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Promotion of *Beauveria bassiana* Mushroom on Different Growing Media and Its Pathogenicity on Insects

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ABSTRACT

One type of entomopathogenic fungus that is known to be effective in controlling important plant pests is the Beauveria bassiana fungus. The fungus *B. bassiana*, has a high reproductive capacity, is easy to produce and under unfavorable conditions can form spores that can last a long time in nature. The purpose of this study was to determine the density and viability of B. bassiana spores on corn and rice media and to investigate the process of B. bassiana fungal infection in the test insects. The method used is; Fungal propagation test on Rice and Corn Media, Microscopic Observation of Fungus from Corn and Rice Media, Calculation of Fungus Spore Density, Calculation of Fungus Viability and Pathogenicity Test. The research results obtained are as follows; Results The propagation of B. bassiana mushroom grew on the seventh day, the fungus growth was faster in rice media than in corn media. The spore density of the fungus *B. bassiana* clove bondowoso in corn media was 3.75x108 spores/ml, rice media was 4.58x108 spores/ml, *B. bassiana* banyuwangi coffee medium was 3.5x108 spores/ml, rice media was 3.75x108 spores/ml. The viability of the fungus *B. bassiana*, clove bondowoso and banyuwangi coffee on rice and corn media was obtained on average above 60%. The results of the pathogenicity test of *B. bassiana* on insects began to appear on the tenth day after the baiting process.

Keywords: Entomopathogen, Beauveria bassiana, viability, pathogenicity

Introduction

Plant pest control is an effort to control plant pests that attack cultivated plants in order to minimize losses. However, actors in agriculture, both small and industrial-scale farmers, often control plant pests using chemical control techniques. Among these impacts are affecting the environment and causing the death of non-target insects (Widiastuti & Kalimah, 2016). The residue left behind from these pesticides pollutes the environment and pollutes the environment endanger human health. Recent research on the dangers of pesticides to the safety of human life and health is extraordinary. Jenni et al. (2014) in their case study stated that 95.8% of vegetable and fruit farmers in Batu, Malang, East Java experienced pesticide poisoning based on measurements of cholinesterase levels in their blood. Biological control is carried out to reduce the residue produced by using chemical pesticides.

The use of biological agents is a safe control method and does not pollute the environment. The use of biological agents in suppressing the development of pests continues to be developed and socialized to the public, especially farmers. Biological agents commonly used for pest control are usually called entomopathogens. Biological control using fungi that are pathogenic for insect pests has the potential to be developed. The group of entomopathogens that can be used as biological agents

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are entomopathogenic fungi. One type of entomopathogenic fungus that is known to be effective in controlling important plant pests and has been tested both at the laboratory level and directly in the field and socialized to the public is the *Beauveria bassiana* mushroom (Trizelia et al., 2015).

B. bassiana fungus, has a high reproductive capacity, is easy to produce and under unfavorable conditions can form spores that can last a long time in nature. Both of these media were able to produce high conidia. With the increase in the price of rice and corn and in the context of utilizing organic waste, it is necessary to find alternative media (substrate) that can be used as a fungus propagation material with high sporulation ability. The purpose of this study was to determine the density and viability of *B. bassiana* spores on corn and rice media and to determine the process of *B. bassiana* fungal infection in the test insects.

Material and Methods

Insect baiting

The soil is put into the mortar first to be pounded until smooth, then sieved, the results of a sieve of 300gr are put into a 500 ml jar, add 250 ml of water and leveled (not too wet), then put the insects into the jar then cover with clear plastic that has been perforated with the aim that the temperature in the jar is maintained and tied with rubber until tight

Beauveria bassiana mushroom propagation

Materials and tools for the propagation of *B. bassiana* mushrooms are corn, rice, trays, water, pans, basins, gas stoves, stirrer, plastic, Bunsen, alcohol, ent needles, isolates of B. bassiana mushrooms, cloves and coffee. With the following steps:

Corn medium

The corn used is corn that has been ground. Corn that has been milled is washed clean of dirt and then drained. The corn is steamed half-baked in a pot for 30 minutes, after which the corn is cooled. The cooled corn is put into a heat-resistant plastic as much as 100 grams per plastic then the plastic is rolled tightly until the air in the plastic is no longer there. The corn medium was then sterilized in an autoclave for 1 hour at 121oC and 2 atm pressure. After being sterilized from the autoclave the media was cooled. After the corn media was cold, pure isolates of *B. bassiana* were inoculated in a sterilized inoculation chamber.

Rice medium

The rice is washed until it is clean of dirt and then drained. Half-cooked rice is steamed in a pot for 30 minutes, after which the rice is cooled. The cold rice is put into heat-resistant plastic as much as 100 grams per plastic, then the plastic is rolled tightly until the air in the plastic is no longer there. The rice media was then sterilized in an autoclave for 1 hour at a temperature of 121oC, a pressure of 2 atm. After being sterilized from the autoclave the media was cooled. After the rice media was cold, pure isolates of *B. bassiana* were inoculated in the inoculation chamber which had been sterilized beforehand. Fungal growth was observed after 2 days, if the media was contaminated with fungi or other bacteria, set aside and then discarded.

Calculation of spore density

Calculation of spore density was carried out by filling four test tubes with 10 ml, 9 ml, 9 ml, and 9 ml of distilled water, respectively. Then, the isolates will be counted, taken using a needle and put into test tube 1 and vortexed for 3 minutes, then 1 ml of liquid is taken using a syringe and put into test tube 2 (10-1 dilution). Test tube 2 was vortexed for 2 minutes, then 1 ml of liquid was taken using a syringe and inserted into test tube 3 (dilution 10-2) then vortexed for 2 minutes, then 1 ml of liquid was taken using a syringe and inserted. to test tube 4 (10-3 dilution).

The Neubauer haemocytometer was placed on the microscope object table and covered with a cover glass. Observations were made with a magnification of 400x to obtain the calculated field on the Haemocytometer. Furthermore, the spore suspension at a dilution of 10-3 was taken using a syringe and then slowly transferred to the counting field through 2 sides of the Haemocytometer until it filled the canal and then allowed to stand for a stable position. Then the counting field is focused to make it easier to count the number of spores.

The spores contained in the counting box (a+b+c+d+e) in Figure 3 were counted with a magnification of 400x using a hand counter and the results of the spore density in the a, b, c, d, and e count boxes in fields 1 and 2 were recorded. Spores located on the boundary line of the counting box are only counted on the left and top of the counting box. The spore count was repeated three times for each fungus. After completion, the Haemocytometer was cleaned with 70% alcohol and tissue. Calculation of the number of spores/ml is done by the following formula:

$$S = \frac{X}{L\ x\ t\ x\ d}\ 10^3$$

Information:
S: number of spores/ml
X: number of spores counted
L: area of the counting box (0.04 x 5 mm2)
T: calculated depth of field (0.1mm)
D: dilution factor
103: calculated suspension volume (1 ml = 103 mm3)

Viability calculation

PDA media in petri dishes were cut using a cork drill with a diameter of 0.5 cm and placed on an object glass. Each object glass contains 3 PDA media as replicates for one isolate. The spore suspension from the 10-3 dilution was dripped using a syringe on each of the media in Figure 5.6 (a) then closed using a cover glass and placed into a petri dish filled with tissue that had been sprayed with distilled water and then incubated for 16 hours (Figure 5.6 (b). After being incubated for 18 hours, it was observed using a microscope at 400x magnification and counted the number of germinating and non-germinating spores with the following formula:

$$Viability = \frac{Number\ of\ germinated\ spores}{Total\ spores\ observed}\ x\ 100\%$$

Results and Discussion

The results of Beauveria bassiana mushroom propagation in rice and corn media

Fungal growth on all media, both corn and rice, began to grow 1 week or 7 days after inoculation, but it was seen that in rice media the growth was faster than corn media, it was seen that *B. bassiana* fungus grew more. This is because rice contains carbohydrates that are higher than corn. *B. bassiana* grows faster on rice media than on corn media. The results of previous studies also showed that the use of rice media with an incubation period of 2 weeks could produce conidia around 7.8 x 109 –1 x 1010 conidia/g media. After 1 week later, spores have begun to be produced. Below is the result of *B. bassiana* propagation on corn and rice media. For the first picture, (a-d) is the fungus B. bassiana isolates from cloves bondowoso with corn media and the picture (e-h) is the fungus *B. bassiana* isolates banyuwangi coffee with corn media. The second picture (a) *B. bassiana* mushroom isolates bondowoso with rice media and (b) B. bassiana mushroom isolates banyuwangi coffee with rice media. Figure, b, c, d, f, g, and h there is no difference between each other. Figure E shows more fungal growth. In the rice media from pictures (a) and (b) there is no difference between the two, it can be seen that the

growth of the fungus is uniform. According to Roosheroe et al. (1999) growth and development of fungi are influenced by several factors, namely: substrate, humidity, temperature, pH.

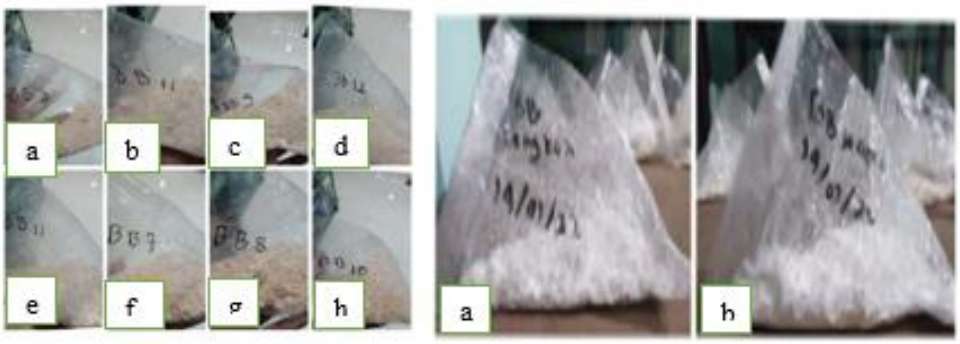

Figure 2. Left; media for corn isolates from Bondowoso cloves (a-b), corn isolates from Banyuwangi coffee (e-h); Right; a media from rice isolates from Bondowoso cloves, b media from Banyuwangi coffee isolates

Spore density results

The results of the calculation of the spore density of the Beauveria bassiana mushroom from cloves bondowoso in corn media obtained 4.58x108 spores/ml, while rice media obtained 3.75x108 spores/ml. The spore density of B. bassiana from banyuwangi coffee in corn media was 3.5x108 spores/ml while rice media was 3.75x108 spores/ml. This calculation was carried out with a Haemocytometer neubareur on a microscope with a magnification of 400 times. It is suspected that the substrate which is the source of nutrients for the fungus in Bondowoso is more than the substrate in Banyuwangi. These new nutrients can be utilized after the fungus excretes extracellular enzymes that can reduce complex compounds from the substrate to simpler compounds. These compounds can be used for the survival of the fungus itself. Dibondowoso itself is a highland which has a humidity of 90%, the humidity is classified as high humidity. This factor is very important for the growth and survival of the fungus, this causes Bondowoso isolates to experience better viability compared to isolates from Banyuwangi because conidia will grow well and are maximum at 80–92% humidity (Roosheroe et al., 1999).

Table 1. Spore density calculation of *Beauveria bassiana* from rice and corn propagation media

Spore Density of Beauveria Bassiana mushroom						
No	Isolate	1	2	3	Amount	Average
1	Corn cloves	9,5	8	5	22,5 x 10^8	3,75 x 10^8
2	Medium cloves of rice	11,5	10	7	28,5 x 10^8	4,58 x 10^8
3	Corn coffee	7	4	5,5	16,5 x 10^8	3,5 x 10^8
4	Rice coffee	9	8	5,5	22,5 x 10^8	3,75 x 10^8

Results of the viability of the fungus B. Bassiana

The results of the calculation of the viability of the fungus B. bassiana from cloves bondowoso corn media obtained results of 64.63%, while the results of rice media obtained results of 66.06%. The viability of the fungus B. bassiana from banyuwangi coffee with corn media was obtained by 60.76% while the yield of rice media was 62.67%. From the calculation of the viability of the fungus B. bassiana as a whole, the percentage results are above 60%.

Table 2. Calculation of viability of *B. bassiana* from rice and corn propagation media

Viability *Beauveria bassiana*			
No	Isolat	Amount	Average
1	Corn cloves	193,88%	64,63%
2	Medium cloves of rice	198,18%	66,06%
3	Corn coffee	182,27%	60,76%
4	Rice coffee	188%	62,67%

Pathogenicity test results

Pathogenicity test of the fungus Beauveria bassiana on insects began to appear on the tenth day after the baiting process with signs that the insects had started to become infected with the fungus *B. bassiana*. On the fifteenth day the insects were completely infected. The results of macroscopic observations showed that *B. bassiana* colonies had a fine white color like flour. This is in accordance with Jannah's statement (2019) which states that *B. bassiana* has the characteristic that it consists of fine white hyphae like flour with microscopic conidia granules but can be seen directly without the aid of a microscope if they are present in large enough quantities. *B. bassiana* penetrates the insect's body through the body wall between the head and thorax and between the body segments. The penetration mechanism begins with the growth of spores in the cuticle (Coppel & Mertins, 2009).

Below is a picture of the results of observations of the pathogenicity test of the fungus *B. bassiana* against the test insects. The first picture of observations from 1-3 there are no signs that show the test insects are infected with the fungus *B. bassiana*. The second picture from the observations on the 4-7th day has not shown any signs of infection. The third picture is the result of observations on days 8-10, there are no signs of infected insects. The fourth picture is the result of observations on 10-15 days, the insects start to show signs of infection with the characteristics of the insect's body starting to turn white, the fifth picture from the observation on the 16th day of the fungus *B. bassiana* has filled the insect's body. The sixth picture is a photo of an insect infected with the fungus *B. bassiana* using a microscope. the process of penetration of the fungus *B. bassiana* into the insect's body requires a supportive environment. The optimum temperature for the growth of *B. bassiana* ranged from 250C to 300C. Germination and growth of *B. bassiana* was best at a relative humidity of 85% to 100%. Jauharlina (1999) stated that the higher the density of B. bassiana spores, the more conidia contained in each ml, so that the conidia could spread more evenly on the insect's body surface. The more spores attached to the insect's body, the greater the opportunity for fungi to grow and develop on the insect's body, subsequently killing the insect (Sudarmadji & Gunawan, 1994).

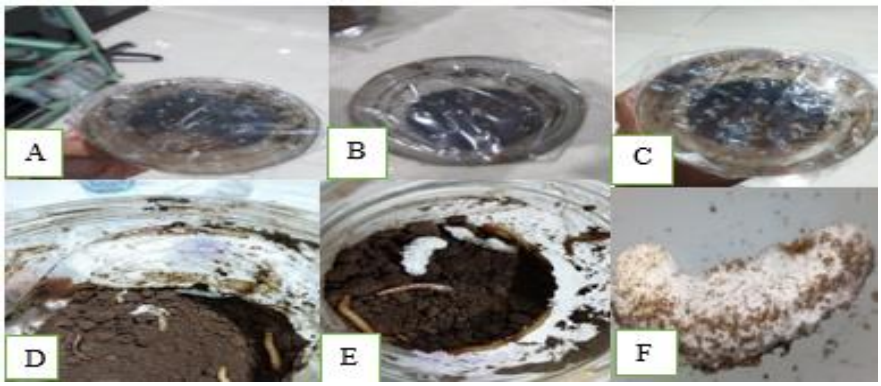

Figure 3. Observation of the pathogenicity of the fungus beauveria bassiana days 1-3 (a) days 4-7 (b) days 8-10 (c) days 10-15 (d) days 16 (e) photos using a microscope

Conclusion

Propagation of the Beauveria bassiana mushroom grew on the seventh day, the growth of the fungus was faster in rice media than in corn media.The spore density of *B. bassiana* clove bondowoso in maize media was 3.75x108 spores/ml, rice media was 4.58x108 spores/ml, *B. bassiana* banyuwangi coffee was maize medium 3.5x108 spores/ml, rice media was 3.75x108 spores/ml .Viability of B. bassiana, clove bondowoso and banyuwangi coffee on rice and corn media was obtained on average above 60%.Pathogenicity test of B. bassiana fungus on insects began to be seen on the tenth day after the baiting process.

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