Insecticidal activity of culture filtrates from liquid medium of *Beauveria bassiana* isolates (at pH 2.5 and 6) ~~of *Beauveria bassiana* isolates~~ originating from South Sumatra wetland soil against larvae of *Spodoptera litura*

Abstract. The obstacle in utilizing entomopathogenic fungi to control pest insects in wetlands is the inability ~~to~~ of their isolates to survive during the saprophytic phase due to the soil being very acidic. Therefore, the exploration of fungi being able to survive in the ~~acidid~~ acidic soil was utilized in the acidic ecosystem. This study aimed to test the toxic activity of *Beauveria bassiana* culture filtrates from liquid medium at pH 2.5 and 6 against *Spodoptera litura* larvae. Total ten isolates collected from ~~There were 10 isolates used from~~ South Sumatra ~~and~~ were grown in liquid media at pH of 2.5 and 6 for six weeks. The result showed that the culture filtrate from liquid medium at pH 6 was more toxic against the larvae than that at pH 2.5. The mortality of the larvae at pH 6 reached 92%, while that at pH 2.5 it reached 13.33% only. The findings of present study revealed ~~is~~ that the culture filtrate isolates of BPdR, BJgTs, BSwTd2, BSwTd3, BSwTd4, BKKPp2 from pH 2.5 media still caused high larvae mortality (6.67-13.33%). These seven isolates are superior due to their ~~still being~~ toxic nature at pH 2.5. Consequently, present study has increased the chances of success in utilizing these isolates for biological control in acidic ecosystems, such as peatlands, tidal lowlands, and freshwater swamps.

Key words: *Capsicum annuum*, entomopathogenic fungus, mortality, LT_{50}, LT_{90}

Running title: Insecticidal activity of *Beauveria bassiana*

INTRODUCTION

Indonesia ~~Wetlands~~ wetlands are characterized by an ecosystem covering ~~in the forms of~~ 9.2 million hectares of freshwater swamps, 11 million hectares of tidal lowlands, and 14.9 million hectares of peatlands ~~freshwater swamps, tidal lowlands, and peatlands~~ and spreading in Sumatra, Kalimantan, and Papua islands ~~covering 9.2 million hectares of freshwater swamps, 11 million hectares of tidal lowlands, and 14.9 million hectares of peatlands~~ (Mulyani and Sarwani 2013). ~~-~~Soil of wetlands is generally submerged during certain months of the year and the difference in immersion time causes the specific cultivated plants (Safitri et al. 2018). ~~-~~Freshwater swamps are generally planted with rice, chili and other vegetables, and tidal lowlands are also planted with rice but more intensive (two−three indexes) compared to that of freshwater swamps (one index) (Herlinda et al. 2018b). While peatlands are rarely planted with annual crops, they are usually used for oil palm ~~plantation (~~plantation (Dohong et al. 2018), forestry and conservation areas.

The Indonesia wetland soil is generally varied, particularly viewed from the aspects of soil moisture, texture, and pH. Soil texture of freshwater swamps is silt dominant (mud) which is silt sedimentation from the river flow, and contains balanced clay and sand (Kartika et al. 2018), the tidal lowlands contain silt of a mixture of river and sea water deposits, the clay and sand content remains balanced (Marlina et al. 2016), the peat soil does not contain clay, sand, and the silt contains only organic matter (Mulyani and Sarwani 2013). ~~-~~The freshwater swamp soil contains pH ranging from 4 to 4.5 (Kodir and Juwita, 2016), the tidal lowlands containing pH ~~ranging from~~ between 4.17–5.35 (Marlina et al. 2016), and in the peatlands ~~containing~~ pH ranging from 3.60-3.95 (Utami et al. 2009).

Entomopathogenic fungi are those of soil parasitizing and killing insects pest ~~insects~~ and prooved superior in biological control~~,~~ ~~for~~ Hoever, ~~example,~~ *Beauveria bassiana* and *Metarhizium anisopliae* are two examples of entomopathogenic fungi (Herlinda et al. 2018a), whose utilization is often constrained by external factors (Sumikarsih et al. 2019). *B. bassiana* is facultative parasites that can be parasitic ~~in~~ on insects and saprophytes in soil or organic matter. Soil pH variations of each ecosystem can affect the ability of adaptation of entomopathogenic fungi as saprophytes (Bugeme et al. 2008). The too acidic pH medium, though ~~that~~ grows both fungi, but can reduce the viability and density of conidia, ~~and~~ even kill the fungi (Rizkie et al. 2017) and reduce the fungal biodiversity ~~of fungi species~~ in the soil (Safitri et al. 2018). However, there are still species or isolates of the fungi whose conidities still survive at pH 2 (Rizkie et al. 2017). ~~-~~According to

(Pinnamaneni et al. (2010), the minimum pH 4 is generally required by for *B. bassiana* which enables this fungi capable of to produceing chitinase enzymes that can kill host insects is at pH 4. The pH range of the culture medium used for growing *B. bassiana* under acidic is at (4.0), neutral at (7.0), and basic at (11.0) pH (Qazi 2008). The results of the previous study studies on wetland soils in South Sumatra showed depicted that the acidic pH potential of inoculum of *B. bassiana* and *M. anisopliae* from peat soil had the most acidic pHas compared to those of freshwater swamps and tidal lowlands (Safitri et al. 2018), yet the effectiveness of these fungi against insect pests was notis still unknown. The main insect pests mostly attack chili (*Capsicum annuum* L.) and other specific vegetables grown in wetlands (Johari *et al.*, 2014, 2016). -Isolates of entomopathogenic fungi able to survive and still effective at extreme low pH are superior isolates that have the potential as biological agents that can be utilized and expected to survive saprophytically on the soil of the wetlands ecosystem. The objective of this research was to test the toxic activity of *B. bassiana* culture filtrates from liquid medium at pH 2.5 and 6 against the larvae of *Spodoptera litura.*

MATERIALS AND METHODS

Study area

The study was conducted at the Laboratory of Entomology, Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Sriwijaya, Indralaya from October 2018 to March 2019. The average temperature during the experiment was 29.60 °C and relative humidity was 82.34%. -All *B. bassiana* isolates used in this experiment were isolates explored from the wetlands South Sumatra, Indonesia (Figure 1) as the results of the exploration carried out by Safitri et al. (2018). -In this study, the *Spodoptera S. litura* were selected as tested insects because they found to attack the vegetable plants commonly grown on wetlands most dominantly. attacked the vegetable plants commonly grown on wetlands.

Test insect preparation

The larvae of *Spodoptera*S. *litura* were collected from chili and other vegetables grown without the application of synthetic insecticides at the Experimental Farm of the Faculty of Agriculture, Universitas Sriwijaya. Then the larvae of *S. litura* were taken to the laboratory and taken care of kept in a plastic jar (15 x 25 cm) carefully at room temperature. a chili plant was inserted Iinto the jar was inserted a chili plant for theto feed of *S. litura* larvae which were then maintained until the second generation.

The growing larvae were transferred into another jar already provided with fresh chili everyday. Every day the larvae were moved into another jar already provided with fresh chili. When the final instar larvae entered the pupa phase, they were moved into a plastic jar having been provided with soil (10 cm thick) which was already sterilized in the oven for 1 hour at 100ºC. In this jar, cChili plants were also provided in this jar for theto facilitate egg laying of *S. litura* adults which started to appear from the pupae.

The eggs were placed laid by the adults on the underside of the leaves. Four days later the egg colonies were moved transferred into other jars that were already provided with new fresh chili leaves for to feed newly hatched larvae feed. The mass-rearing was carried out to obtain the second generation culture. The one day second instar larvae were used for test insects of this study.

Preparation of *Beauveria bassiana* isolates

The isolates were first made fit using a modified method of Herlinda (2010). For preparation of culture media, in 16.2 g of Sabouraud Dextrose Agar (SDA, Merck) 250 ml aquadest was added, sterilized in an autoclave for 120 minutes at a pressure of 1 atm and poured was poured into a 10 ml Petri dish (9 cm diameter) under aseptic conditions of laminar air flow and allowed to solidify. Then *B. bassiana* was cultured on solidified culture media (SDA) along with the *Tenebrio molitor* larvae.

with the *Tenebrio molitor* larvae in the media culture, Sabouraud Dextrose Agar (SDA, Merck). Then, culturing *B. bassiana* used SDA as much as 16.2 g plus 250 ml aquadest, then sterilizing it in an autoclave for 120 minutes at a pressure of 1 atm. On laminar air flow, the SDA solution was poured into a 10 ml Petri dish (9 cm diameter).

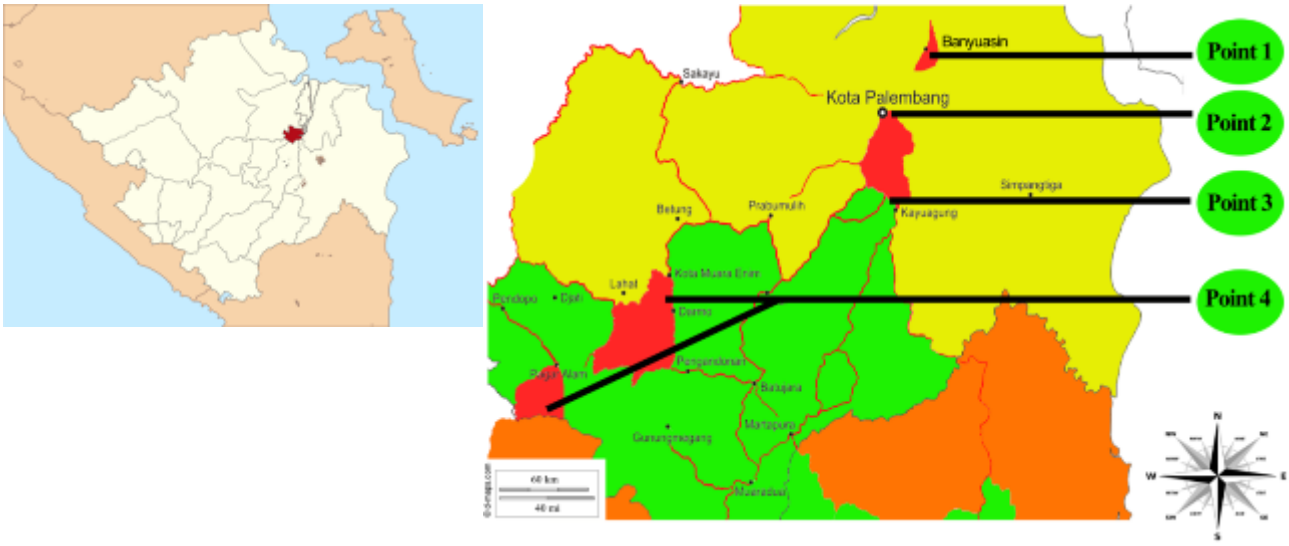

Figure 1. Locations of *Beauveria bassiana* isolates exploration from wetland soil in South Sumatra, Indonesia: point 1 = tidal lowlands, point 2 = freshwater swamps, point 3= peatlands, and point 4 = highlands (Safitri et al. 2018).

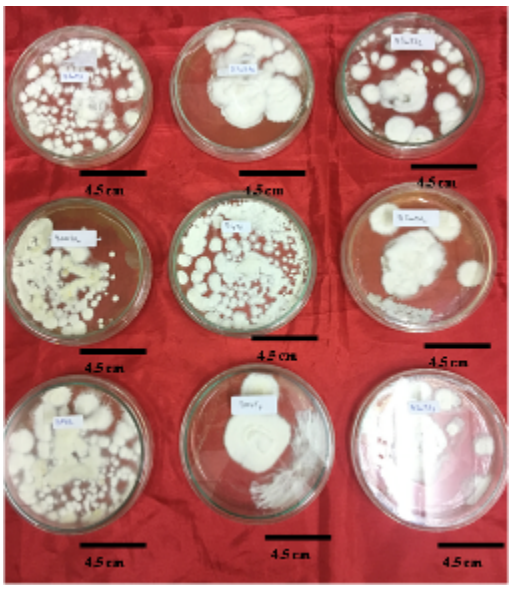

Figure 2. *Beauveria bassiana* SDA culture incubated for 7 days

Table 1. Isolates identity of *Beauveria bassiana* used in this research

Code of isolate	Ecosystems	Vegetation or crop plants	Village or city
BPdR	Freshwater swamps	Paddy	Rambutan
BJgTs	Tidal lowlands	Corn	Telang Sari
BSmMs	Tidal lowlands	Watermelon	Mulya Sari
BSwTd1	Peatlands	Oil palm	Talang Dabok
BSwTd2	Peatlands	Oil palm	Talang Dabok
BSwTd3	Peatlands	Oil palm	Talang Dabok
BSwTd4	Peatlands	Oil palm	Talang Dabok
BKKPp2	Highlands	Rubber and coffee	Pulau Pinang
BKbTp	Highlands	Cabbage	Talang Patai

Preparation of culture filtrate

~~The a~~About one week old isolates of entomopathogenic fungi ~~obtained~~ isolated from SDA media (Figure 2) were then grown in PDB (Potato Dextrose Broth) media ~~as follows~~. The media composition of PDB consisted of 20 g dextrose monohydrate, 200 g peeled potatoes, and 1,000 ml aquadest. Before being extracted, the potatoes were first cut into cubes

with a size of ± 2 cm, and boiled using 1,000 ml aquadest for 20 minutes. Then, the pH of PDB medium was regulated using the method of (Rizkie et al. 2017) by dripping the medium with 32% HCl as many as 1 to 8 drops ~~of~~ with 10 µl micropipette until reaching the pH 2.5. Meanwhile, to get pH 6, the PDB medium was dripped with NaOH 1 mole as many as 1 to 8 drops ~~of~~ with 10 µl micropipette until it reached the pH 6. ~~The measurement of pH used a pH meter.~~ Based on the consideration of the soil in the wetlands, the determination of the treatment of pH 2.5 and 6 was pH <3, whereas the pH 6 was the ideal pH for fungal growth (Qazi 2008). About 150 ml ~~The~~ liquid medium was poured ~~as much as 150 ml~~ into a heat-resistant glass bottle (volume 300 ml) and sterilized into an autoclave for 120 minutes at a pressure of 1 atm~~,~~. ~~after~~ After being cooled~~,~~ ~~in 1 x 1 cm² *B. bassiana* from the SDA medium. The~~ *B. bassiana* culture ~~broth~~ was inoculated in this PDB medium ~~was incubated~~ for 6 weeks.

Production of culture filtrate

Six weeks old ~~The~~ culture broth of each *B. bassiana* isolates ~~from the PDB medium which was already incubated for 6 weeks was~~ were then filtered to separate extracts from hyphae, mycelia, and conidia. ~~by performing t~~ Two stages of ~~filtering~~ filtration method was adapted with ~~by modifying the method~~ slight modification (Cheong 2015). The purpose of separation from hyphae, mycelia, and conidia was to get a culture filtrate containing toxic insecticidal compounds. ~~The first~~ In first stage of filtration~~,~~ ~~was that the~~ 100 ml of *B. bassiana* culture on the SDB was poured into an erlenmeyer tube (volume of 500 ml) whose tube mouth ~~had the~~was fitted with Whatman filter paper number 42 cotton-coated 1 cm thick and produced ± 70 ml coarse culture filtrate. ~~The second stage was that the~~The coarse culture filtrate was filtered using a syringe filter (0.45 µm-25 mm) conducted in second stage filtration as follows: 1 ml of the first stage coarse culture filtrate was sucked using a hypodermic needle (volume of 6 ml) and then the needle was removed and the base of the needle was attached a syringe filter.~~;~~ ~~then~~ Then the needle was attached back to the hypodermic needle and 1 ml of crude culture filtrate was filtered using a syringe filter to obtain the culture filtrate. The culture filtrates were used at a concentration of the original preparation without dilution by ~~steril~~ sterile water.

Isolates activity insecticidal test of *Beauveria bassiana*

~~The result of the second filtration of~~ 1 ml culture filtrates obtained in second filtration was ~~dripped~~ dropped onto the chili leaves; then these leaves were infested with test insects (the second instar of *S. litura* larvae) as many as 25 test insects per isolate for 6 hours and repeated three times. Before being treated, the test insects were left unfed for 2 hours and weighed using a Portable Jewelry Scale (capacity of 30 g x 0.01 g). After 6 hours of infestation and ensuring that all larvae ate the leaves moistened with the culture filtrate *B. bassiana*, the test insects were moved into a plastic cylinder (the top of which was covered with gauze) in which there were 5 new chili leaves (without culture filtrate of *B. bassiana*). Every 1 x 24 hours the dead larvae were recorded and the chili leaves were replaced and the area of the eaten leaves, the weight of the feces, and the larvae body weight were measured. The observations of these variables were carried out for 12 days.

To measure the percentage of the foliar damage area caused by the insects, a bioleaf application ~~of~~ as given by Machado et al. (2016) was used. The area of chili leaves before being eaten was measured using the formula of Widuri et al. (2017) as follows:

$$ILD = 0.606 \times P \times L$$

ILD = Index of leaf area (cm^2); P = leaf length; L = leaf width

Defoliation is the percentage of leaves eaten by the insects. Defoliation was measured using the following formula:

$$LDD = D \times ILD$$

LDD = area of eaten leaves; D = Defoliation

Data analysis

Differences in data on body weight and feces, and the area of eaten leaves among the isolates are shown in a graphical form. Mortality and time of death of the test insects (LT_{50}) were analyzed using analysis of variance (ANOVA). Tukey's Honestly Significant Difference (HSD) Test was employed to test for significant difference among the treatments (isolates) at P = 0.05. LT_{50} and LT_{90} values were calculated by using probit analysis. All data were analysed using software of SAS University Edition 2.7 9.4 M5.

RESULTS AND DISCUSSION

Insecticidal activity of *Beauveria bassiana*

The culture filtrates of nine *B. bassiana* isolates already produced from liquid media both incubated at pH 2.5 and pH 6 showed different lethal or toxicity capabilities ~~of~~ towards *S. litura* larvae (Table 2). The culture filtrates produced from culture media with pH 6 showed that the larvae mortality increased sharply from day 4 to 12, whereas at pH 2.5 the mortality of new test larvae occurred on day 9 and did not increase sharply (Figure 3). On the twelfth day after being applied with the culture filtrates incubated at pH 6 medium, the mortality of tested larvae reached 92%, while those applied with the culture filtrates incubated at pH 2.5 medium only reached 13.33%. Therefore, the pH on the culture medium of *B. bassiana* could affect the level of toxicity of the culture filtrate. On the last day of

observation, the culture filtrates of nine *B. bassiana* isolates from culture medium at pH 6 showed high toxicity to isolate BPdR, BJgTs, BSmMs, BSwTd2, BSwTd4, BKKPp2 and significantly different from controls (Table 2). However, the culture filtrates of nine *B. bassiana* isolates from the culture medium at pH 2.5, all of which had lower toxicity compared to culture filtrates from culture medium at pH 6. The highest toxicity at pH 2.5 was found in BPdR isolates, BJgTs, BSmMs, BSwTd2, BSwTd3, BSwTd4, and BKKPp2. Yet, although they were cultured on a medium at pH 2.5, the isolates were still quite toxic to the test insects.

Table 2. Mortality of larval *Spodoptera litura* at 4, 8, 12 days after being infested with *Beauveria bassiana* culture filtrates from medium at pH 2.5 and 6

Code of isolate	Mortality of *Spodoptera litura* larvae (%)					
	4 days		8 days		12 days	
	pH 2.5	pH 6	pH 2.5	pH 6	pH 2.5	pH 6
BPdR	0.00	5.33^{a}	0.00	34.67^{bc}	8.00^{b}	73.33^{b}
BJgTs	0.00	5.33^{a}	0.00	46.67^{c}	6.67^{b}	92.00^{b}
BSmMs	0.00	8.00^{b}	0.00	32.00^{bc}	6.67^{b}	85.33^{b}
BSwTd1	0.00	1.33^{a}	0.00	22.67^{b}	0.00^{a}	46.67^{ab}
BSwTd2	0.00	2.67^{a}	0.00	28.00^{bc}	8.00^{b}	73.33^{b}
BSwTd3	0.00	2.67^{a}	0.00	26.67^{bc}	6.67^{b}	40.00^{ab}
BSwTd4	0.00	5.33^{a}	0.00	30.67^{bc}	13.33^{b}	65.33^{b}
BKKPp2	0.00	5.33^{a}	0.00	33.33^{bc}	8.00^{b}	73.33^{b}
BKbTp	0.00	0.00^{a}	0.00	25.33^{b}	0.00^{a}	48.00^{ab}
Kontrol	0.00	0.00^{a}	0.00	0.00^{a}	0.00^{a}	0.00^{a}
ANOVA F-value	-	3.07*	-	19.77*	8.55*	5.33*
P value (0.05)	-	0.02	-	0	0	0
Tukey's HSD test	-	15.5	-	12.8	13.3	50.2

Note: * = significantly different; values within a column (the data of each isolate) followed by the same letters were not significantly different at P < 0.05 according to Tukey's HSD test. Original data were transformed using Arcsin *transformation prior to statistical analysis.*

The color of culture filtrate from culture media ~~of~~ with pH 6 and 2.5 also showed ~~the~~ significant differences (Figure 4). The color of the culture filtrate of pH 6 culture media was generally dark brown, while the pH 2.5 was light brown. The most striking color of the culture filtrate isolates of BPdR, BJgTs, BSmMs, BSwT2, BSwTd4, and BKKPp2 of the pH 6 culture media was more brown than those of pH 2.5. If these data were related to the mortality data (Table 2), the isolates which gave rise to the dark brown color of culture filtrate resulted in a higher mortality rate.

Table 3. LT_{50} and LT_{95} of larval *Spodoptera litura* caused by *Beauveria bassiana* culture filtrates from medium at pH 2.5 and 6

Code of isolate	LT_{50} (days) ± SD		LT_{90} (days) ± SD	
	pH 2.5	pH 6	pH 2.5	pH 6
BPdR	14.83±0.44	9.71±0.81	17.35±0.53	14.11±1.38^{ab}
BJgTs	14.82±0.45	8.28±0.45	17.15±0.58	11.63±0.60^{a}
BSmMs	15.36±0.99	9.19±0.48	18.37±1.68	13.50±1.52^{ab}
BSwTd1	-	11.49±0.54	-	16.67±0.87^{ab}
BSwTd2	15.06±0.34	10.24±1.11	18.10±0.41	14.51±1.94^{ab}
BSwTd3	14.66±0.74	11.89±0.36	17.52±1.20	17.20±0.66^{b}
BSwTd4	14.05±0.17	10.03±0.48	16.76±0.44	15.03±0.37^{ab}
BKKPp2	14.97±0.60	9.59±0.80	17.95±1.05	14.06±1.30^{ab}
BKbTp	-	11.52±1.09	-	16.55±1.56^{ab}
Kontrol	-	-	-	-
ANOVA F-value	0.49^{ns}	2.72^{ns}	0.38^{ns}	2.55*
P value (0.05)	0.80	0.05	0.88	0.04
Tukey's HSD test	-	-	-	6.38

Note: ns = not significantly different; * = significantly different; values within a column (the data of each isolate) followed by the same letters were not significantly different at P < 0.05 according to Tukey's HSD test. Original data were transformed using Arcsin *transformation prior to statistical analysis*

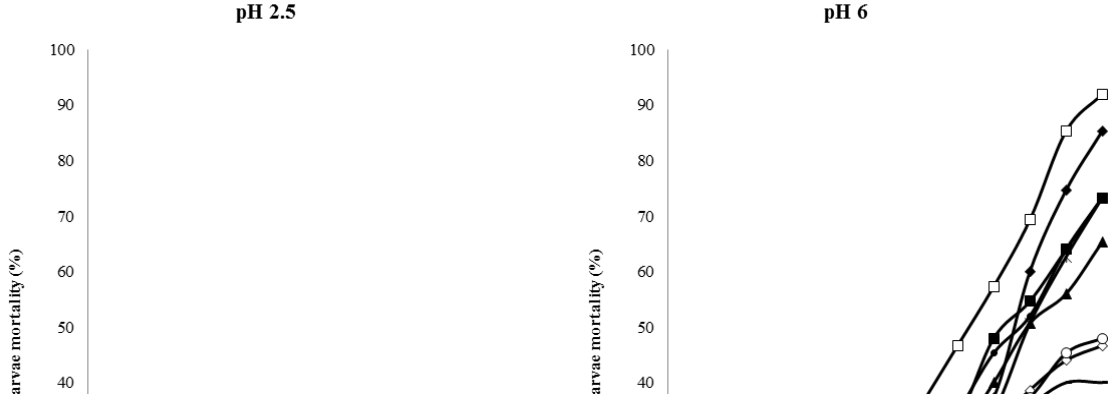

Figure 3. Mortality of *Spodoptera litura* larvae

Figure 4. *Beauveria bassiana* isolates cultured in SDA, PDB, and culture filtrate: BPdR (a), BJgTs (b), BSmMs (c), BSwTd1 (d), BSwTd2 (e), BSwTd3 (f), BSwTd4 (g), BKKPp2 (h), BKbTp (i)

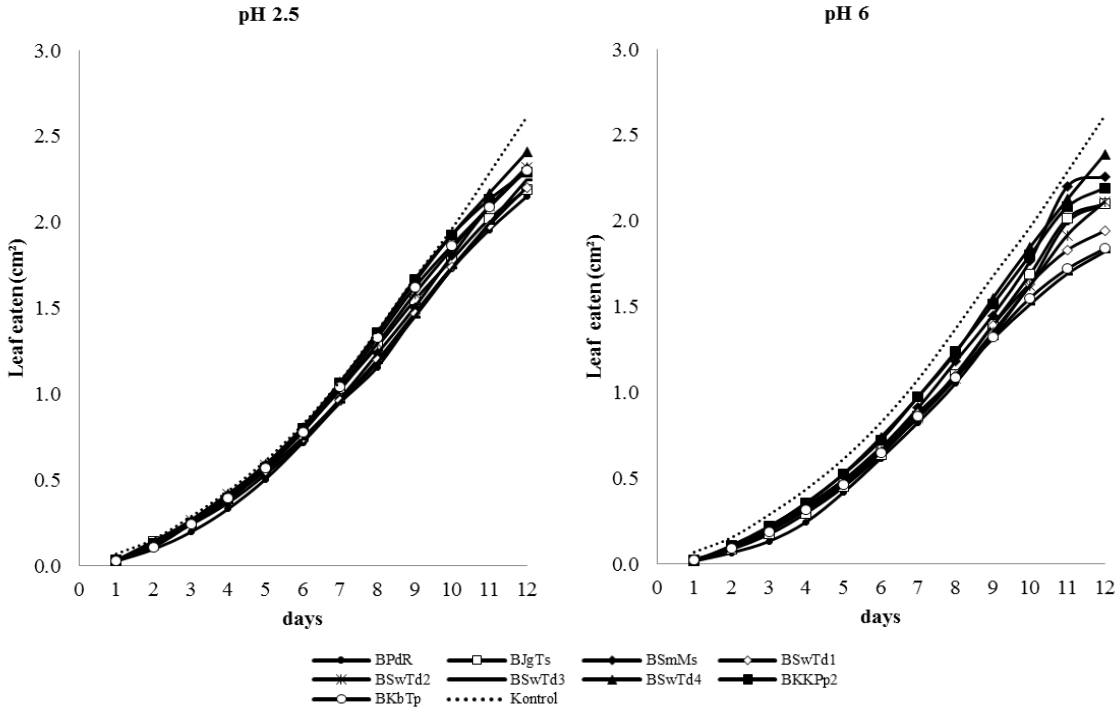

Figure 5. Leaf eaten by *Spodoptera litura*

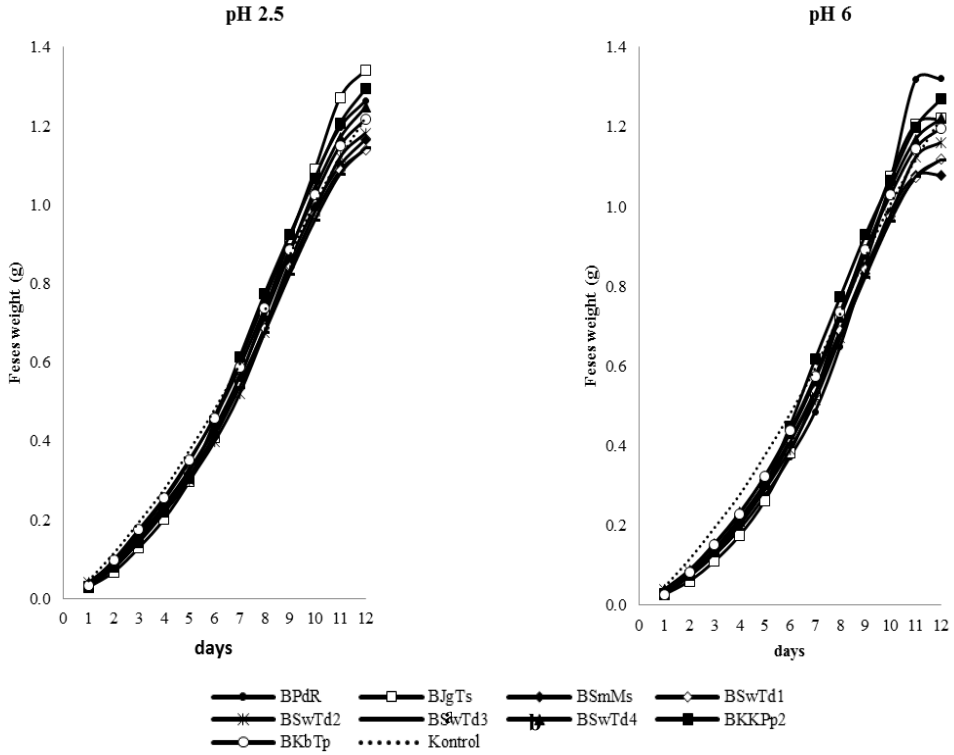

Figure 6. Weight of *Spodoptera litura* feces

a **b**

Figure 7. The dead larvae of *Spodoptera litura* caused by *Beauveria bassiana* culture filtrate (a) and the healthy one (b)

After applying the culture filtrates of nine isolates of *B. bassiana* from the pH 6 medium, the LT_{50} and LT_{90} *S. litura* larvae were shorter than those of pH 2.5 (Table 3). Meanwhile, after applying the culture filtrates from the pH 6 medium, the LT_{50} *S. litura* larvae showed an effect that was not significantly different among the isolates, as well as the LT_{50} which was applied the culture filtrates of pH 2.5 medium. After applying the culture filtrates from pH 6 medium, the LT_{90} of *S. litura* larvae showed significantly different effects among the isolates. The LT_{90} was found the shortest in BJgTs isolates, while the longest one was found in BSwTd3 isolates. After applying the culture filtrates from pH 2.5 medium, the LT_{90} of *S. litura* larvae showed no significant difference among the isolates.

The effects of *Beauveria bassiana* culture filtrates on larval growth

The results of effects of *B. bassiana* culture filtrates from pH 6 or pH 2.5 medium on leaf eaten area (Figure 5) revealed that ~~,~~ weight of feces (Figure 6), and weight of larvae (Figure 7) showed similar trends. The older the larvae age was, the wider the leaves were eaten, and the more weight of feces the larvae per animal becomes. The larvae fed with the controlled leaves (not applied to the culture filtrates *B. bassiana*) ate chili leaves most widely and produced most waste. The weight of all treated larvae, except the control ones, showed a tendency to begin to decline on the sixth day. After 12 days of application, the weight of the larvae treated with the culture filtrates *B. bassiana* from pH 6 medium decreased their weight sharply and the lowest weight reached below 0.5 g/head. The weight of larvae fed with the treatment of culture filtrates from pH 2.5 medium also decreased, but the lowest weight was still above 1g/head after 12 days of application. The larvae weight in the control group, both at pH 6 and pH 2.5 media, continued to increase until the end of the observation (after 12 day application).

The larvae of *S. litura* applied to a culture filtrates *B. bassiana* from a pH 6 or pH 2.5 medium showed the same symptoms; 1 x 24 hours after consuming the treatment leaves, the larvae movement became less active ~~than~~ as compare to larvae ~~eating~~ ate the control leaves. The larvae bodies that were applied to culture filtrates of pH 6 and pH 2.5 tended to be smaller than those of the control. The dead larvae as a result of the applied culture filtrates showed symptoms such as dry, wrinkled, stiff like mummy, and dull integument body (Figure 8), and they did not smell. In addition, the mouth of the dead larvae secreted green liquid. These dead larvae were checked to find out whether their death due to *B. bassiana* spore infection or due to toxic compounds contained in the culture filtrates, by putting these dead larvae into the SDA media. After 7 days of being incubated in the SDA medium, all of the dead insects did not show the infection caused by spores of *B. bassiana*. Therefore, the death of *S. litura* larvae in this study was caused by the toxin compounds contained in the culture filtrates.

Discussion

The data showed that the culture filtrates of *B. bassiana* from ~~the medium of~~ PDB with pH 6 caused mortality of *S. litura* larvae which was higher than the mortality resulted from the culture filtrates of ~~the medium of~~ PDB at pH 2.5. In this study, the culture filtrate produced from the separation of liquid *B. bassiana* culture from its spores was toxic and lethal on larvae of test insects. According to ~~(~~Pinnamaneni et al. (2010)~~. The toxic culture filtrates of *B. bassiana* was caused during the~~ incubation of test fungi in liquid medium~~; the fungus produced~~ produces exochitinase enzyme and the chitinolytic exochitinase enzyme, able to degrade cuticle insects. ~~–~~Based on the resulting mortality, the culture filtrates of *B. bassiana* from medium of PDB (pH 6) were more toxic than that of with pH 2.5. The pH range for chitinase activity of *B. bassiana* was pH 4-6. ~~, and the more~~ More acidic or alkaline nature lowers the activity of culture media. ~~culture was, the lower the activity became~~ (Pinnamaneni et al. 2010). In addition, the pH of culture media less than 3 ~~are~~ is less ideal ~~pH~~ for the growth and viability of spores of~~a~~ *B. bassiana* (Rizkie et al. 2017). The most ideal pH to produce the *B. bassiana* spores is

the pH 5.2 (Pham et al. 2009). The liquid media with a pH less than 3 can cause a decrease in the density and viability of *B. bassiana* spores (Pham et al. 2009; Rizkie et al. 2017).

This study ~~still~~ found that the culture filtrates of *B. bassiana* derived from PDB medium of pH 2.5 were still caused deaths in test larvae. The isolates of BPdR, BJgTs, BSmMs, BSwTd2, BSwTd3, BSwTd4, and BKKPp2 still caused 6.6-13.33% deaths. This proves that the culture filtrates of the isolates are still toxic. Only the superior isolates are still toxic at pH below 3; generally *B. bassiana* isolates are able to produce insecticidal culture filtrates if the liquid media during culture *B. bassiana* has a pH of 3 and above (Cheong 2015). Six of the seven toxic isolates were those from wetland soil (peat soils, tidal lowland, and freshwater swamp soil) in South Sumatra which had a pH below 3 (Safitri et al. 2018). These isolates have the potential to survive as saprophytes in low pH soils. The failure of entomopathogenic fungi to survive as saprophytes in acidid soils, such as soil in peatlands, tidal lowlands, and freshwater swamps spreading across Sumatra, Kalimantan, and Papua can be overcome if the isolates that hold low pH results of this study are applied over there.

In the treatment of with culture filtrates of *B. bassiana* from medium of pH 6 or pH 2.5, the area of the eaten leaves decreased as compared to those of the controls which were given only sterile aquadest. The results of this study showed that the culture of filtrates of *B. bassiana* could reduce the appetite of *S. litura*. The weight of *S. litura* larvae eating the leaves which were applied to thewith culture filtrates of *B. bassiana* from pH 6 or pH 2.5 showed a tendency to lose weight on the sixth day to begin to lose weight, whereas those in the control group on the sixth day until the end of the observation, the larvae weight continued to rise. The decrease in the larvae weight in the culture filtrate treatment resulted from the sick larvae experiencing slowdown growth. The body of the sick larvae continued to decrease in size, shrink, and dried out after and finally they died. The larvae that were sick from eating the leaves applied to culture filtrates showed a difference when compared to the larvae that died from being infected with *B. bassiana* spores. According to Safitri et al. (2018) and Sumikarsih et al. (2019), the larvae that died as a result of being infected with the entomopathogenic fungus spores showed symptoms such as the body decreasing in size, shrinking, drying, and having no smell; however, from the host insect's body grew the fungus in the form of mycelia, hyphae and spores on the surface of the integument. The non-growth of *B. bassiana* in larvae of this study resulted from the loss of mycelia, hyphae, and spores in the culture filtrate. Consequently, the dead death of larvae caused by the toxic compounds contained in the culture filtrate *B. bassiana* did not result from the infection with *B. bassiana* spores.

The color of the culture filtrate of theat pH 6 culture medium is was more brown than the that of pH 2.5. According to Qazi (2008), pH can change the color of culture filtrate because pH it affects *B. bassiana* in releasing protease enzymes and influencing toxin production (Sharma et al. 1992). The protease of *B. bassiana* enzyme functions to dissolve insect body proteins and kill them (Mancillas-Paredes et al. 2019). The optimal pH can cause the fungus to release ammonia and citrate which can increase activity of extracellular enzymes (Khachatourians et al. 2007). Therefore, the data of this study showed that the isolates at pH 6 were more brown and caused mortality of *S. litura* larvae to be higher than those of pH 2.5 due to the more toxic culture filtrate.

The results of this study conclude that the culture filtrates *B. bassiana* of the liquid medium at pH 6 are more toxic against the larvae of the *S. litura* than those at pH 2.5. Still, an important finding was found that the culture filtrates of isolates of BPdR, BJgTs, BSmTd2, BSwTd3, BSwTd4, and BKKPp2 from pH 2.5 media still had high toxicity against the larvae. These isolates have the potential to survive and settle on low pH soils. Therefore, these isolates have the opportunity to be successfully utilized in low pH ecosystems, such as peatlands, tidal lowlands, and freshwater swamps in Indonesia.

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Fwd: [biodiv] Editor Decision

1 message

Siti Herlinda, Prof. Dr. Ir., M.Si. <sitiherlinda@unsri.ac.id> Thu, Jun 17, 2021 at 10:24 AM
To: Suwandi fp <suwandi@fp.unsri.ac.id>

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Dari: **Smujo Editors** <smujo.id@gmail.com>
Date: Sel, 9 Jul 2019 pukul 00.28
Subject: [biodiv] Editor Decision
To: DWI RIZKI AYUDYA <author@smujo.id>, SITI HERLINDA <sitiherlinda@unsri.ac.id>

DWI RIZKI AYUDYA, SITI HERLINDA, SUWANDI SUWANDI:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Insecticidal activity of culture filtrates from liquid medium of Beauveria bassiana isolates from South Sumatra (Indonesia) wetland soil against larvae of Spodoptera litura".

Our decision is to: Accept Submission

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