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Effectivity Of Entomopathogen *Metarhizium anisopliae* On Fall Army Worm (*Spodoptera frugiperda)*

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Abstract

Fall Armyworm (FAW) or armyworm (*Spodoptera frugiperda*) is a major pest of corn plants that was first discovered in West Pasaman, Indonesia since 2019. *Spodoptera frugiperda* causes the highest damage in the larval phase. Symptoms of *Spodoptera frugiperda* larvae attack on corn plants with elongated holes (window panes) and signs of sawdust-like feces on the leaf surface. The level of damage can reach 100% if the population is not controlled. Therefore, environmentally friendly control can be carried out by utilizing fungi that cause insect pathogens, one of which is *Metarhizium anisopliae*. The purpose of this research was to examine the concentration of *Metarhizium anisopliae* on *Spodoptera frugiperda* instar 2 larvae in the laboratory. The research was conducted at the Laboratory of Ecology and Plant Production, Faculty of Animal and Agriculture Science, Universitas Diponegoro, using a 4x5 monofactor Completely Randomized Design (CRD). Treatment based on concentration level of *Metarhizium anisopliae* (control negative, control positif (insecticide with the active ingredient deltamethrin) 10^6, 10^7conidia/ml). The results of the research showed that the highest larval mortality in the deltamethrin treatment was 71% with LT_{50} was 3 days, *Metarhizium anisopliae* had an LC_{50} of 10^{10} conidia/ml and the highest feeding in the deltamethrin wich was 80,45%. Deltamethrin treatment resulted in the highest mortality of *Spodoptera frugiperda* in a short time, while *Metarhizium anisopliae* was less effective and required a long time to infect the larvae.

Keywords: concentration, metarhizium anisopliae, spodoptera frugiperda

Introduction

Corn farming experiences several obstacles that cause a decrease in production. The decrease in production can reach 10% - 50% of the harvest (Maharani et al., 2021). One of the inhibiting factors is pests, namely Fall Armyworm (FAW) or grayak caterpillars (*Spodoptera frugiperda*). This pest originates from the American continent and has spread to various regions of Africa and Asia. *Spodoptera frugiperda* larvae were reported to attack corn plants in Indonesia for the first time in 2019 in the areas of Sumatra (Lihanto, 2019), Sulawesi (Trisyono et al., 2019), West Java (Wardana et al. 2022), East Java (Megasari and Khoiri, 2021) and Yogyakarta (Nurkomar et al., 2021). Corn production in West Sumatra based on data from the Central Statistics Agency in 2019 of 920 thousand tons of pipes has decreased by 7.3% compared to the previous year.

Spodoptera frugiperda larvae if not controlled can cause damage to corn plants of 15% - 73% (Septian et al., 2021) and can reach 100% (Trisyono et al., 2019). An average population density of 0.2-0.8 larvae per plant can reduce yields by 5-20% (Lihanto, 2019). *Spodoptera frugiperda* larvae cause the highest damage in the vegetative phase by attacking young leaves to the growing point of corn plants. Symptoms of *Spodoptera frugiperda* larval attack are indicated by transparent elongated holes leaving a window-like epidermis layer (window pane), then the larvae attack the growing point (Sagar et al., 2020). Signs of larval attack are the discovery of feces like brownish-yellow sawdust on the surface of the leaves to the growing point of the plant. The invasive phase of instar 2 larvae with strong flier imago and high cruising ability (Noer, 2020).

Environmentally friendly control by utilizing fungi that are the cause of insect pathogens, one of which is the entomopathogenic fungus *Metarhizium anisopliae*. The *Metarhizium anisopliae* fungus belongs to the Clavicipitaceae family which can cause death of the larval phase, deformities of the imago, flying behavior, mating, reproduction and the next generation (Sari, 2023). The mechanism of *Metarhizium anisopliae* in infecting target insects through contact with conidia will germinate to form hyphae on the insect's body with the help of the enzymes chitinase, protease, and lipase which can reduce the components of the insect cuticle (Bayu et al., 2021). The insect then undergoes mummification which is coated with green fungal conidia and will die due to disruption of the metabolic system. *Metarhizium anisopliae* also has a toxic compound, namely destructin (Dtx) which can cause insect death (Suroto et al., 2023). Therefore, this study aims to examine the effective concentration of *Metarhizium anisopliae* against *Spodoptera fugiperda* in the laboratory.

Research Method

The tools used in the study were pans, stoves, blenders, stirrers, trays, knives, vials, thinwall, tweezers, cotton, baking paper, imago diameter cages, analytical scales, shakers, meters, hot plate stirrers, Erlenmeyer flasks, measuring cups, stationery, cameras, haemocytometers, and microscopes. The materials used were isolates of *Metarhizium anisopliae*, test insects *Spodoptera frugiperda* instar 2, aquades, jogo beans, nipagin, wheat germ, ascorbic acid, multivitamins, brewer's yeast, 40% formalin, agar, honey, tissue, and insecticide active ingredient deltamethrin.

The study was conducted in August 2024 - December 2024 at the Ecology and Plant Production Laboratory, Faculty of Animal and Agriculture Science, Universitas Diponegoro, Semarang, Central Java. The research stages with bio-assay used a monofactorial Completely Randomized Design (CRD) consisting of 4 treatment levels, each treatment repeated 5 times so that there were 20 experimental units. Each unit contained 20 instar 2 larvae. The treatment levels of *Metarhizium anisopliae* suspension consisted of K0- (Without *Metarhizium anisopliae*/100 ml sterile water), K0+ (Insecticide active ingredient Deltamethrin 0.3 ml/100 ml), K1 (*Metarhizium anisopliae* 10^6 conidia/ml in 100 ml aquades), K2 (*Metarhizium anisopliae* 10^7 conidia/ml in 100 ml aquades).

The research procedure consisted of making a artificial diet for *Spodoptera frugiperda* larvae based on the reference feed ostrinia furnacalis (Taufika et al., 2022), Mass Rearing of *Spodoptera frugiperda*, Preparation of *Metarhizium anisopliae* suspension using the multi-stage dilution method, Calculation of spore density

Metarhizium anisopliae, Bio-Assay by dipping the larvae into the suspension that has been made, and Parameter observations consist of larvae mortality, LC_{50}, LT_{50}, using probit analysis and Inhibition of Feed Activity. Analysis of variance (ANOVA) with a level of 5%. if there was a real effect between treatments, it was continued with the DMRT test with a level of 5%.

Results and Discussion

Mortality of *Spodoptera frugiperda* larvae

The results showed that *Metarhizium anisopliae* suspension significantly affected the mortality of *Spodoptera frugiperda* instar 2 larvae in the laboratory. Total mortality in percent is presented in Table 1.

Table 1. Mortality of *Spodoptera frugiperda* Larvae

Treatments	Mortality Larvae
	---------- % ----------
Control	0^{c}
Deltamethrin	71^{a}
10^{6}conidia/ml	2^{b}
10^{7}conidia/ml	8^{b}

Description: control = not using *Metarhizium anisopliae* (aquades)

The insecticide with the active ingredient deltamethrin on *Spodoptera frugiperda* instar 2 larvae had significantly higher results compared to other treatments. The concentration of *Metarhizium anisopliae* conidia density of 10^{6} conidia/ml in *Spodoptera frugiperda* instar 2 larvae was not significantly different from the concentration of 10^{7}conidia/ml, but was significantly lower compared to the insecticide Deltamethrin and significantly higher compared to the control treatment (without *Metarhizium anisopliae*). Deltamethrin showed the highest mortality of 71% compared to other treatments. The active ingredient deltamethrin is a broad-spectrum insecticide that is not affected by environmental factors with a chemical reaction as a poison that works quickly to kill larvae (Juleha et al., 2022). The condition of *Spodoptera frugiperda* larvae exposed to deltamethrin and infected with *Metarhizium anisopliae* is presented in Figure 1.

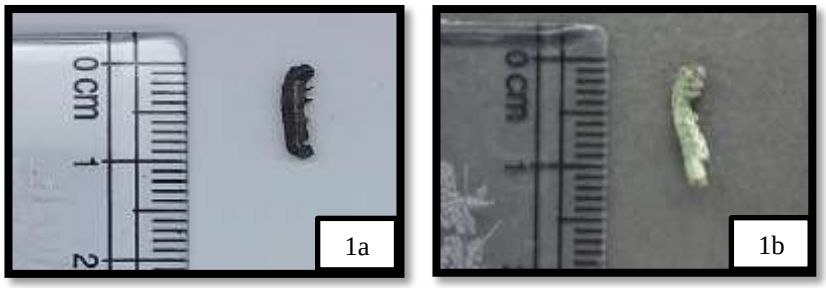

Figure 1. larvae after treatment (a) condition of larvae after exposure to deltamethrin, (b) condition of larvae after infection with *Metarhizium anisopliae*

Larvae exposed to the insecticide deltamethrin and infected with *Metarhizium anisopliae* will experience physical changes. Based on illustration 1a, larvae exposed to Deltamethrin show a change in body color to black, stiff, and dry. According to Ma'wa et al. (2023) morphological changes occur in *Spodoptera frugiperda* larvae exposed to

the synthetic insecticide deltamethrin, changing color to black and dry like burnt. Based on illustration 1b, larvae infected with *Metarhizium anisopliae* will experience symptoms of a stiff larval body, covered in *Metarhizium anisopliae* fungal hyphae from white to green. The mechanism of *Metarhizium anisopliae* spore infection begins with spores that stick to and enter the larva's body, forming hyphae from the epidermis tissue to the entire larval body tissue, then the entire larval body is covered in hyphae, the body is pressed and infected by the toxins produced so that the larvae die (Permana and Dyasti, 2022).

Lethal Concentration 50 Metharizium anisopliae

Probit analysis on Lethal Concentration 50 parameter produces a regression curve. The regression curve of probit analysis of LC_{50} *Metarhizium anisopliae* is presented in Figure 2.

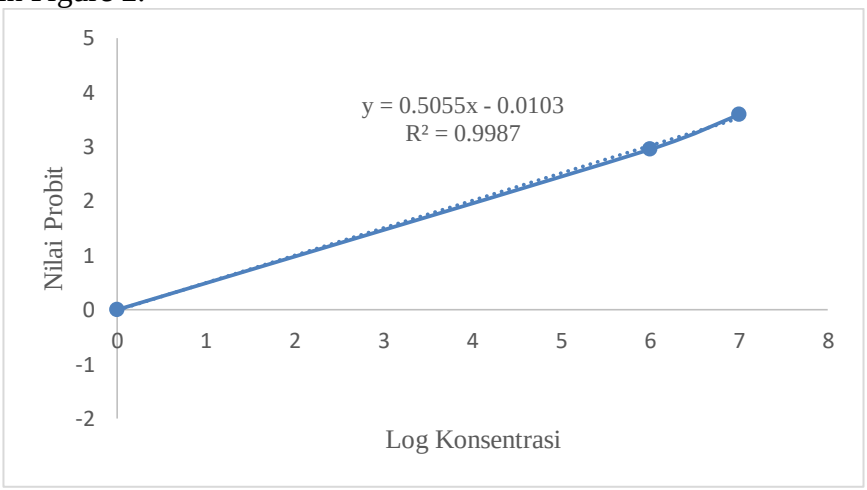

Figure 2. Regression curve of Lethal Concentration 50 *Metahrizium anisopliae*

Regression analysis obtained the equation y = 0.5055x - 0.0103. The calculation results of the LC_{50} equation (concentration that causes 50% mortality) were achieved at a conidia density of 10^{10}conidia/ml. This shows that at this concentration it can kill half of the population of instar 2 *Spodoptera frugiperda* larvae effectively. The LC_{50} value obtained shows the effectiveness of *Metarhizium anisopliae* in causing the death of *Spodoptera frugiperda* larvae through a significant relationship between the concentration given to the larvae (Lutfiyah et al., 2024).

Lethal Time 50 *Metarhizium anisopliae*

The results of the probit regression analysis of Lethal Time 50 *Metarhizium anisopliae* are presented in Table 2.

Table 2. Lethal Time 50 *Metarhizium anisopliae*

Treatments	Mortality Larvae
	---------- days ----------
Control	0
Deltamethrin	3
10^6conidia/ml	2.472
10^7conidia/ml	26

Description: control = not using *Metarhizium anisopliae* (aquades)

Based on the results, it shows that the insecticide deltamethrin has the fastest time to kill 50% of the population of *Spodoptera frugiperda* instar 2 larvae, which is 3 days, the longest time at the concentration of *Metarhizium anisopliae* is 2,472 days (206 months) at a density concentration of 10^6 conidia/ml, and a concentration of 10^7 conidia/ml takes 26 days to kill 50% of the larval population. *Metarhizium anisopliae* can be effective in infecting larvae but for a long time. This is in accordance with Nababan et al. (2022) that *Metarhizium anisopliae* takes time to kill its host insect because the fungal spores attached to the cuticle must germinate to form hyphae first in order to penetrate the cuticle, so it takes a long time and conditions. The control treatment did not cause larval death. This was caused by the control treatment not using *Metarhizium anisopliae*.

Inhibition of Feed Activity

The results of the study showed that *Metarhizium anisopliae* suspension had a significant effect on the feed inhibitory power of *Spodoptera frugiperda* instar 2 larvae. The feed inhibition in percent is presented in Figure 3.

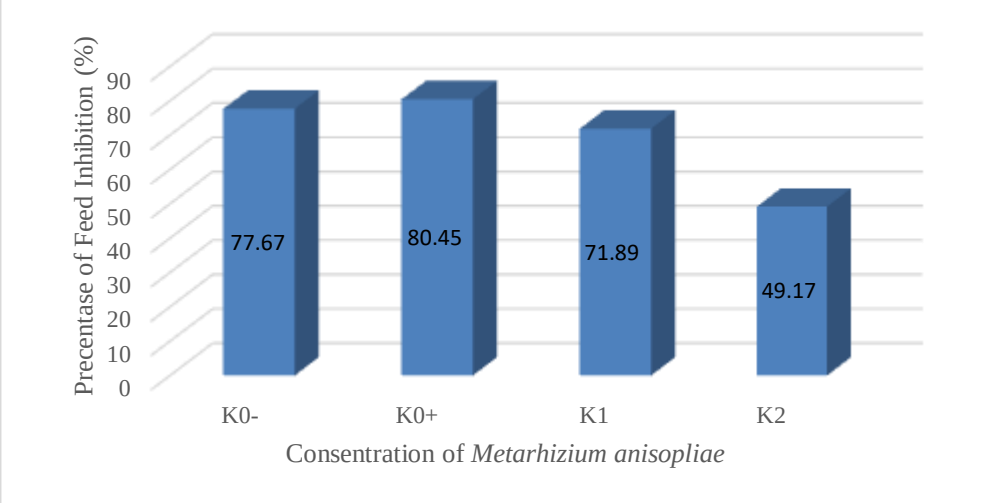

Figure 3. Histogram of Feed Inhibition of *Spodoptera frugiperda* Larvae instar 2

The results showed that the concentration treatment significantly affected the feed inhibition of instar 2 *Spodoptera frugipeda* larvae. The highest feed inhibition activity was in the deltamethrin insecticide at 80.45% compared to other treatments. This showed results that were not significantly different from the feed inhibition activity in the control at 77.67% and the concentration of *Metarhizium anisopliae* and 10^7 conidia/ml at 71.89%. The feed inhibition with the lowest results was in the concentration of *Metarhizium anisopliae* 10^7 conidia/ml at 49.17%, these results also showed a significant difference from other treatments. Deltamethrin had the highest results in inhibiting larval feed which showed results in line with the high mortality of *Spodoptera frugiperda* larvae. Deltamethrin works by affecting the peripheral and central nervous systems of insects through the action of sodium channels, stimulating nerve cells to produce repetitive discharges, which cause paralysis (knockdown in insects) and eventually insect death (Septian et al., 2021). *Metarhizium anisopliae* at a concentration of 10^6conidia/ml is also quite high in inhibiting lava feeding activity, but these results are not in line with the very low larval mortality. This is in accordance with

Dampi et al. (2022) that Metarhizium anisopliae is influenced by the level of virulence so that it only has the potential to inhibit feeding, not to infect which causes the death of *Spodoptera frugiperda* larvae. The concentration of *Metarhizium anisopliae* density 10^7conidia/ml showed the lowest results in feeding inhibition activity with quite low mortality. *Metarhizium anisopliae* can inhibit feeding activity but with low potential. This is in accordance with Bayu et al., (2021) that *Metarhizium anisopliae* has a mechanism by producing chitinase, protease, and lipase enzymes in the body of infected larvae and disrupting their physiology.

Conclusion

The conclusion of this research is deltamethrin produced the highest mortality of *Spodoptera frugiperda* of 71% in a short time, namely LT_{50}= 3 days and the highest feed inhibition of 80.45%, while *Metarhizium anisopliae* was less effective with the highest mortality of 8% and required a long time to infect the larvae.

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