Control of coffee berry borer (*Hypothenemus hampei*) with entomopathogenic fungi

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Abstract: One way to control *H. hampei* is to use biological agents in the form of entomopathogens. This study aims to determine the effect of applying pathogenic fungi *B. bassiana* and *L. lecanii* on population development and attack of the coffee berry borer. The test was carried out by spraying pathogenic fungi on several stalks of infected coffee cherries which were covered with gauze cages and on several sample plants that were left open. The treatments tested were a solution of the fungus *B. bassiana* and the fungus *L. lecanii* with a concentration (1 x 10⁸/µl water), as well as controls. Observations were made on borer attacks before and after treatment until harvest by counting the number of healthy coffee cherries and infected cherries. The results showed that in treatment with *L. lecanii*, the development of the number of hole cherry (attacked) by CBB pests decreased, while healthy cherry (unattacked) showed an increasing trend. Likewise with the treatment using *B. bassiana* showed a decrease in the number of cherries damaged and an increase in healthy cherry. In control, the number of cherries damaged by CBB pests appeared to be stable or tended to increase.

Keywords: plant protection, Beauveria bassiana, Leicanicilium lecanii.

1. Introduction

Coffee plants are one of the estate commodities that have a strategic role in the Indonesian economy. In addition to export, there is also a lot of coffee for domestic consumption. Coffee plants are plant commodities that are mostly cultivated as smallholder plantations which cover 96.6% of the total coffee plantation area in Indonesia that is about 1,252,825 ha with a production of around 755,051 tons [1]. In general, there are 2 types of coffee traded, namely Arabica and Robusta coffee. Indonesian coffee production is still dominated (95%) by the type of Robusta. Robusta coffee is widely grown in areas with an altitude of up to 800 m, while Arabica coffee generally requires an altitude requirement of planting above 1000 m above sea level.

Coffee plants are attacked by many insect pests, one of which has the potential to cause harm is the coffee berry borer (CBB), known as *Hypothenemus hampei*. Apart from Indonesia, this pest is spread throughout the world's coffee-producing countries. The damage causes are in the form of undeveloped cherry, changing colour to reddish-yellow, and finally falling, resulting in a decrease in the number and quality of yields [2]. In Indonesia, this pest is almost always found in coffee plantation centers and causes significant damage to coffee berries/beans. In general, only mated female beetles will burrow coffee berries by making small holes at the basal of the cherry (discus). Female beetles attack coffee berries that are developing to mature cherry. Coffee cherry with hardened endosperm is preferred.
Attacks on the young cherry will cause the cherry not to develop and fall. Meanwhile, attacks on cherry with hardened seeds cause damaged seeds with holes and reduce quality. Female beetles are more attracted to shaded and damp coffee plantations than coffee plantations that are no shelter. *H. hampei* attack on young cherry causes cherry fall. *H. hampei* is known to eat and reproduce only in a coffee cherry. The entire life cycle of the *H. hampei* beetle will be passed in the infested cherry [3].

The average area of CBB attacks on smallholder’s coffee in Indonesia reaches more than 20% with a yield loss of more than 10%. Yield loss by this pest is generally estimated at 500 million USD annually, in Indonesia it is estimated at more than 6.7 million USD per year [4]. This pest attack has reduced crop yields in various countries such as In Uganda it reaches 80%, Colombia 60%, Jamaica 58–85%, Tanzania 90%, Malaysia 50–90% and Mexico 60% [5]. In Indonesia, this pest is one of the main causes of the decline in national coffee production and quality [6]. It is reported that CBB attacks in South Sulawesi have caused yield losses of between 30 and 60% [7]. The intensity of CBB pest attack varies greatly because it is influenced by plant age, land conditions and coffee cultivation systems. CBB includes monophage insects. This is because the plant that is the source of nutrition is only one, CBB live and feed only on the coffee cherry [8].

Control of CBB can be done through garden sanitation, the use of natural enemies such as predators, parasitoids and insect pathogens (entomopathogens) [9]. The fungus *B. bassiana* was effective in controlling CBB [10]. The right application of *B. bassiana* fungus is carried out when the skin of the cherry has hardened [11]. However, until now in the field, coffee pest control is generally carried out by farmers using synthetic pesticides. Control with synthetic pesticides is known to leave residues on crop yields. It also has a negative impact on humans and the surrounding environment. The use of insecticides increases pesticide residues in coffee beans which can affect human health and the environment [12].

One of the relatively safe control methods for the environment is to use biological agents in the form of natural enemies such as the fungus *Beauveria bassiana* and *Lecanicilium lecanii*. The application of the biological agent has proven to be quite effective based on several application tests in field conditions. However, in areas with a dry climate, it is likely to be less effective. In addition, isolates that have high virulence are needed for CBB pests. Results of field trials show that this control technology is quite effective, safe and does not pollute the environment, it is also safe for the coffee products produced.

Several strains of insect pathogenic fungi such as *B. bassiana* have been collected in the Entomology Laboratory of the Indonesian Spices and Medicinal Crops Research Institute. Among these strains, *B. bassiana* ED7 is quite effective in controlling several types of insect pests of plantation crops including white grub, *Lepidiota stigma* (Coleoptera: Scarabaeidae) on a sugarcane plantation. While *L. lecanii* was previously known as quite effective in controlling *Helopeltis* spp. and other insect pests. This study aims to determine the effect of applying pathogenic fungi *B. bassiana* and *L. lecanii* on population development and attack of the coffee berry borer.

2. Materials and method
2.1. Time and location
The location of the activity is a coffee plantation of Kartika 1 variety (Arabica type) in Pakuwon Experimental Station (PES) Sukabumi West Java and its surrounding. The activity was carried out from January to December 2014.

2.2. Materials and equipments
The material used was Coffee plantation, *B. bassiana* and *L. lecanii* biological agents were collected from Entomology laboratory, Indonesian Spices and Medicinal Crops Research Institute, insect cage, sprayer, hand counter.

2.3. Method of study
Testing the effectiveness of the control of weevils in the field was carried out in areas where coffee
borer is endemic to cherry borer attacks. The test was carried out on coffee berries attacked by borers in a closed manner with insects caged and openly, without a cage. The test was carried out by spraying the fungus on coffee berries that showed symptoms of borer attack, where the infected fruit/cherry appeared to have holes in the discs (small holes in the tip of the cherry).

In a closed test using insect caged made of gauze with small holes of less than 1 mm, one branch of a coffee plant with some of its fruit/cherry was attacked by the borer was selected. The number of twigs that were used as replicates was 10 replicates. Before treatment, the number of healthy cherry and cherry infected with CBB (hollow cherry) was counted. The treatments tested were a solution of the fungus \( B.\text{bassiana} \) and the fungus \( L.\text{lecanii} \) with a concentration \( (1 \times 10^8 \text{ / litre of water}) \), and control (without treatment). Observations were made on borer attacks before and after treatment until harvest by counting the number of healthy coffee cherries and infected cherries.

In the open test (without cage), previously selected coffee plantation plots with uniform plantings and attacked by coffee berry borer (CBB). From the planting plots, 4 plants were selected in the center of the plant as treatment points or points, then several plants in the 4 wind directions were selected as observational sample plants. Before treatment, the number of healthy cherry and cherry infected with CBB (cherry with holes) was counted. The treatments tested were a solution of the fungus \( B.\text{bassiana} \) and the fungus \( L.\text{lecanii} \) with a concentration \( (1 \times 10^8 \text{ / litre of water}) \), and control (without treatment). Spraying the fungus solution was carried out on 4 plants located in the middle of the planting. Spraying was carried out 3 times with an interval of 1 week. Observations were made on borer attacks before and after treatment until harvest by counting the number of healthy coffee cherries and infected cherries in the treated sample plants. Observations were also made on each of 2 plants in 4 wind directions with an interval of 2-3 plants.

2.4. Data analysis

Data were analyzed using regression analysis with the formula: \( Y = a + bX \), \( Y \) = number of the coffee cherry, and \( X \) = week of observation. \( a \) = constant/slope, and \( b \) = regression coefficient/intercept. The result of cherry attacked and healthy cherry of coffee treated with biological agents were compared to control.

3. Result and discussion

The results showed that spraying treatment with biological agents \( L.\text{lecanii} \) and \( B.\text{bassiana} \) could reduce the number of damaged cherry and increase the number of healthy cherries.

In the treatment of cherries spraying with the fungus \( L.\text{lecanii} \) on coffee cherries caged in the field, it was shown to be quite effective in suppressing damage to coffee cherries. Table 1 and Figure 1 show a decreasing number of holed cherries (attacked cherry by CBB pests), while healthy cherry (not attacked by CBB pests) shows an increasing trend. Likewise with the treatment using fungus \( B.\text{bassiana} \) showed a decrease in the number of cherries damaged by CBB pests and an increase in the number of healthy cherries. While in control, the number of healthy and damaged cherries by CBB pests appears to be relatively stable or tends to decrease.
Table 1. The percentage of development of the number of healthy coffee cherry and holed cherry by CBB pests on the treatment of the fungus *L. lecanii* on caged branches.

| No. | Treatment of *L. lecanii* | Treatment of *B. bassiana* | Control |
|-----|---------------------------|-----------------------------|---------|
|     | Healthy cherry (%)        | Attacked cherry (%)         | Total cherry (%) | Healthy cherry (%) | Attacked cherry (%) | Total cherry (%) |
| 1   | 14,1                      | 85,9                        | 100,0    | 23,7                | 76,3                | 100,0             | 40,7 | 59,3 | 100,0 |
| 2   | 15,8                      | 84,2                        | 100,0    | 28,1                | 71,9                | 100,0             | 39,0 | 61,0 | 100,0 |
| 3   | 21,0                      | 79,0                        | 100,0    | 29,3                | 70,7                | 100,0             | 35,7 | 64,3 | 100,0 |
| 4   | 26,5                      | 73,5                        | 100,0    | 36,5                | 63,5                | 100,0             | 32,1 | 67,9 | 100,0 |
| 5   | 45,6                      | 54,4                        | 100,0    | 45,5                | 54,4                | 100,0             | 40,4 | 59,6 | 100,0 |
| 6   | 49,0                      | 51,0                        | 100,0    | 40,1                | 59,9                | 100,0             | 37,3 | 62,7 | 100,0 |
| 7   | 51,4                      | 48,6                        | 100,0    | 39,3                | 60,7                | 100,0             | 42,1 | 57,9 | 100,0 |

Figure 1. Development of healthy coffee cherry and attacked cherry by CBB pests in a caged test of *L. lecanii*

Figure 2. Development of healthy coffee cherry and attacked cherry by CBB pests in a caged test of *B. bassiana*
The percentage development of healthy coffee cherry and cherry attacked by CBB in control (not treated with biological agents) in a caged test.

Figure 1. shows the treatment of *L. lecanii* on coffee berries attacked by CBB pests that were caged in the field affecting an increase in the number of healthy cherries by 7.24% per week, and affecting the decrease in the number of damaged cherries by 7.24%. There is a strong correlation between the amount of healthy and damaged cherry and the week of observation. Likewise, the treatment of *B. bassiana* can affect the increase in the number of healthy cherries by 3.14% per week and a decrease in the number of damaged cherries by 3.14% (figure 2). While in the control, the number of healthy cherries decreased by 1.19% and affected the decline in the population of damaged cherry by 1.19% every week. However, the correlation value of the control treatment was low, which was below 20% (figure 3).

Until the end of the observation, the treatment of *L. lecanii* and *B. bassiana* was able to increase the number of healthy cherries by 81.5% and 43.2% respectively with a fairly strong correlation. While in the control increase only 2% with low correlation.

**Table 2.** The percentage development of the number of healthy coffee cherries and holed cherry by CBB pests on the treatment of the fungus *B. bassiana* on opened test

| No. | Treatment of *L. lecanii* | Treatment of *B. bassiana* | Control |
|-----|---------------------------|-----------------------------|---------|
|     | Healthy cherry (%)        | Attacked cherry (%)          | Total cherry (%) | Healthy cherry (%) | Attacked cherry (%) | Total cherry (%) | Healthy cherry (%) | Attacked cherry (%) | Total cherry (%) |
| 1   | 26.5                      | 73.5                        | 100.0    | 20.2                        | 79.8                      | 100.0    | 32.3                        | 67.7                      | 100.0    |
| 2   | 31.8                      | 68.2                        | 100.0    | 29.3                        | 70.7                      | 100.0    | 29.2                        | 70.8                      | 100.0    |
| 3   | 43.9                      | 56.1                        | 100.0    | 41.3                        | 58.7                      | 100.0    | 25.2                        | 74.8                      | 100.0    |
| 4   | 45.6                      | 54.4                        | 100.0    | 45.4                        | 54.6                      | 100.0    | 33.8                        | 66.2                      | 100.0    |
| 5   | 69.8                      | 30.2                        | 100.0    | 61.4                        | 38.6                      | 100.0    | 33.1                        | 66.9                      | 100.0    |
| 6   | 65.6                      | 34.4                        | 100.0    | 60.6                        | 39.4                      | 100.0    | 34.0                        | 66.0                      | 100.0    |
| 7   | 78.1                      | 21.9                        | 100.0    | 59.1                        | 40.9                      | 100.0    | 34.1                        | 65.9                      | 100.0    |
The same results were also shown in the CBB pest control treatment using *L. lecanii* and *B. bassiana* in open field testing. The two biological agents showed that they were quite effective in suppressing the damage to coffee cherries by CBB pests. In *L. lecanii* treatment, the number of healthy cherry increased, while the number of damaged cherry decreased slightly or was relatively stable. The treatment of *B. bassiana* caused an increase in the number of healthy cherries and reduced the number of cherries damaged by CBB pests. While in control the number of cherry damaged by CBB pests increased and healthy cherry was relatively stable.

**Figure 4.** Development of healthy coffee cherry and attacked cherry by CBB pests in an opened test of *L. lecanii*

The treatment of *L. lecanii* on coffee cherries attacked by CBB pests in the field led to an increase in the number of healthy cherries by 8.87% per week and affecting the decrease in the number of damaged cherries by 8.87% with a strong correlation value between the number of healthy cherries and time (figure 4).

**Figure 5.** Development of healthy coffee cherry and attacked cherry by CBB pests in an opened test of *B. bassiana*
Figure 6. Development of healthy coffee cherry and cherry attacked by CBB in control (not treated with biological agents) in an opened test

In the treatment of *B. bassiana* it can increase the number of healthy cherries by 7.12% per week, and affect the decrease in the number of damaged cherries by 7.12% (figure 5). While in population control the number of healthy cherry decreased by 0.82% and affected the decline in the population of damaged cherry by 0.82% per week (figure 5). However, in the control treatment, the correlation value of healthy cherry was low, which is below 30%.

Along the observation shows that the treatment of *L. lecanii* and *B. bassiana* affected the increase in the number of healthy cherries about 67.2% and 64.2% respectively with a strong correlation, compare to control which increase 14.7% with low correlation.

*Lecanicillium lecanii* (=*Verticillium lecanii*) has a wide host range and is cosmopolitan spread in tropical and subtropical areas and is able to infect several types of host insects from the Orders Orthoptera, Hemiptera, Lepidoptera, Thysanoptera, and Coleoptera [13]. *L. lecanii* is a type of entomopathogenic fungus that is ovicidal. However, based on the results of [14] research, *L. lecanii* is able to infest all insect stages (brown planthopper) starting from the egg, nymph and imago stages. The results of observations on samples of coffee berries that were sprayed with fungi at the end of the experiment showed that several CBB insect pests were exposed to fungi (Figure 7.). The pathogenicity of entomopathogenic fungi is influenced by the stage of the host (moult) during the application of the fungus [15]. The mechanism of *L. lecanii* infection is based on direct contact between fungal and insect spores. Once applied, the spores attach to the target insect and germinate. *L. lecanii* is capable of killing insects usually after 7-10 days under proper environmental conditions [16].
Figure 7. The coffee berry borer (CBB) showing symptoms of *B. bassiana* fungus (a), and *L. lecanii* (b) attacks on coffee beans.

The treatment of *B. bassiana* fungus was also able to reduce the attack of CBB pests which was indicated by the increase in healthy cherry and a decrease in damaged cherry. The results of [17] showed that *B. bassiana* was able to suppress CBB pests. The decrease in attacked cherry can be caused by the influence of the application treatment of entomopathogenic fungi that infect and inhibit the movement activity of CBB pests.

The contact or distribution of the fungus on the target insect may be somewhat limited because the insect's activity during its life is mostly in the coffee berries. Almost the entire life cycle of beetle from egg to adult grows and develops inside the coffee cherry. The female beetle after mating in the berries will come out and look for new coffee berries to lay eggs which are usually in the afternoon around 16.00 to 18.00 [18]. Therefore in the application of biological agents should be done in the afternoon, where the female beetle is actively looking for a coffee berry to lay eggs and find food. In addition to avoiding the fungus is not being exposed to sunlight which could reduce the viability of fungus spores.

4. Conclusion

The use of *L. lecanii* and *B. bassiana* as biological agents can suppress the number of the coffee berry borer, *H. hampei* so that the number of coffee berries attacked by the borer is reduced and healthy cherry is increased. The uses of pathogenic fungi *L. lecanii* and *B. bassiana* in the caged test led to an increase in the number of healthy coffee cherries 81.5% and 43.2%, respectively. While in the open control without a caged, the use of pathogenic fungi *L. lecanii*, and *B. bassiana* caused an increase in healthy cherries about 67.2% and 64.2% respectively.

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