Three concentrations were tested for each insecticide: 0.5x recommended concentration, 1x recommended concentration (standard concentration), and 2x recommended concentration. This resulted in a total of nine treatment combinations (3 insecticide types x 3 concentrations) plus one control group (no insecticide). Each treatment combination and the control group were replicated four times, with each replicate consisting of 10 *Spodoptera exigua* larvae, totaling 400 larvae used in the bioassay.

*Spodoptera exigua* larvae were collected from shallot fields in the selected subdistricts and reared under controlled laboratory conditions prior to the bioassay. Insecticides were prepared according to the recommended concentrations for each treatment, based on the label instructions for each insecticide. White mustard (sawi putih) were used as the food source for the larvae. The white mustard greens were dipped in the insecticide solutions for a specific duration (1 minute) to ensure proper insecticide absorption. The control group received white mustard greens dipped in distilled water. The treated white mustard were then placed in Petri dishes, and 10 larvae were introduced into each dish.

The following variables were observed and recorded for each treatment group (Hariri, 2012; Permatasari et al., 2020; Saputra Silaban & Indrawati, 2022):

### 1. Mortality

Mortality rates were recorded after a specific time period (1, 3, 6, 12, 18 and 24 hours) based on the insecticide's mode of action. Larvae were considered dead if they did not show any movement or response to stimuli. Data for the mortality parameter was obtained by counting the number of larvae that died after 1, 3, 6, 12, 18, and 24 hours after treatment. The number of dead larvae in each replicate was averaged across all replicates. Then, the percentage of dead larvae was calculated.

$$\%d = \sum \frac{P_{di}}{P_{ti}} \times 100\% \quad (1)$$

- %d : Percentage of dead larvae affected by treatment
- Pdi : Total of dead larvae affected by treatment of each treatment
- Pti : Total larvae of each replicate

## 2. Feeding Inhibition

The amount of white mustard consumed by each larva was measured at the end of the observation period. This data was used to assess the feeding inhibition caused by the insecticide treatments. Feeding inhibition was calculated by comparing the difference between the amount of leaves eaten in the control treatment and the amount of leaves eaten in the treated group, with the total amount of leaves between the control treatment and the treated group.

$$\%FI = \frac{(Ac - At)}{Ac + At} \times 100\% \quad (2)$$

- %FI : Percentage of feeding inhibition
- Ac : Leaf area eaten by larvae in the control
- At : Leaf area eaten by larvae in the treated group

## 3. Damage Level

The level of damage caused to the white mustard greens by the larvae was assessed using a scale (0-10 scale) based on the severity of leaf damage. The percentage of damage was calculated by comparing the difference between the damage to the white mustard leaves in the control and the treated groups, with the total amount of damage to the white mustard leaves in the control and treated groups.

$$\%DL = \frac{(Dc - Dt)}{Dc + Dt} \times 100\% \quad (3)$$

- %DL : Percentage of damage level
- Dc : Damage level caused by larvae in the control
- Dt : Damage level caused by larvae in the treated group

All research activities were conducted in accordance with ethical guidelines for research involving animal welfare. The use of insecticides in the bioassays was conducted responsibly and in accordance with local regulations.

Data from the bioassays were analyzed using a generalized linear model (GLM) to determine the effects of insecticide type and concentration on larval mortality, feeding inhibition, and damage level. The GLM analysis was conducted using Statistical Package for the Social Sciences (SPSS) software. A significant effect of the insecticide type and/or concentration on the response variable indicated that the factor was influencing the observed outcome.

To identify the specific insecticide treatment with the most significant effect, Duncan's multiple range test (DMRT) was employed. DMRT is a post-hoc test that allows for pairwise comparisons of means between different treatment groups. DMRT was used to determine which insecticide treatment resulted in the highest mortality, lowest feeding inhibition, and lowest damage level, respectively.

### 3. Results and Discussion

The results of the generalized linear model (GLM) univariate analysis revealed a significant effect of insecticide active ingredient on the mortality of *Spodoptera exigua* larvae (Table 3). This effect became significant 3 hours after treatment application. The concentration of the insecticide, however, did not significantly influence mortality. This suggests that increasing the concentration of the insecticide did not result in a significant increase in larval mortality. Furthermore, no interaction was observed between insecticide active ingredient and concentration on larval mortality.

**Table 3.** Analysis of Generalized Linear Model of active ingredient and concentration on mortality of *Spodoptera exigua* larvae

Source	df	Std. Error	Significance					
			After 1 hour	After 3 hours	After 6 hours	After 12 hours	After 18 hours	After 24 hours
Corrected Model	8		0.15	0.01	0.24	0.02	0.70	0.70
Intercept	1		0.03	0.00	0.00	0.00	0.00	0.00
Active Ingredient	2	5.02	0.10	0.00**	0.00**	0.00**	0.02**	0.03**
Concentration	2	5.02	0.34	0.42	0.94	0.71	0.30	0.40
Active Ingredient * Concentration	4	8.70	0.23	0.11	0.22	0.25	0.28	0.15

These findings indicate that the active ingredient of the insecticide plays a more crucial role in determining larval mortality compared to the concentration. This suggests that the mode of action of the active ingredient is more important than the concentration in controlling *Spodoptera exigua* populations (Hariri, 2012; Saputra Silaban & Indrawati, 2022). The lack of a significant effect of concentration on mortality may be attributed to the fact that the tested concentrations were within the recommended range for each insecticide. Higher concentrations might have resulted in increased mortality, but this was not investigated in this study.

The observation that the effect of the active ingredient became significant after 3 hours suggests a delayed response of *Spodoptera exigua* larvae to the insecticide treatments. This delayed response could be due to the time required for the insecticide to penetrate the larval cuticle and reach its target site. Further research is needed to investigate the specific mechanisms of action of the tested insecticides and their effects on *Spodoptera exigua* larvae (Table 4).

**Table 4.** Analysis of Duncan's Multiple Range Test of active ingredient on mortality of *Spodoptera exigua* larvae

Active Ingredient	N	Duncan's Multiple Range Test					
		After 1 hour	After 3 hours	After 6 hours	After 12 hours	After 18 hours	After 24 hours
Isosikloseram	12	0.00	0.00b	7.50b	14.17b	27.50b	35.00b
Chlorantraniliprole + Indoxacarb	12	2.50	5.00b	8.33b	15.00b	28.33b	34.17b
Chlorpyrifos	12	9.17	17.50a	25.83a	36.67a	44.17a	51.67a

A clear trend emerged in the mortality rates observed across the different active ingredients. Chlorpyrifos consistently exhibited the highest mortality rates, reaching 17.50% at 3 hours post-treatment and 51.67% at 24 hours post-treatment. In contrast, both isocycloseram and chlorantraniliprole + indoxacarb displayed significantly lower mortality rates compared to chlorpyrifos throughout the observation period. This consistent pattern suggests that chlorpyrifos possesses a more potent insecticidal effect against *Spodoptera exigua* larvae compared to the other tested active ingredients.

While mortality patterns were primarily influenced by the active ingredient, feeding inhibition exhibited a more complex response, being affected by both the active ingredient and concentration (Table 5). A significant difference in feeding inhibition among the active ingredients and concentrations emerged 18 hours after treatment application and continued until 24 hours post-treatment. Chlorantraniliprole + indoxacarb and chlorpyrifos consistently demonstrated the highest levels of feeding inhibition, while isocycloseram exhibited the lowest (Table 6). Notably, the 200% dose concentration consistently exhibited a greater effect on feeding inhibition compared to other concentrations (Table 7).

**Table 5.** Analysis of Generalized Linear Model of active ingredient and concentration on feeding inhibition of *Spodoptera exigua* larvae

Source	df	Std. Error	Significance					
			After 1 hour	After 3 hours	After 6 hours	After 12 hours	After 18 hours	After 24 hours
Corrected Model	8		0.38	0.45	0.45	0.24	0.19	0.01
Intercept	1		0.00	0.00	0.00	0.00	0.00	0.00
Active Ingredient	2	5.02	0.45	0.42	0.42	0.19	0.03**	0.13**
Concentration	2	5.02	0.19	0.19	0.19	0.20	0.04**	0.03**
Active Ingredient * Concentration	4	8.70	0.46	0.61	0.61	0.39	0.14	0.15

**Table 6.** Analysis of Duncan's Multiple Range Test of active ingredient on feeding inhibition of *Spodoptera exigua* larvae

Active Ingredient	N	Duncan's Multiple Range Test					
		After 1 hour	After 3 hours	After 6 hours	After 12 hours	After 18 hours	After 24 hours
Isosikloseram	12	98.17	96.58	94.25	88.83	80.83b	72.17b
Chlorantraniliprole + Indoxacarb	12	98.58	96.83	94.92	92.92	88.92a	81.92a
Chlorpyrifos	12	99.25	98.67	97.92	96.42	92.42a	90.17a

**Table 7.** Analysis of Duncan's Multiple Range Test of concentration on feeding inhibition of *Spodoptera exigua* larvae

Active Ingredient	N	Duncan's Multiple Range Test					
		After 1 hour	After 3 hours	After 6 hours	After 12 hours	After 18 hours	After 24 hours
50% recommended concentration	12	97.75	95.67	93.75	90.42	83.25b	76.33b
100% recommended concentration	12	99.00	97.58	95.75	90.83	85.58b	78.92b
200% recommended concentration	12	99.25	98.83	97.58	96.92	93.33a	89.00a

In contrast to the observed trends in mortality and feeding inhibition, damage level exhibited a distinct pattern, with both active ingredient and concentration significantly influencing damage levels starting 12 hours after treatment application (Table 8). Chlorpyrifos consistently demonstrated the lowest damage levels, with percentages of 3.58%, 7.58%, and 9.83% at 12, 18, and 24 hours post-treatment, respectively (Table 9). Interestingly, the 200% concentration also showed a consistently lower impact on damage levels, with percentages of 3.08%, 6.67%, and 11% at 12, 18, and 24 hours post-treatment, respectively (Table 10). The combination of chlorpyrifos with the 100% (recommended) concentration emerged as the most effective treatment, minimizing damage to the white mustard greens.

These findings suggest that while chlorpyrifos might be more effective in directly killing larvae and disrupting feeding behavior, it also has a mitigating effect on damage levels. The lower damage levels observed with the 200% concentration, despite its higher feeding inhibition, could be attributed to the rapid mortality of larvae at higher concentrations, limiting their ability to cause significant damage. The optimal combination of chlorpyrifos with the 100% concentration suggests that this specific treatment effectively balances control of *Spodoptera exigua* populations while minimizing damage to the host plant.

**Table 8.** Analysis of Generalized Linear Model of active ingredient and concentration on damage level *Spodoptera exigua* larvae

Source	df	Std. Error	Significance					
			After 1 hour	After 3 hours	After 6 hours	After 12 hours	After 18 hours	After 24 hours
Corrected Model	8		0.38	0.45	0.24	0.02	0.01	0.00
Intercept	1		0.00	0.00	0.00	0.00	0.00	0.00
Active Ingredient	2	5.02	0.45	0.42	0.19	0.03**	0.13**	0.00**
Concentration	2	5.02	0.19	0.19	0.20	0.04**	0.03**	0.02**
Active Ingredient * Concentration	4	8.70	0.46	0.61	0.39	0.14**	0.15**	0.03**

**Table 9.** Analysis of Duncan's Multiple Range Test of active ingredient on damage level

Active Ingredient	N	Duncan's Multiple Range Test					
		After 1 hour	After 3 hours	After 6 hours	After 12 hours	After 18 hours	After 24 hours
Chlorpyrifos	12	0.75	1.33	2.08	3.58b	7.58b	9.83b

Chlorantraniliprole + Indoxacarb	12	1.42	3.17	5.08	7.08ab	11.08b	18.08b
Isosikloseram	12	1.83	3.42	5.75	11.17a	19.17a	27.83a

**Table 10.** Analysis of Duncan's Multiple Range Test of concentration on damage level

Active Ingredient	N	Duncan's Multiple Range Test					
		After 1 hour	After 3 hours	After 6 hours	After 12 hours	After 18 hours	After 24 hours
200% recommended concentration	12	0.75	1.17	2.42	3.08b	6.67b	11.00b
100% recommended concentration	12	1.00	2.42	4.25	9.17a	14.42a	21.08a
50% recommended concentration	12	2.25	4.33	6.25	9.58a	16.75a	23.67a

The observed differences in mortality rates between the active ingredients could be attributed to several factors, including: 1. Mode of Action: Each active ingredient has a unique mode of action, targeting different physiological processes within the insect. Chlorpyrifos, a broad-spectrum organophosphate insecticide, acts by inhibiting acetylcholinesterase, an enzyme crucial for nerve impulse transmission. This disruption of nerve function can lead to paralysis and death in insects. The other active ingredients, isocycloseram and chlorantraniliprole + indoxacarb, may target different pathways, potentially explaining their lower mortality rates (Fernández et al., 2024; Pinto et al., 2024; Sammi et al., 2023); 2. Penetration and Distribution: The ability of an insecticide to penetrate the insect's cuticle and reach its target site can influence its effectiveness. Chlorpyrifos may possess better penetration properties compared to the other active ingredients, allowing it to reach its target site more readily and exert its insecticidal effect (Bolzan et al., 2019; Tarmidzi, 2018; Wei et al., 2019); and 3. Resistance: *Spodoptera exigua* populations may have developed resistance to certain insecticides. The observed lower mortality rates for isocycloseram and chlorantraniliprole + indoxacarb could be indicative of resistance, potentially contributing to their reduced effectiveness compared to chlorpyrifos (Aldini et al., 2021b, 2021a; Aziz et al., 2023; Ma et al., 2021; Shi et al., 2019).

Chlorpyrifos might be more effective in directly killing larvae, both chlorantraniliprole + indoxacarb and chlorpyrifos effectively disrupt feeding behavior, potentially leading to starvation and eventual mortality. The lower feeding inhibition observed with isocycloseram indicates a different mode of action, potentially affecting other physiological processes related to feeding behavior (Hariri, 2012). The higher feeding inhibition observed with the 200% dose concentration suggests a dose-dependent effect on feeding inhibition, implying that higher concentrations may disrupt feeding behavior more effectively, even if they don't necessarily lead to immediate mortality.

These findings highlight the importance of considering both mortality and feeding inhibition when assessing the effectiveness of insecticides. While chlorpyrifos demonstrates a strong direct effect on larval mortality, chlorantraniliprole + indoxacarb and chlorpyrifos exhibit a potent ability to disrupt feeding behavior, which could have significant consequences for larval development and survival. Further research is needed to investigate the specific mechanisms by which these active ingredients influence feeding behavior in *Spodoptera exigua* larvae. These results highlight the importance of considering multiple

factors, including mortality, feeding inhibition, and damage level, when evaluating the effectiveness of insecticides (Hariri, 2012). While some insecticides may excel in one area, such as mortality, they may have less desirable effects on other aspects, such as damage levels. The optimal insecticide treatment will depend on the specific goals of pest management, balancing the need for effective control with minimizing negative impacts on the host plant and the environment (Bird, 2017).

Finally, this study highlights the importance of selecting the appropriate insecticide active ingredient for controlling *Spodoptera exigua* populations. Chlorpyrifos demonstrated superior efficacy in this study, suggesting its potential for use in pest management strategies (Fernández et al., 2024). However, it is crucial to consider the potential for resistance development and the environmental impact of chlorpyrifos before implementing it as a primary control measure (Bolzan et al., 2019). Further research is needed to investigate the mechanisms of action of the tested insecticides and their effects on *Spodoptera exigua* larvae, particularly with respect to resistance development.

#### 4. Conclusion

The results of this study, analyzed using generalized linear models (GLM) and Duncan's multiple range test (DMRT), provide valuable insights into the effects of different insecticide active ingredients and concentrations on *Spodoptera exigua* larvae. The GLM analysis revealed a significant effect of insecticide active ingredient on larval mortality, with chlorpyrifos consistently demonstrating the highest mortality rates. Concentration did not significantly influence mortality, suggesting that increasing the concentration did not result in a significant increase in larval mortality. The DMRT confirmed chlorpyrifos as the most effective active ingredient in terms of mortality.

Both active ingredient and concentration significantly influenced feeding inhibition, with chlorantraniliprole + indoxacarb and chlorpyrifos exhibiting the highest levels of feeding inhibition. The 200% concentration consistently showed a greater effect on feeding inhibition compared to other concentrations. Chlorpyrifos, particularly at the 100% concentration, demonstrated the lowest damage levels to white mustard greens. The 200% concentration also exhibited a lower impact on damage levels, suggesting that higher concentrations may effectively control larval populations while minimizing damage to the host plant.

These findings highlight the importance of considering multiple factors, including mortality, feeding inhibition, and damage level, when evaluating the effectiveness of insecticides. While chlorpyrifos demonstrates a strong direct effect on larval mortality, chlorantraniliprole + indoxacarb and chlorpyrifos exhibit a potent ability to disrupt feeding behavior. Chlorpyrifos, particularly at the 100% concentration, emerges as the most effective treatment for controlling *Spodoptera exigua* populations while minimizing damage to the host plant. Further research is needed to investigate the specific mechanisms by which these active ingredients influence mortality, feeding behavior, and damage levels in *Spodoptera exigua* larvae. Additional studies should also explore the potential for resistance development to these insecticides and the long-term effects of these treatments on the host plant and the environment.

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