Concentration And the Prevalence Of Application Effect Of Fungi Beauveria Bassiana On The Growth Of Soy Pest (Glycine Max, (L) Merr)

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Abstract- This research aims to examine the effect of giving Bauveria Bassiana (K) extract to the pest of soy plant Glycine max, (L) Merr, which include *Spodoptera lidura*, *Bemisia tabacci*, and *Nezara viridulla*. The research was conducted in Gampong Jawa Village, Langsa Kota Sub-dsitrict, Kota Langsa. It was carried out from March until September 2016 at the elevation of 3,4 m above sea level and soil pH was 6. Factorial Randomized Blocked Design was used for the research experiment, testing the hypothesis that concentration, application regime and combination of both factors affect the growth of the pests. Four levels of Bauveria Bassiana (K) concentrations and four levels of application frequency were used during the experiment. The parameter observed were pest population, pest mortality, outbreak prevalence and the percentage of bean deffect. The research shows that all the three source of variations (concentration, frequency and combination of both concentration and frequency) significantly affect the growth of Glycine max (L) Merr pest. Research also suggested that the optimal concentration to be applied to Glycine Max, (L) Merr is 32,5 ml/l water and the concentrate must be given once in every week.

Keyword: *Glycine max*, (L) Merr, *Bauveria Bassiana* (K), Soy pests, Concentration,
Application regime.

INTRODUCTION

Soy (*Glycine max*, (L) Merr) holds an important roles in Indonesians diet. It is one of the plants thas become the main protein source, cheap, and delicacy. The plant the third most cultivated holticultures in Indonesia (Suprapto, 2004). It's production was increased by 2,08% a year within the periods 1978-2012. Land used for soy cultivation was approximately 570 thousands hectares producing 1,4 thon /ha. In Aceh, the production is lower, at 1,3 ton/ha with 35 thousands ha land cultivated for this plant. The statistics for soy production is far below other major soy producers such as Brazil and Argentina who can produce 2,5 ton/ha (BPS, 2013).

One among many factors that has been the reason for the less productiive Indonesian soy farms is the pest that cause diseases (Raden, et al., 2000). The four main pest of soy plant in Indonesia are *Spodoptera litura, Bemisia tabacci, Nezara viridula, and Valanga sp.*

Spodoptera litura

Spodoptera litura or locally called ulat grayak is a type of moth belongs to the family of Noctuidae. The larva has been infamous pest, and highly fecund because one female S. litura can produce 3548 eggs (Tuan et al., 2013). When reaching the age of two weeks, the larvae will have 5 cm length and possesses various colors. The main feature of ulat grayak is the crescent shape in its fourth abdominal segment and is bordered by yellow line on its lateral and dorsal (Pracarya 2008). This larvae live for 20 days and all of its lifetime as larvae is spent as plant pest (Adisarwanto, 2008).

The plants suffered for S. litura infestation wil have leaves with their leaves are torn at the verges (Adisarwanto 2008), and usually the larvae attack one individual plant in a very high population until all the leaves are eaten, and then move to another plant (Pracarya 2008).

Bemisia tabaci

Bemisia tabaci or silfer leaf whitefly or more familiar as white fleas and kutu kebul by local farmers, is one of the white fleas that have become the most disadvantageous flies in agriculture (Adisarwanto 2008). This species is classified into the family *Aleyrodidae* of the order *Homoptera* and was originated from the equatorial region (Faria et al., 2001). Similar to other white fleas, kutu kebul sucks

the liquid from leaves and its excretion results in honey dew which is the media for black smoke disease. This fly is also known to be the vector of viruses (Santosa, 2011).

Female B. tabaci are diploid which come from fertilized egges, while the male are haploid resulted from infertilized eggs. The eggs are usually placed in groups, which innitial color is white, then turn brown until they hatch within five to seven days. After egg phase, the hatchlings develops through four stages of instar and the adults are usually bright yellow with white wings (Adisarwanto 2008). Adults are usually covered with white wax and the size ranges from 1 to 5 mm (Santosa 2011), or four times greater than the eggs size (Adisarwanto, 2008).

Kepik Hijau (*Nezara viridulla*)

Another major pest attacking the soy plant is *Nezara viridulla* or kepik hijau. These species are originated Unlike the two other pest, this pests attck the pod of the plant. The plant suffered from this pest infestation would show suction marks of the flies. The bean can also be empty, showing growth delays thus degrade the quality of beans and even abortion of the pod. The indication of a plant suffered from this pest are the deflation of the bean, the shrink of beans' skin or some brown spots on the beans sometimes it turns black. This pest attack the plants on the stadia nimfa and imago (Grozea, et.al., 2012), where it sucks the liquid of the soybean pod. Therefore the most vulnerable stage of this pests infestation is during the pod and beans development (Pracarya 2008).

The imago of *N. viridulla* is green just like the head with orange to golden yellow and greenish yellow of serna pronotum which has three yellow or green spots. The egggs were placed in groups (10 to 90 eggs/ group) under the leaves. The nimfa has 5 consists of 5 instar stages. The first nimfa stage live in groups on the leaves around where the eggs were placed, and then spreading (Pracarya, 2008). This pest has geographical range et the tropics and subtropical area, and beside attacking soyplants, it also become pest for paddy, corn, tobacco, potato, chilli, cottton, and other plants with pods (Pracarya, 2008).

Valanga sp.

Valanga sp. or gasshoppers belongs to the order Orthoptera and are known to be polifag. This pest attack almost all plants in general. It has straight and hard front wings and the hind wings looks like membrane (Santosa, 2011).

In general, pest controls by farmers is done using chemical insectisides. However, prolonged used of insecticides imposed negative impact such as the elimination of pest natural enemy and the accumulation of insecticides residual. Therefore, it is needed to develop mechanism of perst control in environmentall friendly manner without leaving harm to human and plants. One of the option is using entomopatogen fungi (Hasnah, et al., 2012).

Entomopatogen fungi is microorganism that can be used as biological agent to control pests. Some beneficial aspect of using this type of fungal is having high reproduction rate, short life cycle and withstand extreme environmental gradient. Among potential entomopatogen fungi is Bauveria bassiana (Artanti, et al., 2013) which has been researched for its efficience in controlling insects pests, see Faria et al. 2001; Alvez et al.2002Feng et al., 1994; Tuan et al., 2013 .

Bauveria bassiana possess broad range of hosts and possessing various strain (isolate), and are able to infesting the pests at any pests' lifestages and resulted in natural epizootic (Artanti, et al., 2013). *B. bassiana* has been prooved to be able to compete with chemical insecticides (Feng et al., 1994). This fungi is specially formulated in liquid medium which contains botanical extract and other additive materials which function is as pathogen for pest flies. It can infects the pest flies through direct contact with the pests. The fungi spores will grow and penetrate the skin of the flies, therefore this fungi is avery effective in controlling the plant pests (Driatmoko, 2004 in Santosa, 2011).

Based on the above explanation, the aim of this study is to examine the effect of concentration of B. Bassiana extract and the application frequency (regime) to the development of the soy plants pests.

MATERIALS AND METHODS

Penelitian ini dilaksanakan di Desa Gampong Jawa, Kecamatan Langsa Kota, Kota Langsa, pada bulan Maret 2015 sampai dengan September 2015 pada ketinggian tempat 3,4 m dari permukaan laut dan pH tanah 6 (BPP Kecamatan Langsa Kota, Kota Langsa, 2015).

Study site

This study was conducted in Gampong Jawa Village, Langsa Lama Subdistrict, Kota Langsa. The expeririments was carried out from March until September 2015, at the elevation 3,4 above sea level andground pH was 6 (BPP Kecamatan Langsa Kota, Kota langsa).

Materials

Materials used in this study were soy seed from Anjasmoro variety, B. Bassiana which are obtained from the Universitas Sumatera Utara laboratorium. Water, compost fertilizer, urea fertilizer, TSP, KCl, paint, plastic strap, water, treatment board and plotting board.

Methodology

The research used factorial random blocked design with three levels of B. Bassiana extract concentration (12,5; 22,5 and 32,5 ml B. Bassiana extract for every liter of water) and 1 level as control. The application frequency area every week, every two weeks, every three weeks, and no application as controls. Therefore, there are 16 combinations of treatment during the experiment.

The combination is describe by Table 1.

Table 1. Combination of treatment for the experiment

No	Treatment combinations	Concentration	Spraying frequency
1	$K_0 W_0$	Control	Control
2	$K_0 W_1$	Control	every 1 week
3	$K_0 W_2$	Control	every 2 week
4	$K_0 W_3$	Control	every 3 week
5	$K_1 W_0$	12,5 ml/liter water	Control
6	$K_1 W_1$	12,5 ml/liter water	every 1 week
7	$K_1 W_2$	12,5 ml/liter water	every 2 week
8	$K_1 W_3$	12,5 ml/liter water	every 3 week
9	$K_2 W_0$	22,5 ml/liter water	Control
10	$K_2 W_1$	22,5 ml/liter water	every 1 week
11	$K_2 W_2$	22,5 ml/liter water	every 2 week
12	$K_2 W_3$	22,5 ml/liter water	every 3 week
13	$K_3 W_0$	32,5 ml/liter water	Control
14	$K_3 W_1$	32,5 ml/liter water	every1 week
15	$K_3 W_2$	32,5 ml/liter water	every2 week
16	$K_3 W_3$	32,5 ml/liter water	every 3 week

The model used in this experiment is:

$$\mathbf{Y_{ijk} = \mu + \beta_i + K_j + W_k + (KW)_{jk} + \in_{ijk}}$$

Where:

$\mathbf{Y_{ijk}}$ = the result of observation from concentration (K) at the jth level and application interval (W) at the ith replication

$\boldsymbol{\mu}$ = Mean

$\boldsymbol{\beta}_i$ = effect of ith replication (i= 1 and 2)

$\mathbf{K_j}$ = Effect of concentarion (K) at the jth level (j=1,2 3)

$\mathbf{W_k}$ = effect of application interval (W) at the kth level (k=1,2,3 and 4)

$\mathbf{(KW)_{jk}}$ = affect of interaction pf concentration (K) at the jth level and interval (W) at the kth level.

$\boldsymbol{\epsilon_{ijk}}$ = Effect of the error of concentration K at jth leveland interval application W at kth level.

Soyplants Preparation

The preparation of soyplants includes land preparation, seedings, fertilizing, weed clearings, and harvesting. Preparing the land consisted of land clearing, loosing, and creating 60 x 60 cm plots and distance among plots were 40cm whilst distance among

block were 50 cm. The next step in land preparation was to fertilize each plot. Seedings were done by soaking the seeds for 15 minutes and then left dried for 12 hours before seeded. Three seeds were planted in each hole with the distance 40 x 20 cm, thus each plats have six holes. In total, there are 192 seedlings expected to grow. Furthermore, the plants were fertilized using urea.

Bauveria basiana Preparation

Liquid Bauveria basiana was calibrated before used by spraying water to the plots . The result from innitial calibration was 2l of water/plot. Therefore the concentration of B. Basiana were Ko = Control, K1 = 2,5 ml/l of water , K2 = 45 ml/ml of water and K3 = 65 ml/l of water. B. Basiana extract were sprayed to the plots after 10 days, and then continued as per plan taking the intervals as W0, W1, W2, W3.

The plants were regularly watered as well as clearing the weeds which was done manually. The plants growing abnormally or die were stiched or replaced with other plants before observation period, aproximately seven days after seeding.

Harvesting took place after 75 days when plants has shown sign of seed reaped such as yellowish leaves which was easy to fall and the color of the pods became brownish. Harvesting was done manually using sickle.

Observation

Each plants was then observed for pest population. The observation was carried out when the plants were at age 40, 50, 60 and 70 days. Pest outbreak intensity was then calculated as:

1. Absolute outbreak intensity

$$I = \frac{a}{a+b} 100\%$$

Where:

I = Outbreak intensity (%)

a = The number of pods whith absolute damaged, or considered absolute damaged

b = The number of pods free from pests

2. Inabsolute Outbreak Intensity

$$I = \frac{\sum(nxv)}{Z \text{ x } N} x100\%$$

Where:

I = damaged intensity;

n = the number of plants for each category of attack;

v = The scale value for each attack category;

Z = The scale value of high attack category;

N = The number of dead clumps

3. Pods Damage Percentage

The percentage of damaged soybean pods was calculted as:

$P = \frac{n}{N} x100\%$ (Natawigena, 1993 *dalam* Khasanah, 2008) :

P = Percentage of damaged pods

n = the number of damaged pods

N = the number of observed pods

4. Pest Mortality

Pest mortality was assessed by examining the number of pests those are dead on the 40^{th}, 50^{th}, 60^{th} and 70^{th} day after seeding.

RESULT AND DISCUSSION

Four major pests found during the experiment were (*Spodoptera litura* F.), kepik hijau (*Nezara viridula* L.), kutu kebul (*Bemisia tabacci*) dan belalang (*Valanga* sp). Effect of Bauveria bassiana extract on the pests is given in Table 3.1

Table 3.1 ANOVA table for the effect of B. Bassiana on soyplant pests

Source of Variation	df	SS	MS	F_0	F table	
					0,05	0,01
Kelompok	1	0,78				
K	3	11,30	3,77	144,01**	3,29	5,42
W	3	7,10	2,37	90,53**	3,29	5,42
KxW	9	0,42	0,05	1,79tn	2,59	3,89
Galat	15	0,39	0,03			
Total	31	19,99				

KK= 1,79 %

** : significant

tn : insignificant

As seen in Table 3.1 that Bauveria bassiana concentrations significantly affect the population of the soyplan t, and so with the application frequency.

Pests Mortality

ANOVA result shows that he concentration of Bauveria basiana significanty affect the population of *S. litura* at the age of 50 and 60 HST, *B. tabaci* at the age of 0 and 70 HST, *Nezara viridula* at theage o 50 and 60 and *Valanga sp.* at the age of 50. However, it does not signifcantly affect the population of *S. litura* at the age of 40 HST, and no *B. tabaci* was found at the age of 70 HST, *N. viridula* at the age of 40 and 50 HST, and *Valanga. sp*. at the age of 40 and 70 HST whilst Belalang at the age of 40, 60 and 70 HST. This results were different from the results obtained by Alvez et al., 2002 where they found that conidia and yeast of B. bassiana have been effective in killing L_{50} of insects approximately from the day three or the day seven of the application.

The post hoc least signficant different test shows that the highest intensity of pests outbreak occured at Ko, the control block, and significantly different from other blocks. The lowest intensity of pests outbrak were occured at lock with Bauveria bassiana concentration at 32,5 ml/ liter of water.

As mentioned above, the concentration of B. bassiana significanly afffect pests population. The least sgnificance different shows that average mortality for Ulat grayak occured at the age of 60 HST. Kepik hijau at the age of 60 and 70 HST. Kutu kebulat the age of % HST and Belalang a 5 HST. The highest results given by the block which are applied wih 32,5 ml/Liter of water. The highr the concentration of B. Bassiana, then the higher themortality rate, as explained by David et al. (2014).

From the least significant different test result at 0,05 error type 1, the average of Spodoptera litura occured at the age of 60 HST, Bemissia tabacci at 60 to 70 HST, Nezara viridulla at 50 HST and Valanga sp at 50 HST at the block using the highest frequency of application regime, that is once a week. It was suggested the more often B. Bassiana applied to the plants, the more lethal it is to the pests due its effect on damaging the whole tissue of the pests. Prayogo et al., (2005) said that B. Bassiana application must be given more than once if the pests possessing life cycles consists of several instar stadia to antipate environmental factor that would lead to low success result.

Pod Defect Pecentage

ANOVA results shows the the application regime affect the percentage of pod defect signficantly. The average of pod defec based on the application regime can be seen in Table 2.

Table 2. The average of pod defects based n the applcation regime

Application requency	Pod defect percentage
W_0	22,21d
W_1	17,05 a
W_2	19,30 b
W_3	20,69 c

BNT 0,05	1,31

Keterangan : Angka yang diikuti huruf yang sama pada kolom yang sama berbeda tidak nyata pada uji BNT 0,05

Table 2 suggested that the highest average of pod defects because of B. Bassiana application was obtained by treatment W_0 followed by W_3 and W_2 then lastly W_1. The least significant different test showed the the highest percentage of W0 treatment was higher and significantly different from all other treatments. Meanwhile the treatment with the lowest pod defects was obtained at the W1 treatment. This result adviced that the once a week application of B. Bassiana sp is more effective compared to other regime, which means that the more often B. Bassiana applied to the plants, the percentage of bean defects would be lower and thus, increases production. Riyanti et al., (2013) mentioned that, the higher the frequency of B. Bassiana application, the more effective it is to countrol the pests population.

Interaction between concentration of Beauveria bassiana and application regime

The percentage of Pod defects

ANOVA results for the interaction of concentration and applictaion regimw shows that Bauveria bassiana have significant effect on the bean pod defects. The percentage of bean defects because of the interaction of the both factors can be seen in Table 3.

Tabel 3. The average of bean defects because of the inteaction between concentration and Application regime.

Treatment combination	Bean defect percentation
$K_0 W_0$	25,00 h
$K_0 W_1$	22,72 fgh
$K_0 W_2$	22,93 fgh
$K_0 W_3$	23,74 gh
$K_1 W_0$	22,90 fgh
$K_1 W_1$	18,79 cd
$K_1 W_2$	19,56 cde
$K_1 W_3$	22,07 efg
$K_2 W_0$	20,65 def
$K_2 W_1$	17,34 bc
$K_2 W_2$	18,56 bcd
$K_2 W_3$	19,70 cde
$K_3 W_0$	20,31 def
$K_3 W_1$	9,38 a
$K_3 W_2$	16,15 b
$K_3 W_3$	17,27 bc
BNT 0,05	2,62

Note : The number followed by the same letter in the same row does not significantly effect the result at alfa 0,05 from the least significant different test.

From the least significant difference test, showed that the highest percentage of pod defect because of the interaction between concentration and application regime was found to the treatment K_0W_0 (control site). Which not significantly different with K_0W_1, K_0W_2, K_0W_3 and K_1W_0. However, it significantly different to treatment combination of K_1W_3, K_1W_2, K_1W_1, K_2W_0, K_2W_3, K_2W_2, K_2W_1, K_3W_0, K_3W_3, K_3W_2 dan K_3W_1. This results due to the tratment of K_0W_0 was the tratment without B. Bassiana suspension so that the pests mortality is low and even zero, which in turns results in the the percentage of defects became higher. The lowest pod defects percentage was occured at K_3W_1 (concentration 32,5 ml/liter of water with once a week application). Which means that it is the most effective combination for pest control. This is because K_3W_1 was the application that allows the highest condia density and the highest frequency of application regime. As it was suggested by Riyanti et al., (2013) that the entomopathogen B. Bassiana application frequency affcet the intensity of pest outbreaks . 3 times application in a week resulted in 22,29% of attack. Once a week application would allow 32,29 % attacks and twice a week application would result in 27,8%. This is due to the findings that the more often B. Bassiana were applied, the lower the intensity of pest outbreak.

CONCLUSION

1. B. Bassiana application to the soyplants significantly affect the population of S. Littura, B. Bacci, Nezara viridulla and Valanga sp, and it also affect the pod defects of the plants. The

highest concentration 32,5 ml/liter of water is the most effective in controlling pests population.

2. The application regime significantly affect the pests population where the highest frequency (once a week) application showing the best results as it was shown by the precentage of pod defects.
3. The combination of both factors also affect the population of the pest significantly, where the best results in controlling the pest was demonstrated by the block K_1W_3, that is once a week application with 32,5 ml/liter of water concentration.

Recommendations

As demonstrated by the experiments, the pest control using Beauveria bassiana extract must be given with 32,5 ml/liter of water concentration and must be given once a week.

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