

INFECTIVITY OF *BEAUVERIA BASSIANA* (BALSAMO) AGAINST *TRIBOLIUM CASTANEUM* (COLEOPTERA: TENEBRIONIDAE)

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Abstract

Tribolium castaneum is a major pest in wheat flour but can attack other processed foodstuffs. Control of these pests can be done in various ways, one of them by biological control using entomopathogenic fungus *Beauveria bassiana*. This study aims to determine the infectivity of *B. bassiana* entomopathogenic fungus against *T. castaneum* populations in the laboratory. The experiment was completed with random preparations, each preparation was repeated three times. Imago *T. castaneum* was sprayed with a conidia suspension of *B. bassiana* at a density of 10^6 and 10^7 conidia/mL on three different storage media namely PDA, rice, and corn media. Mortality was calculated based on the percentage of imago that died of *B. bassiana* fungi. One week observation showed the mortality of *T. castaneum* after the application of *B. bassiana* fungus was 55.33% with 10^7 conidia/mL in corn media. The highest spore density was found in rice media with an average of 10.53×10^6 conidia/mL. Meanwhile, the value of the results showed LC_{50} of 2.11×10^7 conidia/mL and the fastest LT_{50} in corn media was 5.31 days.

Key Words : *Beauveria bassiana*, Biological control, Entomopathogenic, *Tribolium castaneum*

Introduction

The main problem with storage warehouses is attack by warehouse pests. Loss of grain products as result of werehouse pest attack reaches 5 to 10% (Syafique *et al.*, 2006). Warehouse pests are included in opportunistic insects, which can quickly use available resources, so they can grow and develop their population quickly, but when resources are no longer supportive these pests will turn to look for new resources or die (Harahap, 2012). One of the most found in food storage in warehouses is *Tribolium castaneum*. *T. castaneum* or red flour beetle (Coleoptera: Tenebrionidae) is a secondary pest that is cosmopolitan and includes an external feeder. Until now, pest control of *T. castaneum* is still very dependent on synthetic pesticides, because this method is easy in application and quickly reduces the pest

population. Also, there have not been found other alternatives that are quite effective. The application of insecticides is usually intensive once a week or 2-3 times a week (Trizelia, 2005). One way to control the *T. castaneum* is environmente friendly natural enemies consisting of entomopathogens. Control of insect pests with pathogens is a process of utilizing pathogens both already in the local ecosystem and by entering pathogens into the ecosystem from the outside through inoculation and inundation techniques. One type of insect pathogen that is widely found in nature and can be used to control insects is the fungus; *Beauveria bassiana*. The utilization of *B. bassiana* for pest control of *T. castaneum* has not been widely supported. The results of preliminary studies that have been carried out indicate that *B. bassiana* isolated from corn as

animal feed can infect Imago and larvae from *T. castaneum* (Sepe et al., 2019). In other insect pests such as *Crocidolomia pavonana* (Lepidoptera: Pyralidae), infection of *B. bassiana* besides causing the death of imago and larvae, also affect thirs activity of feeding and oviposition (Trizelia 2005; Noma and

Materials and Methods

Preparation of *B. bassiana*: Fungi isolated from the collection of Plant Diseases Laboratory, Department of Plant Protection, Hasanuddin University, Indonesia. In order to increase its virulence, *B. bassiana* fungus were reintroduced to insect bodies to complete the infective phase. The host insect used was *T. castaneum*. Suspension of *B. bassiana* isolate was sprayed on *T. castaneum* and allowed to grow mycelium. The mycelia are then isolated on PDA media. Purified isolates on PDA media were propagated on rice and corn media for use in this study.

Testing the growth and production response of *B. bassiana* conidia on the media

Isolate on Rice Media: The rice used as an alternative medium was washed clean. The rice was next poured into boiling water and allowed to stand for an hour. The filtered rice was then dried on a sterile gauze-coated plastic tray (50 grams) of rice were put into HDPE (*High Density Polyethylene*) bag to sterilize using autoclave for 35 minutes at 121 °C. After cold rice *B. bassiana* fungus suspension was induced on rice media in laminar airflow by spraying. Then The rice media was stored in the laboratory at room temperature for 21 days for optimal (Sutadji, 2016).

Inoculation of Isolates on Corn Media: Corn media was white corn that has been cleaned and soaked for 12 hours. The corn (50 grams) of soaked corn was washed again and put in an HDPE (*High Density Polyethylene*) bag and then sterilized with an autoclave at 121 °C for

Strickler (2000). Efforts to increase *B. Bassiana* success as agens of biocontrol *T. castaneum* in the field require high virulence strains, quick killing pest, and able to survive in the warehouse. The three factors refer to a reference to a controlling agent as well.

1 hour. The next step was according to the rice media treatment method (Sutadji, 2016).

Application of *B. bassiana* conidia suspension to *T. Castaneum* : The application was performed by spraying a suspension of *B. bassiana* on the body of *T. castaneum* as much as 3ml/15 imago. Then, *T. castaneum* was fed 5 grams/unit of corn. Each container contains 15 imago.

The percentage mortality of *T. castaneum*: Warehouse pest mortality was seen every 24 hours with the total number of warehouse pests that die in each unit for 7 days of observation, then calculated with the formula on contributions:

$$\text{Percent of mortality (\%)} = \frac{\text{Total } T. \text{ castaneum mortality}}{\text{Total } T. \text{ castaneum tested}} \times 100\%$$

Statistic analysis: Statistical analysis was Analysis of Variance and the experimental design used was Completely Randomized Design, followed by Duncan's test of the parameters analyzed at a 5% level. Each treatment was carried out in 3 replications. Statistical analysis was performed using the SPSS 16.0 program.

Results and Discussion

The density of *B. bassiana* spores in various culture media: The results showed that the media opposed the number of conidia formed (Table 1). The highest number of *B. bassiana* conidia was obtained in rice media, which was 12.53×10^6 per average media. However, the number of conidia in rice media did not correlate with virulence rate when viewed from the number of *T. castaneum* that died below 50% (Table 2).

Table 1. The density of *B. bassiana* spores on various culture media/100 gram media

Replicate	The density of <i>B. bassiana</i> spores on various culture media/100 gram media		
	PDA	Rice	Corn
1	4.2×10^6	13.5×10^6	9.35×10^6
2	5.5×10^6	14.75×10^6	14.6×10^6
3	5.3×10^6	9.35×10^6	6.1×10^6

Average	5 x 10 ⁶ a	12.53 x 10 ⁶ b	10.02 x 10 ⁶ ab
Numbers followed by the same letter (a) in the same column mean significantly different comparison variety-based Duncan's test, $\alpha = 0,05$.			

Data analysis showed that there were significant differences in conidial density in three media treatments namely rice media (12.53 x 10⁶) statistically different from PDA media (5 x 10⁶). The variation in the germination of each isolate was considered to have differences between each isolate. According to Prasad and Pal (2014), growth and conidia production in entomopathogenic fungi was highly dependent on media composition. The amount of conidia produced in alternative media was quite large and capable of tolerance to temperature, but the level of virulence decreases compared to natural media. Updates relating to the presence or absence of nutrient concentrations outside the conidia relating to the surface characteristics of the host species of the isolate were obtained. Pathogenic

isolates in homopterans will germinate better in media that require emissions while pathogenic isolates in coleopterans will germinate in conditions of glucose deficiency (Trizelia, 2005).

Mortality of *T. Castaneum*: The results of the pathogenicity test of *B. bassiana* against *T. castaneum* showed that the total density of conidia used in this study affected the mortality rate of *T. castaneum*. Statistical analysis showed that there was a significant difference between *T. castaneum* applying *B. bassiana* conidia suspension based on differences in media treatment. About 55.56% *T. castaneum* died on corn media with conidia density 10⁶ differing significantly from other conidia densities in the treatment of PDAs and rice media (Table 2).

Table 2. The Percentage mortality of *T. castaneum* (%) against *B. bassiana* for 7 days of monitoring (15 insects/unit)

Treatment Media	Conidia density	Percentage of mortality (%)
Control		2.22a
PDA	10 ⁶	33.33ab
	10 ⁷	44.44bc
Rice	10 ⁶	46.67 bc
	10 ⁷	44.44bc
Corn	10 ⁶	55.56 c
	10 ⁷	53.33 bc
Numbers followed by the same letter (a) in the same column mean significantly different comparison variety-based Duncan's test, $\alpha = 0,05$.		

The results of the analysis of variance in the warehouse pest population showed the application of *B. bassiana* in corn media with 10⁶ conidia/ml spore densities showed a real difference in Duncan's test of 5% level, (Table 2). The application of *B. bassiana* in corn media with a density of 10⁶ conidia/ml results from the death of warehouse pest participation around 55.56%. This relates to the management of *B. bassiana* having the ability to control warehouse pest relationships. Tanada & Rich (1993) stated that virulent lethal isolates kill insects in a short time and non-virulent isolates require a long time to cause chronic infection.

According to Scholte *et al.* (2004), the process of entomopathogenic fungus attack causes the host to die as follows: contact conidia on the integument is followed then stored and germinated and carried out using a sprout tube (appressorium), after entering into the haemocoel, the fungus forms a blastospore that circulates in the hemolymph and forms secondary hyphae to attack other tissues such as the nervous system, trachea, and digestive tract. The occurrence of nutritional deficiencies, toxins produced by fungi, and changes in damage to the body will cause paralysis and death in insects. According to research results Fuquet *et al.* (2004), Valendra *et al.* (2011), and Xu *et al.*

(2008), the toxicity of the *B. bassiana* fungus are determined by the ability of the toxin contained in the movement of insects. The magnitude of trial mortality is indeed a benchmark and fungus isolates that can cause death with mortality reaching 80% are classified as very toxic. Meanwhile, Chong-Rodriguez *et al.* (2011) discuss the toxicity of *B. bassiana* fungus closely related to the type of growing media, growing media containing protein and carbohydrates containing sweet corn produce blastopora which is more toxic than *B. bassiana* fungus grown on media containing only protein (peptone).

The rate mortality of *T. Castaneum*: Virulence of *B. bassiana* is part of the *T. castaneum* imago that dies up to seven days after application. The results showed that the mortality of *T. castaneum* had begun to be seen 24 hours after application, but the highest was seen on PDA media with a spore density of 10^7 which was 11.11% on the 1st day seen. Whereas on the 7th-days view of *T. castaneum* mortality was highest seen in maize media at 55.56% at conidia density 10^6 (Figure 1).

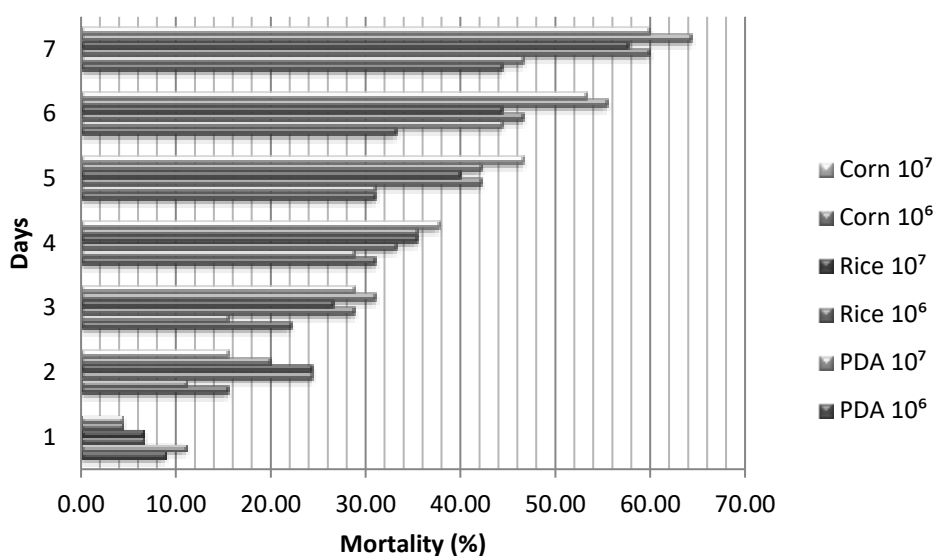


Figure 1. The rate mortality of *T. castaneum* for seven days of observation

The results of this study indicate that the virulence of isolates varies greatly depending on isolates, host insects, and local ecological conditions. According to Utami *et al.* (2014), the cause of death due to *B. bassiana* as a bioinsecticide takes several days after it is applied. Bioinsecticides and target insects have specific biological relationships that are highly protected by the nature of the inoculum that enters the larvae's body which contains bioinsecticides (Pracaya, 2008). Konstantopoulou and Mazomenos (2004), Wang *et al.* (2005), Prasad and Veerwal (2010), Prayogo (2013) reported that the fungus *B. bassiana* produces toxic compounds that are toxic to insect nerves that cause nervous system disturbance and death

in insects. Reports from several researchers indicate that entomopathogenic fungi belonging to the Hyphomycetes group can produce toxin compounds in the form of brassinolide, bassiacridin, oosperin, and cyclosporine which can kill various insect stages (Tikhonov *et al.*, 2002; Mukhtar & Pervaz, 2003; Vega *et al.*, 2008). Judging from the various advantages of these fungi, *B. bassiana* has the opportunity as an alternative for biological control agents *T. castaneum* and as an alternative to chemical insecticides.

The determination of LD₅₀ and LC₅₀:

The results of the *B. bassiana* fungus probit analysis conducted at 7 days after application, obtained LC₅₀ of 2.11×10^7 conidia/mL. That is the density of *B. bassiana* conidia needed

to cause 50% mortality of *T.castaneum* insects is 2.11×10^7 conidia/mL (Table 3).

Table 3 LC₅₀ and LT₅₀ values of *B. bassiana* fungi against *T. castaneum*.

Probit Analysis	Types of media						
		PDA	Rice	Corn	PDA	Rice	Corn
		95% confidence limits for days			95% confidence limits for (days) ^a		
LC ₅₀		2.11×10^7			0.324×10^7		
LT ₅₀	10 ⁶	10.18	6.05	5.31	1.008	0.782	0.725
LT ₅₀	10 ⁷	7.86	6.39	5.44	0.896	0.806	0.736

LT₅₀ value of *B. bassiana* obtained from three types of media at a density of 10⁶ conidia/mL shows that the corn medium has a faster lethal LT₅₀ of 5.31 days. Whereas on rice and PDA media they were 6.05 and 10.18 days, respectively. When compared between the density of 10⁶ and 10⁷ conidia/mL shows that the density of 10⁶ conidia/mL is faster to kill *T. castaneum* imago both on corn and rice media. That is, at a density of 10⁶ conidia/mL 50% of the *T.castaneum* imago population died within 5.31 days due to being infected by *B. bassiana*. Anshori (2017) reports that the probit analysis of LT₅₀ *B. bassiana* values at a density of 10⁹ conidia/mL is 4.67. That is,

at a density of 10⁹ conidia/mL 50% of the test risk population within 4.67 days of being infected by *B. bassiana*.

Summary

B. bassiana fungal spore density on rice media was 12.53×10^6 culture media/100 grams of media. Application of *B. bassiana* fungal suspension resulted in a mortality of *T. castaneum* at 53.56%. The mortality rate differed statistically significantly. The amount of conidia/mL needed to deactivate 50 % of *T. castaneum* was relatively low at 2.11×10^7 conida/mL. Whereas the time it takes about 5.44 days

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