

Efficacy Of Beauveria Bassiana Against Helopeltis Sp. On Cacao (Theobroma Cacao)

Dyah Rini Indriyanti, Siti Nur Faizah, Muji Slamet

ABSTRACT: Cacao (*Theobroma cacao*) is the top five major plantation commodities in Indonesia. Cocoa fruits in Temanggung, Central Java Province were attacked by fruit sucking insect, *Helopeltis* sp. *Helopeltis* sp is also known as a major pest in plantation crops in Indonesia. This study aimed to analyze the mortality of *Helopeltis* sp. after the application of parasitic fungi *Beauveria bassiana* in certain doses. *B. bassiana* used was in form of kaolin powder produced by Plantation Corps Protection Center (BPTBUN) of Salatiga. This study used four doses of treatment : 0 gL⁻¹, 100 gL⁻¹, 150 gL⁻¹, and 200 gL⁻¹. *B. bassiana* used had a spore density of 4.2 x 10⁸ spores/g with viability of 67.2%. Results of the study showed that *B. bassiana* could infect and kill *Helopeltis* sp. The optimum dose to control the population of *Helopeltis* sp. is 150 gL⁻¹. Spraying was conducted two days in a row, then followed by 1 week interval. This study took more than four weeks to completely kill the test insects. The results are useful for the recommendations of biological control agent of *Helopeltis* sp pest in the field.

Keywords: *Beauveria bassiana* , Biological control, Cacao (*Theobroma cacao*), *Helopeltis* sp.

INTRODUCTION

Cacao is the top five major plantation commodities in Indonesia. However, cacao production decreased in 2010-2014. Cacao production reached 839.918 tons (in 2010), while only 817.322 tons in 2014. During the cacao harvest in 2016, Cacao Plantation of Central Java Province found that there was a pest attack on cacao plants in the Karanggedong village. One of the identified pest was fruit sucking insect, *Helopeltis* sp. It is a very destructive pest of cocoa. They were found almost in all the fruit-bearing cocoa trees. The infected fruit was identified by the color change into black patches, fruit shrinks and the fruit falls before ripe. The attacks can be performed by both imago (mature insect) and nymph (immature insect). The nymph attacks have a lighter impact compared to the imago attacks, due to the limited movement of the nymph [1,2]. *Helopeltis* sp. attacks in young fruit may cause the stalled fruit development [3, 4]. Farmers use pesticides to control *Helopeltis* sp. at this time, but there has been insect resistance, so that the doses used should be high. Therefore, it needs another way, that is biological control using Biological Control Agent (BCA). Biological Control Agent (BCA) is a microorganism or organism that has the ability to suppress, obstruct, or kill targeted organisms through certain mechanisms and potentially to be used in controlling the target's population. BCA can be as parasite, predator or pathogen. BCA such as an entomopathogenic fungi have been widely used by farmers as an effective, safe and environmentally friendly pest control [5]. Pest control using biological agents is a good choice in terms of environmental preservation and biodiversity conservation

[6]. The use of BCA as a substitute for chemical pest control or chemical pesticides is potentially reducing the level of environmental damage. Using pesticides can lead to soil degradation, the extinction of certain species and the emergence of resistant species. *B. bassiana* is a pathogenic fungus that can infect and causing the disruption to the body of insects. *B. bassiana* has more than 700 species of host insects, which mean that *B. bassiana* has no specific host [7]. Utilization of *B. bassiana* for controlling the population of *Helopeltis* sp. has been widely reported [3, 8,9]. The application of *B. bassiana* can decrease the *Helopeltis* sp. attacks [3]. The fact that *B. bassiana* can control the *Helopeltis* sp. population leads to an urgency to conduct an adaptation and efficacy test of the *B. bassiana* produced by BPTBUN in purpose of controlling *Helopeltis* sp. This study aimed to analyze the adaptation and efficacy of *B. bassiana* fungus in controlling *Helopeltis* sp. The study results are useful for the recommendations of biological control agent of pest in the field.

METHODS

Efficacy test of *B. bassiana* were conducted from October to November 2016, at one of the Temanggung Cacao plantation The study site has a land area of 7.44 ha, an altitude of 1000 m above msl, a temperature of 25-34.9°C, an air humidity of 66-99%, and rains that fell almost daily during the data retrieval process. *B. bassiana* fungi were obtained from BPTBUN Salatiga in the form of kaolin powder formulations. Tests of spore density and viability were performed prior to the use of *B. bassiana*. The test was conducted at BPTBUN Pest Laboratory, Salatiga. The test of density and viability steps were described as follows by method from Directorate of Plantation Protection [10]. Density Test of *B. bassiana* Spores One gram of *B. bassiana* in the form of kaolin powder was weighed and put into a glass with 100 mL of Aquades, then stirred until blended. The spore suspension plus three drops of Agristic (adhesive compound) were taken with a 0.2 ml pipette stirred until blended and dripped on a hematocytometer. The suspense was left for about 1 minute to set the conidia in stable position. Spores were observed under a microscope with a magnification of 100 or 400 times. The spores' calculation was repeated 4 times for valid results using the following formula:

- Dyah Rini Indriyanti, Biology Department, Faculty of Mathematics and Sciences, Universitas Negeri Semarang, Indonesia
- Siti Nur Faizah, Biology Department, Faculty of Mathematics and Sciences, Universitas Negeri Semarang, Indonesia
- Muji Slamet, Estate Crop Protection Board in Salatiga, Central Java Province. Indonesia
Corresponding: dyahrini36@gmail.com

$$S = (t \times d \times 10^6) / (n \times 0.25)$$

S = The number of spores per gram of medium

t = The number of spores counted on field count (a, b, c, d and e)

d = Levels of dilution

n = The number of boxes observed (5x16 small boxes = 80 boxes)

This formula applies only to the Neubauer Improve hemocytometer.

Viability test of *B. bassiana* spores The liquid agar medium was poured into the petri dish cap aseptically, then left to cool and solid. The thin layer of agar medium was sliced with a sterile scalpel approximately 1 x 1 cm and moved over the object glass. One object glass can be occupied by 2 pieces of agar media. The spore suspension was dripped onto an object glass, covered with a cover glass and then observed under 400 times magnification of microscope to determine the spores' density. The object glass containing the medium with spore suspension was inserted into a large closed petri dish then incubated for 8 hours at room temperature. Growing spores were characterized by the growth of short hyphae or tailed-looking spores. Result of observation was recorded and the treatment was repeated 4 times. The calculation of *B. bassiana* spores' viability was performed by the following formula:

$$VK = \sum KB / (\sum KB + \sum KTB) \times 100\%$$

VK = viability of conidium

KB = the germinated conidium

KTB = the ungerminated conidium

Good Quality Standards of BCA according to the Directorate of Plantation Protection are as follows [10]:

Table 1. Quality Standards of BCA [10]

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

Adaptation Test

The adaptation test was conducted to determine whether *B. bassiana* can adapt well to infect *Helopeltis* sp. in the Temanggung cocoa garden with an altitude of 1000 m above sea level. The efficacy test was intended to determine whether *B. bassiana* can kill *Helopeltis* sp. or not. In the process of the treatment, cocoa fruits were covered with a white color tile fabric with a size of 15x 25 cm. Ten *Helopeltis* sp. was given inside each confinement. This study used four treatment doses of *B. bassiana*: treatment A: 100 gL⁻¹; treatment B=150 gL⁻¹, treatment C=200 gL⁻¹ and control (without any *B. bassiana*) with 10 times repetition for each dose. The covered fruits with *Helopeltis* sp. inside then were sprayed with the formula of *B. bassiana* according to the specified dose. Observation was conducted in every week, by observing at whether or not the insects were infected and dead. Dead insects were taken from the confinement, then incubated by storing on a 5 cm x 5 cm mica box. At the base of the mica was given a

tissue soaked in moist water. Incubation was done for about 2-4 days. Whitish marks appear on the body of an infected insect as the infected mark *B. bassiana*. To ascertain the type of fungus that was attacked *Helopeltis* sp. Fungus identification was conducted at Biology Laboratory of Semarang State University and Laboratory of BPTBUN. The percentage of insect mortality was calculated by the following formula:

$$\text{Percentage of deaths} = \frac{\sum M}{(\sum M + \sum H)} \times 100\%$$

M = dead *Helopeltis* sp.

H = alive *Helopeltis* sp.

RESULTS AND DISCUSSION

From the result of spore density calculation, it was found that the formulation used contained 4.2 x 10⁸ spores/g (Figure 1). According to the Directorate of Plantation Protection [10], spore density of > 10⁶ includes in good category, it means that the kaolin formulation used in this study had a good quality. The number of spores was related to the dose level used. The higher the dose level the more spores contained in it [11].

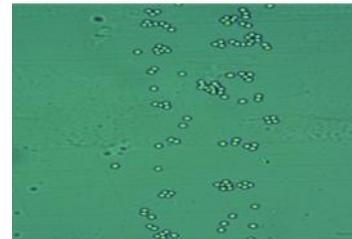


Figure 1. Spores of *B. bassiana* at 400x Magnification

Observations with 400x magnification shows oval rather round shaped of spores. This is in accordance with Ligozzi et al.[12], which described the morphology of *B. bassiana* grown on PDA media possessed features of white color. The oval-shaped spore is slightly round to ovoid with a grape-like structure. The average viability of *B. bassiana* in the applicable kaolin medium was included in deficient category because of its viability after 8 hours incubation was only 67.2%. However, the terms of a pathogen as biological control agents is not only based on its viability, but also some other things such as its ability to produce enzymes and secondary metabolites that functionate as a degradation compound of the cuticle and chitin and also can demolish the nervous system of the insects [13]. The percentage of death *Helopeltis* sp. was obtained from the result of *Helopeltis* sp. mortality observation for four weeks. The percentage of death *Helopeltis* sp. as a result of *B. bassiana* infection is listed in Figure 2.

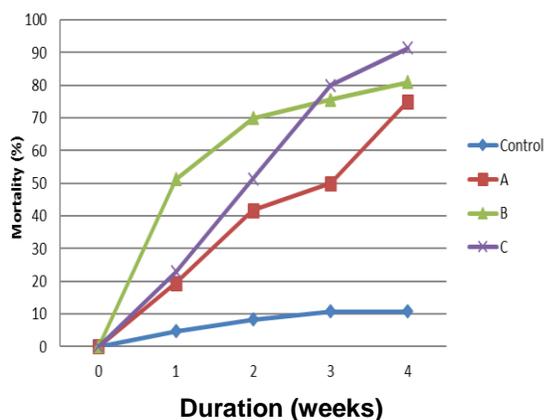


Figure 2. The Percentage of *Helopeltis* sp. Mortality due to the Infection of *B. bassiana*

A: 100 gL⁻¹; B = 150 gL⁻¹, C = 200 gL⁻¹ and control (without any *B. bassiana*).

The results of the adaptation and efficacy of *B. bassiana* fungi (Figure 1), shows that *B. bassiana* can adapt and infect *Helopeltis* sp. at an area of 1000 m above sea level (msl). According to Lecuona et al.[14], the optimum temperature that supports the development of *B. bassiana* is 20-30 ° C. This fungus is capable of developing in the temperature range of 15-35 ° C and the humidity below 95.5%. The air temperature at the site ranges from 25-34.9°C with air humidity of 66-99%. The very high humidity (99%) was due to the rainfall at the time of recording. Environmental factor data indicate that in that location, *B. bassiana* can develop well. At the first week after the application of *B. bassiana*, infected *Helopeltis* sp. in the treatment of B was the highest mortality (51.3%) compared with treatment A (19.5%) and C (22.9%). This was because at the first application of treatment B, the heavy rain fell half an hour after the spraying resulting in the repetition of the treatment on the next day. This action was selected because of the fear that the *B. bassiana* dissolved because of the rain. However, the results showed that B treatment resulted in the highest *B. bassiana* infection compared to A and C. This indicates that despite heavy rains, spores are still present attached to the body of insects, resulting in many insects infected with *B. bassiana*. In treatment A, the number of infected *Helopeltis* sp. was less than in the treatment C because the number of spores in treatment C was more than the treatment A. In the control treatment, some *Helopeltis* sp. were also attacked by fungus. It is due to the contamination during the spraying process because the distance of the locations was not too far (approximately 500 m). However, at the second to fourth week, only the number of infected *Helopeltis* sp. in treatment A, B and C that was increasing. Based on the results of the efficacy test, it can be suggested that second spray can be done the day after the first spray. Spraying can then be done one week later with the intervals of once a week. This is intended to increase the chances of more spores contact with the more insects in the beginning of treatment. The nature of the fungus is not like a deadly insecticide. It takes time to contact with the body of the insect, germinate, then infect the body of the insects. The more spores attached to the body of the insect resulting in the faster of the infection

occurs, so the sooner the insect dies. The following is a microscopic description of *Helopeltis* sp. which was attacked with *B. bassiana* (Figure 3). Incubation of *Helopeltis* sp. in a mica box with the tissue aimed to ascertain whether the death of *Helopeltis* sp. was caused by *B. bassiana* or not. The effectiveness of *B. bassiana* can be seen from the thickness of the mycelium that grows in the body of insects [15]. The observations under a microscope of the mycelium that surrounds *Helopeltis* sp. which has been incubated (Figure 3).

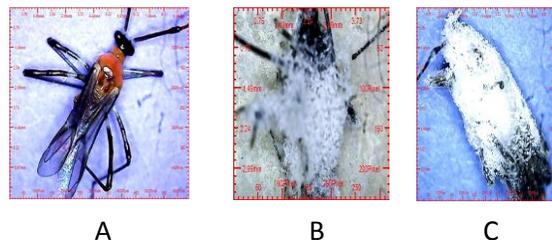


Figure 3. Healthy *Helopeltis* sp. (A), *Helopeltis* sp. infected by *B. bassiana* at 700x Magnification (B &C).

Figure 3 shows the difference between healthy *Helopeltis* sp. and *Helopeltis* sp. infected by *B. bassiana*. This indicates that the death of *Helopeltis* sp. is actually caused by *B. bassiana* infection. Use of BCA is safe for the environment because it does not pollute the environment has no impact on non-pest organism. It is in accordance with Ulin et al.[16] stated that *B. bassiana* does not cause a significant infection on non-pest insects. The virulence of pathogenic fungi takes time to infect to kill the insects, infection begins with the attachment of conidia, germination and penetration [17]. The more conidia attached to the target host the more quickly host insects being infected [18]. The infection is strongly influenced by enzymes produced from metabolism system that have an important role in the mechanism of fungal pathogens in infecting insect [19]. Application of *B. bassiana* should be done when the young fruit of cacao plants begin to grow, so that insect pest populations can be controlled earlier. The results of study by Amalin et al. [20], were proving that covering cocoa fruits with kaolin which contained *B. bassiana* can resist insects to approach cocoa fruit. If the pest populations are high, more frequent spraying and fruit wrapping are required, so that the more fruit damage is expected to be minimized. The recommended application dose of 150 g L⁻¹ should be repeated a day after application and subsequent spraying can be done one week later.

CONCLUSION

B. bassiana used in the study has a spore density of 4.2 x 10⁸ spores/g with viability of 67.2%. Adaptation and efficacy test results of *B. bassiana* on *Helopeltis* sp. showed that *B. bassiana* can adapt well at 1000m above msl at a dose of 150 grams per liter of water on daytime temperatures ranging from 25-34.9 ° C, air humidity of 66-99%, and almost daily rain intensity at the time of the study. In this study, it took more than four weeks to kill all *Helopeltis* sp. The adaptation and efficacy of *B. bassiana* fungus in controlling *Helopeltis* sp. The study results are useful for the recommendations of biological control agent of pest in the field.

REFERENCES

- [1] Srikumar, K.K., & Bhat, P.S. 2013. Biology and feeding behaviour of *Helopeltis antonii* (Hemiptera: Miridae) on Singapore cherry (*Muntingia calabura*) refuge host. *Journal of Entomological Research*, 37(1): 11-16.
- [2] Karmawati, E & Siswanto. 2016. Efektivitas Minyak Jarak Pagar dan Jamur *Beauveria Bassiana* untuk Pengendalian Hama *Helopeltis* Spp Pada Kakao. *Jurnal Litri* 22(4): 189 – 196.
- [3] Kresnawati, I., Budiani, A., Wahab A, & Darmono, T.W. 2010. Aplikasi biokaolin untuk perlindungan buah kakao dari serangan PBK, *Helopeltis* spp. dan *Phytophthora palmivora*. *Menara Perkebunan*, 78(1): 25-31.
- [4] Susi Melina, S., Martono, E., Trisyono, Y.A. 2016. Confirmation that *Helopeltis* species attacking cacao in Yogyakarta is *Helopeltis bradyi* Waterhouse, not *Helopeltis antonii* Signoret (Heteroptera: Miridae). *Jurnal Entomologi Indonesia*, 13(1), 9-20.
- [5] BPTBUN. 2011. Petunjuk pembiakan dan penggunaan jamur *Beauveria bassiana* secara sederhana. Dinas Perkebunan Propinsi Jawa Tengah. Balai Proteksi Tanaman Perkebunan.
- [6] Goebel, N.L., Turk, K.A., Achilles, K.M., Paerl, R., Hewson, I. & Morrison, A.E. 2010. Abundance and distribution of major groups of diazotrophic cyanobacteria and their potential contribution to N₂ fixation in the tropical Atlantic Ocean Environment *Microbiology*. 12: 3272–3289.
- [7] Devi, U.K., Padmavathi, J. Rao, U.M., Khan, C & Mohan, M.C. 2008. A study of host specificity in the entomopathogenic fungus *Beauveria bassiana* (Hypocreales, Clavicipitaceae). *Biocontrol Science Technology*, 18: 975–989.
- [8] Anggrawati, H. S. 2014. Upaya pengendalian hayati *Helopeltis* spp., Hama penting tanaman *Acacia crassiparva* dengan cendawan *Beauveria bassiana* dan *Lecanicillium lecanii*. Tesis. Bogor: Entomologi Institut Pertanian Bogor.
- [9] Gargita, Wayan, D., Sudiarta, I. P., Wirya, G.N.A.S. 2017. Pemanfaatan Patogen Serangga (*Beauveria bassiana* Bals.) untuk Mengendalikan Hama Penghisap Buah Kakao (*Helopeltis* spp.) di Desa Gadungan, Kecamatan Selemadeg Timur, Kabupaten Tabanan. *Jurnal Agrotekologi Tropika*. 8(1): 11-20.
- [10] Directorate of Plantation Protection. 2014. Pedoman Uji Mutu dan Efikasi Lapangan Agens Pengendalian Hayati (APH). Direktorat Perlindungan Perkebunan, Kementerian Pertanian. 115 p.
- [11] Hasyim, A & Aswana. 2003. Patogenitas isolat *Beauveria bassiana* dalam Mengendalikan Larva *Spodoptera litura*. *J. Hort*. 13(2): 120-130.
- [12] Ligozzi, M., Maccacaro, L., Passilongo, M., Pedrotti, E., Marchini, G., Koncan, R., Cornaglia, G., Centonze, A.R. and Lo Cascio, G.. 2013. Utility of molecular identification in opportunistic mycotic infections: a case of cutaneous *Alternaria infectoria* infection in a cardiac transplant recipient. *Journal Clin Microbiol* 42: 5334–5336.
- [13] Pramesti, R.N., Himawan, T. & Rachmawati R.. 2014. Pengaruh Pengkayaan Media dan Suhu Penyimpanan Terhadap Kerapatan dan Viabilitas Konidia Jamur Patogen Serangga *Beauveria Bassiana* (Balsamo) Vuillemin (Hypocreales : Cordycipitaceae). *Jurnal Hama Penyakit Tanaman*, 2(3): 42-50.
- [14] Lecuona R.E., Edelstein, J.D. Berretta, M.F. La Rossa, F.R. & Arcas, J.A. 2001. Evaluation of *Beauveria Bassiana* (Hyphomycetes) strains as potential agents for control of *Triatoma infestans* (Hemiptera: Reduviidae). *Journal Med Entomol*, 38: 172–179.
- [15] Navik O.S., Godase, S.K. Turkhade, P.D. & Narangalkar, A.L. 2015. Evaluations of entomopathogenic fungi and botanicals against tea mosquito bug *Helopeltis antonii* Signoret. *Current Advances in Agricultural Sciences*, 7(2): 203-204.
- [16] Ulin G., Reitz, S.R. Wang, J., Tamez-Guerra, P., Wang, E., Xu, X. & Lei, Z.. 2012. Potential use of the fungus *Beauveria bassiana* against the western flower thrips *Frankliniella occidentalis* without reducing the effectiveness of its natural predator *Orius sauteri* (Hemiptera: Anthocoridae), *Biocontrol Science and Technology*, 22(7): 803-812.
- [17] Hasyim, A., Nuraida & Trizelia. 2009. Patogenisitas Jamur Entomopatogen terhadap stadia telur dan larva hama kubis *Crociodomia pavonana* Fabricius. *J. Hort* 19(3): 334-343.
- [18] Sianturi, N.B., Pangestiniingsih, Y. & Lubis, L.. 2014. Uji Efektifitas Jamur Entomopatogen *Beauveria bassiana* (Bals.) dan *Metarrhizium anisopliae* (Metch) terhadap *Chilo sacchariphagus* Boj. (Lepidoptera: Pyralidae) di Laboratorium. *Jurnal Online Agroekoteknologi*, 2(4): 1607-1613.
- [19] Sun M., Ren, Q., Guan, G., Li, Y., Chao-Ma, X.H., Yin, H., & Luo, J.. 2013. Effectiveness of *Beauveria bassiana* sensu lato strains for biological control against *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae) in China, *Parasitology International*, 62(5):412-415.
- [20] Amalin, D.M., Averion, L., Bihis, D., Legaspi, J.C. & David, E.F. 2015. Effectiveness of kaolin clay particle film in managing *Helopeltis collaris* (Hemiptera: Miridae), a major pest of cacao in The Philippines. *Florida Entomologist*, 98(1):354-355.