Journal of Biotechnology and Natural Science
Vol. 6, No. 1, June 2026, pp. 01-08
ISSN 2808-2508

Effects of *Metarhizium anisopliae* and Red Ginger Extract (*Zingiber officinale var.rubrum*) on Feeding Inhibition and Mortality of *Spodoptera litura* Larvae

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ARTICLE INFO

Article history
Received: February 3th 2026
Revised: March 3th 2026
Accepted: April 6th 2026

Keywords
Feeding inhibition
Metarhizium anisopliae
Mortality
Red ginger

ABSTRACT

The management of *Spodoptera litura* as the main pest of vegetable crops is largely dependent on synthetic pesticides. Excessive use raises concerns about environmental safety and human health. The purpose of this study was to test the effectiveness of red ginger extract (*Zingiber officinale var rubrum*) and the entomopathogenic fungus *Metarhizium anisopliae* propagated in corn media against feeding inhibition and mortality for *S. litura* larvae. The test procedure is carried out in the laboratory using the leaf dip method. Feeding inhibition activities were assessed using choice and non-choice tests. The results showed that red ginger extract caused 48% of larval mortality at a concentration of 2 ml/50 ml of water, while *M. anisopliae* extract caused 28% of larval mortality at a conidia density of 2.45 x 10^4 conidia/ml. Meanwhile, the results of the feeding inhibition test on red ginger extract showed a weak category of 10.20% at a concentration of 2 ml/50 ml while *M. anisopliae* extract did not show any effect of inhibition on the feed activity of *S. litura* larvae.

How to Cite: Mendes, J.A., Kusumah, R., Sembiring, J., Resubun, M., & Kurniawan, A. (2026). Effects of *Metarhizium anisopliae* and Red Ginger Extract (*Zingiber officinale var.rubrum*) on Feeding Inhibition and Mortality of *Spodoptera litura* Larvae. *Journal of Biotechnology and Natural Science*, 6(1):01-08.

1. Introduction

Spodoptera litura is one of the main pests of several agricultural crops in Indonesia, such as vegetables, ornamental plants, and food crops. Larval infestation begins in the early stages of the instar and continues until the final instar larvae usually feed on the leaf surface, leaving the leaf epidermis. The fast life cycle, and aggressive feeding behavior of *S. litura* contribute to its status as a primary pest (Ramadhan et al., 2016)

Pest control of *S. litura* relies heavily on the use of synthetic insecticides because the effects are quickly noticeable. However, excessive use excessively high and untargeted application frequency can result in environmental pollution, risks to human health, and negative impacts on non-target organisms, one of which is a natural enemy. These concerns are the driving factors for determining other control strategies that are environmentally friendly, effective in suppressing pest populations while maintaining ecological balance, as emphasized in the framework of Integrated Pest Management (IPM) (Indiati, 2017) The alternative is to utilize biological control agents, especially entomopathogenic fungi, namely *Metharizium anisopliae* and botanical insecticides from red ginger extract.

Metarhizium anisopliae is an entomopathogenic fungus that is known to infect a variety of insect hosts and cause significant mortality in a variety of agricultural pests (Uge *et al*., 2021). Some studies on red ginger extract (*Zingiber officinale var.rubrum*) explain that the extract contains bioactive compounds such as gingerol (Sholikhati *et al*., 2023), flavonoids, alkaloids, and saponins (Putri *et*

al., 2022) that exhibit antifeedant, repellent, and toxic effects on insects and are particularly relevant in influencing eating behavior (Asfi *et al.*, 2015;Qatrinida *et al*., 2021). The integration of entomopathogenic fungi and botanical extracts in this study is expected to provide information that their activity can weaken larvae and reduce feeding activity, thereby increasing susceptibility to fungal infections, while entomopathogenic fungi provide a sustained lethality effect. Therefore, this study aims to evaluate the effects of *M. anisopliae* and red ginger extract against mortality and feeding inhibition for *S. litura* larvae in the laboratory.

2. Methods

This research was conducted at the agrotechnology laboratory, faculty of agriculture, Musamus University, from July to December 2025. The experiment consisted of four treatments plus controls with five replicates. The materials used are larvae *of S. litura*, Dextrose Agar, Potato, Alcohol, *M. anisopliae* isolate, Corn, Tween 80 and water. The tools used are Vortex, Micropipette, hemocytometer, cover glass, cotton, heat-resistant plastic, glass bottle, aluminum foil, petri dish, headcounter, steamer, microscope, and scale.

2.1. Test insect propagation

S. litura larvae are collected from agricultural land and then raised in laboratories. Larvae that enter the pupa stadia are given sawdust media so that pupae are formed. The image that appeared was fed with a 10% honey solution. The image of the female laying eggs is then collected and placed in a plastic container with a gauze window. The larvae of instar 1 that appear is fed in the form of pakcoy leaves. This process is carried out continuously until the population of larvae suitable for treatment is obtained.

2.2. Extraction red ginger

Red ginger extract was prepared by distillation process using water as the solvent. A total of 30 kg of red ginger rhizomes is washed and thinly sliced. Furthermore, the distillation process is carried out for 4-5 hours to obtain the extraction results.

2.3. Propagation of *M. anisopliae* isolate in PDA media and corn media

The isolation *of M. anisopliae* using Potato Dextrose Agar (PDA) media with a composition consisting of 200 grams of potatoes, 20 grams of sugar, 20 grams of gelatin and 1000 ml of distilled water. The working procedure is that potatoes are cut into small cubes and boiled for 15 minutes then filtered to obtain the extract. The media is sterilized in an autoclave at 121°C for 15 minutes. The sterile medium was then poured into a sterile petri dish and isolated part of the *structure of M. anisopliae* then the PDA medium was isolated for 7-21 days (Figure 1A).

The propagation of isolates in corn is carried out by a procedure; the corn is cleaned and then dried using a sieve for 20 minutes. Next, the corn is steamed for 30 minutes. A total of 100 grams of corn is put in glass bottles and heat-resistant plastic. Next, the media is sterilized using an autoclave for 15 minutes. The transfer of the isolates is carried out by taking the mycelium *M. anisopliae* using an Ose needle. The isolation was incubated for 7-21 days (Figure 1B).

Figure 1. (A) *M. anisopliae* Isolated was cultured on PDA; (B) Isolated propagation using corn after incubating in 21 days.

2.4. Suspension and conidia density test

20 grams of *M. anisopliae* conidia corn media dissolved in 50 ml of water + 0.1% Tween 80 then whipped using a vortex for 1 minute. Dilution is carried out three times by transferring 25 ml of solution into 25 ml of water until the desired concentration is obtained, while the control treatment uses water. Conidia density was calculated using a hemocytometer where 1 ml of suspension was dripped on a hemocytometer and observed using a microscope. Calculation of spore density using the formula:

$$\text{Spore density (SD)} = N \times F \times 10^4 \quad (1)$$

Description:
N = Average number of spores
F = Dilution factor
10^4 = Conversion factor

2.5. Feeding inhibition test.

The feeding inhibition test used choice and non-choice methods. The leaf pieces are weighed and then dipped in the test solution of each concentration. In the method of choice, the treatment leaves and control leaves are placed in the same petri dish while in the method without choice, the treatment leaves and control leaves are placed in different petri dishes. Ten test larvae were used and left to feed for 24 hours. After 24 hours, the remaining leaves are oven-dried at 105°C for 2 hours and weigh the final weight. The percentage of feeding inhibition was calculated using formula in Mendes *et al.*, (2017):

$$\text{Feeding inhibition (FI) (choice) (\%)} = \frac{C - T}{C + T} \times 100 \quad (2)$$

$$\text{Feeding inhibition (FI) (no choice) (\%)} = \frac{C - T}{C} \times 100 \quad (3)$$

The value of feeding inhibition that has been calculated will be determined by the feeding inhibition criteria, where the FI value of ≥ 80 is strong; $61 \leq x < 80$ including the moderate; $40 \leq x < 60$ weak; < 40 is very weak.

2.6. Mortality test

Leaf pieces measuring 3 x 3 cm are dipped in suspense and control leaf pieces are dipped in a control solution. Next, the treatment leaves are dried for a few minutes until the surface moisture is reduced. Ten larvae were then placed in each petri dish lined with tissue paper as its base and two pieces of treatment leaves. Each treatment is repeated five times. Mortality observation was carried out after 24 JSP to 72 hours after treatment. The percentage of deaths is calculated using the formula:

$$M\ (\%) = n/N \times 100 \quad (4)$$

Description:
M = Mortality
n = Number of test insects killed
N = total test insects used

2.7. Data analysis

Analysis mortality larvae using polo PC program for LC_{50} and LC_{90}.

3. Results and Discussion

This study evaluated two control agents with different characteristics. Red ginger can trigger behavioral disorders through antifeedant effects and metabolic toxicity that are faster but do not always cause the death of test larvae. Meanwhile, *M. anisopliae* works through an infectious process that involves cuticle penetration, internal colonization, and toxin production so that death can occur before the appearance of internal mycosis symptoms.

3.1. Feeding inhibition

3.1.1. Red ginger extract

The results test on choice method showed that red ginger extract was able to inhibit the feeding activity of larvae at a concentration of 2 ml/50 ml of water with a feeding inhibition percentage of 10.2% and was categorized as weak. Meanwhile, other concentrations showed very weak eating inhibition activity (Table 1). Inhibition of feeding activity of *S. litura* larvae in non-selective methods, showed that there was no inhibitory effect of feeding and was categorized as very weak at all concentrations (Table 2).

Table 1. Feeding inhibition test of red ginger extract (*Zingiber officinale var. rubrum*) against *S.litura* using choice test

Concentration	FI (%)	Criteria
A	-60.00	very weak
B	-9.35	very weak
C	-24.68	very weak
D	10.20	weak

footnote: FI = Feeding Inhibition; A=0.25 ml/50 ml of Water; b=0.50 ml/50 ml of Water; C= 1 ml/50 ml of water; D=2 ml/50 ml of water

Table 2. Feeding inhibition test of red ginger extract (*Zingiber officinale var. rubrum*) against *S.litura* using no-choice test.

Concentration	FI (%)	Criteria
A	-24.00	very weak
B	-51.90	very weak
C	-54.70	very weak
D	-47.50	very weak

footnote: FI = Feeding Inhibition, A=0.25 ml/50 ml of Water; b=0.50 ml/50 ml of Water; C= 1 ml/50 ml of water; D=2 ml/50 ml of water

3.1.2. *M. anisopliae* extract

The results of the feeding inhibition test showed that *M. anisopliae* extract was very weak in inhibiting larval feeding at all concentrations (Tables 3 and 4).

Table 3. Feeding inhibition test of *M.anisopliae* against *S.litura* using choice method

Concentration	FI (%)	Criteria
A	-9.80	very weak
B	-12.94	very weak
C	-28.83	very weak
D	-50.00	very weak

footnote: FI = Feeding Inhibition; A=$2.45x10^4$, B=$1.23x10^4$, C=$6.13x10^3$, D=$3.06x10^3$

Table 4. Feeding inhibition test of *M.anisopliae* against *S.litura* using no-choice method

Concentration	FI (%)	Criteria
A	-52.50	very weak
B	-45.45	very weak
C	-28.13	very weak
D	-93.55	very weak

footnote: FI = Feeding Inhibition; A=$2.45x10^4$, B=$1.23x10^4$, C=$6.13x10^3$, D=$3.06x10^3$

The relatively low feeding inhibition value in the treatment of red ginger extract (10.20%) indicates that the bioactive compounds contained in it are likely to only cause changes in the taste or aroma of the leaves without providing a strong repellent effect. These changes are thought to be tolerated by larvae so that they do not have a significant feeding inhibition effect. In contrast, treatment of *M. anisopliae* did not show an inhibitory effect on eating. This is explained by the mechanism of action of fungi that indirectly affects eating preferences but works through the infection process after the conidia attaches and penetrates the larval cuticle. Thus, these differences in responses reflect fundamental differences in the mechanism of action between antifeedant agents and entomopathogenic agents.

3.2. Mortality

3.2.1. Red ginger extract

The results showed a relationship between increased extract concentration and larval mortality percentage. The highest mortality (48%) was recorded at a concentration of 2 ml/50 ml of water on day 8. Meanwhile, the lowest mortality (8%) was observed at a concentration of 0.25 ml/50 ml of water (Fig.2).

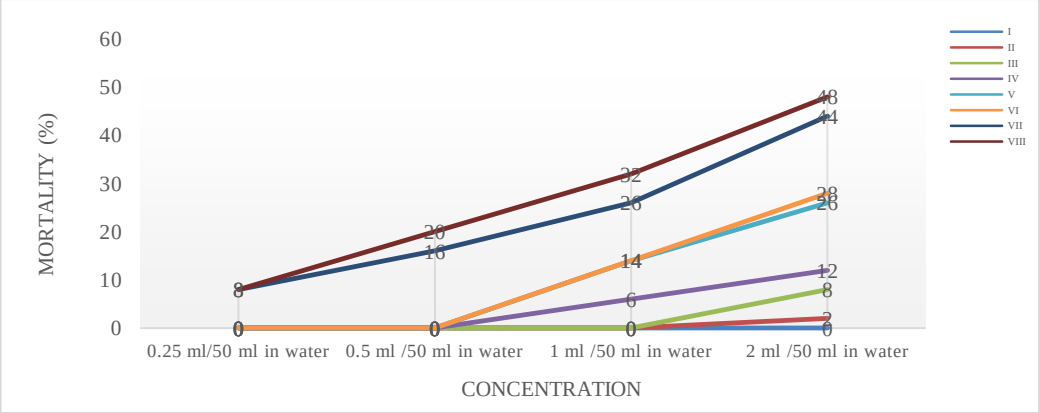

Figure 2. Larval mortality (%) of *S. litura* treated with red ginger extract (*Zingiber officinale var rubrum*) at different observation times.

The toxic effects of red ginger extract work slowly which is likely related to the presence of bioactive compounds such as gingerol (Sholikhati *et al*., 2023), flavonoids, alkaloids, and saponins (Putri *et al*., 2022). These compounds exhibit antifeedant, repellent, and toxic activity against insects and mainly affect feeding behavior (Asfi *et al.*, 2015), (Qatrinida *et al*., 2021). As a result, larval mortality was low during the observation period at baseline but gradually increased due to the cumulative effects of repeated exposure. This increase in mortality may be due to the accumulation of chemical compounds that interfere with the digestive system and the metabolic processes of larvae. Due to this physiological disorder, it causes the death of larvae and changes in body color to brownish-yellow, dullness, and body stiffness shown in Figure 3. These changes occur after the consumption of treatment leaves, so it is suspected that these changes are caused by tissue damage and physiological disorders that cause larval death.

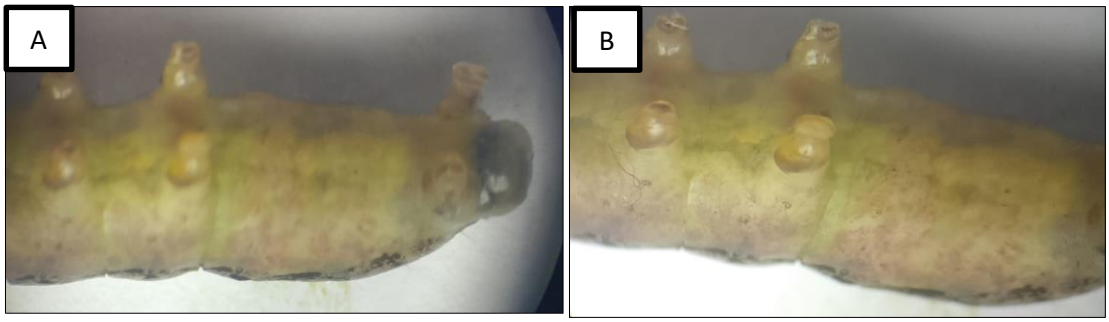

Figure 3. Physical changes of *S. litura* larvae following treatment with red ginger extract (*Zingiber officinale var rubrum*)

The relationship between red ginger extract concentration and *S. litura* larval mortality was analyzed using probit analysis to estimate LC50 and LC90 values at each observation time. As shown in table 5, no larval mortality was recorded during observations I-IV; therefore, the LC50 and LC90 values cannot be determined for this period. The very high slope values (b) and standard error (SE) observed during observations I-III indicate instability in parameter estimation due to low and uneven mortality across treatments. In contrast, the toxicity of red ginger extract increased during V-VIII observations. On day VIII, the LC50 value was 2.11 (95% CI: 1.44-4.60), while the LC90 value reached 16.25 (95% CI: 6.51-146.71). These results suggest that the toxic effects of red ginger extract are cumulative, causing progressive disruption of the physiological processes of the larvae and requiring a longer period of exposure to cause death. One of the limitations of this bioassay is leaf damage treated after soaking in extract suspensions, especially at higher concentrations, which may affect the

larval feeding activity. Nevertheless, a consistent increase in larval mortality over time suggests that red ginger extract exhibits toxic effects on *S. litura* larvae.

Table 5. Probit analysis parameters of *S. litura* larval mortality in response to red ginger extract concentration at different observation times.

Observation time (days)	b ±SE	LC_{50} (CI 95) (%)	LC_{90} (CI 95) (%)
I	$0.000 \pm 3.39x10^{6}$	-	-
II	$21.34 \pm 1.62x10^{6}$	-	-
III	$21.39 \pm 4.97x10^{6}$	-	-
IV	2.50 ± 0.92	-	-
V	2.03 ± 0.50	3.90 (2.45 – 13.00)	16.60 (6.80 – 200.10)
VI	1.93 ± 0.47	3.81 (2.40 – 12.11)	17.51 (7.04 – 208.32)
VII	1.38 ± 0.32	2.70 (1.71 – 7.61)	22.84 (7.93 – 357.65)
VIII	1.45 ± 0.31	2.11 (1.44 – 4.60)	16.25 (6.51 – 146.71)

Footnote: b = Slope; SE= Standard Error; LC= Lethal Concentrate; CI=Confidence Interval; (-) = not estimable

3.2.2. *M. anisopliae* extract

The effect of *M. anisopliae* spore density on larval mortality showed that treatment A and B caused relatively low larval mortality of 2% and 6%. Treatment C had the highest mortality rate of 12% and treatment D had a mortality rate of 6%, 16% and 28% (Figure 4). Infection began to be detected on the 96 HAT mortality in treatment A and treatment B. In treatment B, only 1 larva was infected out of a total of 3 larvae that died. In treatment C, the number of infected larvae up to 144 HAT reached 4 larvae out of 6 dead larvae. Meanwhile, treatment D showed a higher number of infected larvae, namely 8 larvae out of 16 dead larvae (Figure 5). The development of a greenish hyphae mass spreading over the surface of the larvae is a symptom indicated by infection by *M. anisopliae* (Figure 6A). The infected larvae were re-isolated from PDA media and incubated for 7 to 21 days. The incubation results showed that the growth of white mycelium spread on the surface of the PDA media (Figure 6B). Microscopic observations of fungal isolates revealed round-shaped conidia with a greenish-yellow color (Figure 6C) and septate hyphae structure (Figure 6D).

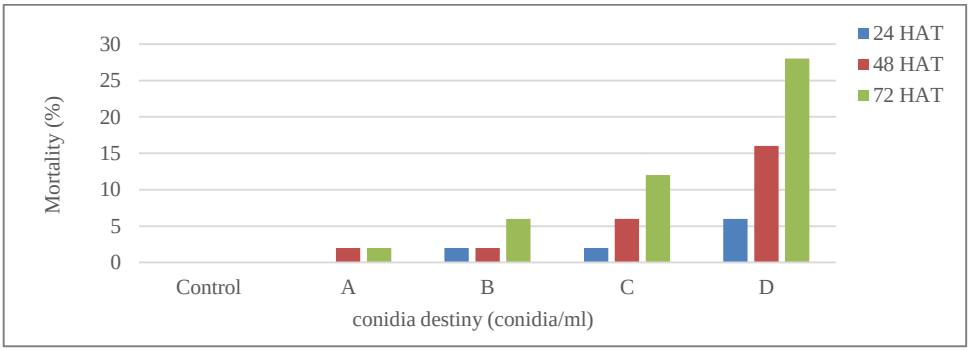

Figure 4. Relationship between *M. anisopliae* conidia destiny (conidia/ml) and larval mortality at 24 HAT, 48 HAT, and 72 HAT

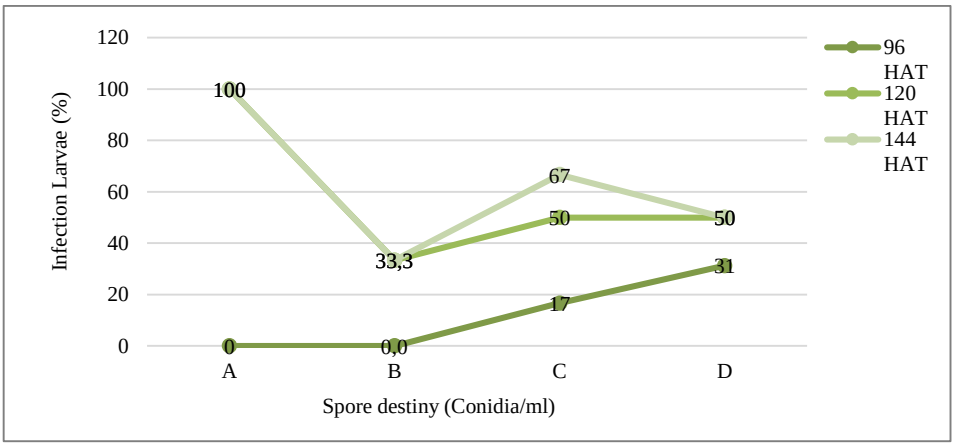

Figure 5. The percentage of infected larvae after being given treatment *M. anisopliae*

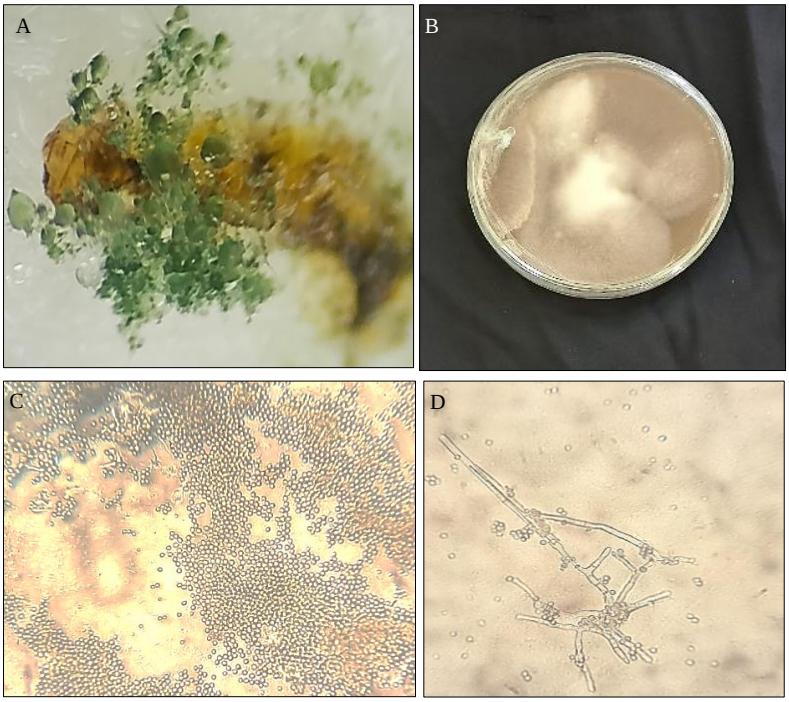

Figure 6. (A) *S. litura* larvae infected with *M. anisopliae*; (B) infected larvae cultured on PDA medium; (C) microscopic appearance of *M. anisopliae* spores isolated from infected larvae; (D) Microscopic appearance structure of *M. anisopliae* hypae.

Table 6. Parameters of probit analysis describing the relationship between *M. anisopliae* concentration and larval mortality *S. litura*.

Observation time (HAT)	**b ±SE**	**LC_{50} (CI 95) (%)**	**LC_{90} (CI 95) (%)**
24	1.23 ± 0.72	-	-
48	1.21 ± 0.46	-	-
72	1.63 ± 0.42	47.45 (26.54 – 238.602)	290.40 (92.00 – 8892.9)

Footnote: b = Slope; SE= Standard Error; LC= Lethal Concentrate; CI=Confidence Interval; (-) = not estimable

The relationship between *M. anisopliae* concentration and larval mortality *S. litura* was analyzed using probit analysis; however, LC_{50} and LC_{90} values could not be estimated at all observation times. As shown in table 6, larval mortality at 24 HAT and 48 HAT was too low to allow the determination of LC_{50} and LC_{90} values. In contrast, at 72 HAT the estimated LC_{50} value was 47.45 (CI 95%: 26.54 – 238,602), while the LC_{90} value reached 290.40 (CI 95%: 92.00 – 8892.9).

The results of this study show that both entomopathogenic mushrooms and red ginger extract have different biological response characteristics to *S. litura* larvae. This information is important as a scientific basis for designing more effective combination strategies in follow-up research. The high mortality of larvae is not followed by a high proportion of infections. This is suspected to be influenced by several factors, namely too low spore density, this conjecture is in line with the results of previous studies which showed that the highest mortality percentage of 100% was found in *the treatment of M. anisopliae* 108/ml with the fastest time of death on the second day after application (Tobing *et al*., 2015). In addition, according to Aw and Hue (2017), conidia formulation plays an important role in increasing contact between pathogens and hosts. On the other hand, formulations that consist only of water are suspected to cause conidia not to spread, thus reducing the chance of contact with larvae. Differences in host feeding behavior and environmental conditions may further influence the observed variability in mushroom performance.(Pertiwi & Nanang Tri Haryadi, 2022).

4. Conclusion

The results showed that red ginger extract and the entomopathogenic *M. anisopliae* did not act as an effective feeding inhibiting agent against *S. litura* larvae. Nonetheless, the larval mortality rate was relatively high, especially in the treatment of red ginger extract with repeated exposure, which indicates that mortality had more to do with cumulative physiological or toxic effects compared to decreased feeding activity. In addition, the high mortality that occurs in the treatment of *M. anisopliae* is not accompanied by the appearance of symptoms of infection.

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