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Colony Growth, Sporulation, and Viability of Entomopathogenic Fungus *Beauveria bassiana* (Balsamo) Vuillemin on Various Agricultural Waste as Growing Media

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KEYWORDS

Beauveria bassiana; Sporulation; Viability; Agricultural waste.

Abstract. Beauveria bassiana is an entomopathogenic fungus that is widely produced for the benefit of biological control agents of various insect pests. The current production that is expected is to use cheap media but produce quality isolates. This study aims to determine colony growth, sporulation and viability of Beauveria bassiana conidia in several agricultural waste media. The experiment was compiled in a complete randomized design and replicated four times. B. bassiana in rice bran media added with cricket flour had the fastest colony growth while B. bassiana in rice husk media was the lowest, lower than the control treatment. In wheat bran media added cricket flour has slower colony growth than rice bran with cricket flour media similar to control treatment but has the second highest sporulation after rice bran with cricket flour media. The highest viability belongs to B. bassiana which is grown on rice bran with cricket flour media. The production in each treatment medium has a real effect but the addition of cricket flour has a significant influence in the production of quality isolates.

Introduction

Common entomopathogenic fungi are produced worldwide as biological control agents in various insect pests. Among the various biocontrol methods used to control insect pests, the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Clavicipitaceae) is very promising. The fungus *B. bassiana* has been shown to cause high mortality in countries where there are food crops (Afandhi *et al*., 2020; Erawati *et al*., 2018; Kaur & Padmaja, 2008; Moorthi, 2011; Petlamul & Prasertsan, 2012; Rai *et al*., 2022; Sayuthi *et al*., 2022; Wargane *et al*., 2020; Wu *et al*., 2019; Xu *et al*., 2020) Production media as a source of nutrition for is a determining factor in strains that produce quality isolates that are adequate for mass production and field applications. Currently, solid media that are often used are semi-synthetic media such as PDA (Potato Dextrose Agar) and SDAY (Saubaraud Dextrose Agar Yeast) or alternative media such as rice and corn media. While the liquid media uses GPE (Glucose Potato Extract). Production using alternative media by utilizing agricultural waste is considered to reduce large costs for mass production. Low-cost natural substrates from agricultural by-product yields have been evaluated to produce effective yields as a medium of production of *B. bassiana* (Geetha *et al*., 2018). Most of the production of *B. bassiana* spores in Indonesia for plant pest insect biocontrol agents is carried out using semi-synthetic media as mentioned above. Media requires expensive costs for mass production and the resulting isolates do not necessarily produce virulent isolates. This type of propagation medium has a noticeable effect on the sporulation of entomopathogenic fungi. The main composition of the media must have C/N content, Carbon is needed in the fungal metabolic process while nitrogen is needed in

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stimulating the growth and protein synthesis process of fungi (Puspaningrum, 2013).

Rice husk media containing C/N 22.67 has been shown to produce a significant increase in spore production (Mishra *et al*., 2016). Successful control using entomopathogenic fungi must have the ability to produce large amounts of inoculum. Characteristics used for mass production must have high sporulation in artificial media, high virulence against target organisms, and the ability to survive in the environment where the pest is located (Mutmainah, 2015). Good indicators of virulence of entomopathogenic fungi include high germination, high growth and sporulation (Chandler *et al*., 1997). Uruilal *et al*., (2018) showed that sago pulp can be used as a growth medium for the fungus *Neurospora sitophila*, and *Gliocladium* sp. Meanwhile, Novianti (2017) reported that rice polish, husk and rice bran media produced the best growth for the fungus *Metarhizium anisopliae*. Good quality rice bran has an average protein in dry matter is 12.4%, fat is 13.6% and crude fiber is 11.6%. Rice bran provides higher quality protein compared to corn due to its high content of thiamin and niacin. This study aimed to obtain the most suitable agricultural waste media for the production of the entomopathogenic fungus, *Beauveria bassiana*.

Materials and methods

Sources of Isolates

The pure culture of *Beauveria bassiana* was obtained from the collection of entomopathogenic fungi in the biological control laboratory of the HPT Department of the Faculty of Agriculture, University of Brawiijaya (FP UB). The study was conducted from July to November 2022 at the FPUB Biological Control Laboratory.

Research Design and Treatment

The study used a Complete Randomized Design with 5 treatments and 4 replications. The factors studied were production media factors consisting of: SDAY synthetic media as control media; rice husk media; rice polish media; wheat bran medium+ 15% cricket flour; rice bran media + 15% cricket flour.

Production Media Preparation

The manufacture of media was carried out by means of rice husks, rice polish, wheat bran, rice bran and cricket flour mashed and then sifted using a sieve with a density of 35 mesh (0.5 mm). Furthermore, the manufacture of media was carried out by means of each media weighed as much as 60 g and soaked using aquades that have been heated as much as 200 ml, then the media was filtered and put into heat-resistant plastic and steamed for 30 minutes and put in a petri dish as much as 25 g of wet media then wrapped using plastic wrap (Novianti, 2017) . For treatment with the addition of cricket flour to the base media, 15% of the cricket flour is added and mixed thoroughly before soaking in hot aquades (Sari & Khobir, 2020). It was then sterilized using a 121°C autoclave, a pressure of 15 psi, and constant for 60 minutes, then lifted and cooled. The sterile medium was then inoculated with *B. bassiana isolates* using a 0.5 cm diameter cork borer in the LAFC. Then the inoculated medium of *B. bassiana* was incubated at a temperature of 26-28 °C for 20 days until the media was covered by the mycelium of the entomopathogenic fungus.

Data Collection Techniques

The observation parameters carried out include testing nutritional characteristics on the production medium, observing the radial growth of *B. bassiana* colonies, calculating the density and viability of *B. bassiana* conidia. Described as follows:

Testing the characteristics of agricultural waste production media, each production media was weighed as much as 100 grams then the media was tested by organic matter content analysis at

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the UPT Laboratory for the Development of Agribusiness of Food Crops and Horticulture.

Radial growth measurements, the results of isolation of fungi bred on the production medium of each treatment, were incubated for 20 days at a temperature of 26-28 °C. The colony growth rate of each isolate on each treatment was measured every 5 days until the 20th day. The calculation of the growth of the diameter of the mycelia of the fungus *B. bassiana* was carried out by measuring the diameter of the radial direction as many as four straight lines using the following formula:

$$Radial\ diameter = \frac{\emptyset W + \emptyset X + \emptyset Y + \emptyset Z}{4}$$

Where:
ØW = axis diameter W
ØX = axis diameter X
ØY = axis diameter Y
ØZ = the axis diameter Z

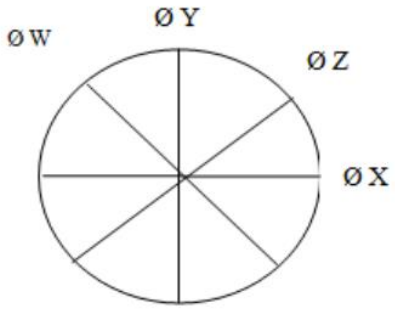

Figure 1. Colony diameter sampling of entomopathogenic fungi

Calculating the density of conidia of B. bassiana, isolates that had been incubated for 20 days were harvested and then observed the density and viability of conidia using a haemocytometer. First, fungi in the production media of each treatment was harvested as much as 1 gram then mixed with sterile aquades as much as 10 ml (Mutmainah, 2015) and 0.05% Tween80 (Khaerati *et al*., 2020) and divortex for 1 minute then filtered using a 70 mesh sieve. Then, the conidia suspension was taken as much as 10 µl using a micropipette slowly dripped on the haemocytometer canal. Finally, the density of the conidia contained in the haemocytometer was calculated, with a magnification of 400x. Measurements were made twice at each observation treatment.

Calculation of conidia viability of B. bassiana, the calculation of viability is carried out by means of SDAY media in the form of slabs with an area of about 1 cm 2 and a thickness of 1-2 mm placed on a glass of sterile objects. On top of the medium dripped 10 µl of conidia suspension containing 10^7 conidia/ml and put in a sterile box filled with moist wipes and incubated for 24 hours at 25°C. Each treatment is repeated twice. Percentage viability was calculated from 100 conidia. Conidia were declared germinated when the length of the sprout tube has exceeded the diameter of the conidia.

Data Analysis

Data from the observation of the selection test of agricultural waste production media for the growth of *B. bassiana* fungus were analyzed statistically using One Way Analysis of Variance (ANOVA) to determine whether there was a real difference. If the results of the F test differ markedly, it is followed by Duncan's Multiple Range Test (DMRT) test at a significant level of α = 5%.

Results and discussion

Nutritional Characteristics of B. bassiana Production Media

Rice husks, rice polish, wheat bran, and rice bran are agricultural by-products that can be used as a medium for mass production of entomopathogenic fungi (Sahayaraj & Namasivayam, 2008). In this study, the agricultural by-product was used for the propagation medium of *B. bassiana.* Each medium has different nutritional characteristics as compounds that fungi need to grow. The nutrient content in each medium used for the

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production of *B. bassiana* is shown in (Table 1). The carbon content in each production medium ranges from 14-24%, the nitrogen content ranges from 1.22-1.61% of the carbon content and nitrogen causes a C/N ratio ranging from 10.37-19.67%. The main energy sources that entomopathogenic fungi such as *B. bassiana* need to grow are carbon and nitrogen (Liu *et al*., 2013). The highest carbon content is in rice husk media, followed by wheat bran + cricket flour, rice bran + cricket flour, and rice polish. Meanwhile, the highest nitrogen content is found in wheat bran + cricket flour media, followed by rice bran + cricket flour, rice polish, and rice husks.

Currently, several nutritional studies are known for the production and sporulation of entomopathogenic fungi the importance of carbon-nitrogen (C:N) in culture substrates as one of the most critical parameters for increasing conidia production (Shah & Pell, 2003). Carbon is needed in the metabolic processes of entomopathogenic fungi. According to Puspaningrum (2013), carbon sourced from monosaccharide sugars is used as an energy source for metabolism (anabolism and catabolism) and is also needed in the form of pentose sugar as a component of nucleic acids that play a role in the process of regulation and gene expression through the process of protein biosynthesis and cell division. Protein and chitin are necessary in the formation of conidia and hyphae as well as increasing the virulence of entomopathogenic fungi. The nitrogen content is necessary in stimulating the growth and process of protein synthesis of entomopathogenic fungi. According to Kalsum *et al*. (2011), nitrogen is needed in synthesizing purines and pyrimidine which are components of RNA and DNA that play a role in protein synthesis and cell division, as well as stimulating the growth of shoots in the asexual reproductive phase of entomopathogenic fungi.

Jackson & Bothast (1990) found that the medium strongly influenced the sporulation of Colletotrichum truncatum, especially the carbon concentration and C/N ratio significantly affected the amount of conidia produced and the attributes of C. truncatum conidia in cultures. Cultures grown in a medium with a C:N ratio of 15:1 produce more conidia than cultures grown with a C:N ratio of 40:1 or 5:1 (Schisler, 1990). An increased C/N ratio from 5:1 to 15:1 led to increased spore yield for the biocontrol agent Talaromyces flavus (Engelkes *et al*., 1997). All these studies have shown that carbon and nitrogen sources, along with carbon concentrations, the C/N ratio of culture media have a great impact on fungal growth and sporulation.

All of the aforementioned studies show their conclusions with a culture medium that is replaced with a source of carbon or nitrogen, and or carbon concentration along with a C/N ratio by the method of one factor at a time. Based on the results of the corresponding carbon concentration, the C/N ratio, this effort will require selecting the most preferred combination of carbon and nitrogen sources for mycelia to grow in the solids medium for mass production of this biocontrol agent as well as considering the cost of each isolate for the lowest concentration that gives high yields, and jointly helping to develop this promising fungus as a practical biopesticide.

Table 1. Nutritional characteristics of different types of agricultural waste media

Media	Carbon (%)	Nitrogen (%)	C/N
Rice Husks	24	1.22	19.67
Rice Polish	14	1.35	10.37
Wheat Bran + Cricket Flour	22	1.61	13.66
Rice Bran + Cricket Flour	20	1.46	13.70

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Growth of B. bassiana Fungus Colonies on Various Production Media

The results of observations on the growth of colonies of the fungus *B. bassiana* on various production media after 5, 10, 15, and 20 days of incubation period showed a noticeable difference (Table 2). The highest diameter growth was found in rice bran+cricket flour media treatment, followed by rice polish, rice husk media and lowest in wheat bran+cricket flour media. There is uniformity of colony growth at the beginning of the incubation period until the 5th day of observation. After 5 days of incubation the diameter of the colony reaches 0.56-1.2 cm depending on the medium of production and growth begins to increase markedly after 10 days of incubation. The average increase in the diameter of *B. bassiana* colonies per day ranges from 1.46 – 3.12 mm/day. The results of the research of Varela & Morales (1996) showed that the growth rate of colonies of *B. bassiana isolates* on SDAY media ranged from 2.17 – 2.72 mm/day. The growth of the highest and lowest diameters shows a significant difference. The growth of *B. bassiana* colonies at each treatment is influenced by substrates or media containing nutritional components for the growth of entomopathogenic fungi. Each substrate treated has a C/N ratio content that is not much different so that it supports the growth of fungal colonies. Wheat bran+cricket flour medium has the lowest colony diameter, but the colony growth is thicker, compact and dense, the conidia are easier to harvest from the surface of the medium. The growth of *B. bassiana* colonies in other treatments was rice husks that looked higher than those of *B. bassiana* colonies on WBMC media the colony growth was thinner, and spread in all directions and more difficult to harvest from the surface of the media. The growth of *B. bassiana* colonies on rice polish and rice bran+cricket flour media shows similarities, the colonies are relatively diffuse and quite thickened. However, rice bran+cricket flour media has the highest colony growth among others. Cricket flour is a source of chitin, the addition of nutrients such as chitin also supports the growth of *B. bassiana* colonies. Chitin substrates are the main source of carbon and nitrogen needed for mycelium growth and the formation of fungal conidia (Barreto *et al*., 2004). The carbon and nitrogen present in this medium are the main constituents of carbohydrates, nucleic acids, proteins and lipids. Chitin is considered a carbon source for chitinolitic organisms and is able to increase its growth rate and multiplication (Gerding-González *et al*., 2007).

Based on the criteria put forward by Rayner & Boddy (1988), the texture characteristics of fungal colonies can be seen in Table 3. The colony of the fungus *B. bassiana* has several texture characters, namely: (1) Plumose (mycelium pile with short or display hyphae, groups of hyphae emerging from the middle and fan-shaped), (2) Pellicular (colonies growth in the form of thin mycelium), (3) Farinaceous (likeflour), and (4) Velvety (hifa short, straight and thick) (Tabel 3). The character of the colony is related to other physiological characters such as virulence and the production of conidia. Colony character can be used as one of the potential benchmarks of biological agents in the selection of virulent isolates of entomopathogenic fungi. According to Rehner *et al*. (2011) the virulent fungus *B. bassiana* has the character of a farinaceous colony with cotton-shaped and starchy colonies, the colony being white then turning pale yellow.

According to Feng *et al*. (2000) isolates of *Lecanicillium lecanii* which have a thick, dense, and compact colony texture, so the conidia produced are easier to harvest from the surface of the medium with a larger number of

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inoculum so that the time required for propagation is only a small amount. In addition, the fungus is better able to compete with other microorganisms and in the field the isolate is faster in the process of transmission to healthy host insects so that epizootics are easier to create (Atkinson & Durschner-Pelz, 1995).

Table 2. The average diameter of *B. bassiana* colonies on production media at the age of 5, 10, 15, and 20 days

Treatment	Average colony diameter (cm) ± SD			
	5 hsi	10 hsi	15 hsi	20 hsi
SDAY	1.11±0.19**b**	1.48±0.29**a**	1.94±0.36**a**	2.21±0.57**a**
Rice Husk	0.56±0.10**a**	1.92±0.52**ab**	2.75±0.58**bc**	3.99±0.67**b**
Rice Polish	0.99±0.39**b**	1.72±0.27**ab**	2.83±0.32**c**	4.39±0.70**b**
Wheat Bran+Cricket Flour	1.11±0.08**b**	1.64±0.15**a**	2.16±0.23**ab**	2.44±0.38**a**
Rice Bran+Cricket Flour	1.20±0.23**b**	2.23±0.32**b**	3.09±0.55**c**	4.45±0.24**b**

The number followed by the same letter showed no noticeable difference on the 5% Duncan test (p=0.05); x̄: average; SD: Standard Deviation.

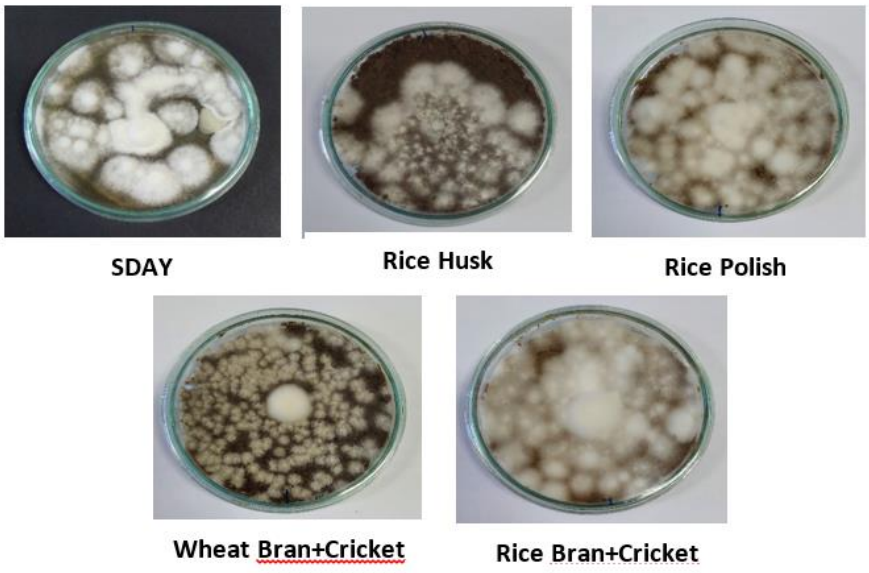

Figure 2. Growth of *B. bassiana* colony after 15 Days of incubation period

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Table 3. Texture characteristics of *B. bassiana* colonies on treatment media

Treatment	Characteristics of the colony	Description
SDAY	Plumose	Piles of mycelium with short or display hyphae, groups of hyphae emerge from the middle and take the form of a fan
Rice Husk	Pellicular	Colony growth in the form of thin mycelium
Rice Polish	Farinaceous	Like flour
Wheat Bran+Cricket Flour	Velvety	Hyphae are short, straight and thick
Rice Bran+Cricket Flour	Farinaceous	Like flour

Sporulation of B. bassiana

The amount of conidia *of B. bassiana* produced in each production medium after 20 days of incubation period showed markedly different results (Table 4). *B. bassiana* fungus in rice bran+cricket flour culture media has the highest sporulation ability of 25.79 x 10^7 conidia/ml and is significantly different from fungi bred in other production media. The lowest production of conidia *B. bassiana* is in rice husk culture media, which is 17.66 x 10^7 conidia/ml. Meanwhile, the control mediia, namely SDAY, showed a spore production value of 17.66 x 10^7 conidia/ml greater than in rice polish media, namely 14.74 x 10^7 conidia/ml. All five media contain the types of nutrients required by *B. bassiana* for their growth, especially carbon and nitrogen sources, but there may be differences in the amount of nutrients resulting in different conidia densities. Various efforts to utilize rice husks, rice polish and rice bran as the only source of nutrition have yielded satisfactory results in terms of the production of good quality inoculum *B. bassiana* (Pham *et al*., 2009; Sahayaraj & Namasivayam, 2008; Seema *et al*., 2013). Cricket flour provides additional nutrients in the form of chitin so that it triggers growth in the fungus *B. bassiana.* Baretto (2004), exposing chitin powder to the growing medium makes it denser and triggers the fungus to form more conidia. The results showed that the media added with cricket flour, namely wheat bran+cricket flour and rice bran+cricket flour, had the highest conidia production capability. The production of conidia in medium enriched with cricket flour will increase because the rich nutrient content affects the growth and germination of conidia (Kim *et al*., 2014).

Visually, rice husk media looks drier and rougher than SDAY, rice bran, wheat bran+cricket flour and rice bran+cricket flour media. The growth of fungi on rice husk media is slower and the growing fungi look less than other media. According to Afifah & Saputro (2020), that narrow or clumpy media surfaces can reduce conidia production. The number of fungal spores is influenced by various factors such as surface area and nutrient content in the propagation medium used. The surface area overgrown with mold in rice bran+cricket flour, wheat bran+cricket flour, and rice polish media is higher than that of rice husk media in equivalent volumes. Entomopathogenic fungi need a medium with a high content of glucose and protein. The nutritional content (protein, fat, minerals, calcium, iron and thiamine) in

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rice-wheat bran and rice polish is higher than that of husks (Brotodjojo *et al*., 2020). The ability of fungi to form conidia has an important meaning because conidia are propagule entomopathogenic fungi that play a major role in transmitting and infection (Wraight *et al*., 2001). If there is little sporulation, then the transmitting will be limited and its ability as a biological control agent will also be reduced. Poor conidia production in rice polish may be due to the absence of sufficient carbohydrates and proteins to support sporogenesis. Rice polish contains phenolic compounds that can act as growth inhibitors (Das *et al*., 2012). Whereas in SDAY media the presence of dextrose and peptone to the production media, media productivity increases significantly along with the increase in potential and significant growth. In terms of potential, rice husks and rice polish alone are the worst media. According to Jenkins *et al*. (1998) The amount of conidia produced per gram of media by entomopathogenic fungi is the main information that is needed for the mass propagation of fungi to be produced as bioinsecticides.

Viability of conidia B. bassiana

The results showed that the germination of conidia *B. bassiana* on various production media differed markedly (Table 5). The viability of *B. bassiana* conidia in wheat bran+cricket flour and rice bran+cricket flour production media reaches >80% significantly different from *B. bassiana* in other production media. Viability in the control treatment is similar to the rice husk and rice polishtreatment, which is in the range of 64.95 – 68.97%. Germination of conidia *B. bassiana* in wheat bran+cricket flour and rice bran+cricket flour media is high at above 70%. Conidia of all isolates germinated after incubation for 24 hours at rates ranging from 70% - 100% including high, germination is an important characteristic of entomopathogenic fungi necessary for infection and the rate of occurrence often correlates with virulence (Kalesanwo *et al*., 2019). Lei *et al*. (2022) adding the percentage of conidia viability of entomopathogenic fungi determined after 24-36 hours of incubation of at least 70% conidial viability is considered suitable for insect pest control test measures. This shows that isolates in wheat bran+cricket flour and rice bran+cricket flour media have good germination. Isolates produced with these media have a great chance of causing infection and killing test insects.

Table 4. The average density of the colony of *B. bassiana* on the medium at 20 days

Treatment	Conidia Density (10^7 conidia/ml)
	$\bar{x}$ ± SD
SDAY	17.66±1.64**bc**
Rice Husk	9.85±4.75**a**
Rice Polish	14.74±0.48**b**
Wheat Bran+Cricket Flour	20.48±1.26**c**
Rice Bran+Cricket Flour	25.79±3.22**d**

The number followed by the same letter showed no noticeable difference on the 5% Duncan test (p=0.05); $\bar{x}$: average; SD: Standard Deviation.

Isolates produced on SDAY, rice husk, and rice polish media have a germination range of 64.95 – 68.97%. Although it is lower than the isolates bred in the other two production media, in this case it is still classified as good germination. According to Sumikarsih *et al*. (2019) thegermination of conidia *B. bassiana* 60.74-70.29% at 25°C can kill *Nilaparvata lugens* up to a mortality value of 96.67%. Borisade *et al*. (2016) and Yeo *et al*. (2003) reported that the failure of entomopathogenic fungi to germinate in a 24-hour incubation period does not mean that spores cannot live, since the optimal conditions necessary for

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germination and growth are well distinguished among species and strains. Time lags known to vary among entomopathogenic fungi and similar species often show wide differences in abiotic needs, especially temperature and water activity that allow germination and growth (Borisade & Magan, 2014).

Table 5. Average viability of colonies of *B. bassiana* at 20 days

Treatment	Viability of Konidia (%)
	$\bar{x}$ ± SD
SDAY	64.95±2.42**a**
Rice Husk	67.51±4.85**a**
Rice Polish	68.97±1.86**a**
Wheat Bran+Cricket Flour	82.76±2.55**b**
Rice Bran+Cricket Flour	84.39±1.64**b**

The number followed by the same letter showed no noticeable difference on the 5% Duncan test (p=0.05); x̄: average; SD: Standard Deviation.

The difference in germination of each isolate is thought to be caused by differences in nutrients available in the production medium. According to Hatzipapas *et al*. (2002) Conidia germination is highly dependent on environmental conditions such as humidity, temperature and light as well as nutrition. In this test, all isolates on the production media were placed under homogeneous conditions, of course, in the lab, so that the environmental conditions should be relatively the same. The trigger for germination relates to the nutritional characteristics of the culture medium. The addition of cricket flour as a source of chitin for isolates is a difference in germination between isolates bred in the treatment medium. Chitin is an important compound in increasing the virulence level of fungi. In this study, chitin derived from 15% cricket flour mixed with 100 grams of rice bran and wheat bran which is a mushroom growth medium has a positive effect on the growth rate of fungi because it can be used as a source of energy needed by the fungus. In addition, fungal growth in chitin-fed media produces better quality conidia compared to chitin-free media (Saputro *et al*., 2019). Entomopathogenic fungi require a medium with a high content of sugars and proteins (Agus *et al*., 2015). *Beauveria bassiana* needs carbon to support germination. Carbon sources can come from glucose, N acetylglucosamine, glucosamine, chitin, starch, lanolin, hydrocarbons, and some long-chain fatty acids. In addition, it requires a source of nitrogen for hyphae growth (Smith & Grula, 1981). Conidia germination plays an important role in the ability of fungi to penetrate and infect hosts (Alavo *et al*., 2002) and becomes one of the most decisive factors in the development of diseases in insects (Hatzipapas *et al*., 2002). Entomopathogenic fungal conidia are declared to germinate when the length of the sprout tube has exceeded the diameter of the conidia *(*Inglis *et al*., 1999). Evaluation of the germination of entomopathogenic fungal conidia needs to be carried out especially if the fungus is to be developed as a bioinsecticide. The speed of germination of isolates must be considered, isolates that germinate faster have the potential to cause infection, because they avoid dryness and the influence of other microorganisms and are detached from the cuticle of insects at the time of ecdysis (Trizelia, 2005).

Conclusions and suggestion

The production medium of rice bran plus cricket flour proved to be the best medium for the propagation of *B. bassiana.* Rice bran plus cricket flour media achieved the highest spore count (25.79 x 10^7 conidia/ml), in stark contrast to SDAY (17.66 x 10^7 conidia/ml), and the highest viability (84.39%) was also achieved rice bran plus cricket flour media. It is necessary to

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test the test insects to determine the level of virulence produced by *B. bassiana* in each production medium.

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