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Effect of potential of *Beauveria bassiana* and *Metarrhizium* sp. to control the *Plutella xylostella* pest in South Kalimanatan

Akhmad Rizali*[1], Noor Laili Aziza[2]

[1]*Lambung Mangkurat University, Faculty of Agriculture, Department of Agroecotechnology, A. Yani Street, Banjarbaru, South Kalimantan, Indonesia*

[2]*Banua Botanical Garden, Regional Research and Development Agency, Aneka Tambang Street, Banjarbaru, South Kalimantan, Indonesia*

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Abstract

Plutella xylostella is an insect pest with a wide range of hosts and the resulting losses are enormous, so control is very necessary. Biological control is one of the most widely used control strategies with active ingredients of microorganisms such as entomopathogenic fungi. This study aimed to analyze the ability of two entomopathogenic fungi, namely *Beauveria bassiana* and *Metarrhizium* spp. in South Kalimantan. The experimental design used was completely randomized design. Observation of symptoms of caterpillar larvae mortality *P. xylostella* placed on the trays showed the presence of hypa *B. bassiana* and *Metarrhizium* spp. growing on the caterpillar, fungi attacks are characterized by the insect body becoming stiff and hard, and the insect body will come out as a white hypa, this shows the conidia of the two entomopathogenic fungi. The results of this study indicated that the two entomopathogenic fungi have the potential to control *P. xylostella* in South Kalimantan with a concentration of 1.5g of entomopathogenic fungi un 100ml of distilled water.

* **Corresponding Author:** Akhmad Rizali ✉ arizali25@yahoo.com

Introduction

Plutella xylostella is a pest insect that has a wide host range, the host of these insect pests are cultivated plants and wild plants. The greatest damage was felt by farmers who cultivated the Brassicaceae family plants, and it was estimated that the cost of controlling *P. xylostella* insects globally, amount to 4-5 billion USD per year (Zalucki *et al*., 2012).The majors of controling *P. xylostella* insects include crop cultivation control such as crop rotation, intercropping, planting of trap crops and resistant varieties, biological control (utilizing parasitoid, predators, and pathogens) from the insect pests *P. xylostella*, and chemical control (Philips *et al*., 2014). Chemical control is widely used by farmers, this is due to its easy application and quick results. However, one of the negative effects of the use of chemical pesticides is that it can lead to pest resistance (Kotta *et al*., 2018).

The level of pest resistance for each region is different, however, *P. xylostella* has the ability to increase its resistance (Philips *et al*., 2014). If the use of these chemical pesticides is continued, it will ultimately increase the cost of controlling the pests. To prevent this condition, the other two controls, namely cultivation control, and biological control, are considered.

Research on pathogens against *P. xylostella,* commonly known as entomopathogen has been widely used. This is because it is widely known that these insect pest pathogens have the potential and ability to replace chemical pesticides when controlling insect pests. Besides, people's awareness of their healthy life is now getting better so that producers will continue to innovate and provide free chemicals for healthy foods. Application of these insect pathogens (entomopathogenic *Beauveria bassiana* and *Metarrhizium* sp.), especially the entomopathogenic fungi is one option. Both of these entomopathogenic fungi have a mortality potential of up to 100% (Duarte *et al*., 2018).

Entomopathogenic fungi are strongly influenced by environmental factors such as temperature and humidity (Indrayani, 2011), as well as the resistance of *P. xylostella* to its pathogens. In addition to its ability factor, the concentration factor of its application is also influenced by environmental factors, hence the research on its effective application in a given area. This study aimed to analyze the ability of two entomopathogenic fungi, namely *B. bassiana* and *Metarrhizium* sp. at various concentrations against the insect pest *P. xylostella* in South Kalimantan.

Materials and methods

Materials used in this study are *Beuveria bassiana and Metarrhizium* sp. isolates, *Plutella xylostella* insect 3rd instar, mustard healthy leaves, PDA, sterile distilled water, newspaper, and 70% alcohol. The materials used are petridish, pipette, needle nose, aluminum foil, cotton, cling wrap, autoclave, bunsen burner, microscopes, glass beaker, tweezers, scissors, analytical balance, calipers, a scalpel blade, spatulas, measuring cups, and, Erlenmeyer, thermometer, magnetic stirrer, hot plate, oven, pan heater, brush, and glucose.

Sterilization Equipment

Glass wares that were used in this study was placed in running water and washed until clean and then dried. We plugged the mouth surface apparatus with cotton. Then, we used newspaper to wrap all the tools and used autoclave to sterilize them at 121^{o}C for ten minutes.

PDA Media Preparation

We washed thoroughly 200 g of potatoes, and diced them into smaller pieces, then boiled until tender in 1000ml of distilled water. Then we add 20 g agar and 20 g dextrose and stirred until blended. We poured PDA into the Erlenmeyer after boiling, and the mouth was plugged with cotton and wrapped with aluminum foil. Furthermore, the media was sterilized using autoclave at 2 atm, 121^{0}C for 30 minutes.

Fungi and Insect Culture

Cultures of *B. bassiana* and *M. anisopliae* that are already established were used in the bioassays. To check the hyphal (mycelium) viability, petri plates (9-cm diameter) were used to grow the collected cultures on potato dextrose agar (PDA) media, they were incubated at a temperature of 28°C, 65±5% relative humidity (RH), and a photoperiod of 12:12 (L:D) h for 15 days. We extracted conidia from each cultured plates by adding 20ml sterile distilled water with 0.05% Tween-80 suspension. *P. xylostella* was taken from the Agroechotechnology Department of the Faculty of Agriculture, Lambung Mangkurat University. We maintained the colony mortality in a pathogen-free environment. Larvae were kept at 25 ± 1◦C with alight: dark cycle of 16:8 h and 60-70% relative humidity [Xu *et al*., 2017].

Selection of Isolate

The doses of Lethal and sublethal was calculated using the pre experimental data. The least isolate with LC50 and high mortality was picked for the downstream application.

Experimental Validation of Lethal (LC50) Concentrations

Calculated lethal concentrations were validated. We used larvae (3rd instar neonates) for each treatment including 15 of *P. xylostella*. We replicated the experiment four times.

Statistical analysis

We carried out statistical analysis in four replicates without control. The method used in this study was a Completely Randomized Design (CRD) with double factors (10 treatments). The data was analyzed using the one-way analysis of variance (ANOVA) followed by Turkey's test, Duncan's multiple range test for the average value of the parameter among the three treatments, and also to compare the mean values between each treatment.

The first factor is a different entomopathogenic fungi:

(a_1) *Beauveria bassiana*

(a_2) *Metarrhizium* spp.

The second factor is a different concentration:

(b_1) 0, 5 g entomopathogenic fungi in 100ml of distilled water

(b_2) 1 g entomopathogenic fungi in 100 of distilled water

(b_3) 1, 5 g entomopathogenic fungi in 100ml of distilled water

(b_4) 2 g entomopathogenic fungi in 100ml of distilled water

(b_5) 2.5 g entomopathogenic fungi in 100ml of distilled water

Plutella xylostella insects was taken from the field, then cultured in a cage to get 400 caterpillar larvae. Observations were carried out by counting the number of *P. xylostella* caterpillar larvae that died during each treatment, starting from day one after application until one of the treatments mortality reached 100%. Mortality is calculated according to Patahuddin and Hidayati (2013):

$$\mathrm{Po} = \frac{r}{n} x 100\%$$

Explanation:

Po = mortality (%)

r = the number of caterpillar larvae that died

n = the total number of life caterpillar larvae

While the meantime of death is calculated using formulas:

$$\text{Time mortality of } P.xylostella \text{ L.} = \frac{\Sigma \text{ (the number of } P.xylostella \text{ L. die day to} - \text{i x day to} - \text{i after application)}}{\text{the number of } P.xylostella \text{ L. die every treatment from day to} - \text{i up to} - \text{n}}$$

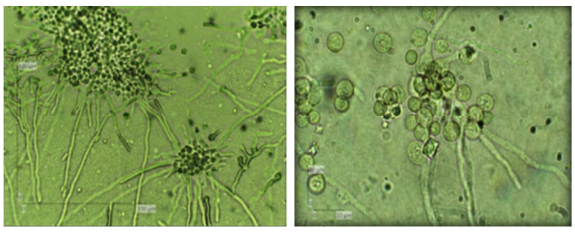
Result

Mortality

This study used two entomopathogenic fungi, namely *B. bassiana* and *Metarrhizium* spp. microscopically, the *B. bassiana* fungi colonies are white, while the *Metarrhizium* spp. colonies were initially white and then these fungi colonies turned them to green. Microscopic imprinting of these two fungi is presented in Fig. 1.

Fig. 1 shows the conidia of the two entomopathogenic fungi. Conidia of the fungus *Metarrhizium* spp. are spherical and hyaline

cylinders, while the conidia of *B. bassiana* are round to oval, hyaline and solitary at the tip of the conidiophore. The results of this study indicated that statistically, the combined treatment of entomopathogenic fungi applied to various concentration does not affect the percentage of *P. xylostella*. It can be seen that the percentage of mortality between both treatments are the same and not different (Fig. 2).



(A) **(B)**

Fig. 1. (A). *B. bassiana* and (B) *Metarrhizium* spp.

Fig. 2 shows the percentage of mortality *P. xylostella* applied to two types of entomopathogenic fungi at various concentrations that were quite high, not less than 82.50%. There are two combined treatments with the percentage of mortality rate of *P. xylostella,* the lowest is in the treatment of 0.5g of *B. bassiana* in 100ml distilled water and 0.5g of *Metarrhizium* spp. in 100ml distilled water. A single application of treatment in the study of an entomopathogenic fungal dose has an effect on the parameter of treatment, the percentage of mortality between one treatment and another (Fig. 3).

Fig. 3 shows that the application of entomopathogenic fungi at the concentration of 2.5g in 100ml of distilled water resulted in higher mortality when compared to the application of entomopathogenic fungi at a concentration of 0.5g in 100ml of distilled water. However, this treatment was not different from the application of entomopathogenic fungi at a concentration of 2g in 100ml of distilled water, a concentration of 1.5g in 100ml of distilled water, and a concentration of 2g in 100ml of distilled water. The study showed that statistically, the combined treatment of entomopathogenic fungi that was applied at various concentrations did not affect the mortality rate of *P. xylostella* (Fig. 4).

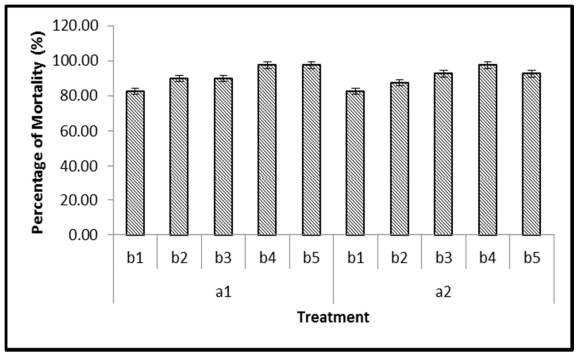


Fig. 2. Percentage of mortality *P. xylostella* applied to the combined treatment of entomopathogenic fungi at various concentrations.

a_1= *Beauveria bassiana*, a_2 =*Metarrhizium* spp., b_1 = 0.5 g entomopathogenic fungi in 100ml of distilled water, b_2 = 1 g entomopathogenic fungi in 100 of distilled water, b_3 = 1.5 g entomopthogenic fungi in 100ml of distilled water, b_4 = 2 g entomopathogenic fungi in 100ml of distilled water, b_5 = 2.5 g entomopathogenic fungi in 100ml of distilled water

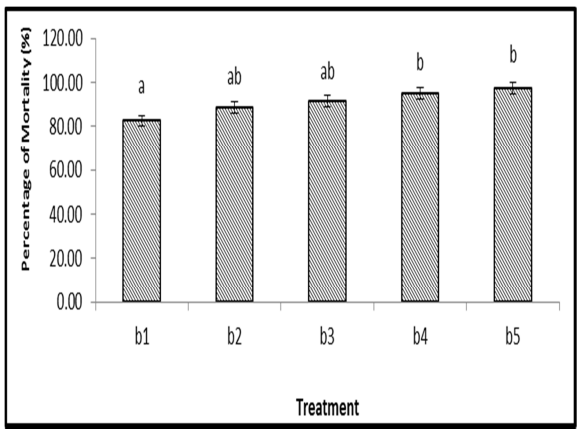


Fig. 3. Percentage of mortality *P. xylostella* applied with a single treatment of the entomopathogenic fungi concentration.

b_1 = 0.5 g entomopathogenic fungiin 100ml of distilled water, b_2 = 1 g entomopathogenic fungi in 100 of distilled water, b_3 = 1.5 g entomopathogenic fungi in 100ml of distilled water, b_4 = 2 g entomopathogenic fungi in 100ml of distilled water, b_5 = 2.5 g entomopathogenic fungi in 100ml of distilled water[abc] Columns with different letter are different by Turkey's test ($p \leq 0.05$)

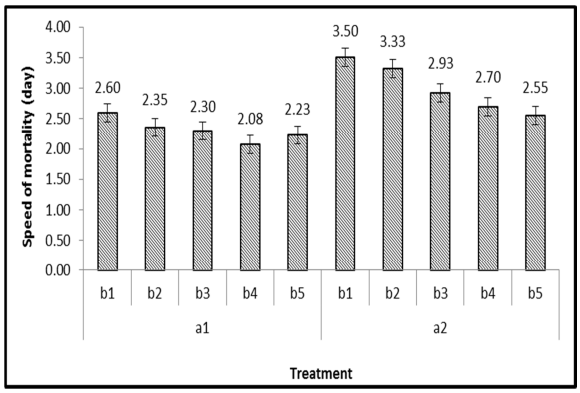


Fig. 4. Mortality rate of *P. xylostella* which was applied by combined treatment of entomopathogenic fungi at various concentration. a_1 = *Beauveria bassiana*, a_2 = *Metarrhizium* spp., b_1 = 0.5 g entomopathogenic fungiin 100ml of distilled water, b_2 = 1 g entomopathogenic fungi in 100 of distilled water, b_3 = 1.5 g entomopthogenic fungi in 100ml of distilled water, b_4 = 2 g entomopathogenic fungi in 100ml of distilled water, b_5 = 2.5 g entomopathogenic fungi in 100ml of distilled water

Speed of mortality

The fastest of *P. xylostella* in numbers was shown in the treatment of 2 g of *B. bassiana* in 100ml of distilled water for 59.92 hours, while the longest mortality rate of P. xylostella was shown in 0.5 g treatment, *Metarrhizium* spp. in 100ml of distilled water for 84 hours. The results showed that the type of entomopathogenic fungi depend on its influenced on the mortality rate of *P. xylostella*. This indicates that there is a difference in the rate of killing power of the two types and the difference is presented in Fig. 5.

Based on Fig. 5, the entomopathogenic fungi *B. bassiana* has a 23% killing power compared to the entomopathogenic fungi *Metarrhizium* spp. The single factor concentration of the entomopathogenic fungi also affectsthe rate of mortality of *P. xylostella*when applied to various concentrations (Fig. 6).

Fig. 6 shows that the application of entomopathogenic fungi at a concentration of 2.5 g in 100ml of distilled water could result in the mortality of *P. xylostella* which was faster than the application of entomopathogenic fungi at the concentration of 0.5 g in 100ml of distilled water and a concentration of 2 g in 100ml of distilled water and the concentration of 1 g in 100ml distilled water. However, this treatment was not different from the application of entomopathogenic fungi at a concentration of 1.5g in 100 of distilled water and a concentration of 2g in 100ml of distilled water. Observation of the symptoms of caterpillar larvae of the mortality of *P. xylostella* placed on the trays, shows the presence of hypa *B. bassiana* and *Metarrhizium* spp. growing on the caterpillar.

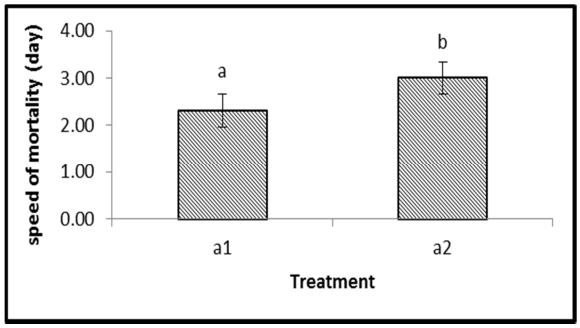


Fig. 5. Speed of death *P. xylostella* which was applied by treatment.

a_1=*B. bassiana* and a_2= *Metarrhizium* spp.

^{abc} Columns with different letter are different by Turkey's test ($p \leq 0.05$)

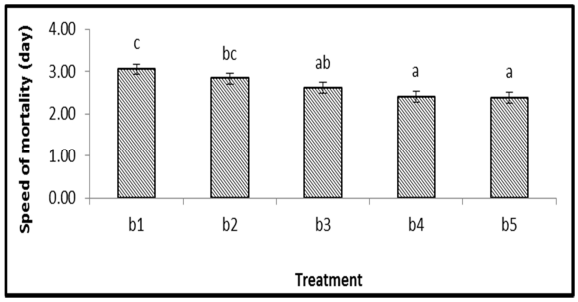


Fig. 6. The rate of mortality *P. xylostella* was applied using a single treatment of the entomopathogenic fungi concentration.

b_1 = 0.5 g entomopathogenic fungiin 100ml of distilled water, b_2 = 1 g entomopathogenic fungi in 100 of distilled water, b_3 = 1.5 g entomopthogenic fungi in 100ml of distilled water, b_4 = 2 g entomopathogenic fungi in 100ml of distilled water, b_5 = 2.5 g entomopathogenic fungi in 100ml of distilled water ^{abc} Columns with different letter are different by Turkey's test ($p \leq 0.05$)

(A) **(B)**

Fig. 7. (A) Symptoms of infected *P. xylostella* by *B. bassiana* and (B) by *Metarrhizium* spp.

Observation of the symptoms of caterpillar larvae of the mortality of *P. xylostella* placed on the trays, shows the presence of hypa *B. bassiana* and *Metarrhizium* spp. growing on the caterpillar.

Discussion

The results of the percentage of *P. xylostella* mortality in all treatments in this study were almost equivalent to the application of *B. bassiana* and *Metarrhizium* spp. at a concentration of 4 x 20^8 spore'sml^{-1} with the percentage mortality of *P. xylostella* which is above 80% in the study of Zafar *et al*. (2020). This shows that the potential for the development of entomopathogenic fungi as *B. bassiana* and *Metarrhizium* spp. in South Kalimantan is quite high. The indication of this is the entomopathogenic fungi ability to develop well with the high toxicity of the two entomopathogenic fungi against caterpillar *P. xylostella* from South Kalimantan because LC50 from entomopathogenic fungi population is 0,12 g of entomopathogenic fungi in 100 of distilled water. The two fungi at various application concentrations can be used as recommendations for controlling *P. xylostella*, because it fulfills the criteria as a bioinsecticide with a control of above 72% (Utami 2014) and it also fulfills high toxicity with IC_{50} under 1 g in 100 of distilled water (Ihsan *et al*., 2018).

This research shows that the number of entomopathogenic fungi population will determine the effectiveness its control over *P. xylostella*, because the higher the entomopathogenic fungi concentration, the higher the mortality percentage of *P. xylostella*. The results of this study are the same as the results of Zafar (2020), which also shows that the greater the concentration of entomopathogenic fungi that is applied, the higher the larval mortality of *P. xylostella*. The higher the entomopathogenic fungi concentration, the higher the mortality of *P. xylostella*. The effectiveness of entomopathogenic fungi causing the mortality of *P. xylostella* is in how well the fungus can infect its host and the infection is influenced by the density of the conidia (Nurnilahwati 2013). Therefore, the cost of controlling *P. xylostella* application at a concentration of 1 g to 2 g of entomopathogenic fungi in 100 of distilled water is recommended.

The mean mortality of *P. xylostella* applied by *Metarrhizium* spp. was 72 hours after application, while that of *P. xylostella* applied by *B. bassiana* was 55.44 hours. The duration of mortality of these two isolates were faster than the time of mortality of *P. xylostella* which was applied using *B. bassiana* in the study of Utami *et al*. (2014), with the range of mortality rates of *P. xylostella* from 123-158.4 hours.

The rate of pest mortality is one indicator of the success of bioinsecticides, the faster the mortality caused by entomopathogenic fungi, the less damage will be generated by these pests. The ability of the entomopathogenic fungi to quickly cause mortality in pests is the difference in species of entomopathogenic fungi and their virulence which is related to the ability of entomopathogenic fungi to grow and develop, produce toxins, and induce systemic resistance of host plants. The speed of *B. bassiana* in causing mortality in *P. xylostella*, because *B. bassiana* can produce a toxin called Beauvericin (Utami *et al*., 2014).

The working principle of entomopathogenic fungi is not as fast as synthetic insecticides which can

directly kill the targeted insects. The entomopathogenic fungi takes longer in killing the targeted insects. During the process of infection, *B. bassiana* produces several enzymes for its development in the insect body, such as proteases, chitinases, and lipases which degrade the host cuticle and facilitate the attachment of conidia *B. bassiana* to the host cuticle (Aw & Hue 2017, Brady 1979). In addition, Tanada & Kaya (1993) stated that entomopathogenic fungi produce several types of toxins which in their working mechanism cause an increase in hemolymph pH, hemolymph clotting, and cessation of hemolymph circulation. Some of the toxins produced by *B. bassiana* are beauvericin, beauverolit, basianolit, isorolit, and oxalic acid. Then, according to the statement of (Ayudya *et al*., 2019), attacking fungi are characterized by the insect body becoming stiff and hard.

Conclusion

Conidia of the fungus *Metarrhizium* spp. are spherical and hyaline cylinders, while the conidia of *B. bassiana* are round to oval, hyaline, and solitary at the tip of the conidiophores.*B. bassiana* and *Metarrhizium* spp. growing on the caterpillar, fungi attacks are characterized by the insect body becoming stiff and hard. This study concludes that there is a potential development of bioinsecticide from two entomopathogenic fungi, such as *B. bassiana* and *Metarrhizium* spp. which are effective for controlling insect pests (*P. xylostella*) in South Kalimantan. The recommended application, which is the concentration of *B. bassiana* and *Metarrhizium* spp. is effective in virulence and economic of insect pests (*P. xylostella*) in South Kalimantan with a 1.5 g of entomopathogenic fungi in 100ml of distilled water.

Competing interests

Authorshavedeclared that no competing interests exist.

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