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## Effectiveness of *Beauveria bassiana* Vuill. isolate on various culture media and its pathogenicity against *Tribolium castaneum*

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# 1 Effectiveness of *Beauveria bassiana* Vuill. isolate on various culture media and its pathogenicity against *Tribolium castaneum*

I D Daud, A Gassa and A Rizwaldy

Plant Pest and Disease Department, Faculty of Agriculture, Universitas Hasanuddin, Makassar, 90245, Indonesia.

E-mail: itfir@yahoo.com

**Abstract.** One of the selected microbes that has been widely used for production of bioinsecticides is *B. bassiana*. The fungus is very effective in suppressing larvae of pest insects Lepidoptera and Coleoptera. However, data on the potential of *B. bassiana* as bioinsecticides against Coleoptera are not widely available in Indonesia. This study aims to determine the effectiveness of *B. bassiana* against warehouse pests from Coleoptera *Tribolium castaneum*. Culture of *B. bassiana* on Potato Dextrose Agar media was propagated on corn media, corn liquid media, PDA media, and rice media. *Tribolium castaneum* test insects are derived from the remains of rice mills and then kept until imago lay eggs and have a uniform age. Then *B. Bassiana* was applied to *Tribolium castaneum*. Observations were made every 24 hours on the mortality of *Tribolium castaneum*. The results showed that rice media is the best media used for propagation of *Beauveria bassiana* when viewed from spore density where the average spore density is  $12.53 \times 10^6$ . The fungus isolates that were propagated using liquid corn media, rice media, and corn media were the best isolates that could be used to kill *T. castaneum* pest in warehouse.

## 1. Introduction

Agricultural development is an important element in Indonesian economy. Various types of pest insects can attack agricultural crops resulting in large economic losses. To control these pests, chemical insecticides are generally used. However, the use of chemical insecticides widely and continuously can indeed reduce damage due to pest attacks. Nevertheless, there are also problems with pest insects that occurs resistance, resurgence, killing of natural enemies of pest insects, the emergence of secondary pests, killing of pollinating insects and other dangers to livestock and humans [1].

Integrated pest control (IPM) is a control that combines several ways, namely technical culture, physical mechanical control and use of biological agents [2], but this method is rarely done by farmers. The IPM component that has been developed and researched is controlling using natural enemies. Pest control using entomopathogenic fungi has several advantages including relatively easy to produce, organisms used are available in nature, have high reproductive capacity, short life cycle, less likely to occur resistance [3, 4]. Many entomopathogenic fungi have been tested for virulence and tested in pest control both laboratory and field scale, one of which is the fungus *Beauveria bassiana* (Balsamo) Vuillemin. *Beauveria bassiana* belongs to the phylum Ascomycota, subfilum Pezizomycotina, class Sordariomycetes, order Hypocreales [5].



One of the selected microbes that has been widely used for production of bioinsecticides is a strain of member species of *B. bassiana*. This research is expected to contribute as preliminary data about the ability of entomopathogenic fungal infection. Especially about the age of insect death after infection states that entomopathogenic fungi that match the host will produce a good combination of enzymes to be able to penetrate that depends on several pathogenicity factors including host suitability and physiological properties of the fungus [6]. This study aims to determine the effectiveness of *B. bassiana* fungus against warehouse pests from the order Coleoptera, *Tribolium castaneum*.

## 2. Materials and methods

### 2.1. Preparation of *B. bassiana*

*B. bassiana* used in this study was an isolate taken from the Laboratory of Plant Pest and Diseases, Faculty of Agriculture. Isolates are made in pure culture by being transferred using cork borer to a new PDA media.

### 2.2. Media preparation of *B. bassiana*

2.2.1. *Corn media*. Corn kernels are soaked for 1 x 24 hours and drained for approximately 2 hours. Furthermore, corn is put into 100 grams of plastic, closed tightly, after that media is sterilized by autoclave for 2 hours at 121°C at a pressure of 1 atm.

2.2.2. *Liquid media*. Liquid media is made by separating the corn from the cob and then boiled until the juice comes out about 1 hour. Then the liquid media is put into the Erlenmeyer for 2000 ml. Then close tightly using aluminium foil and glued using wrapping. Then sterilized for 2 hours at 121°C at a pressure of 1 atm.

2.2.3. *Potato dextrose agar (PDA) media*. Potatoes are cut into small pieces and boiled with distilled water. Then the extracted potato is filtered and put into an Erlenmeyer that already contains sugar with agar. After that the distilled water is added to reach a volume of 1000 ml. Then stir all the mixture. The media is sterilized for 2 hours at 121°C at a pressure of 1 atm.

2.2.4. *Rice media*. Rice is washed 3 times to separate the rice from the dirt that is still attached. After washing the rice is then soaked for 1 X 24 hours, drained for approximately 2 hours. Rice is put into 100 grams of sugar plastic, then closed tightly, after that the media is sterilized for 2 hours at 121°C at a pressure of 1 atm.

### 2.3. Inoculation of *B. bassiana* in culture media

*B. bassiana* is transferred to each culture media, namely PDA, corn media, rice media, liquid media and then tightly closed. The media is stored in a storage room, except for liquid media that shaken for approximately one month.

### 2.4. Preparation of *Tribolium castaneum* test insects

*Tribolium castaneum* which is used as a test insect is taken from the rest of the rice mill. Then maintained until the imago lay eggs. Imago with a uniform age are used as test insects.

### 2.5. Spore density of *B. bassiana*

Pure culture of *B. bassiana* in the petri dish was given 2 ml of distilled water and flamed with a brush. Then stored in an erlenmeyer containing distilled water. The solution was taken as much as 1 ml and then diluted in a test tube containing 9 ml of distilled water and homogenized. Furthermore, *B. bassiana* was calculated its spore density using a hemocytometer to obtain a concentration of 10<sup>6</sup> spores/ml. The results obtained are calculated based on the formula Gabriel and Riyanto (1989), namely:

$$C = \frac{T}{N \times 0.25} \times 10^6$$

C = Spore density per ml of solution.

T = Total number of spores in the total sample observed.

N = Number of sample boxes (5 big boxes X 16 small boxes)

0.25 = Correction factor for the use of a small-scale hemocytometer sample box

## 2.6. Application of *B. bassiana* on imago *Tribolium castaneum*

Suspension of *B. bassiana* fungus was applied to *Tribolium castaneum* imago in the Laboratory of Plant Pests and Diseases, Faculty of Agriculture, Universitas Hasanuddin. Suspension was dissolved into a sterile distilled water solution in accordance with the required concentration. The suspension was obtained from four culture media along with a control solution. 5 g flour was taken and stored in a container. Spore density of 5 ml were then put into the sprayer, keeping a day until dry. Subsequently, *Tribolium castaneum* imago was released into container.

## 2.7. Observation

Every twenty-four hours after application, the percentage of *Tribolium castaneum* mortality was calculated using the formula proposed by Basle (1985).

$$P = \frac{r}{n} \times 100 \%$$

P = Percentage of mortality

r = Number of dead test insects

n = Number of test insects observed

## 2.8. Data analysis

The data obtained were analyzed statistically with variance by difference test at the 5% level.

# 3. Results

## 3.1. Spore density of *B. bassiana* from various culture media

The highest average spore density was found in rice media with an average spore density of  $12.53 \times 10^6$  while the largest spore density for each repetition in rice media for 100 g of media was found in the second treatment which was  $14.75 \times 10^6$  (table 2). While the second largest average density after rice media is found in maize media with an average spore density of  $10.02 \times 10^6$ , and spore density at each test for corn media is found in the second test with spore density of  $14.6 \times 10^6$ . The third sequence is PDA media with an average spore density of  $5 \times 10^6$  with the highest spore density for each repetition found in the third treatment with spore density of  $5.3 \times 10^6$ . Media that has the smallest spore density of the four media used that is, it is found in liquid media made from corn mites with an average spore density of  $1.2 \times 10^6$ , with the largest spore density for each test found in the first treatment with a spore density of  $3.6 \times 10^6$ . Rice media is a good medium used for propagation of *Beauveria bassiana* fungus, this is in accordance with the opinion of [7-9], macroelements such as oxygen, sulfur, and phosphate are the main components of nutrients needed by fungi.

5  
**Table 1.** *Beauveria bassiana* spore density on several culture media/100 gram media

Treatment	Spore density of <i>B. bassiana</i> spores in various media			
	PDA	Corn	Rice	Liquid
1	4.2 X 10 <sup>6</sup>	9.35 X 10 <sup>6</sup>	13.5 X 10 <sup>6</sup>	3.6 X10 <sup>6</sup>
2	5.5 X 10 <sup>6</sup>	14.6 X 10 <sup>6</sup>	14.75 X 10 <sup>6</sup>	1.1 X10 <sup>6</sup>
3	5.3 X 10 <sup>6</sup>	6.1 X 10 <sup>6</sup>	9.35 X10 <sup>6</sup>	1.6 X 10 <sup>6</sup>
Average	5 X 10 <sup>6</sup>	10.02 X 10 <sup>6</sup>	12.53 X 10 <sup>6</sup>	2.1 X 10 <sup>6</sup>

### 3.2. Mortality of *B. bassiana* against *Tribolium castaneum*

On the first day observation the highest average mortality of test insects died was in the liquid media that is equal to 16.67% at the density of spores 10<sup>7</sup>. In addition to the liquid media, the death of the most test insects at the first observation was also found on the treatment of PDA media at the spores density 10<sup>7</sup> which has an average mortality equal to mortality in liquid media that is equal to 16.67 insects. Whereas the lowest mortality rate of dead test insects was found in corn media treatment, which was only 6.67 insects in the two spore density treatments applied. Then for the best total observation mortality was found in maize and rice media because almost all observations of all treatments applied for test insect mortality never touched zero. The treatment can be seen significantly different in the sixth day of observation this is evident from the results of further tests that have been done. Imago of *T. castaneum* has little susceptibility to *B. bassiana*, even in powdered *B. bassiana* only contains 9.4 X 10<sup>10</sup> conidia per gram even in 2000 mg/kg only can control about 2.5% *Tribolium castaneum* after 7 days of application [10, 11].

**Table 2.** Average mortality of *T. castaneum* on each observation day (10 insects/unit)

Media		Observation						
		1 <sup>th</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup>	7 <sup>th</sup>
PDA	10 <sup>-6</sup>	13.33	20.00 <sup>c</sup>	33.33	36.67 <sup>d</sup>	46.67 <sup>d</sup>	50.00 <sup>c</sup>	66.67 <sup>c</sup>
	10 <sup>-7</sup>	16.67	16.67 <sup>c</sup>	23.33	46.67 <sup>cd</sup>	50.00 <sup>d</sup>	70.00 <sup>b</sup>	73.33 <sup>bc</sup>
Corn	10 <sup>-6</sup>	6.67	30.00 <sup>bc</sup>	46.67	53.33 <sup>bcd</sup>	63.33 <sup>cd</sup>	83.33 <sup>ab</sup>	96.67 <sup>a</sup>
	10 <sup>-7</sup>	6.67	23.33 <sup>bc</sup>	43.33	56.67 <sup>bc</sup>	70.00 <sup>bc</sup>	80.00 <sup>ab</sup>	90.00 <sup>a</sup>
Rice	10 <sup>-6</sup>	10.00	36.67 <sup>ab</sup>	43.33	50.00 <sup>cd</sup>	63.33 <sup>cd</sup>	70.00 <sup>b</sup>	90.00 <sup>a</sup>
	10 <sup>-7</sup>	10.00	36.67 <sup>ab</sup>	40.00	53.33 <sup>bcd</sup>	60.00 <sup>cd</sup>	66.67 <sup>bc</sup>	86.67 <sup>ab</sup>
Liquid	10 <sup>-6</sup>	13.33	50.00 <sup>a</sup>	56.67	76.67 <sup>a</sup>	86.67 <sup>a</sup>	93.33 <sup>a</sup>	96.67 <sup>a</sup>
	10 <sup>-7</sup>	16.67	30.00 <sup>bc</sup>	36.67	66.67 <sup>ab</sup>	83.33 <sup>ab</sup>	83.33 <sup>ab</sup>	93.33 <sup>a</sup>
Control		10.00	10.00	16.67	30.00	36.67	36.67	36.67

6  
According to [10] the results of his research show that *B. bassiana* can be used successfully against pests that are stored in wheat barns. The long-term use of this formulation as well as other formulations of entomopathogenic fungi is recommended in grain storage conditions. The insecticide efficacy of *B. bassiana* is very high influenced by several factors such as insect behavior, population density, age, nutrition and genetic information, also, physiology and morphology of the host effect on their sensitivity to biological control agents such as entomopathogenic fungi [12]. So the difference in susceptibility of beetle storage to *B. bassiana* cannot be explained as a function of the concentration of conidia used [12].

### 3.3. Mortality of insect test

The highest mortality for *Tribolium castaneum* test insects was found in the treatment of liquid media at the density of spores  $10^6$  that is 77.14% and the lowest mortality was in the treatment of rice media at spores density  $10^7$  on the third replication with an average mortality value of 31.43. While the comparison for each test in each treatment there is not much difference. The highest difference was found in the treatment of rice media at the spore density of  $10^6$ , the proof in the first test had a mortality of 1.25 while in the second test there was 1.38 and in the third test there were 1.50 dead average test insects.

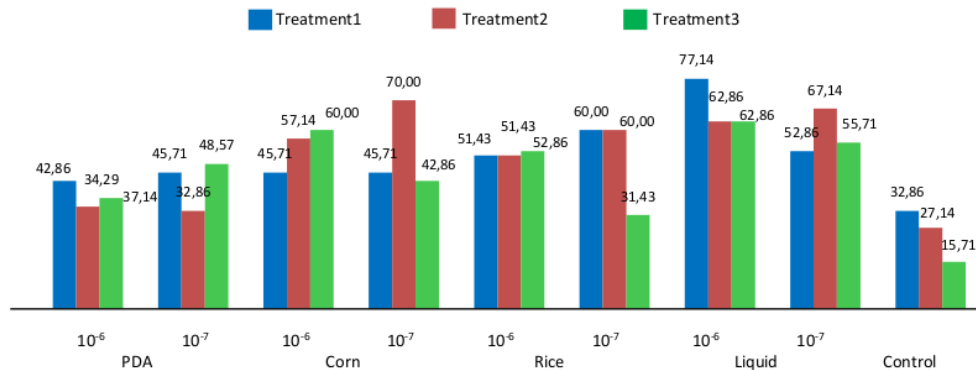


Figure 1. Average mortality of *Tribolium castaneum* for each treatments (10 insects / unit)

## 4. Discussion

Rice media is the best media used for the propagation of *Beauveria bassiana*, when viewed from the spore density of the three replications with an average spore density of  $12.53 \times 10^6$  this is better than corn media, PDA media and liquid media. When viewed from the observed spore density it can be concluded that liquid media is a poor medium used for the propagation of *Beauveria bassiana* spores because the amount of spore density obtained is  $2.1 \times 10^6$ . This is in accordance with the opinion of [12] which states that the surface area of the growing media also affects amount of conidia produced. The more surface area of the media, the more conidia are produced. Media that tend to clot will have a narrow surface area, so that conidia production is also small. The density of conidia of *B. bassiana* from rice media was higher, because the surface of rice media was wider than the surface of PDA media and liquid media.

*B. bassiana* on PDA media and rice media began to grow at 2-3 days after inoculation [13]. Fungi grow and develop enveloping rice media, whereas on PDA media the fungus grows in certain parts. This is caused by the nature of the *B. bassiana* isolates grown. Media with high sugar levels would increase the virulence of entomopathogenic fungi. The media used to grow entomopathogenic fungi greatly determines the rate of colony formation and the number of conidia during growth. Fungi media must contain organic substances as a source of C, a source of N, inorganic ions in sufficient quantities as a supplier of growth as well as a source of vitamins. Numerous studies show that the use of high carbohydrates promotes the growth of vegetative fungi. Apart from that, mushrooms also need micronutrients (calcium, iron, copper and manganese) which are usually found in raw materials. Media material that tends to clot will have a narrow surface area, so that conidia production is also small. Ideal media are media that not only have particles with a wide surface, but also which can maintain the integrity of particles during the production process.

While the results of research on the mortality of the fungus *B. bassiana* against *Tribolium caastaneum* obtained data that between the treatment of liquid media, corn media and rice media did not significantly affect the mortality value. This is supported by the average data of the number of mortality of test insects in the range of 1.24 to 1.83 which is very significantly different from the treatment of PDA media which has an average of 0.95 to 1.00 in both spore density treatments. The insecticide efficacy of *B. bassiana* is very high influenced by several factors such as insect behaviour, population density, age, nutrition and genetic information, also, physiology and morphology of the pest effect on their sensitivity to biological control agents such as entomopathogenic fungi. The entomopathogenic fungus, *B. bassiana* Vuillemin, has proven efficacy for many insect pests from stored grain and grain products but is not considered a commercially independent option for controlling *T. castaneum* [10].

Most entomopathogenic fungi have a two-phase biological cycle, the vegetative and generative phases using mycelium as a growth unit. The spore or conidia type consists of asexual type (anamorpha) and sexual type (telemorpha), both of which play an important role in their life cycle, especially when environmental conditions are less supportive and when appropriate host limitations. Because its main function is to infect the host, conidia is the most likely fungus propagule to be produced. Conidia of Deuteromycetes fungi can generally be reproduced on solid or liquid media through the fermentation process. However, the propagation of *Beauveria bassiana* is mostly done in solid media, such as rice, wheat, or corn [14]. Propagation of *B. bassiana* on a small scale and for a short duration (<1 year) of storage is sufficient to use Sabouraud Dextrose Agar (SDA) media. This media can maintain the viability of *B. bassiana* conidia for up to 6 weeks before being used as a source of inoculum in mass propagation. To maintain virulence, purification on artificial media should be done four times [15].

## 5. Conclusion

Rice media is the best media used for propagation of *Beauveria bassiana* when viewed from the spore density of  $12.53 \times 10^6$ . The fungus isolates which were propagated using liquid media, rice media, and corn media were the best isolates that could be used to kill *T. castaneum* pest in the warehouse.

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