Application of biofertilizer and indigenous *Beauveria bassiana* Vuillemin on growth, resistant and production of rice (*Oryza sativa* L.)

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Submission: 4 August 2021 Revised: 7 May 2022 Accepted: 10 June 2022

ABSTRACT

Rice (Oryza sativa L.) is the most important food crop in Indonesia. Increased rice production can be achieved if fertilization and pest control are carried out. The growth and production of rice plants will also be optimal if rice plants are healthy, and the intensity of pest attacks is controlled. The research was conducted by combining the application of biofertilizer and indigenous entomopathogenic fungi Beauveria bassiana to support the growth and production of environment-friendly plants so that it is expected to minimize the use of inorganic fertilizers and chemical pesticides. The research aimed to determine the effect of the application of biofertilizer and indigenous B. bassiana on the growth, resistance, and production of rice plants and their interaction. This research was experimentally arranged according to a factorial completely randomized design with two factors. The first factor was the timing of biofertilizer application consisting of 4 times, namely: no applications of biofertilizer, one application at 14 days after planting (DAP), two applications at 14 DAP and 28 DAP, and three applications at 14 DAP, 28 DAP, and 42 DAP. The application of biofertilizer was carried out by watering it into the rice planting soil using a 10 mL container. The second factor was the timing of indigenous B. bassiana application consisting of 4 times, namely: no applications of indigenous B. bassiana, one application at 14 DAP, two applications at 14 DAP and 28 DAP, and three applications at 14 DAP, 28 DAP, and 42 DAP. Application of indigenous B. bassiana was done by watering it on rice plants until the plants become wet (at 50 mL for the first, at 100 mL for the second, and at 150 mL for the third watering). The results showed that there was no interaction between the application of biofertilizers and indigenous B. bassiana in each observation. The application of biofertilizer had a significant effect on plant height, maximum number of tillers, and number of productive tillers (P < 0.05). The application of indigenous B. bassiana had a significant effect on the intensity of pest attack (P < 0.05). The combination of the application of biofertilizer two times and indigenous B. bassiana two times could be able to provide good grain yields, but the combination of three times biofertilizer and three times indigenous B. bassiana tended to give better results in all observations, especially in the production of rice plants, because production can determine the success of the cultivation.

Keywords: Rice, biofertilizer, environment-friendly plants, indigenous Beauveria bassiana

Thai J. Agric. Sci. (2022) Vol. 55(1): 34-44



INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important food crops worldwide, especially in Indonesia. The need for national rice continues to increase along with the increasing population in Indonesia. Rice plays an important role in providing food to achieve food security. Increased production of rice plants can be achieved if the rice plants are healthy and grow optimally, so it is necessary to fertilize and pest attack control.

Fertilization aims to provide nutrients needed by plants so that they can increase plant growth and production. Fertilization is generally using high doses of inorganic fertilizers because one of the advantages of inorganic fertilizers is that the nutrients are easily decomposed and absorbed by plants. The use of inorganic fertilizers with excessive doses continuously can make the soil harden and lose its porosity also the synthetic chemicals in inorganic fertilizers change the pH of the soil and make it acidic. The result of Frimawaty et al. (2013) showed that the analysis of the sustainability of rice farming in multidimensional revealed that rice farming carried out by farmers is less sustainable. Therefore, the use of inorganic fertilizers needs to be reduced by using environmentally friendly fertilizers, one of which is biofertilizer.

The application of biofertilizer is expected to reduce and streamline the use of inorganic fertilizer doses, so as to increase plant growth and production in a more environmentally friendly. Soil fertility will increase due to the application of complementary biofertilizers in increasing crop yields and can also stimulate plant growth (Setiawati, 2016). The growth and production of rice plants will be optimal if the rice plants are healthy, and the intensity of pest attacks is controlled. Currently, pest control is generally carried out using pesticides. The use of chemical pesticides can cause residues and resurgence, and cannot always suppress pest attacks due to many factors such as inappropriate dosage, inappropriate application time, wrong application method, and others. Therefore, it is necessary to have alternative pest control that is environmentally friendly and sustainable, one of which is the use of entomopathogenic fungi.

Entomopathogenic fungi were among the first organisms to be used for the biological control of pests. More than 700 species of fungi from around 90 genera are pathogenic to insects (Sanjaya et al., 2014). The entomopathogenic fungi used was indigenous Beauveria bassiana which came from agricultural land. According to Herlinda et al. (2008), the use of B. bassiana as a pest control has several advantages, including having a high reproductive capacity, being able to form spores that are durable in nature, environmentally friendly, and having high pathogenesis of target pests.

The research was conducted by combining the application of biofertilizer and indigenous entomopathogenic fungi B. bassiana to support the growth and production of environmentally friendly plants so that it is expected to minimize the use of inorganic fertilizers and chemical pesticides. Research on the production of environmentally friendly rice plants has also been carried out by Hapsoh et al. (2020) by utilizing a consortium of cellulolytic microbes and several organic materials that are used as basic ingredients for making biofertilizers. The results showed that the biofertilizer based on rice washing water and a consortium of 10 mL cellulolytic microbes per plant produced the highest weight of 1,000 upland rice grains of 29.87 g. Research by Saothongnoi et al. (2014) implied that rice straw induces soil fertility which is useful for plant growth. Doni et al. (2013) also found that some microbes are also involved in cell regulation and signaling in rice plants, there are significant influences on the growth and yield of rice plants by microbes. Therefore, this research aimed to determine the effect of the application of biofertilizer and indigenous B. bassiana and their interaction on the growth, resistance, and production of rice plants.

MATERIALS AND METHODS

The materials used in this study were rice seeds of the Inpago 9 variety, rice washing water, a consortium of microbes *Proteus mirabilis* TKKS3 and *Proteus mirabilis* TKKS7, *Bacillus cereus* JP6 and *Bacillus cereus* JP7, *Providencia vermicola* SA1 and *Providencia vermicola* SA6 (private collection of

Hapsoh, Wawan, and Isna), indigenous *B. bassiana* isolate BBDS 2 (Desita Salbiah's personal collection), 70% alcohol, aquades, molasses, water, chicken manure, urea fertilizer, TSP, KCI fertilizer, crushed corn, and sugar.

Ethics Statements

This research and all procedures were approved and financially supported by a basic research grant from the Directorate General of Higher Education, Ministry of Research, Technology and Higher Education, Indonesia (Contract No. 484/ UN.19.5.1.3/ PT.01.03/2021).

Management and Data Collection

Management

The production of biofertilizer was carried out with the basic ingredients of rice washing water, nutrient broth (NB) media, and cellulolytic microbes that have been consorted. The manufacture of biofertilizer began with growing a consortium of cellulolytic microbes on NB media and incubating for 2 days. The incubated cellulolytic microbial consortium was then put into rice washing water and incubated for 14 days. The results of soil analysis that have been applied with biofertilizers also show that the content of N elements in the soil is moderate (0, 3%), P elements are very high (31, 21 mg/100g) and K is moderate (20, 97 mg/100g) based on the criteria for assessing soil chemical properties of the Soil Research Institute (2009).

Isolate of indigenous *B. bassiana* BBDS 2 (Desita Salbiah's personal collection) was made in the form of a starter which was propagated on broken corn media and then incubated for 7 days. The indigenous *B. bassiana* fungi that had been propagated was taken as much as 30 g and mixed with 1 L of sterile water using a shaker, then filtered using gauze. One liter of suspension of *B. bassiana* was added with 15 g of granulated sugar. The indigenous *B. bassiana* solution for treatment was taken from the stock solution with a conidia density of 3.64 × 10⁷.

The application of biofertilizer was carried out by watering it into the rice planting soil using a 10 mL container. The dose of biofertilizer application

was 10 mL per plant (Hapsoh *et al.*, 2020), so the dose of biofertilizer for one plot measuring 1.5 m × 1.5 m was 240 mL for each application. Application of indigenous *B. bassiana* by watering it on rice plants. Calibration was carried out before each application to determine the volume of flush to be used by filling 1,000 mL of water into the Erlenmeyer, then watering the rice plants until the plants became wet and calculating the volume of water remaining in the Erlenmeyer. Flush volume was the product of the initial volume minus the final volume. Indigenous *B. bassiana* application was carried out in the afternoon according to the treatment.

Data collection

Plant height (cm) was measured using a ruler or tape measure. Rice plants were measured from a benchmark made as high as 10 cm from the ground. The measurement results were then added by 10 cm so that the plant height is obtained. Observation of the maximum number of tillers began 4 weeks after planting and subsequent observations were made at intervals of once a week until the number of tillers had begun to decrease. The maximum number of tillers was the highest number of tillers in the observation of the sample plants. The number of productive tillers was observed by counting the number of each tiller that produced panicles in each sample plant clump. The intensity of pest attack (%) was observed by counting the number of sample plants that were attacked by plant pests. The observation of flowering age was carried out by counting the number of days from the beginning of planting until ± 50% of one bed had flowered. The harvesting age of rice plants was calculated from the day of planting until the rice plants have physiologically matured 90%. The panicle length was measured from the base to the tip of the panicle in each sample plant clump, then averaged. Observation of the percentage of pithy grain was carried out by threshing the grain on each sample plant first, then separating the pithy grain from the empty grain and calculating the total pithy grain. The weight of 1,000 grains was observed by calculating the weight of 1,000 grains of rice grain



sampled in each bed using a digital scale. The weight of milled dry grain was observed by drying the grain of the sample plants in each experimental unit under the sun for an average of 8 hours per day for 2 days, then weighed using a digital scale.

Experimental Design

This research was experimentally arranged according to a factorial completely randomized design with two factors. The first factor was the application of biofertilizer consisting of 4 times, namely: 0 applications of biofertilizer, 1 application at 14 days after planting (DAP), 2 applications at 14 DAP and 28 DAP, and 3 applications at 14 DAP, 28 DAP, and 42 DAP. The second factor was the application of indigenous B. bassiana consisting of 4 times, namely: 0 applications of indigenous B. bassiana, 1 application at 14 DAP, 2 applications at 14 DAP and 28 DAP, and 3 applications at 14 DAP, 28 DAP, and 42 DAP. The number of levels of application factors for biofertilizer and indigenous B. bassiana resulted in 16 experimental combinations and was repeated 3 times, resulting in 48 experimental units. Each experimental unit consisted of 24 plants and 5 plants were sample plants.

Data Analysis

The data obtained were analyzed statistically using analysis of variance. The factorial completely randomized design linear model was used. The data obtained were then compared with Duncan's new multiple range test (DNMRT) at a level of 5% using SPSS software.

RESULTS AND DISCUSSION

Rice Plant Growth

The results of data analysis showed that there was no interaction between the application of biofertilizer and indigenous B. bassiana on plant height, maximum number of tillers, flowering age, and harvest age of rice plants (P > 0.05). Biofertilizer application showed a significant effect on plant height and maximum tiller number (P < 0.05), while indigenous *B. bassiana* application had no significant effect on plant height, maximum number of tillers, flowering age, and harvest age of rice plant (Table 1).

Table 1 Application of biofertilizer and indigenous Beauveria bassiana Vuillemin to the observation of rice growth

Application of biofertilizer	Application of indigenous Beauveria bassiana Vuillemin					
	Zero times	One time	Two times	Three times	Average	
Plant height (cm)						
Zero times	112.12	110.07	107.11	112.13	110.38 ^B	
One time	114.54	114.63	108.29	110.25	111.92 ^{AB}	
Two times	108.82	112.14	118.03	114.33	113.33 ^{AB}	
Three times	113.82	112.96	119.41	115.91	115.52 ^A	
Average	112.34	112.45	113.15	113.21		
Maximum number of	f tillers (tiller)					
Zero times	23.20	22.27	23.80	24.27	23.38 ^B	
One time	22.07	24.60	23.67	24.67	23.75 ^B	
Two times	23.87	22.20	25.87	26.07	24.50 ^{AB}	
Three times	25.93	25.67	26.07	26.40	26.02 ^A	
Average	23.76	23.68	24.85	25.35		

Table 1 Cont.

Application of	Application of indigenous Beauveria bassiana Vuillemin						
biofertilizer	Zero times	One time	Two times	Three times	Average		
Flowering age (DAP)							
Zero times	81.33	80.33	80.33	79.00	80.25		
One time	78.00	77.67	78.33	80.33	78.80		
Two times	79.67	77.67	78.67	78.33	78.58		
Three times	80.67	79.33	77.33	76.00	78.33		
Average	79.92	78.75	78.67	78.42			
Harvest age (DAP)							
Zero times	111.00	110.33	110.67	109.33	110.30 ^B		
One time	109.67	108.00	110.67	108.33	109.17 ^{AB}		
Two times	109.67	109.33	110.33	110.33	109.92 ^{AB}		
Three times	107.67	109.67	110.33	108.00	108.92 ^A		
Average	109.50	109.33	110.50	109.00			

Note: Means within the column followed by the same capital letter were not significantly different according to Duncan's new multiple range test at the 5% level. DAP = day after planting

The application of biofertilizer and indigenous *B. bassiana* did not interact with the observation of rice plant growth. Although there was no interaction, the combination of three times biofertilizer and three times indigenous *B. bassiana* tended to produce better plant growth such as the maximum number of tillers (26.40 tillers) and flowering age (76.00 DAP) compared to rice plants on single factors and other combinations.

Factors affecting plant growth include genetic factors and environmental factors. Environmental factors that affect plant growth such as temperature, water, sunlight, nutrients, and others. An optimal plant growing environment can support the success of plant growth and development. One way to get an optimal growing environment is the application of fertilizer, such as the application of biofertilizer. The biofertilizer used is based on selected potential cellulolytic microbes that have been studied by Hapsoh et al. (2016), cellulolytic microbes came from empty fruit bunches of oil palm, acacia litter, and rice straw. The six isolates were *Proteus mirabilis* TKKS3 and *Proteus mirabilis* TKKS7 from empty

oil palm fruit bunches, *Bacillus cereus* JP6 and *Bacillus cereus* JP7 from rice straw, *Providencia vermicola* SA1 and *Providencia vermicola* SA6 from consortium acacia litter.

The application of biofertilizer for three times can increase the nutrient content of the soil so that the nutritional needs of rice plants are fulfilled. Sufficient plant nutritional needs can support the growth of rice plants, nutrients that play an important role in the growth of rice plants, such as nitrogen and phosphorus elements. From the report of Patti *et al.* (2013), nitrogen has an important role for rice plants, such as increasing plant growth and improving yield and grain levels through increasing the number of tillers, leaf area development, grain formation, grain filling, and protein synthesis.

The application of biofertilizer had a significant effect on plant height, harvest age, 1,000-grain weight, and yield (P < 0.05). Harvest age and grain yields showed a positive response with the addition of 25% biofertilizer and or 50% inorganic fertilizer at the recommended dose. This is thought to be due to an increase in physiological



mechanisms resulting from the availability and absorption of nutrients by a consortium of microbes that are transferred into plant tissues (Mulyaningsih et al., 2015).

The good growth of rice plants is also thought to be influenced by the intensity of pest attacks on plants. Rice plants that were applied to indigenous B. bassiana three times gave better growth compared to plants that were not applied to indigenous B. bassiana. This is because indigenous B. bassiana is an entomopathogenic fungi that can attack various types of insects, thus pest attacks on upland rice plants are controlled. Controlled pest attacks cause the intensity of pest attacks to be lower so that the growth of rice plants is not disturbed.

Application of B. bassiana in the afternoon (after 4.00 pm) was able to cause the death of pests. The death of pests will reduce the intensity of pest attacks on rice plants. The success of the application of B. bassiana can suppress body

damage and plant death so that the plant growth process is not disturbed. Rice plant pests infected with B. bassiana will experience symptoms of sluggish movement, decrease appetite and stop, then the pest dies and the pest's body turns pale (Soetopo and Indrayani, 2007). Trizelia et al. (2007) also stated that one of the advantages of B. bassiana in controlling pest attacks is that it can be used to control various stages of development of pests ranging from egg, larva, pupa, and imago stages.

Insect Pest Resistance of Rice

The results showed that there was no interaction between the application of biofertilizer and indigenous B. bassiana on the intensity of pest attack. The application of biofertilizer had no significant effect on the intensity of pest attacks, but the application of indigenous B. bassiana had a significant effect on the intensity of pest attacks (P < 0.05; Table 2).

Table 2 Application of indigenous biofertilizer and Beauveria bassiana Vuillemin on the intensity of rice pest attack (%)

Application of biofertilizer	Application of indigenous Beauveria bassiana Vuillemin						
	Zero times	One time	Two times	Three times	Average		
Zero times	18.33	15.00	10.00	15.00	14.58		
One time	15.00	18.33	13.33	10.00	14.17		
Two times	16.67	15.00	10.00	11.67	13.33		
Three times	13.33	11.67	13.33	8.33	11.67		
Average	15.83 ^B	15.00 ^B	11.67 ^A	11.25 ^A			

Note: Means within the row followed by the same capital letter were not significantly different according to Duncan's new multiple range test at the 5% level.

Although there was no interaction between the application of biofertilizer and indigenous B. bassiana, the combination of biofertilizer and indigenous B. bassiana three times tended to produce a lower intensity of rice pest attack than the intensity of rice pest attack on a single factor and other combinations, namely 8.33%. Rice plants that were applied with a combination of biofertilizer and indigenous B. bassiana three times resulted in a lower intensity of pest attacks because rice plants were more tolerant of pests. The application of biofertilizer helps provide macronutrients and micronutrients so that the nutritional needs of plants are fulfilled. The application of indigenous *B. bassiana* helps in controlling pest attacks, so the combination of biofertilizer and B. bassiana causes rice plants

to be healthy and tolerant of pests. The results of research by Hasnah and Susanna (2010) also showed that the application of biofertilizer had a significant effect on the percentage of affected plants because biological fertilizers containing phosphate solubilizing microbes had already played a role in the soil, thus P and K nutrients could be absorbed by plants and functioned in the tissue growth process crops and plants become more resistant to pest attacks.

Application of *B. bassiana* in the afternoon can cause insect death. The death of these insects

will reduce the intensity of pest attacks on rice plants (Isrin and Fauzan, 2018). The indigenous entomopathogenic fungi *B. bassiana* consumes the internal parts of the insect's body so that the nutrients in the hemolymph are utilized for the growth of the entomopathogenic fungi which results in decreased insect activity and the insect will die (Salbiah and Rumi'an, 2014). The types of pests found in upland rice plants such as *Locusta migratoria*, *Spodoptera frugiperda*, *Phillophaga helleri*, *Leptocorisa acuta*, *and Nezara viridula* (Figure 1).

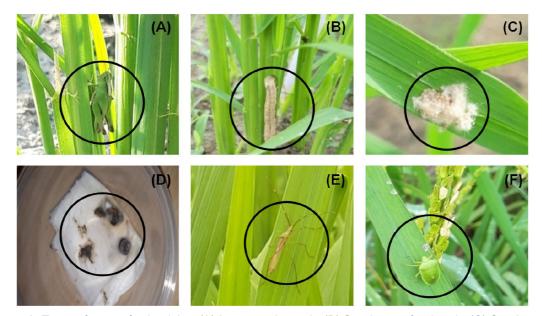


Figure 1 Types of pests of upland rice: (A) Locusta migratoria, (B) Spodoptera frugiperda, (C) Spodoptera frugiperda's egg, (D) Phillophaga helleri, (E) Leptocorisa acuta, and (F) Nezara viridula

Rice Production

The results of the analysis of variance showed that there was no interaction between the application of biofertilizer and indigenous *Beauveria bassiana* on the number of productive tillers, panicle length, percentage of pithy grain, 1,000-grain weight, and dry milled grain weight per clump of rice plants. The application of biofertilizer significantly affected the number of productive tillers of rice plants (P < 0.05) but the application of indigenous *B. bassiana* had no significant effect on the observation of rice production

(Table 3). Although, there was no interaction between the application of biofertilizer and the application of indigenous *B. bassiana* to the observation of rice production. The combination of the application of biofertilizer and indigenous *B. bassiana* three times tended to give more production components, such as the number of productive tillers (18.20 tillers), panicle length (28.25 cm), percentage of pithy grain (71.81%), and dry milled grain weight per clump (53.22 g) better than rice plants on a single factor and two factors.



Good growth of rice plants can support the production of rice plants, such as the maximum number of tillers which will cause the number of productive tillers to increase, and good plant height can also support the panicle length of rice plants. Panicle length can affect the difference in the amount of grain, so the longer the rice panicle, it is expected that more grain will be formed. Increased production of rice plants can be achieved if the plant nutrient needs are fulfilled. The application of biofertilizer three times can increase the nutrient content so that rice plants that were applied three times biofertilizer tended to give better yields.

Biofertilizers are inoculants made from active living organisms that can bind certain nutrients or facilitate the availability of nutrients in the soil for plants (Suriadikarta and Simanungkalit, 2006). Lack of nutrients in the soil is mostly caused by the number of macronutrients being small or in unavailable form as bound by minerals in the soil. The combination of biofertilizers with inorganic fertilizers and varieties had a significant effect on the components of rice yields, such as the weight of 1,000 grains. The combination of biofertilizers with inorganic fertilizers in addition to increasing rice yields, this combination can also reduce the use of inorganic fertilizers by 25% (Malahayati et al., 2019).

Table 3 Application of biofertilizer and indigenous *Beauveria bassiana* Vuillemin to the observation of rice plant production

Application of	Application of indigenous Beauveria bassiana Vuillemin					
biofertilizer	Zero times	One time	Two times	Three times	Average	
Number of productiv	e tillers (tiller)					
Zero times	14.87	15.13	15.73	15.80	15.38 ^{BC}	
One time	15.07	15.13	15.07	15.00	15.06 ^c	
Two times	14.87	15.33	17.87	17.60	16.41 ^{AB}	
Three times	15.47	17.93	16.00	18.20	16.90 ^A	
Average	15.07	15.83	16.17	16.65		
Panicle length (cm)						
Zero times	25.98	27.68	27.44	26.57	26.92	
One time	26.81	26.42	27.19	27.73	27.04	
Two times	26.44	27.70	26.72	27.73	27.15	
Three times	27.65	27.04	28.03	28.25	27.74	
Average	26.72	27.21	27.35	27.57		
Percentage of pithy	grain (%)					
Zero times	68.85	69.65	70.16	70.71	69.84	
One time	69.83	69.75	70.28	70.92	70.20	
Two times	69.87	70.48	70.58	71.67	70.65	
Three times	70.42	71.12	71.74	71.81	71.28	
Average	69.74	70.25	70.69	71.28		
1,000-grain weight (g)					
Zero times	25.80	26.51	26.31	27.12	26.43 ^B	
One time	27.82	26.25	27.73	26.62	27.10 ^{AB}	
Two times	28.04	26.79	26.97	28.14	27.48 ^A	
Three times	26.79	27.05	28.22	27.90	27.49 ^A	
Average	27.11	26.65	27.30	27.44		

Table 3 Cont.

Application of biofertilizer	Application of indigenous Beauveria bassiana Vuillemin					
	Zero times	One time	Two times	Three times	Average	
Dry milled grain weig	ght per clump of rice	plants (g)				
Zero times	40.00	39.83	43.15	48.28	42.81 ^B	
One time	48.36	43.83	47.12	40.99	45.07 ^{AB}	
Two times	41.26	49.48	51.52	44.39	46.66 ^{AB}	
Three times	44.81	49.15	48.16	53.22	48.84 ^A	
Average	43.60	45.57	47.49	46.72		

Note: Means within the column followed by the same capital letter were not significantly different according to Duncan's new multiple range test at the 5% level.

Rice production can also be affected by pest attacks on plants, especially pests that attack the generative phase. Three times indigenous *B. bassiana* application tended to give better production compared to non-indigenous *B. bassiana* application. This is because the plants applied to indigenous *B. bassiana* are more resistant to pests. Pests that commonly attack rice plants in the flowering phase until the filling of rice grains is *Leptocorisa acuta*.

The use of the fungi *B. bassiana* as a pest control has several advantages, including having a high reproductive capacity, being able to form spores that are durable in nature, environmentally friendly, and having high pathogenesis of target pests (Herlinda *et al.*, 2008). Entomopathogenic fungi as biological agents obtained from indigenous land will have higher adaptation and performance on pests to be controlled compared to biological agents introduced from other areas (Prayogo, 2006).

CONCLUSION

The application of biofertilizer and indigenous Beauveria bassiana Vuillemin did not interact with plant height, maximum number of tillers, number of productive tillers, pest attack intensity, flowering age, harvest age, panicle length, percentage of pithy grain, 1,000-grain weight, and dry milled grain weight per clump of rice plants. The application of biofertilizer can support plant height, maximum tiller number, and number of productive tillers of rice plants. The application of indigenous Beauveria bassiana Vuillemin can reduce the intensity of rice pest attacks. The combined application of biofertilizer and indigenous Beauveria bassiana Vuillemin two times was able to provide grain yields that matched the average yield in the Inpago 9 rice description, but the combination application of biofertilizer and indigenous Beauveria bassiana three times tended to give better results in all observations. This research recommended applying a combination of bifertilizer and indigenous Beauveria bassiana Vuillemin two to three times on upland rice plants in order to reduce the use of inorganic fertilizers by 25%.

ACKNOWLEDGEMENTS

Thank you to those who have helped carry out this research, Directorate General of Higher Education, Agrotechnology Department, lecturers, soil science laboratory assistant, and plant pest laboratory assistant.



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