Entomopathogen *Beauveria bassiana* as an environmentally friendly alternative for control of brown planthopper (*Nilaparvata lugens*) and rice black bug (*Scotinophara coarctata*)

S Kurniawati, P N Susilawati, Y Astuti, E Y Susanti and Y S Hidayat
Assessment Institute for Agricultural Technology (AIAT) Banten, Indonesian Agency for Agricultural Research and Development (IAARD), Banten, 42182, Indonesia

E-mail: jilan_hafizhah@yahoo.com

Abstract. Pest was one of the limiting factors for rice production including the presence of brown plant hopper (BP) and Rice Black Bug (RBB). The use of entomopathogen fungi such as *Beauveria bassiana* to control these pests has been reported to be quite effective and has other advantages, namely being friendly to the environment. This study aims to determine the potential of *Beauveria bassiana* in controlling BP and RBB. The research was conducted in January-July 2018 in Pulo Kencana Village, Pontang District, Serang Regency, Banten Province on irrigated rice fields. The environmental design used was a split plot design with *Beauveria bassiana* application treatment, chemical control based on pest populations with 50% active ingredient propoxur and farmer control technology (pesticide mixture intervals). Each treatment consisted of 2 varieties Mekongga and Inpari 33, with 6 replications. The test of farmers’ preferences for *Beauveria bassiana* application technology was carried out on 30 respondents and then analyzed descriptively. The results showed that the population of BP and RBB was generally lower in the existing chemical treatment of farmers. The pest preference was lower in Inpari 33 variety. Furthermore, the production in the *Beauveria bassiana* application was 12.06-24.48% lower than the existing and controlled chemical control of farmers. Meanwhile, farmers’ preference for *Beauveria bassiana* application is 31.93% and chemical control is 40.76%.

1. Introduction
The government’s efforts to increase rice production and productivity continue to be made considering that there is still a wide gap between real production/productivity and its potential. The national average rice productivity in 2019 was 5.11 t/ha and in Banten was 4.84 t/ha [1]. The productivity potential of a superior variety is 6-10 t/ha [2]. One of the limiting factors in rice production is the presence of brown plant hoppers/BP (*Nilaparvata lugens*) and Rice Black Bug/RBB (*Scotinophara coarctata*) pests.

Symptoms of BP and RBB attack on plants have in common, namely yellowing and drying of leaves like burning (hopperburn/bugburn) and the plant becoming stunted. BP apart from directly damaging the plant's liquid can also act as a vector for Grassy Stunt (Rice Grassy Stunt Virus/RGSV) [3] and Ragged Stunt (Rice Ragged Stunt Virus/RRSV) [3, 4]. Plants that have been attacked by the disease will not produce panicles/grain.

The average area of BP attacks in Indonesia in 2012-2017 reached 74,929.5 ha and an area of 1,415 ha experienced crop failure [5]. If the average yield loss due to BP attacks is 1-2 t/ha [6], the yield loss for the period 2012-2017 will be 74,929.5-149,859 tons of grain, equivalent to 2.99 - 5.99 trillion rupiah. Furthermore, the average RBB attack area in 1997-2006 was 6,162 ha [7]. Extensive information on
RBB attacks in the last decade has not been widely reported, but in some areas, it has caused many losses.

Efforts to control BP and RBB include using BP resistant varieties and the use of chemical pesticides. BP-resistant varieties have been produced by Indonesian Center for Rice Research (ICRR) such as IR 64, Ciherang, Mekongga, Inpari 13, Inpari 31, and Inpari 33 [2]. Furthermore, insecticides to control BP that are recommended and have a distribution permit in Indonesia, there are 28 types of active ingredients [8] including imidaclorpid, amitraz, buprofezin, BPMC, fipronil, carbofuran, carbosulfan, metocarb, MIFCI, propoxure, and others [9]. The recommendations for insecticides to control RBB include propuxur, carbosulfan, carbofuran, BPMC, carbaryl, carbosulfan, profenophos, acephate, fipronil, fention, and imidacloprid [10]. The weakness of using chemical insecticides if not carried out wisely will cause negative impacts on the environment such as the resistance of insect pests to a pesticide. BP has been reported to be resistant to imidacloprid, thiamethoxam, and buprofezin in China [11], imidacloprid in Central and East Java [9]. Other disadvantages as a result of pesticide application are resurgence, death of non-target organisms, environmental pollution, and high chemical residue content in rice which is harmful to human health. Based on this, it is necessary to control efforts that are friendly to the environment, one of which is the use of the entomopathogen Beauveria bassiana.

Beauveria bassiana is an entomopathogenic fungus in several insect pests such as BP and RBB. The use of Beauveria bassiana was reported to be effective in controlling BP [12, 13, 14] and effectively controlling RBB [15]. The Ministry of Agriculture, through the Plant Pest Organism Forecasting Center and the Center for Food and Horticulture Protection, has succeeded in developing the propagation technology for Beauveria bassiana isolates using rice media. This will make it easier for farmers to adopt the technology because the entomopathogenic fungi isolates can be reproduced independently by farmers. However, farmers' confidence in using these isolates to control rice pests, especially BP and RBB, was still low. The purpose of this study was to determine the potential of Beauveria bassiana in controlling BP and RBB.

2. Materials and methods

2.1. Research site and materials

The research was carried out on irrigated rice fields, which is one of BP's endemic areas, namely in Pontang District, Serang Regency, Banten Province, Indonesia in January-July 2018. The materials used are Mekongga and Inpari 33 varieties of rice seeds, 2 tonnes/ha of organic fertilizer, urea. 165 kg/ha, NPK Phonska 120 kg/ha, KCl 20 kg/ha, Beauveria bassiana culture with a spore density of $10^6$-$10^8$/ml, insecticides (active ingredient: 5% propoxure, 100 g/l cypermethrin, 18 g/l abamectin and chlorphiripos 500 g/l + cypermethrin 50 g/l). Beauveria bassiana cultured using rice media was obtained from the PHP laboratory of Serang Regency, Banten Province.

2.2. Design experiment

The environmental design used was a split-plot design with control treatment as the main plot and varieties as subplots (table 1). Each treatment was repeated 6 times and for each experimental unit, 5 clumps of samples were taken so that the total sample size was 180 clumps. Observation parameters consisted of the total population of BP and RBB at the age of 21-63 DAS (days after seeding), plant height, and the number of tillers at 70 DAS, and productivity.
Table 1. The treatments for application of BP and RBB control.

| Treatments | The application of BP and RBB control |
|------------|--------------------------------------|
| **Main plots:** |                                        |
| P1         | Implemented Beauveria bassiana (Beauveria application) 4 times (21, 35, and 49 DAP/days after planting) by spraying it onto plants, the dose used was 2 kg/ha per one application and the spray volume was 400-500 l/ha. |
| P2         | The application of chemical insecticides (Controlled of Chemical) with active ingredient propoxur 50% based on the presence of BP and RBB (21 and 35 DAP) populations, dose 2 ml/l with a spray volume of 400-500 l/ha. |
| P3         | Regular application (Farmers existing) of mixed chemical insecticides once every 1-2 weeks with the type of active ingredient%, cypermethrin 100 g/l, abamectin 18 g/l and chlorphiripos 500 g/l + cypermethrin 50 g/l), dosage 2-4 ml/l, spray volume 200-300 l/ha. |
| **Sub plots:** |                                        |
| V1         | Mekongga                               |
| V2         | Inpari 33                               |

2.3. Management of rice cultivation
Land preparation was carried out with complete tillage and application of organic fertilizers before planting. Seedlings aged 18 DAS were planted by planting legowo rows 4: 1 with a spacing of 25 x 18 x 50 cm. The application of urea fertilizer was carried out 3 times, at the age of 7, 21, and 35 DAS. Phonska NPK fertilizer application was carried out 2 times at the age of 7 and 21 DAS and KCl was carried out once, namely at the age of 7 DAS. Weed control is carried out twice, namely before the second fertilization and before the third fertilization. Irrigation is carried out intermittently. Harvest is done when the grain is physiologically ripe.

2.4 Farmers preference test
The test of farmer preference on plant performance and application of control technology was carried out on 30 respondents. The test was carried out when the plants have entered the ripening phase. Sampling was done deliberately (purposive sampling). The method of data collection was carried out using a questionnaire with an ordinal scale, the respondents chose the answers to really dislike, dislike, somewhat like, like, and really like the column provided. The plant performance variables observed were the number of tillers, number of panicles, number of grain, and resistance to pests and diseases. The observed variables for the response of control technology consisted of aspects of easiness, effectiveness, cost, availability of materials, and the interest of farmers to apply these technologies.

2.5. Data analysis
The data were analyzed using the Anova test and Duncan multiple range test for determining the performance of different various treatments on a level of 5%. The analysis used SPSS 20 software. Meanwhile, the results of data collection on farmer preferences on plant performance and responses to control technology applications were processed and analyzed descriptively.

3. Results and discussions
Population observations of BP and RBB began at the age of 21 DAP. In general, the population of BP and RBB was lower in Inpari 33 varieties. This shows that Inpari 33 has better resistance to BP and RBB compared to Mekongga. The population of BP already exists in the Beauveria bassiana plot (P1) of 1.70-1.77 individual/family and chemical control (P2) is 1.47-1.50 individuals/family, while in the existing farmers there was no BP population (figure 1). This was because, in the existing treatment of farmers, pesticide applications have been carried out at the age of 10-14 DAS using the insecticides cypermethrin and abamectin, then routinely continue every 1-2 weeks using other insecticides, namely chlorpyrifos + cypermethrin. The application of Beauveria bassiana at (P1) was carried out after observation at 21 DAS. On the observation of 28 DAP, the BP population at P1 decreased. This shows
that the control performance using *Beauveria bassiana* is quite effective in reducing BP by 49.02 - 64.15%. The decrease in BP population occurred in the chemical control treatment (P2) of 100% after application of the propoxur insecticide. The goal of control is not to control BP because the population is still below the control threshold. The BP control threshold was 10 individuals/hill for young plants and 40 individuals/hill for plants with more than 40 DAP [16].

![Figure 1. BP population 21-63 DAP. P1V1 = Beauveria bassiana + Mekongga, P1V2 = Beauveria bassiana + Inpari 33, P2V1 = Controlled of Chemical + Mekongga, P2V2 = Controlled of Chemical + Inpari 33, P3V1 = Existing Technology + Mekongga, P3V2 = Existing Technology + Inpari 33.](image)

The BP population increases at 35 DAP. The BP found is a population of migrants from other places. This can be seen from the shape of the long wings on the imago (macropterous). The application of *Beauveria bassiana* on P1 was again carried out and the effectiveness of control reached 75.12-77.26%. At 63 DAP, the BP population increased again, up to 9.5 individuals/family in P1 and 13.17 individuals/family in P2. The existing farmer treatment plots (P3) tend to be stable due to routine insecticide applications. However, the BP population was still below the control threshold. The application of propoxur insecticide to P2 was due to the high RBB attack of up to 8.03 individuals/family (figure 2).

Application of *Beauveria bassiana* to RBB on 21 DAP reduced the population from 72.79-79.92% and application on 35 DAP was able to reduce the RBB population by 95.83%. Furthermore, the application of propoxur to RBB was able to reduce the population from 94.94 to 97.19% for 21 DAP and 86.67-99.04% for 35 DAP. As for the existing farmer control (P3), the RBB population tends not to change at 21, 28, and 35 DAP even though the control is carried out almost once a week. The ineffective control was due to the application of farmer insecticides by mixing 2-3 types of insecticides with a spray volume of 50-60% of the recommended dose.

Control using the entomopathogen *Beauveria bassiana* effectively suppressed the nymphs population at 10⁸ conidia/ml and a concentration of 10¹⁰ conidia/ml was effective in suppressing adult-phase RBB [17]. Another report stated that a concentration of *Beauveria bassiana* of 10⁷ conidia/ml was effective as an ovidical for controlling egg BP [12]. The mechanism of *Beauveria bassiana* in controlling insect pests begins with the attachment of conidia to the insect's skin, then the conidia will germinate to form hyphae and continue to grow as well as produce chitinase and protease enzymes that destroy the cuticle. Due to the damage to the cuticle, hyphae will penetrate and develop in the insect's body. The insects will die because their entire body has been filled with the mycelia *Beauveria bassiana*. Furthermore, the hyphae will penetrate out and grow on the outside insect bodies and produce conidia which will be spread to the environment and infect other insects [18].

Apart from producing chitinase and protease enzymes, *Beauveria bassiana* to attack insect pests is to produce toxins. The resulting mycotoxin is beauvericin which has insecticidal, antimicrobial, antiviral, and cytotoxic activities [19]. The toxin will inhibit insect mitochondrial ATPase [20]. The application of toxins in the pesticide industry can be in the form of fungal propagules or bioactive compounds. However, applications in the field are still widely used in the form of fungal propagules.
The advantage of using these propagules is that the fungus will attach itself to and multiply on the insect's body and will spread (propagate) along with the movement of insects [19].

Control treatment did not affect plant height and the number of tillers (figure 3). In general, the Inpari 33 variety has a lower plant height compared to Inpari 33. This is consistent with the characteristics of each variety, where Inpari 33 has a plant height of ± 93 cm and Mekongga 91-106 cm [2]. Meanwhile, the number of tillers tended to be the same between Inpari 33 and Mekongga.

The productivity between Mekongga and Inpari 33 varieties had no interaction and was not significantly different between treatments (table 2). The highest productivity was obtained in the chemical control treatment (P2), but it was not significantly different from the existing control (P3). The Beauveria bassiana (P1) treatment had 12.06-24.48% lower productivity compared to P2 and P3.
Table 2. Productivity performance mekongga and inpari 33 on various control technologies.

| Treatment                          | Harvested dry grain | Milled dry grain |
|------------------------------------|---------------------|------------------|
| Varieties                          |                     |                  |
| - Mekongga (V1)                    | 5.57 tn             | 5.10 tn          |
| - Inpari 33 (V2)                   | 5.62 tn             | 5.16 tn          |
| Technologies                       |                     |                  |
| - Beauveria bassiana (P1)          | 4.84 a              | 4.43 a           |
| - Controlled of chemical (P2)      | 6.20 b              | 5.66 b           |
| - Existing control (P3)            | 5.75 ab             | 5.30 ab          |

In Table 3, the results of preference tests based on plant performance and the aspect of technology implementation that are most preferred are controlled chemical control (P2) with a score of 433 (40.76%). The preference score for control using Beauveria bassiana was 159 (31.93%).

Table 3. Preference test results.

| Parameter                              | Beauveria bassiana | Chemical control | Existing |
|----------------------------------------|--------------------|------------------|----------|
| Plant performance                      |                     |                  |          |
| a. Number of tillers / clumps          | 30                 | 51               | 43       |
| b. Number of panicles / clump          | 37                 | 54               | 42       |
| c. Number of grain / panicle           | 44                 | 55               | 40       |
| d. Plant resistance                    | 35                 | 44               | 38       |
| Total                                  | 146                | 204              | 163      |
| Technologies performance               |                     |                  |          |
| a. Convenience                         | 38                 | 54               | 34       |
| b. Effectiveness                       | 36                 | 54               | 40       |
| c. Cost                                | 51                 | 48               | 20       |
| d. Availability of materials           | 34                 | 47               | 42       |
| Total                                  | 159                | 203              | 136      |
| Interest in applying technology        | 1                  | 26               | 4        |
| Total number                           | 306                | 433              | 303      |

n = 30

Based on the aspect of interest in implementing Beauveria bassiana technology in controlling BP and RBB, only 3.33%. This shows that from the experimental plot and the explanation of technology farmers are starting to be interested in this information but are not yet interested in applying it to the next planting season. Farmers still believe in and choose chemical control for the implementation of BP and RBB controls from the aspects of ease, effectiveness, and cost. However, these results can convince farmers that the use of chemical pesticides can be managed better and wisely so that negative impacts on the environment and humans can be reduced.
4. Conclusion

In general, the population of BP and RBB were lower in the chemical treatment and existing farmers. Meanwhile, the pest preference was lower in Inpari 33 variety. Furthermore, the production in the Beauveria bassiana application was 12.06-24.48% lower compared to the controlled and existing chemical control by farmers. Meanwhile, farmers’ preference for Beauveria bassiana application was still low (31.93%) and control of chemical (P2) was still the most preferred choice, in the amount of 40.76%.

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