Virulence of Beauveria bassiana (Bals.) Vuill. (Deuteromycotina: Hyphomycetes) on Tribolium castaneum (Tenebrionidae: Coleoptera)

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Abstract— *Tribolium castaneum* is one of the pests that can cause serious damage and great losses on products in the warehouse. *Beauveia bassiana* is one of the entomopathogenic fungi that can be used to control *T. castaneum*. The purpose of this study was to determine the relationship between the type of growth media and the level of concentration on the mortality of *T. castaneum*. Application tests were carried out with 3 types of media, namely PDA, rice and corn media, while dilution was carried out at 5 media dilution levels, namely 10^4, 10^5, 10^6, 10^7, and 10^8 at a dose of 3 mL (5 times spray/repetition). This study uses a Factorial Complete Randomized Design (RALF). Testing 10^7 levels of dilution on PDA media resulted in *T. castaneum* mortality (71.11%), 10^6 on rice media dilutions (51.11%), and 10^5 dilutions on corn media (31.11%). LC_{50} value of 2.23 x 10^8 conidia / mL. The LT_{50} value at 10^8 conidia / mL density is 8,315 (8 - 9 days).

Keywords: Bioinsecticides, Entomopathogenic, Fungus, Mortality, Rate of dilution.

1 INTRODUCTION

Warehouse pest *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) causes product damage to warehouses in Indonesia and around the world [1]. Imago and larvae *T. castaneum* damage flour during storage and cause physical and chemical changes in flour [2]. Besides, *T. castaneum* emits toxic quinones which are carcinogenic and cause risks to human health. The level of economic damage to flour during storage ranges from 34-40% [3].

Biological control with pathogens is an alternative solution in suppressing *T. castaneum* populations in storage warehouses [4][5]. Among the invertebrate fungal pathogens, *B. bassiana* has taken a key role in the management of several agricultural, veterinary, and forestry arthropod pests [6]. This fungus is widely used as a biocontrol agent for a number of insect pests [7][8] and the efficiency of *B. bassiana* has been proven against several Lepidoptera, such as *Castnia licus* Drury [9], *Ostrinia nubilalis* Hübner [10], *Plutella xylostella* L. [11], *Spodoptera frugiperda* Smith [12], *Cylas formicarius* (Coleoptera: Curculionidae) [13], *Frankliniella occidentalis* (Thysanoptera: Thripidae) [14], *Spodoptera litura* (Lepidoptera: Noctuidae) [15], *Crocidolomia pavonana* (Lepidoptera: Crambidae) [16] dan *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) [17].

However, data on the potential of *B. bassiana* entomopathogenic fungi as bioinsecticides against *T. castaneum* are not widely available in Indonesia. This research is expected to contribute to providing preliminary data on the ability of the entomopathogenic fungal infection. The study was conducted to test the effectiveness of *B. bassiana* isolate against *T. castaneum* (Herbst) (Coleoptera: Tenebrionidae) in the Laboratory.

2. MATERIALS AND METHODS

The research was carried out at the Plant Pest and Disease Laboratory, Department of Plant Protection, Faculty of Agriculture, Hasanuddin University. This research took place from May to October 2019.

2.1 Preparation of test insects

The insect pest of the warehouse of *T. castaneum* (Coleoptera: Tenebrionidae) was collected from the animal feed storage shed. Insects are put into plastic containers in which corn seeds are ground which has been ground as food and stored in a laboratory at room temperature. The warehouse pest insect used as a test insect is imago.

2.2 Exploration of *B. bassiana* isolates

Isolation of fungi was carried out by the method of insect baiting (insect bait method), ie the feed sample in the Laboratory area of livestock was first regulated with moisture by providing sufficient water (15-50% humidity). Insects used as fishing insects are larvae and imago from *T. castaneum*. larvae and imago from *T. castaneum* are placed in plastic containers filled with corn feed. Furthermore, the container is closed using gauze so that the larvae and imago do not come out of the container, then wait for 1-2 weeks in a dark place so that the larvae move actively until it is in contact with the entomopathogens fungus that is in the performance of the feed sample [18].

2.3 Propagation of fungi on PDA media

In this study, *B. bassiana* isolates used were from the main host of *T. castaneum*. The composition of the PDA media used is 200 g potatoes, 20 g dextrose, 1 g chloramphenicol, 20 g agar, and 1 L distilled water [19]. The PDA media is compacted in a 9 cm diameter petri dish. The fungus was incubated for 14 days at room temperature.

2.4 Propagation of fungi on rice and corn media

The results of a pure culture of *B. bassiana* which is 14 days old are grown again on rice media. It aims to get a higher conidia density. Washed rice thoroughly, half-cooked then cooled in a plastic tray. Rice media as much as ± 50 g were put into HDPE (high-density polyethylene) clear plastic bags, sterilized in an autoclave (for 35 minutes at 121^{o}C and 1 atm pressure). After cooling, the media were inoculated with *B. bassiana* each. Inoculation was carried out by spraying the fungus conidia suspension into a rice bag. All stages are carried out under sterile conditions in laminar airflow. This fungus culture was incubated for 21 days which is the optimum age for testing [19]. Whereas for isolate inoculation on corn media white corn was washed with clean water and then soaked for 12 hours. Furthermore, the process of filling into HDPE plastic sheet, sterilization, and inoculation was carried out according to the method of inoculation on rice media [20].

2.5 Preparation of Boletus Suspension for Testing

The results of *B. bassiana* culture on rice and corn media were made as a suspension. Medium rice/corn that has been overgrown with fungi taken as much as 2 bags (50 g / bag), added 100 ml of sterile distilled water and Tween 20 solution of 0.025 ml per 50 ml of water (0.05%) and crushed in a mortar. The mixture is filtered with fine gauze, then put into a 100 ml Erlenmeyer flask and shaken with vortex for 30 seconds until homogeneous. The conidia density of each suspension was calculated with a Neubauer hemocytometer improved until the highest conidia density was 10^8 conidia/mL. The required conidia density is obtained by making multilevel dilution with a mixture of sterile aquades + tween [19].

2.6 Application of Fungi on *T. castaneum*

Suspension of *B. bassiana* isolates which had been calculated for conidial density was then applied to *T. castaneum* imago. The conidia density used was 10^4, 10^5, 10^6, 10^7 and 10^8 conidia/mL. Application is done by spraying 3 ml of conidia suspension on the *T. castaneum* imago. Test insects were put into plastic containers measuring 20 cm x 15 cm x 5 cm (p x l x t) which had been given corn as a source of imago feed and covered with gauze. As a control, test insects were sprayed with distilled water with the same volume of the tween. Each unit of experiment consisted of 15 individual test insects and was repeated 3 times. Variables observed were the number of imagoes that died due to infection with *B. bassiana* which was calculated from the time of application up to 9 days after application.

The percentage of imago mortality is calculated using the formula:

$$M = \frac{A}{D} \times 100\%$$

Information:
M = Percentage of mortality (%)
A = Number of insects that have died due to fungal infections
D = Number of insects tested

This research uses a completely randomized factorial design. The middle value was tested by Least Significance Different (LSD) with a 5% significance level.

2.7 Data analysis

Data were analyzed using Microsoft Excel 2010, SPSS 13.0 for Windows and tested further with a test of significantly different honest (α = 0.05). Death concentration (LC_{50}) and time of death (LT_{50}) were analyzed by probit analysis using SPSS 13.0 for windows.

3.RESULTS AND DISCUSSION

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3.1 Mortality of *T. castaneum* to the level of dilution of *B. bassiana* in different media

In general, the application of entomopathogenic *B. bassiana* shows that the type of media and the level of conidia density significantly affect the mortality of test insects. Testing the dilution rate of 10^7 on PDA media resulted in *T. castaneum* mortality of 71.11%. In rice media, the highest mortality rate of test insects occurred at dilution of 10^6 (51.11%), whereas in maize media the highest mortality occurred at the dilution rate of 10^5 (31.11%) (Table 1). The application at the lowest dilution rate, which is 10^4 conidia/mL resulted in the number of test insects experiencing mortality of 40% on PDA and Rice media, while on Corn media it was 26.67%. The percentage of totality is significantly different from the control and can be said to be effective in suppressing

T. castaneum populations in storage. The mortality rates of *T. castaneum* on PDA (10^7 and 10^8) and rice (10^5) media have exceeded 50% so that the probit analysis is then performed to determine the LC_{50} and LT_{50} values. The results of probit analysis showed that LC_{50} and LT_{50} values on PDA media were getting smaller with increasing levels of kodia density (Table 2). This is in accordance with the increase in mortality starting from 10^4, 10^5, and 10^6 and the increase in mortality is higher at 10^7. While in the corn media the mortality rate is ≤ 50%.

Table 1. Mortality of *T. castaneum* treated with *B. bassiana* dilution levels in different media

Conidia density level (mL)	Mortality rates (%) test insect of medium type					
	PDA		Rice		Corn	
Control	6.67	h	4.44	h	6.67	h
10^4	40.00	bcd	40.00	bcd	26.67	defg
10^5	48.89	bc	37.78	bcd	31.11	cdef
10^6	46.67	bc	51.11	b	20.00	efgh
10^7	71.11	a	35.56	bcde	17.78	efgh
10^8	53.33	ab	13.33	fgh	11.11	gh
LSD (0.05%)			19.301			

* The average mortality percentage followed by the same letter shows no significant difference based on the LSD test level of 5%.

The mortality rates of *T. castaneum* on PDA (10^7 and 10^8) and rice (10^5) media have exceeded 50% so that the probit analysis is then performed to determine the LC_{50} and LT_{50} values. The results of probit analysis showed that LC_{50} and LT_{50} values on PDA media were getting smaller with increasing levels of kodia density (Table 2). This is in accordance with the increase in mortality starting from 10^4, 10^5, and 10^6 and the increase in mortality is higher at 10^7. While in the corn media the mortality rate is ≤ 50%.

3.2 Effect of conidia density on test insect mortality

The level of conidia density influences the mortality of *T. castaneum*. The relationship between *T. castaneum* mortality and conidia density is shown by the regression equation (Figure 1).

The results of tests conducted on PDA media showed an increase in mortality in conidia density 10^7. The relationship between mortality of test insects with *B. bassiana* conidia density as indicated by a regression equation y = 0.25x - 0.65. That is, if the conidia density increases by one unit, then mortality will go up by 0.25%. Conidia densities 10^4, 10^5, 10^6, 10^7, and 10^8 conidia/mL indicate a linear relationship with the value $R^2 = 0.424$ (Figure 1a).

the mortality rate of test insects on Rice media showed that the density of 10^6 was high mortality with a regression equation y = 0.35x - 1.55. That is, if the density increases by one unit, then mortality will go up by 0.35%. The regression line obtained shows a linear relationship with the value of $R^2 = 0.438$ (Figure 1b). While the mortality of test insects on the highest corn media at conidia density 10^5 with a regression equation Y = 0.25x - 1.65 with a linear relationship with the value $R^2 = 0.833$ (Figure 1c)..

3.3 LC_{50} and LT_{50} values in the Insect Mortality Test

The results of the *B. bassiana* fungus probit analysis conducted on the 9th day after treatment obtained LC_{50} values for PDA media of 2.23 x 10^8 conidia / mL, Rice of 0.75 x 10^8 conidia / mL, and Corn of 0.24 x 10^8 conidia / mL (Table 2).

Table 2 LC_{50} values on the 9th day and LT_{50} at a density of 10^8 conidia / mL of *B. bassiana* fungi from PDA, Rice and Corn media against *T. castaneum* imago

Media	LC_{50} 10^7 (conidia/mL)[1]	LT_{50} (Days) [2]
PDA	2.23 x 10^8	8.315
Rice	0.75 x 10^8	19.953
Corn	0.24 x 10^8	40.574

[1]Lethal Concentrate 50
[2]Lethal Time 50

The LC_{50} (Lethal Concentrate 50) value indicates the amount of conidia density needed by *B. bassiana* fungi to kill 50% of the *T. castaneum* population tested. Comparison of LC_{50} values of PDA, Rice and Corn media is much different, LC_{50} values of rice and corn media are smaller than the LC_{50} value of PDA media, which is the difference of 1.48 x 10^8. This shows that the virulence of *B. bassiana* fungus from PDA media and other media is different, so it can be said that the use of *B. bassiana* fungus from PDA and other media has a different level of effectiveness in suppressing *T. castaneum* populations.

Lethal Time 50 (LT_{50}) is the time of day needed to kill 50% of the insect population tested. The results of the *B. bassiana* fungus probit analysis conducted at 10^8 conidia/mL densities with observations on days 1 to 9 obtained LT_{50} values for *B. bassiana* fungi from PDA media at 8,315 days, and B. bassiana fungi from rice media and the rates are 19,953 and 40,574 days, respectively (Table 2).

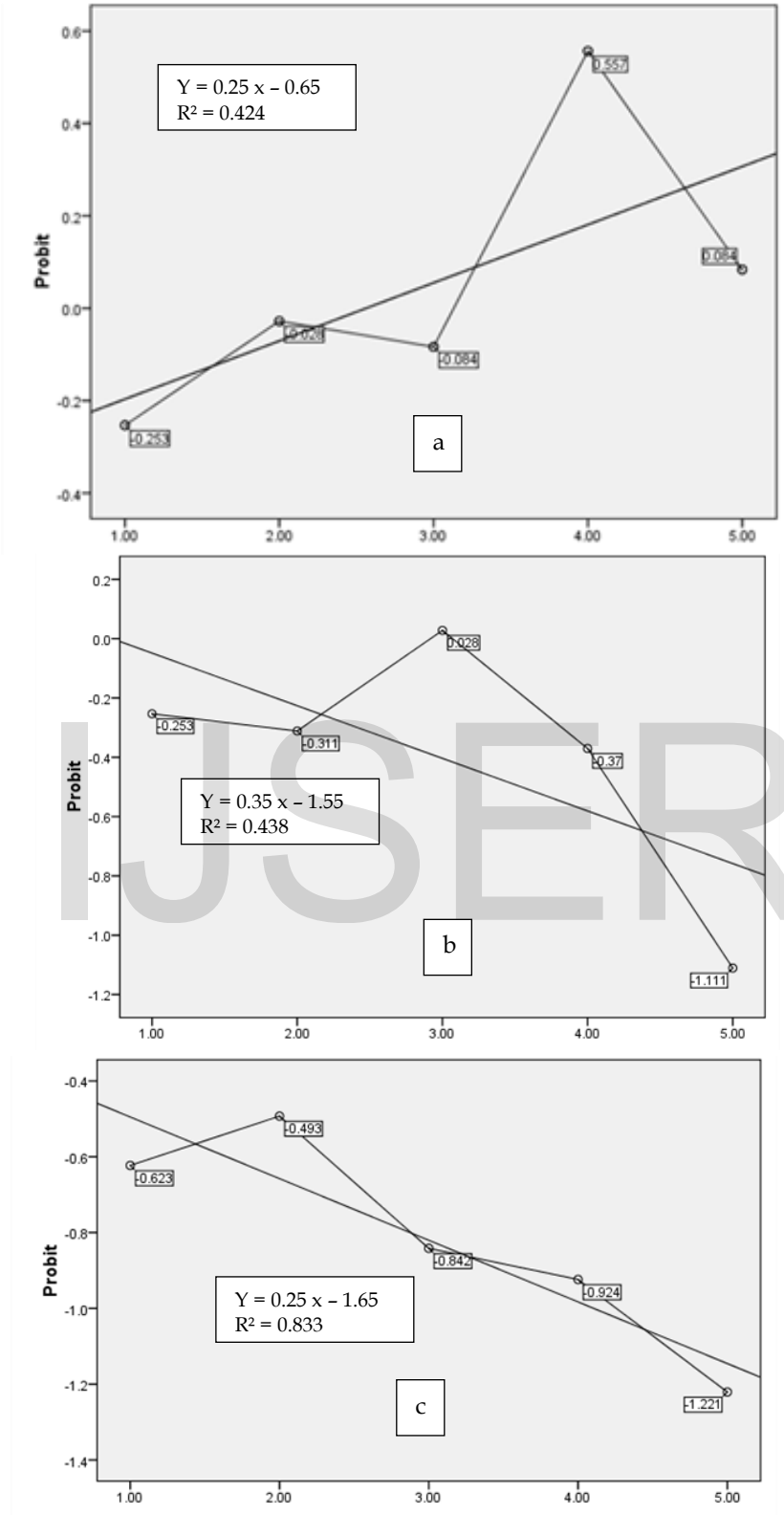

Figure 1 Relationship between conidia density and the percentage of mortality of *T. castaneum* infected with *B. bassiana* fungi from (a) PDA, (b) Rice, and (c) Corn media on the 9th day

Discussion

Test insect mortality occurred from day 1 of observation. According to [21] insect death due to entomopathogenic fungus usually occurs 2 to 14 days after infection, but death can also occur in less than 24 hours. The effectiveness of insect pathogenic fungi to control target pests is very dependent on the age of the insect, development stage, cuticle surface, and spore density [22]. According to [23] to kill test insects for *Cinara atlantica* (Hemiptera: Aphididae), the density of the entomophatogen conidia used must be 10^8 /ml, Ashouri et al. (2004) also reported that a density of 10^7-10^8 /ml can suppress *Myzus persicae* (Hemiptera: Aphididae) up to 100%.

Treatment using *B. bassiana* fungi from PDA, Rice and Corn media showed that *T. castaneum* mortality increased every day. Factors that influence the production of conidia of *B. bassiana* fungi in mass culture are the type and amount of nutrients contained in the growing media used, especially the ratio of content between carbon and nitrogen. Besides, according to [24], macroelements such as oxygen, sulfur, and phosphate are the main components of nutrients needed by fungi. The surface area of the growing media also influences the amount of conidia produced. The more surface area of the media, the more conidia are produced. According to [25], the germination of conidia of fungi grown in natural hosts is higher than that of conidia of fungi grown in alternative media. This is thought to cause the mortality of *R. linearis* imago in rice media slow until the 4th day and starts to increase sharply on the 5th day.

Lethal Time 50 (LT_{50}) is the time of day needed to kill 50% of the insect population tested. The comparison of LT_{50} values from PDA media, rice media, and corn media are different, although the time needed for rice and corn media is a little longer than PDA media. This shows *that B. bassiana* fungus from natural and alternative media can kill *T. castaneum* imago and the speed of germination of *B. bassiana* fungus from PDA media is relatively the same compared to rice and corn media.

4. CONCLUSION

The entomopathogenic fungus *B. bassiana* successfully caused *T. castaneum* mortality by 71.11% on a ninth day after treatment at conidia density 10^7 /mL in the laboratory. LC_{50} value of 2.23 x 10^8 conidia / mL. The LT_{50} value at 10^8 conidia/mL densities is 8,315 (8 - 9 days).

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