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The Effectiveness of Entomopatogen Fungi of Beauveria Bassiana Ferr. for Handling the Spodoptera Litura F. Caterpillar on Soybean Plant (Glycine max L. Merr)

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***Abstract**: Soybean is a source of vegetable protein, fats, minerals, and vitamins, currently its position as a very important raw material industry. Indonesia's soybean need reaches 2.20 million tons/year; its domestic production is sufficient 35-40%, so the shortage (60-65%) is met from import (Ministry of Agriculture 2008). The domestic need continues to increase to 9.55% per year. The entire body of S. litura is almost obscured by the white-colored mycelia. With a 109/mL density to shut down up to 50% of the population takes 5 days and 95% of death takes 10 days. B. bassiana infects the insects through the cuticle and multiplies in the body of the insect, while producing beauverisin toxins which can damage the structure of the insect cell membrane to death.*

***Keywords**: fungi; soyabean, b; conventional; hymenoptera parasitoid*

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I. Introduction

Soybean (Glycine max L. Merr) is one of the food commodities that have long been cultivated in Indonesia. Soybean is a source of vegetable protein, fats, minerals, and vitamins, currently its position as a very important raw material industry. Indonesia's soybean need reaches 2.20 million tons/year; its domestic production is sufficient 35-40%, so the shortage (60-65%) is met from import (Ministry of Agriculture 2008). The domestic need continues to increase to 9.55% per year. The high demand is influenced by the development and the improvement of people's welfare (Adisarwanto and Wudianto, 2008).

The Ministry of Agriculture continues to increase the domestic soybean production, but not apart from the problems. One limiting factor that inhibits the increase of soybean production in Indonesia is larval attack (Marwoto & Suharsono, 2008). Grayak caterpillar (Spodoptera litura) is one of the important larvae in Soybeans plant that can lead to the failure of crop. The larvae attack the soybean plants begin in the larval phase, by eating the leaves until they are damaged, perforated and appear transparent (Suyanto, 1994). Adie (2012) states that S. litura is one of the important larvaes that damages the soybean leaves compared with other leaf-damaging larvae. Furthermore Jadhav & Teli (2009) add that S. litura causes very serious damage because they are greedy in eating the leaves and the attacks can continue to damage the pods of S. litura, attacks the plants at various growth phases such as vegetative phase (11-30 HST), flowering phase and initial filling pod (31-50 HST), and the growth as well as the development phases of pods and seed filling (51-70 HST) (Tengkano & Soehardjan, 1985). The loss of the results due to S. Litura attack reaches 80%, even can worse if not controlled. The Grayak caterpillar attacks at night, and during the day the caterpillar enters the moist soil (Marwoto & Suharsono, 2008).During this control of S. litura, synthetic insecticides is more often used that has a negative impact on the environment and surrounding communities (Jaramillo et al., 2006), moreover it also causes the resistance of larvae, the larvae resurgence, leaving no residue on the product, the danger for the users, and others (Ramlan, 2010). The increased human awareness towards the sustainable agriculture systems replaces the conventional farming systems and spur on biological control, one of which is by using entomopathogenic fungi (Untung, 2007).

According to Yasin (2005), one of the potential entomopathogenic fungi is the B. bassiana fungus. This fungus infects its host through the cuticle and digestion. Konidia B. bassiana sprouts germinate to form hyphae penetrant (Apresorium) to penetrate into the internal tissue of the host (hemosel) to the surface of the host integument. In Canada, Todorava et al., (2003) proved that B. bassiana very effectively kill the larvae of Choristoneura rosaceana Harris (Lepidoptera: Tortricidae) leaf roller larvae and pupae. The death of larvae and pupa reached more than 85% at doses of 107 conidia/ml up to 60 days after the treatment. The high reproduction capacity of B. bassiana is easy to obtain and easy to reproduce so that it can lower the cost of larval control (Sudarmadji, 1994). Until now, the information on controlling the S. litura larvae by making use of

entomopathogenic fungi is still very small, therefore it needs to do a further research to determine the effectiveness of the fungus B. bassiana to control the S. litura larvae on soybean plants.

II. Research Method

2.1 Place and Time

This research was conducted at the Laboratory of Pest and Disease of Agriculture Faculty of Syiah Kuala University and continued in the experimental garden of Agriculture Faculty of Jabal University Ghafur, Sigli from August 2015 to completion.

2.2 Tools and Materials

The tools used are sterile roospace, petridish, microscope Nikon Eclipse 50i, refrigerators, autoclaves, incubators, erlenmeyer, measuring cups, spatulas, Haemocytometer superior made in Germany, thermometer, gauze, wipes, jars, knife, sterile cotton, aluminum foil, poly And stationery. The materials used are larva S. litura, B. bassiana fungus collection of Forestry and Plantation UPTD-Hall Plantation Crop Protection Banda Aceh, Anjasmoro varieties of soybean seeds, potato dextrose agar (PDA), distilled water, alcohol, methylated spirits and detergents.

2.3 Research Methods

The research in invitro laboratory uses non factorial Randomized Complete (RAL) with 5 levels and 4 replications to get 20 units of experiment, and continued research in the field by using non factorial Random Group Plan (RAK). The research data on each treatment was analyzed by variance, if there was a significant difference then continued with the Smallest Different Difference Test (BNT) at the 0.05 level (Gomez & Gomez, 1995). The composition of conidial density of entomopathogen B. bassiana fungus can be seen in Table 1.

Table. 1 Composition of Treatment

Entomopatogen Fungi of B. bassiana (B)	Density of Konidia(mL)
B	- 10^5/mL
C	- 10^6/ mL
D	- 10^7/ mL
E	- 10^8/ mL
	- 10^9/ mL

2.4 Implementation of Invitro Research

2.4.1 Revirulency of B. bassiana

The Fungus of B. bassiana collection from UPTD-Balai Proteksi Plantation Plant Banda Aceh, re-divirulensi on S. litura larvae is to restore its toxicity. This process is conducted by dipping the larvae S. Litura into conidia suspension B. bassiana for 4 seconds (Desyanti, 2007). Furthermore, S. litura larvae are replaced in Petridish with moist filter paper, and then the Petridish is wrapped with aluminum foil and kept at 20-30ºC.

2.4.2 Preparation of PDA media

A total of 10 grams of PDA are mixed with 250 mL of aquades were stirred evenly in Erlenmeyer and sterilized in Autoclave for 30 minutes at 121 ° C, then sterile PDA media was poured into Petridish.

2.4.3 Preparation of conidial B. bassiana density

Conidia fungus of B. bassiana which are the results from revirulence on S.litura larvae are refined and reproduced on PDA media at Pest Laboratory, Faculty of Agriculture, Syiah Kuala University. To obtain the proper conidia density of B. bassiana, it can be calculated by using Haemocytometer.

2.4.4 Preparation of S. litura larvae

The S.litura larvae are collected from soybean plants, then selected to be cultured to obtain a homogeneous sample of S. litura larvae as test larvae, the test larvae used in this study were the third instar larvae. The collected S. litura are placed on the jar as a medium for conducting the research.

2.4.5 Suspension Preparation

The suspension preparation of B. bassian fungus with conidia density 105, 106, 107, 108, and 109/mL then each jar each is invested 10LLsva S. litura, then B. bassiana fungus are applied in accordance with the specified conidia density.

2.4.6 Observation of Invitro Test

In this study the variables observed include:

a. Symptomatology

The observations are performed based on the symptoms seen in S.litura larvae after the application of B. bassiana fungus by using a microscope.

b. Mortality Rate S. litura

To calculate the mortality percentage of S. Litura larvae, it is used the formula:

$$P = \frac{a}{b} \times 100\%$$

Note
p = Percentage of the dead Larvae
a = Number of the dead Larvae
b = Total Number of the dead Larvae

2.4.7 Percentage of emerging Pupa

The observation of the emerging pupa percentage is conducted to see the S.litura larva which is able to continue its life cycle from larva to pupa after being applied the b. bassiana fungi.

2.4.8 Percentage of emerging Imago

The observation of the emerging pupa percentage is conducted to see the S.litura larva which is able to continue its life cycle from larva to imago after being applied the b. bassiana fungi.

2.5 Implementation of Field Research

2.5.1 Preparation of host plants

The preparation of soybean crop is conducted by cultivating the soil with a ratio of 3: 1 between the soil and the organic fertilizer, and then the soil is put into the poly bag of 8 kg volume. The seeds are planted with 3 - 5 cm depth, each hole contains 3 seeds. The land arrangement is conducted after 7 HST leaving two plants per hole.

The fertilizer provided is SP-18 at 100 Kg dose per Ha, KCl at 100 kg dose per Ha, and urea at 75 kg dose per Ha. The Fertilizers of SP-18, KCl, and urea are given at planting time. The urea fertilizer is given twice at planting time and the plant age is 30 days after planting. The weeding is conducted at the plant age of 2 - 4 MST and 6 MST. The harvest is conducted when the plant has been ripe physiologically which is characterized by 95% of the pods are brown and the leaves begin to dry out.

2.5.2 Propagation of S. litura

The S. litura larvae are collected from soybean cultivation of Gle Gapui Sigli. These larvae are preserved in plastic boxes and fed with fresh soy leaves. The larvae food is replaced after it is gone or not fresh anymore. At the time of the larvae will be pupa, sawdust is given on the base of the box. All the imagos which are out of the pupa are kept in bulk in insect cages that have been given fresh soy leaves as a place to lay eggs. As the food, imagos are used honey with 10% concentration. The egg group is placed then moved to another box and used for testing.

2.5.3 Application of B. bassiana in the Field

After obtaining the most effective density of fungi conidia of B. bassiana from the laboratory test results, it is followed by the field testing by applying directly to soybean crops each of which has been invested 10 S. litura larvae per experimental poly bag.

2.6 Observation in the Field

In this study the variables to be observed include:

2.6.1 Attack Intensity of S.Litura Larvae

The observation of the leaf damage caused by the attack of S.Litura larvae on soybean crop is conducted at 1-12 weeks after the implementation of B. bassiana. The observation of the leaf damage is conducted by scoring method based on the percentage of the attack width of the S. litura larvae on the leaves.

$$I = \frac{(n \times v)}{N \times V} \times 100\%$$

Note:
I = attack intensity of the larvae
n = number of the affected plants
v = score value in each attack category
N = the highest attack score value
V = number of plants observed

2.6.2 Mortality percentage of S. litura

To calculate the mortality percentage of S.litura larvae is used the formula:

$$P = \frac{a}{b} \times 100\%$$

Note:
P = Percentage of the dead larvae
a = Number of the dead larvae
b = Total Number of larvae

2.6.3 Data analysis

The data of attack intensity of S. litura and the component results obtained from the observation are analyzed by using t test at the level of 1% and 5%.

III. Results and discussion

3.1 Laboratory Observation

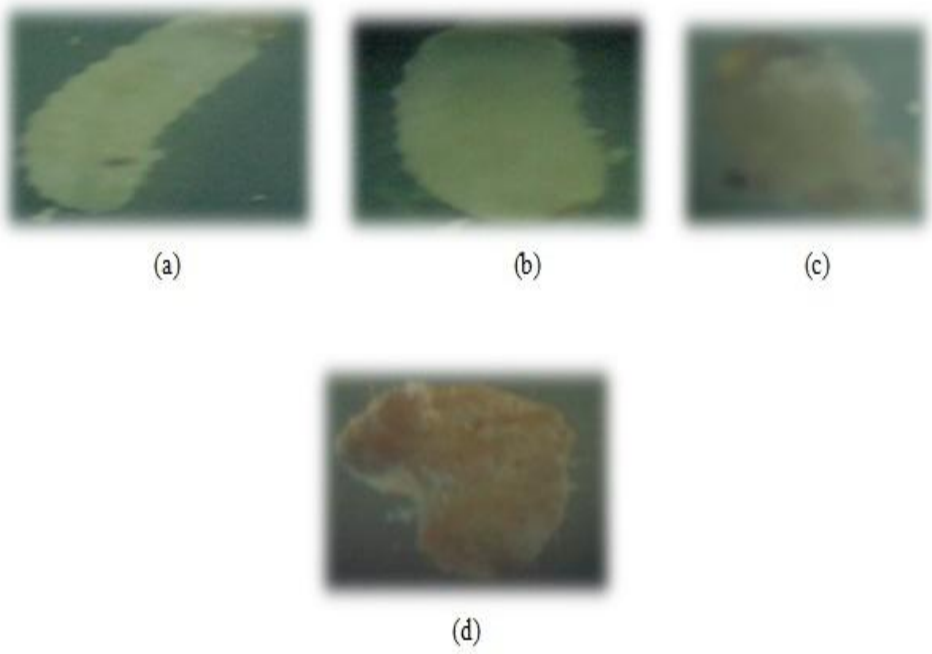
3.1.1 Symptomatology

The oberservaton visually on the 1st day of the S. litura larvae movement becomes slow, it is thought as a result of the B bifana hypha activity begins to penetrate into the tissues of the host body and interfere with the physiological processes of the larvae (Fig. 2a). According to Neyes and Alves (2004) the B. bassiana fungi requires several stages of process to infect the host, i.e. inoculation, conidia attachment and penetration. Infected host will become weak, inactive and eventually die (Tanada & Kaya, 1993).

The observation on day 2, the death occurs, with the body of the larvae begins to change the color to yellowish white and mycelia is not visible on the surface of S. litura larvae's integument. It is thought that B. bassiana fungi are still in the invasion stage to damage the internal tissue of the host body (Fig. 2b). In accordance with Sayuthi's (2011) statement that before the penetration penetrates its host integument, the entomopathogenic fungi will form a sprocket tube in the form of penetrating hyphae (apresorium) and enter into the hemosel by mechanical and chemical means through secreting the enzymes and the toxins. According Desyanti (2007) at the beginning of the host's death, mycelia has not been seen clearly.

The observations on the 3rd and 4th days, B. bassiana fungi begin to appear in the integum part of the host's body, especially on the abdomen (Figs. 2e and 2d). It is thought that the mycelia began to densely grow inside the host's body and the food source begins to decrease so that it penetrates to the outside of the host's body to get a new food source. In accordance with the opinion of Steinhaus (1967) the mycelia will pass through the host's integument between 24-48 hours after its host died and the fungus begins to damage other tissues by forming the reproductive organs. According to Sayuthi, (2011) the mycelia passes through the host's integument by using penetrant hyphae (apresorium), when the unfortunate environment of the mycelia will not pass through the integument and it takes place in the host's body only.

Figure. 1 Being Observed by Using a Microscope of Nikon Se Type 102 with L0 X Magnification

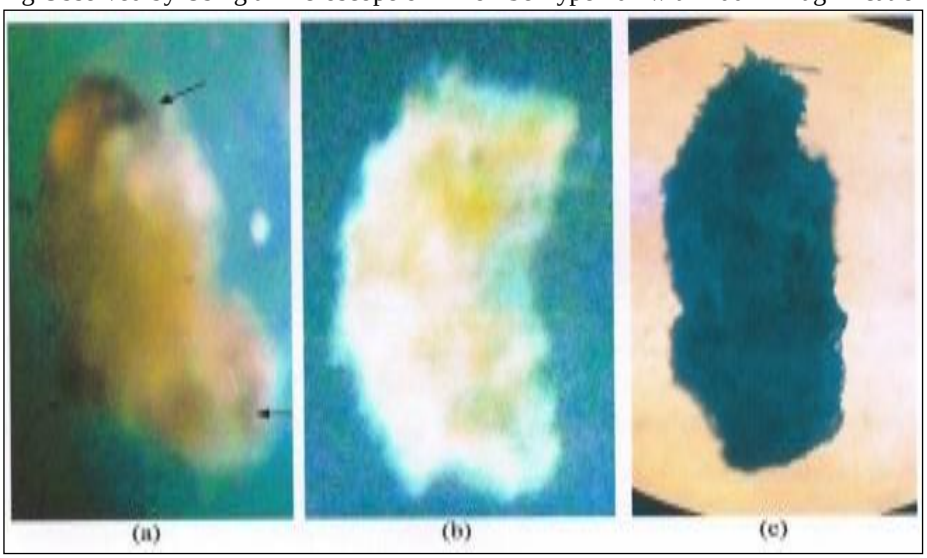


(A) Observation day 1, (b) Observation day 2, (c) Observation day 3, (d) Observation day 4.

The observation on the 5th day, the host appears to swell on the edge, allegedly B. bassiana fungus began to damage in this part. According to Mahr (2003) in Pujiastuti, (2006) due to the attack of B. bassiana fungus, the host body swells and looks dry. On the larvae's body there are black spots which is thought as the starting point of penetration and B. bassiana fungi (Fig. 1 a). In accordance with Thomas's (1984) statement that the infected host with the fungus may change its color and the cuticle appears to be black spot as the starting

point of entomopathogenic penetration and fungus. The penetration point is influenced by the configuration of integument morphology (Bidochka et al., 2000 in Sayuthi, 2011).

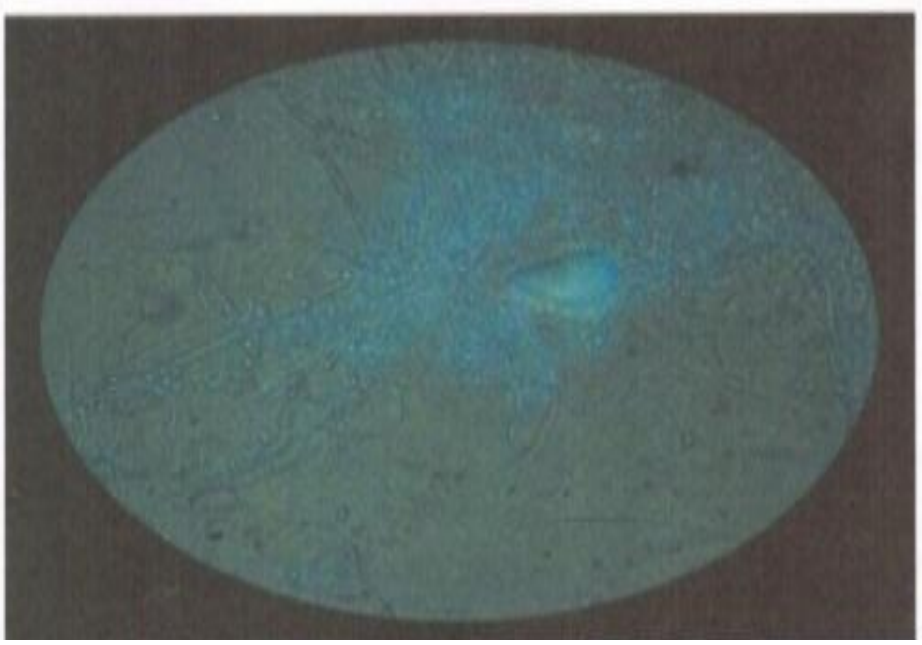
Figure. 2 Being Observed by Using a Microscope of Nikon Se Type 102 with L00 X Magnification



(a) Black spot where the penetration begins on day 5, (b) Observation day 6, (c) Observation day 7.

The observation visually on the 6th day, the color of the larvae body begins to turn into yellowish brown and the entire surface of the host's integument has been masked by the white myelia of B. bassiana fungi (Fig. 2 b), the mycelia appears to pass through the cuticle to the integral surface of the host using penetrant hyphae (apresorium) which looks like a thread (Figure 3).

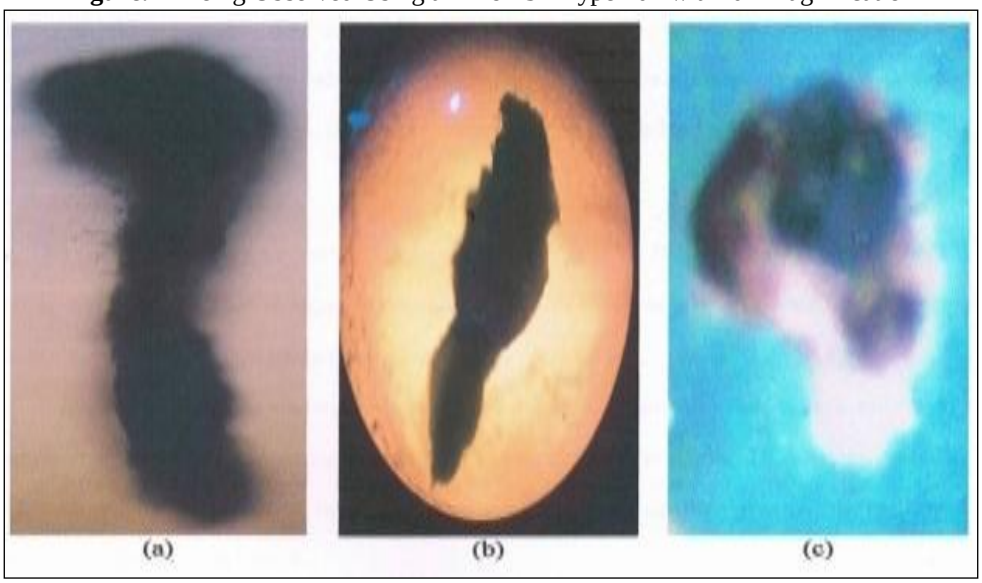
Figure. 3 Mycelia of B. bassiana fungus penetrates the S. litura larva's integument observed by Microscope of Nikon Eclipse 50i by 100 X Magnification



On Figure 3, the Hifa is located on branches can be seen, arranged in a circle (verticillate) and bubbling or thickening. The conidia sticks tomteh branches or the edges side of conidiophores. The oval conidia shaped slightly (globose) to ovoid (obovate) of the conidiophore group, while below the mycelium bubbles. According to Wiryadiputra (1994) the zig-zag conidiophores form is a characteristic and B. bassiana genus. The observation visually on the 7th day, the color of the host body appears to begin to darken in the last segment of the abdomen starting to crease and the mycelia appears denser. It is presumably the crimped on the abdominal

end segment of S. larvae's limbs has been absorbed by the B. bassiana fungus, with the larvae body begins to change which is due to fluid loss and the effects of toxins and B. bassiana fungi.

Figure. 4 Being Observed Using a Nikon SE Type 102 with l0x Magnification



(a) Observation day 8, (b) Observation day 9, (c) Observation day 10.

On the 8th day, the host appears dry and stiff with the body getting smaller, especially on the abdomen (Figure.4 a) because the host's body fluid has been absorbed by B. bassiana fungus. According to Feron (1981) the insects are dead and hard as the results the fluids of the host's body have been absorbed by the entomopathogenic fungus of Nutritinya. The day-9 observation is not significantly different from the observation day-10 that is the host's body which has been covered entirely by mycelia B. bassiana fungus become dry, black colored, the smaller and the fountain looks not to Shaped (Figures 4 b and 4 c). According to Haris (2005) the insects infected by B. bassiana fungus in the final stages will harden (mummification) and mycelia B. bassiana fungus will cover the entire body of the host.

On the development in the insects' body, B. bassiana will secrete a toxin called beauvericin which causes paralysis of the limbs of insects. The paralysis leads to loss of coordination of the motion system, so that the movement of the insects is irregular and gradually weakens, then stops altogether. After more than five days there is total paralysis and death. The toxin also causes a tissue damage, especially in the gastrointestinal tract, muscles, nervous system, and respiratory system (Wahyudi, 2008). This is related to the results of Jauharlina's (1998) research on the of S. litura larvae and Harmiyanti's (2006) research on the crocidolomia binotalis larvae that infected with B. bassiana fungus which is characterized by a color change in the larvae body and the slow activity of the larvae.

The entomopathogenic fungi begin to infect the insects if conidia have attached to the insect's body, the supported conditions then the infected insects will be covered by mycelia of B. bassiana fungus. The entomopathogenic fungi begin to infect the insects if conidia have been attached to the insect's body, the supported conditions then the infected insects will be covered by fungi mycelia (Sulistyowati, 1993). According to Kershawet al. (1999) at relatively low concentrations, the infected insects can survive, but failed to experience the gradual formation of the pupa and the slowly experienced the death. The effectiveness of entomaptogen to control the pest depends on the type of isolates, conidia and age of pests stadia (Widayat and Riyanto 1993 in Trisawa and Profit, 2006).

3.1.2 Percentage of Larva Mortality

The results of observation on larval mortality percentage of S.Litura larvae on 10 HSA with conidia density and the application technique of B bassiana can be seen in (Appendix 1). The analysis of variance shows that the conidia density of B. bassiana fungi significantly impact on the mortality of S. litura larvae. Next the mortality percentage of emerging S. litura can be seen in Fig. 5.

Figure. 5 Percentage of Mortality Formed Due to the Infection of B. bassiana fungi with Different Conidia Density

Based on the data in Fig. 5 it can be explained that the conidia density of 109/mL bassiana fungus resulted in higher mortality than 105/mL. The average mortality percentage of the highest S. litura is found in the treatment (E) with a density of fungus (109/mL) of 90% which was not significantly different from the treatment (D), whereas the percentage of low liters S. mortality is found in the treatment (A) with the density of the fungus (105/mL) is 13% which is not significantly different from the treatment (B), but it is significantly different from other treatments. According to Atmadjaet al. (2000), the higher the conidia concentration of B. bassiana, the mortality rate of the insect is always higher than the other concentrations.

The effectiveness difference which is due to the application density of conidia suspension of B. bassiana is presumably due to the different number of conidia attached to the larvae. The suspension application of the 109/mL produces the highest number of conidia on the surface of the larvae compared to the 105/mL application. The conidia density affects the percentage of larval mortality, the higher the application volume then the higher the mortality percentage produced. Furthermore, it decreases successively on the application volume of conidia suspension of B. bassiana at the lowest concentration.

This is due to the dose of application and virulence of isolates is a decisive factor against the mortality (Neves & Alves 2004). Every 46 species of entomopathogenic fungus has a virulence level and a way of attacking its host. To paralyze the host defense, entomopathogenic fungi produce secondary metabolites of toxin, such as destruxins which is released by the B. bassiana fungus (Amiri-Besheli et al. 2000). Generally, the death of host is caused by the infection of entmopathogenic fungus which occurs between 5 to 8 days, and is highly dependent on the host size.

3.1.3 Percentage of Emerging Pupa

The observation results on the percentage of emerging pupa of S. litura on 10 days after the application (HSA) with conidia of B. Bassiana. The analysis of variance shows that the conidia density of B. bassiana fungi has a very significant effect on the pupa percentage of S. litura larvae. Furthermore, the number of percentages of the emerging S. litura larvae can be seen in Fig. 6.

Figure.6 Pupa Percentage Formed by Infection of B. bassiana Fungus with Different Conidia Density

Based on the data in Fig.6, it can be explained that, the density of bassiana 109/mL fungus can decrease the percentage of emerging pupa. The average percentage of the lowest pupa S. litura is found in the treatment (E) with the fungus density (109/mL) that is 10% which is not significantly different from the treatment (D), while the highest is found in the treatment (A1) with the fungus density (105/ml) that is 87% which is significantly different with all other treatments. The effectiveness of B. bassiana is influenced by the application density; the higher the application volume the higher the percentage of pupa mortality produced. Furthermore, it decreases consecutively on the conidia application volume of B. bassiana at the lowest concentration. The high conidia density affects the strength of the B. basiana fungus that contain conidia which makes it much easier for penetration, development and infection by the fungus become more quickly cause the death than with low concentrations (Hasnah et al., 2012).

This corresponds to B. bassiana to kill the pupa of S. litura. The high density of B. bassiana fungus and spray volume in the opinion of Prayogo et al. (2005) that, the effectiveness of B B. bassiana is influenced by the volume of application, the higher the application volume the higher the percentage of deaths produced and the lower the pupa becomes imago. In addition, by the strength of insect skin, the harder and stronger the skin of the insects, it will be more difficult to be infected by the fungus B. bassiana (Malauet al., 2010).

Fig.6 shows that the percentage of pupa emerges the highest due to be infected by B. bassiana fungus that found in the treatment A (105/mL) followed by treatment B and C, and the lowest is encountered in the treatment E (109/mL) and not significantly different D (108/mL). The percentage of pupa to be imago of S. litura is closely related to the pupa mortality. The higher the pupa mortality, the lower the imago appears (Herlinda et al., 2012).

3.1.4 Percentage of emerging Imago

The observation result on the imago percentage of S.Litura appears at 10 days after the application (HSA) with conidia density of B. Bassiana fungus can be seen in (Appendix 5). The analysis of variance indicates that the conidia density of B. bassiana fungus has a very significant effect on the imago percentage of S. litura. Next, the number of imago percentage of S. litura which emerges can be seen in Fig. 7

Figure.7 Imago Percentage Infected by B. bassiana Fungus with Different Conidia Density

Based on the data in Fig.7, it can be explained that the conidia density of B. bassiana fungus can decrease the percentage of S. litura. The average imago percentage of the lowest S. litura is found in the treatment (E) with the density of the fungus (109/mL) that is 10% which is not significantly different from the treatment D (108/mL), but significantly different from other treatments, while the imago percentage of the highest S. litura is found in the treatment (A) with the density of the fungus (105/mL) that is with the imago percentage of 87% which is not significantly different from the treatment B (106/mL), but significantly different from other treatments.

The effectiveness difference due to the suspension application of conidia density of B. bassiana is thought due to the difference in the number of conidia attached to the larva. The suspension application of the 109/mL produced the highest number of conidia on the surface of the larvae compared to the application of 105/mL. Conidia density affects the percentage of pupa mortality, the higher the application volume the higher the imago percentage produced. Furthermore, it decreases consecutively on the volume of suspension application of conidia density of B. bassiana at the lowest concentration. The pupa percentage to imago of S. litura is closely related to pupa mortality. The higher the pupa mortality, the lower the imago emerges (Herlinda

et al., 2012). The difference in the percentage of mortality between the results of this study compared with Shurtikanti and Yasin (2009) is as attributable to the following factors. The first factor s thought to be a more sensitive difference than the pupa. Stadia larva is more vulnerable compared to stadia pupa and imago (Gomies, 2009). The second factor is the conidia density used for pupa applications is higher than to the larvae. The conidia density used for the application against the percentage of pupa mortality is higher than that of the application used for larvae (Huang et al., 2012). The higher the conidia density of B. bassiana, the lower the percentage of epidemic emerges (Herlinda et al., 2008).

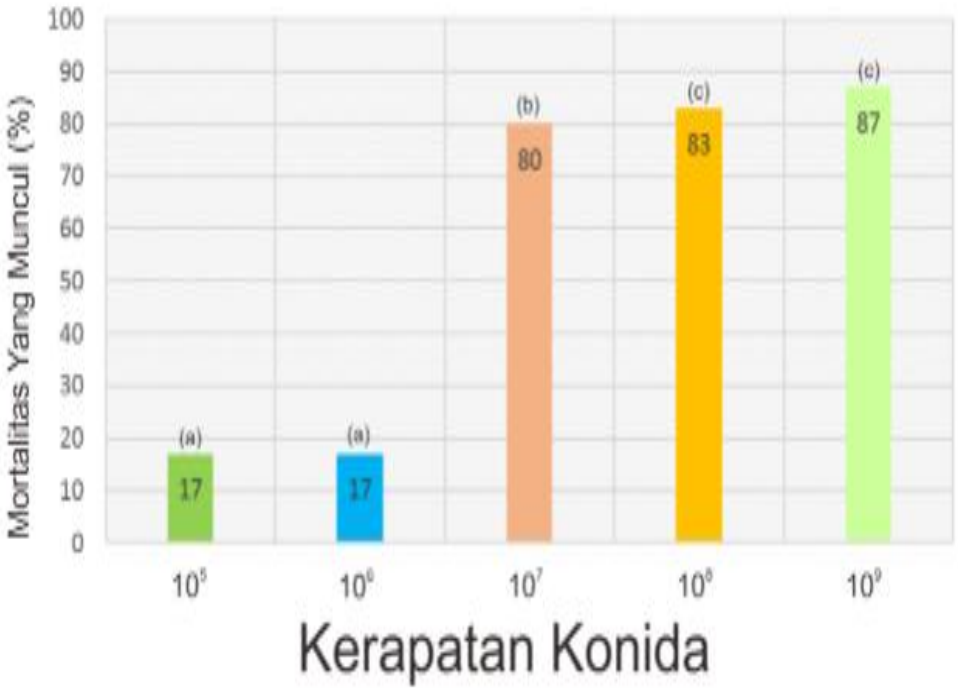
This is supposedly due to the increasing number of conidia attached to the body of the larvae; the faster the mortality will be again supported by the conditions of temperature and the humidity in accordance with the desired entomopathogenic fungus. The number of conidia of entomopathogenic fungi is related to the concentration level used, because the higher the concentration the higher the conidia is, and the higher the mortality is (Hashim and Azwana, 2003). This is consistent with the research of Malau et al., (2010), the spray volume of 10 ml with 108 conidia/mL is able to control the adult fruit flies by 50% different from the armyworm, which reached 100%. In contrast to the research of Senewe and Manengkey (2011), the volume of 20 ml, the B. bassiana applications of similar density is only able to suppress the imago of Leptocorisa oratorius by 30.03%. The conidial spraying is different106/mL - 1010/mL with 25 ml spray volume on cabbage plants can control the larvae of Plutella xylostella. L with the percentage range of deaths 16.67% - 50%.

3.2 Tests in the Field

3.2.1 Mortality Percentage of S. litura

The observation results on the mortality percentage of S litura larvae at 10 days after application (HSA) with conidia density of B. Bassiana fungus can be seen in (Appendix 7). The analysis of variance indicates that the conidia density of B. bassiana fungus has significant effect on the mortality of S. litura larvae.. Next is the amount of mortality of the emerging S. litura can be seen in Figure 8.

Figure. 8 Mortaliity Percentage of S. litura Larvae Due to the Infection of B. bassiana Fungus with Different Conidia Density



Based on data of Figure 8, it can be explained that, the more conidia of B. bassiana fungus can reduce the percentage of the average number of the emerging larvae. The average percentage of the highest mortality of S. litura is found in the treatment (E) with the density of the fungus (109/mL) that is 87%. While the lowest percentage of S. litura mortality is found in the treatment (A) with the fungus density (105/mL) that is 17% which is not significantly different from the treatment B.

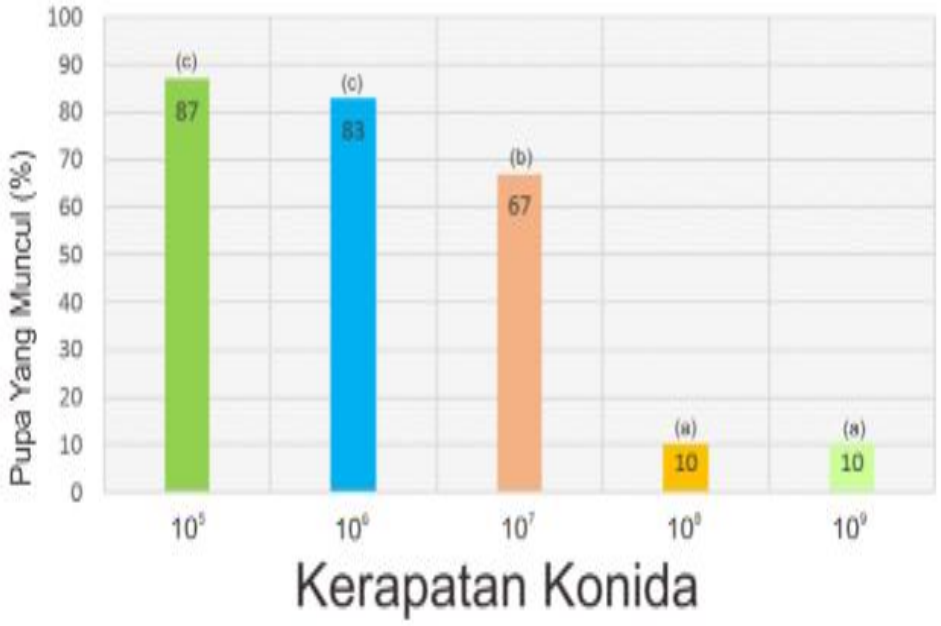
The effectiveness differences of the conidia suspension application volume of B. bassiana is thought due to the difference in the number of conidia attached to the suspension larva. The application of 109/mL produced in the highest number of conidia on the larvae surface compared with the application of 105/mL. The

conidia density impacts on the mortality percentage of larvae. This is accordance with Patahuddin's research (2005), the different conidia density (104-109/ml) is able to produce the death of Spodoptera exigua larvae which is different from the death of average percentage of 3.33% - 100%. According to Hashim and Azwana (2003), the B. bassiana fungus from Baso with the different conidia density (3.2 x 104 to 3.2 x107) can control the imago of Cosmopolite sordidus which is different from the average mortality percentage 56.67% -96.67% . The Conidia application of B. bassiana on different conidial density (10 -108/ml) is able to kill the pupa of Bacrocera. The effectiveness of B. bassiana is influenced by the application volume, the higher the application volume the higher the percentage of mortality produced. Next, it decreased successively on the volume of suspension application of conidia density of B. bassiana at the lowest concentration. This happens because of conidia density range used in this research shows that the higher the density of conidia, the faster the death in the insects, and vice versa the less conidia density, the longer the event of the insects' death. This is made possible by the many conidia chance to germinate and infects the insects (Sapdi, 1998). Furthermore, Atmadja et al., (2000), state that the higher conidia density of B. bassiana, the mortality rate of the insects is always higher than other concentrations.

3.2.2 Attack Intensity

The observation results on the attack intensity of S. litura larvae at the age of 10 HSA. The various analyses indicated that the conidia density of B. bassian fungus is very significant to the attack intensity of S. litura larvae on the plant age at 10 HST. Furthermore, the intensity number of S. liturary appears can be seen in Fig. 9.

Figure.9 Intensity Percentage due to the infection of B. bassiana fungus with different conidia density



Based on the data in Fig. 9, it can be explained that the addition of conidia density of B. bassiana fungus can decrease the attack intensity of S. litura. The average attack intensity of the lowest S. litura larvae is found in the treatment (E) with the fungus density (109/ml) that is 20.47% which is not significantly different from the treatment (D). While, the highest attack intensity is found in the treatment (A) with the fungus density (105/ml) that is 49.13%.

This is in accordance with the opinion of Surtikanti et al. (1997) that, the density that cause the optimal killing power is at the suspension sporadic level (conidia/ml), if the spore value is below the value, it can be expected that its effectiveness will be lower. The attack intensity data showed that the conidia density has an effect on the attack intensity with the percentage of leaf area indicated by Grayak caterpillar larvae as shown in Table 1. The high attack intensity occurs when the low conidia density is applied. Similarly, the low attack intensity occurs when the conidia density is applied so that the conidia density given should be appropriate for

the control of Grayak caterpillar with the optimal function, if excessive (such as conidia density 109), there will be decreased effectiveness (Prayogo, 2004) .

According to Saleh et al. (2000) this is thought to be related to the disturbed eating activity of larvae. Plants sprayed on the plants, in addition to entering the larvae through sensitive larvae skin, are also directly consumed by larvae through the leaves they eat. The treatment with a thickened suspension containing more conidia and the development of fungus in the larvae body will be faster and cause the eating activity of the larvae is disturbed. The reduced eating activity and the ability to eat food from larvae in plants sprayed with higher concentrations of fungus can also be seen from the extent of crop damage. The attack intensity of larvae on sprayed plants with higher fungi suspensions will show lower results. There have been several changes in the behavior and condition of Grayak larvae as a result of entomopathogenic fungi, besides the effect on the larvae movement activity (Saleh et al., 2000).

IV. Conclusion

The mycelia of the insect body begin to be visible on the 4th day, and enter the 7th day. The entire body of S. litura is almost obscured by the white-colored mycelia. With a 109/mL density to shut down up to 50% of the population takes 5 days and 95% of death takes 10 days. B. bassiana infects the insects through the cuticle and multiplies in the body of the insect, while producing beauverisin toxins which can damage the structure of the insect cell membrane to death. The result of laboratory research on the highest mortality percentage of S. litura larvae is on conidia suspension of B. bassiana with conidia density of 109 spores/mL with the percentage of larvae 90%. The result of laboratory research on the highest pupa percentage of S. litura is on conidia suspension of B. bassiana with conidia density of 109 spores/mL with percentage of pupa emerges 10%. The result of laboratory research on the lowest imago percentage is on the conidia suspension of B. bassiana with conidia density of 109 spores/mL with the percentage becomes 10% of imago. The result of field research on the highest mortality percentage of S. litura larvae is on conidia suspension of B. bassiana with conidia density of 109 spores/mL with the mortality percentage of 90%. Conidia density of B. bassiana 109/mL is effective against the attack intensity of S. litura larvae with the attack intensity of 20.47%.

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