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RESEARCH PAPER

Joint application of *B. bassiana* and *M. anisopliae* bioinsecticides for controlling rice bugs and improving rice yields

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ABSTRACT

The rice bug (*Leptocorisa oratorius* F.) is a serious pest of head rice (*Oryza sativa* L.). It feeds on the developing kernels of rice, starting from the heading phase when the panicle is exposed from the boot until the end of the ripening phase, which makes it susceptible to indirect yield loss. Multiple insecticide applications are often made during the hard drought growth stage due to high densities of rice bugs that frequently migrate from nearby harvested fields or as a result of eggs laid by subthreshold populations during the first two weeks of heading. The research was conducted at The Food Crops and Horticulture Laboratory Trimurjo, Central Lampung, from August to October 2020, using a randomized complete block design (RCBD) with 7 bioinsecticide treatments. The objective of the research was to determine the efficacy of integrating biopesticides *B. bassiana* and *M. anisopliae* on rice bug mortality and rice yields. The results showed that rice bug mortality increased by 100% with a lethal period of 72 hours after exposure to *B. bassiana* 5 g L^{-1} + *M. anisopliae* 5 g L^{-1}. Additionally, there was a 19.73% increase in the percentage of rice grain maturity (hard dough) and a reduction of 99.4% in damage (empty grain) compared to the control.

Key words: biological agents, rice, pest control

INTRODUCTION

Rice (*Oryza sativa* L.) is the most important food crop in Indonesia. More than half of Indonesia's population consumes rice produced from rice plants (Manopo et al., 2013). The rice bug (*Leptocorisa oratorius*) is the most important pest of rice in heading stages. Rice attacked by *L. oratorius* has an impact on empty grain, reducing the size and quality of rice seeds (Sihombing & Samino, 2015).

The rice bug feeds on the developing kernels of rice. Feeding that occurs from the flowering growth stage through the beginning portion of the milk growth stage can result in direct yield loss from blanked kernels and reduced kernel weights. The rice bug feeds by inserting piercing-sucking stylets into kernels to extract nutrients, so the density of *L. oratorius* on the panicle can reduce the yield and the quality of the grain. Hutagalung et al. (2013) showed that a density of *L. oratorius* of 5 per 9 clumps of rice would reduce yields by 15% and 10 per 9 clumps reduced yields by up to 20%. Furthermore Baehaki (1993) reported that the pest attack can reduce yields by up to 10% or even 40%. Heavy attacks can reduce yields to 100%.

The pest control of the rice bug mainly depends upon chemical insecticides. Even though it is contrary to principles of integrated pest control and causes pest resistance in some populations of Hemiptera (Blackman et al., 2015). Additionally, the use of chemical insecticides has negative impacts on natural enemies and other non-target organisms (Lidova et al., 2019). All these issues necessitate the investigation of other eco-friendly control tactics to develop sustainable natural enemies, such as parasitoids, predators, and pathogens.

Beauveria bassiana and *Metarhizium anisopliae* are classified as entomopathogenic fungi that caused disease in insects. *B. bassiana* is saprophytic, obtaining food osmotically from dissolved organic material, and parasitic, obtaining nutrients from its host. *Metarhizium* spp. is an entomopathogenic fungus that has a wide host range, infecting insect pests and beneficial insects as predators (Permadi, 2012). The combination of biopesticides *B. bassiana* and *M. anisopliae* is an important study for further research against rice bugs and rice yields.

Entomopathogenic fungi infect insect bodies through contact and penetrate the host's body, then spread to new hosts through spore adhesion. Infected insects will die with a hardened body resembling a

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mummy, covered in white fungus. Moreover, this situation can lead to environmental contamination, either through spores penetrating the host's cuticle or through contaminated feces. The application of entomopathogenic fungi can effectively reduce the population and attacks of rice bugs. Therefore, it is necessary to further study the types and concentrations of these bioinsecticides for pest control.

Aditya et al. (2019) demonstrated that a concentration of 25 g L^{-1} of fungal bioinsecticide effectively controlled the pest population, with 90% dead pests at 7 days after application (dsa), 100% at 14 dsa, and 100% at 21 dsa, with an attack intensity of 8.65%. The effectiveness of entomopathogenic fungi in killing insects is influenced by the timing of application (Lacey & Goettel, 1995).

MATERIALS AND METHODS

Study site. The experiment was conducted at the Food Crops and Horticulture Protection Laboratory in Trimurjo, Central Lampung in October 2020.

Research design. The experiment was arranged in a Randomized complete block design (RCBD) with three repetitions. Spraying the rice bugs with seven treatments of a combination of types and concentrations of bioinsecticides was applied. The seven treatments were as follows:
A = Control;
B = *B. bassiana* 5 g L^{-1};
C = *B. bassiana* 15 g L^{-1};
D = *M. anisopliae* 5 g L^{-1};
E = *M. anisopliae* 15 g L^{-1}';
F = *B. bassiana* 5 g L^{-1} + *M. anisopliae* 5 g L^{-1};
G = *B. bassiana* 15 g L^{-1} + *M. anisopliae* 15 g L^{-1}.

Biopesticide. *B. bassiana* and *M. anisopliae* were produced by CV Pandawa located in Yogyakarta, Indonesia, and packed in rice flour media.

The rice bugs preparation. In this study, the imago rice bugs were quarantined in a cage located within the rice plot area (Figure 1A) that met the following criteria: the abdomen covered by wings, a bright yellowish-green color, and they were 60 days old. The imago rice bugs were caged using a wood-framed tile screen with dimensions of 200 × 100 × 100 cm (Figure 1A). A total of 630 imago rice bugs were quarantined for a duration of 7 days.

Field experiment. The Inpari 42 variety was used as it is susceptible to *L. oratorius* F. The rice plants, which were 60 days old, were grown in plastic buckets with a diameter of 25 cm. All the plants were then caged using a wood-framed tile screen with dimensions of 40 × 40 × 150 cm (Figure 1B). Two days prior to the application of bioinsecticides, 10 imago rice bugs from the quarantine were released into the experimental rice plants.

Application of bioinsecticides. The bioinsecticide suspension was directly sprayed onto the imago rice bugs on the caged rice plants at 03:00 PM (Figure 1C). The dose of 4 mL per plant was applied according to the treatment groups: control, *B. bassiana* 5 g L^{-1} and 15 g L^{-1}, *M. anisopliae* 5 g L^{-1} and 15 g L^{-1}, and *B. bassiana* + *M. anisopliae* with each at 5 g L^{-1} and 15 g L^{-1}. The mortality of the rice bugs was observed every morning at 08:00 AM, and the lethal period was defined as the time when 50% of the rice bugs were dead after the application of bioinsecticides. The percentage of filled grains, empty grains, and empty grains attacked by rice bugs were evaluated at grain maturity.

Data collection. The data collected includes the following:
Mortality rate of the rice bugs. This was determined

Figure 1. Cage of stink bugs used during experiment. A. Imago quarantined cage; B. Cage field experiment; C. Rice plant + Imago stink bugs in cage.

by counting the number of dead rice bugs that met the following criteria: their originally greenish-yellow color turned to yellowish-brown or black, their bodies became stiff, and they did not respond when touched for up to seven days.

Lethal period of the rice bugs. This refers to the time it took for 50% of the rice bugs to die after the application of the bioinsecticides.

Percentage of filled grain. This was obtained by counting the number of filled grains in the rice panicles.

Percentage of non-filled grain. This was determined by counting all the empty grains in the rice panicle.

Percentage of grain punctures. This was calculated by counting all the empty grains in the panicles that were attacked by rice bugs after the application of the bioinsecticides, indicated by black spots where the bugs punctured the grains.

Data analysis. The homogeneity of the data was tested using the Bartlett test, and the non-additivity was tested using the Tukey test before analyzing the variance data. The effects among the treatments were analyzed using the Least significant difference test (LSD) at a significance level of 5%.

RESULTS AND DISCUSSION

The fungus *B. bassiana* and *M. anisopliae* infecting rice bugs are characterized by their bodies being covered with their respective in white and green-colored fungi (Figure 2). This study demonstrates the synergistic effect in pest control through the combination of different types and concentrations of bioinsecticides, resulting in more effective pest control. The evidence of synergism is indicated by an increase in efficacy. Among the treatments, the combination of *B. bassiana* 5 g L^{-1} + *M. anisopliae* 5 g L^{-1} showed the best results, with a rice bug mortality rate 143.25% higher than the control group. It also had the fastest lethal period of 72 hours, with a bioinsecticide efficacy of 100% (Table 1). The control group, without any bioinsecticide treatment, recorded the lowest rice bug mortality rate at 41.11%. This can be attributed to the rice bugs' failure to adapt to the cage with a wood-framed tile screen shape, which is related to the rice bug's life cycle of 80 days (Siwi et al., 1981). It is suspected that there is a synergistic

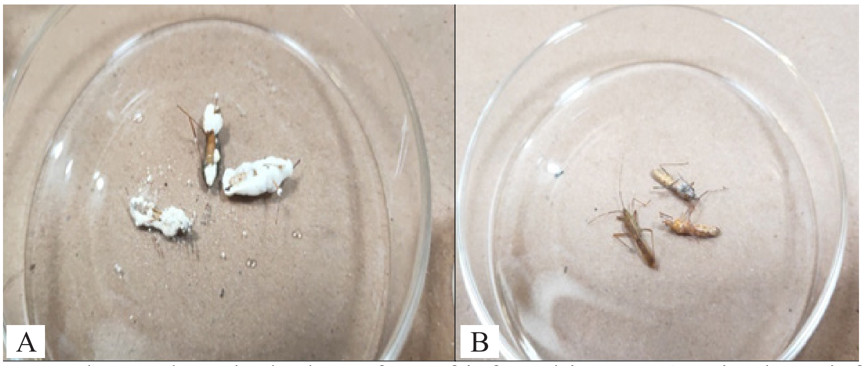

Figure 2. Mycelia was observed on the body surface of infected insects. A. Rice bugs infected by *B. bassiana*; B. Rice bugs infected by *M. anisopliae.*

Table 1. Means percentages of mortality, efficacy, lethal period of rice bugs treated with *B. bassiana* and *M. anisopliae* at different types and concentrations

Treatments	Mortality (%)	Lethal Period (hours)
Control	41.11 a	184.00 d
B. bassiana 5 g L^{-1}	83.34 b	136.00 c
B. bassiana 15 g L^{-1}	93.33 c	104.00 b
M. anisopliae 5 g L-1	75.56 b	120.00 bc
M. anisopliae 15 g L^{-1}	96.67 c	72.00 a
B. bassiana 5 g L^{-1} + *M. anisopliae* 5 g L^{-1}	100.00 c	72.00 a
B. bassiana 15 g L^{-1} + *M. anisopliae* 15 g L^{-1}	96.67 c	80.00 a
LSD 5%	9.43	19.68

Note: Means followed by the same letter in the same column do not differ significantly (LSD= 0.05).

effect between *B. bassiana* and *M. anisopliae*. The use of a biocontrol consortium for safe and sustainable pest control is more effective than using a single organism. A consortium of microorganisms is a collection of similar organisms that form a community with different populations (Kurniawati et al., 2016).

B. bassiana is capable of producing secondary metabolites in the form of beauvericin toxin, which disrupts the hemolymph function of insects, leading to swelling, hardening, and damage to insect body tissues. According to Hasnah et al. (2012), *B. bassiana* can produce several toxins, including beauvericin, beauvericolid, bassianolide, and oxalic acid. These toxins work by increasing the pH of the insect's blood, causing blood clots and cessation of blood circulation. Humairoh et al. (2013) stated that the hyphae of *M. anisopliae* spores enter the host insect's body with the assistance of enzymes and mechanical pressure.

The concentration of bioinsecticides has a significant effect on pest mortality and can cause appreciable damage to grain (Rusly & Trizelia, 2009). A concentration of 20 g L^{-1} of *Beauveria* sp. resulted in the highest mortality rate for *L. oratorius* compared to concentrations of 5 g L^{-1}, 10 g L^{-1}, and 15 g L^{-1} (Koswanudin & Wahyono, 2014). Salaki & Pelealu (2015) reported that after 7 days of applying each entomopathogenic fungus (*Metarhizium* sp. and *Beauveria* sp.) to *L. acuta*, the average mortality ranged from 83.34 to 93.33 and was significantly different from the control. According to Prayogo et al. (2005), the effectiveness of entomopathogenic fungi in infecting the host can be influenced by conidia density, frequency of application, host age, and storage time of the entomopathogenic fungi.

In this study, the treatments *M. anisopliae* 15 g L^{-1}, *B. bassiana* 5 g L^{-1} + *M. anisopliae* 5 g L^{-1}, and *B. bassiana* 15 g L^{-1} + *M. anisopliae* 15 g L^{-1} have the shortest lethal periods (Table 1). It is evident that a shorter lethal time implies a higher ability of the pathogen to kill the host (pathogenicity). The lethal period of rice bugs is 29.5 hours for *B. bassiana* and 22.4 hours for *Metarhizium* sp. at a concentration of 20 g L^{-1} (Effendy et al., 2010). Each isolate (*Metarhizium* sp. and *Beauveria* sp.) showed a short lethal period of 22.4 and 29.5 hours, respectively, to kill rice bugs (Salaki & Pelealu, 2015). Herlinda et al. (2006) stated that the lower the lethal period, the more virulent the isolate is in killing insects. Previous studies have also found that differences in the time of insect death are attributed to variations in the infectivity of each fungus, including penetration, enzyme usage, and growth rate (Freimoser et al., 2003). The time of host insect death can be influenced by various factors, such as environmental conditions, where fungi suitable for the environment will exhibit faster pest control.

The effects of treatments on the quality of rice were recorded as percentages of filled grains, empty grains, and empty grains attacked by rice bugs for each rice sample per cage. According to Table 2, the treatment *B. bassiana* 5 g L^{-1} + *M. anisopliae* 5 g L^{-1} resulted in 19.73% more filled grains compared to the control. It is known that the application of *B. bassiana* was able to inhibit the growth of rice bugs, resulting in lower grain damage compared to the control (Budiyono et al., 2005). In a study by Tabudlong et al. (2016), it was found that the application of *B. bassiana* resulted in significantly higher yields (4.08 tons ha^{-1}) compared to the control group (3.39 tons ha^{-1}) in the second growing season, while yields were comparable (ranging from 3.19 to 3.63 tons ha^{-1}) in the first growing season.

This study revealed that the percentage of grain punctures by rice bugs in the control group (11.32) was higher than in the other treatments. This damage is a result of a reduction in grain content and damage to flowers, where flower abortion can lead to completely empty kernels. Feeding during the milk to soft and

Table 2. Means percentages of filled grain, non-filled grain, grain punctures of rice treated with *B. bassiana* and *M. anisopliae* at different types and concentrations

Variable	Filled grain (%)	Non-filled grain (%)	Grain punctures (%)
Control	72.03	16.65	11.32
B. bassiana 5 g L^{-1}	84.91	8.07	7.02
B. bassiana 15 g L^{-1}	84.37	8.73	6.91
M. anisopliae 5 g L^{-1}	80.91	9.27	9.82
M. anisopliae 15g L^{-1}	83.46	8.17	8.37
B. bassiana 5 g L + *M. anisopliae* 5 g L^{-1}	86.24	8.35	5.40
B. bassiana 15 g/L + *M. anisopliae* 15 g L^{-1}	82.65	9.41	6.37

hard dough growth stages can result in a loss of quality associated with broken, chalky, or discolored kernels. The combinations of types and concentrations of bioinsecticides did not show significant differences. However, the treatment *B. bassiana* 5 g L^{-1} + *M. anisopliae* 5 g L^{-1} showed a tendency to have the lowest percentage of non-filled grains and percentage of grain punctures.

CONCLUSIONS

The yield components of rice (*Oryza sativa* L.) were dependent on the rice bug biopesticides. The combination of *B. bassiana* 5 g L^{-1} + *M. anisopliae* 5 g L^{-1} proved to be more effective in causing rice bug mortality and reducing the lethal period. The mortality rate of the rice bug reached 100% with a lethal period of 72 hours after exposure to *B. bassiana* 5 g L^{-1} + *M. anisopliae* 5 g L^{-1}. Additionally, it increased the percentage of rice grain maturity (hard dough) by 19.73% and reduced damage (empty grains) by 99.4% compared to the control group.

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AUTHORS' CONTRIBUTIONS

EP, NK and R considered and planned the experiment. P and Z arranged field experiment, collected of sting bugs, pathogenicity tests including maintenance of rice plants. EP and NK collected data on areas of crop damage caused by rice bugs. R performs analysis and interpretation of crop damage and weather data. P prepares the script. The authors provided feedback and comments on the research flow, data analysis and interpretation and the form of the manuscript. All authors have read and approved the final manuscript.

COMPETING INTEREST

The authors have no conflict of interest to declare.

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