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Efficacy of Emprit Ginger Extract Containing Shogaol Compounds Armyworm (*Spodoptera Litura*) Larvae

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Abstract. *Spodoptera litura is a polyphagous pest that causes serious damage to various agricultural commodities. Control methods that still rely on synthetic pesticides negatively influence the environment and health, necessitating more environmentally friendly control alternatives. This study aims to determine the efficacy of ginger extract (Zingiber officinale var. amarum), which contains shogaol compounds, on the mortality of third instar S. litura larvae. The study was conducted experimentally using a one-factor completely randomized design (CRD) with six treatment levels: a positive control (fenite 150 OD), a negative control (Aquades), and ginger extract concentrations of 2%, 4%, 6%, and 8%, each with four replicates. Mortality tests were conducted using the topical application method and observed for 72 h. Data were analyzed using ANOVA, Tukey's test, and probit analysis to determine the LC_{50}, LC_{90}, LT_{50}, and LT_{90} values. The results showed that ginger extract treatment significantly influenced the mortality of S. litura larvae (Sig. = 0.032). The 8% concentration resulted in the highest mortality of 82.5% at 72 h and the highest efficacy value of 82.5%, approaching that of the positive control. The LT_{50} and LT_{90} values were 37.15 hours and 64.81 hours, respectively, while the LC_{50} and LC_{90} values were 0.815% and 23.134%. The extract treatment also caused morphological changes in the larvae, including changes in body color, shrinkage, and tissue damage. Emprit ginger extract containing 6.8% shogaol, particularly at an 8% extract concentration, has the potential to serve as an effective environmentally friendly biopesticide in controlling S. litura.*

Keywords: *biopesticide; ginger; topical application; shogaol; Spodoptera litura.*

Type of the Paper: Regular Article.

1. Introduction

The armyworm (*Spodoptera litura*) is a pest with various host plants (polyphagous), including food crops, horticultural crops, and even non-cultivated plants. Armyworm attacks are characterized by leaves that are slightly white and have holes, which disrupt the photosynthesis process. Severe infestation can cause up to 90% damage to plants if control measures are not implemented immediately [1]. The continuous use of synthetic pesticides to control *S. litura* has various negative impacts, such as pest resistance, secondary pest outbreaks, environmental pollution, and the accumulation of chemical residues that are harmful to human health and non-target organisms [2,3].

Biopesticides containing plant-based active ingredients offer an environmentally friendly and sustainable alternative for controlling *S. litura*. They work specifically against target pests,

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minimizing harm to natural enemies, have easily degradable residues, and supporting SDGs (Sustainable Development Goals) 2, 3 and 12 [4,5,6]. Ginger extract has been proven effective in controlling pests such as *Spodoptera litura F., Plutella xylostella, Blatella germanium, Aedes* spp., and brown planthoppers [7,8]. Emprit ginger extract contains various bioactive compounds such as flavonoids, alkaloids, terpenoids, and phenols that effectively controls pest populations by disrupting pest metabolism and triggering rapid death [9,10]. The research by Dwiyanti & Salbiah shows that the application of ginger extract on *S. exigua*, which is in the same genus as *S litura*, at a concentration of 60 mL/L water is effective in controlling *S. exigua* with an initial time of death of 7 hours, a Lethal Time 50 (LT50) of 40.25 hours, and a mortality rate of 82.50% [11].

Shogaol is one of the major bioactive compounds in ginger and is structurally related to gingerol. While previous studies have primarily evaluated the insecticidal activity of crude ginger extracts, the contribution of individual bioactive compounds, particularly shogaol, remains unclear. Shogaol is a monomethoxybenzene belonging to phenols and enones [12]. Keosaeng reported that the compound 8-shogaol is the most active component against *S. frugiperda* with LC_{50} values of 7.68 and 3.96 µg/larva at 24 and 48 hours after treatment [13]. Although ginger extract has been reported to be effective against several insect pests, including *Spodoptera* species, most previous studies have focused on crude ginger extracts rather than specific bioactive compounds such as shogaol. Moreover, while the insecticidal activity of shogaol has been demonstrated against *S. frugiperda*, evidence of its efficacy against *S. litura* remains limited. Therefore, it remains unclear whether shogaol can effectively increase the mortality of *S. litura*, highlighting the need for further investigation. Therefore, this study aims to evaluate the efficacy of shogaol derived from ginger in increasing the mortality of armyworm (*Spodoptera litura*).

2. Materials and Methods

2.1. Collection and Propagation of Spodoptera litura

Spodoptera litura larvae were collected from pest-infested vegetable crops in Kopeng Village, Getasan Subdistrict, and Bandungan Subdistrict, Semarang Regency, with morphological characteristics of lateral black spots on each abdomen and body colors varying from pale green to dark green and brown, depending on larval development [14]. The larvae obtained were kept in jars containing cabbage leaf pieces as food and covered with gauze. Rearing was conducted in an environmental chamber maintained at 25 ± 2°C, a relative humidity of 60–65% RH, and a photoperiod of 14:10 hours (Light:Dark). The larvae were fed fresh cabbage leaves until they reached instar 5 and reared to pupal stage. The formed imagoes were then transferred to a maintenance container and fed a 10% honey solution. Moths were allowed to lay eggs on cabbage leaves, after which the eggs were transferred to jars lined with tissue paper and fed fresh cabbage leaves. The larvae that hatched after 3 life cycles were reared until they reached instar 3 before being used as samples [15,16].

The collection of *Spodoptera litura* in agricultural fields was initiated by identifying the symptoms of attack and the morphology of *S. litura* larvae. Fig. 1 shows the symptoms of *S. litura* larval attack on cabbage plants, characterized by numerous holes in old leaves and the presence of green larvae with black longitudinal stripes and black spots on their bodies. According to Khamid and Siriyah [17], *S. litura* infestation is characterized by holes in cabbage leaves because the larvae eat the leaf tissue, leaving only the epidermis and leaf veins. *S. litura* larvae are generally characterized by lateral black dots on each abdomen segment and body color ranging from green to greenish brown, depending on the larval stage and food source [15].

Fig. 1. Cabbage plants infested with *Spodoptera litura* larvae in a cultivated field. Scale bar = 1 cm.

2.2. Extraction of Emprit Ginger

The ginger rhizomes were cleaned, cut into small pieces, and dried in an oven at 70 °C. The moisture content of the simplisia was measured using a moisture analyzer. The dried simplisia were then ground using a grinder until it became a fine powder. Extraction was conducted using the maceration method with 96% ethanol as the solvent. A total of 100 g of ginger sample was macerated with 500 mL of 96% ethanol for 24 hours at room temperature. After maceration, the sample was filtered to separate the residue (pulp) from the filtrate (macerate). The resulting filtrate, referred to as crude extract, was concentrated using a rotary evaporator. The concentrated extract was then dried with a nitrogen gas flow to obtain a dry extract [18]. The gingerol and shogaol compound content were validated using HPLC analysis to obtain ginger extract suitable for efficacy testing [13].

High-performance liquid chromatography (HPLC) analysis was performed using a Vertex Europher 100-5 C18 reversed-phase column (150 × 4.6 mm). Separation was conducted under isocratic conditions using a mobile phase consisting of acetonitrile and 0.1 M sodium acetate (3:97, v/v). The flow rate was maintained at 1.0 mL/minute with an injection volume of 20 µL. The analysis was conducted at ambient temperature, and analytes were detected using a UV detector set at 254 nm. The operating pressure was maintained at approximately 8.4 MPa. Before analysis, all sample solutions were filtered through a 0.45 μm nylon membrane filter and transferred into HPLC vials. A 6-shogaol standard solution was prepared in methanol to obtain a stock solution of 1000 mg/L and subsequently diluted to the required working concentrations. Calibration was performed using standard solutions at different concentrations to construct a calibration curve based on peak area versus concentration. Compounds were identified by comparing the retention time of sample peaks with that of the 6-shogaol standard, and quantification was performed using the corresponding calibration curve.

2.3. Analytical methods

i. Mortality Test

Mortality testing was conducted using the topical application method by applying 1 μL of each test solution to the ventral thorax of third-instar larvae with a micropipette [19]. The mortality test consisted of six treatments: a 0% treatment (distilled water) as the negative control; Fenite 150 OD (Produce by PT Petrokimia Kayaku), containing emamectin benzoate (75 g/L) and lufenuron (75 g/L), as a positive control; and ginger extract containing shogaol at concentrations of 2%, 4%, 6%, and 8%, each dissolved with pure distilled water. Each treatment was tested on 10 *Spodoptera litura* instar 3 larvae with 4 replicates (groups) per concentration. Mortality was assessed every 24 hours for 3 days. *Spodoptera litura* larvae were considered dead when they exhibited decreased movement, decreased feeding activity, darkening of the body, and fluid discharge from the abdomen [20]. The mortality rate was calculated using the Abbott formula [21] and expressed as a percentage (%), as shown in Eq. (1).

$$M = \frac{a}{b} \times 100\% \qquad (1)$$

Description

M : Mortality percentage (%)

a : Number of *Spodoptera litura* larvae that died

b : Number of *Spodoptera litura* larvae used

The mortality data were then analyzed using probit to determine LT50, LT90, LC50, and LC90. LC50 (Lethal concentration) is the concentration of biopesticide required to kill 50% of the test organism population under specified experimental conditions and is measured in ppm [22]. The LT50 (Lethal time) was determined to identify the time required to kill 50% of the test animals from the application of biopesticides [23]. The probit analysis is calculated using Eq. (2).

$$\mathrm{P} = \mathrm{a}\ \mathrm{Log}\ \mathrm{C} + \mathrm{B} \qquad (2)$$

Description

P : Probit value at 50% mortality

a : Slope
B : Intercept

ii. Morphological Changes

Morphological changes in the shape and color of the *S. litura* larvae were observed concurrently with mortality data collection. Observations were conducted every 24 h for 3 days by photographing and recording the morphological changes in dead larvae. The observed morphological changes were then described qualitatively.

iii. Efficacy Test

Efficacy tests were conducted along with mortality tests on third instar *Spodoptera litura* larvae. The efficacy value was calculated based on the number of larvae that died after 3 days of application in each treatment and control and expressed as a percentage (%).A pesticide is considered effective if it achieves an efficacy value greater than 50% [24,25].

The efficacy test was calculated using Abbott's equation [21], as shown in Eq. (3).

$$\text{Efficacy} = 1 - \left(\frac{Ca}{Ta} \times \frac{Tb}{Cb}\right) \times 100\% \quad (3)$$

Description
T_a : Number of larvae in the treatment that are still alive after application
T_β : Number of larvae in the treatment that are alive before application
C_a : Number of larvae in the control that are still alive after application
C_β : Number of larvae in the control that are alive before application.

2.4. Statistical Analysis

Prior to statistical analysis, data normality was assessed using the Kolmogorov-Smirnov test. Homogeneity of variances was evaluated using Levene's test. The data were analyzed using ANOVA to determine significant differences in the mean differences between two sources of variation: the treatment group and the *Spodoptera litura* larval mortality group. When a significant difference was detected, a Tukey's post hoc test (BNJ) was used to compare all pairs of treatment means. Data were analyzed using SPSS software with 95% confidence level.

3. Results and Discussion

3.1. HPLC Shogaol Compound Ginger Extract Emprit

The chromatogram results (Fig. 2.) obtained from HPLC using 6-shogaol standards showed shogaol extract concentrations of 6.8% (6.815 μg/mL). Chromatographic analysis identified shogaol as the most dominant active compound at the third peak, exhibiting a retention time of 4.817 minutes and a peak area of 5,610,161. This high peak area value indicates that shogaol is the primary component, possessing the highest relative abundance than the other active compounds within the extract. Srikandi et al. [26] reported that red ginger extract prepared using hexane as the solvent contained 4.71% 6-shogaol. This shows that the solvent and ginger used produced different shogaol concentrations from the extraction performed. Firdausni and Kamsina [27] reported that ginger has higher shogaol compounds than red ginger. The 96% ethanol solvent used in this study produced more shogaol compounds than the hexane solvent used in previous studies. Ethanol can extract shogaol compounds optimally with a concentration adjusted to the solvent percentage [28].

Shogaol is a phenolic compound resulting from the dehydration of gingerol during the drying or heating process of ginger rhizomes. The greater stability of shogaol in dry conditions compared to gingerol increases the proportion of shogaol compounds in the extract produced due to the simplisia undergoing heating during drying at 60–80 ºC [18]. The use of a temperature of 70 ºC in this study was optimal for producing ginger extract with a higher shogaol content [29]. In ginger *(Zingiber officinale* var. amarum), the relatively high shogaol content is influenced by post-harvest conditions, drying temperature, and the extraction method used [30-32]. Furthermore, the use of ethanol as a polar solvent enhances solvent penetration into the ginger matrix, facilitating the dissolution and efficient isolation of shogaol.

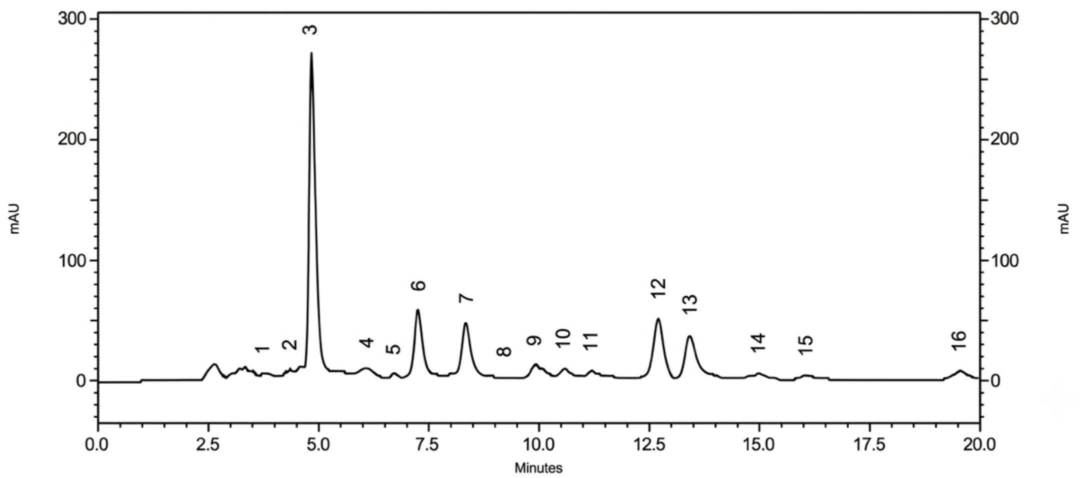

Fig. 2. HPLC chromatogram of ginger extract at a dilution factor of 50×: shogaol (peak 3, rt = 4.833 minutes, area = 3,113,828 mAU·minutes). Analysis was performed at λ = 254 nm.

Table 1. Results of one-way ANOVA test on mortality data of third instar *Spodoptera litura* larvae treated with ginger extract containing shogaol.

Mortalitas	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7.879.167	5	1.575.833	3.596	0.032
Within Groups	5.258.333	12	438.194		
Total	13.137.500	17			

3.2. ANOVA Test

The ANOVA test (Table 1) showed that the treatment significantly influenced the mortality of Spodoptera litura instar 3 larvae (Sig. = 0.032). This effect is attributed to the shogaol content of the ginger extract, which disrupts insect metabolism, including chitin synthesis, respiratory system, central nervous system, sexual communication, larval shrinkage, and mortality or insect death rate [7,33].

3.3. Mortality

The administration of ginger extract containing shogaol significantly increases larval mortality with increasing observation time and concentration (Table 2). At 24 hours, the highest mortality rate was observed at concentrations of 6% and 8% at 32.5%, while the lowest mortality (27.5%) occurred at the 2% and 4% concentrations. At 48 hours, mortality continued to increase, reaching 57.5% at the 8% concentration, and the lowest mortality (45%) was recorded at the 4% concentration. The peak was at 72 hours, where all treatments showed maximum effectiveness, with the 8% concentration giving the highest mortality (82.5%), closely approaching the positive control (90%). Conversely, the lowest mortality at 72 hours was found at a concentration of 2% (65%). This indicates that the shogaol compound in ginger exhibits toxic activity and requires sufficient contact time to paralyze the biological system of the larvae. Rustam et al. [34] reported a mortality rate of 84% for *S. litura* using a 1% ginger extract without validating its shogaol content. In the present study, the ginger extract was confirmed to contain shogaol, and the highest mortality was achieved at an 8% concentration, producing a mortality rate comparable to that reported in the previous studies.

Table 2. Mortality of third instar *Spodoptera litura* larvae treated with ginger extract containing shogaol at 24, 48, and 72 h.

Treatment	Mortality %		
	24 h	48 h	72 h
Positive Control	$37.5^{b} \pm 12.58$	$62.5^{c} \pm 9.57$	$90^{d} \pm 8.16$
Negative Control	$0^{a} \pm 0$	$0^{a} \pm 0$	$0^{a} \pm 0$
2%	$27.5^{b} \pm 15$	$47.5^{b} \pm 10$	$65^{b} \pm 10$
4%	$27.5^{b} \pm 17.08$	$45^{b} \pm 10$	$70^{bc} \pm 8.16$
6%	$32.5^{b} \pm 15$	$55^{bc} \pm 10$	$77.5^{cd} \pm 9.57$
8%	$32.5^{b} \pm 5$	$57.5^{bc} \pm 9.57$	$82.5^{d} \pm 5$

Note: Data are presented as mean ± SD. Numbers in the same column and followed by the same letter are not significantly different according to Tukey's test at a 95% confidence level.

The shogaol compound in ginger extract exhibits insecticidal properties through its larvicidal effects on various insects, disrupting the respiratory and digestive systems of insect pests, causing mortality in pests [2,35]. Subahar et al. [35] reported that shogaol extracted from ginger inhibits the growth of lice (*Pediculus humanus capitis* De Geer) by reducing the activity of the enzymes AChE and GST. Shogaol inhibits AChE activity, causing the accumulation of acetylcholine in the synapses. This accumulation triggers excessive stimulation of nerve cells, causing convulsions, paralysis, and ultimately death of the larvae [36]. Salim [37] reported that significant mortality of *Spodoptera litura* larvae was observed after the AChE genes (i.e., Ace1 and Ace2) in these pests were silenced. Shogaol can reduce GST activity, which affects the pest's ability to detoxify foreign compounds/toxins, causing toxic compounds to accumulate in the body and cause systemic cell damage [38]. The enzyme glutathione S-transferase (GST) plays an important role in metabolizing or neutralizing insecticide compounds before they reach their physiological targets. Increased GST enzyme activity is a response to exposure to various insecticides, serving as the main defense system of insects against the toxicity of these compounds. Inhibition of SlGSTe11 gene expression significantly increases the mortality of *Spodoptera litura* [39].

3.4. Morphological changes

The mortality of *Spodoptera litura* larvae was characterized by morphological changes in the larvae's body condition and color. Treatment with ginger extract containing shogaol on third instar *Spodoptera litura* larvae (Fig. 3) caused noticeable morphological changes compared to the control larvae. These changes included a body color change from creamy white to brown, indicating disruption of internal tissues and physiological processes. The caput becomes blackened due to tissue damage or a toxic reaction of the active compound to the nervous system and cuticle. In addition, the larvae's body shape shrank and appeared wrinkled, followed by a lysed and mushy body condition, indicating cell damage and tissue structure degradation. Permatasari and Asri [40] reported that the body colour of *S. litura* changes from greenish-yellow to blackish-brown and is accompanied by body stiffening, drying, and shrinkage. Similarly, Rustam et al. [34] reported that the active compounds in ginger act as a contact poison, disrupting plasma membrane permeability and causing lysis in *S. litura*.

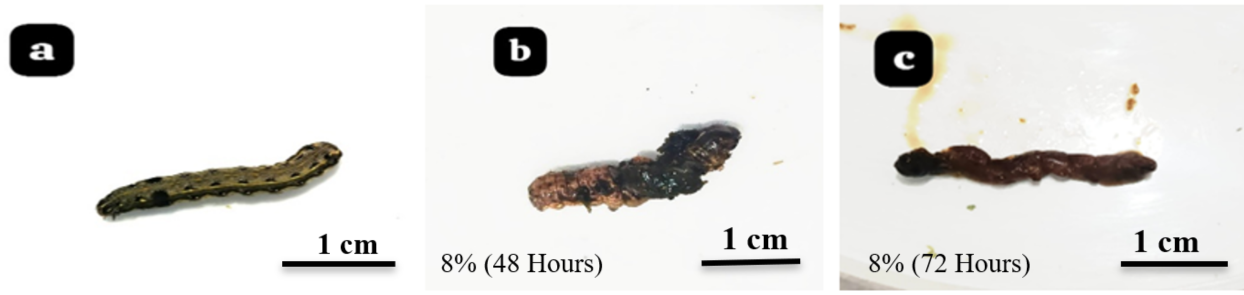

Fig. 3. Morphological changes in third instar *Spodoptera litura* larvae after treatment with ginger extract containing shogaol (a: before treatment, b and c: after treatment).

The chemical structure of shogaol, which contains an α, β-unsaturated ketone group, makes it highly reactive to biological molecules in insects [37]. This group allows shogaol to interact with important proteins and enzymes, disrupting vital functions such as the nervous system, respiration, or metabolism of insects. This effect is often mediated through oxidative stress or inhibition of key enzymes, ultimately leading to insect death and morphological changes. In addition, the lipophilic nature of shogaol facilitates its penetration through the insect cuticle, enabling it to act quickly as a contact poison without requiring ingestion [41]. Although the topical application method used in this study requires a higher concentration of ginger extract to achieve a mortality rate similar to that of the spray method, the active compounds only enter through the insect cuticle without involving the digestive tract. The hydrophobic nature of the cuticle limits the penetration of compounds, resulting in lower toxic effectiveness than the spray method. However, the topical application method has advantages in terms of dose accuracy, exposure homogeneity, and its ability to evaluate specific contact toxicity [19,42].

3.5. Lethal concentration (LC) dan lethal time (LT) 50/90

Table 3. Lethal concentration of ginger extract containing shogaol against third instar larvae of *Spodoptera litura.*

LC	Concentration	Minimum (%)	Maximum (%)
50%	0.815	0.031	1.658
90%	23.134	11.14	681.92

Table 4. Lethal time of ginger extract containing shogaol against third instar larvae of *Spodoptera litura.*

LT	Time (Hour)	Minimum (Hour)	Maximum (Hour)
50%	37.148	31.930	41.705
90%	64.810	55.931	83.649

The LC50 and LC90 values (Table 3) show that ginger extract containing 0.815% shogaol could eliminate 50% of the test larvae. Meanwhile, to achieve 90% mortality, the required dose increased significantly to 23.134%. Ikawati [43] reported that ginger extract tested on *Spodoptera litura* had an LC50 value of 29%. Compared with the previous study, the ginger extract confirmed to contain shogaol in the present study exhibited a much lower LC_{50} value, indicating greater toxicity against *S. litura* larvae. This is because the higher shogaol compound has a much higher and more concentrated active compound concentration, while ginger extract contains a more complex and diluted mixture of compounds. The higher shogaol concentration enhances the toxic effect on the target insect [44]. The LT50 and LT90 values (Table 4) show that ginger extract containing shogaol against *Spodoptera litura* instar 3 larvae take about 37 hours to eliminate half of the larval population and 65 hours to eliminate 90% of the population. Research by Rustam et al. [34], using ginger extract without confirmed shogaol content showed an LT50 of 33 hours for *S. litura*. In this study, ginger extract confirmed to contain shogaol had a higher LT50 value.

3.6. Efficacy

Treatment with ginger extract containing shogaol showed that an increase in concentration was directly proportional to the mortality rate of *Spodoptera litura* larvae. The 8% test concentration provided the highest efficacy value of 82.5%, closely approaching the positive control (90%) (Table 5). This was followed by a concentration of 6% with an efficacy of 77.5%, the concentration of 4% with 70%, and the lowest efficacy was at a concentration of 2% with 65%. The effectiveness of botanical insecticides is determined based on the efficacy value obtained. If the efficacy value is ≥ 50%, the insecticide is declared effective against the target pest. Conversely, if the efficacy value is ≤ 50%, the insecticide is considered ineffective. Moreover, higher efficacy values reflect greater effectiveness in pest control [45,46]. This indicates that all test concentrations were effective because they have an efficacy value ≥ 50%, with the 8% test concentration being the most effective of the several test concentrations used.

Table 5. Efficacy of ginger extract containing shogaol.

Treatment	Efficacy % (Mean ± SD)
Positive Control	$90^{d} \pm 8.16$
Negative Control	$0^{a} \pm 0$
2%	$65^{b} \pm 10$
4%	$70^{bc} \pm 8.16$
6%	$77.5^{cd} \pm 9.57$
8%	$82.5^{d} \pm 5$

Note: Numbers followed by the same letter are not significantly different according to Tukey's test at a 95% confidence level.

4. Conclusions

The application of ginger extract containing shogaol at concentrations of 2%, 4%, 6%, and 8% under laboratory conditions demonstrated effective biopesticidal activity against third-instar *Spodoptera litura* larvae. The highest efficacy value was achieved at a test concentration of 8%, with an efficacy value of 82.5%. Further research should use more appropriate methods to obtain high concentrations of shogaol, direct application in the field, and the use of lower test concentrations.

Abbreviations

ANOVA Analysis of Variation

Data Availability Statement

Data will be shared upon request by the readers.

CRediT Authorship Contribution Statement

Yabez: Data collection, data analysis, data interpretation, and article writing. **Ruth Meike Jayanti**: Conception, design, and article writing. **Maria Marina Herawati**: Data analysis and article writing.

Declaration of Competing Interest

The authors of this manuscript declare no conflict of interest or competing interest.

Declaration of Use of AI in the Writing Process

Nothing to disclose.

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