Effects of the entomopathogen concentration of Beauveria Bassiana [Bals] Vuil for biological control of Helicoverpa armigera Hubner [Lepidoptera : Noctuidae]

N Nelly¹, R Reflin¹, and Y A Hidayat¹
¹Department of Plant Protection, Agriculture Faculty, University of Andalas. Padang. West Sumatera, Indonesia.

E-mail: novrinelly@yahoo.com

Abstract. Beauveria bassiana is an entomopathogenic fungus found in the rhizosphere, known to affect the mortality of H. Armigera. The research was conducted at the Laboratory of Biological Control, Department of Plant Protection, Faculty of Agriculture, Andalas University, from January 2019 to March 2019. The aim of this study, therefore, is to obtain an effective concentration with the optimal characteristics of controlling H. armigera, utilizing a completely randomized design, with 4 treatments and 5 replications. In addition, the treatment required the application of B. bassiana suspension, at a concentration of [A] 5x10⁷ conidia/ml, [B] 1x10⁸ conidia/ml, [C] 5x10⁸ conidia/ml and [D] control [distilled water]. The results showed the treatment with the highest concentration [5x10⁸ conidia/ml] possessed the ability to increase the mortality rate by 68% in the larvae of H. armigera, at an LT₅₀ of 5.82 days. Hence, a higher concentration affects the lifespan, and nature of pupae, and adult pests formed.

1. Introduction
The corn cob borer, Helicoverpa armigera [Lepidoptera: Noctuidae], is a major pest known to attack corn plants, which normally occurs at the age of 45-65 days, along with the appearance of cob hairs, leading to a reduction in the quantity and quality of yield. The Directorate-General for Food Crops, Ministry of Agriculture established that potential loss in yields, due to attack by caterpillars reduced production by 40%, and similar events have been identified in plantations within Indonesia [1].

Efforts have been made by farmers to control H. armigera by using chemicals. However, such control is identified as unfriendly to the environment. Moreover, synthetic varieties have been speculated to initiate the occurrence of numerous negative impacts, encompassing environmental pollution by chemical residues, pest resistance, and blasting. Hence, the need for alternative control measures, including the conduction of biological approaches [2].

The biological control method requires entomopathogenic fungus, e.g. Beauveria bassiana fungus [3], of the Lepidoptera order. These possess the ability to infect insect pests, by penetrating the host cuticle and causing diseases, especially where there is direct contact between the fungal conidia.

B. bassiana fungus is a biological agent, which has been proven to possess the capacity to control various types of plant pests. Furthermore, it does not cause any form of phytotoxins [poisoning] in plants, as well as pollution [4], and the broad range of host affected has prompted its wide adoption in pest insect control [5]. This fungus has great potential as a biological pest control agent, and it is also an important component in an integrated system [6].
A study showed the possibility of applying *B. bassiana* as a biological control agent, especially against insects in the order Lepidoptera, which encompasses the larvae borers *Helicoverpa armigera*. Conversely, applying this treatment at a concentration of 2.5x10^5 conidia/ml has been proven to cause mortality of about 36-48% on the 7th day after treatment [7]. Suharto *et al* [8] also reported the high propensity of directly using a density of 1x10^6 conidia/ml on *H. armigera* larvae, to demonstrate a mortality rate of 52%, within 14 days.

Study indicating the effective concentration of *Beauveria bassiana* adopted in the control of *H. armigera* has not been identified. Therefore, such investigation is necessary. This study aims to obtain an effective concentration with the optimal characteristics of controlling *H. armigera*.

### 2. Materials and methods

The research was conducted from January to March 2019, at the Insect-Biology and the Biological Control Laboratories, Faculty of Agriculture, Andalas University, Padang, West Sumatra.

#### 2.1. Material and tools

The materials used including corn cobs and leaves, the larvae of *H. armigera*, and the pure isolates of *Beauveria bassiana* were isolated from corn root soil, at the Biological Control Laboratory. Also, *Saubouraud Dextrosa Agar Yeast* [SDAY] aqua dest, 70% alcohol, tween 80, honey, labels, filter papers, wrappings, tissue, aluminum foil were provided.

Equipment and digital scales used including electric stoves, knives, ovens, Erlenmeyer, spatulas, binocular microscopes, tweezers, glass petri dishes, plastic cups [6 cm diameter and 7 cm high], drop pipettes, bunsen lamps, *autoclaves*, *laminary air flow* [LAF], *cork borrer* [diameter 0.8 cm], *object glass*, *cover glass*, *haemocytometer* [Neubauer Improved], plastic box [20 cm x 14 cm x 10 cm] [19 cm diameter and 16 cm high], small brush, hand spray bottle [size 15 ml], *scoot* bottles, scissors, pans, *vortex mixers*, micropipettes, documentation tools, and stationery.

#### 2.2. Design of study

This study adopted a Complete Random Design [CRD] consisting of 4 treatments and 5 replications. Each unit consisted of 12 larvae, placed in plastic cups.

The treatment is *B. bassiana* suspension involved the use of different concentrations, encompassing:

A. *B. bassiana* 5 x 10^7 conidia/ml.
B. *B. bassiana* 1 x 10^8 conidia/ml
C. *B. bassiana* 5 x 10^8 conidia/ml
D. Aquades [Control]

#### 2.3. The procurement of *Beauveria bassiana* entomopathogen isolate

*B. bassiana* fungus was collected from a corn planting area [rooting land] located in the Biological Control Laboratory, Faculty of Agriculture, Andalas University. The isolates were rejuvenated by the addition of fungus conidia using a *cork borrer* [d = 0.8 cm], transferred with the aid of a spatula to the *Saubouraud Dextrosa Agar Yeast* [SDAY] medium in a petri dish. This was subsequently incubated at room temperature for ± 21 days, and up to the point where the conidia in a petri dish were filled.

#### 2.4. Rearing of *H. armigera*

*H. armigera* larvae were obtained from the cobs and corn silk, in the corn planting areas of Java Gadut, Limau Manis Village office, Padang City, then placed into plastic cups, before the laboratory procedure. Furthermore, each larva was provided with a piece of corn [1.5 cm long] as feed, which was replaced daily. At the pupae stage, they were separated into plastic containers containing sawdust. Besides, the imago that emerged from the pupa was moved into a plastic maintenance box [20 cm x 14 cm x 10 cm], with its top lid covered with gauze. Moreover, feeding at this stage requires the provision of 10% honey, infused into cotton and placed on the top of the box lid. The environmental condition was maintained until the egg production phase.
The *H. armigera* eggs were transferred into a plastic container [height 19 cm x diameter 16 cm] before the hatching process into larvae, reserved up to the instar 3 [as a test subject], where they were fed with corn leaf. Furthermore, the categories to be treated were then placed in plastic cups, with the placement of 1 in each containment.

2.5. *The making of suspension of entomopathogenic B. Bassiana fungi*

Pure culture of *B. bassiana* placed on *Saubouraud Dextrosa Agar Yeast* [SDAY] media incubated for ± 21 days was provided, then 10 ml of distilled water and 2 drops of *Tween 80* were added. This was performed to release the conidia fungus from the media surface, with the assistance of a small brush. Furthermore, the spores of the fungus present in the suspension were sieved, using filter paper and placed into an Erlenmeyer; therefore, the homogenization process was initiated, employing a vortex for 20-30 seconds. Besides, a serial dilution of $10^{-1}$ and $10^{-3}$ was conducted, followed by calculating the conidia density, using a Haemocytometer, leading to the preparation of a suspension with a density of $5 \times 10^7$, $1 \times 10^8$, $5 \times 10^8$ conidia/ml.

2.6. *Application of B. bassiana entomopathogenic fungi in H. armigera larvae*

*B. bassiana* entomopathogenic fungus, suspended at a density of $5 \times 10^7$, $1 \times 10^8$, $5 \times 10^8$ conidia/ml, was applied to *H. armigera* stadia larvae instar 3. This was performed by direct spraying technique, which occurred 3 times [0.21 ml] for each, while the control required the use of sterile aqua dest.

2.7. *Larvae maintenance after application*

The treated larvae were fed with corn [1.5 cm long] up to the pupae stage, where it is then moved to the specific container containing sawdust until it becomes an imago. These were then separated and placed in a plastic maintenance container, where cotton soaked in a 10% honey solution was used as feed to promote egg production.

2.8. *Observation*

2.8.1. *Larvae mortality and LT$_{50}$ value*

The number of dead larvae in each treatment after applying *B. bassiana* were counted daily and observed for the subsequent 12 days. Therefore, the percentage of mortality was calculated using the following formula:

$$M = \frac{n}{N} \times 100\%$$

Information:

- M : Percentage of larvae mortality
- N : Number of dead larvae
- N : Number of larvae treated

The lethal time value for 50% [LT$_{50}$] *B. bassiana* concentrations applied to the *H. armigera* larvae was calculated based on the observed mortality rate, and then probit analysis was performed to determine the specific value.

2.8.2. *Pupae Formed [%]*

The number of pupae formed was counted daily and observed for complete development. Therefore, the percentage was calculated using the following formula:

$$P_p = \frac{P}{N} \times 100\%$$

Information:

- $P_p$ : Percentage of pupae formed
P : Number of pupae formed
N : Number of larvae treated

2.8.3. Adult formed [%]
The number of male and female imago formed in all treatments was determined and observed daily until all had attained complete development. Then, the percentage formation was calculated with the formula as follows:

\[ PI = \frac{I}{N} \times 100\% \]

Information:
P_i : Percentage of imago formed
I : Number of imago formed
N : Number of larvae treated

2.8.4. Live duration of adult
The determination of lifespan was conducted by calculating the time taken for the adult to emerge from the pupae

2.9. Data analysis
All data were evaluated using Analysis of Variance [ANOVA] and further tested with LSD at 5% level.

3. Results and discussions

3.1. Effect of B. bassiana concentration on the mortality of H. armigera larvae
Application of B. bassiana at different concentrations was observed to have affected the mortality rate of H. armigera larvae [P = 0.00], and the 5x10^8 conidia/ml was not significantly different from 1x10^8 conidia/ml, although there was significant dissimilarity with 5x10^7 conidia/ml. Furthermore, all treatments were experimentally distinct from the controls, and every increase in the concentration of B. bassiana provided also increased the occurrence of larvae mortality, and also accelerates the time of death. Hence, the highest rate was identified at 5x10^8 [68.0%], with a time of death [LT50] of 5.82 days [Table 1].

Table 1. Mortality of H. armigera larvae to which different B. bassiana concentrations were applied [12 days after application]

| B. bassiana Concentration | Larvae Mortality [%] ± SD | LT50 [days] |
|---------------------------|--------------------------|-------------|
| 5 x 10^8 conidia/ml       | 68.0 ± 10.86 a           | 5.82        |
| 1 x 10^8 conidia/ml       | 63.0 ± 13.94 ab          | 6.64        |
| 5 x 10^7 conidia/ml       | 48.2 ± 13.69 b           | 11.57       |
| Control                   | 0.0 ± 0.00 c             | -           |

The numbers attached with similar lowercase letters in the same column and rows are not significantly different according to the LSD 5% test.

The cumulative mortality rate of H. armigera larvae after applying B. bassiana suspension is seen in Figure 1, where the death was initiated on the first day of treatment, up to day 12. Furthermore, the observations also showed a positive correlation between the increase in B. bassiana concentration and the mortality rate.
Days after application

**Figure 1.** The mortality rate of *H. armigera* larvae after applying different concentrations of *B. bassiana*.

*H. armigera* larvae infected with *B. bassiana* showed abnormal external symptoms, and they also tend to die eventually, characterized by the hardening of the body, and the dead larvae were covered in white fungus hyphae [Figure 2].

**Figure 2.** Instar 3 of *H. armigera* larvae [a. Normal and b. infected Larvae *H. armigera*]

### 3.2. Effect of larva treatment on formed pupae and imago [%]

The application of *B. bassiana* to larvae caused a decline in the percentage of pupae and imago formed, which was prominent at higher concentrations. Therefore, a significant difference was also observed in the results obtained for each treatment [P = 0.00], although minimal disparity was identified between 5x10^7 and 1x10^8 conidia/ml. Furthermore, there was also a marked difference between the pupae and imago formed from the different concentrations of *B. bassiana* conidia in contrast with the control [Table 2].
Table 2. Percentage of pupae and adult formed after applying *B. bassiana* at varying concentrations

| Concentration       | Pupae formed [%] ± SD | Adult formed [%] ± SD |
|---------------------|-----------------------|-----------------------|
| Control             | 89.6 ± 6.97 a         | 79.6 ± 9.50 a         |
| 5x10⁷ conidia/ml    | 43.0 ± 12.35 b        | 36.2 ± 10.86 b        |
| 1x10⁸ conidia/ml    | 33.2 ± 10.20 bc       | 29.6 ± 9.51 bc        |
| 5x10⁸ conidia/ml    | 24.6 ± 10.18 c        | 21.4 ± 9.50 c         |

Numbers followed by the same lowercase letters in the same column and row are not significantly different according to the LSD 5% further test.

Furthermore, the application of *B. bassiana* was observed to initiate the formation of normal *H. armigera* pupae, although the abnormal types were also seen. Therefore, it was established that the differences in concentration administered affects the percentage of normal and abnormal [deformed] pupae formed [Table 3].

Table 3. The percentage of pupae formed normal and abnormal [defective] after applying *B. Bassiana*

| *B. bassiana* Concentration | Normal [%] ± SD | Abnormal [%] ± SD |
|-----------------------------|-----------------|-------------------|
| 5x10⁷ conidia/ml            | 34.4 ± 10.87 b  | 8.0 ± 5.89 a      |
| 1x10⁸ conidia/ml            | 26.4 ± 13.69 bc | 6.4 ± 6.97 ab     |
| 5x10⁸ conidia/ml            | 16.2 ± 10.20 c  | 8.0 ± 5.89 a      |
| Control                     | 89.6 ± 6.97 a   | 0.0 ± 0.0 b       |

The numbers followed by the same lowercase letters in the same column and row not significantly different according to the LSD 5% test.

The perfectly formed pupae displayed normal shapes, characterized by a shiny brown coloration, and a marked movement when touched. However, the abnormally formed types do not transit when they are subjected to the same stimuli, and they tend to occur as brownish-black coloration. A covering with an overgrowth of *B. bassiana* hypha fungus is seen on the pupa body part as shown in Figure 3.

**Figure 3.** *H. armigera* pupae [a. Normal and abnormal pupae]
The application of \textit{B. bassiana} caused most of the \textit{H. armigera} imago formed to remain normal, although an increase in concentration reduced the percentage value obtained. Meanwhile, the number of abnormal types was not significantly different amongst treatments, except the interaction between the 1x10⁸ conidia/ml concentration and the control [Table 4].

\textbf{Table 4.} Percentage of \textit{H. armigera} adult formed normal and abnormal [defective] after applying \textit{B. bassiana}

| \textit{B. Bassiana} concentration | Normal [%] | Abnormal [%] |
|----------------------------------|------------|--------------|
| 5x10⁷ conidia/ml                 | 32.8 ± 14.44 b | 6.4 ± 6.97 ab |
| 1x10⁸ conidia/ml                 | 21.4 ± 9.50 bc | 8.0 ± 5.89 a  |
| 5x10⁸ conidia/ml                 | 16.2 ± 10.20 c | 4.8 ± 4.56 ab |
| Control                          | 79.6 ± 9.50 a  | 0.0 ± 0 b     |

Numbers followed by the same lowercase letters in the same column and row are not significantly different according to 5% LSD test.

The normal-shaped adult was observed to exhibit a perfect shape and size [Figure 4], while the abnormal forms had imperfect development [defective], with damaged wings or legs.

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{figure4.png}
\caption{\textit{H. armigera} adult [a. Abnormal, b. normal]}
\end{figure}

3.3. \textit{Effect of treatment with B. bassiana on H. armigera adult life span}

The administered \textit{B. bassiana} tends to influence the longevity of adults, as the average life span of the control [no-treated larvae] was 9 days longer than those receiving treatment, which was limited to about 6.4-7.7 days [Table 5].

\textbf{Table 5.} The average life span of adults after treatment with \textit{B. Bassiana}

| \textit{B. bassiana} concentration | Life span of adult [days] ± SD |
|----------------------------------|-------------------------------|
| 5x10⁷ conidia/ml                 | 7.7 ± 0.54 ab                 |
| 1x10⁸ conidia/ml                 | 6.4 ± 1.42 b                  |
| 5x10⁸ conidia/ml                 | 6.4 ± 1.36 b                  |
| Control                          | 9.0 ± 0.21 a                  |

Numbers followed by the same lowercase letters in the same column and row are different according to the 5% of LSD test.
Table 5 shows the lifespan during the adult phase with $1 \times 10^8$ and $5 \times 10^8$ conidia/ml treatments, which was estimated to be 6.4 days. These values are not significantly different from one another. Conversely, the lifespan of the $5 \times 10^7$ conidia/ml concentration [9 days] did not vary significantly from the control [7.7 days].

The results obtained showed a significant positive relationship between the application of *B. bassiana* and larva mortality, thus, an elevation in the concentration administered resulted in higher percentage death. This event is assumed to occur because of the increase in the number of *B. bassiana* fungus conidia sticking to the body of the larvae. Sitompul and Lazuardi [9] reported that the success of infecting fungus against insects is determined by conidia density, because direct contact with the host tends to cause infection, thus, a higher attachment percentage leads to faster infection of the target host.

Entomopathogenic *B. bassiana* fungi did not cause a decline in the number of pupae formed, nor did it initiate the incidence of infection. Therefore, a higher concentration tends to decrease the percentage of formation, as Herlinda *et al.*, [3] reported that larvae treated with the *B. bassiana* die, while the live forms tend to develop into pupae, where some suffer damages in the form of imperfect organs [defective].

The infected pupae often die or fail to develop into adults, characterized by changes in body color to black on some surfaces, and fungal hyphae tend to appear in the affected areas. This event occurs due to the continued *beauvericin* toxin production process initiated by *B. bassiana* fungi, possessing the capability to damage the host's body tissue, and inhibit its metabolic processes, therefore, causing abnormal development. Rosmiati *et al.* [10] also explained the increased propensity of hypha growth in the body of an insect to release toxic active compounds for this purpose.

The application of *B. bassiana* affects the number of adults formed, in contrast with the control. Thus, a higher concentration reduces the percentage of development. Also, the quantity present in a control group was observed to be much higher than in the treatment, as seen in the successful development of pupa into imago, as well as the normal growth pattern. Meanwhile, physical disability and death were prominent in the treatment category.

The adult formed exhibited both normal and abnormal [deformed] development properties, as seen in Figure 4. This event is assumed to occur during the growth of *B. bassiana* hyphae in the host body, and the production of *beauvericin* toxin, which leads to paralysis, subsequently damaging tissues and organs. As reported by Sodiq and Dwi [11], these fungi possess the capacity to confer damage, due to the production of chitinase, lipase and proteinase enzymes, which tend to decompose components of the insect cuticle, causing death as a result of tissue and organ impairment.

It was also established that this application shortens the life span of *H. armigera* adult, based on its ability to deplete the nutrients required for development, thus, disrupting development. Saleh *et al.*[12] stated that a high propensity for the toxin produced by entomopathogenic fungi to directly damage the main functions of a hosts’ body, especially via hormone replacement, and the disruption of new skin formation processes, as a result of nutritional deficiencies. Therefore, the development does not ensure perfectly during the time where it is expected to turn into an adult, leading to a reduction in the capacity to survive longer. *B. bassiana* that was used previously was from the rhizosphere of corn [13]. It is thought that *B. bassiana* derived from corn rhizosphere can control *H. armigera* if it is made in the right concentration.

4. Conclusion

Based on the results and the discussion, it is concluded that treatments of *B. bassiana* at the highest concentration [$5 \times 10^9$ conidia/ml] cause mortality of about 68% in the *H. armigera* larvae, with $LT_{50}$ of 5.82 days. Also, a higher concentration negatively affected development based on lifespan, and the nature of pupae and adult form.
References

[1] Tuliabu R, J Pelealu, and MF Dien 2015 Populasi Hama Peenggerek Tongkol Jagung Helicoverpa armigera Di Kabupaten Bone Bolango Provinsi Gorontalo Jurnal Eugenia 21 1-5

[2] Adnan AM 2011 Manajemen Musuh Alami Utama Tanaman Jagung Seminar Nasional Serealia. Maros: Balai Penelitian Tanaman Serealia 388-405

[3] Herlinda S, Y Pujiaustuti, J Pelawi, A R尼亚ita, E Nurmayati and Suswandi 2005 Patogenesitas Isolat-Isolat Beauveria bassiana (Bals.)Vuill. Terhadap Plutella xylostella (L.) (Lepidoptera: Plutellidae) di rumah kaca Inovasi 2 85-92

[4] Wagiman 2013 Meningkatnya peran agen hayati dalam pengelolaan ekosistem secara kuantitatif. Jurusan Hama dan Penyakit Tumbuhan Yogyakarta- Fakultas Pertanian UGM.

[5] Reddy NP, APA Khan, KU Devi, SV John, and HC Sharma 2008 Assessment of the suitability of Tinopal as an enhancing adjuvant in formulations of the insect pathogenic fungus Beauveria bassiana (Bals.) Vuillemin J. Pest Management Science 3 121-132

[6] Pangestiningsih Y 2011 Uji Efektifitas Beberapa Jamur Entomopatogen dan Insektisida Botani terhadap Spodoptera exigua Hubn. pada Tanaman Bawang Merah (Allium ascalonicum L) Jurnal Ilmu Pertanian Kultivar 5 (2) 83-87

[7] Indrayani IGAA, D Soetopo, J Hartono 2013 Efektivitas formula jamur entomopatogen beuveria bassiana dalam pengendalian Helicoverpa armigera Jurnal Littri 19 178-85

[8] Suharto, BD Endang, and P Hari 199. Kajian Aspek Fisiologik Beauveria Bassiana dan Virulensinya Terhadap Helicoverpa armigera Jurnal perlindungan Tanaman Indonesia 4 120-119

[9] Sitompul UC and Lazuardi 2014 Pengaruh Jamur Beauveria bassiana Sebagai Pengendalian Hati Terhadap Mortalitas Hama Ulat Katung (Metisa plana Walker) Seminar Nasional Biologi dan Pemelajarannya, 23 Agustus 2014

[10] Rosmiati A, H Cecep, F Efrin, and S Yati 2018 Potensi Beauveria cassiana Sebagai Agen Hayati Spodoptera litura Fabr Pada tanaman kedelai Jurnal Agrikultura 29 (1) 43-47

[11] Sodiq M and M Dwi 2009 Pengaruh Beauveria bassiana Terhadap Mortalitas Semut Rangrang Oecophylla smaragdina F. (Hymenoptera : Formicidae) Jurnal Entomologi 6 53-59

[12] Saleh RM, T Rosdah, and Suprapti 2000 Pengaruh pemberian (Beuveria bassiana Vuill) terhadap kematian dan perkembangan larva (Spodoptera litura Fabricus) di rumah kaca Jurnal Hama dan Penyakit Tumbuhan Tropika 1 7-10

[13] Nelly N, M Syahrawati, H Hamid, T Habazar, and DN Gusnia 2019 Diversity and characterization of entomopathogenic fungi from rhizosphere of maize plants as potential biological control agents Biodiversitas 20 1435-41

Acknowledgment
The author is grateful to the Rector of Andalas University for funding the research through Research Professor Cluster Grant with contract number: T/36/UN/17.18/ PP-KP-RP1GB/LPPM/2019.