Isolation *Beauveria Bassiana* Vuill. Entomopathogen Local From Plant Agriculture Rhizosphere in Riau Province, Indonesia with Insect Bait *Tenebrio Molitor* Larvae

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Abstract. *Beauveria bassiana* is an entomopathogen that has the ability to control agricultural crop pests. The purpose of this study was to find local *B. bassiana* from various rhizosphere of agricultural plants in Riau Province, Indonesia using insect baiting techniques. Isolation is carried out in several rhizosphere in plants such as oil palm, chili and rice. The results showed that isolate *B. bassiana* from oil palm plantations showed a faster time of infection in the test larvae of 3 days and 100% mortality, time to grow on PDA medium 2 days and conidia density $4.87 \times 10^7$ con/ml.

1. Introduction

The attack of plant pests until now remains a dominant problem in every agricultural business. Control by using synthetic pesticides that are a mainstay increasingly shows a decrease in effectiveness. As a result there are several types of pests that become resistant to chemical pesticides. This can cause poisoning and environmental pollution for humans due to excessive and unwise use. Therefore it is necessary to take action to minimize the use of chemical pesticides to comply with the concept of integrated pest control (IPM). Appropriate control is pest control with consideration of ecological aspects / maintaining environmental, social and economic sustainability. The use of microorganisms for pest control is an option that can be used in plant protection. Microorganisms that can be used to control pests and have enormous potential, including the entomopathogenic *Beauveria bassiana*.

*Beauveria bassiana* is one of the most widely used pathogenic for plant pests control. Entomopathogenic *B. bassiana* is suitable for pest control because it is selective against target insects so that it does not harm other non-target insects, such as predators, parasitoids, pollinating insects, and useful insects (honey bees). The advantage of using *B. bassiana* is that it does not leave toxic residues on agricultural products, in the soil or in watercourses. It also does not cause phytotoxins (poisoning) in plants and is easily produced with simple techniques. [1] utilize *B. bassiana* to control Ceratitis capitata in fruit fly. The technique of using bait is a way to get entomopathogenic *B. bassiana* [2]. In addition entomopathogenic *B. bassiana* from Riau province will be more effective in controlling pest insects in Riau province because the environment is the same, making it easier for *B. bassiana* to attack its host. *B. bassiana* entomopathogen s were obtained from the soil at a depth of 5-15 cm from the soil surface, because of this depth it is estimated that there are many *B. bassiana* inoculum. One of the techniques for acquiring *B. bassiana* entomopathogenic s from the soil is to use hongkong caterpillars (*Tenebrio molitor*) as a bait [3].

Hongkong caterpillars have economic value and benefits. *T. molitor* caterpillars are used as bird feed, but in this study Hong Kong caterpillars are used as bait to obtain entomopathogenic *B. bassiana*.
, this is because *T. molitor* larvae are easily obtained in bird markets in the city of Pekanbaru as the capital of Riau province. The purpose of this study was to obtain entomopathogenic isolate *B. bassiana* from the rhizosphere of agricultural crops in the Indonesian Riau Province using larvae bait *T. molitor*.

2. Methodology
The source soil of *B. bassiana* isolates was taken from the rhizosphere of oil palm, chilli and rice plants in Langsat Permai village, Bunga Raya sub-district, Siak Regency, Riau Province, Indonesia. Soil is taken by determining three points, sampling diagonally on a plant plot, the area of one plot of agricultural crops for example about 2 x 2 m. Soil is taken as much as 2 kg using a shovel at a depth of 5-10 cm, then put into plastic measuring 2 kg and labeled the origin of the soil, the date of collection and location of soil collection.

**Breeding *T. molitor* larvae:** *T. molitor* larvae are bought on the market and then bred in the Plant Pest Laboratory until they become imago. Eggs hatch into larvae on instar 3 to be used as test larvae. The larvae used were whitish color as the test larvae. Each soil container was given 10 larvae of *T. molitor*.

**Isolate entomopathogenic *B. bassiana* from soil:** Isolation is carried out using the insect bait method [3]. Soil that has been taken is weighed as much as 400 g and put into each container. *T. molitor* test larvae as bait were put into containers containing 10 tons of soil each, then covered with a thin layer of soil and sprayed with sterile aquades so that moisture could be maintained. Spraying using a hand sprayer is done every day, then the container is covered with a black cloth. Observations on the test larvae were observed every day to determine whether there were larvae infected with local *B. bassiana* entomopathogenic.

The larvae infected with local *B. bassiana* first surface sterilize with 1% sodium hypochlorite for 3 minutes. Then rinse with distilled water 3 times and air dried on filter paper. Furthermore, the sterilized larvae were placed on petri dishes which had been dialed with filter paper that had been moistened and tightly closed, then incubated for seven days to accelerate the growth of local *B. bassiana* entomopathogenic.

After the growth of *B. bassiana* in the test larvae, observations were carried out under a microscope to see the characteristics of local *B. bassiana* such as the color of the *B. bassiana*, the shape of the colony and the shape of mycelium. If the observations show that is *B. bassiana*, the mycelium that comes out of the larva’s body is then taken using an ose needle and cultured on a PDA (Potato Dextrose Agar) medium and incubated for 7 days.

**Calculation of Conidia:** Before conidia are counted, conidia multiply on the corn media as a starter for up to 5 days. Calculate conidia by taking 35 g of *B. bassiana* starter and mixed with 1000 ml of distilled water, then knead and filtered using gauze. Then dilution is carried out. Dilution is done by taking 1 ml of the suspension, then put in 9 ml of distilled water. Dilution is carried out 7 times. Conidia are calculated using a haemocytometer under a microscope.

3. Results and Discussion
*B. bassiana* are common found on the ground and can be found all over the world. Based on the results of research in the Laboratory with a temperature of 27°C and humidity of 79%, from the three origins of agricultural land, namely oil palm, rice and chilli plantations, local *B. bassiana* found Riau.

**Observation of microscopic morphology of isolates**
Microscopic observations with microscopy showed that the isolates found from rhizosphere soil of oil palm, rice and chilli plants were entomopathogenic *B. bassiana* which were characterized by hyphae, dividing, branching and hyaline color, hyaline conidiophores, insulated and unbranched and conidia round and clustered on hyphae. In accordance with the opinion of [4], *Beauveria* sp. has hyphae insulated, hyaline straight, and thick. A single-celled hyaline conidia formed solitary at the end of the conidiophor. The observations can be seen in Figure 1.
According to [5] single-celled conidia, the shape is oval rather round to ovoid, colored hyalin, smooth walls with a diameter of 2-3 µm. Conidia and conidiophores are arranged symmetrically, these zig-zag-shaped conidiophores are a hallmark of the genus *B. bassiana* (Figure 2).

Transmission back to *T. molitor* larvae

Transmission of isolates obtained from the soil using $10^7$ conidia/ml suspensions. The results of transmission of *T. molitor* larvae showed that the percentage of larval mortality between 80-100% can be seen in Table 1.

| Isolate Source | Time of Infection (day) | Mortality (%) |
|----------------|-------------------------|---------------|
| Palm oil soil  | 3                       | 100           |
| Rice soil      | 5                       | 80            |
| Chili soil     | 10                      | 100           |

Table 1 shows local *B. bassiana* were able to infect larvae at different times between three soil origins. The entomopathogenic *B. bassiana* which is in the soil infects the test larvae which are placed on the ground by entering the body of the larvae through the skin, digestive tract, spiracles and other natural holes. Inoculum attached to the body of the test larvae will germinate and develop to form a germination tube, then enter the skin of the test larvae. Penetration is done mechanically and chemically by removing enzymes and toxins. will develop in the body of larvae and attack all larval body tissue so that the larvae die. The mycelium penetrates outside the body of the growing larva to cover the body of the larva and produce conidia [6].

The entomopathogenic *B. bassiana* needs a moist growing medium and the availability of sufficient nutrients in the soil. Laboratory conditions during the study with a temperature of 27°C and humidity of 79% are suitable for growing *B. bassiana* in the test larvae. *B. bassiana* naturally occurs in the soil...
as a saprophytic, the growth of *B. bassiana* in the soil is strongly influenced by soil conditions such as organic matter content, temperature, humidity, eating habits of insects, the presence of synthetic pesticides, and application time. In addition, fertilization is always carried out at the location of the soil so that the soil becomes fertile and the pH of the soil increases, thereby fulfilling the nutritional requirements of the *B. bassiana* in the soil. The application of pesticides can break the life cycle of *B. bassiana*, because chemical pesticides contain toxic substances that can kill plants that are nearby, including *B. bassiana* which is in the soil. Rice and chili plants also do not have a wide canopy, so that the soil in rice and chili plantations is exposed to direct sunlight which causes high temperatures and low humidity. Such environmental conditions cause entomopathogenic isolates of *B. bassiana* from rhizosphere soil of oil palm plantations to grow and develop more quickly than soil origin of rice and chilli rhizosphere. Baits of insects that are attacked by the larvae appear to harden, turn brownish black and the white mycelium can be seen in Figure 3.

![Figure 3. T. molitor larvae infected by local B. bassiana at the age of 7 days.](image)

**Observation of macroscopic morphology**

The time needed for *B. bassiana* to grow on the PDA medium to 1 cm in diameter can be seen in Table 2.

| Isolate source | Rate |
|----------------|------|
| Palm oil soil  | 2.0  |
| Rice soil      | 3.0  |
| Chili soil     | 3.0  |

Table 2 shows the time needed for *B. bassiana* to grow on a PDA medium up to 1 cm in diameter is not much different from the land of oil palm, rice and chilli crops. The fastest time of *B. bassiana* to grow on a PDA medium up to 1 cm in diameter is the soil from which oil palm is planted, then followed by the soil from which rice and chillies are planted. PDA medium can meet *B. bassiana* nutrition during growth. Because the medium contains carbohydrates and sugars that serve as food during *B. bassiana* mushroom growth in PDA medium. At 28°C and 92% humidity suitable for the growth of *B. bassiana*, the will grow quickly. The results showed that *B. bassiana* isolates from oil palm plantations were faster and more effective in utilizing nutrients on PDA medium than isolates from rice and chilli plants (Figure 4).
Figure 4. B. bassiana colonies on PDA medium at 3 days of age and 7 days

The shape of a colony on the PDA medium
Observation of the shape of the colony is done by looking directly with the eyes when the B. bassiana is 3 days old, while the shape of the B. bassiana colony is white round with a diameter of 1-3 cm, the direction of its growth upwards and then widens laterally within a week at temperature of 28°C and 92% humidity. According to [7], the diameter of the B. bassiana colony reached 0.6-2.3 cm in 8 days at a temperature of 20°C, the colony's appearance was like wool, sometimes resembling fine flour because of its abundant conus.

Colony color
Observation of the color of the colony is done by looking directly with the eyes. The color of the colony is white to slightly yellowish or cream. This is in accordance with the research of [8], that the color of the B. bassiana colony is chalk white, light brown to light yellowish white. This is also supported by the opinion of [9] stating that the colony is white like cotton on a PDA medium.

Conidia density (con/ml)
Conidia density calculation is done using a haemocytometer under a microscope can be seen in Table 3.

| Isolate source | Rate (con/ml) |
|----------------|---------------|
| Palm oil soil  | 4.87 x 10^7   |
| Rice soil      | 3.92 x 10^7   |
| Chili soil     | 4.55 x 10^7   |

Table 3 shows the density of B. bassiana conidia from oil palm soils is higher than that of conidia from rice and chilli soil. On the soil of oil palm plantations there are more isolates of B. bassiana compared to the origin of rice and chilli. The home environment of oil palm plantations is more suitable for the growth of B. bassiana than that of rice and chilli. Oil palm plants also have a large canopy, so that the land around the crop is protected, while the rice and chili plantations are short-lived plants so that the land is often cultivated and pesticides are often applied to overcome pest and disease attacks.

4. Conclusion
Agricultural planting land in the Riau province of Indonesia, namely oil palm, paddy and chilli land, has been found in Riau's local entomopathogenic B. bassiana using T. molitor larvae. Soil from oil palm plantations showed a faster time of infection in the test larvae of 3 days and 100% mortality, time to grow on PDA medium 2 days and conidia density 4.87 x 10^7 con/ml.
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