

Potency Of *Beauveria Bassiana* And Sex Pheromone In Controlling *Spodoptera Exigua* (Lepidoptera: Noctuidae), An Important Pest Of Onion

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Abstract: The use of insecticide is a common practice to control armyworms (*Spodoptera exigua*) an important pest of onion, but the result is less effective due to the habit of this pest that lives inside the leaf. Therefore, it is necessary to find an alternative way of controlling it by using Sex Pheromone in combination with *Beauveria bassiana*, the natural enemy of this pest. The objective of this research is to investigate the potency of combining sex pheromone and *B. Bassiana* in controlling *Spodoptera exigua* on onion. The experiment was arranged according to a Split Plot design with the main plot is sex pheromone (with or without sex pheromone) and the subplot is the concentration of *B. bassiana* (at the rate of 0, 2, 4, 6, or 8 g/L). The experiments were conducted in the field and each combination treatments were repeated 4 times. The results showed that the development of pest attack intensity as shown by their Area Under the Disease Progress Curve (AUDPC) value was significantly higher when sex pheromone is applied compared to those without sex pheromone. Meanwhile, the rate of *B. bassiana* used influenced the pest attack intensity in which the higher the rate of the *B. bassiana* applied the lowest the pest attack intensity. However, *B. bassiana* at the rate of 4 g/L was not significantly different from the higher rate. Therefore, we concluded that *B. bassiana* at the rate of 4 g/L in combination with sex pheromone could suppress the intensity of *S. exigua*.

Keywords: *Beauveria bassiana*, Onion, Pheromone, *Spodoptera exigua*

I. Background

Onion is one of the most important horticulture commodity in West Nusa Tenggara (WNT) province. Most farmers in WNT grow onion after harvesting rice. However, the yield and productivity of onion in WNT, especially in Lombok Island is not optimum. It is caused by many factors such as climate change, pest and disease attacks. One of the main pests on onion is armyworm (*S. exigua*). The armyworm attack can decrease the yield by up to 20 – 57% (Dinas Pertanian Provinsi NTB, 2014; Sastrasiswojo, 1992). In the last 4 years, the attack of *S. exigua* has been fluctuating with attacking as large as 452.02 hectare/year (BPTPH, 2013 *Unpublished data*). This pest feeds on the leaves and causes loss in yield because the larva from the first instar to the end instar can destroy the leaves (Wulansari, 1996).

The existence of *S. exigua* on the onion is caused by the farmers who practice cultivation unwisely. According to Tarmizi (2008), the use of pesticide has been causing instability of habitat that stimulates the development of *S. exigua*. The use of pesticide is generally caused by the wrong understanding on how to control the pests. Pests are considered as organisms that bring disadvantage economically so that it has to be destroyed. The partial understanding on the pest from the economic point of view is wrong, rather the pest is an ecologic component that needs to be considered, therefore it is necessary to control the pests ecologically in order to keep the balance of ecosystem in the cropping areas.

B. bassiana is an entomopathogenic fungus that is widely used to control insect pests and becomes the most developed species as biopesticide (Butt *et al.*, 2001; Zimmerman, 2007). According to Nasir (2004), the application of *B. bassiana* on onion twice a week is effective to suppress the attack of *S. exigua* on onion. In addition, the fungus applied at the rate of 10×10^5 conidia/ml can kill *S. exigua* in its first instar by 73% (Wafa, 2004).

Pheromone is a substance that is released by an insect and able to affect the same kind of insect by giving particular physiological response. On armyworm, sex pheromone is produced by female insect especially at night to attract the male insect. Sintetic pheromone is commonly used as male insect trap (Yahya, 2004). A research by Nurawan and Haryati (2011) showed that sex pheromone was effective to control armyworm on onion and trapped around 41 to 83 insects per trap per week. It could reduce the number of armyworm and reduce the percentage of attack of armyworm on the plant. Based on the ability of *B. bassiana* and sex pheromone in suppressing the attack of *S. exigua*, an experiment was conducted on the potency of *B. bassiana* and sex pheromone in controlling the attack of *S. exigua* on onion.

II. Materials And Methods

This experiment was conducted in Denggen Village, East Lombok and Laboratory of Microbiology, Faculty of Agriculture, Mataram University on November 2015 to March 2016. The plot used in this research was 2500 m². Raised beds were made on the plots, each bed was 5x1 m² wide and separated by 0.4 meter wide drainage canal. There were 40 beds used in this experiment. The variety of Onion used in this experiment was Philipine variety with the planting space of 20x20 centimeters so that in one raised bed there were 125 onion plants. Fifteen plants were taken as sample for each raised bed. The treatment for sex pheromone was placed in modified glass container. The strainer was filled by soap water around ± 3 cms from the bottom of the container. There were 3 traps placed around 15-20 cms above the onion plant using bamboo stands and set on the 1st week after planting. *B. Bassiana* application was diluted in water according to the dose for each treatment. The method used in this experiment was split plot design. The main plot was Pheromone with 2 treatments, i.e. : F0 (without pheromone), and F1 (with pheromone); and the sub plot was the rate of *B. bassiana* application, consisted of 5 treatments i.e.: B1 (without *B. bassiana*), B2 (*B. bassiana* at the rate 2 gr/l), B3 (*B. bassiana* at the rate 4 gr/l), B4 (*B. bassiana* the rate 6 gr/l) and B5 (*B. bassiana* at the rate 8 gr/l). Treatment is combination of pheromone and *B. bassiana* in certain rates. There were 4 replications for each treatment so that there were 40 experimental units.

Observation Parameters

Spodoptera exigua Population

Observation of *S. exigua* population was conducted by observing the number of affected foliages by *S. exigua* with the assumption that there was one pest insect population in one affected foliage. Observation was conducted six times when the plants were at the age of 10, 18, 26, 34, 42, and 50 days after planting. The rate of *S. exigua* population development was calculated to discover how fast the population grow.

Attack Intensity of *Spodoptera exigua*

Observation of attack intensity was conducted when the plants were at the age of 10, 18, 26, 34, 42, and 50 days after planting by observing the symptom on the leaves. Attack intensity was calculate using absolute attack intensity formula (Ditlintan, 2008):

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

I : attack intensity (%)

a : numbers of affected foliage

b : numbers of unaffected foliage

Area Under Disease Progress Curve (AUDPC) was used to compare the development of attack intensity of pest that was observed continually in 8 days interval. AUDPC formula is shown below (Campbell and Madden, 1990) :

$$AUDPC = \sum_{i=1}^{n-1} \left(\frac{X_i + X_{i+1}}{2} \right) (t_{i+1} - t_i)$$

Where:

AUDPC : Area Under the Disease Progress Curve

X_i : Percentage of affected foliage on the (i) day of observation

X_{i+1} : Percentage of affected foliage on the (i+1) day of observation

t_i : Time of observation at the itime

t_{i+1} : Time of observation at the i+1time

n : Number of observation

Data Analysis

Observation data from each treatment combination was analyzed by ANOVA with 5% significance level and means test with HSD at the same significance level.

III. Result

Sex pheromone treatment did not show any significant effect on pest population, but application of various rates of *B. bassiana* gave significant result. AUDPC calculation on development of attack intensity of *S. exigua* on onion showed that there was non significant interaction (p>0.01) between pheromone and various rates of *B. bassiana*. However, there was significant effect were observed on each factor to the attack intensity parameter (Table 1).

Table1. Analysis of Population and Attack Intensity of *S. exigua* on Onion

Source	Parameter	
	Pest Population	Attack Intensity
Main Plot		
Replication	0.22 NS	0.99 NS
Pheromone	0.18 NS	0.01 *
Sub Plot		
Rates of <i>B. bassiana</i>	0.004 ***	0.01 *
Pheromone* <i>B. bassiana</i>	0.15 NS	0.24 NS

Note : NS (Non Significant), * Significant

Population of *S. exigua*

The development of population of *S. exigua* in one living cycle of onion is shown on Picture 1 below:

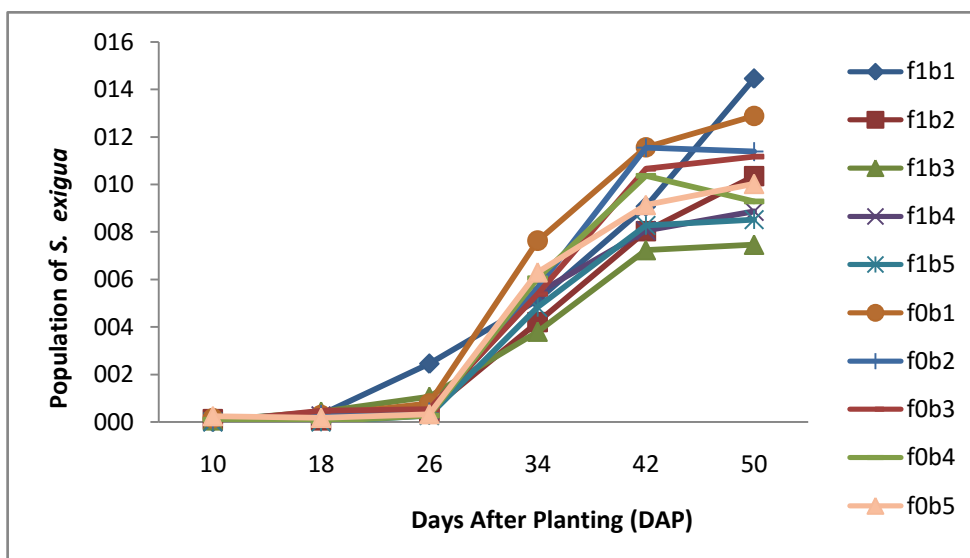


Figure 1. Population of *S. exigua*

Figure 1 shows the development of population number of *S. exigua* in the treatments used in this experiment. On 10 DAP to 26 DAP, the number of population of the pest in all treatments were approximately at the same numbers. Meanwhile, the significant increase of population of the pest is shown on 26 DAP to 50 DAP where Treatment F1B1 shows higher number pest population than other treatments. FIB3 Treatment shows the least pest population. Table 2 shows significant difference on numbers of population and the highest number of population among the treatments.

Table 2. Analysis of Rate of Population Development of *S. exigua* in one living cycle of Onion

Treatment	Rate of Population Development of <i>S. exigua</i>
F1	2.19 a
F0	2.59a
HSD	0.54
B1	2.94 a
B2	2.48 ab
B3	2.16 b
B4	2.21 b
B5	2.18 b
HSD	0.62

Note : Numbers followed by the same letter are non significant on HSD Means Test with 5% of significance level

Pheromone treatments shows non significant result on Rate of Population Development of *S. exigua* on onion. Meanwhile, Application of *B. bassiana* shows significant effect for each treatment. Treatment B1 and B2 did not any significant effect but treatment B3, B4 and B5 shown significant lower rate of population development (Table 2).

Development of Attack Intensity of *S. exigua*

Development of Attack Intensity of *S. exigua* in one cycle of onion increased continually from 10 DAP to 42 DAP. On 50 DAP, the attack intensity started to decrease and stabilized for several treatments excluding F1B1 and F1B2 that kept increasing. The highest attack intensity rate was discovered in F0B1 as shown in Figure 2.

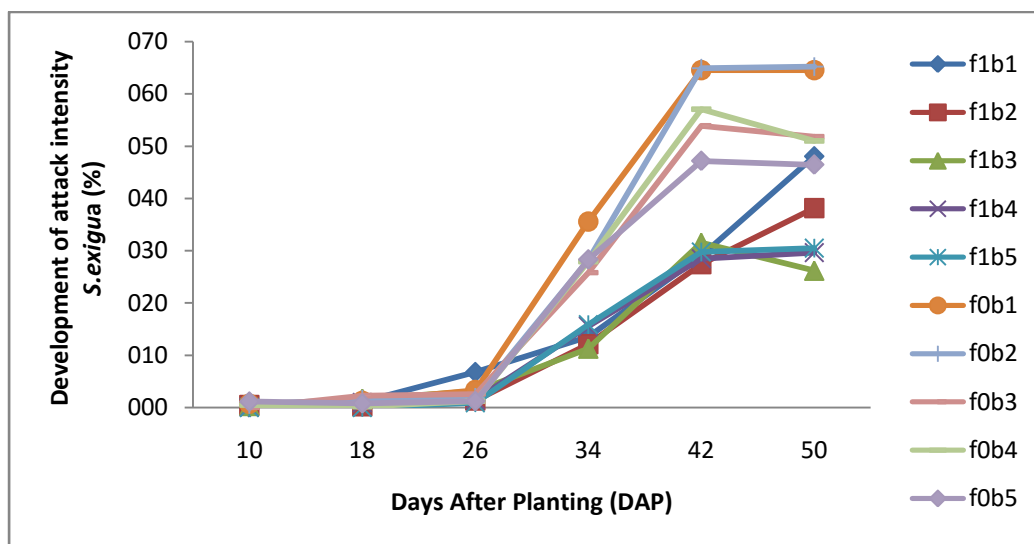


Figure 2.Development of Attack Intensity of *S. exigua*

The fastest development of attack intensity is shown on AUDPC table below Table 3. Analysis of Development of Attack Intensity of *Spodoptera exigua* calculated by AUDPC

Treatments	Development of Attack Intensity of <i>Spodopteraexigua</i>
F1	509.41 a
F0	944.41 b
HSD	302.64
B1	847.03 a
B2	758.77 ab
B3	684.22 b
B4	692.92 ab
B5	651.72 b
HSD	161.17

Note : Numbers followed by the same letter are non significant on HSD Means Test with 5% of significance level

Data in Table 3 showed that attack intensity of *S. exigua* in pheromone treatment is less than without pheromone. The development of attack intensity of *S. exigua* on pheromone treatment was 509.41 and without pheromone was 944.41. According to AUDPC calculation, pheromone treatment could suppress AUDPC value by 394.95. Application of various rates of *B. bassiana* showed that the least attack intensity was discovered on B5 at the rate 8 g/l as shown by its lowest AUDPC value of 651.72, followed by B3 at the rate 4 g/l AUDPC value of 684,22 and the highest was B1 at the rate 0 g/l with AUDPC value of 847.04.

IV. Discussion

Attack of *S. exigua* is found nearly at every growing phase of onion. Group of eggs can be found on the early growing phase of onion. The population of pest kept increasing at 3 weeks after planting and reached the highest population on week 4 to 6 after planting. Sutarya (1996) described that the population of *S. exigua* increases on week 2 after planting and would reach the highest number of population on week 4 and 5 after planting. The increase of pest population is linear with the increase of attack intensity. It is related with the development of *S. exigua* where on the first week the pest laid the eggs and the second week the eggs would hatch and became larvae of instar 1 and 2. The third week observation showed that larva *S. exigua* had reached instar 3 according to some characteristics, i.e. : the body of larva was brownish green and the larva feed on the

foliages fastly. According to Haryaksono (1989), vegetative phase is the growing phase where *S. exigua* attacks intensively. The newly hatched larva (instar 1) feeds on the outer side of foliage, makes a hole inside and then stays there until it reaches instar 3 phase. When the larva has reached instar 4 phase, the larva would feed on the foliages. The damaged foliages affect the productivity of onion plants. It is called as Critical Period because every leaf contains chlorophyll for photosynthesis.

The treatments with and without pheromones showed non significant difference on the rate of pest populations. However, the treatments affected in suppressing the attack intensity. The pheromone attracted the male insects migrated to the plots where pheromone was placed. There was still possibility of mating occurrence with female insects outside the trap. That made the populations of *S.exigua* showed non significant difference between both treatments. Beside that, there were spaces filled by tobacco, grasses and egg plants around the experimental plot that acted as hosts and food sources of the mature pest of *S. exigua*. It caused the increase of population of *S. exigua* shown by the number of trapped pests inside the pheromone-filled glass container since 10 DAP to 50 DAP with average population of 17 pests. French (1969), Southwood (1972) , and Mitchell (1981) described that beside the food source in abundance, the other main factor related to pest population on seasonal plants was pest migration into the inner part of planting area. The migration of *S. exigua* to the planting plot caused sincronization of developments of eggs population followed by the population of larva. However this is in contrast with the study by Nurawan and Haryati (2011), showed that sex pheromone was effective in controlling armyworms and trapped 41 to 83 pests per trap per week, so it can suppressing the pest population and attack percentage on plants.

Treatments of various rates of *B. Bassiana* showed significant result on population and attack intensity. It was caused by the difference of concentration of conidia applied on the treatments. The higher concentration of conidia, the higher mortality of larva would be and it decreased attack intensity. However, treatment B2, B3, B4, and B5 showed non significant difference. It was assessed by the failure of conidia emergence in unsuitable micro climate of the experiment. The temperature when the experiment was conducted was 35°C from 0 to 32 DAP. Meanwhile on 33 to 55 DAP, the temperature in experiment plot was 27°C where it rained during that period. According to Junianto and Sukamto (1995), conidia emergence needs relative humidity above 90% and optimum temperature around 20°C to 30° C. Trizelia (2005) added that conidia of *B. bassiana* on the leaves that were exposed to direct sunlight would lose its viability and virulence by 50-100% in 24 hours and several days. Beside that, the amount of rain was very potential in the loss of conidia from the leaf surface (Ferron *et. al.*, 1991)

Data showed that treatment with pheromone could suppress AUDPC value by 394.95. It was presumed that the amount of pheromone would interfere mating process due to the trapping of male insects. Winoto (2009) described that female butterflies would spread pheromone when they flapped their wings. That was how the pheromone could be spreaded in the air and attracted the male insects. Sex pheromone has specific characteristic for reproductive activity where male or female from one species are not attracted and do not respond to the pheromone released by other species. The use of pheromoid (sintetic pheromone) is not only to control the population of pest but also to disrupt the mating. By disrupting the mating process of pest, there will not be many eggs that can successfully hatch so the population is suppressed. Allison and Carde (2007) explained that generally mating process in insects is influenced by sex pheromone produced by the female insects to attract male insects.

Application of various doses of *B. bassiana* showed that treatment B5 at the rate 8 g/l had the least development of attack intensity with the AUDPC value of 651.72, followed by B3 at the rate 4 g/l with the value 684,22. The highest attack intensity was discovered on B1 at the rate 0 g/l with the AUDPC value of 847.04. Several studies showed that virulency of *Beauveria* spp. to the host insect varied. The different of virulency is influenced by several factors i.e.: environment and different strains of *Beauveria* spp. Prayogo (2006) explained that to maintain the effectivity of *B. bassiana* and to increase success rate in controlling the pest in the field, the application of *B. bassiana* should be done in the evening as well as rising the frequency of application. The low virulency of *B. bassiana* caused the attack intensity if armyworm is increasing.

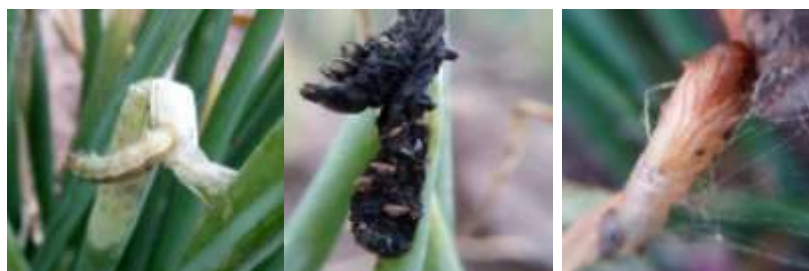


Figure 3. *S. exigua* were infected with *B.bassiana*

V. Conclusion

Based on the data analysis and discussion, there are three points that can be concluded, i.e. :

1. Sex pheromone could suppress the attack intensity of *Spodopteraexigua* on onion plants.
2. *Beauvariabassiana* at the rate 4 g/l could suppress attack intensity of *Spodopteraexigua* on onion plants.
3. There was not interaction between pheromone and various rate of *Beauveriabassiana* in suppressing the attack intensity of *Spodopteraexigua* on onion plants.

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