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Application of Riau local entomopathogen fungi *Beauveria bassiana* toward *Spodoptera litura* pest and plants growth red chili

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Abstract

Red chili is a horticultural crop that is widely cultivated commercially. The need for red chili is increasing, in accordance with the increasing variety of types of food that use red chili as a raw material. Pest attacks on red chili plants are an obstacle in red chili cultivation. *Spodoptera litura* is a red chili pest that can cause yield reductions of up to 80%. Control using the Riau local entomopathogenic fungi *Beauveria bassiana* is an effort to control *S. litura* using environmentally friendly materials. This study aims to determine the effect of the application of the Riau local entomopathogenic fungi *B. bassiana* to contol *S. litura* pests and the growth of red chili plants. The research was carried out at the Plant Pest Laboratory and Experimental Garden, Faculty of Agriculture, Riau University from July to August 2020. This study was designed in a completely randomized design with five treatments, namely the concentration of Riau local Riau *B. bassiana* 20 gl-1 water, 25 gl-1 water, 30 gl-1 water, 35 gl-1 water and 40 gl-1 water and four replications to obtain 20 experimental units. The results showed that the concentration of Riau local *B. bassiana* 30 gl-1 of water containing a conidia density of 4.1×10^8 con.ml⁻¹ was a concentration that affected *S. litura* pests and chili plant growth by causing the intensity of *S. litura* pests which could be categorized as lower at 4.15% and can cause a peak in larval death on the fourth day after application.

Keywords: Beauveria bassiana; Spodoptera litura; Capsicum annum; Riau local entomopathogenic

1. Introduction

Red chili (*Capsicum annum* L.) is a horticultural crop that is widely cultivated commercially. The need for red chili is increasing, in accordance with the increasing variety of types of food that use red chili as a raw material. The increasing demand for red chili plants needs to be balanced with an increase in red chili production.

Red chili production in Riau Province in 2017 was 15,813.4 tons with a harvested area of 2,236 ha, and in 2018 it decreased to 1,732.39 tons with a harvested area of 2,309 ha [1]. The decrease in red chili production was caused by *S. litura* attack.

S. litura pests attack red chili plants in the vegetative and generative phases. *Spodoptera litura* causes defoliation, at a severe level *S. litura* eats up the entire leaf and leaves only the leaf bones, thus disrupting the photosynthetic ability of plants [2].

The control of *S. litura* pests on red chili plants is generally done by using chemical insecticides, continuous use of chemical insecticides can cause resistance, resurgence, increased residues carried with crop yields, destruction of natural enemies and environmental pollution.

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Spodoptera litura pest control using bioinsecticides is an effort that can be done to reduce the use of chemical insecticides. The Riau local entomopathogenic fungi *B. bassiana* is a bioinsecticide that has a wide host range, easy application, can be applied to various phases of insect life, is safe for the environment and does not interfere with the survival of natural enemies [3].

Nurani *et al* [4] stated that the entomopathogenic fungi *B. bassiana* at a concentration of 30 g.l-1 was able to control *S. litura* pests with a mortality of 75% with a conidia density of 1×10^7 con.ml⁻¹. The Riau local Riau *B. bassiana* fungi was able to control the pod borer Maruca testualis with a mortality of 86% at a concentration of 30 g.l⁻¹ water with a conidia density of 5.12×10^4 con.ml⁻¹ [5]. Steinhaus [6] declare that entomopathogenic fungi can be categorized as biopesticides if they are successful in controlling 72% - 95% of insect pests.

2. Material and methods

2.1. Preparation of Research Media

The research was carried out at the Plant Pest Laboratory and Experimental Garden, Department of Agrotechnology, University of Riau from July to Agust 2020. The research was carried out experimentally using a completely randomized design consisting of 5 treatments and 4 replications, obtained 20 experimental units with each treatment being an application. concentration of Riau local entomopathogenic fungi *B. bassiana* 20 g.l⁻¹ water, 25 g.l⁻¹ water, 30 g.l⁻¹ water, 35 g.l⁻¹ water.

2.2. Provision of Armyworm Caterpillars (Spodoptera litura)

Spodoptera litura larvae were taken from the papaya plant at Experimental Garden, Faculty of Agriculture, Riau University and brought to the Plant Pest Laboratory. Larvae are kept in a jar with a diameter of 15 cm and a height of 7 cm and given fresh spinach leaves as food. Spinach leaves are changed every day until the larvae become pupae. The pupae were transferred to a jar with a diameter of 18 cm and a height of 20 cm filled with sawdust and maintained until they became imago. Imago is fed with a 10% concentration of honey solution by dipping it with cotton and hanging it in a jar. The side of the jar is given a rough tissue to place the eggs in. The egg groups on the tissue are transferred to a 13 cm x 9 cm jar. Larvae are reared until they reach third instar and are ready to be used as test insects.

2.3. Provision of Riau local Beauveria bassiana

The soil used comes from the rhizosphere of the red chili plant in Langsat Permai Village, Bunga Raya District, Siak Regency, Riau Province. The isolation of *B. bassiana* was carried out by the insect bait method using fifth instar larvae of *Tenebrio molitor*. *T. molitor* suspected of being infected with *B. bassiana* was transferred to filter paper and incubated for seven days. *B. bassiana* was isolated in PDA media and incubated for seven days.Identification of Riau local *B. bassiana* was carried out macroscopically and microscopically based on its morphology using a book written by Barnett and Hunter [7].

Riau local isolates of *B. bassiana* growing on PDA media were isolated on crushed corn medium and incubated for five days. Starter *B. bassiana* broken corn was weighed according to treatment, namely 20 g, 25 g, 30 g, 35 g and 40 g respectively mixed with 1000 ml of water then stirred and filtered. The suspension is put in a bottle and shaker for 24 hours. The suspension is then carried out dilution with a ratio of one ml of suspension and nine ml of water with a dilution of 10⁻⁷. Conidia were seen using a haemocytometer under a microscope and conidia density was calculated by handcounter. Conidia density is calculated by the formula:

$$J = \frac{t x d}{0.25 x n} \times 10^6$$

Where:

J: the number of spores in one gram of media

t: the number of spores in all the counted squares

d: dilution factor if it must be diluted (d = 1 means not diluted; d = 10 means diluted 1:10)

0.25: kostanta

n: the number of squares counted

2.4. Application

Riau local application of Riau local suspension *B. bassiana* was carried out using a 100 ml sprayer. Each red chili plant is sprayed evenly according to the volume of spray. The application of Riau local suspension *B. bassiana* was carried out in the afternoon at 18.00 WIB.

2.5. Data Analysis

Daily mortality data were analyzed descriptively and displayed in the form of graphs, while the intensity of attack, red chili plant growth data were statistically analyzed using variance and displayed in tabular form. Data from variance was analyzed using Duncan's New Multiple Range Test (DNMRT) at 5% level.

3. Results

3.1. Daily Mortality (%)

The results of observations of the daily mortality percentage of *S. litura* larvae with the application of several different concentrations of Riau Riau local *B. bassiana* on red chili plants showed fluctuations in the mortality of *S. litura* larvae. Daily mortality fluctuations of *S. litura* after application of several concentrations of Riau local Riau *B. bassiana* can be seen in Figure 1.

3.2. Attack intensity (%)

Application of concentration of Riau local entomopathogenic fungi *B. bassiana* on red chili significantly affected the intensity of attack of *S. litura* larvae (Table 1). The results of the DNMRT follow-up test at the 5% level can be seen in Table.

3.3. Growth of red chili plants

Application of concentration of Riau local entomopathogenic fungi *B. bassiana* in red chili has a significant effect on the growth of red chili plants. The results of the DNMRT follow-up test at the 5% level can be seen in Table 2.



Figure 1 Daily mortality fluctuations of *S. litura* larvae after application of Riau Riau local *B. bassiana* on red chili plants

Riau local concentrations of <i>B. bassiana</i> (conidia density)	Attack intensity (%)	
20 g.l ⁻¹ air (3,3 × 10^8 kon.ml ⁻¹)	41,63 a	
25 g.l ⁻¹ air (3,8 × 10^8 kon.ml ⁻¹)	34,75 a	
30 g.l ⁻¹ air (4,1 × 10 ⁸ kon.ml ⁻¹)	4,15 b	
$35 \text{ g.l}^{-1} \text{air} (4,6 \times 10^8 \text{ kon.ml}^{-1})$	2,23 b	
40 g.l ⁻¹ air (5,0 × 10^8 kon.ml ⁻¹)	2,23 b	

Table 1 Attack intensity of *S. litura* pest on red chili plants after the application of of Riau Riau local *B. bassiana*

The numbers in the row followed by lowercase are significantly different according to the DNMRT test at the 5% level.

Table 2 Growth of red chili plants after application of the of Riau Riau local *B. bassiana*

Riau local concentrations of <i>B. bassiana</i> (conidia density)	Plant Height (cm)	Dichotomous Height (cm)	Flowering Age (days)
20 g.l ⁻¹ air (3,3 × 10^8 kon.ml ⁻¹)	26,40 d	14,12 c	41,0 a
25 g.l ⁻¹ air (3,8 × 10^8 kon.ml ⁻¹)	33,55 c	15, 37 b	40,0 b
30 g.l^{-1} air (4,1 × 10^8 kon.ml^{-1})	36,47 bc	16,87 a	39,5 bc
35 g.l ⁻¹ air (4,6 × 10 ⁸ kon.ml ⁻¹)	39,37 ab	17,15 a	38,5 c
40 g.l ⁻¹ air (5,0 × 10^8 kon.ml ⁻¹)	41,57 a	17,75 a	37,0 d

The numbers in the row followed by lowercase are significantly different according to the DNMRT test at the 5% level.

4. Discussion

4.1. Daily Mortality (%)

The first day after the application of Riau local *B. bassiana* no dead *S. litura* larvae were seen, presumably because Riau Riau local *B. bassiana* needed time to make contact with the larval integument and secreted beauvericin toxin which would damage the tissue and cause death of *S. litura* larvae. This is in accordance with the statement of Riningrum *et al.*, [8] which states that *B. bassiana* takes 2-4 days to kill the test insects.

Daily mortality of *S. litura* larvae only occurred on the second day after application of 5% according to the initial time of death for the first time, which was 43 hours after application (concentration 40 g.l-1 water). Concentration of Riau local *B. bassiana* 35 g.l-1 water, 30 g.l-1 water, 25 g.l-1 water, and 20 g.l-1 water on the second day after application has not been able to cause mortality in *S. litura* larvae. This is presumably because at this concentration the Riau local conidia density of *B. bassiana* applied was lower than the concentration of 40 gl-1 of water and the beauvericin toxin produced by Riau local *B. bassiana* was less, which resulted in the larval body of *S. litura* still being able to tolerate infection from *B. bassiana*. Riau locally so that the larvae can still survive. Prayogo [2] states that the tolerance response carried out by insects is an effort to defend their lives from pathogen attacks. Observations on the third day showed that all Riau local concentration of 35 gl-1 water showed the percentage of dead *S. litura* larvae was 22.5%, the concentration of 20 gl-1 of water by 10%. The difference in the daily mortality percentage of *S. litura* larvae was caused by differences in the concentration of Riau local *B. bassiana* applied, the higher the Riau local *B. bassiana* concentration, the higher the infection power of *S. litura* larvae. Turnip *et al.*, [9] stated that insect mortality is largely determined by the concentration applied, the higher the conidia density, the higher the mortality.

Observation of the fourth day of daily mortality of *S. litura* larvae experienced the highest peak mortality of any Riau local *B. bassiana* concentration except the concentration of 20 g.l-1 water. The peak mortality of Riau local *B. bassiana* was 40 g.l-1 water, 35 g.l-1 water, 30 g.l-1 water was 27.5% and the concentration of 25 g.l-1 water was 22.5%. The Riau local *B. bassiana* concentration of 20 g.l-1 only experienced an increase in daily mortality on the fifth day by 20%. Riau local *B. bassiana* concentrations of 20 g.l-1 took longer to reach peak daily mortality than other Riau local *B. bassiana* concentrations. The difference in the peak time of death of *S. litura* larvae was due to differences in the concentration of Riau local *B. bassiana* applied to *S. litura* larvae so that the level of pathogenicity was also different. Mardiana [5] stated that the amount of concentration applied would determine the effectiveness of *B. bassiana* in controlling the test insects.

Application of various concentrations of Riau local *B. bassiana* caused a decrease in the percentage of daily mortality of *S. litura* larvae on the sixth day. Daily mortality fluctuations of *S. litura* larvae were caused because Riau local *B. bassiana* had infected and developed in the body of *S. litura* larvae by secreting toxins and absorbing body fluids of *S. litura* larvae which resulted in many *S. litura* larvae dying, so the number of *S. litura* larvae died. The litura that died the next day was reduced. The difference in daily mortality for each treatment was due to the different concentrations of entomopathogenic fungi that were applied to *S. litura* larvae so that their virulence was also different.

4.2. Attack intensity (%)

Table 1 menunjukkan bahwa aplikasi Table 1 shows that the application of higher concentrations of Riau local Riau *B. bassiana* tends to cause the intensity of attack of *S. litura* larvae on red chili plants in the range of 2.23%-41.63%. The concentration of *B. bassiana* in Riau local 30 gl-1 of water caused the intensity of attack of 4.15% and was significantly different from the concentration of 25 gl-1 of water and 20 gl-1 of water but not significantly different from the concentrations of 35 gl-1 of water.

The difference in pest attack intensity was caused by differences in the concentration of Riau local Riau *B. bassiana* which was applied to red chili plants. This was due to the activity of *S. litura* which was affected by infection with the fungi *B. bassiana*. *S. litura* pests that have been infected with the *B. bassiana* fungi will experience metabolic disorders, the respiratory system, and the digestive system, so that the armyworm's appetite is reduced resulting in the caterpillar becoming less active [4], so that the armyworm's activity will have an effect on the intensity of the damage did not increase.

Application of concentration of *B. bassiana* Riau local Riau 30 g.l-1 water, 35 g.l-1 water and 40 g.l-1 water can be classified as very low attack intensity because it is less than 10%. This is in accordance with Syahrawi and Busniah [10] which states that the intensity of pest attacks can be categorized as very low if it is less than 10% and can be categorized as low if it is between 10-50%.

4.3. Growth of red chili plants

Table 2 shows that the application of Riau local concentrations of *B. bassiana* on plant height parameters tends to cause higher chili plant height growth of 41.57 cm. The concentration of 40 g.l-1 water had no significant effect with the concentration of 35 g.l-1 water but significantly different with the concentration of 30 g.l-1 water, 25 g.l-1 water, 20 g.l-1 water. It is presumed that the higher the concentration of Riau local Riau *B. bassiana* applied, the higher the red chili plant height.

The dichotomous height parameter of the concentration of 30 g.l-1 water was not significantly different from the concentrations of 35 g.l-1 water and 40 g.l-1 water, but was significantly different with the concentrations of 20 g.l-1 water and 25 g.l-1 water. This is because the Riau local concentration of *B. bassiana* that is applied affects the growth of red chili plants. The higher the concentration of Riau local *B. bassiana* applied, the higher the dichotomous level.

Flowering age of red chili plants after application of Riau local Riau *B. bassiana* concentration ranged from 37-41 days. The concentration of 40 g.l-1 water was significantly different from other concentrations. because the higher the concentration of Riau local Riau *B. bassiana* applied, the faster the flowering period in chili plants. Concentration of 30 g.l-1 water showed no significant effect with concentrations of 35 g.l-1 water and 25 g.l-1 water.

5. Conclusion

The Riau local Riau application *B. bassiana* within six days is able to caused the mortality of *S. litura* larvae by 90%, attack intensity 2.23%. The intensity of attack affects the growth of red chili plants, because low intensity can provide opportunities for red chili plants to grow optimally.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors declare there is no conflict of interest in this paper.

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