Effect Of Beauveria Bassiana Doses On Spodoptera Litura Mortality

Dyah Rini Indriyanti, Siti Mahmuda, Muji Slamet

ABSTRACT: Beauveria bassiana is a parasitic mold for insect, it is commonly used as a control agent. Spodoptera litura is insect pest attacked tobacco plants in Salatiga. This studied would give analysis the effectiveness of B. Bassiana on S. litura larvae mortality with various doses. B. bassiana was obtained from Estate Crop Protection Board (BPTBUN) in Salatiga, Central Java as dust formulation. The S. litura. larvae were obtained from tobacco farm, then adapted to laboratory environment for two days before used for Bioessay. There were five different doses treatment: 1g 100 mL^{-1}, 2g 100 mL^{-1}, 4g 100 mL^{-1}, 8g 100 mL^{-1} and 0 g 100 mL^{-1} (as control). Each treatment used 10 larvae and repeated five times. The result showed that B. bassiana with 8 g100 mL^{-1} concentration was more effective to kill S.litura larvae than others doses. The important finding of this research is that B. Bassiana can be used to control S. litura larvae safely and not pollute the environment.

Keywords: Beauveria bassiana; parasitic mold for insect; Spodoptera litura

INTRODUCTION

Spodoptera litura is an insect pest which attacks various cultivated plants [1]. One of cultivated plants with a high economical value is tobacco [2]; [3]. It is one of valuable plantation commodities in Indonesia. It spreads widely in Sumatera, Java, Bali, West Nusa Tenggara, and Sulawesi. In Central Java, it is mostly planted in Salatiga, but recently the productivity of tabacco plant decreased because of S.litura attack. The S. litura larvae control in Salatiga regency only relies on synthetic insecticide. Synthetic pesticides leads to physiological resistance and adverse environmental effects to insect pest [4]. This method gives negative effects on other biological enemies, such as the predators and parasitoids. Insecticide utilisation in Salatiga might cause larvae resistance. Hence, it needs an alternative environmental friendly pest control which gives no negative effects and resistance problems. One of the efforts is by utilising a parasitic mold, B. bassiana. This mold is one of potential natural agents to control some pests [5]. B. bassiana can kill the hospes by destroying its organic struture and dehydrate the inner cell [6] . B.bassiana was succeed to kill all Helicoverpa armigera larvae after 14 days of application with concentration 25 g L^{-1} (10^7 con g^{-1} conidial density) [7].

- *Dyah Rini Indriyanti, Siti Mahmuda, Muji Slamet*
- *Biology Department, Faculty of Mathematics and Sciences, Universitas Negeri Semarang, Indonesia D6 Building Floor 1 Sekaran Campus, Gunungpati, Semarang, 50229 Indonesia E-mail: dyahrini36@gmail.com*
- *Biology Department, Faculty of Mathematics and Sciences, Universitas Negeri Semarang, Indonesia D6 Building Floor 1 Sekaran Campus, Gunungpati, Semarang, 50229 Indonesia E-mail: smahmudah@.gmail.com*
- *Estate Crop Protection Board in Salatiga, Central Java Province. Indonesia BPTBUN, Salatiga, Central Java Province. Indonesia Email: bproteksi@gmail.com*

Application of B.bassiana on M. testulalis larvae with concentration of 47.2 x 10^6 con g^{-1} can cause 36% mortality of S. litura in fifth day of application [8]. B. bassiana with concentration of 4 g mL^{-1} also kill Conopomorpha cramerella larvae in fifth day of application [9]. B.bassiana effectively controls cotton leafworm (Spodoptera littoralis) larvae [10], Spodoptera litura [11]. Tobacco farmers in Salatiga have never used B. Bassiana to control S.litura. Therefore, it is a worth to have a laboratory analysis of B. bassiana effectiveness on S.litura larvae before disseminated. This article will inform about the B. bassiana application and larvae mortality in a laboratory. The benefit of this research is, it can be used for S. litura control recommendations.

METHODS

Density and viability of B. Bassiana conidia

B.Bassiana was obtained from Estate Crop Protection Board in Salatiga, Central Java Province as dust formulation (kaolin powder mix with conidia). Conidial density and viability of B. Bassiana were determined before used in the laboratory. This research was conducted at Laboratory of Estate Crop Protection Board Salatiga, Central Java, June -August 2015.

The conidial density was observed by counting the number of conidia using a microscope with the magnification of 400x. The conidial density was observed as follow: 1 g of mold was solved in 100 mL of aquadest, then stirred by magnetic strirer for 1 minute. A drop of conidia suspension then was put on haematocytometer and covered by coverslip. The suspension was left for a minute to stabilize conidial position. The number of conidia on haemacytometer (a+b+c+d+e) was counted by microscope with 400x magnification. The extrapolation was repeated 3-5 times to get valid data. The number of conidia on counting chamber was observed by this following formula [12].

$$S = (t \times d)\ (n \times 0.25)^{-1} \times 10^6$$

S = The number of spores per gram of medium
t = The number of spores counted on field count (a, b, c, d and e)
d = Levels of dilution

n = The number of boxes observed (5x16 small boxes = 80 boxes)
This formula applies only to the Neubauer Improve hemocytometer.

The viability of conidia

The viability of conidia was observed by the germination of conidia on the Potato Dextrose Agar (PDA) media. The liquid PDA was poured on a small petri dish cover aseptically, then let it cold and densed. The thin layer of the media then cut in size of 1 x 1 cm then moved on an object glass. An object glass stuffed by 2-3 media. Suspension of the conidia was dropped on cuts of media then covered by coverslip and put it on bigger petri dish, covered and incubated for 8 hours in room temperature. The object glass was removed from petri dish and dripped by lactophenol cotton blue to stop the grow and clarify microscopic observation (colouring the conidia). The germinated conidia were observed under microscope with 400x magnification. Both germinated and non germinated conidia was counted by this following formula [12].

$V = (g)\ (g + u)^{-1} \times 100\%$
V= Viability of conidia
g = the number of germinated conidia
u = the number of ungerminated conidia
Good Quality Standards of BCA according to the Directorate of Plantation Protection are as follows [12].

Table 1. *Quality Standards of BCA [12]*

No	Criteria	Good	Moderate	Poor
1	Spores density (g/ml)	$>10^6$	10^6	$< 10^6$
2	Spores viability (%)	86 – 100	75 – 85	< 75

Larvae of S. litura

There were 250 larvae of S. litura were obtained from tobacco plantation. The larva had 2-3 cm length (third instar), brown and brown-black colored. Larva was fed by pesticide-free tobacco for two days in the laboratory before testing.

Bioessay

The larvae was laid in a transparent containner with Ø 15 cm, and 10 cm height. There were ten larvae in each containner and fed by pesticide-free tobacco. B. bassiana solution of each concentration was sprayed on S. litura larvae by sprayer. The containner were covered by white tulle cloth and tied, then observed until all larvae died. This research were consisted of five treatment levels and repeated 5 times, total 25 experimental units. B. bassiana concentrations used were as follow: 1 g 100 mL^{-1} (P1), 2 g 100 mL^{-1} (P2), 4 g100 mL^{-1} (P3); 8 g100 mL^{-1} aquades (P4), and 0 g 100 mL^{-1} (P0) as control. The behavior larvae after B. bassiana application and the number of dead larvae were observed every day. Abiotic data in the research laboratory (temperature, humidity and light intensity) were also recorded.

RESULTS AND DISCUSSION

Density and viability of B. bassiana conidia

The average of B. bassiana conidial density were 3.06 $x10^8$ conidia g^{-1} (Table 2), based on quality standart of Biological control agent, this density was categorized on good criteria (with more than 10^6 conidia). The average of B.bassiana conidial viability was 92.92% (Table 3), based on quality standart of Biological controller agents, this viability was categorized on good criteria, (range 86-100 %).

Table 2. *Density of B. bassiana conidia*

Repeti-tion	Number of conidia on Haemacytometer counting beds					Total	Density (conidia gr^{-1})
	a	b	c	d	e		
1	25	35	15	25	20	120	3.00×10^8
2	28	34	14	26	20	122	3.05×10^8
3	21	24	24	26	29	126	3.15×10^8
Average	24.6	31	17.6	25.6	23	122.6	3.06×10^8

Table 3. *Viability of B. bassiana conidia*

Repeti-tion	Number of conidia			Viability (%)
	Non germinated	germinated	Total number	
1	4	63	67.00	94.02
2	7	67	74.00	90.50
3	5	82	87.00	94.25
Average			70.66	92.92

Observation on germinated and non germinated B.bassiana conidia with 400x magnification (Figure 1).

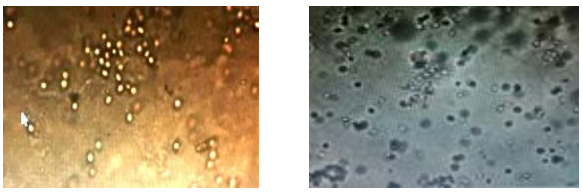

Figure 1. *Conidia of B. bassiana before (left) and after germinated (right) with 400x magnification.*

Egg & larvae [13] Pupae [14] Imago S.litura [15]

Figure 2. *Metamorphosis of Spodoptera litura (egg, larvae, pupae and imago)*

S. litura has metamorphosis: egg, larva, pupa and imago (Figure 2). A female can produce 1500 eggs [13]. Life cycle from egg to moth ranges from 30-61 days. Egg (2-4 days), larva (20-26 days), pupa (10-14 days), imago or moth (5-9 days). Based on the observation S. litura larvae sprayed with B. bassiana show the following symptoms. S. litura larva started to change the behaviour since first day after treatment by lowering its movement. Eating activity was also decrease on day 3 and there were many larvae which stucked on the wall of containner (day 5). In accordance to Dinata [16] stated that there is a behaviour known as "summit disease" where the insect which died by entomopatogenic mold will move to the tip of the plants and stick theirself. Actitivity of S. litura larvae was declining because of B. bassiana infection. The mold infection starts when conidia reach the larvae body. The attached conidia will form a a tube of germination and produce chytinase to penetrate the cutticle and reach haemolymph. The mold grows inside the larvae and produces beauvericin, a toxic to destroy larvae body tissues [17]. B. bassiana produced chytinase, lipase and proteinase ezymes. The toxin produced is beauvericin, an antibiotic which disturbs haemolymph function and nucleus of the insect then stiffen the infected insect [18].

Figure 3. *Larvae of S. litura eat the leaves (left) and died larvae infected by B. bassiana (right)*

The dead S. litura larvae cause of B. bassiana, identified by a white colour powder on the outer surface of S. litura body. It shows growth of the hyphae and produce conidia which would covered nearly the entire surface of larval body. Micellium originally appears on the segment of larval abdoment. Then, the white coloured micellium covers the infected larval body surface. Generally the hyphae will grow on the surface of pest's body through spiracles, mouth and membran between its body segments [19]. In the concetration of 8 g 100mL^{-1}, it showed the existence of B. bassiana micellium which grew on the larvae on day 9. In concentration of 1 g 100mL^{-1} and 2 g 100mL^{-1} , micellium growth of the mold started to be visible on day 11. It shows that the higher dose, the more conidia will germinate and the more faster growth of the hyphae. This is in line with Pinem [20] who stated that the mold's micellia penetrates from the outside into the host's body and produce conidia. Within a few days, the insect would then finally die. The insect which are infected by B. bassiana will die with a hardened body like a mummy and be covered by the white coloured mold.

Effect of B. bassiana on S. litura larvae mortality

The dead S. litura larvae after B. bassiana spraying (Figure 4) as follow :

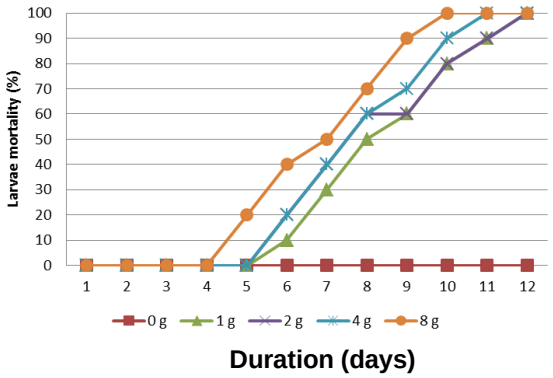

Figure 4. *Percentage of dead S. Litura larvae after sprayed B. Bassiana for 12 days observation*

There was no larvae died on day 1 until 4 (Figure 4) . It was the incubation phase of B. bassiana on the insect body. Mold need few days to infect and grow on S. litura body. On day 5, with dose of 8 g 100 mL^{-1}, Mold started to kill larvae (20%). On day 6, larvae died 10-40% and all larvae finally died on day-12. It shows that B. bassiana could kill S. litura with 3.06 x 10^8 con/g density and 92.92% conidia viability (Table 2 & 3). Some researchers report that the number of conidia of entomopathogenic fungi is related to the mortality of larvae. Conidia density 12.24 x 10^8 con g^{-1} in B. bassiana concentration could kill all larvae on 10th day [21]. The density of B. bassiana conidial 10^8 con g^{-1} could kill 50% Maruca testulalis larvae in 13th day [22]. B. bassiana conidia with density of 1.3 x 10^8 con g^{-1}, cause 65% Nezara viridula larvae mortality in 14th day [23]. In this research proofed that B. bassiana conidia density of 3.06 x 10^8 con g^{-1} and 92.92% viability were effective to kill all S. litura larvae for 10-12 days. B. bassiana invades the hospes through its skin, disgestive tract, spiracle and other cavities. Attached mold innoculum on the hospes then germinates and grows as tube then penetrates to the skin [24]. The mold will reproduce inside the hospes then grow, attack all body tissues and kill it [25]. The mold will fasten its reproduction to strive with insect immunity. At the same time, antibiotic toxin produced by the mold weakens the insect and fastly kill the insect at once. The hyphae will grow and cover the entire insect's body. The mold starts to grow, the insect shows sick symptom, such as uncontrolable movement until it finally died [26]. The density and germination ability of conidia will determine entomopatogenic mold effectiveness in controlling pest [27, 31]. Figure 2 shows that on day 6, there were some larva die caused of B.bassiana. Further statistical analysis of larvae motality because of B.bassiana on 6th day (Table 3).

Table 3. *Mortality of S.litura larvae on 6th day after infected by B.bassiana*

B.bassiana (g 100 mL^{-1})	Larvae mortality (%)
0	0 ^{a}
1	10^{b}
2	20^{b}
4	20^{b}
8	40^{c}

Anova showed (F= 52.56; df=24; P=0.000; P<0.05), that there is a significant difference among B.bassiana treatments. Tukey test showed that the control was significanly different from all treatments of B.bassiana doses. Treatment of 1 g dose was not significally different from dose of 2g, and 4g (P= 0.58 and P= 0.21 > 0.05). Treatment of 8 g dose was significally different from all treatments (P=0.000 <0.05). On day 6, the mortality of S. litura larvae increased up to day 11. It indicated infection and reproduction of B. bassiana which caused the more larvae died everyday. The surviving larvae, would eventually weaken and die 100% on day 12. The other hand there was no dead larvae in the control until the end of observation (12 days). This is because the larvae were not infected with B. bassiana (Figure 4). The fastest early larval mortality was in B. bassiana concentration of 8 g100 mL^{-1} on day 5 after application. It shows that 8 g100 mL^{-1} concentration was the most effective dose compared to others. On day 10 it had reached 100% mortality. It shows that the higher concentration conidia applied, the higher toxins produced by mold to kill larvae so increased the larval mortality rate. The concentration will affect entomopathogenic mold effectiveness in controlling test insect [27]. More conidia density will be increasing contact pathogen and the host, so it will be increasing larvae mortality [28]. In the concentration of 1 g100 $mL^{-1}$and 2 g100 mL^{-1} reached the longest mortality rate (12 days). The reason is mostly because the lower concentration of B. bassiana the lower its infection. Temperature, humidity, and light intensity also greatly affect the effectiveness of B. bassiana in controlling pests. High relative humidity is associated with high mortality in insects due to the infection with entomopathogens [29]. The entomopathogenic mold B. bassiana are able to grow in the range of 15-35^{o}C and less than 95.5% humidity [30]. The temperature of the laboratory research was in range of 22-27^{o}C and humidity of 59-61% with the light intensity of 14.6 – 27.2 Lux. It was the optimal condition for B. bassiana to grow and reproduce. The important finding in this study is that dosage of 8 g100mL^{-1} was the best for using B. bassiana application on S. litura. This dosage can be used for the recommendation of B. bassiana controlling S. litura.

CONCLUSION

The formulations of B. bassiana used in this study contain 3.06 x 10^{8} con g^{-1} conidia density and 92.92% viability. The symptoms S. litura larva infection by B. Bassiana was decreasing movement and eating activity. The dose of 8 g100 mL^{-1} B. bassiana was the most effective dose compared to others and can be used to control S. Litura.

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