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The Sublethal Effects of *Blatella germanica* Infected with *Metarizhium anisopliae* and *Beauveria bassiana* on Its Offspring

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ABSTRACT

The sublethal effect of *Blatella germanica* infected with *Metarhizium anisopliae*, *Beauvaria bassianna,* and *Spicaria* on its offspring has been carried out. It is known that the LC_{50} value of each fungus is 10^6. This study aims to determine the extent of the sublethal effect on the duration of ootheca formation, the number of ootheca, and the number of ootheca produced. The 'Dipping method' was used with three treatments and five replications. The results showed that the sub-lethal effect influenced the duration of ootheca formation. *Metarhizium anisopliae* and *Beauveria bassiana* affected the start of ootheca formation when compared to the control (25.95 days), the length of time needed for ootheca that produced nymphs in *Metarhizium anisopliae* and *Beauveria bassiana* exposure compared to the control (6 days) and the number of nymphs caused by *Metarhizium anisopliae* and *Beauveria bassiana* produced fewer nymphs than the control (29.25 days).

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1. INTRODUCTION

In developing countries like Indonesia, various diseases are easily transmitted and widespread. In addition to direct contact with disease sufferers, vectors are also one of the causes. Vectors of numerous diseases are usually animals. Insects are one of the animals that act as vectors of varied diseases.

Among the various insects, the German cockroach is one of the insect vectors of disease. The German cockroach (*Blatella germanica*) that produces many nymphs is resistant to diverse conditions and has the smallest body, so it can easily occupy hard-to-reach spaces. The German cockroach (*Blatella germanica*) can be a vector for several diseases in humans, including salmonella infection, dysentery, gastritis, and infection with other organisms that can cause stomach disorders by spreading through feces and secretions from the digestive tract (Anonymous, 2007). In addition, the German cockroach is also a host for a variety of worms and can also cause allergenic reactions in humans.

Biological control methods are increasingly considered as sustainable alternatives to chemical pesticides, especially in combating pests with high resistance levels such as the German cockroach. Entomopathogenic fungi, including *Metarhizium anisopliae* and *Beauveria bassiana*, have shown potential as biocontrol agents due to their ability to infect and kill insect hosts through fungal growth and toxin production. While the lethal effects of these fungi on cockroach populations have been widely studied, there is limited knowledge about their sublethal effects, particularly on the offspring of infected individuals. Understanding these sublethal effects could provide insights into the long-term population dynamics of *Blattella germanica* and enhance the efficacy of integrated pest management (IPM) strategies (Zimmermann, 2007; Meyling & Eilenberg, 2007). This study aims to investigate the sublethal effects of *Metharizium anisopliae* and *Beauveria bassiana* infections on the reproductive performance of *Blatella germanica*.

2. METHODS

The method used is experimental research conducted by manipulating the object of study and the existence of controls (Nazir, 1983).

2.1. Research Design

The research method used is a completely randomized design (CRD) because the experiment was homogeneous with controlled environmental factors. Five treatments for male cockroach imago and five repetitions occurred.

The number of repetitions is based on the method of Gomez and Gomez (1995), as follows:

$(txr) - 1 \geq 20$

$5\ r - 1 \geq 20$

$5\ r \ \geq 21$

$nr \ \geq 4{,}2$

Note: t= treatment; r= replication

With five fungal treatments, including one control and five replications, the research design used as follows: 5x5 =25 blocks, as below:

D4	C4	C3	B5	A5
E3	A4	D2	A2	A3
D1	B5	E2	A1	C1
D5	E5	D3	B3	C2
E1	B2	E4	B1	C5

Note:
A : Control, NaCl (0.85%)
B : Spore concentration 10^4 conodia/ml
C : Spore concentration 10^5 conodia/ml
D : Spore concentration 10^6 conodia/ml
E : Spore concentration 10^7 conodia/ml

2.2. Population and Sample

A population is a collection of individuals with predetermined qualities and characteristics. In this case, the population measured is male *Blattella germanica* at the PGSM RISET Laboratory, University of Education Indonesia.

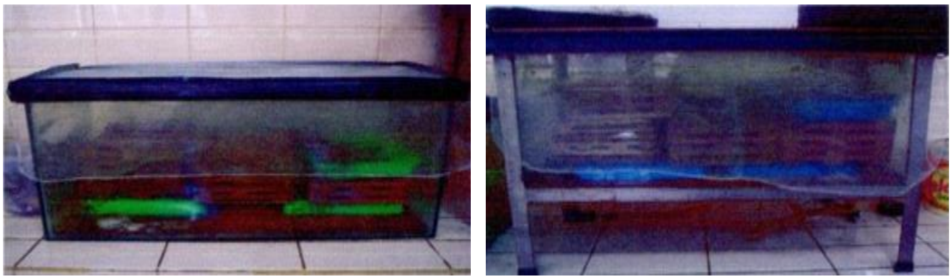

Figure 1. German cockroach cage side view

Figure 2. German cockroach cage top view

The samples observed were dead *B. germanica* infected with the entomopathogenic fungus *B. bassiana,* which was retrieved with the probability sampling technique based on possibility. Sample size were 4 individuals x 25 sample blocks = 100 individuals.

2.3. Research Location

This research was conducted in the Biology Research Laboratory of PGSM and the Microbiology Laboratory of FPMIPA Universitas Pendidikan Indonesia, Dr. Setiabudhi Street No.229 Bandung from September 2007 to January 2008.

2.4. Work Flow

a. Preparation of tools and materials,
b. Preparation of 1000 ml Potato Dextrose Agar (PDA) medium,
c. Sterilisation in an autoclave at 121oC pressure 17 psi 15-20 minutes,
d. Fungal subculture
e. Preparation of fungal spore suspension
f. Bioassay
g. Koch's postulates
h. Climatic Factor Measurement

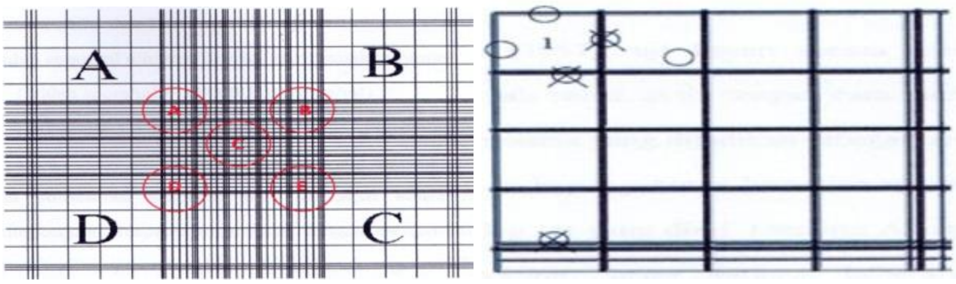

Figure 3. Haemocytometer count area
(source: Cell count using improved Neubauer Haemocytometer Prepared by Santiago Perez, 3/22/2006)

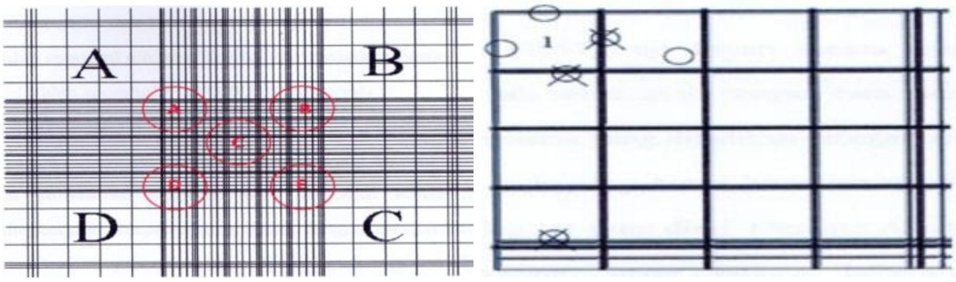

Figure 4. Spore count of each box
(source: Cell count using improved Neubauer Haemocytometer Prepared by Santiago Perez, 3/22/2006)

2.5. Data Processing Techniques

The number of *Blattella germanica* died was recorded for each treatment and its repetition. The total and average number of dead *Blattella germanica* were counted after 20 days of observation. Data analysis was carried out on the average mortality of *Blattella germanica* that died. To determine the LC50, PROBIT analysis testing was carried out with the POLO-PC LeOra Software 1987 program.

Data on environmental factors such as temperature, humidity, and light intensity were tested using the Linear Regression Test to determine its effect on *Blattella germanica* mortality. Santoso (2002) states that the R square ranges from 0 to 1. The smaller the R square, the weaker the relationship between the independent and dependent variables. This means that other factors affect the mortality of *Blattella germanica*. The test was using SPSS version 11.5 for Windows.

This test was carried out at concentrations below the LC50 and the development of the German cockroach will be seen when compared to the control.

3. RESULTS AND DISCUSSION

The results of the sublethal and vertical effects on *Blatella germanica* can be seen in Table 1-3.

Table 1. Sublethal and vertical effects of the fungus *Metarhizium anisopliae* on the Development of *Blatella germanica*

	10^5	10^4	Control
Ooteca age (day)	27,25 a	26,67 a	25,95 b
Ooteca formation	9,6 a	6.5 b	6 b
Number of nymphs	27,25 a	26,67 a	29,25 b

Note: Numbers in the same column marked with the same letter mean not significantly different according to Duncan's multiple range test at 5% significance.

Table 2. Sublethal and vertical effects of the fungus *Beauveria bassiana* on the development of *Blatella germanica*

	10^5	10^4	Control
Ooteca age (day)	29,8 a	26,67 a	25,95 b
Ooteca formation	9 b	7 a	6,5 a
Number of nymphs	23,67 a	26,5 a	29,25 b

Note: Numbers in the same column marked with the same letter mean not significantly different according to Duncan's multiple range test at 5% significance.

Table 3. Sublethal and vertical effects on *Blatella germanica* development

	Metarhizium 10^5	Beauveria 10^4	Metarhizium 10^5	Beauveria 10^4	Control
Ooteca age (day)	27,25 b	29,8 b	26,67 a	26,67 a	25,95 a
Ooteca formation	9,6 b	9.0 b	7,1 a	7.0 a	6,5 a
Number of nymphs	27,25 b	23,67 a	26,67 b	26,5 a	29,25 b

Note: Numbers in the same column marked with the same letter mean not significantly different according to Duncan's multiple range test at 5% significance.

From Table 2, it can be seen that *Beauveria bassiana* treatment affects the length of the ooteca period, the start of ooteca formation, the number of nymphs, and the number of offspring both at concentrations of 10^5 and 10^4 when compared to the control. *Metarhizium* *anisopliae* produced a longer ooteca period, ooteca formation, and produced death in a greater number of *Blatella germanica* offspring.

Effects on offspring were also reported by Nielsen (2004), who researched the impact of *Metarhizium* on houseflies, which can cause 50% mortality in fly larvae. Vertical infection also affects the time of ooteca formation, the duration of ooteca, and the number of larvae that reach instar three.

It was found that vertical transmission of entomopathogenic fungi in a population occurred, with the rate of vertical transmission depending on the age or stadia of the first-generation (parental) larvae at the time of treatment or exposure to the virus. That vertical transmission is transovum, characterized by the presence of traces of entomopathogens on the surface of the eggs. There is a positive correlation between the frequency of fungal presence in female moths and the mortality rate of fungus-induced progeny, supporting maternal transmission.

The mortality rate of progeny from egg batches placed during oviposition up to 3 days after oviposition in a fungal-contaminated environment, where epizootic had occurred, was higher than that from oviposition up to three days after oviposition in an uncontaminated environment (laboratory). The mortality rate did not differ between progeny from parents who survived fungal infection exposure during larval stadia and those from parents who were not exposed. Likewise, the mortality rate of progeny derived from eggs produced by parents from a contaminated environment and from parents from an uncontaminated environment (laboratory) during oviposition until three days after oviposition is placed in a contaminated environment, apparently gives a progeny mortality rate that is not different. It can be argued that in a contaminated environment, the egg mass will acquire a higher level of contamination if compared to contamination obtained vertically from infected (exposed) parents. Contamination of the egg mass due to its presence in a contaminated environment is a major component of the fungus transmission from generation to generation.

4. CONCLUSION

This study reveals that infections by *Metarhizium anisopliae* and *Beauveria bassiana* have significant sublethal effects on *Blattella germanica* and its offspring, primarily through vertical transmission mechanisms, such as transovum contamination. While fungal exposure affects ootheca development and larval survival, environmental contamination plays a larger role in increasing progeny mortality compared to direct vertical transmission. These findings suggest that entomopathogenic fungi can influence cockroach population dynamics not only by direct lethality but also through generational impacts, making them promising candidates for integrated pest management strategies

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6. AUTHORS' NOTE

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

7. REFERENCES

Barnett, H.L. dan B.B. Hunter. (1972). *Illustrated Genera of Imperfect Fungi*. Fourth edition. Macmillan Publishing Company, New York. Collien Macmillan Publishers, London.

Burges, H.D. (1970), *Microbial Control of Pests and Plant Diseases*, *1970-1980.* Academic Press, London.

Cindawati. (1996). *Pengendalian Jamur Patogen Fusarium oxysporum, Schlecht. dengan Menggunakan Jamur Antagonis Trichoderma* sp*. Persoon ex Fries di Laboratorium*. Laporan Kerja Praktek. Jurusan Biologi ITB, Bandung.

Departemen Pertanian Direktorat Jenderal Perkebunan Direktorat Bina Perlindungan Tanaman. (1994). *Pedoman Pengembangan Spicaria* sp. *Secara Sederhana*. Jakarta: Departemen Pertanian.

Departemen Pertanian Direktorat Jenderal Perkebunan Direktorat Bina Perlindungan Tanaman. (1994). *Pedoman Pengembangan Beauveria bassiana Secara Sederhana*. Jakarta: Departemen Pertanian.

Departemen Pertanian Direktorat Jendral Perkebunan Direktorat Bina Perlindungan Tanaman. (1994). *Pedoman Pengembangan Trichoderma* sp. *Secara Sederhana*. Jakarta: Departemen Pertanian.

Departemen Pertanian Direktorat Jendral Perkebunan Direktorat Bina Perlindungan Tanaman. (1994). *Pedoman Pengembangan Metarhizium anisopliae Secara Sederhana*. Jakarta: Departemen Pertanian.

Departemen Pertanian Direktorat Jendral Perkebunan Direktorat Bina Perlindungan Tanaman. (1993). *Baku Operasional Pengendalian Terpadu Hama Kumbang Kelapa (Oryctes rhinoceros L.)*. Jakarta: Departemen Pertanian.

Direktorat Bina Perlindungan Tanaman Perkebunan. (1993). *Baku Operasional Pengendalian Terpadu Penyakit Busuk Batang Panili Fusarium oxysporum* f. sp. *Vanillae* Wr. Jakarta: Departemen Pertanian.

Huffaker, C.B dan Messenger, P.S. (Eds). (1989). *Teori dan Praktek Pengendalian Biologis*. UI Press. Jakarta.

Irianti, A.P.P., Wagiman FX, Martoredjo, T. "Faktor-faktor yang Mempengaruhi Patogenitas *Beauveria bassiana* terhadap *Hypothenemus hampei*". *AGROSAINS*.

Proyek Pengembangan dan Penerapan Pengendalian Hama Terpadu. (2001). *Laporan Teknis*. Bandung: Departemen Pertanian.

Rollianty, V. (2002). *Pengaruh Ekstrak Biji Nimba (Azadirachta indica) terhadap Kadar Glukosa Darah Tikus Putih Rattus norvegicus Galur WISTAR jantan*. Sripsi Sarjana Biologi ITB, Bandung.

Soesanto, L. (2001). "Pemanfaatan Agensia Hayati dalam Mewujudkan Keseimbangan Ekosistem Pertanian". Dalam *BIOSAINS* Unsoed. (2001). Purwokerto Jawa Tengah.

Suhajati. (1996). *Jamur Kontaminan Dodol Garut*. Skripsi Sarjana ITB, Bandung.

Zimmermann, G. (2007). Review on safety of entomopathogenic fungi for non-target organisms and the environment. Biocontrol Science and Technology, 17(9), 879-920.

Zurek L., D. W. Watson, and C. Schal. 2002. Synergism between Metarhizium anisopliae (Deuteromycota: Hyphomycetes) and boric acid against the German cockroach (Dictyoptera: Blattellidae). Biological Control 23: 296-302.