Characterization of an Indigenous *Beauveria bassiana* from Samarinda and its In-Vitro Effectiveness on The Rice Bug (*Leptocorisa acuta* Thunberg.)

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Abstract. The rice bug (*Leptocorisa acuta*) is an important pest of rice plants that can significantly reduce rice production. One effective control technique is biological control, which utilizes the entomopathogenic fungus, *Beauveria bassiana*. This study aimed to test the mortality rate of *L. acuta* exposed to the entomopathogenic fungus *B. bassiana*. The study employed a completely randomized design with six treatments, each repeated ten times. The treatments involved varying concentrations of *B. bassiana* ($g.L^{-1}$): 0 (control 1), 5, 10, 15, and 20, and deltamethrin of 2 $mL.L^{-1}$ as control 2. The data obtained were analyzed using analysis of variance. Further examinations using the least significant difference (LSD) test at 5% were conducted for the data showing significant differences. The results showed that on the tenth day, the highest mortality percentage was found in the concentration of *B. bassiana* 20 $g.L^{-1}$ with a rate of 78%, whereas insecticide (deltamethrin) showed a mortality rate of 87%. The results of the probit analysis showed that the LT_{50} value at a concentration of *B. bassiana* 20 $g.L^{-1}$ reached on days 7^{th}; 15 $g.L^{-1}$ reached on days 8^{th}; 10 $g.L^{-1}$ reached days 10^{th}; 5 $g.L^{-1}$ reached days 11^{th}, with an LC_{50} value of 5.77 or at a concentration of 20 $g.L^{-1}$. The virulence of *B. bassiana* infected with *L. acuta* occurred on the second day after application.

Keywords: *Beauveria bassiana*, Rice Bug, Mortality, Insecticide

1. Introduction

Like most provinces in Indonesia, East Kalimantan prioritizes rice as its main food commodity, with the majority of its population relying on it as a staple food source. In 2023, rice productivity in East Kalimantan reached 3.98 tons per hectare, which is still below the national rice productivity average of 5.28 tons per hectare [1]. One significant factor affecting the decline in rice productivity is the attack by plant-disturbing organisms, particularly pests.

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The rice bug (*Leptocorisa acuta*) is one of the most common pests that attacks rice during the generative phase, occurring in both the dry and rainy seasons [2]. When rice bugs attack, they cause rice grains to grow imperfectly, leaving them empty or hollow. Yield losses due to rice bug infestations can reach up to 50% [3], and in cases of severe attacks with very high pest populations, production can be reduced by up to 100% [4].

In general, the rice bug control technique applied by farmers involves the use of synthetic chemical pesticides with the active ingredient deltamethrin [4]. The use of synthetic insecticides in pest control has caused several problems, such as the emergence of resistance to insecticides, pest resurgence, a surge in secondary pests, and a decrease in the natural enemies of pests. Based on this, there is a need for an alternative to pest control, such as the use of entomopathogenic fungi as biological agents for pest control.

There are several reasons for choosing entomopathogenic fungi for pest control. These include entomopathogenic fungi with a high reproductive capacity, a short life cycle, and spores that can survive for a long time in nature. The use of entomopathogenic fungi is also relatively safe, selective, compatible with various control components in integrated pest management (IPM), relatively easy to produce, and the possibility of causing resistance is very small. Beauveria bassiana (Balsamo) Vuillemin has the potential to control pests [5].

Beauveria bassiana is an entomopathogenic fungus that has been widely developed as a biological agent to control various types of insect pests [5]. The success of biological control using *B. bassiana* as a biological agent can kill up to 96% of all stages of insects and has a fairly wide host range, including the Orders Homoptera, Hemiptera, Orthoptera, Coleoptera, Lepidoptera, Diptera, Isoptera and Hymenoptera, and does not cause resistance in target insects [6].

In addition, *B. bassiana* can produce secondary metabolites that effectively suppress the intensity of diseases caused by soil-borne pathogens by up to 99% [6]. *B. bassiana* fungi are currently also easy to obtain because they are packaged as biological pesticide products both in online stores and sometimes in agricultural stores. However, the *B. bassiana* product that has been packaged for isolates used as an active ingredient is not clear from the isolate and does not come from Kalimantan. Indigenous *B. bassiana* isolates are more likely to be effective in controlling pests than non-indigenous ones because their ability to adapt is better than the conditions of the area where the pest is controlled.

Therefore, research on the characterization of *Beauveria Bassiana* Indigenous Samarinda from East Kalimantan and its effectiveness on rice bug pests in vitro needs to be conducted. This study aimed to analyze the virulence of the *B. bassiana* fungus in controlling rice bug pests and to analyze the concentration of *B. bassiana* and insecticides as a comparison that can control rice bugs.

2. Methods

The research was conducted over five months starting from sampling to the analysis process. Experiments on *B. bassiana* trapping, isolation, and in vitro effectiveness testing of *B. bassiana* against rice bugs were conducted at the Laboratory of Plant Pests and Diseases, Faculty of Agriculture, Mulawarman University, Samarinda.

The research began by conducting rhizosphere soil sampling around rice fields in Lempake Village, North Samarinda District, Samarinda City, East Kalimantan, which aimed to explore *B. bassiana* in the field using the baiting trap method with mealworms (*Tenebrio molitor*) in the laboratory.

Exploration was carried out by taking 1 kg of soil at depth of 0-10 cm and 10-20 cm, and the soil samples were air-dried until the soil reached the field capacity limit, or the soil was not too dry or too moist. The soil sample was then put into a plastic cup measuring 20 cm high and 10 cm deep as much as half the size of the cup, then put 10 mealworms into the plastic cup container, then the plastic cup was closed and given an oxygen hole and incubated in a closed room and observed for one week [7].

Mealworms were attacked by *B. bassiana* and showed symptoms of white hyphae threads from *B. bassiana* on the body of the larvae, then taken using an ose needle and isolated on Potato Dextrose Agar (PDA) media in Petridishes to grow isolates from *B. bassiana*.

2.1. Macroscopic and microscopic observations

Isolated *B. bassiana* was then observed, both macroscopically and microscopically, which was then identified based on identification guide books, namely the Introductory mycology book by Alexopoulos [8], Illustrated Genera of Imperfect Fungi by Barnett and Hunter [9] and Insect Pathology by Tanada and Kaya [10]. Macroscopic observations included colony color, shape, texture, and growth. Microscopic observations included septate or non-septate hyphae, hyphal growth (branched or unbranched), hyphal color (dark or transparent hyaline), conidia color (dark or transparent hyaline), and the presence or absence of conidia and conidia shape (round, oval, chained, or irregular).

2.2. Conidia Density Test

Conidial density was calculated using a Neubauer-type hemocytometer by taking 1 g of *B. bassiana* that had been isolated for 7 days and diluted using distilled water at a dilution of 10^{-5}. The conidial density was calculated using the Gabriel and Riyatno formula [11]:

$$C = \frac{t}{n \; x \; 0{,}25} \text{ x } 100\%$$

Description:
C = density of conidia per ml of solution
t = number of conidia counted in the observed sample box
n = number of sample boxes (5 large boxes x 16 small boxes)
0.25 = correction factor for the use of small-scale sample boxes in the hemocytometer.

2.3. Application of B. bassiana on rice bugs

The image of rice bugs as the test insect is a host of rice fields and *Echinochloa crus-galli* weeds taken from Lempake Village, North Samarinda District, Samarinda City, East Kalimantan. Insect capture was carried out using a net by swinging it on rice stalks and collecting 500 adult insects.

The application used *B. bassiana* was grown on 200 g of rice medium (3 weeks old) and mixed with 1 liter of water according to the experimental design. The application was performed by spraying the *B. bassiana* suspension five times for each repetition using a hand sprayer, with the suspension released every fifth spray, namely 4.8 ml. Observations of the test insects were carried out every day for 10 days, starting on the first day after application.

This experiment used a Completely Randomized Design (CRD) with six treatments each and was repeated 10 times. The treatments were as follows.

p_0 : without treatment (control 1)
p_1 : *B. bassiana* 5 $g.L^{-1}$
p_2 : *B. bassiana* 10 $g.L^{-1}$
p_3 : *B. bassiana* 15 $g.L^{-1}$
p_4 : *B. bassiana* 20 $g.L^{-1}$
p_5 : Deltametrin 2 $mL.L^{-1}$ (control 2)

The observation parameters in this experiment were mortality, lethal time 50 (LT_{50}), and lethal concentration 50 (LC_{50}) against *L. acuta*. The data obtained were analyzed using analysis of variance, and if there was a significant difference, then the least significant difference (LSD) test was continued at the 5% level.

$$\text{Mortality} = \frac{\text{number of dead insects}}{\text{number of insects tested}} \times 100\%$$

3. Results and Discussion

3.1. Morphology of Indigenous Beauveria bassiana from Samarinda

Identification of fungal isolates obtained from mealworms (*T. molitor*) suspected of being infected with *B. bassiana* was performed based on macroscopic and microscopic morphology. Macroscopic observation aims to observe the characteristics of the fungal part either directly or with the naked eye. Microscopic observation aims to observe the identified fragment, which is a specific component used to identify the fungus through microscopy [12].

The results of the observation that the macroscopic morphology on PDA media in petri dishes show that the surface of the colony has a mycelium shaped like fine white threads, a scattered and dense growth pattern, a round colony shape, a smooth texture, and a convex elevation (Fig. 1A).

Microscopic observation of *B. bassiana* stained with methylene blue on slides at a magnification of 40x showed branched hyphae and formed conidiogenous cells and long branches. Single-celled *B. bassiana* conidia were round, tending to be oval,

clear, or hyaline in color (Fig. 1B), comparable to previous studies showing that *B. bassiana* on media such as flour and white in color is a microscopic fungus with a body in the form of fine threads (hyphae) that can form colonies called mycelia and have a slightly round to oval conidia shape with a hyaline color [13].

The results of the conidia calculation on the hemocytometer that has been carried out on the *B. bassiana* fungus at a dilution of 10^{-5} obtained data on the calculation of the density of conidia of indigenous *B. bassiana* fungi from Lempake rice fields (Samarinda) had number of conidia of 7.05 x 10^5/g.

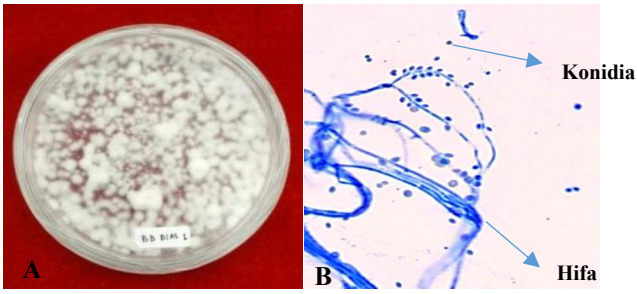

Fig 1. *Beauveria bassiana* colony macroscopically (A), and Conidia and hyphae microscopically (40x) (B)

3.2. Effect of *B. bassiana* on rice bugs

The results of laboratory observations on the application of *B. bassiana* to *L. acuta* in the treatment of 20, 15, and 10 g.L^{-1} began to show the death of rice bugs two days after spraying. Meanwhile, the treatment of *B. bassiana* at 5 g.L^{-1} began to cause insect death in rice bugs three days after application. In the treatment with deltamethrin insecticide 2 mL.L^{-1} (control 2) began to cause the death of rice bugs 1 d after application.

The death of rice bugs showed the same early signs after infection with *B. bassiana*, such as a stiff body and movement that seemed to slow down slightly. Dead rice bugs will be covered with white hyphae from *B. bassiana* either in certain parts of the body surface, partially, or almost completely (mummification) (Fig. 2). Hyphae appeared almost evenly throughout the body of the insect, and white hyphae appeared on the body of *L. acuta* [14].

On the fourth day after application, the mycelium from *B. bassiana* has begun to be seen at concentrations of 20 g.L^{-1} and 15 g.L^{-1}, while for concentrations of 10 g.L^{-1} and 5 g.L^{-1}, mycelium from *B. bassiana* can be seen on the fifth day after application. The mechanism of B. bassiana infection is that spores or inoculum of *B. bassiana* enter the body of the host insect through the cuticle, digestive tract, spiracles, and other holes [15]. Penetration of the insect cuticle involves mechanical pressure, releasing various enzymes (chitinase, lipase, protease, etc.) to degrade the cuticle and specific infection structures produced by hyphae (appressoria) that can penetrate host cells and reproduce [16]. This fungus then releases the toxin beauvericin [16], [17], which causes damage to insect body tissue so that in a matter of days the insect will

die. In the insecticide treatment, deltamethrin 2 mL.L^{-1}showed symptoms such as stiff body and lack of insect movement. Dead insects showed a slight blackish color change.

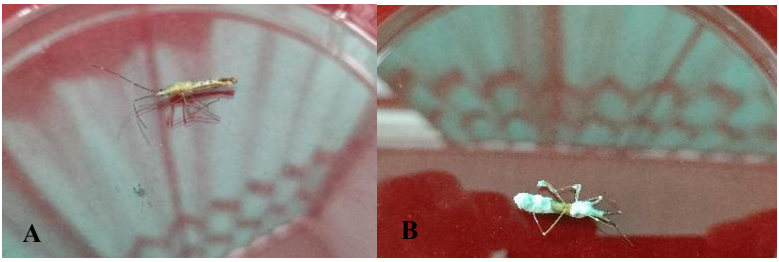

Fig 2. Rice bug that has not been covered with *B. bassiana* (A), and Rice bug that has been covered with *B. bassiana* (B)

B. bassiana is an obligate entomopathogenic fungus that relies on external sources for nutrition. One of the most notable features of this fungus is the appearance of white mycelia in the infected insects. Fungal growth occurs internally within the host, leading to the death of the insect and subsequent mummification, which is characterized by a hardened exoskeleton [10]. The white fungal mycelium begins to penetrate the cuticle out of the insect's body in the most easily attacked parts, namely, the body segments and mouthparts, and finally covers the body of the insect. The occurrence of *B. bassiana* fungal infection in insects causes the insect's body to be covered with white fungal mycelia and penetrates the larval integument [18]. The growth of the fungus causes the rice bug insect to exhibit slow, restless behavior, seizures, motor disorders, respiratory failure, and death. The fungus that develops in the body tissue of the insect forms conidiospores [10].

Treatment with *B. bassiana* at a concentration of 20 g.L^{-1} (p_4) resulted in the highest increase at 10 DAP, followed by concentrations of 15 g.L^{-1} (p_3), 10 g.L^{-1} (p_2) and 5 g.L^{-1} (p_1). In comparison, the insecticide (deltamethrin) at 2 mL.L^{-1} (p_5) killed the rice bugs faster and experienced the highest increase from the first day to the 10th *DAT*. This shows that *B. bassiana*, as a biological agent, cannot be compared with chemical insecticides in terms of the speed of killing rice bugs. However, when comparing its effect on the percentage of rice bug mortality, it almost equals the ability of chemical insecticides seven days after application, where this is achieved at the highest concentration of *B. bassiana* (p_3 and p_4) (Fig. 3). Therefore, the higher the concentration of *B. bassiana* used, the greater the effect on the mortality rate because the concentration of the conidia suspension affects the host to be infected.

3.3. Mortality Percentage

The results of the analysis of variance showed that the *B. bassiana* concentration treatment showed no significant difference from control 1 (without treatment) on the mortality of *L. acuta* pests at 1 *DAT (day after treatment)* and 2 *DAT*, whereas at 3

DAT, 4 *DAT*, 5 *DAT*, 6 *DAT*, 7 *DAT*, 8 *DAT*, 9 *DAT*, and 10 *DAT*, the results were significantly different (Table 1).

Table 1. Effect of *B. bassiana* application on the Mortality Percentage of *L. acuta* after application on the 1st to 10th day

Treatment	Day After Treatment (DAT, %)									
	1	2	3	4	5	6	7	8	9	10
B. bassiana Conc. (g.L-1)										
0 (p_0)	0.71a	0.71a	0.71a	0.71a	0.71a	0.71a	0.71a	0.71a	0.71a	0.71a
5 (p_1)	0.71a	0.71a	1.47ab	1.47ab	1.47a	1,97ab	2.67b	4.81b	5.86b	6.41b
10 (p_2)	0.71a	1.21a	1.47ab	1.47ab	1,97ab	2.41b	3.53b	5.59bc	6.51bc	6.84c
15 (p_3)	0.71a	1.21a	2.23b	3.37c	3.63bc	4.70c	6.49c	7.67c	8.08cd	8.32c
20 (p_4)	0.71a	1.21a	2.10b	2.10b	3.09c	4.90c	7.04cd	7.21cd	7.87d	8.81cd
Deltametrin 2 $mL.L^{-1}$ (P5)	3.92b	4.61b	4.84c	5.25d	6.91d	8.58d	8.70d	8.83d	9.19d	9.31d

Note: Data were transformed using the square root transformation of (x + 0.5).

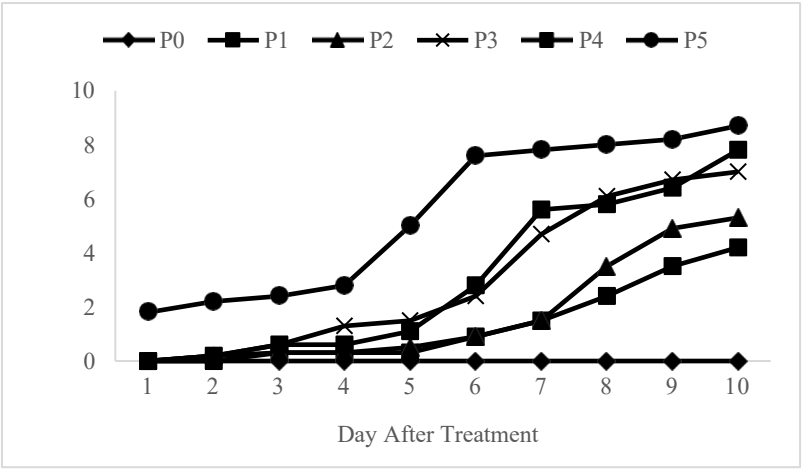

Fig. 3. Mortality (%) of *L. acuta* after *B. bassiana* application Treatment p_{0-4}: *B. bassiana* concentration of 0 (control 1), 5, 10, 15, and 20 $g.L^{-1}$, and p_5: Deltamethrin 2 $mL.L^{-1}$ (control 2).

Various studies have shown that *B. bassiana* is very effective in controlling pest populations in target species; however, its pathogenicity varies from species to species [19]. The application of *B. bassiana* effectively reduces the population of rice bug pests, thereby reducing attacks and yield losses. *B. bassiana* can be used as a biological (natural) pest control because it is effective in controlling insect pest populations at a mortality rate of 82.5% [20]. Furthermore, the highest mortality rate caused by *B. bassiana* can be attributed to the higher pathogenic properties of the insect, such as the presence of conidia adhesion, germination rate, growth conditions, or production of enzymes or secondary metabolites released by it [21]. The way *B. bassiana* conidia work when exposed to rice bugs begins with the presence of fungal

infection with the occurrence of conidia attachment, where the strength of conidia attachment is an important indicator of the virulence of entomopathogenic fungi. The attachment of fungal cells to the cuticle can involve specific ligand-receptors and/or nonspecific hydrophobic and electrostatic mechanisms [22; 23; 24]. If the adhesion strength of the entomopathogenic fungus weakens, it can cause the conidia to be washed off from the host, thereby preventing infection [25]. The fluctuation in virulence among different *B. bassiana* isolates may be caused by differences in the level of hydrophobicity or biochemical properties [21].

Conidia shaped like sprout tubes penetrate the integument mechanically and chemically in the insect's body tissue. This process is carried out using enzymes that decompose components of the insect cuticle [26]. These enzymes are hydrolytic enzymes, such as proteases, chitinases, and lipases, which are secreted by the fungus [27]. These enzymes hydrolyze the insect integument, which is composed of proteins and chitin [16].

The death of rice bugs infected with *B. bassiana* occurs because of the activity of toxins produced by the fungus in the insect's body. After penetration into the insect's body, the fungal hyphae develop and enter the blood vessels, producing several toxins such as oosporein, tenellin, bassianin, beauvericin, bassianolide, and oxalic acid [28], whose working mechanisms cause an increase in blood pH, blood clotting, and cessation of insect blood circulation. These toxins also cause mechanical damage to hemocoel tissues, such as the digestive tract, muscles, and nervous and respiratory systems. As a result of the entire process, rice bugs die.

3.4. *Lethal Time* 50 (LT_{50}) and *Lethal Concentration* 50 (LC_{50}) of *Beauveria bassiana* against the rice bugs

The toxicity of *B. bassiana* against rice buds was estimated by examining the LC_{50} or LT50 values. The LC_{50} value is the concentration that can cause the death of 50% of the insect pests tested in a particular observation, whereas the LT_{50} value is the time (hours) needed to kill 50% of the test insects. Based on the analysis results, the LT_{50} values of *B. bassiana* against rice bugs in the various treatments are presented in Table 2. The longest LT_{50} value was obtained in the *B. bassiana* concentration treatment of 3.5 x 10^6, namely 11.65 days (fiducial limit 10.38-13.07) and the fastest was obtained at a *B. bassiana* concentration of 14.0 x 10^6, namely 7.36 days (fiducial limit 7.02 - 7.73). This means that in determining the LC_{50} value in the *B. bassiana* concentration experiment of 14.0 x 10^6, it was able to cause the death of test larvae by 56% of the total population on the 7th day. Infected insects showed early symptoms before death, such as a stiff body and movement that seemed to slow slightly. The lower the LC_{50} value of the *B. bassiana* concentration, the higher the virulence of the concentration. The length of exposure to *B. bassiana* affects the LC_{50} value because the longer the exposure to *B. bassiana*, the smaller the LC_{50} value. This shows that the longer the exposure time to *B. bassiana*, the smaller the concentration required to kill 50% of the rice bug population. When associated with the concentration of *B. bassiana* conidia applied, a higher concentration of the extract accelerated the LT_{50} value.

Table 2. LT_{50} value of *B. bassiana* isolate treatment against rice bugs

Beauveria (conidia/ml)	LT_{50}		Chi Square	Slope
	days	Fiducial Limit		
3.5 x 10^6	11.65	10.38 - 13.07	16.91	3.53
7.0 x 10^6	10.19	9.36 - 11.10	27.80	3.23
10.5 x 10^6	7.51	7.11 - 7.92	12.64	3.89
14.0 x 10^6	7.36	7.02 - 7.73	21.75	4.19

The relationship between the y value (probit percentage of mortality) of rice bugs and the x value (log, concentration) of *the B. bassiana* suspension can be expressed by the linear probit regression equation y = 2.44x + 2.29. The results of LC_{50} probit analysis of *B. bassiana* against rice bugs are shown in Table 3.

The LC_{50} value of *B. bassiana* against rice bugs on the 7th day was 12.58%, with a fiducial limit of 10.80-14.65%. From the test results, the chi-square value (8.66) was obtained, which means that at each concentration of *B. bassiana* suspension used, the test insects showed homogeneous mortality data because the rice bugs used were quite uniform in age.

Table 3. LC_{50} value of *B. bassiana* isolate treatment against rice bugs

Day	*B. bassiana* concentration		Chi-Square	Slope
	Conidia per mL (x 10^6)	Fiducial Limit		
7	12.58	10.80 – 14.65	8.66	2.43
8	9.47	7.92 – 11.32	4.74	1.71

From the calculation results of the LC and LT values, the density of *B. bassiana* 10^6 conidia/ml has a high level of pathogenicity against rice bugs, so it is recommended for application in the field. During observations after the application of *B. bassiana*, sick and slow-moving rice bugs were observed.

The toxicity of a type of insecticide, including entomopathogenic fungi, can be expressed as LC_{50} the median lethal concentration) and LT_{50} (median death time), namely the concentration and time needed to kill 50% of the test insect population [29].

LT_{50} refers to the time required to cause death in 50% of the tested pest population. In pest control using *B. bassiana* against rice bugs, LT_{50} will provide information on the effectiveness of the time needed by the fungus to achieve a significant level of death in the test insects. The time required to cause death of larvae is determined by various factors, including pathogen virulence, host resistance, and environmental conditions [18].

The LC_{50} is the concentration of a substance or agent required to cause death in 50% of the tested pest population. LC_{50} provides information on the effectiveness of *B. bassiana* in killing rice bugs at a certain concentration. According to several studies, *B. bassiana* produces host-specific secondary metabolites that, in low amounts, can cause death of up to 50% [30, 31].

Inoculum concentration greatly affected the infection time of rice bugs. In addition to the inoculum concentration, the viability of conidia also affects the duration of infection. Conidial viability is the initial stage of fungal growth before penetrating the insect integument. The mortality rate was higher when the concentration was higher [32].

B. bassiana produces ribotoxins which have a deterrent effect on insects so that infected insects will reduce their feeding activity [33]. Ribotoxins also cause cytotoxicity by breaking down proteins in the host body and are utilized in fungal metabolism. In addition, ribotoxins inhibit the immune response of insects to facilitate infection by blocking host translation [34].

4. Conclusion

Beauveria bassiana isolate Samarinda is effective in controlling the rice bug *L. acuta* with a mechanism through conidia or *B. bassiana* inoculum that enters the host insect's body through the cuticle spiracles and other holes. The fastest LT_{50} value of *B. bassiana* at a P4 concentration of 20 g.L-1 reached 7 days and the LC_{50} value at a P4 concentration of 20 g.L-1 with a value of 5.77.

In the P4 (*B. bassiana* 20 g.L-1) and P3 (15 g.L-1) treatments, it caused higher mortality compared to other treatments starting from 3 HSA to 10 HSA, with mortality on the last day of observation (10 *DAT*) of 8.81% and 8.32%, respectively. However, the Samarinda isolate of *B. bassiana* has not been able to kill rice bugs at the same rate as chemical insecticides, but its ability to kill at the percentage level of rice bug mortality is almost the same as that of chemical insecticides 7 days after application, especially at higher concentrations (P4 and P3).

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