Toxicity of entomopathogenic fungi, Beauveria bassiana, and clove oil-based pesticide to the main pests of black pepper

Wiratno*, P Maris, M P Sari and T E Wahyuno

Indonesian Spice and Medicinal Crops Research Institute, Indonesian Agency for Agricultural Development, Jalan Tentara Pelajar No. 3, Cimanggu, Bogor 16111, West Java, Indonesia.

*Email: wiratno02@yahoo.com

Abstract. In order to get the healthy black pepper plant, the presence of various types of pest organisms should be controlled below the economic threshold. One of the efforts to obtain an environmentally friendly control strategy is by using natural pesticides i.e. entomopathogenic fungi, Beauveria bassiana and clove oil-based botanical pesticides. The insects tested in this study were pepper stem borer, Lophobaris piperis, and pepper fruit sucker, Dasynus piperis. The results of the laboratory bioassays showed that 20 gr/l of B. bassiana could kill some of the tested insects, but its toxicity in the field tended to be low. Mortality of L. piperis and D. piperis at the end of laboratory assay was 20% and 33.3%, respectively. Therefore, to get maximum impacts, field application must be repeated several times at intervals of 1 to 2 months. The botanical pesticide formula at the highest concentration (20 cc/l) was able to kill 43.3% of L. piperis. Meanwhile, it was very effective against D. piperis at a concentration of 2.5 cc/l, which was able to kill all the tested insects. The botanical pesticides and B. bassiana have potential to control D. piperis and L. piperis.

Keywords: control strategy, Dasynus piperis, Lophobaris piperis

1. Introduction

The existence of various pests and diseases is one of the obstacles in the cultivation of black pepper plants. The attacks of two main insect pests of pepper plants, namely stem borer, L. piperis, and fruit suckers, D. piperis can interfere with crop production and often cause plant death. In Bangka, the attack intensity of D. piperis can reach 36% [14]. Meanwhile, L. piperis was reported to cause damage to branches and stems of plants up to 42.8% [15]. Therefore, to obtain healthy and high-producing black pepper plants, the existence of main pest should be controlled below its economic threshold.

Pests are generally controlled using synthetic insecticides. However, the continued use of synthetic pesticides can cause the environmental degradation and pest resistance [16]. In this regard, it is necessary to apply other comparative technologies that are effective in controlling pests in black pepper plants as well as being friendly to the environment and surrounding living organism, one of which uses biopesticides and botanical pesticides.

B. bassiana is an entomopathogenic fungus with the potential to be widely used as a biological control of plant pest and considered as one of the alternatives to protect plants that are efficient and
environmentally friendly. *B. bassiana* is one of major fungal pathogens that can be exploited for biocontrol, mainly because of their ubiquitous, cosmopolitan distribution and easy mass production using artificial media [18]. This fungus grows naturally in the soil and causes white muscardine disease. Moreover, *B. bassiana* produces spores that are resistant to extreme environmental influences. Species of the genus Beauveria (Moniliales; Moniliaceae) have been reported to produce secondary metabolites such as bassianin, bassiicridin, beauvericin, bassianolide, beauverolides, tenellin and oospore in which can cripple and cause insect death [1,2,3]. *B. bassiana* is reported to be effective in suppressing mite pest populations by 80-100% [4] and *D. hewetti* up to 93% [5].

Insects infected with *B. bassiana* are covered in white hyphae and their bodies hardened or mummified. In Indonesia, *B. bassiana* has been proven capable of attacking and killing *Helopeltis antonii* [6]. Laboratory result showed that the use of alkyl aryl alkoxylate and oleic acid adhesives against *B. bassiana* caused the highest mortality in *H. antonii* by 90%, whereas the addition of alkyl glycerol phthalate adhesive could kill 88% of the insect. Meanwhile, the addition of 1 ml / l alkyl glycerol phthalate adhesive into the *Verticillium lecanii* conidia suspension before application can increase the effectiveness of the fungus up to 20% [7].

Basically, botanical pesticides use plant secondary compounds as their active ingredients. These compounds have function as repellents, attractors, pest killers, and as appetite inhibitors for pests. Several examples of plant secondary compounds classified as insect repellents are geraniol and citronellal, both of which are contained in citronella oil (*Andropogon capillaris*) [17]. These two compounds are reported to be able to resist various types of mosquitoes. Five constituents were detected in essential oils derived from clove oil by GC-MS. The main constituents are eugenol (88.61%) and eugenol acetate (8.89%) [19]. Eugenol compounds contained in clove oil (*Syzygium aromaticum* L.) are reported to be able to repel *Sitophilus zeamais* Motsch [8], mites that attack livestock, *Dermanyssus gallinae* (De Geer) [21], and parasites in cattle, *Iodesricinus* (L) [9].

One of plant secondary compound that is attractive to insects is methyl eugenol contained in *Melaleuca brachetata*, plant oil that is able to attract male fruit flies (*Dacus dorsalis*). This fly attacks various types of fruit plants such as guava, jackfruit, starfruit, and mango. The secondary compound that act as an insect killer is nicotine contained in tobacco plants (*Nicotiana tabacum*). This compound effectively kills *Clavigralla mentoscollis* (Stat) and *Riptortus dentipes* (Fab.) [10]. Another secondary compound is pyrethrum contained in the pyrethrum, *Chrysanthemum ceanerarum*. This compound has been proven to effectively kill several warehouse pests such as *Sitophilus granarius* (L) [11], *Rhysophertadominica* (F) and *Tribolium confusum* (DuVal) [12]. Azadirachtin compounds from neem plant (*Azadirachta indica* A Juss) effectively inhibit the feeding of Lepidoptera larvae [13].

Given the magnitude of the role of this fungus and secondary compounds, bioassays are needed to test the formula of *B. bassiana* and botanical pesticides to control black pepper pests. This research aimed to find out the efficacy of *B. bassiana* and botanical pesticides to *D. piperis* and *L. piperis* by performing in vitro and in vivo test. The results of the study are expected to support the provision of healthy pepper seeds as well as to increase black pepper production.

2. Materials and methods

2.1. In Vivo Test of *B. bassiana*

The experiment started by multiplying *B. Bassiana* as a starter on Potato Dextrose Agar (PDA) media, and then propagated using corn media for 2 weeks. Afterwards, the fungus was inoculated into carrier material sconsisted of corn flour, rice and granulated sugar in a certain proportion and stored at room temperature for 1 month until it was ready touse.

The ideal carrier composition of *B. bassiana* was corn flour, rice flour, and flour granulated sugar. Corn flour and rice flour serve as a medium to grow fungi shortly after inoculation was carried out so that the fungus can grow and develop optimally. Meanwhile, granulated sugar acts as the main energy source for the fungi shortly after the formula was dissolved in water and ready for use. The carrier
inoculated with *B. bassiana* solution using a dry spraying system. The carrier was then placed in a circular container and rotated using an electric motor (Figure 1). When the motor was turned on, the container will rotate and at the same time a solution containing *B. bassiana* fungus spores was sprayed. The process of rotating the container causes the fungus inoculation distributed evenly and the the carrier forms measuring wad of 0.3 to 0.5 cm. The solubility test of the formula in water showed that the carrier could be completely dissolved so that when used it will not clog the nozzles (Figure 2). The powder formula was then ready to be tested for its pathogenicity to the main black pepper pests.

![Figure 1. The carrier was ready to be inoculated with *B. bassiana* (A), Formulation of *B. bassiana* using a dry spraying system (B)](image)

Laboratory assay was carried out by dissolving 5 g, 10 g, 15 g, 20 g and 25 g of the formula into 1000 ml of water and incubated for 1 hour. The solution applied by spraying on 10 insects tested and repeated 5 times. Observations were carried out every week for 5 weeks after the application. Dead insects infected by entomopathogen *B. bassiana* characterized by the growth of white hyphae.

Field-testing was carried out in the experimental station of Bangka Belitung Assessment Institute for Agricultural Technology (AIAT). Shoots / flowers / young black pepper fruit covered with black gauze, and then each gauze was inserted 10 test insects (*L. piperis* or *D. piperis*) (Figure 3). Each plant was

![Figure 2. Powder formula of *B. bassiana* (A), The formula perfectly dissolved in water (B)](image)
hung with three gauzes, each of which contained one type of test insect. The treatment was repeated 10 times. Application was completed by spraying formulas that has been dissolved in water. The amount of spray solution used in this test was about 2 liters per plant and sprayed evenly on all canopies of black pepper plants (Figure 4).

![Figure 3. Inserting the test insects into the cage](image1)

2.2. **In Vivo Test of Botanical Pesticides**

The laboratory bioassay was conducted to determine the effective dose of botanical pesticides in killing test insects. The concentrations tested were 0 cc/l, 2.5 cc/l, 5 cc/l, 10 cc/l, and 20 cc/l of water and repeated 2 times.

The field tests were conducted at the Experimental station of the Bangka Belitung AIAT. Preparation has been conducted to test *B. bassiana* to *L. piperis* and *D. hewetti*. Shoots / flowers / young pepper fruit covered with black gauze, then 10 insects were put in each gauze. Each plant was hung with three cages containing 10 test insects of *L. piperis* and *D. hewetti*. Each treatment was repeated 10 times. Application...
was completed by spraying formulas that has been dissolved in water at the most effective concentration of test the results in the laboratory. The amount of solution used in this test was 2 liters per plant and sprayed evenly up to the maximum black pepper canopy at a height of 2 m. The parameters observed were pest mortality at 1, 2, 3, 4, 5 days after application.

3. Results and discussion

3.1. Preliminary Test of B. bassiana

Bioassay of B. bassiana formula on adults of L. piperis, and D. piperis was carried out in the laboratory. The results presented in Table 1 and Table 2 below:

| Dosage (gram) | Observation time (weeks) | 0 | I | II | III | IV | V |
|---------------|--------------------------|---|---|---|----|----|---|
| 5             |                          | 0 | 0 | 0 | 0  | 7.0| 10|
| 10            |                          | 0 | 0 | 0 | 0  | 10 | 30|
| 15            |                          | 0 | 0 | 0 | 0  | 30 | 30|
| 20            |                          | 0 | 0 | 3 | 0  | 47 | 73|
| 25            |                          | 0 | 3 | 3.3| 47 | 80|

| Dosage (gram) | Observation time (weeks) | 0 | I | II | III | IV | V |
|---------------|--------------------------|---|---|---|----|----|---|
| 5             |                          | 0 | 40| 40| 40 | 40 | 40|
| 10            |                          | 0 | 37| 37| 37 | 37 | 37|
| 15            |                          | 0 | 47| 47| 47 | 47 | 47|
| 20            |                          | 0 | 70| 70| 70 | 70 | 70|
| 25            |                          | 0 | 83| 90| 90 | 90 | 90|

Preliminary test showed that the effect of B. bassiana on L. piperis was shown 4 weeks after application and a dose of 25 g was the most effective dosage. Meanwhile, in D. piperis, mortality of D. piperis was shown 1 week after application and the dose of 25 g was the most effective dosage (Table 2 and 3).

3.2. Field Test

Based on the preliminary test results, the dosage for the field of B. bassiana formula was determined to be 20 gr/l. The test results showed that mortality of L. piperis and D piperis after fungi application was very low, different from the results obtained in the preliminary test in laboratory. The mortality of L. piperis and D. Piperis at the 4th week of observation were 20 and 33.3%, respectively. The low mortality of the test insects might be due to the treatment carried out during the dry season, so that B. bassiana spores did not last long and die from exposure to sunshine. Therefore, to obtain maximum results, the application of this formula should be conducted several times with a grace period of 1 or 2 months. Continuous treatment expected in the long term to be able to suppress populations and pest attacks in black pepper plants. The environment have big impact to B bassiana effectiveness, most conidia can disintegrate quickly in the environment and only small proportions will succeed in infecting new hosts [20].
3.3. Formulation and Preliminary Test of Botanical Pesticides

Botanical pesticides formulated by dissolving clove oil and citronella oil in a solvent mixed with carriers. The solubility formula was very good, in which all solutions can dissolve completely in water. The results of the chemical analysis revealed that the formula contained 5.63% eugenol, 0.17% Citronellal, and 0.014% Geraniol.

Preliminary test formula of Botanical pesticides (Figure 4) has not shown satisfactory results because the death of test insects still ranges from 20 to 40%. Therefore, pesticide was reformulated with higher active ingredients, i.e. 8.80% eugenol, 1.68% Citronellal, and 0.54% Geraniol. Further tests revealed that the formula produced a significant mortality of *D. piperis* but not in *L. piperis*. The concentration and percentage of mortality listed in the following tables 3 and 4:

| Table 3. Average mortality of *L. piperis* after botanical pesticide application(%) |
|-----------------------------------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| Concentration (cc/l) | Observation day of .... | 1   | 2   | 3   | 4   | 5   | 6   |
|---------------------|--------------------------|-----|-----|-----|-----|-----|-----|
| 0                   | 0                        | 0   | 0   | 0   | 0   | 0   | 0   |
| 2.5                 | 0                        | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| 5                   | 10.3                     | 10.3| 10.3| 20.3| 20.3| 20.3| 20.3|
| 10                  | 47.3                     | 47.3| 50  | 57.3| 57.3| 57.3| 57.3|

| Table 4. Average mortality of *D. piperis* after botanical pesticide application(%) |
|-----------------------------------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| Concentration (cc/l) | Observation day of .... | 1   | 2   | 3   | 4   | 5   | 6   |
|---------------------|--------------------------|-----|-----|-----|-----|-----|-----|
| 0                   | 0                        | 0   | 0   | 0   | 0   | 0   | 0   |
| 2.5                 | 70                       | 80.0| 80.0| 80.0| 80.0| 80.0| 80.0|
| 5.0                 | 70                       | 87.3| 87.3| 87.3| 87.3| 87.3| 87.3|
| 10                  | 80                       | 90.0| 90.0| 90.0| 90.0| 90.0| 90.0|
| 20                  | 100                      | 100 | 100 | 100 | 100 | 100 | 100 |

Preliminary test showed that the effect of botanical pesticide on *L. piperis* was shown on first week after application, the optimum dosage is 20 cc/l. Meanwhile, in *D. piperis*, mortality of *D. piperis* also shown 1 week after application and the optimum dosage is 20 cc/l, the mortality of tested insect can reach 100% (Table 2 and 3).

3.4. Field Test

Based on the preliminary test it was determined that the concentration of botanical pesticides for further research on *L. piperis* was 20 cc/l while that for *D. piperis* was 2.5 cc/l (Table 3 and 4). The field test results revealed that percentage of mortality of *L. piperis* at 7 days after application (DAP) was relatively low, reaching only 43.3%, while that of *D. piperis* at 1 DAP was 86.7% and reaching 100% at 2 DAP.

The low mortality of *L. Pipersis* due to the very strong skin of these insects that causes the active compounds of the pesticide were difficult to penetrate. In addition, the weather during conducting the experiment was warm with low humidity. These conditions accelerated the degradation of pesticides, so that the efficacy effect of the pesticide decreased.

The opposite situation occurred in *D. piperis* which in these same weather conditions, its physiology became not optimal because berries of the plant was quite rare. It made the insects were weaker compared to the conditions where the food was abundant. So that even though the efficacy of pesticides was also decreased, the toxicity to *D. piperis* remain high.
4. Conclusions and recommendations

Powder formula of *B. bassiana* at a concentration of 20 gr / l although it was able to kill the main pest of pepper, its toxicity in the field tends to be low. The test insect mortality at the end of the observation for *L. piperis* and *D. piperis* was 20 and 33.3%, respectively. In order to get maximum results, application should be repeated several times at intervals of 1 to 2 months.

The formula for botanical pesticides at the test concentration of 20 cc/l turned out still not very effective for controlling *L. piperis* because it was only able to kill 43.3% of the test insects. On the contrary, the pesticide turns out effective to control the pest that at the concentration of 2.5 cc /l and has the potential to be used to control the population of *D. piperis*.

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