SUSCEPTIBILITY OF GERMAN COCKROACH, *Blattella germanica* TO ORGANIC INSECTICIDES BASED ON THE ENTOMOPATHOGENIC FUNGI *Beauveria bassiana*

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Abstract. German cockroaches have been reported to have developed resistance to 42 active insecticide ingredients. One effort to minimize the development of resistance in German cockroaches is to use organic insecticides, one of which is a secondary metabolite from the entomopathogenic fungus *Beauveria bassiana* (B10). The purpose of this study was to determine the susceptibility of German cockroaches to secondary metabolites of *B. bassiana* B10 as organic insecticide. This study used an experimental method with a completely randomized design consisting of ten treatments, namely concentrations of 0, 0.1, 0.5, 1, 5, 10, 20, 30, 40, and 50% (v / w). Each treatment unit used ten male German cockroaches which were repeated three times. Mortality data were analyzed using ANOVA followed by Duncan's test at a significance level of $p < 0.05$. The results showed that the application of organic insecticide *B. bassiana* B10 was quite effective in controlling German cockroaches as indicated by the mortality of German cockroaches. The application of *B. bassiana* B10 secondary metabolites affect the mortality of German cockroaches with a concentration of 40 % with a mortality percentage of 50%.

Keywords: beauveria bassiana, controlling, entomopathogenic fungi, german cockroach, secondary metabolites

A. Introduction

The German cockroach, *Blattella germanica* is one of the four most common cockroach species infesting urban areas. Research shows that the infestation trend of the German cockroach ranges between 40-70%. In addition to being a vector of various organisms that cause gastrointestinal diseases in humans, cockroaches can also trigger allergic reactions and asthma [1]. Due to its harmful presence, especially in terms of health, population control efforts are necessary.

The most common control method used is synthetic insecticides; however, the prolonged use of synthetic insecticides has led to resistance in German cockroaches against various types of insecticide active ingredients. German cockroaches have been reported to be resistant to 42 insecticides [2]. One technique to slow down and minimize resistance is by using biological control agents, one of which is the entomopathogenic fungus *Beauveria bassiana*, which has been reported to be effective in controlling several types of insect pests [3]. The use of biological agents to control German cockroaches in sensitive areas such as hotel kitchens, restaurants, supermarkets/malls, food and feed industries, office buildings, public areas, transportation modes, as well as hospitals and healthcare facilities is safer compared to synthetic insecticides [4].

B. bassiana is an entomopathogenic fungus that has been widely reported as effective for controlling agricultural insect pests such as brown planthoppers, rice bugs, and pod suckers [5, 6, 7]. *B. bassiana* can be used in both its spore and secondary metabolite forms. However, based on studies, the use of secondary metabolites is more effective, has longer shelf life, and is not dependent on seasons or isolate sources [8]. The use of secondary metabolites from entomopathogens is more practical in terms of production, storage, and application, and is also more economical since the secondary metabolites can be diluted and require only a small amount [9].

The secondary metabolites of *B. bassiana* include bassianin, bassiacridin, beauvericin, bassianolide, cyclosporin A, oxalic acid, beauverolides, beauvericin, enniatins, isarolides, and bassianolide (which has insecticidal properties) [8]. The application of *B. bassiana* B16 secondary metabolites caused 52% mortality in *Nilaparvata lugens* with a time-to-death of 6.57 days after application [7]. Until now, the use of *B. bassiana* secondary metabolites has been more widely applied to control agricultural and plantation insect pests, while their use for controlling urban pests in Indonesia has been limited.

Secondary metabolites of *B. bassiana* have been reported to be effective as larvicides to control *Aedes aegypti* larvae in urban areas[10]. Therefore, the effectiveness of *B. bassiana* secondary metabolites should be tested against German cockroaches as an alternative method to minimize resistance in this species. The purpose of this study was to determine the susceptibility of German cockroaches to secondary metabolites of *B. bassiana* B10 as organic insecticide

B. Methods

1. Time and Place

This research was conducted at the Laboratory of Entomology and Parasitology, Faculty of Biology, Jenderal Soedirman University. The research was conducted from January-.August 2024.

2. Tools and Materials

Male German cockroaches of Vector Control Research Unit (VCRU) strain were obtained from Vector Control Research Unit School of Biological University Sains Malaysia. This VCRU strain has been reared in the Entomology and Parasitology Laboratory, Faculty of Biology, Jenderal Soedirman University, Purwokerto, since 2020. Secondary metabolites of *B. bassiana* B10 as organic insecticides were obtained from the Plant Protection Laboratory, Faculty of Agriculture, Jenderal Soedirman University, Purwokerto. Cat food as cockroach feed, aquades, vegetable oil, vaseline, and water.

The tools used in this research include a tube-shaped arena with sizes 25 L and 1 L, single chamber (tub) with size p x l x t = 40 x 25 x 15 cm, plastic bucket, small plastic pot, cardboard, plastic cups, rubber belts, rubber bands, gauze, cotton, oil containers, scissors, labels, bottle caps, spons, teapots, microscope, camera and stationery

3. Rearing of German Cockroaches

The rearing of German cockroaches was conducted in the Entomology and Parasitology laboratory following the method described by Ahmad & Suliyat [11]. The cockroaches were kept in a 25 L plastic container with dimensions of 27.5 cm in height and 30 cm in diameter. To prevent the cockroaches from escaping, the rim of the container was coated with a mixture of Vaseline and oil. Food and water were provided once a week on an ad libitum basis. The top of the container was covered with a thin cloth. Cat feed was used as the feed during the maintenance period.

4. Research Design

This study used an experimental method with a completely randomized design consisting of ten treatments, namely concentrations of 0, 0.1, 0.5, 1, 5, 10, 20, 30, 40, and 50% (v / w). Each treatment unit used ten male German cockroaches which were repeated three times. The parameters measured were German cockroach mortality percentage.

5. Efficacy Test of Organic Insecticide *B. bassiana* B10 Against Mortality of German Cockroach by Baiting Method

Testing using the baiting method. Gel bait weighing 1 g contains secondary metabolites of *B. bassiana* with concentrations of 0%, 0.1%, 0.5%, 1%, 5%, 10%, 20%, 30%, 40%, and 50% (v/b).

German cockroaches were fasted from food for 3x24 hours in the test arena. After that, 1 g of treatment bait was inserted into the test arena. Mortality observations were carried out every 24 hours. The percentage of cockroach mortalities was calculated using the following formula

$$M = \frac{a}{b} \times 100\%$$

Note: M = mortality, *a* = number of dead test animals(individuals), and *b* = number of test animals observed (individuals)

6. Data Analysis

Mortality data were analyzed using ANOVA followed by Duncan's test at a significance level of $p < 0.05$.

C. Results And Discussion

The results of secondary metabolite testing of the entomopathogenic fungi B. *bassiana* on German cockroach mortality are shown in Fig. 1 below.

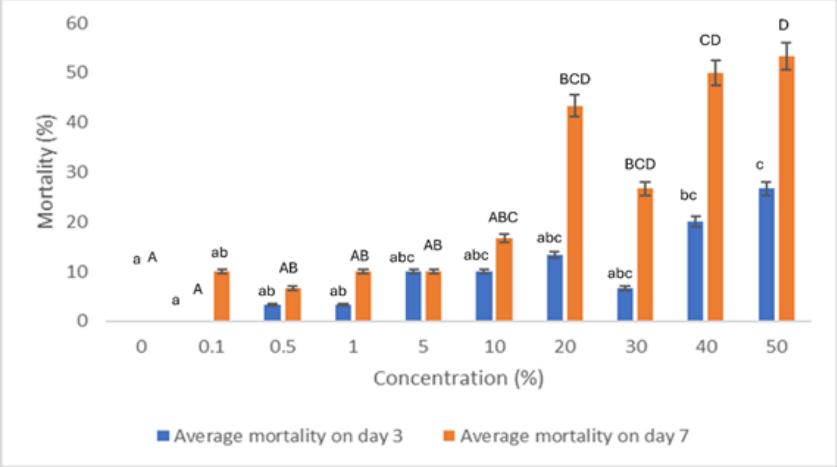

Fig 1. Effect of secondary metabolites of entomopathogenic fungi *B.bassiana* on mortality of german cokhroaces (%)

The results showed that the treatment of secondary metabolites of *B.bassiana* fungus affected the mortality of German cockroaches ($p<0.05$) both on the third and seventh days after exposure. Concentrations of 40% and 50% (v/w) caused mortality on the third day of 20%, respectively, and could cause mortality of 50 and 53.3% on the seventh day. As seen in Figure 1, the higher the concentration of *B. bassiana* secondary metabolites, the higher the percentage of mortality of German cockroaches. The variation in concentration can affect the toxicity and the number of German cockroach mortality. This indicates that the mechanism of action of secondary metabolites works slowly and does not immediately cause mortality after

application. The treatment of secondary metabolites of *B. bassiana* shows that German cockroach mortality occurs gradually as the observation time increases.

Differences in mortality in German cockroaches occur due to differences in the concentration levels of secondary metabolites of *B. bassiana* that are applied. Mortality will occur faster at high concentrations because more active ingredients enter the insect's body so that the toxin can work faster because the content of the active ingredients is increasing, thus increasing effectiveness and inhibiting growth and causing insect death faster [12]. This statement is in accordance with [13] which states that the treatment of secondary metabolites of *B. bassiana* fungi against *Toxoptera citricida* test insects can cause mortality above 70% at a concentration of 12 ml/L, and mortality is caused by toxins that can cause disturbances in the insect hemolymph system. The higher the concentration of secondary metabolites of *B. bassiana*, the faster the German cockroaches will die [14].

Secondary metabolites of *B. bassiana* have a mode of action as stomach poisons. The mechanism of absorption of insecticides, in addition to through the cuticle, can also be through the digestive tract, which is the effect of stomach poison, where food consumed then enters the midgut, which consists of two parts, namely the gastric sac, which secretes digestive enzymes and the ventriculus [15]. The absorption of organic insecticides that have a stomach-toxic effect mostly occurs in the midgut. The midgut is the main organ of digestion in insects because this part of the digestive tract is the organ of nutrient absorption and secretion of enzymes. If enzyme secretion is disrupted, the food digestion process will also be disrupted, so the insect will lack energy and eventually die. Active compounds contained in organic insecticides accumulated in the body of cockroaches will act as toxicants. The insect circulatory system (hemolymph) will distribute this toxicant to all body cells. The mechanism of killing cockroaches depends on the active compounds contained in organic insecticides [16].

Each entomopathogenic fungi produces metabolite compounds that act as toxins. This greatly affects the effectiveness of entomopathogenic fungi in infecting insects. *B. bassiana* is one of the toxin-producing fungi that is deadly to host insects [13]. Secondary metabolite compounds could increase pH and blood clots so that mechanical hemocoel damage occurs, such as the digestive tract, muscles, nervous system, and respiratory system, so that in a certain period of time, it causes death in test insects [17]. These processes cause sterility, paralysis, and death of infected insects. Secondary metabolite compounds produced by entomopathogenic fungi at high concentrations can reduce insect-feeding activity and cause death. Beauvericin compounds are the most influential in killing these insects because they are cytotoxic and ionophoric. *B. bassiana* produces a toxin called beauvericin. This toxin can cause disturbances in the function of hemolymph and insect nucleus, resulting in swelling accompanied by hardening of the body of infected insects. Apart from contact, *B. bassiana* can also infect insects through inoculation or feed contamination. Each insect infected with *B. bassiana* will effectively become a source of infection for healthy insects around it [18].

D. Conclusion

German cockroaches are susceptible to the secondary metabolites of *B. bassiana*. The application of *B. bassiana* secondary metabolite affect the mortality of German cockroaches with a concentration of 40 % with a mortality percentage of 50%. Environmentally friendly control of German cockroaches is highly recommended to be applied in sensitive areas such as the kitchen area. The main advantages of using biological agents in vector control are that they can specifically target pests, are safe against non-target organisms, do not cause pathogenic effects on the environment and human health, and can be used to control vectors that experience resistance to conventional insecticides, so they are very compatible with the ideal components of integrated vector control management.

E. Acknowledgement

Thank you to Jenderal Soedirman University for the BLU research funding for the 2024 applied research scheme with contract number: 26.340/UN23.35.5/PT.01/II/2024

F. References

It contains only a list of related literature cited by the authors in the paper. The reference list should be written in Vancouver styles.

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