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THE EFFECT OF BEAUVERIA BASSIANA ON THE EFFECTIVENESS OF NICOTIANA TABACUM EXTRACT AS BIOPESTICIDE AGAINST HYPOTHENEMUS HAMPEI TO ROBUSTA COFFEE

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ABSTRACT

This study examined the effect of the addition of *Beauvaria bassiana* on the effectiveness of *Nicotiana tabacum* extract as a biopesticide against coffee berry borer (CBB) or *Hypothenemus hampei*. A six months field experiment was carried out to examine the combined biopesticide effect on robusta coffee plantation. Randomized complete block design (RCBD) was used as a model for this experiment that consists of two factors. The first factor was the composition of *Beauveria bassiana* in water (0 to 320 g), while the second factor was *Nicotiana tabacum* extract composition in water (10 to 30 mL). The results showed that the dosage of *Beauveria bassiana* had a significant effect on tobacco biopesticide extract on the percentage and the intensity of CBB attacks. The combination of 320 g *Beauveria bassiana* /8 L of water and 30 mL *Nicotiana tabacum* extract/10 L of water, in our experiments (B_4T_3) could reduce the percentage and intensity of coffee fruit attacked by CBB to 1.54% and 0.33%, respectively.

Keywords: *Beauveria bassiana; Hypothenemus hampei*; *Nicotiana tabacum;* Robusta Coffee

1. INTRODUCTION

Coffee is one of the important plantation commodities that have high economic value in Indonesia (Rahardjo, 2012). The major problems in coffee plantation are low productivity and quality. Coffee production is influenced by the suitability of the growing environment, cultivation techniques, varieties, and the presence of plant-disturbing organisms. Coffee berry borer (CBB) is one of the causes in low production and quality. In addition, CBB attacks the coffee when it is stored. CBB attacks the young to old berries, causing perforated seeds. This also affects the quality of coffee produced.

Beauveria bassiana is one of the entomopathogenic fungi that potentially developed as an alternative biological control for CBB. *B. bassiana* infection develops causing cell function damage. The toxins produced by *B. bassiana* are beauvericin, beauveroulite, bassianaliti, isorolite, and oxalic acid. The toxins play a role in damaging the digestive tract, muscles, nervous

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system, and respiration of the CBB (Mahr, 2003). The resulting enzymes, such as proteases, lipolytic, amylases, and chitinase, play a role in hydrolyzing protein complexes in the integument.

The use of synthetic pesticides is more effective; however, it is harmful to the environment and human body. Nowadays, the biological control of CBB pests is developed in Indonesia as a result of increasing demand for bio-coffee. Biological control agents are able to control CBB pests, relatively safe, and easy to use. Tobacco biopesticide (Fauzantoro et al., 2017; Wijanarko et al., 2017) is one of the biological control agents that expected to control the CBB attacks.

The advantages of biopesticides are that it is easily degraded, leaves no long-term residue, is fast acting, has low mammalian toxicity, and low phytotoxicity. Tobacco can be used as insecticide because it contains nicotine, D-limonene, and pyridine (Gozan et al., 2014). Pyrolysis and reflux extraction methods have been used to increase the yield of bio-oil as biopesticide from *Nicotiana tabacum* (Gozan et al., 2014; Purwanto et al., 2015; Supramono et al., 2016; Fauzantoro et al., 2017). Nicotine alkaloid, nicotine sulfate, and other nicotine content are used as contact poison, fumigant, and stomach toxins (Hasanah et al., 2012). Nicotine as the fumigant will evaporate and penetrate into the integument of insects causing death (Matsumura, 1975).

The content of tobacco extract (Fathi et al., 2018) is an active compound, such as terpenoids. Terpenoids have a bitter taste and antifeedant that can inhibit normal feeding behavior of insects (Gozan et al., 2014). Terpenoids are also insect repellents. This compound acts as a stomach poison through food into the gastrointestinal tract (Jumar, 2000). These compounds affect the function of nerves because they inhibit cholinesterase enzymes, causing impaired transmission that decreases the coordination of muscle work and then death (Foster et al., 2011; Afifah et al., 2015). Terpenoid mechanisms start by inhibiting the arrangement of calcium ions (Ca^{2+}) in the insect muscle system, then stimulate muscle contraction resulting in paralysis and death (Pu’u, 2010).

2. MATERIALS AND METHODS

2.1. Materials

The *Beauveria bassiana* was obtained and donated by Plantation Office, Central Java Province. The extract tobacco biopesticide was provided by Biorefinery research group from Universitas Indonesia and was produced using the pyrolysis method (Gozan et al., 2014).

2.2. Coffee Plantation

The study was conducted in Kalibening residence, Kebondalem village, Jambu district, Central Java province, Indonesia (South Latitude 7°16'12.4" and East Longitude 110°20'03.4"), 870 m above sea level and about 40.7 km to the south of the Ahmad Yani International Airport. This residence is surrounded by four mountains, namely Ungaran, Merbabu, Sumbing, and Sindoro. The location is shown in Figure 1.

This area is a community-based coffee plantation. The research was conducted from March to August 2017 during wet season or monsoon season in this area. The type of coffee plantations in this area produce robusta coffee (*Coffea canephora*) which is a common type cultured in the highlands. The observed plants averaged 12–15 years of age.

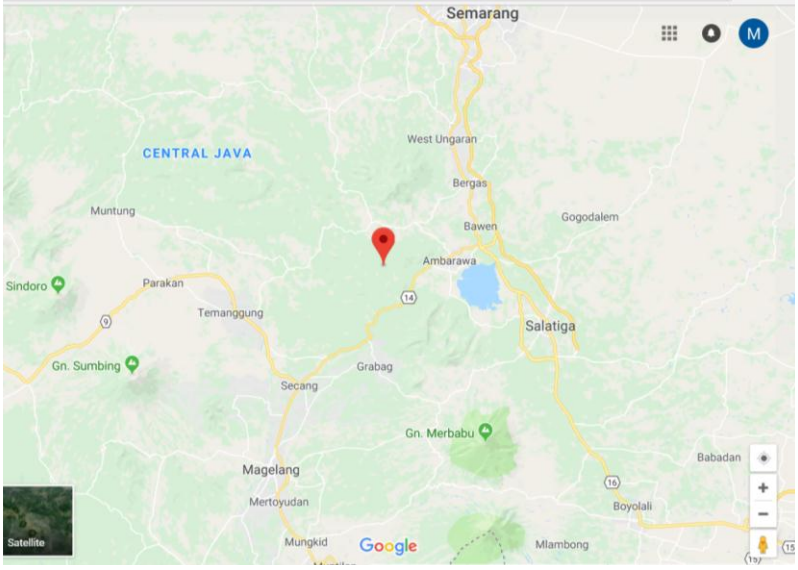

Figure 1 Geographical location of observation: Kalibening residence, Kebondalem village, Jambu district, Central Java province, Indonesia

2.3. Randomized Complete Block Design

This research was conducted by using Randomized Complete Block Design (RCBD) consisting of two treatments and repeated three times. The first factor was five level doses of *Beauveria bassiana* (B) powder ranging from 0 to 320 g in 8 L of water. The second factor was three level doses of tobacco extract (T) ranging from 10–30 mL in 10 L of water. The composition used in the Randomized Complete Block Design (RCBD) experiments is illustrated in Table 1.

Table 1 Composition of treatment

Treatment (level dose)	Amount of *Beauveria bassiana* (g/8 L of water)	Treatment code
Beauveria bassiana	0	B_0
	80	B_1
	160	B_2
	240	B_3
	320	B_4
	Amount of Tobacco extract (mL/10 L of water)	Treatment code
Tobacco Extract	10	T_1
	20	T_2
	30	T_3

The combination of B and T were applied to coffee trees. Each coffee tree contained many twigs which contained coffee berries and were subjected to spraying. Observations were carried out on three randomized twigs in each tree. The application of water spraying without *Beauveria bassiana* nor tobacco extract (B0T0) acted as the control. The first spraying to each combination was applied when the seed coffee began to harden in early March. The sprayings were continued fortnightly. Thus, between March and May there were five sprayings applied to each tree.

The data obtained from the observations were analyzed by diversity analysis. The significantly different data were then followed by Duncan's Multiple Range Test (DMRT) with a significant difference of 5%.

2.4. Percentage and Intensity of CBB attack

The percentage of CBB attacks (P) was calculated using the formula:

$$P = \frac{n}{v}.100\% \tag{1}$$

where n is the number of affected trees and v is the number of observed trees.

The intensity of pest attack (I) was calculated using the formula:

$$I = \frac{\sum n.v}{Z.N} x100\% \tag{2}$$

where n is the number of berries attacked by CBB; v is the attack category scale; Z is the scale value of the highest attack category; and N is the total number of berries observed. Scale category of CBB pests. The score is 0 when there is no attack; 1 if the attacks are between 0–24%; and 2 if the attack 25–49%; 3 if the attacks are between 50–74%, and 4 if the attacks are between 75–100%.

3. RESULTS AND DISCUSSION

The observation of CBB attacks on the coffee trees and intensity of the CBB attacks was conducted during the monsoon season in the Kalibening residence, Jambu district, Central Java province. The area is a highland with an altitude of 870 m above sea level.

3.1. Percentage of CBB Pest Attack

The number of trees attack by CBB or *Hypothenemus hampei* during the observation is shown in Figure 2. The addition of *Beauveria bassiana* to the tobacco extract reduced the CBB attacks. Without the addition of *B. bassiana*, i.e. B0 as the control, the figure shows that 16.0%, 13.6%, and 11.0% of the trees were attacked by CBB at the concentration of *N. tabacum* extract 10, 20, and 30 mL/10 L water, respectively. An addition of 80 g of *B. bassiana* to the tobacco extract (B1) lowered the CBB attacks to 10.7%, 9.7%, and 9.4%. The addition of 320 g of *B. bassiana* to the tobacco extract (B4) reduced the CBB attacks to less than 2% for all tobacco formulations. Therefore, the addition of this biological control fungi consistently increased the protection of the robusta coffee tree from CBB attacks.

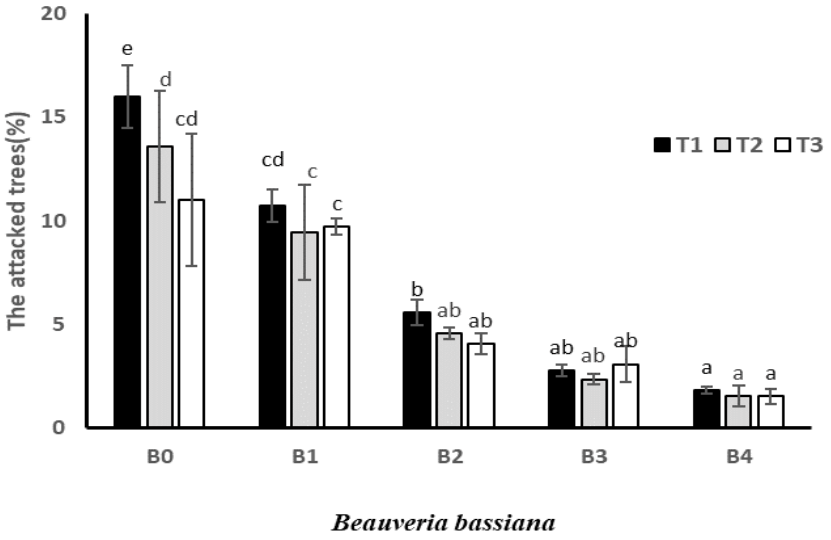

Figure 2 The effect of mixed *Beauveria bassiana* and tobacco extract on the percentage of CBB attacks. The letter a, b, c, d, and e represent the results from statistical DMRT analysis

The treatment using a combination of tobacco extract and *B. basiana* showed remarkably good results against CBB attacks. Figure 2 shows the attacked trees with T3B0 treatment decreased from 11.01% to 1.54% in T3B4. This is because the extracts of tobacco biopesticides and fungi *Beauveria bassiana* contacted directly with CBB at the time of spraying or through residues located on the surface of the coffee berry (Wiryadiputra, 2012).

The results of the DMRT analysis are given by the notation a to e in Figure 2. From this figure, the DMRT analysis shows that B1T2, and B1T3 share the same notation (c). Although the values are different, this means that, statistically, there is no significant difference. Likewise, B3T1, B3T2, and B3T3 are not significantly different because they share the same notation.

After harvesting, the coffee bean is a good breeding ground for CBB; each seed can produce around 75 insects. CBB survive for about a year in coffee beans in a closed state (Khalshoven, 1991). The female insect of CBB lay eggs to produce 35–50 larvae and then hatch at the endosperm of young berries to grow and harden into adulthood in the coffee hole. Figure 3 shows the symptoms and CBB infections in coffee. The coffee infected with CBB can be a source of infection in the next year (Wiryadiputra, 2012).

Figure 3 Symptoms of Coffee Berry Borer (*Hypothenemus hampei* abbreviated CBB) on Robusta Coffee

The tobacco leaf extract also contains active compounds, such as terpenoids. Terpenoids have a bitter and antifeedant taste that can inhibit insect feeding activity (Gozan et al., 2014). Triterpenoids are also insect repellents because of its unfavorable smell for insects. This compound acts as a stomach poison that is absorbed by the digestive tract (Jumar, 2000). The compound can affect the function of the nerve that inhibits the enzyme, cholinesterase, causing impaired transmission excitatory, decreased coordination of muscle work, convulsions, and insect death (Afifah et al., 2015; Foster et al., 2011).

Terpenoids contained in tobacco leaf extracts kill insect pests by inhibiting the arrangement of calcium ions (Ca^{2+}) in insect muscles, stimulating muscle contraction in insects and causing paralysis and death. From this mechanism of death, the pesticide group is classified as a new group (novel pesticide) because it works in contrast to previously active pesticide groups (organochlorines, carbamates, organophosphates, pyrethroids, and nicotinamides). The previous active group of insecticide is excellent for dealing with insect resistance (Pu'u, 2010).

One of the entomopathogenic fungi potentially developed as an alternative to CBB pest control is *Beauveria bassiana*. This entomopathogenic fungus has a high reproductive capacity, short life cycle, is relatively safe, selective, compatible with several types of insecticides, and easy to produce. *B. bassiana* produces a toxin called beauvericin. Several studies explain the killing mechanisms of these antibiotics. Direct contact and inoculation or feed contamination of the produced antibiotics cause disruption to the functioning of the insect nucleus, thus rendering the swelling accompanied by hardening of infected insects (Tanada & Kaya, 1993). Thus, according to that study, the mechanism of death is initiated by behavioral, shape and color changes of the larvae caused by the growth of the fungus in the larval body part. This also means that *B. Bassiana* infects and develops in the body of insects by removing enzymes that cause damage to the cell function of the larval tissue. According to Mahr (2003), toxins produced by *B. bassiana*—beauvericin, beauveroulite, bassianaliti, isorolite, and oxalic acid—can damage the digestive tract, muscle, nervous system, and respiration of the insect. The resulting enzymes—such as proteases, lipolytics, amylases, and chitinases—will hydrolyze the protein complexes in the integument.

3.2. Intensity of CBB Pest Attacks

Figure 4 shows the treatment combination of tobacco extract and *B. Bassiana*. When the doses of biopesticides and *B. bassiana* fungi were increased, the intensity of CBB attacks decreased. Tobacco biopesticide extract is quite effective in suppressing the population of CBB pests in coffee plants. The average intensity of CBB pest attacks on the treatment of tobacco biopesticide extract T1B0 decreased from 12.51% to 0.40% on treatment T1B4. Increased dosage of tobacco extract and *B. bassiana* biopesticide were significantly different to mean percentage of CBB attacks. Intensity of CBB attacks on T2B0 reached 9.96% and tended to decrease with increasing T and B. Intensity of CBB attacks on T2B1 reached 5.37% and tended to decrease to 0.33% on T2B4. The combination of T3B0 treatment reached 6.32% and decreased to 0.34% in T3B4. The treatments of T1B2, T2B2, T3B2 are not different and tended to decrease with T1B3, T2B3, T3B3, T1B4, T2B4, T3B4. Therefore, the dosage of 200 mL/ha and 300 mL/ha with the combination of *Beauveria bassiana* dosage 160g, 240g, and 320g can be said to be effective to control the level of CBB pest attacks.

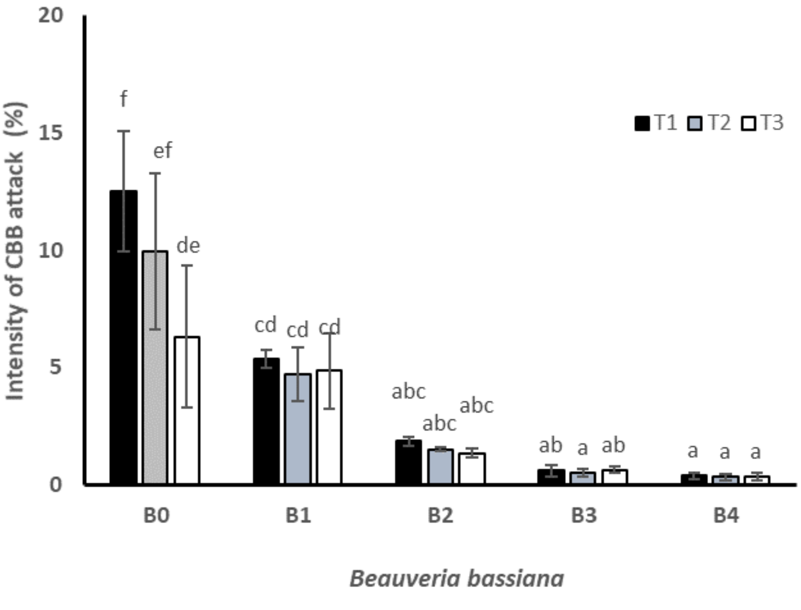

Figure 4 Effect of interaction of tobacco biopesticide extract and *Beauveria bassiana* on intensity of CBB pest attacks. The letters a, b, c, d, e, and f represent the results from statistical DMRT analysis

This is due to the extract of tobacco biopesticide and the *B. bassiana* fungi working together in contact against CBB insect larvae and beetles. *B. bassiana* produce toxins that can lead to aggressive paralysis of larvae and imago insects. Some toxins isolated from the fungus *B. bassiana* are beauvericin, beauverolide, isorolide, and dyestuffs and oxalic acid (Mahr, 2003). They lead to decreased appetite of the larvae, causing the larvae to be less active and the rigid larvae body color to be masked by white hyphae (Muslim et al., 2007). The mortality of *C. sacchariphagus* larvae was 66.67% with *B. bassiana* treatment of 30 g/L (Sianturi et al., 2014). *B. bassiana* penetrate into the body of the insect by contact with the skin between the joints of the body. Its penetration mechanism begins by attaching conidia to the cuticle or insect mouth.

The mechanism of tobacco biopesticide extract as repellent and as antifeedant is initiated by interfering with the digestive process in the insect body (Indrarosa, 2013). Tobacco biopesticide extract contains 13.1% nicotine (Gozan et al., 2014). Nicotine as a neurotoxin, contact poison (Afifah et al., 2015), fumigant, and stomach poison is capable of killing the insect (Hasanah et al., 2012). The symptoms of nicotine intoxication in insects are indicated by seizures followed by death (Matsumura, 1975).

4. CONCLUSION

Our observation showed that the highest percentage of coffee beans attacked by CBB was 15.98% (B0T1); while the highest intensity of CBB attacks was 12.51% (B0T1). The combination of 320 g Beauveria bassiana /8L of water and 30 mL Nicotiana tabacum extract /10L of water, in our experiments (B4T3) could reduce the percentage and intensity of coffee fruit attacked by CBB to 1.54% and 0.33%, respectively

5. ACKNOWLEDGEMENT

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