

Entomopathogenic infection ability of low land rice (*Oryza sativa* L.) cultivation from Ogan Komering Ilir regency



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ABSTRACT

Entomopathogenic fungi are biological agents important in suppressing or regulating pest populations in agricultural ecosystems. This study aimed to determine the ability to infect entomopathogens from lowland rice (*Oryza sativa* L.) cultivation from the Ogan Komering Ilir District. This research was conducted in the Pest and Plant Diseases Laboratory, Faculty of Agriculture, the University of Palembang, from February to May 2022. The study used a Completely Randomized Design (CRD) method consisting of 5 treatments, namely PS1 = mortality of bait insects three days after infestation (dai), PS2 = bait insect mortality six dai, PS3 = bait insect mortality nine dai, PS4 = bait insect mortality 12 dai, PS5 = bait insect mortality 15 dai and five replicates. This study concluded that the entomopathogenic fungus found in lowland rice (*O. sativa* L.) from Ogan Komering Ilir Regency was *Beauveria bassiana* and could infect the highest bait insect mortality on day nine after infestation.



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Introduction

Microorganisms in the plant rhizosphere play a role in plant growth¹ and provide insect pathogenic fungi or entomopathogens^{2,3}. Entomopathogenic fungi are widely used as biological control agents^{4,5} because they are environmentally friendly and can survive unfavorable environmental conditions⁴. In addition, entomopathogenic fungi have a high reproductive ability in a short life cycle. Using entomopathogenic fungi to control insect pests in the field can reduce the use of chemical insecticides and protect biodiversity in ecosystems⁶⁻⁸ and as a biopesticide in sustainable integrated pest management⁹⁻¹¹. Entomopathogenic fungi have over 100 genera and 750 species with a wide host range⁸. Several soil-derived, including *Penicillium*, *Paecilomyces*, *Trichoderma*¹², *Aschersonia*, *Aspergillus* sp, *Beauveria bassiana*, *Cordyceps*, *Fusarium* sp, *Hypocrella*, *Metharizium* sp, *Ophiocordyceps*, *Penicillium* sp¹³⁻¹⁷. Efforts to exploit the ability of entomopathogenic fungi to parasitize their hosts can use bait insects⁸.

Researchers have conducted many studies and evaluations using the insect bait method to obtain entomopathogenic fungi from soil, including the bait insects *Galleria mellonella* (Lepidoptera: Pyralidae), *Omphisa fuscidentalis* (Lepidoptera: Pyralidae), *Tenebrio molitor* (Coleoptera: Tenebrionidae)^{8,18-21}. Important factors in describing entomopathogenic fungi include spore and colony morphology characteristics, extracellular proteins, pathogenicity, growth and nutrient availability⁸.

Efforts to increase rice production in Ogan Komering Ilir Regency include suppressing pest attack populations which can reduce the quality and quantity of rice production. The use of biological agents in the form of entomopathogenic fungi is one of the solutions to reduce the use of chemicals in controlling paddy rice pests. Therefore, it is important to know the biological agents contained in paddy rice soil in Ogan Komering Ilir as information for the management of lowland rice pest control using environmentally friendly and sustainable biological agents. According to¹⁷ the benefits that can be obtained from exploring entomopathogenic fungi are selecting microbes that can adapt to environmental changes. The aim of this study was to determine the ability to infect entomopathogen from lowland rice (*Oryza sativa* L.) cultivation from Ogan Komering Ilir District.

Method

This research was carried out in the Pest and Plant Diseases laboratory of the Faculty of Palembang University Agriculture from February to May 2022. Research using a completely randomized design (CRD) method consisting of 5 treatments and 5 repetitions (Table 1).

Table 1. Chart of non-factorial completely randomized design with 5 treatments and 5 replications

Treatment and repetition design				
PS1R1	PS2R2	PS5R2	PS2R3	PS3R2
PS3R3	PS4R3	PS2R5	PS2R4	PS2R1
PS4R2	PS3R4	PS3R5	PS3R1	PS5R3
PS5R1	PS1R2	PS5R4	PS5R5	PS1R5
PS4R5	PS4R1	PS1R4	PS4R4	PS1R3

Note:

PS1= mortality of bait insects 3 days after infestation (dai)

PS2= mortality of bait insects 6 dai

PS3= mortality of bait insects 9 dai

PS4= mortality of bait insects 12 dai

PS5= mortality of bait insects 15 dai

R1 = repetition 1

R2 = repetition 2

R3 = repetition 3

R4 = repetition 4

R5 = repetition 5

Soil sampling

Sample soil as a source of entomopathogenic fungi was taken from rice fields owned by farmers in Ogan Komering Ilir Regency. The location of the sample soil is determined, namely five sample points diagonally. Each soil sample was taken as much as 2 kg with a depth of 5-10 cm, then put into a plastic bag measuring 3 kg and labeled with information about the time and place of sampling. The soil samples were brought to the laboratory to be air-dried for 3 days until the soil became crumbly.

Preparation of treatment soil

The soil was mashed by sifting using a 600 mesh sieve, then put into a plastic tray measuring 35x28x7 cm² with a soil thickness of 3 cm. The plastic container containing the

sample soil was infested with 10 bamboo caterpillars (*Omphisa fuscidentalis*) as bait insects for entomopathogenic fungi. The bait insect method can be used to determine the ability of fungi to infect their hosts⁸. The bamboo caterpillars were spread evenly on the sample soil and then covered with a thin layer of soil, then sprayed with a little water using a hand sprayer to maintain soil moisture. The treatment tray was then covered with black puring cloth, tied with a rubber band, and then sprayed with water on top. Spraying on the surface of the tray is done every day to maintain soil moisture. The treatment soil was prepared in 5 trays as a treatment replication.

Bait insect mortality. The ability of entomopathogenic fungi infection can be seen from the mortality of bait insects, which are characterized by stiff and immobile bodies. Observations were made at 3; 6; 9; 12 and 15 dai. The percentage of mortality is calculated using equation²².

$$M = \frac{A}{A + B} \times 100\%$$

Information:

M = percentage of bait insect mortality

A = number of dead bait insects (heads)

Macroscopic entomopathogenic fungi

Observation of the body of the bamboo caterpillar as a bait insect was carried out simultaneously with the observation of mortality. The bodies of the dead caterpillars were observed, the symptoms were recorded and documented using a camera.

Data analysis

The ability of entomopathogenic fungal infection was determined by analyzing the mortality data of bamboo caterpillars using Analysis of Variance. If there is a difference between treatments, then it is continued with the smallest significant difference test (BNT) at a significant level of 5%. Macroscopic entomopathogenic fungi are described and shown in the form of images.

Results and Discussion

The results of observations of entomopathogenic fungal infections in bamboo caterpillar bait insects (*Omphisa fuscidentalis*) caused various symptoms and modes of infection. The initial symptoms are stiff bait insects, slow movement, and changes in body color, and then mycelia and conidia will grow. Observation of PS1 (3 dai) showed that the movement of the bait insects was stiff and there was a change in color on the body surface. The body color which was originally bright white changed to brownish-white, namely on PS2 (6 dai) and blackened on PS3 (9 dai) then entomopathogenic fungi, namely on PS4 (12 dai) will grow (Fig 1).

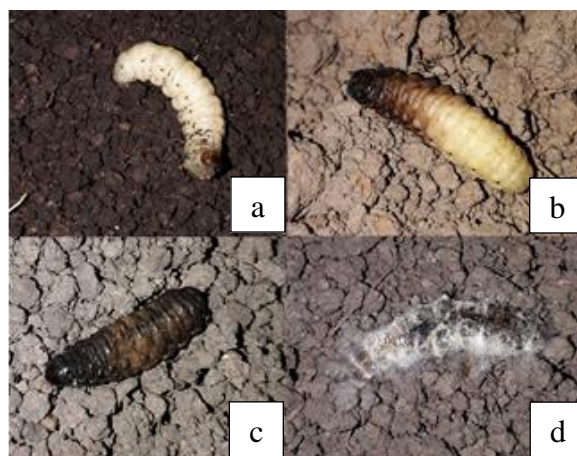


Fig 1. Symptoms of bait insect (*Omphisa fuscidentalis*) infected with entomopathogenic fungi (a. infection 3 dai; b. infection 6 dai; c. infection 9 dai d. infection 12 dai)

The second symptom and infection is a change in the color of the body of the bait insect to brown and then a mass of conidia will grow. The results showed that the first to be infected were the mouth, tail and sides of the body (*abdomen*) (Fig 2). Entomopathogenic fungi that infect insects do not have to be ingested but can pass through the cuticle in the presence of a spore tube or appressorium. The cuticle penetration of the host insect by the spore appressorium occurs due to mechanical stress and the activity of hydrolytic enzymes, assimilation, lipase/esterase enzymes, catalase, cytochrome P450s, proteases, chitinases and secondary metabolites that facilitate infection. The next process will occur colonization of fungal hyphae in hemoceol^{18,23,24}.



Fig 2. Entomopathogenic fungal infection of the mouth (a), abdomen (b) and tail (c) of the bait insect (*Omphisca fuscidentalis*)

The favorable environmental conditions and high virulence cause the development of entomopathogenic fungi so rapidly that they can cover the entire body of the bait insect. At 9, 12 and 15 dai observations, the mycelia and conidia mass covered the entire body surface of the bait insect. The color of the conidia mass ranged from white, green, brownish and yellowish (Fig 3). In bait insects, namely bamboo caterpillars (*O. fuscidentalis*), entomopathogenic fungi appear to be very favored by entomopathogenic fungi because they are softer and have a large body surface so they are easy to infect. Abbas stated that entomopathogenic fungi prefer fat or soft bodies for hyphal invasion²⁴.

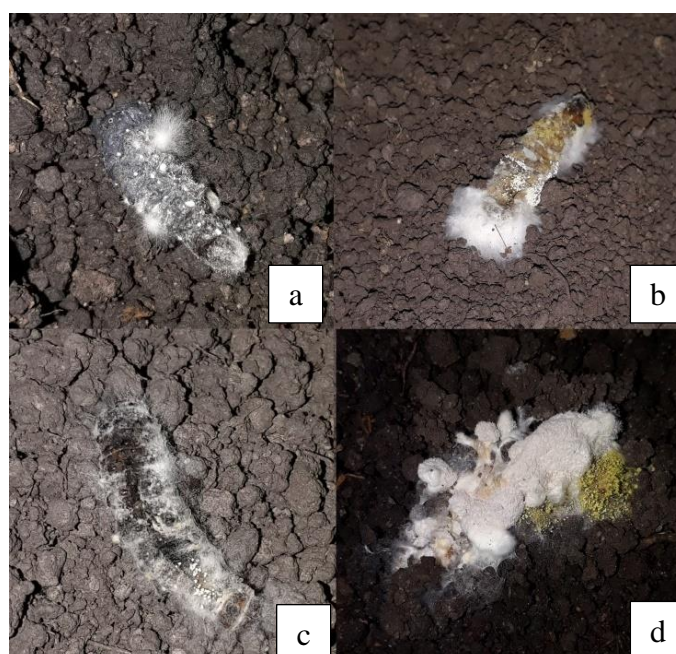


Fig 3. Conidia mass color ranges from white (a), green (b), brownish (c) and yellowish (d) in *Omphisca fuscidentalis* larvae

It is suspected that the entomopathogenic fungus that infects the bait insects is *Beauveria bassiana*. Gunawan et al. stated that insect pest larvae attacked by the fungus *B. bassiana* showed early symptoms of the slow movement, and then caused death. Insects that die are hard and stiff, but the shape of the insect's body does not change²⁵.

The results of the study showed that armyworms infected with the entomopathogenic fungus *Beauveria bassiana* on day 9 grew white fungal colonies²⁶, as well as Deka et al¹⁷ which stated that bait insects infected with entomopathogenic fungi on the 7th day died and on the 14th day white and moss-green hyphae grew. The results of the analysis of the variance of the treatment time of observation of entomopathogenic fungal infection on bait insects were very significant at the 5% and 1% test levels (Table 2).

Table 2. Analysis of variance in the ability of entomopathogenic fungal infections to bait insects

SV	df	SS	MS	F_value	p_value	
					0.05	0.01
Treatment	4	43.28	10.82	7.45**	2.87	4.43
Error	20	29.06	1.45			
Total	24					

Note: ** = very significant difference

The mortality of bait insects due to entomopathogenic fungal infection from lowland rice in Ogan Komering Ilir Regency in PS1 observations was still low. PS2 observations increased mortality until PS3 observations. On PS4 and PS5 observations there was a decrease in bait insect mortality. The PS1 treatment was not significantly different from PS5 and very significantly different from PS2, PS3, and PS4, while the PS2 treatment was not significantly different from PS4 and very significantly different from PS1, PS3, and PS5. PS3 treatment was significantly different in all observed treatments. The lowest mortality was seen in the PS1 treatment, which was 2.23 (22.3%) while the highest was in the PS3 treatment, which was 6.03 (60.3%) of the observed population average (Table 3).

Table 3. Mean mortality of bait insects due to entomopathogenic fungal infection with the time of observation

Observation time	Average \pm SD
PS1 (3dai)	2.23 \pm 1.39 a
PS2 (6dai)	4.15 \pm 1.25 b
PS3 (9dai)	6.03 \pm 0.46 c
PS4 (12dai)	5.12 \pm 0.54 b
PS5 (15dai)	3.45 \pm 1.81 a

Note: Numbers with the same letter in the same column are not significantly different (BNT5% = 1.590)

This shows that entomopathogenic fungal infection is related to the time to kill the bait insects. The results of the research conducted showed that the highest average mortality occurred in PS3 observations (9 dai) (Table 3). The results of the study²⁷, the fungus *B. bassiana* takes 3 weeks to infect *L. stigma* larvae. It show that entomopathogenic fungal infections are related to the time to kill bait insects. Several factors that cause differences in mortality due to the presence of susceptible bait insects so that they are easily infected, as well as environmental factors, especially temperature and humidity, can stimulate entomopathogenic fungi to grow on the bodies of bait insects. In this study, the bait insects used were *O. fuscidentalis* larvae which have a soft body so they are susceptible and easily infected by entomopathogenic fungi. The soil medium where the treatment was treated was also sprayed with a small amount of water using a hand sprayer to maintain the temperature and humidity of the soil. The fungal conidia

will germinate under special environmental conditions such as high humidity and then infect bait insects^{6,21}. Furthermore, soil organic matter content and feeding habits also affect the mortality of bait insects¹⁷. Research conducted by Gunawan²⁵ found that in vegetable fields that contain a lot of organic matter and rarely use synthetic pesticides, entomopathogenic fungi were found to be more abundant.

The highest bait insect mortality occurred at 9 days after the bait insect infestation, which was 60.3%. To cause death in pest insect larvae, some entomopathogenic fungi take several days after infecting pest insect larvae. Based on the results of research conducted by Masyitah et al²⁸ that the mortality percentage of *Spodopteralitura* larvae due to the application of the entomopathogenic fungi *M. anisopliae* and *B. bassiana* was high after 7 days and there were no deaths until the third day of observation. In addition, environmental factors at the time of the study were kept moist and at a temperature suitable for the development of entomopathogenic fungi. Also added by Abbas²⁴ that a suitable environment will affect the virulence of entomopathogenic fungi. During the study, it was observed that the cause of death of bait insects was due to infection by entomopathogenic fungi from the soil. Death is also caused by infection from dead or sick bait insects with high pathogenicity. According to Mora²⁹ entomopathogenic fungi must be able to produce enough spores to infect their hosts in an effort to maintain the population.

Conclusion

The results of this study found that the entomopathogenic fungus from lowland rice (*O. sativa* L) from Ogan Komering Ilir Regency was *Beauveria bassiana* and had an infectious ability that caused the highest bait insect mortality on day 9 after infestation.

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Author contributions

All authors contributed to the study's conception and design. Material preparation, data collection and analysis were performed by [**Haperidah Nunilahwati**], [**Dewi Meidalima**], [**Yani Purwanti**], [**Laili Nisfuriah**] and [**Chuzaimah**]. The first draft of the manuscript was written by [**Haperidah Nunilahwati**] and [**Dewi Meidalima**]. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.