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Pathogenicity of Entomopathogenic Fungi *Beauveria bassiana* on Armyworm Larvae (*Spodoptera litura)*

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Spodoptera litura is a polyphagous pest that attacks various types of plants, especially in the Brassicaceae family. Intensive use of synthetic chemical pesticides has a negative impact on the environment, pest resistance, and resurgence in non-target organisms. Control techniques that are more environmentally friendly are an alternative in pest control such as the use of the entomopathogenic fungus Beauveria bassiana. The purpose of this study was to determine the concentration level of Beauveria bassiana fungus that is effective on Spodoptera litura mortality. The research was conducted at the Laboratory of Plant Ecology and Production, Faculty of Animal and Agriculture, Diponegoro University, using a monofactor experiment with a 4x5 completely randomized design (CRD). Application of Beauveria bassiana fungus by dipping Spodoptera litura larvae in suspensions according to concentration levels (control, 10^4, 10^6, and 10^8 conidia/ml). The results showed that the highest larval mortality reached 7%, the highest larval feeding inhibition reached 22,85%, the fastest incubation period of Beauveria bassiana was 117.5 hours, the LC_{50} value was 10^{12} conidia/ml and the LT_{50} was 18,5 days. The use of the entomopathogenic fungus Beuveria bassiana with a concentration of 10^8 conidia/ml can produce mortality of 6%.

Keywords : *beauveria bassiana, spodoptera litura, mortality*

Introduction

Spodoptera litura is a polyphagous pest that attacks various types of plants, such as cabbage, mustard greens, tobacco, soybeans, tomatoes and many other plants. Data from the Central Statistics Agency in 2023 showed a decrease in mustard greens production of 73,732 tons from the previous year. One of the problems in mustard greens cultivation is the attack of the *Spodoptera litura* pest which is the main pest of the plant. The attack of the *Spodoptera litura* pest can reduce mustard greens production by up to 10.31% (Sutarman *et al*., 2022).

Symptoms of an attack by *Spodoptera litura* are that the leaves of the plant will have holes and the leaf veins remain if the attack intensity is high (Rafu *et al*., 2023). Signs of an attack by *Spodoptera litura* are the presence of something left behind from its activities, for example a collection of eggs, feces, saliva that is like fiber, and others. *Spodoptera litura* attacks the leaves of mustard greens which can reduce production by up to 88% (Sikah, 2018) and even cause 100% crop failure if not controlled properly.

The use of synthetic chemical pesticides is still the main choice for farmers because it shows effective and efficient results. However, the negative impact of excessive use of synthetic chemicals is the occurrence of resistance in target pests and resurgence in non-target organisms. One alternative control technique that is more environmentally friendly is biological control using entomopathogens. Biological control using entomopathogens such as bacteria, fungi, and viruses that are pathogenic to insects (Yenny and Rafika, 2022).

Beauveria bassiana is one example of an entomopathogenic fungus that has been widely used in pest control. Research on the use of *Beauveria bassiana* fungus on *Spodoptera litura* larvae has been tested by Afandhi *et al*., (2020) stating that the density of *Beauveria bassiana* conidia 10^8 can produce mortality of 42% in instar II larvae of *Spodoptera litura*. Biological control, especially using the entomopathogen *Beauveria bassiana*, has several advantages, namely it does not cause pest resistance, does not have a negative impact on humans or the environment. The purpose of this study was to determine the level of concentration of *Beauveria bassiana* fungus that is effective against *Spodoptera litura* mortality, and to answer farmers' reasons for using synthetic chemical pesticides.

Research Method

The research was conducted in November 2024 – March 2025 at the Laboratory of Ecology and Crop Production and Screenhouse, Agrotechnoprak, Faculty of Animal Husbandry and Agriculture, Diponegoro University, Semarang.

Research Materials

The materials used in this study consist of tools and materials. The materials used include *Beauveria bassiana* isolates from the collection of BPTPH (Center for the Protection of Food Crops and Horticulture) Central Java, *Spodoptera litura* instar II larvae, 70% alcohol, artificial feed. The tools used include microscopes, hemocytometers, Erlenmeyers, analytical scales, magnetic stirrers, test tubes, vortex mixers, plastic jars, thinwalls, vial bottles, tweezers.

Experimental Design

This study was conducted using a monofactor experiment with a Completely Randomized Design (CRD) consisting of 4 treatments with 5 replications. The treatments used were: B0 (Control, without giving *Beauveria bassiana* (soaked in distilled water)), B1 (Density of 10^4 conidia/ml), B2 (Density of 10^6 conidia/ml, B3 (Density of 10^8 conidia/ml). There were 20 experimental units, each experimental unit contained 20 instar II *Spodoptera litura* larvae, so the number of *Spodoptera litura* larvae needed was 400.

Making Artificial Feed for Spodoptera litura

The preparation of artificial feed is done by mixing 120 grams of red beans (Vigna angularis), 60 grams of cornstarch, 36 grams of fermipan, 3.65 grams of ascorbic acid, 2.2 grams of nipagin, 5 grams of multivitamins, 3 ml of 40% formalin. After that, the feed ingredients are mixed with 10 grams of boiled agar-agar and 500 ml of aquadest with a blender until it forms a dough. The artificial feed mixture is poured into a cleaned tray, then the artificial feed is waited for to harden (Pinto *et al*., 2019). The plastic tray containing the artificial feed is put into a transparent plastic bag and put into the refrigerator. The nutritional content of the artificial feed is still optimal and can be used up to 14 days after the date of manufacture (Taufika *et al*., 2022).

Mass Rearing

Spodoptera litura larvae were obtained from mustard greens farmers' gardens or other plants that were their hosts, then taken to the Ecology and Plant Production Laboratory. The larvae obtained were put into a vial bottle. The larvae were fed with artificial feed that had been made and replaced every 2 days. When they were almost entering the pre-pupa stage, the larvae were transferred into a plastic jar that had been lined with straw paper and given honey and then left until the imago stage. Then the imago was allowed to lay eggs in the jar area, the eggs laid by the imago were then transferred into a plastic jar lined with straw paper and covered with gauze until the eggs hatched and developed into instar II larvae which were used as test insects (Silvani *et al*., 2022).

Making Beauveria bassiana Suspension

Beauveria bassiana fungus in corn media was taken as much as 1 gram then put into a test tube and added 10 ml of distilled water and shaken using a vortex. Before being applied, the resulting suspension was calculated for conidia density first.

Calculation of Beauveria bassiana conidia density

The density of *Beauveria bassiana* conidia can be calculated with the help of a hemocytometer. Beauveria bassiana isolate on corn media is weighed 1 gram then dissolved with 10 ml of sterile distilled water and homogenized using a vortex for 1 minute. The conidium suspension is dropped as much as 0.2 ml slowly until the counting field is filled with the suspension, then left for 1 minute and then observed under a binocular microscope with a magnification of 400x. Then calculate the density of the conidia in the counting box (a + b + c + d + e). If the density calculation result is too high, then a serial dilution is carried out, by taking 1 ml of conidia solution and putting it into a test tube containing 9 ml of sterile distilled water. The density of the conidium is calculated using the following formula:

$$S = \frac{X}{L \times t \times d} \times 10^3$$

Information :
S = Spore density (conidia/ml)
X = Average number of spores in planes a,b,c,d,e
L = Area of counting box 0.04 mm^2
t = depth of counting plane 0.1 mm
d = dilution factor

Bio – assay Test

It is done by dipping the second instar larvae of *Spodoptera litura* for 15 seconds in a 100 ml *Beauveria bassiana* suspension (10 grams of *Beauveria bassiana* isolate + 100 ml of distilled water) according to the treatment. Then drained and placed in a vial bottle.

Research Parameters

Mortality (%)

Pest mortality is the percentage of pests that die. Observations start from 1 HSA (Day After Application) for 7 days. Pest mortality can be calculated using the formula:

$$M = \frac{a}{b} \text{ x } 100\%$$

Description :

M = Mortality percentage
a = Number of dead test insects
b = Total number of test insects

Incubation Period of *Beauveria bassiana* (Hours)

The incubation period is the time required by *Beauveria bassiana* to infect the body of *Spodoptera litura* until it shows initial symptoms. Symptoms that appear are usually white hyphae on the insect's body and changes in insect activity that are reduced. Observations of the incubation period were carried out from 1 hour after application (JSA) until initial symptoms appeared for 24 hours for 7 days.

***Lethal Concentration* (LC_{50})**

To obtain the LC50 value, it was calculated using Microsoft Excel with a probit model analysis calculation to determine the dose that obtained the death of *Spodoptera litura* instar II larvae reaching 50%.

***Lethal Time* (LT_{50}) (Days)**

To obtain the LC50 value, it was calculated using Microsoft Excel with a probit model analysis calculation to determine the time at which the death of *Spodoptera litura* instar II larvae reached 50%.

Feeding inhibition (gram)

Feeding inhibition is a parameter to determine the activity of *Spodoptera litura* larvae. Larvae attacked by *Beauveria bassiana* are characterized by decreased feeding activity and weakened movements which result in death. Observation of feeding inhibition is carried out since 12 hours after application (JSA) for 7 days and calculated using the formula:

$$DHM = \frac{(Ba-Bs)}{Ba} \text{ x } 100 \%$$

Description :

DHM = Feeding inhibition
Bk = Initial Feed Weight
Bp = Final Feed Weight

Data Analysis

The data obtained will be analyzed using Analysis of Variance (ANOVA) with a significance level of 5% to determine the effect of treatment on the observed parameters, if the results allow for continuation, then it will be continued with the further BNT (Smallest Real Difference) test and to determine the maximum point of treatment using the Orthogonal Polynomial test.

Results and Discussion

Mortality of *Spodoptera litura* (%)

Based on the results of the bio-assay test that has been carried out, it shows that the mortality rate of *Spodoptera litura* larvae due to *Beauveria bassiana* fungus is very low. The mortality rate of *Spodoptera litura* larvae in the bio-assay test due to the treatment of entomopathogenic fungus *Beauveria bassiana* is presented in Table 1.

Table 1. Mortality of *Spodoptera litura*

Beauveria bassiana Concentration	Mortality of *Spodoptera litura*
	--------------------------------%--------------------------------
Control	0^{ns}
10^4 conidia/ml	0^{ns}
10^6 conidia/ml	1^{ns}
10^8 conidia/ml	7^{ns}

Description: superscript with symbol *: significant; ns: not significant

Based on Table 1. it can be seen that the highest mortality value of *Spodoptera litura* larvae was found at a *Beauveria bassiana* concentration of 10^8 conidia/ml of 7%. While the lowest mortality value was found in the treatment of 10^6 conidia/ml, which was 1%. The *Beauveria bassiana* concentration treatment did not affect the mortality value of *Spodoptera litura* larvae.

The low virulence of the *Beauveria bassiana* fungus causes the mortality value of *Spodoptera litura* larvae to be very low. The factor that causes the low virulence of the *Beauveria bassiana* fungus is that the *Beauveria bassiana* colony has been in the artificial growth medium for too long, so that it forgets its host. In addition, the low adaptation factor of *Beauveria bassiana* in infecting *Spodoptera litura* larvae is also a challenge in using *Beauveria bassiana* as a biological control (Jeong *et al*. 2023).

Incubation Period of *Beauveria bassiana* (Hours)

Based on the results of the bio-assay test that has been carried out, it shows that the incubation period of Beauveria bassiana against Spodoptera litura larvae is very long. The incubation period of Beauveria bassiana against Spodoptera litura larvae is presented in Table 2.

Table 2. Incubation Period of *Beauveria bassiana*

Beauveria bassiana Concentration	Incubation Period of *Beauveria bassiana*
	-------------------------------Jam------------------------------
Control	$0{,}00^{tn}$
10^4 conidia/ml	$0{,}00^{tn}$
10^6 conidia/ml	144^{tn}
10^8 conidia/ml	$117{,}5^{tn}$

Description: superscript with symbol *: significant; ns: not significant

Based on Table 2, it can be seen that the fastest incubation period of *Beauveria bassiana* against *Spodoptera litura* larvae was at a concentration of 10^8 conidia/ml, which was 117.5 hours. The longest incubation period of *Beauveria bassiana* was at a concentration of 10^6 conidia/ml, which was 144 hours, while at the control and 10^8 conidia/ml treatment levels, no *Spodoptera litura* larvae were attacked by *Beauveria bassiana*.

(a) (b) (c)

Figure 1. Symptoms of mummification in *Spodoptera litura*,
(a) and (b) *Beauveria bassiana* concentration 10^8 conidia/ml, (c) *Beauveria bassiana* concentration 10^6 conidia/ml

The factors that cause the low incubation period of *Beauveria bassiana* are the low viability of *Beauveria bassiana* conidia, temperature and humidity that are not in accordance with the growth of *Beauveria bassiana* fungi. This is in accordance with the statement of Hidayah *et al*. (2019) which states that the optimal temperature for the level of virulence and growth of *Beauveria bassiana* fungi is 25^0C - 27^0C. In addition, spore viability must also be considered, the higher the spore viability value, the greater its potential as an entomopathogen. This is in accordance with the statement of Herlinda *et al*. (2006) which states that the high viability of *Beauveria bassiana* fungus results in the highest mortality in *Plutella xylostella* larvae of 78.33%.

***Lethal Concentration* (LC$_{50}$) and *Lethal Time* (LT$_{50}$)**

Based on the results of the bio-assay test that has been carried out, it shows that the Lethal Concentration value of Beauveria bassiana fungus is 10^{12} conidia/ml and the fastest Lethal Time is 18.5 days. Lethal Concentration (LC50) and Lethal Time (LT50) of *Beauveria bassiana* fungus are presented in Table 3 and Table 4.

Table 3. Lethal Concentration (LC$_{50}$) *Beauveria bassiana*

Lethal Concentration 50%	Concentration
entomopathogenic fungus *Beauveria bassiana*	10^{12} conidia/ml

Table 4. Lethal Time (LT$_{50}$) *Beauveria bassiana*

Beauveria bassiana Concentration	Lethal Time (LT$_{50}$)
	----------------------days----------------------
Control	0
10^4 Conidia/ml	0
10^6 Conidia/ml	104,3
10^8 Conidia/ml	18,5

Based on Table 3. it can be seen that the Lethal Concentration value of 50% of *Beauveria bassiana* fungus is 10^{12} conidia/ml. This means that *Beauveria bassiana* fungus is able to kill 50% of the population of *Spodoptera litura* test larvae, namely at a concentration of 10^{12} conidia/ml. Meanwhile, the fastest Lethal Time value of 50% is 18.5 days at a concentration of 10^{8} conidia/ml. This means that *Beauveria bassiana* fungus at a concentration of 10^{8} conidia/ml is able to kill 50% of the population of *Spodoptera litura* test larvae, requiring 18.5 days.

Based on the LC50 and LT50 values, the challenge in using *Beauveria bassiana* fungus as an entomopathogen is to obtain a high concentration of up to 10^{12} conidia/ml. The factor that causes the low LC50 and LT50 values is due to the *Beauveria bassiana* fungus isolate being in the artificial media for too long. *Beauveria bassiana* fungal isolates that have been in artificial media for too long can affect the virulence of the fungus in infecting its host.

Feeding Inhibiton (%)

Based on the results of the bio-assay test that has been carried out, it shows that Beauveria bassiana treatment affects the inhibition of Spodoptera litura larvae feeding. The inhibition of Spodoptera litura larvae feeding is presented in Figure 2.

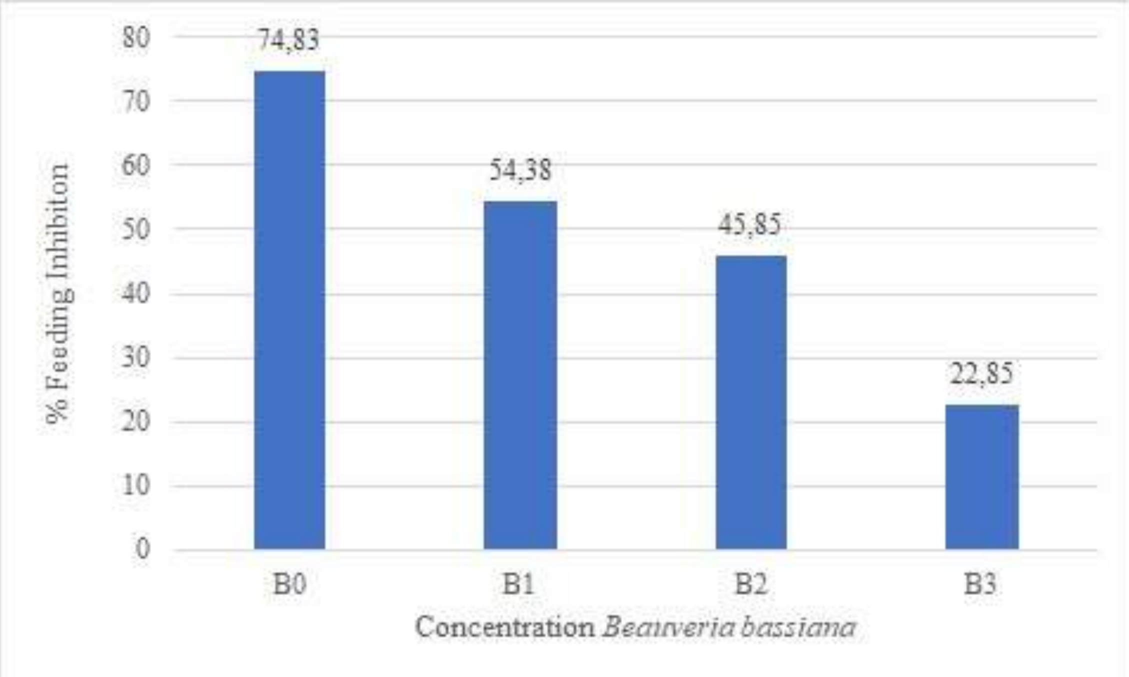

Figure 2. Feeding Inhibition of *Spodoptera litura* Larvae

Based on Figure 2. It can be seen that the *Beauveria bassiana* concentrate treatment of 108 conidia/ml was able to inhibit the feeding activity of *Spodoptera litura* larvae by 22.85%, meaning that only 22.85% of the feed was eaten by *Spodoptera litura* larvae, while at the lowest concentration of 10^{4} conidia/ml *Spodoptera litura* larvae at 54.38% of the feed. *Beauveria bassiana* fungus was only able to inhibit the feeding activity of *Spodoptera litura* larvae, but it took a long time to cause death in *Spodoptera litura* larvae.

Conclusion

The conclusion of this study is: 1). The highest mortality of *Spodoptera litura* larvae was found at a concentration 10^8 conidia/ml but does not provide effective results. 2) *Beauveria bassiana* is able to inhibit the feeding activity of *Spodoptera litura* larvae, but it takes longer to cause death.

The suggestion for future research is that the growth media of Beauveria bassiana fungus needs to be studied further, in order to obtain a fungus with a high level of virulence.

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