JPT: Jurnal Proteksi Tanaman (Journal of Plant Protection)
p-ISSN : 2580-0604, e-ISSN: 2621-3141
Accredited: Sinta 3, DOAJ
Publisher: Universitas Andalas, Website: https://jpt.faperta.unand.ac.id/index.php/jpt
Vol. 10 No.1 (2026): 71 – 82

Bioinsecticidal Activity of a Local *Metarhizium anisopliae* Isolate against *Spodoptera litura* Larvae on Green Mustard

Hafiz Fauzana*, Pajri Ananta Yudha, Rini Afriani

Agrotechnology Study Program, Faculty of Agriculture, Universitas Riau, Indonesia

*E-mail: fauzana_hafiz@yahoo.co.id

Received: 20 December 2025 | 1st Revised: 10 April 2026 | Accepted: 05 June 2026 | Published: 15 June 2026

Abstract

Tobacco cutworm, *Spodoptera litura* Fabricius is a polyphagous pest that attacks green mustard and may cause substantial yield losses. Entomopathogenic fungi, including *Metarhizium anisopliae* Metsch., offer an environmentally friendly alternative to synthetic insecticides. This study aimed to evaluate the bioinsecticidal activity of a local isolate of *M. anisopliae* against third-instar *S. litura* larvae on green mustard under laboratory conditions. The experiment was arranged in a completely randomized design with six suspension treatments (0, 10, 20, 30, 40, and 50 g/L in distilled water) and five replications, with 10 larvae in each experimental unit. The observed variables included isolate identification, total mortality, initial time of death, LT_{50}, LC_{50}, LC_{95}, daily mortality, and behavioral and morphological changes in infected larvae. This study confirmed that the local isolate of *M. anisopliae* is pathogenic to *S. litura* larvae on green mustard, causing high larval mortality, and a shorter lethal time at higher concentrations. Although the highest mortality was recorded at 50 g/L, the 30 g/L treatment showed comparable effectiveness to higher concentrations, indicating its potential as an efficient concentration for larval control.

Keywords: *Brassica juncea*, concentration, entomopathogenic fungi, mortality, tobacco cutworm

Introduction

Green mustard (*Brassica juncea* Linnaeus) is one of the most widely consumed vegetable crops in Indonesia, and its demand continues to increase annually. However, the production of this vegetable has declined over the last three years. In 2021, national production reached 727,467 tons, decreased to 706,305 tons in 2022, and further declined to 686,876 tons in 2023 (Badan Pusat Statistik, 2024). One factor contributing to the decline in green mustard production is infestation by the tobacco cutworm, *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae).

S. litura is a polyphagous insect pest that attacks various horticultural, food, and industrial crops, making its management particularly challenging (Syah and Purwani, 2016). The larvae damage plants by feeding on the leaves, causing perforations, irregular cuts, and severe defoliation that may leave only the epidermal layer and leaf veins. Heavy infestation can reduce crop quality and marketability (Mega et al., 2019). Previous studies have reported that yield losses from this pest on mustard plants may reach 85% (Ganul et al., 2021).

Pest management practices among farmers still rely heavily on synthetic

insecticides. The unwise use of synthetic insecticides may cause several ecological problems, including resistance and resurgence of target pests, mortality of non-target organisms, and environmental contamination due to pesticide residues in water and surrounding ecosystems (Septariani et al., 2019). In addition, the active ingredients in synthetic insecticides may pose risks to human health (Pamungkas, 2016). Therefore, more environmentally friendly pest management strategies are urgently needed. In the context of sustainable pest management, biological control agents, such as entomopathogenic fungi, have received increasing attention because they can reduce dependence on chemical insecticides and support integrated pest management programs (de Miranda et al., 2024; Albana et al., 2025; Syahrawati et al., 2025).

One promising alternative is the use of the entomopathogenic fungus *Metarhizium anisopliae* Metsch. This species has been widely studied as a microbial control agent due to its ability to infect diverse arthropod pests and its relevance to sustainable pest management (de Miranda et al., 2024; Bui et al., 2025). This fungus is an effective biological control agent because it can infect several developmental stages of insects, including eggs, larvae, pupae, and adults (Yunizar et al., 2018). Susandi et al. (2023) also reported the pathogenic activity of *M. anisopliae* against the green leafhopper, *Nephotettix virescens*. *M. anisopliae* produces bioactive metabolites, such as destruxins, which may disrupt the midgut, hemocytes, Malpighian tubules, and muscular tissues of infected hosts (Sari et al., 2023). Destruxins produced by *Metarhizium* spp. can also interfere with hemocytin-mediated hemolymph immunity, damage hemocytes, and facilitate fungal colonization in the host body (Wang et al., 2024). The advantages of entomopathogenic fungi include their relatively high environmental safety, reproductive capacity, host selectivity, and lower risk of resistance development compared with synthetic insecticides (Justa, 2019). Recent studies have also emphasized that the pathogenicity of *Metarhizium* spp. is influenced by fungal strain, conidial density, host species, larval instar, and environmental conditions; therefore, each local isolate needs to be evaluated before being recommended as a biological control candidate (Riaz et al., 2026).

The use of local isolates is considered important for improving the effectiveness of biological control agents. Local isolates may have greater ecological compatibility with target pests and better adaptation to local environmental conditions, thereby enhancing their ability to suppress pest populations. The use of local isolates in biological control is also recommended because they are expected to have lower impacts on non-target organisms and better adaptation to the soil or habitat from which they were isolated, particularly under fluctuating temperature and humidity conditions (Gürlek et al., 2018). However, the virulence of entomopathogenic fungi is often isolate-dependent; therefore, each local isolate should be evaluated against a specific target pest, host plant system, larval stage, and application method before it is recommended as a biological control candidate (Riaz et al., 2026).

Kusmiadi et al. (2017) reported that the application of a local isolate of *M. anisopliae* at 40 g/L caused 100% mortality of *S. litura*. Fauzana and Destari (2024) reported that *M. anisopliae* at 40 g/L (0.90×10^6 conidia mL^{-1}) was the most effective concentration against *Nilaparvata lugens*, causing 72.50 ± 9.57% mortality after application. Sonune et al. (2019) reported that a suspension of a local isolate of *M. anisopliae* at 1×10^9 spores mL^{-1}, applied using the leaf-dip method, caused

91.66% mortality of *S. litura* larvae. El Husseini (2019) also reported that the mortality of *Spodoptera littoralis* larvae decreased with decreasing conidial concentration, while maximum mortality was recorded at higher inoculum concentrations. Different *M. anisopliae* isolates showed wide variation in LC_{50} and LT_{50} values against *S. litura*, confirming the need to screen and characterize each isolate before practical application.

Steinhaus (1969) stated that an entomopathogenic fungus can be categorized as a bioinsecticide when it causes insect mortality of approximately 72–95%. To date, information on the pathogenicity and bioinsecticidal potential of local soil-derived *M. anisopliae* isolates from Riau against third-instar *S. litura* larvae on green mustard remains limited. In particular, data integrating total mortality, time to death at the initial stage, LT_{50}, LC_{50}, LC_{95}, and behavioral and morphological changes in infected larvae are still needed. Therefore, this study aimed to determine the most effective concentration of a local isolate of *M. anisopliae* as a bioinsecticidal candidate against *S. litura* larvae on green mustard under laboratory conditions, based on total mortality, initial time of death, LT_{50}, LC_{50}, and LC_{95} values, and changes in larval behavior and morphology.

Methods

This research was conducted at the Plant Pest Laboratory, Faculty of Agriculture, Universitas Riau, Pekanbaru, Indonesia, from May to July 2025. The experiment was carried out under laboratory conditions, with an average temperature of 25.5°C and an average relative humidity of 75.5%.

Research Design

The experiment was arranged in a completely randomized design (CRD) consisting of six treatments and five replications, resulting in 30 experimental units. Each experimental unit consisted of 10 third-instar larvae of *Spodoptera litura*. The treatments consisted of six levels of *Metarhizium anisopliae* suspension prepared from fungal cultures grown on cracked corn medium and diluted in distilled water. The treatments were 10 g/L (0.51×10^8 conidia mL^{-1}), 20 g/L (0.57×10^8 conidia mL^{-1}), 30 g/L (0.65×10^8 conidia mL^{-1}), 40 g/L (0.69×10^8 conidia mL^{-1}), 50 g/L (0.73×10^8 conidia mL^{-1}), and a control treatment without *M. anisopliae* suspension. The g/L concentration refers to the mass of *M. anisopliae* culture grown on cracked corn medium used to prepare 1 L of suspension, while the values in parentheses indicate the corresponding conidial density measured using a hemocytometer.

Preparation of Isolate

The local isolate of *M. anisopliae* was obtained from soil collected from the rhizosphere of oil palm plants at the Experimental Farm of the Faculty of Agriculture, Universitas Riau, altitude approximately 35 meters (about 115 feet) above sea level, latitude 0°28’27.73”N, GPS coordinates 0.4744° N, 101.3804° E. Soil samples were collected from a depth of 10 cm and transported to the laboratory. The fungus was isolated using the insect bait method with *Tenebrio molitor* larvae. A total of five trays were prepared, and 10 *T. molitor* larvae were placed in each tray as bait insects.

Infected larvae were identified based on the appearance of white mycelia that subsequently turned dark green within 2–3 days. The infected larvae were incubated for 5–7 days. Fungal isolation was conducted using the moist chamber, and the fungus was grown on potato dextrose agar (PDA). The isolate was identified macroscopically and microscopically based on morphological characteristics following Barnett and Hunter

(1972). The identified isolate was subsequently propagated on cracked corn medium.

Preparation of *Metarhizium anisopliae*

The *M. anisopliae* starter culture grown on cracked corn medium was weighed according to each treatment level, namely 0, 10, 20, 30, 40, and 50 g. Each amount was mixed with 1 L of distilled water and supplemented with 2 g of granulated sugar. The suspension was homogenized using a rotary shaker for 24 h. Serial dilution was conducted up to 10^{-8} to determine conidial density. Conidial viability was not assessed in this study. The conidial density was counted using a hemocytometer under a microscope and calculated using the formula of Nuryanti et al. (2012):

$$J = \frac{t \times d}{0.25 \times n} \times 10^6 \quad \text{..... (1)}$$

where J is the conidial density per mL, t is the total number of conidia counted, d is the dilution factor, n is the number of counted squares, and 0.25 is the correction constant.

Rearing of *Spodoptera litura*

Larvae of *S. litura* were collected from cabbage fields in Tanah Datar District, West Sumatra, Indonesia. The larval colony was maintained in the laboratory and fed with green mustard leaves until pupation. The pupae were placed on sawdust medium. Newly emerged adults were transferred into separate plastic jars in pairs. Cotton soaked in a honey solution at a ratio of 1:10 was placed on the jar lid as adult food. Taro leaves were placed inside the plastic jars as an oviposition substrate. After egg hatching, the larvae were reared and fed with green mustard leaves. The larvae were maintained until they reached the third instar, which was used for the bioassay. The third-instar stage was determined based on two molting events.

Application of Treatments

Each experimental unit consisted of 10 third-instar larvae placed in a plastic jar. The larvae were starved for 1 h before treatment and then provided with green mustard leaves. The fungal suspension was applied by spraying both the larvae and the green mustard leaves using a 100 mL hand sprayer. The spray volume was 3.8 mL per experimental unit, equivalent to 30 sprays. Spraying was performed from a distance of 15–20 cm. After spraying, the larvae and treated leaves were air-dried for 10 min. The treatment was applied in the afternoon at 18:00 Western Indonesian Time.

Observed Variables

The observed variables included identification of the local *M. anisopliae* isolate, total mortality, initial time of death, lethal time 50 (LT_{50}), lethal concentrations (LC_{50} and LC_{95}), daily mortality, and changes in larval behavior and morphology.

Identification

The local isolate of *M. anisopliae* was identified based on macroscopic and microscopic morphological characteristics. Macroscopic observations included colony color and growth appearance on PDA, while microscopic observations included the morphology of conidiophores and conidia.

Mortality (%)

Total mortality was calculated by counting the cumulative number of dead *S. litura* larvae in each experimental unit on the seventh day after treatment application. Total mortality was calculated according to Natawigena (1993) using the following formula:

$$M = \frac{b}{a+b} \, x \, 100 \quad \text{..... (2)}$$

where M is the total mortality of *S. litura* larvae, a is the number of surviving larvae, and b is the number of dead larvae.

The initial time of death was determined by recording the time required

for *M. anisopliae* to cause the first larval death in each treatment unit. Observations were conducted every 3 h after application.

Daily mortality (%)

Daily mortality was calculated by counting the number of dead *S. litura* larvae each day after treatment application. Daily mortality was calculated according to Natawigena (1993) using the following formula:

$$\mathrm{Md} = \frac{\mathrm{a}}{\mathrm{b}}\, x\, 100 \quad \text{..... (3)}$$

where Md is the daily mortality of *S. litura* larvae, a is the total number of larvae tested, and b is the number of dead larvae recorded on each observation day.

Lethal time 50 (LT_{50}) (h)

LT_{50} was determined as the time required for each *M. anisopliae* suspension to kill 50% of the tested *S. litura* larvae in each experimental unit. Observations were conducted every 3 h after application.

Lethal concentration 50, 95 (LC_{50}, LC_{95}) (%)

LC_{50} and LC_{95} were determined as the concentrations of *M. anisopliae* required to kill 50% and 95% of the tested larvae, respectively. Larval mortality was recorded every 24 h after application for each treatment. LC_{50} and LC_{95} values were estimated using probit analysis with POLO-PC software.

Data Analysis

Data were tested for normality before analysis. Data that did not meet the normality assumption were transformed using arcsine √(y + 0.5). Mortality data were not corrected using Abbott's formula because no mortality occurred in the control treatment. Data on initial time of death, LT_{50}, and total mortality were analyzed using analysis of variance (ANOVA) with SAS version 9.1. When significant differences were detected, means were compared using the honestly significant difference (HSD) test at the 5% significance level. LC_{50} and LC_{95} values were analyzed using probit analysis with POLO-PC software. Daily mortality data were analyzed descriptively and presented graphically.

Results

Macro- and Microscopic Characteristics of *Metarhizium anisopliae*

The fungal isolate was identified based on macroscopic and microscopic characteristics. Macroscopically, the colony of *M. anisopliae* grown on PDA initially appeared white and later turned green as conidia developed. Microscopic observation showed that the isolate had hyphae, oval to cylindrical conidia, and branched conidiophores. These morphological characteristics confirmed that the local soil-derived isolate used in this study belonged to *M. anisopliae* (Figure 1).

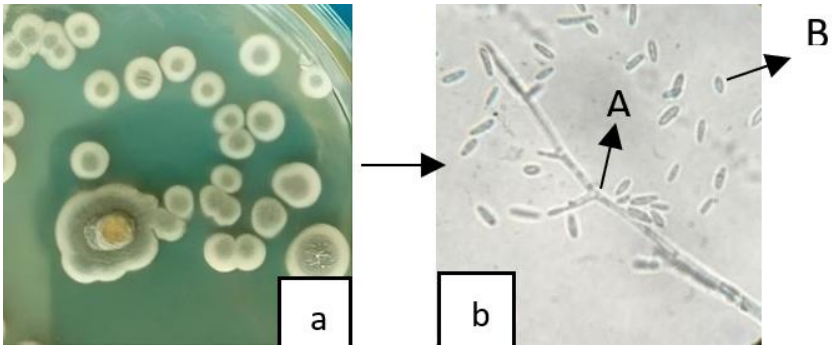

Figure 1. Macro- and microscopic morphology of *Metarhizium anisopliae*: (a) colony growth on potato dextrose agar (PDA); (b) microscopic structures observed under microscope: (A). conidiophore, (B). conidia.

Mortality of *Spodoptera litura* Larvae

The local soil-derived *M. anisopliae* isolate showed measurable bioinsecticidal activity against third-instar *S. litura* larvae on green mustard under laboratory conditions. The application of different concentrations of *M. anisopliae* significantly affected the total mortality of *S. litura* larvae. No larval mortality was observed in the control treatment. In contrast, all treatments containing *M. anisopliae* caused larval mortality, ranging from 60% to 88%. The lowest mortality among fungal treatments was recorded at 10 g/L (60%), while the highest mortality was observed at 50 g/L (88%). Mortality increased with increasing concentration. However, the 30 g/L treatment, which caused 78% mortality, was the lowest concentration within the highest mortality group and did not differ significantly from the 40 and 50 g/L treatments.

A similar concentration-dependent trend was observed for the initial time of death. The first larval death occurred more slowly at lower concentrations, whereas higher concentrations accelerated the onset of larval mortality. At 30, 40, and 50 g/L, the first larval death occurred at 28.80, 27.60, and 27.00 h after application, respectively. These three concentrations were not significantly different from one another, indicating that increasing the concentration above 30 g/L did not significantly accelerate the initial mortality response. Therefore, 30 g/L can be considered the most efficient concentration under laboratory conditions because it provided strong bioinsecticidal activity while requiring a lower amount of fungal inoculum. The detailed data are presented in Table 1.

Table 1. Mortality of *Spodoptera litura* larvae after application of different concentrations of *Metarhizium anisopliae*

Concentration (conidia mL^{-1})	Total mortality (% ± SD)	Initial time of death (h ± SD)
0 g/L (0.00×10^8)	0 ± 0 c	168.00 ± 0 a
10 g/L (0.51×10^8)	60 ± 7.07 b	53.40 ± 13.81 b
20 g/L (0.57×10^8)	68 ± 8.37 b	43.20 ± 10.73 bc
30 g/L (0.65×10^8)	78 ± 4.47 a	28.80 ± 13.35 c
40 g/L (0.69×10^8)	82 ± 8.37 a	27.60 ± 4.93 c
50 g/L (0.73×10^8)	88 ± 8.37 a	27.00 ± 14.70 c

Values in the same column followed by different lowercase letters are significantly different according to the HSD test at the 5% level after arcsine √(y + 0.5) transformation

Daily Mortality

The daily mortality of *S. litura* larvae showed a fluctuating pattern after treatment. Mortality increased gradually during the first two days and reached its peak on the third day, with daily mortality ranging from 24% to 34%. After this peak, daily mortality declined progressively until the seventh day. In general, higher concentrations were associated with higher daily mortality, with the 50 g/L treatment showing the highest peak. However, the mortality pattern at 30 g/L was relatively close to those at 40 and 50 g/L, indicating that 30 g/L may be an efficient concentration for suppressing *S. litura* larvae (Figure 2).

Lethal Time 50 (LT_{50})

The LT_{50} values of *S. litura* larvae decreased as the concentration of *M. anisopliae* suspension increased. The control treatment did not reach 50% mortality during the observation period, whereas all fungal

treatments shortened the time required to kill 50% of the larvae. The lowest concentration, 10 g/L, produced an LT_{50} of 114.00 hours, while the highest concentration, 50 g/L, produced the shortest LT_{50} of 63.00 hours. However, the LT_{50} values at 30, 40, and 50 g/L were statistically similar, indicating that 30 g/L was sufficient to induce rapid mortality. This result suggests that the local isolate of *M. anisopliae* exhibits strong pathogenicity against *S. litura* larvae, particularly at concentrations of 30 g/L or higher. The novelty of this finding lies in determining the effective lethal time of a local *M. anisopliae* isolate and identifying 30 g/L as a potentially efficient concentration for bioinsecticide application against *S. litura* (Table 2).

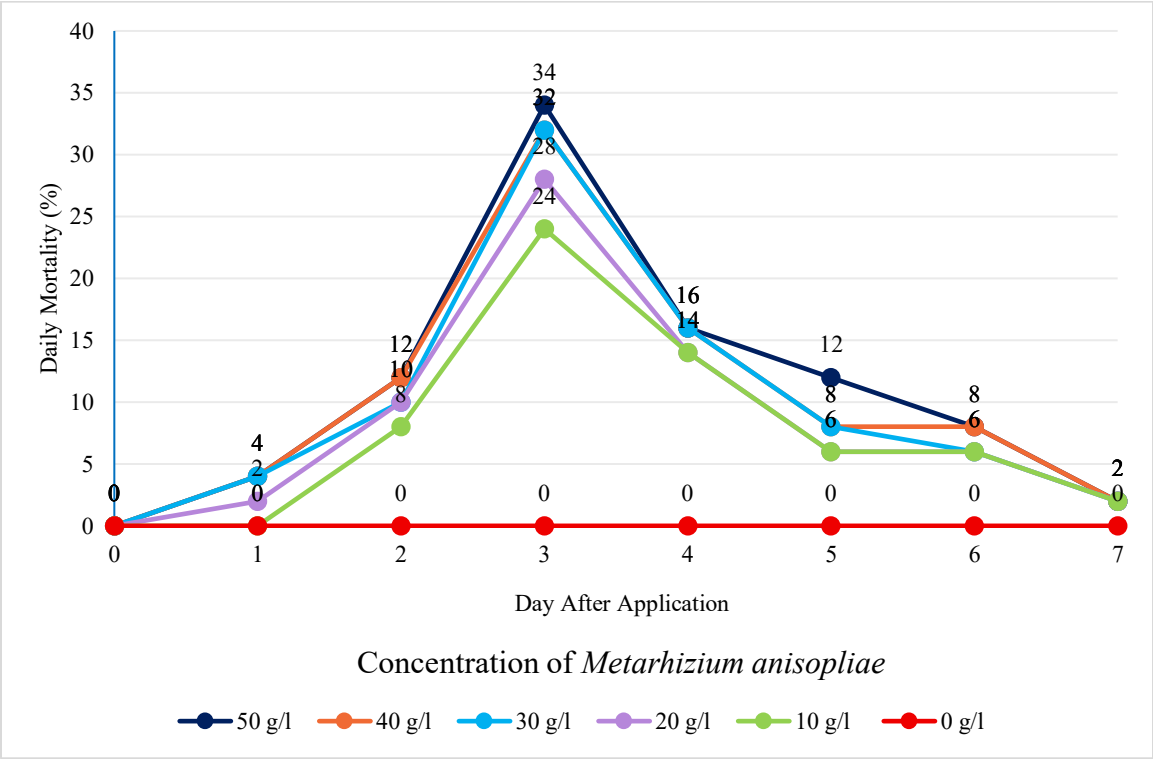

Figure 2. Daily mortality pattern of *Spodoptera litura* larvae after application of different concentrations of *Metarhizium anisopliae* suspension

Table 2. Lethal time 50 (LT_{50}) of *Spodoptera litura* larvae after treatment with different concentrations of *Metarhizium anisopliae*

Concentration (conidia mL^{-1})	*Lethal time* 50 (h ± SD)
0 g/L (0.00×10^8)	168.00 ± 0 a
10 g/L (0.51×10^8)	114.00 ± 30.52 b
20 g/L (0.57×10^8)	95.40 ± 1.34 b
30 g/L (0.65×10^8)	67.20 ± 7.82 c
40 g/L (0.69×10^8)	64.80 ± 9.63 c
50 g/L (0.73×10^8)	63.00 ± 20.12 c

Values in the same column followed by different lowercase letters are significantly different according to the HSD test at the 5% level after arcsine √(y + 0.5) transformation

Lethal Concentration 50, 95 (LC_{50}, LC_{95}) (%)

M. anisopliae was effective at relatively low concentration for causing 50% larval mortality, but a substantially higher concentration would be needed to achieve near-complete control. Based on probit analysis, the LC_{50} indicated that a concentration of 0.70% was required to kill 50% of *S. litura* larvae. Meanwhile, the LC_{95} indicated that a much higher concentration, 14.53%, was required to kill 95% of the larval population. The confidence interval for LC_{50} ranged from 0.15% to 1.17%, suggesting that the estimated concentration required to kill 50% of larvae was relatively narrow and more precise. In contrast, the LC_{95} confidence interval was much wider, ranging from 7.29% to 138.25%. This wide interval indicates greater uncertainty in the LC_{95} estimate, due to variation in larval response at higher concentrations or limited mortality data near the upper response range (Table 3).

Table 3. Lethal concentration 50 and 95 (LC_{50} and LC_{95}) of *Metarhizium anisopliae* against *Spodoptera litura* larvae

Lethal concentration (LC)	Concentration (%)	CI 95 (%)
LC_{50}	0.70	0.15-1.17
LC_{95}	14.53	7.29-138.25

Note: CI = Confidence interval

Discussion

The local fungal isolate used in this study was morphologically identified as *Metarhizium anisopliae*. The colony initially appeared white on PDA and gradually turned green as conidia developed, while microscopic observation showed branched conidiophores and oval to cylindrical conidia (Figure 1). These characteristics are consistent with the general morphology of *M. anisopliae*, as described by Barnett and Hunter (1972), Sopialena et al. (2022), and Elham et al. (2018). However, molecular confirmation would be useful in future studies to strengthen species-level identification.

The application of *M. anisopliae* significantly increased the mortality of third-instar *S. litura* larvae. All fungal treatments caused mortality ranging from 60% to 88%. Mortality tended to increase with increasing fungal concentration, indicating a concentration-dependent response. There was no difference between 50 g/L, 40 g/L and 30 g/L. Therefore, 30 g/L can be considered the most efficient concentration under laboratory conditions because it was the lowest concentration, causing 78% mortality (Table 1). This result showed the potential of the local *M. anisopliae* isolate as a bioinsecticide candidate against *S. litura*, but its efficacy still needs to be validated under greenhouse or field conditions.

The concentration-dependent mortality observed in this study may be related to the number of conidia that contacted and infected the larval cuticle. Masyitah et al. (2017) also demonstrated that fungal species and conidial density affected the mortality of *S. litura* larvae on tobacco, supporting the importance of inoculum density in determining treatment effectiveness. Higher concentrations generally provide more infective propagules, increasing the probability of conidial attachment, germination, penetration, and subsequent colonization of the host. Harun et al. (2022) stated that the infection process of entomopathogenic fungi involves sequential stages, including inoculation, penetration, infection, and invasion. Similarly, Aw and Hue (2017) explained that *Metarhizium* spp. use cuticle-degrading enzymes, such as

chitinases, proteases, and lipases, during host penetration. The higher mortality recorded at 30–50 g/L was likely associated with a greater number of viable infective units and more intensive infection pressure on the larvae.

The initial time of larval death also reflected the effect of concentration. Lower concentrations resulted in slower initial mortality, with the first death occurring at 53.40 h after application at 10 g/L and 43.20 h at 20 g/L. In contrast, the first larval death occurred faster at 30, 40, and 50 g/L, namely 28.80, 27.60, and 27.00 h after application, respectively (Table 1). These three concentrations were statistically similar, suggesting that increasing the concentration above 30 g/L did not substantially accelerate the onset of larval mortality. The results showed that 30 g/L was sufficient to initiate infection and induce early mortality under the laboratory conditions.

The LT_{50} further support the effectiveness of the 30 g/L treatment. The LT_{50} decreased from 114.00 h at 10 g/L to 63.00 h at 50 g/L, indicating that higher concentrations shortened the time required to kill 50% of the larval population (Table 2). However, the LT_{50} values at 30, 40, and 50 g/L were not significantly different. The 30 g/L treatment produced an LT_{50} of 67.20 h, which was statistically comparable to 40 g/L and 50 g/L. Therefore, 30 g/L may be regarded as an efficient concentration because it achieved relatively high mortality and rapid lethal activity without requiring the highest amount of fungal inoculum. Similar trends have been reported by Sonune et al. (2019) and El Husseini (2019), who found that higher concentrations of *M. anisopliae* generally resulted in greater mortality and faster larval killing by *Spodoptera*.

The probit analysis showed that the LC_{50} of *M. anisopliae* against *S. litura* larvae was 0.70%, with a 95% confidence interval of 0.15–1.17% (Table 3). This indicates that a relatively low concentration was sufficient to kill 50% of the tested larvae under laboratory conditions. In contrast, the LC_{95} value was 14.53%, with a very wide confidence interval of 7.29–138.25% (Table 3). The wide confidence interval indicates low precision of the LC_{95} estimate, likely because the tested concentration range did not yield mortality close to 95%. Therefore, the LC_{95} value should be interpreted cautiously and not recommended directly as an application concentration. Additional bioassays using a wider concentration range are needed to obtain a more reliable LC_{95} estimate.

Daily mortality showed a delayed but clear infection pattern. Mortality was low on the first day after application, increased on the second day, and peaked on the third day, with daily mortality ranging from 24% to 34% (Figure 2). After the third day, daily mortality gradually declined until the seventh day. This pattern is consistent with the biology of entomopathogenic fungi, which require time for conidial attachment, germination, penetration, multiplication within the host, and toxin production. Nasution et al. (2021) reported that *M. anisopliae* can produce destruxin toxins during host colonization, which may contribute to tissue damage and insect death. The decline in daily mortality after the peak may be associated with a decrease in surviving larvae and the possible influence of laboratory humidity, which averaged 75.5% in this study. This humidity was slightly below the optimal range reported for *M. anisopliae* development by Ratmawati (2020).

Infected larvae showed progressive behavioral and morphological changes following exposure to *M. anisopliae*. Larval movement and feeding activity gradually decreased, followed by immobility and death. Morphological changes included dark brown

discoloration, softening of the body, shrinkage, and the development of fungal mycelia on the larval surface. These symptoms are consistent with infection by entomopathogenic fungi. Harun et al. (2022) reported that insects infected by entomopathogenic fungi commonly exhibit reduced feeding activity, slower movement, and changes in body color. The dark brown coloration observed in this study may be associated with melanization, a component of the insect's immune response to invading pathogens. Erawati and Wardati (2016) explained that melanization is mediated by phenolic compounds through phenoloxidase activity. Some dead larvae did not show external mycelial growth, which may occur when external conditions are less favorable for fungal outgrowth after host death, as reported by Rahmawati (2013).

Conclusions

This study confirmed that the local isolate of *Metarhizium anisopliae* is pathogenic to *Spodoptera litura* larvae on green mustard, causing high larval mortality, and a shorter lethal time at higher concentrations. Although the highest mortality was recorded at 50 g/L, the 30 g/L treatment showed comparable effectiveness to higher concentrations, indicating its potential as an efficient concentration for larval control.

Declaration

Author contributions

Hafiz Fauzana designed and supervised the study, analyzed the data, and drafted the manuscript. Pajri Ananta Yudha collected samples, conducted the experiments, and curated the data. Rini Afriani contributed to methodology, morphological identification, and manuscript revision. All authors read and approved the final manuscript.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Competing interest

The authors declare that they have no financial or personal relationships that could have influenced the work reported in this article.

References

Albana H, NAI Mohd Zainudin, N Abu Bakar, N Saputra, DD Mailvaganam, WM Syazwan, M Syahrawati. 2025. Effects of entomopathogenic fungi on natural enemies: a systematic review of their use in biological control. Malaysian Applied Biology 54(4): 25–39. DOI. 10.55230/mabjournal.v54i4.3566.

Aw KMS, SM Hue. 2017. Mode of infection of *Metarhizium* spp. fungus and their potential as biological control agents. Journal of Fungi 3(2): 30. DOI. 10.3390/jof3020030.

Badan Pusat Statistik. 2024. Produksi Tanaman Sayuran. Badan Pusat Statistik. Jakarta. https://www.bps.go.id/id/statistics-table/2/NjEjMg%3D%3D/pro. [19 Januari 2025].

Barnett HL, BB Hunter. 1972. Illustrated Genera of Imperfect Fungi. Third Edition. Burgess Publishing Company. Minneapolis.

Bui KD, Hidrayani, Nurbailis. 2025. Biological control of *Aphis gossypii* on red chili pepper using locally isolated *Beauveria bassiana* and *Metarhizium anisopliae*. Jurnal Proteksi Tanaman (Journal of Plant Protection) 9(2): 95–107. DOI. 10.25077/jpt.9.2.95-107.2025.

de Miranda RPR, TKA Soares, DP Castro, FA Genta. 2024. General aspects, host interaction, and application of *Metarhizium* sp. in arthropod pest and vector control. Frontiers in Fungal Biology 5: 1456964. DOI. 10.3389/ffunb.2024.1456964.

El Husseini MMM. 2019. Efficacy of the entomopathogenic fungus, *Metarhizium anisopliae* (Metsch.), against larvae of the cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae), under laboratory conditions. Egyptian Journal of Biological Pest Control 29: 50. DOI. 10.1186/s41938-019-0156-2.

Elham MSH, PK Kin, GLE Lin, I Ishak, WA Azmi. 2018. Occurrence of entomopathogenic fungus, *Metarhizium anisopliae* isolated from island, BRIS and coastal soils of Terengganu, Malaysia. Journal of Sustainability Science and Management Special Issue 5: 179–189. https://jssm.umt.edu.my/wp-content/uploads/sites/51/2020/05/Bab-14.SI5_.pdf.

Erawati DN, I Wardati. 2016. Teknologi pengendali hayati *Metarhizium anisopliae* dan *Beauveria bassiana* terhadap hama kumbang kelapa sawit (*Oryctes rhinoceros*). Prosiding Seminar Nasional Hasil Penelitian dan Pengabdian Masyarakat 2016. Politeknik Negeri Jember. Jember. pp. 1–5. https://sipora.polije.ac.id/10500/.

Fauzana H, I Destari. 2024. Test concentrations of Riau local rice field isolate entomopathogenic fungus *Metarhizium anisopliae* toward mortality brown planthopper (*Nilaparvata lugens* Stal.) in the laboratory. BIO Web of Conferences 99: 04007. DOI. 10.1051/bioconf/20249904007.

Ganul T, H Alang, ADR Ainulia. 2021. Pengaruh ekstrak rimpang jeringau (*Acorus calamus* L.) terhadap larva ulat grayak (*Spodoptera litura*) pada sawi putih (*Brassica pekinensis*). Celebes Biodiversitas 4(1): 6–16. DOI. 10.51336/cb.v4i1.255.

Gürlek S, A Sevim, FM Sezgin, E Sevim. 2018. Isolation and characterization of *Beauveria* and *Metarhizium* spp. from walnut fields and their pathogenicity against the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae). Egyptian Journal of Biological Pest Control 28: 50. DOI. 10.1186/s41938-018-0055-y.

Harun Y, AK Parawansa, A Haris. 2022. Kajian patogenisitas *Beauveria bassiana* dan *Metarhizium* sp. terhadap larva ulat grayak (*Spodoptera frugiperda*) pada tanaman jagung. Agrotek: Jurnal Ilmiah Ilmu Pertanian 6(2): 81–93. DOI. 10.33096/agrotek.v6i2.238.

Justa NS. 2019. Pengaruh aplikasi cendawan entomopatogen *Metarrhizium anisopliae* (Metch) dengan metode perendaman dan penyemprotan pada tanaman padi terhadap mortalitas wereng hijau, *Nephotettix virescens* (Distant). [Skripsi]. Universitas Hasanuddin. Makassar. https://repository.unhas.ac.id/id/eprint/5088/.

Kusmiadi R, SN Aini, R Apriyadi, Ciko. 2017. Uji efektivitas agensia hayati *Metarizhium anisopliae* terhadap hama ulat grayak (*Spodoptera litura* Fabr.) secara in vitro. AGROSAINSTEK: Jurnal Ilmu dan Teknologi Pertanian 1(2): 86–94. DOI. 10.33019/agrosainstek.v1i2.12.

Masyitah I, SF Sitepu, I Safni. 2017. Potensi jamur entomopatogen untuk mengendalikan ulat grayak *Spodoptera litura* F. pada tanaman tembakau in vivo. Jurnal Online Agroekoteknologi 5(3): 484–493. DOI. 10.32734/ja.v5i3.2211.

Mega ENP, D Supriyatdi, A Sudirman. 2019. Pengaruh ekstrak buah mengkudu terhadap mortalitas ulat grayak (*Spodoptera litura* F.). Jurnal Agrosains dan Teknologi 4(2): 95–101. DOI. 10.24853/jat.4.2.95-101.

Nasution L, AR Cemda, S Isnaini, M Afrillah, P Filsa. 2021. Pemanfaatan jamur *Metarhizium anisopliae* berasal dari isolat *Brontispa longissima* mengendalikan larva *Oryctes rhinoceros* secara in vitro. Jurnal Agrica Ekstensia 15(2): 132–141. DOI. 10.55127/ae.v15i2.101.

Natawigena H. 1993. Dasar-dasar Perlindungan Tanaman. Trigenda Karya. Bandung.

Nuryanti NSP, L Wibowo, A Azis. 2012. Penambahan beberapa jenis bahan nutrisi pada media perbanyakan untuk meningkatkan virulensi *Beauveria bassiana* terhadap hama walang sangit. Jurnal HPT

Tropika 12(1): 64–70. DOI. 10.23960/j.hptt.11264-70.

Pamungkas OS. 2016. Bahaya paparan pestisida terhadap kesehatan manusia. Bioedukasi 14(1): 27–31. https://bioedukasi.unej.ac.id/index.php/bioed/article/download/4532/3355.

Rahmawati R. 2013. Aplikasi *Metarhizium anisopliae*, nematoda entomopatogen dan kombinasi keduanya terhadap mortalitas larva *Oryctes rhinoceros* di lapangan. [Skripsi]. Universitas Negeri Semarang. Semarang.

Ratmawati I. 2020. Jamur *Metarhizium anisopliae* senjata alami atasi larva kwangwung. POPT Perkebunan. Jawa Timur.

Riaz M, L Kafle, TY Chang, WH Chen. 2026. Characterization and virulence of *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae) isolates from ecologically distinct soils against *Spodoptera litura* (Lepidoptera: Noctuidae). Journal of Insect Science 26(1): ieaf113. DOI. 10.1093/jisesa/ieaf113.

Sari DE, R Faridah, M Adiaman. 2023. Formulasi cendawan entomopatogen *Beauveria bassiana* dan *Metarhizium anisopliae* terhadap *Leptocorisa acuta*. Jurnal Agrotan 9(2): 41–44. https://ejournals.umma.ac.id/index.php/agrotan/article/view/2009.

Septariani DN, A Herawati, Mujiyo. 2019. Pemanfaatan berbagai tanaman refugia sebagai pengendali hama alami pada tanaman cabai (*Capsicum annum* L.). PRIMA: Journal of Community Empowering and Services 3(1): 1–9. DOI. 10.20961/prima.v3i1.36106.

Sonune BD, MJ Patil, RB Kothikar, HR Sawai, VS Wargane, KK Mane. 2019. Pathogenicity of *Metarhizium anisopliae* against *Spodoptera litura* and its compatibility with insecticides. International Journal of Chemical Studies 7(5): 1637–1640. https://www.chemijournal.com/archives/2019/vol7issue5/PartAC/7-5-2-829.pdf.

Sopialena, A Sahid, J Hutajulu. 2022. Uji efektivitas jamur *Metarhizium anisoplae* dan *Beauveria bassiana* Bals lokal dan komerisial terhadap hama kutu daun (*Aphis craccivora*) pada tanaman kacang panjang (*Vigna sinensis* L.). AGRIFOR: Jurnal Ilmu Pertanian dan Kehutanan 21(1): 147–160. DOI. 10.31293/agrifor.v21i1.5939.

Steinhaus EA. 1949. Principles of Insect Pathology. McGraw-Hill. New York.

Susandi Y, CL Salakia, JF Watung. 2023. Aplikasi *Metarhizium anisopliae* dan *Azadirachta indica* A. Juss untuk mengendalikan *Nephotettix virescens* D. sebagai serangga vektor penyakit tungro pada tanaman padi. Jurnal MIPA 12(2): 68–73. DOI. 10.35799/jm.v12i2.49072.

Syah BW, KI Purwani. 2016. Pengaruh ekstrak daun belimbing wuluh (*Averrhoa bilimbi*) terhadap mortalitas dan perkembangan larva *Spodoptera litura*. Jurnal Sains dan Seni ITS 5(2): E23–E28. https://www.neliti.com/id/journals/jurnal-sains-dan-seni-its/browse/volume/5/2.

Syahrawati M, N Saputra, Trizelia, E Sulyanti. 2025. Entomopathogenicity of endophytic fungi isolated from Payo rice variety in Kerinci, Indonesia. Egyptian Journal of Biological Pest Control 35: 25. DOI. 10.1186/s41938-025-00859-y.

Wang J, H Hu, S Pang, X Yin, B Cao, J Huang, X Xu, Q Weng, Q Hu. 2024. Destruxin A inhibits the hemocytin-mediated hemolymph immunity of host insects to facilitate *Metarhizium* infection. Cell Reports 43(2): 113686. DOI. 10.1016/j.celrep.2024.113686.

Yunizar N, Rahmawati, Kustiati. 2018. Patogenitas isolat jamur entomopatogenik *Metarhizium anisopliae* terhadap lalat rumah *Musca domestica* L. (Diptera: Muscidae). Protobiont 7(3): 77–82. DOI. 10.26418/protobiont.v7i3.29090.

How to cite: Fauzana H, PA Yudha, R Afriani. 2026. Bioinsecticidal Activity of a Local *Metarhizium anisopliae* Isolate against *Spodoptera litura* Larvae on Green Mustard. Jurnal Proteksi Tanaman (Journal of Plant Protection) 10(1): 71 – 82.