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The Growth of *Beauveria bassiana* on Corn Waste Medium

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Abstract. The purpose of this study was to analyze the density and viability of *Beauveria bassiana* grown on corn waste medium with 1% NPK (Nitrogen Phosphate Potassium) supplementation. This experimental study used a completely randomized design with 3 treatments and 9 repetitions. Each growth medium consisted of 25 grams of corn waste supplemented with NPK 1% with a dose of 0 mL (P0), 2mL (P1), and 3 mL (P2). The results of this study showed that the highest fungal density ($16.72x10^8$ conidia/mL) and viability (44.66%) were obtained from P2 treatment. It can be concluded that 1% NPK on corn waste medium affect the density and viability of *Beauveria bassiana*.

Key words: *B. bassiana*, NPK, Density, Viability.

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INTRODUCTION

Beauveria bassiana is an entomopathogenic fungi that can be utilize as a Biological Control Agent (BCA). *B. bassiana* have a wide range of host i.e. almost all types of insect especially from Coleoptera, Lepidoptera, Diptera, Homoptera, and Hymenoptera order (Gul *et al.*, 2014), and including controlling *Spodoptera litura* (Indriyanti *et al.*, 2017a) and *Helopeltis* sp. (Indriyanti *et al.*, 2017b).

In order to fulfill the requirement of BCA in the field, BCAs are cultivated using various growth media. Common medium used for the culture is solid medium such as corn, rice, etc. (Zulfiana *et al.*, 2020). Corn is a good medium due to its high nutritional content. According to Agus *et al.*, (2015), fungi need macronutrients such as carbohydrate and protein to support their growth.

B. bassiana cultivations usually use ground or cracked corn medium with 14 days of incubation before harvesting the conidia. Corn medium used in Plantation Plant, Horticulture, and Agriculture Protection Center (*Balai Perlindungan Tanaman Pertanian, Hortikultura dan Perkebunan*/ BPTPHP) Salatiga is only used once and resulted in a lot of waste. The discarded corn waste still looks intact, but the nutrition is reduced. Therefore, efforts are needed to utilize the corn waste as a cultivation medium for another batch. In order to make an optimum *B. bassiana* cultivation results, additional nutrients are needed for the corn waste culture medium.

The results of previous studies stated that the addition of nutrients such as yeast as a nitrogen source in the culture media can increase conidial production (Bharati *et al.*, 2007) and mycelium growth (Mustafa & Kaur, 2009). In this study, the nutrient added to the corn waste was NPK (Nitrogen Phosphate Potassium) fertilizer (15:15:15). The fertilizer contains 15% Nitrogen, 15% Phosphate (P2O5), and 15% Potassium (K2O). The presence of nitrogen, phosphate, and potassium elements is expected to meet the nutrient needs of *B. bassiana* during its growth process. The purpose of this study was to analyze the growth of *B. bassiana* on corn waste medium supplemented with NPK fertilizer.

METHODS

The pure culture of *B. bassiana* used in this study was obtained from the BPTPHP Laboratory in Salatiga, Central Java, in a solid form grown on PDA (Potato dextrose agar) medium. The NPK used was red NPK contained N (15%), P (15%) and K (15%). The solution used was 1% NPK (1 gram of NPK dissolved in 100 mL of distilled water).

The corn waste was washed and then air-dried to reduce the water content. Each corn waste was weighed as much as 25 grams, then added with 1% NPK solution with various doses (0, 2, 3 mL). The media were then sterilized by autoclaving for 20 minutes at a temperature of 121˚C and a pressure of 2 atm. After cooling, each medium was inoculated with one ose of *B. bassiana* isolate aseptically in the *enkas* (steril room). The cultures were incubated for 14 days at a temperature of ± 26-27°C and humidity of 84-94%. Conidial density was calculated with haemocytometer. A total of 0.2 mL of the suspension solution was loaded through 2 channels of the haemocytometer using a syringe (each channel was 0.1 mL of

suspension). Only conidia that were in 5 medium-sized squares diagonally (a,b,c,d,e) were counted. Conidial density was calculated using the following formula (BBPPTP, 2014).

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

Description:
S = conidial density
X = number of conidia
L = area of haemocytometer counting chamber (0.04 x 5 = 0.2 mm^2)
t = depth of haemocytometer counting chamber (0.1 mm)
d = dilution factor
10^3 = volume of suspension counted (1 ml = 10^3 mm^3)

Conidial viability was the ability of conidia to germinate on PDA medium after 17 hours of incubation. The conidial viability was calculated using the following formula (BBPPTP, 2014):

$$V = \frac{g}{g+u}\ x\ 100\%$$

Description:
V = conidial viability
g = number of conidia germinated
u = number of conidia that have not germinated

The data of conidial density and viability were analyzed by using One Way ANOVA and followed by LSD test.

RESULTS AND DISCUSSION

The density of *B. bassiana* cultivated on NPK-supplemented medium was higher that without the supplementation (Figure 1). This condition indicates that the nutrient in P0 treatment has been reduced. Supplementation of 1% NPK allows the fungal mycelia to grow thicker. This proves that the nutrient supplementation plays an important role in the growth of *B. bassiana*.

P0 P1 P2

Figure 1. The growth of *B. bassiana* on various medium 14 days after inoculation

Note: P0: 25 g corn waste + *B. bassiana*; P1: 25 g corn waste + 2 mL of 1% NPK + *B. bassiana*; P2: 25 g corn waste + 3 mL of 1% NPK + *B. bassiana*

Corn contains various kinds of nutrients, such as protein, fat, carbohydrates, calcium, phosphorus, and iron (Diah, 2022). Corn waste used in this study obviously has decreased in nutrition so that the nutrient supplementation is needed. In this study, 1% NPK was used as the supplementation. The addition of 1% NPK by 2-3 ml/25 gram of corn waste medium resulted in a higher fungal density than the control (P0) (Figure 1).

The calculation of conidial density in each treatment (Table 1) shows that P2 has more conidia than P1 and P0. This shows that the addition of nutrients can increase the density of conidia. The application of 1% NPK solution in corn waste media affected the density of *B. bassiana* conidia. The addition of 1% NPK solution plays a role in replacing and increasing the nutrients in the corn waste media that had been lost, especially the N, P, and K elements.

The results showed that the highest conidial density reached 16.72x108 conidia/mL (P2) and 11.72x108 (P1) in the 1% NPK-supplemented media. Based on the provisions of the Directorate of Plant Protection (2014) the minimum conidial density value is 106 conidia/mL, so that the conidial density results obtained has reached the minimum value (Table 1).

Conidial viability was calculated after 24 hours of incubation. The conidial viability obtained from P0 and P1 was categorized as low by 44.66% and 60.07% respectively, while, the conidial viability of P2 was categorized as medium by 78.52% according to the BBPPTP standard (2014). The highest conidial viability is due to the supplementation of 1% NPK.

Tabel 1. Average of *B. bassiana* conidial density and viabity, and water content of corn media supplemented with 1% NPK

Treatment	Average Conidial Density (x 10^8conidia/mL)	Average Conidial Viability (%)	Water Content (%)
P0	5.67^a	44.66^a	35.68
P1	11.72^b	60.07^b	41.08
P2	16.72^c	78.52^c	48.20

Note: Different superscripts indicate the significant different results (P<0.05) of LSD test

Note: P0: 25 g corn waste + *B. bassiana*; P1: 25 g corn waste + 2 mL of 1% NPK + *B. bassiana*; P2: 25 g corn waste + 3 mL of 1% NPK + *B. bassiana*

The formation of entomopathogenic fungi conidia is determined by the culture medium (Islam *et al.*, 2019 & Daud *et al.*, 2020). The culture media must contain nutrients without any inhibitory substances (Permadi *et al.*, 2020). The nutrients in the culture media determine the growth and virulence of the fungus. According to Safavi *et al.* (2010), the nutrient content in the growing media is used by fungi for biosynthesis and energy release as the main factors supporting viability, sporulation, fungal survival, and helping the growth process of hyphae and mycelium (Afifah *et al.*, 2020; Senthamizhselvan *et al.*, 2010). Conidial production of a fungus will be higher when the growing medium contains sufficient and appropriate nutrients (Rajnikanth *et al.*, 2010).

The NPK fertilizer used is NPK with a ratio of nitrogen, phospate, and potassium composition of 15:15:15. Nitrogen together with carbon plays a role in determining the final morphology of the culture, the formation of fungal cell walls, and as constituents of fungal hyphae and conidia cells (Hartl *et al.*, 2012;). According to Swach & Jaipal (2004), the nitrogen in the growing media is used to increase the growth of the fungus. Nitrogen is used for the process of forming fungal cell walls and helping the process of hyphal formation (Hartl *et al.*, 2012). Nitrogen also plays a role in increaseing the fungal viability and hyphal formation (Mustafa & Kaur, 2009 & Hartl *et al.*, 2012).

The other components of the NPK fertilizer are phosphate and potassium. Phosphate is used to help the sporulation process (Cannon, 2010; Martín & Lopez-Aviles, 2018). The addition of potassium elements into the media can increase the level of virulence or entomopathogenic activity of the fungus (Iskandarov *et al.*, 2006).

The phosphate content of NPK is absorbed by the fungus in the form of H^2PO_4- and HPO_4^{2-}. Phosphate is used to help the sporulation process (Cannon, 2010; Martín & Lopez-Aviles, 2018). Potassium is also an excellent source of nutrients for the growth of entomopathogenic fungi. The results showed that the NPK solution added to the corn waste media is able to increase the density of *B. bassiana* conidia. The addition of nitrogen, potassium, and phosphate elements in the corn waste media cause the conidial density to be higher than the control. This shows that the natrium, phospate, and potassium elements from the NPK fertilizer are proven to increase the production of conidia.

According to this study, *B. bassiana* conidia can grow after incubation process at a temperature of ± 26-27°C and humidity of 84 – 94%. These conditions are suitable for the growth of *B. bassiana*.

Water content is one of the main parameters affecting conidial production. The availability of water in the culture media affects the transfer of nutrients and oxygen. Water content that is too low causes respiratory problems of the fungi and if it is too high it can inhibit the growth of the fungi. Corn waste media with the addition of 1% NPK solution can be used as an alternative medium for *B. bassiana* growth.

CONCLUSION

The growth of *Beauveria bassiana* on corn waste media supplemented with 1% NPK showed a higher conidial density and viability than on the corn waste medium without any supplementation.

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