

Pathogenicity Efficacy of Entomopathogen Fungus *Beauveria bassiana* Against In Vitro Rice Stem Borer (*Scirpophaga innotata*)

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ABSTRACT

White rice stem borer (*Scirpophaga innotata*) is one of the dangerous pests for rice (*Oryza sativa*) because it attacks rice stems that can cause 50% loss of total production. The purpose of this study was to determine the pathogenicity of Entomopathogen Fungus *Beauveria bassiana* at different conidia dilutions (J), namely J1 (10^5), J2 (10^6), J3 (10^7), J4 (10^8), and J5 (10^9). The mortality of *S. innotata* larvae was tested with the density of *B. bassiana* of 2.08×10^6 conidia/ml. The effect of *B. bassiana* on the success transformation of *S. innotata* larvae to pupae for 14 observation day was also observed at the same *B. bassiana* density. This research resulted in the optimal pathogenicity of *B. bassiana* at dilution of 10^7 (treatment J3) which caused 100% death and 100% successful pupation. The lowest treatment was J5 (10^9 dilution) with a mortality rate of 50% and a successful pupation of 20%. The results of the probit analysis showed that the fastest average lethal time (LT_{50}) value was found in treatment of 10^7 (J3) which reached 11.11 h.

1. INTRODUCTION

The white stem borer (*Scirpophaga innotata*) is a major pest for rice crop that has not been resolved and can cause losses to farmers up to 50% of the total production. The intensity of pest attacks recorded from 1998 to 2021 in Indonesia reached 20.5% in an area of 151,577 ha. This stem borer has a clustered distribution pattern, resulting in higher yield losses compared to other borers (Olden *et al.*, 2021). The highest attack of *S. innotata* was reported in Luwu Regency with an intensity of up to 609 ha and North Luwu with an area of land affected of 1035 ha (Rahmawasih *et al.*, 2022).

Based on field surveys at rice production centers in Deliserdang Regency in 2023, control of white stem borer at the farmer level still use Vayego as a synthetic insecticide. The use of synthetic insecticides causes various environmental problems, such as water and soil pollution and the danger of poisoning in humans who come into direct contact with chemical insecticides. In addition, the practice can also cause resistance and resurgence of this potential pest (Dewata & Danhas, 2023). Based on analysis of extracellular enzyme activity, *Bemisia tabaci* contains chitinase, lipase and protease of 3.41, 4.45 and 5.44, respectively. Meanwhile, pathogenicity testing of *Bemisia tabaci* showed that its effectiveness ranged from 71.67-98.33% (Gebremariam *et al.*, 2022). Testing the effectiveness of the *B. bassiana* suspension against the pest *Euschistus heros* with a conidia density of 10^9 conidia/mL showed a mortality of 80-90% (Silva-Santana *et al.*, 2022).

One potential biological control technique is through the use of entomopathogenic fungi. One potential fungus in controlling several species of insect pests is *Beauveria bassiana* (Dannon *et al.*, 2020). In several previous studies, the

B. bassiana fungus was developed as a biological control agent against several ordoes such as *Coleoptera*, *Lepidoptera*, *Hemiptera*, and *Orthoptera* (Baron *et al.*, 2019; Mahankuda & Bhatt, 2019). Larvae in these ordoes are important pests in rice cultivation and one of them is *Scirpophaga innotata* (Bayu *et al.*, 2020). The use of *B. bassiana* in rice plantations is reported as effective in inhibiting the development of the stink bug (Telaumbanua *et al.*, 2020).

Research on differences in *B. bassiana* conidia density levels on *S. innotata* larvae mortality is very necessary to see different levels of virulence. Testing the effectiveness of entomopathogenic fungi requires several conidia densities as a treatment to test their ability to infect (Erika *et al.*, 2023). Therefore, research was carried out to test the pathogenicity of the entomopathogenic fungus *B. bassiana* against *S. innotata* larvae at several different density levels. So it was found that the density level was effective in causing the death of *S. innotata* larvae. This research was conducted to test the pathogenicity of the fungus *B. bassiana* at density levels of 10^5 , 10^6 , 10^7 , 10^8 , and 10^9 conidia/ml against *S. innotata* larvae and its effect on the success of *S. innotata* larvae becoming pupae.

2. MATERIAL AND METHODS

This research was carried out at the Microbiology Laboratory, Faculty of Mathematics and Natural Sciences (FMIPA), University of North Sumatra, Medan, from March 2023 to April 2023. The tools used are an Olympus CX43 microscope, autoclave, Haemocytometer, VS-8480SN incubator shaker, 1000 µl micropipette, hand sprayer, stirrer, vortex, vision mixer, tweezers, Bunsen, hot plate (Thermo scientific), digital scales (Sartorius), filter, petri dish, Erlenmeyer flask, knife, plastic wrapping, stationery, wet and dry tissue, cells calculator application.

The material that will be used in this research is white rice stem borer originating from Inpari 32 variety rice plantations in East Sambirejo Village, Subdistrict of Percut Sei Tuan, Deli Serdang Regency, North Sumatra Province. The entomopathogenic fungus isolate *B. bassiana* was obtained from the Balai Besar Perbenihan dan Proteksi Tanaman Perkebunan, Medan. Chemical ingredients include 20 grams of dextrose, Potato Dextrose Agar (PDA), 20 grams of Mutiara brand agar, chloramphenicol, 200 grams of potatoes, 25 ml of Potato Dextrose Broth (PDB), 70% alcohol and sterile distilled water. Other materials include masks, gloves and paper labels.

2.1. Preparation of Test Insects

Collection of white stem borers was done by picking them directly from the affected rice. The borer was taken along with a piece of rice straw where the larvae are located and then put it in a bottle that has air circulation (by making a small holes in the bottle). At the bottom of the bottle there was tissue paper to maintain moisture before being taken to the laboratory for further research. The larvae sampled had characteristic of white color with a length of 10 to 25 mm. The larvae were collected from a rice field in the phase of milk ripe (81-90 DAP). The time it takes for test insects to be taken to the laboratory is around 2 h.

2.2. Preparation of Entomopathogenic Fungi

The *B. bassiana* fungus was obtained from the Balai Besar Perbenihan dan Proteksi Tanaman Perkebunan (BBPPTP) Helvetia, Medan. This fungus was available in culture form in PDA. Then the fungal isolate was multiplied using PDA media which had been made previously and was placed in a petri dish which had been sterilized by autoclaving. After inoculating the fungus from the old media to the new media, it was then kept in a shaker incubator for 24 h at a temperature of 30 °C and a speed of 130 rpm so that the culture develops.

2.3. Dilution of Entomopathogenic Fungus

Before dilution into several treatments, a *B. bassiana* culture suspension with a PDB of 25 ml was first made in order to change the form of the isolate, which was initially a solid, to a solution. The isolate was transferred from PDA to PDB in a sterile manner (close to Bunsen) using tweezers and then placed into a test tube containing 25 ml of PDB. Then the test tube was homogenized with a Vortex Mixer Vision for 1 min. Next, the PDB was incubated at 31 °C for 24 h according to the procedure described by (Kirana *et al.*, 2014). The use of PDB is as a liquid medium to ensure the availability of nutritional sources for cultures in this liquid medium. It was then shaken using shaker incubator for 24 h at a speed of 130 rpm and temperature of 30 °C. Then 1 ml of the pure isolate was taken using a micropipette and put

into a test tube containing 9 ml of distilled water. After that, homogenization was carried out using a vortex for 1 min. Then 1 ml of this solution was taken using a micropipette and then put into a new test tube containing 9 ml of distilled water and homogenized. This dilution was carried out 9 times to have a concentration of up to 10^{-9} . Then take fungus samples according to the dilution treatment required.

2.4. Calculating Conidia Density

The solution that has been diluted was taken with a micropipette and dropped onto a haemocytometer and then covered with a deck glass so that the conidia density is 2.08×10^6 . The spore density (C) was calculated using a light microscope using the formula described by (Indriyanti *et al.*, 2016):

$$C = t/n \cdot 0.25 \times 10^6 \quad (1)$$

where t is total number of conidia in the observed sample box, and n is number of sample boxes observed; 0.25 is a correction factor for the use of a small-scale sample box in a hemocytometer.

2.5. Applications of Entomopathogenic Fungi

As much as 1 ml of the entomopathogenic fungal solution that had been diluted was taken and transferred to a petri dish that had been prepared and marked for each treatment with two replications due to limitations in the process of providing uniform samples. Then, after placing it in a petri dish, it is then leveled with a stirrer so that the treatment spreads throughout the PDA (evenly). Every time a job is moved at each treatment level, the stirrer is sprayed with 70% alcohol to prevent mixing and contamination. The control treatment was only sprayed with sterile distilled water. The application of entomopathogenic fungi was carried out in vitro by inoculating 5 larvae of *S. innotata* placed in spread in a Petri dish with solution containing *B. bassiana* conidia. Six different treatment was selected according to the *B. bassiana* conidia addition and solution dilution, namely J0 (Control, no *B. bassiana* conidia), J1 (*B. bassiana* conidia, dilution 10^5), J2 (*B. bassiana* conidia, dilution 10^6), J3 (*B. bassiana* conidia, dilution 10^7), J4 (*B. bassiana* conidia, dilution 10^8), and J5 (*B. bassiana* conidia, dilution 10^9). All tests were performed with 2 duplicates.

2.6. Mortality Percentage

The death of *S. innotata* larvae was observed every 3 h for 48 h after inoculation. Observations were carried out until an average of 100% was obtained, namely at 30 h after inoculation. The percentage of larva mortality (P) was carried out by counting the dead larvae using the formula:

$$P = (a/b) \times 100\% \quad (2)$$

where a is the number of dead larvae, and b is the number of larvae observed. After obtaining the mortality data, a probit analysis was then carried out which was intended to estimate the effective dose by determining the mortality concentration and determining the LT50 in this study.

3. RESULTS AND DISCUSSION

3.1. Pathogenicity of *B. bassiana* Isolates on *S. innotata* Mortality

Figure 1 (left) shows microscopic observation of *B. bassiana* using a haemocytometer which was then viewed with a microscope with a magnification of 40x. It could be confirmed that the fungus used was *B. bassiana* with the characteristic conidia being oval, slightly round to round like an egg and thread-colored or hyaline (Kastilong, 2021). According to (Halwiyah *et al.* (2019), the mycelium of the *B. bassiana* fungus is shaped like fine threads with a powdery shape and is white in color accompanied by oval (slightly round) to egg-shaped conidia with a hyaline color. From Figure 2, it can be seen that the spread of mycelium is thought to be due to dilution activities first before being tested with a haemocytometer and then the density is calculated using the CellsCalculator application, resulting in a density of 2.08×10^7 conidia/ml (not from the initial PDB suspension). In line with research (Pelawi *et al.*, Handayani, 2021) that microscopic observations of isolates of RTM3(3) *Aspergillus* sp. looks spread and is only guided by identification through the round to semi-round shape of the spores.

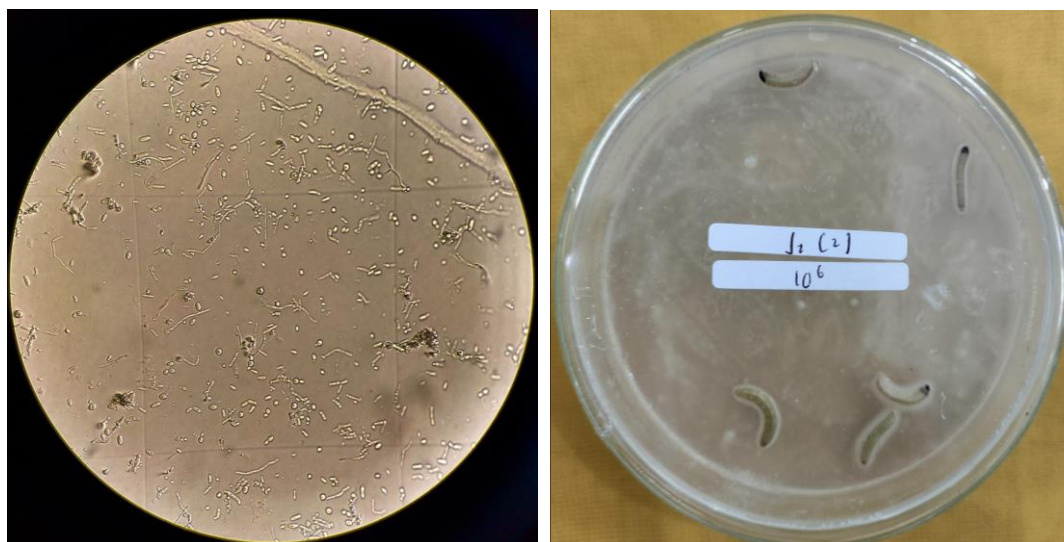


Figure 1. Microscopic visualization of *B. bassiana* suspension (left), and Visualization of *S. innotata* larva mortality (right)

Mechanical identification carried out personally showed that the characteristics of larval death can be observed macroscopically, namely the absence of movement of the larva's body nodes. Furthermore, there is a white line around the larva that neatly follows the shape of the larva's body (Figure 1, right). This is in line with the observation of [Susanto *et al.* \(2015\)](#) that if the larva still shows movement in its body then it is said to be alive, but if no movement then it has already died. There is literature regarding other physiological characteristics, namely color change to black and hardening. According to [Ni'mah *et al.* \(2021\)](#), larvae infected with *B. bassiana* will show hardened body changes and a change in color to blackish brown. However, during the observation, the dead larvae that experienced a change in color, only a small number of which was blackish brown, while majority turn to pink. The pink pigment is an indication of prodigiosin compound, which is a secondary metabolite from *S. innotata* ([Kahar *et al.*, 2019](#)).

Table 1. Average death percentage of *S. innotata* larvae due to *B. bassiana* fungus in different conidia density treatments

<i>B. bassiana</i> Treatment	Conidia Dilution	Death of <i>Scirpophaga innotata</i> larvae (%) after inoculation time (h)						
		3	6	12	15	18	24	30
J1	$\times 10^5$	20	40	30	10	-	-	-
J2	$\times 10^6$	30	20	10	-	-	40	-
J3	$\times 10^7$	40	30	30	-	-	-	-
J4	$\times 10^8$	-	20	20	30	20	10	-
J5	$\times 10^9$	-	-	-	-	20	30	50

The mortality percentage of *S. innotata* larvae due to inoculation of *B. bassiana* isolates is presented in Table 1. It can be seen that density of *B. bassiana* conidia had a significant effect on the mortality of *S. innotata* larvae. Observations carried out every 3 h showed that the highest death rate for *S. innotata* larvae was at 3 concentrations, namely 10^5 , 10^6 , and 10^7 , amounting to 100%. However, among the 3 concentrations, the best mortality results were obtained in treatment 10^7 . This can be seen from the number of dead larvae, namely 4 larvae with 1 larva in replicate sample 1 and the other 3 in replicate sample 2 in the first 3 h of observation. Identification of death is based on biochemical and mechanical indications of death. An indication of biochemical death is that the *Beauveria bassiana* fungus attached to the larva's body has penetrated the larva's body chemically with the chitinase enzyme. Infection of the larva's body causes an increase in blood pH, blood clots, and cessation of blood circulation in the larvae which causes death and then the dead larvae will grow mycelium from the fungus *B. bassiana* ([Fadhilah *et al.*, 2019](#)). In addition, the fungus will release beauverin poison which damages the insect's body tissue so that within days the insect will die ([Kastilong & Elhanan, 2021](#)). Toxic peptide compounds are also effective in controlling the growth of mosquito larvae ([Jelenić *et al.*, 2021](#)). Bauvarin from *B. bassiana* has also been reported to affect root nodes caused by the parasitic nematode, namely *Meloidogyne*, and trigger plant growth ([Pant *et al.*, 2018](#)). This compound is also

reported to be found in *Trichoderma* sp. N4-3 isolated from the rhizosphere of banana plants is effective for controlling the fungus that causes banana root rot (*Fusarium oxysporum* f. sp.) (Long *et al.*, 2022).

The level of pathogenicity of *B. bassiana* on the mortality of *S. innotata* larvae is classified as high. The pathogenicity classification can be divided into three levels, namely the highest with a mortality percentage of more than 64.49%, moderate pathogenicity with a mortality of 30.99% to 64.49%, and a mortality percentage of less than 30.99% classified as low pathogenicity (Budi *et al.*, 2013). In some samples with low pathogenicity, it may be due to a decrease in the virulence ability of isolates from culture. According to (Alfian *et al.*, 2022), there is another strong factor that causing the low mortality, namely the decrease in virulence ability due to cultures that have been repeatedly transferred to new media so that the infection mechanism does not work optimally. The *B. bassiana* isolate used in this study was provided by the BBPPTP, and was not a pure isolate in the host insect but was only propagated via PDA media. This can trigger random mutations that can affect or even reduce the virulence ability of *B. bassiana* isolates.

After 3 h of observation (post-inoculation), 1 larvae in treatment 10^5 (J1) from both replications began to show no movement so it could be concluded that they were death (Figure 1). Then at the same hour followed by treatment J2 with 2 larvae showing signs of no movement (an indication of death). Then the J3 treatment showed better results because in the first 3 h of observation after inoculation it was found that 2 larvae died. Meanwhile, in treatments J4 and J5 there were no dead larvae, as did the controls.

From observations made on all treatments except control, the one that experienced the fastest mortality was treatment J3 with maximum observations in the first 12 h reaching 100%. Then the longest time in this mortality test was the J4 treatment at the 30th h after inoculation. Based on personal identification, the characteristics of the last larva to die were recorded after 30 h of observation, showing that there was no movement in the body parts, meaning already died. From the observations above, it can be concluded that treatment J3 resulted in the fastest and most potential pest mortality, followed by treatments J1 and J2 in sequence, while treatments J5 and J4 provided relatively longer mortality.

The *B. bassiana* fungus is still capable of providing up to 100% mortality in each dilution treatment. This potential has also been proven in previous research, such as (Alfian *et al.*, 2022) who conducted experiments with 3 treatment levels, namely 10 g/L, 20 g/L, and 30 g/L. The results showed that the fungus in the 10 g/L treatment had a higher pathogenicity effect than the control. Testing the pathogenicity of the fungus *B. bassiana* on *S. innotata* larvae showed better results than testing on armyworm larvae. As stated by (Harun *et al.*, 2022), the fungal infection process of *B. bassiana* against *frugiperda* armyworm larvae (*Spodoptera frugiperda*) takes a minimum of 2 d and a maximum of 10 d. However, they have similarity on the level of pathogenicity which has an average capacity of above 45%.

3.2. Lethal Time (LT₅₀) of *S. innotata* Larvae

Lethal time (LT₅₀) is interpreted as the time required to kill 50% of the total number of insects tested. Based on the calculations, the higher the density of *B. bassiana* conidia applied to *S. innotata* larvae, the faster the larva mortality will be. This is due to the accumulation of toxic compounds produced from *B. bassiana* which are destructive to *S. innotata* larvae. Calculation results showed that the average mortality rate that occurred in larvae was between 11.11 h after inoculation and a maximum of 20.29 h after inoculation (Table 2). The calculation for LT₅₀ was based on regression equation resulted from probit analysis as presented in Figure 3.

Table 2. Average value of LT₅₀ of *B. bassiana* on the *S. innotata* larva according to different dilution of conidia solution

Treatment	Conidia dilution	Regression Equation	LT ₅₀ (hour)
J1	10^5	$Y = 2.921x + 1.382$	17.32
J2	10^6	$Y = 2.796x + 1.345$	20.29
J3	10^7	$Y = 3.490x + 1.350$	11.11
J4	10^8	$Y = 3.880x + 0.379$	15.52
J5	10^9	$Y = 2.922x + 1.418$	16.82

Note: Observation was conducted for 14 days.

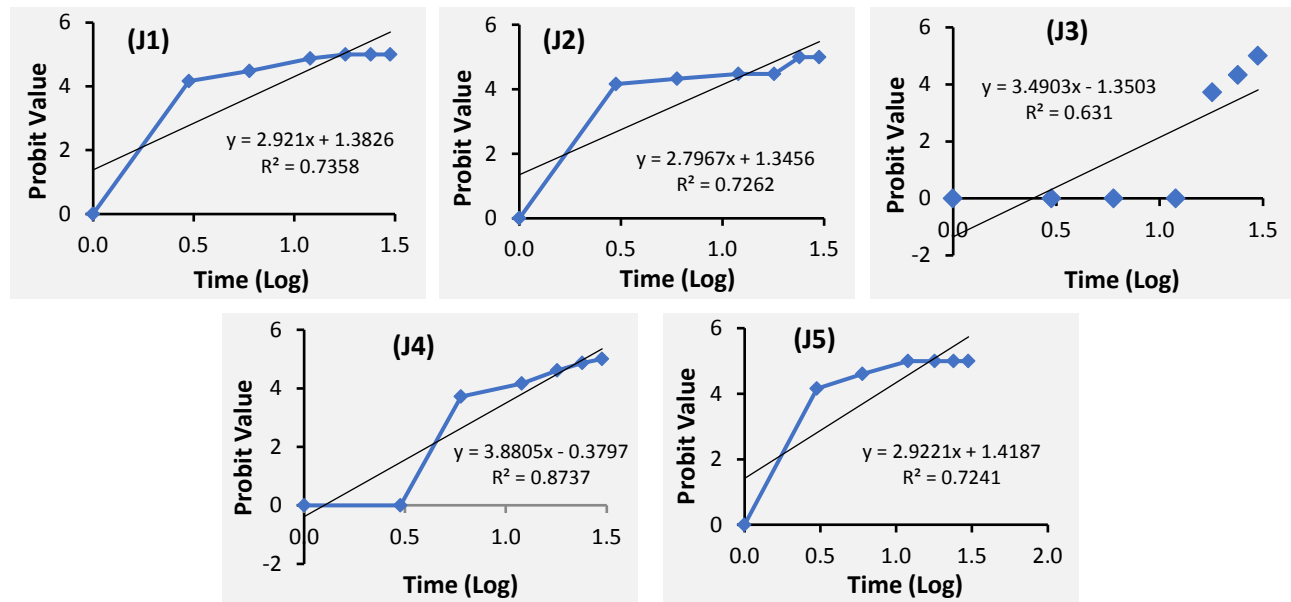


Figure 3. Probit analysis to find LT_{50} for conidia dilution treatments J1 – J5

From Table 2, it can be seen that the density of the *Beauveria bassiana* fungus which causes LT_{50} at a density of 2.08×10^6 conidia/ml is at the fastest time, namely treatment J3 (10^7) for 11.11 hours. The data showing differences in LT_{50} in each treatment is based on the assumption that it is caused by the number of conidia of the *Beauveria bassiana* fungus that attach to the larva's body with different abilities. This is in line with (Budi *et al.*, 2013) that the higher the attack, the faster the larvae die from infection.

3.3. Percentage of *S. innotata* Larvae that Successfully Pupae

Figure 3 shows the transformation of *S. innotata* larvae that successfully change into pupae under J3 treatment (conidia dilution of 10^7). Based on the analysis carried out, it showed that the conidia density treatment of the *Beauveria bassiana* fungus had an effect on the percentage of success in turning *Scirpophaga innotata* larvae into white pupae (Table 3). From observations made over 14 days, the appearance of pupae started from treatment J1 (10^5) on day 3, then on day 4 the appearance of pupae on the body segments of larvae in treatment J2 (10^6) and J3 (10^7). Treatments J1 and J3 had pupa emergence up to 100%. However, the best development and pupa form was in the J3 treatment with a dilution level of (10^7). This is characterized by the development of white hyphae which then cover the

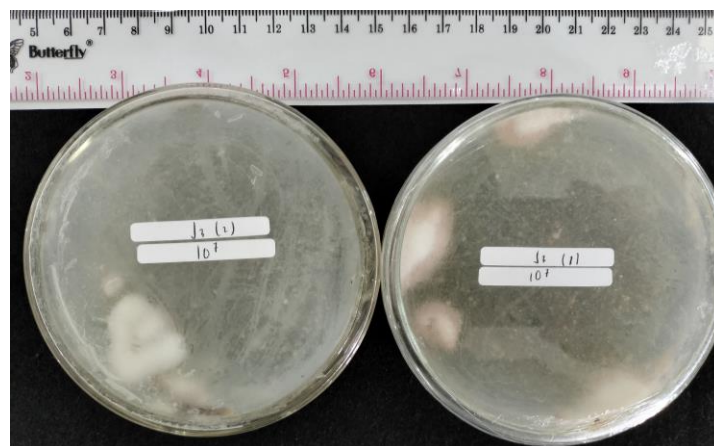


Figure 4. Treatment sample J3 (10^7) on the 14th day of observation

Table 3. Average percentage on the successfully transformation of *S. innotata* larvae into pupae

Treatment	Conidia dilution	Pupa change (%)	Corrected change (%)
J1	10 ⁵	100	100.0
J2	10 ⁶	60	55.6
J3	10 ⁷	100	100.0
J4	10 ⁸	50	44.4
J5	10 ⁹	20	11.1

body segments of the larva until they cover all parts of the body of the *S. innotata* larva (Figure 3). Meanwhile, the treatment with the lowest rate of transformation into pupae was treatment J5 (10⁹) with 2 larvae. This figure is almost the same as that of the control which did not change into a pupa. This treatment was good in terms of color change, mortality and change to pupa which was relatively low compared to the previous treatment.

4. CONCLUSIONS

From the research that has been conducted, several conclusions can be drawn, including the following:

1. Dilution of the *Beauveria bassiana* fungus which can cause up to 100% death of *Scirpophaga innotata* larvae, there are three types of treatment levels, namely treatment J1 (dilution 10⁵), treatment J2 (dilution 10⁶), and treatment J3 (dilution 10⁷).
2. The treatment that caused the highest hyphae to appear was treatment J1 (dilution 10⁵) and treatment J3 (dilution 10⁷), whereas the treatment that gave rise to the lowest hyphae was treatment J5 (dilution 10⁹), which was 20%.
3. The density of the entomopathogenic fungus *Beauveria bassiana* which resulted in the fastest lethal time (LT₅₀) on *Scirpophaga innotata* larvae, namely treatment J3 (dilution 10⁷) at the same isolate density of 2.08×10^6 conidia/ml within 11.11 h.

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