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# Auto Dissemination Effect from Female German Cockroach Exposed by *Metarhizium anisopliae* and *Beauveria bassiana* to Male German Cockroach

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## ABSTRACT

Cockroach control should be based on Integrated Vector Pest Management connected to healthy food. This research investigated the auto dissemination phenomenon from infected females to males by the mating process. The method used is a randomized design experiment with 4 treatments and 5 replications. The result showed that the auto dissemination effect occurred from infected females to males during the mating process. The highest concentration caused a higher effect while *M. anisopliae* has a potential effect to control effective and safe cockroaches.



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## 1. INTRODUCTION

Development of science and technology today has helped to overcome the environmental problems of controlling the use of biological agents, such as the use of fungi and bacteria that are pathogenic, and other insects that can control *Blatella germanica* cockroaches (Zurek *et al.,* 2002). According to Greathead (1992), a biological control program using natural enemies and pathogens organisms to environmentally friendly pest control numbers. The effort is expected to be stabilizing agriculture and safe for human life.

Currently for Integrated Vector Control attempted to use entomopathogen fungi (Maina *et al.,* 2018). Entomopathogen fungi commonly used as *Beauveria bassiana*, *Verticillium lecanii* and *Metarhizium anisopliae* (Mascarin & Jaronski, 2016; AIAVO, 2018; Vivekanandhan, 2022). *M. anisopliae* can infect several insects of the Order Coleoptera, Lepidoptera, Hemiptera and Isoptera (Khun *et al.,* 2021; Ullah *et al.,* 2022). Spreading of the fungi can be horizontal or vertical. To reduce the population in the houses, *M. anisopliae* and *B. bassiana* were infected to females, and afterward observe influence on male cockroach cockroaches when they were mating. This phenomenon may reduce the population of cockroaches in the house.

## 2. METHODS

This research was conducted in FPMIPA Biology Education University of Indonesia department with an auto dissemination test by Complete Random Design (RAL) with 4 treatments and 5 replications. Treatment is the concentration of entomopathogen spores $10^7$, $10^6$, and $10^5$ spores per gram.

### 2.1. German Cockroach Rearing (*Blatella germanica*)

The test insect used in this study was a *Blatella germanica* collected from hotels, restaurants, trains, and buses. Insect rearing was done in the Ecology laboratory-UPI on condition of temperature 26°C ± 2°C, 70-80% relative humidity, and dark-light periods of 12: 12 (Berthold and Wilson, 1967). Adult *B. germanica* consisting of 2 pairs of males and females placed in plastic containers, then fed and given water wafers. Shelter was given pieces of paper for the cockroach's eggs to be placed in tissue paper. The setup is shown in **Figure 1**.

**Figure 1.** German cockroach rearing place.

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### 2.2 Cultivation of pure fungal isolates entomopathogen

To create a pure isolate of the fungus or reproduce entomopathogen *Metarrhizium anisopliae* against insects are taken from the German cockroach insects that attack each fungi. The method is as follows: All materials/substances making up this medium are weighed and measured according to the rule. Potatoes that have been washed clean cut small dice-shaped size 1x1 cm inserted into a 500 ml aquadest are heated to boil and continue for 1 hour (until the potatoes are soft and out of extract). Meanwhile, enter into the stem 300 ml aquadest and simmer until become liquid. During that time the volume of potato extract and order is maintained. Next sterile water added to extract the remaining potatoes and so while still heated to boiling. Potato extract liquid was obtained to be filtered separately in hot conditions. After that extract the filtrate into dextrose including potatoes and yeast extract, stirring until dissolved homogeneous then inserted to dilute the results of screening. Mixture of potato extract and so if the volume is less than 1 liter plus aquades up to 1 liter in volume. The mixture is heated over a low heat, stirring frequently, after all the soluble material, at the time of the fire is almost boiling off, the solution put into tubes and sterilized in the autoclave (121°C temperature, pressure of 15 PSI for 30 minutes). If using a powder-ready PDA, the following PDA's powder is ready to use as much as 39 grams dissolved in 1000 ml aquades, then heated to boiling in a bath, stirring frequently. The solution was inserted into a test tube each of 5 ml, then covered with cotton and aluminum foil in a petri dish, while the PDA was poured as much as high ¼ petri dish or 10 ml and then sterilized in the autoclave temperature of 121°C, the pressure of 15 PSI for 30 minutes. After a test tube then sterilize and slant it (Patrick *et al.,* 1993).

### 2.3 Auto Dissemination Test

In research, trials will spread horizontally infected female German cockroaches dipped by over 60 seconds at petri dish spore suspension-sided *M. anisopliae*. Then infected female cockroaches were placed in pairs of male guinea pigs in 5 replications, while the concentrations used were 107, 106, and 105. Ten visits resulted in the death of a cockroach (Hussein *et al.,* 2012). Data was analyzed by ANOVA followed by Duncan's multiple range test.

## 3. RESULTS AND DISCUSSION

Testing the effect of horizontal by female infection cockroaches then seen the effects on male cockroaches, as cockroaches are going to do male copulation with a female cockroach. Observational data horizontal effects of *Metarhizium anisopliae* and *Beauveria bassiana*-infected *B. germanica* can be seen in **Table 1**.

**Table 1.** Horizontal effects of *Metarhizium anisopliae* and *Beauveria bassiana* against uninfected *Blatella germanica*.

| Concentration of entomopathogen (spores/g) | *Metarhizium anisopliae* | *Beauveria bassiana* |
|---|---|---|
| $10^5$ | 0 a | 0 a |
| $10^6$ | 60 ± 2.5 b | 30 ± 3 b |
| $10^7$ | 100 c | 60 ± 5 c |
| Control | 0 a | 0 a |

*Note: The value in the column and lines are marked with the same letter means do not differ according to Duncan's multiple range test on the real level of 5%*

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In **Table 1**, it can be seen that both the treatment of *Metarhizium anisopliae* and *Beauveria bassiana* can affect male cockroach death. Cockroaches previously healthy males will even die because females infected by the male cockroach are to penetrate. Cockroaches females are the first infected and only male cockroaches infected fungi, this is one of the phenomenon insect fungi can infect horizontally (Mkiga *et al.,* 2020). This is as well as Sholtecresearch (2004) which examined the infected female mosquito then paired with healthy males and healthy male mosquitoes will be infected with *M. anisopliae* and *Beauveria bassiana* due process of copulation.

Fungal dissemination within a host population can occur within activities and movements of the host. The fungus can exploit host behaviour like gathering, communication, grooming (in social insects) and mating to spread through a host population (Roy *et al.,* 2006). The physiological state of the females and the natural display of behaviour at the time of the bioassays, it is assumed that the observed auto dissemination of *M. anisopliae* and *B. bassiana* from female to male *B. germanica* was the result of mating (Reyes-Villanueva *et al.,* 2011). This is deeply supported by the findings from experiment where none of the males that had stayed on the surface area where fungus-contaminated females had rested previously acquired an infection. The average age at death of fungus-infected mosquitoes was quite high when compared to mosquito survival in Scholte *et al*. This is probably due to the relatively low level (105) of inoculum transferred. From those cockroaches that were checked under the microscope for the presence of conidia, four out of one male contained conidia. It is thus likely that many males become contaminated, but that only a relatively low proportion of these males will succumb to the infection: in many cases the number of conidia was low, resulting in marginal infections that were successfully countered by the immune responses staged by the males.

**Figure 2.** Horizonal effects *Beauveria bassiana* against *Blatella germanica*.

In order to achieve the highest possible impact of the fungus on the mosquito population, it is desirable that other pathways besides the primary mode of (direct) contamination are utilized. The results of this study show that under laboratory conditions horizontal transmission can occur, which suggests that it may occur in the field. When these experiments were carried out, it was presumed that predominantly females would be infected directly from the indoor resting targets.

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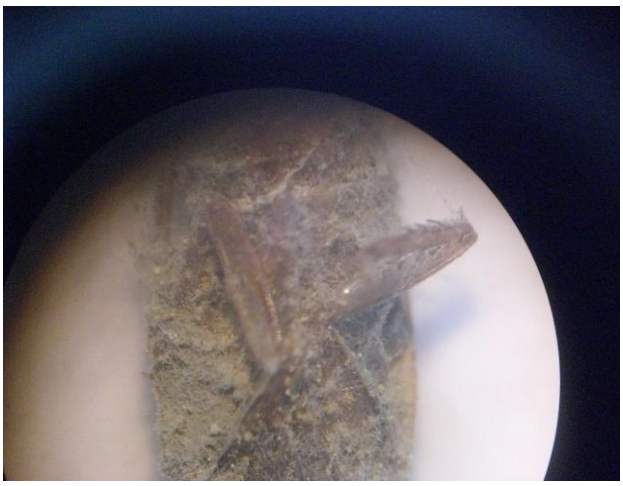

**Figure 3.** Horizonal effects *Metarhizium anisopliae* against *Blatella germanica.*

Based on the data and the picture above can be seen *that M. anisopliae* and *Beauveria bassiana* can be spread with the spread of auto dissemination from females to males. This could be a reference for controlling cockroaches in homes.

## 4. CONCLUSION

The highest concentration caused a higher effect while *M. anisopliae* has a potential effect to control effective and safe cockroaches.

## 5. AUTHORS' NOTE

The authors declare that there is no conflict of interest regarding the publication of this article. Authors confirmed that the paper was free of plagiarism.

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