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Insecticidal activity of filtrate of *Beauveria bassiana* cultures incubated under the temperatures of 25 °C and 34 °C against larvae *Spodoptera litura*

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Abstract. *Beauveria bassiana* has been widely used to control lepidopteran larvae, such as *Spodoptera litura*. The objective of this research was to observe the mortality and median Lethal Time (LT₅₀) of larval *S. litura* after exposure of filtrate of *B. bassiana* cultures incubated under temperatures of 25° and 34°C. The isolates used were 10 isolates of *B. bassiana* and they were incubated under temperatures 25 and 34 °C in liquid media for 6 wk. The results showed that the highest mortality caused by *B. bassiana* was found on the fungus incubated under 25 °C and was significantly different from that of temperature 34 °C. The shortest LT₅₀ of larval *S. litura* was found on the fungus incubated under temperature 25 °C and was significantly different from that of temperature 34 °C. The highest mortality was found on isolate BTmPc (96%) incubated under temperature 25 °C with the shortest LT₅₀ (7.51 d), which was not significantly different from isolate BTmTr (93.33%) incubated under temperature 25 °C with the LT₅₀ (7.31 d). Therefore, isolate BTmTr incubated under temperature 25 °C was the most effective on killing *S. litura* larvae. However, isolate of BMkMs incubated under 34 °C was still caused the highest percentage of larval mortality (18.67%) and the shortest LT₅₀ (16.20 d). Therefore, the BMkMs isolate can be developed into bio-insecticide active ingredients for controlling *S. litura* in tropical ecosystem.



1. Introduction

Beauveria bassiana was proven to have controlled various pest insect species, for example *Nilaparvata lugens* [1], *Spodoptera litura* [2], and *Aphis gossypii* [3] on chili. In addition, the fungus did not endanger the natural enemy arthropods [4, 5].

B. bassiana although proven effective in controlling various species of pest insects, the success of controlling insect pests in the field is generally influenced by many external factors, including the pH of culture media [2], temperature [6, 7]. Most entomopathogenic fungi tolerate in temperature ranged from 0 – 40 °C [6], but certain strains are only able to survive at temperatures below 35 °C [8]. Optimal temperatures for the growth of the entomopathogenic fungi are generally around 25-30 °C [9] and temperature at 25 °C is ideal for the fungal germination [10]. Extreme high temperatures can cause the death of the fungus [11]. In addition, the production of colony and conidial density of *B. bassiana* decreases significantly when the temperature during fungal incubation is increased from 30 – 35 °C [11], and all isolates die at 36 °C [12, 11]. Fungal germination also drops at temperatures above 30° C [12] and the highest up to 33 °C [13]. Virulence of the entomopathogenic fungi is also affected by the temperature due to decreased production of toxins produced by fungus [14, 15]. Each strain or species of the entomopathogenic fungi has different optimal and temperature tolerance. Entomopathogenic fungal strains able to survive in extreme high temperatures above 33 °C [13] are superior strains that can be come candidates to control insect pests in a tropical ecosystem. Therefore, this study aimed to find out the mortality and median Lethal Time (LT₅₀) of larval *S. litura* after exposure of filtrate of *B. bassiana* cultures incubated under temperatures of 25 °C and 34 °C.

2. Methods

The study was conducted in the Laboratory of Entomology, Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Sriwijaya, Indralaya, from May 2018 to March 2019. The average temperature during the experiment was 29.69 °C and the relative humidity was 82.95%.

2.1. Preparation of test insects

The test insects chosen were *Spodoptera litura* attacking chili and various types of plants in freshwater swamps. *S. litura* larvae were obtained from chili plants grown in the experimental farm of Faculty of Agriculture, Universitas Sriwijaya, and the chili was not applied with synthetic pesticides. Then, the *S. litura* larvae were kept in the laboratory for 2 generations and fed with a mixture of mulberry leaves and water spinach which were grown without the application of synthetic pesticides and the feed was changed every 2 times a day. Mass rearing was carried out to get the 2nd generation of culture. The 2nd instar larvae of one day old were used for test insects in this experiment.

2.2. Preparation of isolates *Beauveria bassiana* and culture filtrate

B. bassiana isolates used in this study (Table 1 and Fig. 1) were the result of exploration by [16] from the wetlands land, South Sumatera, Indonesia. Before being used for the study, all isolates were made fit using the method of [3] which modified the enrichment of the culture medium, Sabouraud Dextrose Agar (SDA, Merck) used smashed *Tenebrio molitor* larvae. The flour acted as a source of protein.

One week-old *B. bassiana* culture derived from SDA media was then grown in PDB (Potato Dextrose Broth) media containing 20 g dextrose monohydrate, 200 g potatoes, and 1000 mL aquadest. The liquid culture (culture broth) of *B. bassiana* in the PDB medium was incubated for 5 wk at room temperature. After that, it was incubated at 25 °C and 34 °C according to the treatment for 7 d (1 wk). The treatment used ideal temperature (25 °C) as a control and an extreme high temperature (34 °C) aiming to test whether the extreme temperature affected the virulence of culture filtrate.

2.3. Production of culture filtrate

The liquid culture (culture broth) *B. bassiana* derived from PDB medium that was given a temperature treatment was filtered to separate the solution from the pulp (hyphae, mycelia, and conidia) by performing 2 stages of filtering by modifying the method of [17]. The separation of hyphae, mycelia,

and conidia from the culture was to produce culture filtrate containing insecticidal toxic compounds. The first stage of filtering: 100 mL of liquid culture of *B. bassiana* of 6 wk-old already given a temperature treatment was poured into an erlenmeyer tube (volume of 500 mL) in which the mouth of the tube was installed filter paper of Whatman no. 42 covered with cotton of 1 cm thick. This filtering process produced ± 70 mL of crude culture filtrate. The second stage: the crude culture filtrate was filtered using a syringe filter (0.45 μm -25 mm). This second filtering was carried out by means of 1 mL of coarse culture filtrate which was filtered first using a hypodermic needle (volume 6 mL), after that the needle was removed and the base was attached to a syringe filter, then the needle was reassembled into the hypodermic needle and the 1 mL of coarse culture filtrate was filtered using a syringe filter to get the culture filtrate. The culture filtrates were used at concentration of the original preparation without dilution by sterile water.

Table 1. Isolates of *Beauveria bassiana* used in the experiment.

Isolate	Ecosystems	Village or city
BTmPc	Freshwater swamp	Indralaya
BtmGa	Freshwater swamp	Gandus
Blepd	Freshwater swamp	Pagardin
BMkMs	Highlands	Muara Siban
BPCmS	Highlands	Muara Siban
BTmkt	Freshwater swamp	Kenten
BTmTr	Freshwater swamp	Telang Rejo
BTmTs	Highlands	Mulia Sari
Ts1d3	Peatlands	Talang Dabok
Ts1d2	Peatlands	Talang Dabok

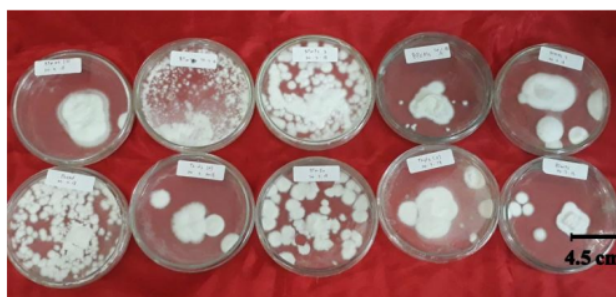


Figure 1. *Beauveria bassiana* SDA culture incubated for 7 days.

2.4. Test of insecticidal activity isolates of *Beauveria bassiana*

The culture filtrates of the second filtering results of as much as 1 mL was dripped to wet the whole chili leaves, then they were infested with the 2nd instar larvae of *S. litura* as many as 25 larvae per isolate for 6 hr and repeated 3 times for each isolates. Before being treated, *S. litura* was first fasted for 24 hr. After 6 hr of infestation and having confirmed that all larvae ate the leaves that were dipped in the culture filtrate *B. bassiana*, then the test insects were transferred into a plastic cylinder in which there were 5 sheets of new chili leaves (without culture filtrate of *B. bassiana*) already placed. Every 1 x 24 hr the dead larvae were recorded and chili leaves were replaced.

2.5. Data analysis

Mortality and the time of death of test insects (LT₅₀) were analyzed using analysis of variance (ANOVA). Turkey's Honestly Significant Difference (HSD) Test was used to test the effect of temperature and isolates (factorial). LT₅₀ values were calculated by using probit analysis. All data were analyzed using SAS University Edition software 2.7 9.4 M5.

3. Results and discussion

The results showed that the culture filtrates of *B. bassiana* with incubation temperatures of 25 °C and 34 °C for 7 d affected the mortality of *S. litura* larvae (Table 2). The mortality caused by the culture filtrates incubated at 34 °C showed a significant decrease and LT₅₀ treatment was longer than the culture filtrates incubated at 25°C.

B. bassiana with incubation temperatures of 25 °C and 34 °C for 7 d affected the mortality of *S. litura* larvae. The mortality caused by *B. bassiana* decreased after the larvae incubated at 34 °C. However, some isolates still caused high mortality after applied with *B. bassiana* at 34 °C, such as BMkMs. The mortality caused by culture of *B. bassiana* filtrate with incubation temperature of 34 °C was decreased because the temperature decreased the ability of conidia production and decreased the viability of conidia germination [1].

The optimal temperature for conidia produced by entomopathogenic fungi is at 25 °C – 30 °C [9], while the optimal temperature for conidia fungus germination is at 25 °C [10]. If the incubation temperature of the fungus is raised to 35 °C, a decrease in the production of colony and conidial density of *B. bassiana* and extreme high temperatures can cause fungal death [11].

Table 2. Mortality of *S. litura* larvae at 12 days after infested with *B. bassiana* culture filtrates from medium at 25 °C and 34 °C.

Incubation temperature	Mortality (%) ± SD ^a	LT ₅₀ (days) ± SD ^a
25 °C	70.8±8.26 ^b	8.40±0.32 ^a
34 °C	13.1±1.69 ^a	19.27±0.72 ^b
ANOVA F-value	313.8*	363.47*
P value (0.05)	0.01	0.01
Tukey's HSD test	3.2	1.15

^a Significantly different; values within a column followed by the same letters were not significantly different at P < 0.05 according to Tukey's HSD test.

The effect of isolates of *B. bassiana* culture filtrates on the mortality of larval *Spodoptera litura* showed significant differences (Table 3). The isolate caused the highest mortality (54.67%) was found in BTmkt isolates, but not significantly different from the mortality by BMkMs (49.33%). The weakest isolate of lethal ability was found in Ts1d3 isolate (34.67), but when compared to the aquadest drops, the mortality caused by Ts1d3 isolate was significantly higher. The shortest LT₅₀ was found in isolates BMkMs (11.96 d), while the longest occurred in isolates Ts1d3 (16.44%). Thus, the deadliest culture of filtrate of isolate was BMkMs.

The effect of temperature interaction and isolates of *B. bassiana* showed a significant interaction (Table 4). BTmPc culture filtrates incubated at 25°C caused the highest mortality (96%) with the shortest LT₅₀ of only 7.51 d. For incubation at 34°C, BMkMs isolates caused the highest mortality (18.67%) with the shortest LT₅₀ (16.20 d, while Ts1d2 isolates indeed caused the mortality reaching 18.67% but longer LT₅₀ (22.08 d).

Local isolates of BMkMs originated from wetland soils of South Sumatera, Indonesia continued to cause high mortality, indicated that these isolates were superior to other isolates. This isolate was more resistant to high temperatures and able to produce culture filtrate which was toxic to insect pests. The local isolates of this experiment which were still toxic culture filtrate at 34 °C had the potential to be

developed as active ingredients bio-insecticides to control insect pests of the Lepidoptera order in a high-temperature agro-ecosystem, such as in freshwater swamps and tidal lowlands in South Sumatera, Indonesia.

Table 3. The effect of isolates of *Beauveria bassiana* culture filtrates from medium incubated at 25 and 34 °C on mortality of larval *Spodoptera litura*.

Isolate	Mortality (%) ± SD ^a	LT ₅₀ (days) ± SD ^a
BTmPc	52.7±43.33 ^{de}	12.86±5.43 ^{ab}
BtmGa	41.33±33.33 ^{bcd}	13.56±5.68 ^b
BlepD	51.33±39.33 ^{de}	13.49±5.43 ^b
BMkMs	49.33±30.67 ^{cde}	11.96±4.23 ^a
BPCmS	45.33±30.67 ^{bcd}	12.50±3.72 ^{ab}
BTmkt	54.67±36.00 ^e	13.08±5.47 ^{ab}
BTmTr	54.00±39.33 ^e	13.08±5.76 ^{ab}
BTmTs	38.00±23.00 ^{bc}	15.59±6.04 ^c
Ts1d3	34.67±20.00 ^b	16.44±6.36 ^c
Ts1d2	40.00±21.33 ^{bcd}	15.79±6.28 ^c
Control	0 ^a	-
ANOVA F-value	14.8*	2.91*
P value (0.05)	0.01	0.01
Tukey's HSD test	10.5	4.00

^a Significantly different; values within a column followed by the same letters were not significantly different at P < 0.05 according to Tukey's HSD test.

The symptoms of dead insects caused by culture filtrates of *B. bassiana* indicated typical symptoms (Fig. 2). One day (1x24 hr) after the *S. litura* larvae fed on leaves already dipped in culture filtrate solution, the larvae began to be lazy to move. On the second day the integument of the larvae started to look dull, on the next day the larvae's body started to shrink and contract, and on the fifth day there were some larvae beginning to die. The dead larvae had characteristics, namely its body was dry, shrunken, and black, but did not smell.

The colors of culture filtrate produced by isolates of *B. bassiana* showed a tendency that the isolates causing high mortality of culture filtrates colors were darker than those causing the low mortality. However, the color of culture filtrates incubated at temperatures 25 °C and 34 °C did not show any real difference; the tendency of having different color of culture filtrates was due to the differences in isolates rather than in temperature.

Symptoms of dead insects caused by culture filtrate of *B. bassiana* was typical and different from those of the death caused by *B. bassiana* conidia. The symptoms of insect pests that died of culture

filtrate of *B. bassiana* caused their bodies to be dried, shrunk, and black, but did not stink [2], while the symptoms of the death caused by conidia of *B. bassiana*, the insect body was dry and wrinkled, but covered with hyphae and mycelia fungus [2]. The insects that died of the culture filtrate did not have hyphae or mycelial fungus due to the fact that *B. bassiana* did not grow in larva body because the mycelia, hyphae, and spores were no longer present in the culture filtrate. As a result, *S. litura* larvae died due to the toxic compounds contained in the culture filtrate of *B. bassiana* and not because of being infected with conidia of *B. bassiana*.

Table 4. The effect of interaction between incubation temperature and isolates of *Beauveria bassiana* culture filtrates on mortality of larval *Spodoptera litura*.

Isolates x Temperature	Mortality (%) \pm SD ^a	LT ₅₀ (days) \pm SD ^a
BTmPc x 25 °C	96.00 \pm 2.31 ^f	7.51 \pm 0.59
BtmGa x 25 °C	74.67 \pm 8.11 ^{cde}	7.87 \pm 0.79
Blepd x 25 °C	90.57 \pm 5.81 ^{ef}	8.06 \pm 0.49
BMkMs x 25 °C	80.00 \pm 10.58 ^d	7.72 \pm 0.49
BPCmS x 25 °C	76.00 \pm 12.22 ^d	8.78 \pm 0.39
BTmkt x 25 °C	90.67 \pm 4.81 ^{ef}	7.60 \pm 0.56
BTmTr x 25 °C	93.33 \pm 6.67 ^f	7.31 \pm 0.59
BTmTs x 25 °C	61.33 \pm 8.11 ^{cd}	9.54 \pm 0.71
Ts1d3 x 25 °C	54.67 \pm 9.33 ^c	10.08 \pm 0.86
Ts1d2 x 25 °C	61.33 \pm 5.81 ^c	9.51 \pm 0.21
Control	0 ^{ab}	-
BTmPc x 34 °C	9.33 \pm 1.33 ^b	18.21 \pm 1.41
BtmGa x 34 °C	8.00 \pm ^{ab}	19.24 \pm 2.75
Blepd x 34 °C	12.00 \pm 2.31 ^b	18.93 \pm 0.81
BMkMs x 34 °C	18.67 \pm 1.33 ^b	16.20 \pm 1.34
BPCmS x 34 °C	14.67 \pm 3.53 ^b	16.23 \pm 0.009
BTmkt x 34 °C	18.67 \pm 1.33 ^b	18.56 \pm 0.61
BTmTr x 34 °C	14.67 \pm 2.67 ^b	18.85 \pm 2.09
BTmTs x 34 °C	14.67 \pm 3.53 ^b	21.64 \pm 3.09
Ts1d3 x 34 °C	14.67 \pm 3.53 ^b	22.81 \pm 1.42
Ts1d2 x 34 °C	18.67 \pm 3.53 ^b	22.08 \pm 1.77
Control	0 ^{ab}	-
ANOVA F-value	5.9 [*]	0.89
P value (0.05)	0.01	0.54
Tukey's HSD test	16.4	-

^a Significantly different; values within a column followed by the same letters were not significantly different at $P < 0.05$ according to Tukey's HSD test.

The darker color of culture filtrate produced by *B. bassiana* isolates indicated a link with high mortality caused by the isolates. The darker color of culture filtrate was due to the higher quantity of protease enzymes produced by fungus compared to the light-colored culture filtrate, whereas an increase in protease enzymes causes an increase in fungus produced by fungus [18]. The protease enzyme produced by *B. bassiana* was to dissolve the insect's body protein resulting in dead insects

[19]. At optimal temperature and pH, the fungus can release ammonia and citrate which could increase the activity of extracellular enzymes [20]. Therefore, in this study the data showed that BMkMs isolates were much brown and caused mortality of *S. litura* larvae higher than any other isolate due to the more toxic culture of filtrate produced by the isolates.

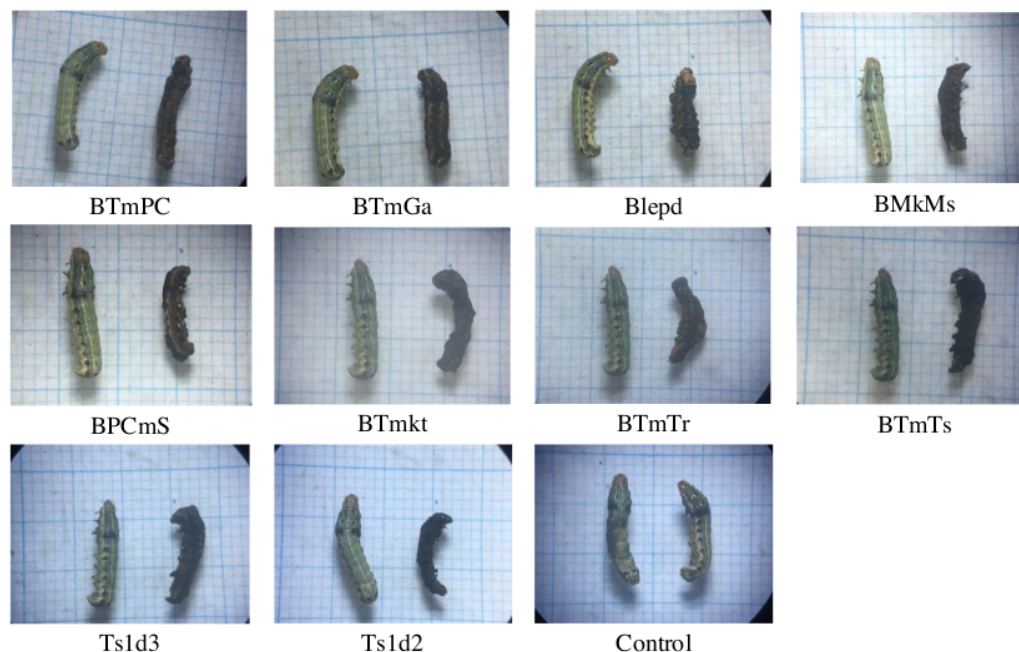


Figure 2. The dead larvae of *S. litura* caused by *B. bassiana* culture filtrate (right) and the healthy one (left).

4. Conclusion

Based on the data of this study, it concludes that the most lethal isolate is BMkMs and these BMkMs isolates have the potential to survive and settle in agro-ecosystems with temperatures ranges from 25 °C to 34 °C. Consequently, the isolates will have a chance to succeed if they are developed for bioinsecticide active ingredients which suitable to be applied in lowland agro-ecosystems with temperatures higher than those of highlands.

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