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Combination of plant growth-promoting bacteria and botanical pesticide increases organic red rice yield and reduces the *Leptocorisa acuta* population

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Abstract. *Hoesain M, Prastowo S, Suharto, Pradana AP, Asyiah IN, Alfarizy FK, Adiwena M. 2021. Combination of plant growth-promoting bacteria and botanical pesticide increases organic red rice yield and reduces the Leptocorisa acuta population. Biodiversitas 22: 1686-1694.* Organic red rice production faces similar constraints as conventional rice production, namely pest attacks, especially rice earhead bugs (*Leptocorisa acuta*). The use of synthetic chemical inputs is also not allowed in organic red rice production. This problem can be overcome with the application of botanical pesticides, but the use of botanical pesticides does not promote plant growth. As a solution, a combination formula of plant growth-promoting bacteria and botanical pesticides is required. This study aimed to examine the effectiveness of a combination of plant growth-promoting bacteria with botanical pesticides in increasing the growth and yield of organic red rice plants and reducing the rice earhead bug population. The research was conducted on organic agricultural land in Jember Regency, Indonesia. The research began with the extraction of botanical pesticides from the leaves of *Azadirachta indica*, *Aglaia odorata*, and *Ageratum conyzoides* using 96% ethanol. The bacterial isolates obtained and characterized in previous studies were tested for their compatibility and cell viability in the combination formula. The field experiment was conducted with 8 treatments, 3 replications, and each replication consisted of 150 rice plants. The randomization pattern followed a randomized block design pattern. The results showed that all bacteria were compatible when combined with 20% botanical pesticides. In addition, cell viability at 3 months of storage decreased, but not significantly. In the organic rice growth variable, all combinations of bacteria with botanical pesticides showed good performance to increase plant growth. Increased plant growth occurred in the number of tillers, plant height, and the number of productive panicles. Furthermore, yield variables, fresh seed weight per panicle, dry seed weight per panicle, and weight of 1000 seeds also increased significantly after being treated with a combination of plant growth-promoting bacteria and botanical pesticides. The decline in the rice earhead bug population also occurred in all plants treated using botanical pesticides either singly or in combination. The best treatments in this study were BS01 and PD01, which showed consistent results. This study provides information that the combination of plant growth-promoting bacteria and botanical pesticides is compatible, does not cause a high number decrease of bacterial cells at a shelf-life of 3 months, and can stimulate the growth and yield of organic red rice, and can suppress rice earhead bug populations.

Keywords: *Bacillus*, extraction, compatibility, *Pseudomonas*, viability

INTRODUCTION

Rice is the staple food consumed by people in most countries of the Asian continent, including Indonesia (Dawe and Timmer 2012). The demand for rice in Indonesia is reported to be increasing year by year (Asngari et al. 2020). As one of the rice producers, Indonesia certainly faces various challenges in rice production, some of which are pest attacks and plant diseases, soil quality degradation, and climate change (Thorburn 2015). These problems are complex and require holistic prevention and response efforts. One effort that can be done is through organic rice cultivation (Prasertwattanakul and Ongkunaruk 2018).

Organic rice is currently gaining the public's great interest, especially health-conscious consumers who are aware of the importance of consuming healthy products (Jitrawang and Krairit 2019). Besides, the demand for red rice is also increasing along with the many campaigns about the benefits of consuming red rice for long-term health (Hegde et al. 2013; Niu et al. 2013). Several standard rules and requirements must be met in organic rice cultivation, including not using synthetic chemicals in the cultivation process, either fertilizers or pesticides (Hazra et al. 2018). Therefore, the use of organic fertilizers and pesticides is an essential factor in an organic farming system (Bhattacharjee and Dey 2014).

Similar to conventional rice cultivation, organic rice cultivation also encounters many problems. A common problem in rice cultivation is the presence of pests and plant diseases (Sukristiyonubowo et al. 2011). The common pest found in rice cultivation, particularly before the harvest season, is the rice earhead bug (*Leptocorisa acuta*). These pests reduce yields and reduce grain quality, such as brown spots on grain due to liquid suction by the pests on rice's milk stage (Litsinger et al. 2015; Ane and Hussain 2016). These insects can also suck the liquid of rice stalks. Unlike ladybugs, rice earhead bugs do not perforate the rice grains during sucking but pierce through the cavity between lemma and palea (Huang et al. 2014; Bewke 2018).

Although, the nymph of *L. acuta* is more active than the imago, however, the imago can do more damage because of its longer life (Mandanayake et al. 2014). The loss of seed fluid causes the rice seeds to shrink but rarely become empty because the rice earhead bug cannot completely empty the growing seeds' contents (Girish and Balikai 2015). If the milk stage ripe grain is unavailable, rice earhead bugs can still attack or suck in the grains, which have begun to harden by releasing enzymes that can digest carbohydrates (Singh and Singh 2014). Rice earhead bug was also reported to contaminate the seeds with microorganisms, which leads the seeds to become discolored and brittle (Anand and Jagadiswari 2000). The damage in this phase is more of a qualitative nature. Meanwhile, the grains of rice going through the milling process will be brittle and break easily (Jahn et al. 2004). Rice earhead bug can also be a vector for the pathogen *Helminthosporium oryzae* (Lakshmanan et al. 1992).

Various attempts to control the rice earhead bug population have been performed, but the rice earhead bug attack has not been fully resolved. The use of botanical pesticides is considered as a quite effective solution in reducing the rice earhead bug population (Rahman et al. 2016). Several previous studies have reported that the use of neem leaf extract (*Azadirachta indica*) effectively controls rice earhead bug attacks on a greenhouse scale and a field-scale (Benelli et al. 2017). Several other botanical ingredients that have the potential to control rice earhead bugs are *Ageratum conyzoides* leaf extract, *Aglaia odorata* leaf extract, *Cymbopogon nardus* leaf extract, *Carica papaya* leaf extract, and extracts from various other plants (Rioba and Stevenson 2017; Dougoud et al. 2019). These materials can be extracted using various solutions such as H_2O, acetone, ethanol, and methanol. Extraction can be done through easy means such as immersion or by maceration followed by an evaporation process using a rotary evaporator (Pavela 2016; Isman 2020).

The plant extracts have the opportunity to be developed into a source of botanical insecticides because its toxic compounds, anti-feeding, and hormonal properties for insects (Kedia et al. 2015). Neem contains azadirachtin compounds, and lemongrass containing citronellal and eugenol, soursop (*Annona muricata*), sugar apple (*Annona squamosa*) have been shown to be active as plant-based insecticides, as antifeedants, inhibitors of development, and inhibitors of transmission (Mpumi et al. 2016; Hikal et al. 2017; Lengai et al. 2020). Soursop leaves contain acetogenin compounds, including asimycin, roundacin, and squamosin (Gajalakshmi et al. 2012). At high concentrations of acetogenin compounds, it will be antifeedant to insects, thus causing insects to refuse to eat/no longer eager to devour plants' parts. At low concentrations, the oral administration may cause stomach poison and in severe conditions may lead to death (Khater 2012). Although botanical pesticides are effective in controlling rice earhead bugs, it cannot stimulate plant growth (Isman and Grieneisen 2014). Based on these facts, an effective and efficient solution is urgently needed; thus the botanical pesticides can play a dual role as pest control and promote plant growth.

In previous studies, we have isolated and characterized rhizobacteria and endophytic bacteria from various locations. All the bacteria isolated were reported to be able to increase the growth of various plants. The isolates characterization results showed that the isolates were able to dissolve P, bind N, and produce several extracellular enzymes such as proteases and chitinases. Even though it was reported to have the potential to stimulate plant growth, nevertheless these isolates had never been tried to promote rice plant growth (Asyiah et al. 2015; Asyiah et al. 2018). Rhizobacteria and endophytic bacteria are reported to be more effective when they were combined with other materials such as organic materials or other carrying materials (Kumar et al. 2019; Panpatte et al. 2021). However, there have been no reports lately of its effectiveness combined with botanical pesticides. This study aimed to evaluate the effectiveness of the combination of plant growth-promoting bacteria with botanical pesticides in promoting the growth of organic red rice and evaluates its ability to suppress rice earhead bug populations on organic rice fields.

MATERIALS AND METHODS

Time and research site

The research was carried out at the Laboratory of Plant Pest and Disease Control Technology, Plant Protection Study Program, Faculty of Agriculture, Jember University, and organic agricultural land in Rawasari Village, Sumberjambe Subdistrict, Jember Regency, East Java, Indonesia. Research in the laboratory and the field was performed from September to December 2020.

Botanical pesticides production

Botanical pesticides were produced from the leaf extracts of three types of plants, namely *Azadirachta indica*, *Aglaia odorata*, and *Ageratum conyzoides*. These three leaves were collected from the surrounding area of Jember University. The leaves were dried in the greenhouse for 4 days or until the leaves' water content reached ± 20%. The drying process is carried out carefully to avoid direct sunlight exposure on the sample. The dried leaf samples were then mashed and filtered using a 150-mesh sieve.

The extraction process was carried out by weighing 50 g of powder from each available plant leaf to obtain 150 g of powder from three types of plants. The powder was soaked using 750 mL of 96% ethanol. The soaking suspension was then mixed with 3 mL of Tween 80 and stirred until evenly distributed. The suspension was incubated for 24 hours, then filtered using filter paper. The supernatant formed was evaporated using a rotary evaporator at a temperature of 40 ˚C until the solvent (ethanol) evaporated and the suspension turned into a paste. The paste formed was then stored till used for further testing. Before being used, 1 g of paste was dissolved in 10 mL of sterile distilled water (Iram et al. 2013).

Source of bacterial isolates

The bacterial isolates used in this study were two rhizobacterial isolates and four endophytic bacterial isolates that had been isolated and characterized in previous studies. The details of the bacterial isolates used are presented in Table 1.

Compatibility test of endophytic bacteria with botanical pesticides

The compatibility test was performed by using the paper disk diffusion method. Bacteria were grown on Tryptic Soy Broth (TSB, HiMedia, India) medium for 24 hours at 30°C. A total of 200 µl of bacterial suspension was then grown on Tryptic Soy Agar (TSA, HiMedia, India) medium in a petri dish with a diameter of 8 cm. After that, 300 µl of the botanical pesticide formula suspension was dropped onto a sterile filter paper with a diameter of 5 mm. Combinations are compatible if no clear zone is formed. The concentration of botanical pesticides used was 20%, sterile distilled water was used as a negative control, and 70% ethanol was used as a positive control. Each treatment was replicated four times (Chandra et al. 2016).

Viability of bacterial cells after being combined with botanical pesticides

Cell viability was measured following the total plate count method. A total of 80 mL of bacterial suspension was mixed with 20 mL of botanical pesticide suspension filtered using a Millipore filter with Ø 12.25 mm and pore size of 0.2 µm. Measurement of the number of cells was carried out at the beginning of mixing and once a month since the mixing. Measurements were carried out for 3 months (Berninger et al. 2018).

Field experiment

The test was carried out on organic rice fields that have been certified by the organic agriculture certification body of PT. LeSOS (Indonesia). The red rice used was the 'Padi Merah Bali' (Bali Red Rice) variety. The design used in this study followed a randomized block design pattern with 8 treatments, 3 replications, and each replication consisting of 150 rice plants. The treatments used in this study are presented in Table 2.

The application of the combination formula of bacteria and botanical pesticides was carried out six times, namely at 15 days after planting (DAP), 30 DAP, 45 DAP, and 60 DAP, 75 DAP, and 90 DAP. The application technique was carried out by spraying 500 mL of combination formula according to each block treatment; thus, it requires 1500 mL of combination formula for 3 blocks.

Observational variables measured in this study consisted of plant growth variables (number of tillers, plant height, number of productive panicles), yield variables (seed fresh weight per clump, dry seed weight per clump, and fresh weight of 1000 seeds), and number of rice earhead bug populations. Observation of the rice earhead bug population was performed before applying a combination of bacteria and botanical pesticides and morning a day later. The number of earhead bug was calculated manually by observing the existing population using a hand counter (Islam et al. 2013). Growth and yield data were analyzed for diversity, if there was a difference, it was followed by a further test following the Duncan's Multiple Range Test (DMRT) method with 95% confidence level. The analysis was performed using the *DSAASTAT version 1.101* program.

Table 1. Sources of plant growth-promoting bacteria isolate used in this study

Isolate code	Bacterial species	Status	References
PD01	*Bacillus subtilis*	Rhizobacteria	Asyiah et al.
BS01	*Pseudomonas dimunita*	Rhizobacteria	(2015; 2018)
SK14	*Bacillus* sp.	Endophyte	
SK15	*Bacillus* sp.	Endophyte	
KB11	*Bacillus antrachis*	Endophyte	
KB14	*Bacillus* sp.	Endophyte	

Table 2. Treatments used in the study

Code	Treatment
K-	1500 mL water
K+	20 mL botanical pesticide + 1480 mL air
PD01	20 mL botanical pesticide + 100 mL bacterial suspension PD01 + 1380 mL water
BS01	20 mL botanical pesticide + 100 mL bacterial suspension BS01 + 1380 mL water
SK14	20 mL botanical pesticide + 100 mL bacterial suspension SK14 + 1380 mL water
SK15	20 mL botanical pesticide + 100 mL bacterial suspension SK15 + 1380 mL water
KB11	20 mL botanical pesticide + 100 mL bacterial suspension KB11 + 1380 mL water
KB14	20 mL botanical pesticide + 100 mL bacterial suspension KB14 + 1380 mL water

RESULTS AND DISCUSSION

Endophytic bacteria compatibility with botanical pesticides

Based on the compatibility test results, it has been shown that all bacteria are compatible when combined with 20% botanical pesticides. This is indicated by the absence of a clear zone formed 24 hours after the test was carried out. The same pattern also occurred in the negative control (K-). In the positive control (K+), the results showed a clear zone around the paper disk, which means that the bacterial cells are unable to grow or incompatible.

Furthermore, bacterial compatibility data are presented in Table 3.

Viability of bacterial cells

Based on the analysis results, it was found that at the beginning of the combination, all the tested bacteria had a cell density of 10^8. After one month of storage, the bacterial density decreased slightly, but not significantly; all bacteria still had a cell density of around 10^8. For example, PD01 + 20% botanical pesticides, on the first day of combination, the cell density reached 4.2×10^8, then after one month of storage, the density decreased slightly to 3.7×10^8. The decrease in cells had a further decrease in the 2nd month. The observation results showed that in the 2nd month, the average density of bacterial cells was 10^7. Furthermore, in the 3rd month, the density of bacterial cells decreased not significantly in some isolates (PD01, BS01, SK07, and KB14), it was shown that the cell density was still around 10^7. However, there was a decrease in cell density in other isolates to 10^6 (SK14 and SK15 and 10^5 (KB11). The bacterial viability data after being mixed with botanical pesticides are presented in Figure 1.

Organic red rice plant growth performance

Based on observations, several combinations of plant growth-promoting bacteria and botanical pesticides effectively increase organic red rice plants' growth. In the number of tillers, it was obtained that the number of tillers in rice without any treatment (K-) had an average number of tillers of 13.78, and on the average number of tillers of rice treated by K+ (only sprayed using 20% botanical pesticides) was 14.67. The highest number of tillers was shown by plants treated with BS01 (16.65), followed by SK15 (16.27), SK14 (16.26), PD01 (15.85), KB14 (15.54), and KB11 (15.33). All treatments except KB11 showed an average value for the number of tillers significantly different from control plants.

Plant height treated with K- showed an average plant height of 83.16 cm, and treated with K+ showed an average plant height of 83.70 cm. Statistically, K- and K+ treatments did not show any significant differences. Best performance on plant height was shown by the treatments of BS01 with an average plant height of 100.14 cm, followed by PD01 (99.92 cm), SK14 (97.77 cm), KB14 (97.53 cm), KB11 (96.34 cm), and SK15 (91.60 cm). In addition, the number of productive panicles showed the average number of productive panicles from treatment K- was 8.68, and K+ was 9.32. The highest average number of productive panicles was shown by the treatments of PD01 with the number of productive panicles 14.31, followed by KB14 (14.17), BS01 (13.62), KB11 (13.4), SK15 (13.28), and SK14 (13.15).

In general, two treatments show consistency in increasing organic red rice plants' growth, namely the BS01 and PD01 treatments. The two treatments always showed high performance, and the variable plant height and number of productive panicles between BS01 and PD01 showed no significant difference between the two. Furthermore, the growth data for organic red rice in the various treatments tested are presented in Table 4.

Organic red rice yield performance

Based on the observations, it is shown that the application of a combination of plant growth-promoting bacteria with botanical pesticides can increase the yield variable of organic red rice. The fresh weight of seeds per panicle with K treatment showed the average seed fresh weight of 25.70 g, and in the K+ treatment was 25.07 g, not statistically different. The highest average seed fresh weight per panicle was obtained in plants treated by BS01 (37.22 g), followed by PD01 (34.67 g), KB14 (33.15 g), KB11 (32.66 g), SK15 (31.26 g), and SK14 (31.11 g). Statistically, all treatments showed significant differences with K- and K + treatments; this indicates that all treatments had a significant positive effect on seed fresh weight per panicle. Data regarding seed fresh weight per panicle is presented in Figure 2.

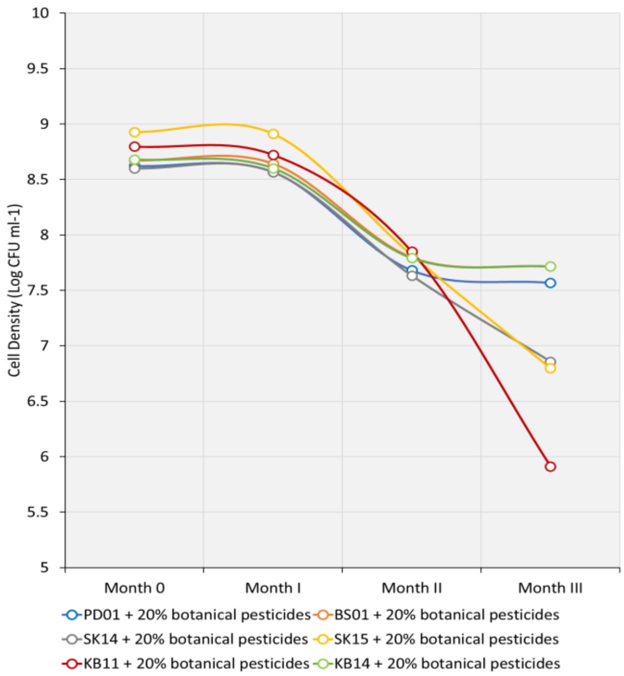

Figure 1. The viability of bacterial cells after being mixed with botanical pesticides

Table 3. Compatibility of plant growth-promoting bacteria with botanical pesticides

Code	Treatment		
	20% Botanical pesticide	Sterile water	70% Ethanol
K-	-	-	+
K+	-	-	+
PD01	-	-	+
BS01	-	-	+
SK14	-	-	+
SK15	-	-	+
KB11	-	-	+
KB14	-	-	+

Note: (-) no clear zone is formed/compatible; (+) a clear zone is formed/incompatible

Table 4. Growth variables of organic red rice

Treatment	Number of tillers (∑ ± SD)	Plant height (cm ± SD)	∑ Productive panicle (∑ ± SD)
K-	$13.78^{a} \pm 0.23$	$83.16^{a} \pm 0.72$	$8.68^{a} \pm 0.10$
K+	$14.67^{b} \pm 0.61$	$83.70^{a} \pm 0.34$	$9.32^{a} \pm 0.40$
PD01	$15.85^{cd} \pm 0.64$	$99.92^{c} \pm 2.99$	$14.31^{b} \pm 0.44$
BS01	$16.65^{e} \pm 0.29$	$100.14^{c} \pm 1.19$	$13.62^{b} \pm 0.67$
SK14	$16.26^{de} \pm 0.50$	$97.77^{c} \pm 3.75$	$13.15^{b} \pm 0.71$
SK15	$16.27^{de} \pm 0.22$	$91.60^{b} \pm 4.19$	$13.28^{b} \pm 1.36$
KB11	$15.33^{bc} \pm 0.35$	$96.34^{c} \pm 1.85$	$13.40^{b} \pm 0.31$
KB14	$15.54^{cd} \pm 0.62$	$97.53^{c} \pm 1.12$	$14.17^{b} \pm 0.61$

Note: The number followed by each different letter shows a significant difference in each line based on the DMRT test results with a 95% confidence level. SD = standard deviation

The results of observations on seed dry weight per panicle showed that plants treated with K- showed an average dry weight of 19.62 g, and K+ showed an average dry weight of 22.03 g. The best performance treatment on seed dry weight per panicle was BS01 (28.32 g), followed by KB11 (25.42 g), SK14 (25.35 g), PD (25.19 g), SK15 (25.06 g), KB14 (24.46 g). Based on the results of statistical analysis, it is found that the combination of plant growth-promoting bacteria and botanical pesticides provides significantly different results compared to control plants. The BS01 treatment shows the best performance. Data regarding the seed dry weight per panicle is presented in Figure 3.

Based on the results of observations on the weight of 1000 seeds in each treatment, it is found that the average weight of 1000 seeds in K- treatment was 27.85 g, and K+ treatment was 28.76 g. Both K- and K + treatments did not show statistically significant differences. The treatment with the best performance on the variable weight of 1000 seeds was BS01 weighing 37.68 g, followed by KB11 (35.84 g), SK14 (35.39 g), KB14 (33.97 g), SK 15 (33.80 g), and PD (32.81 g). Statistically, all combination treatments of plant growth-promoting bacteria and botanical pesticides resulted in significantly different results, indicating that all the combinations tested gave significant positive results. The data of the weight of 1000 seeds is presented in Figure 4.

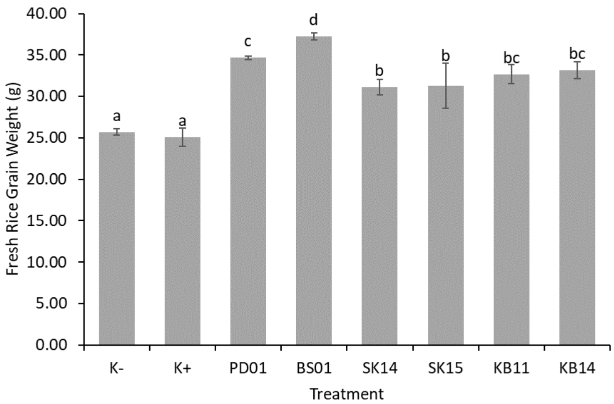

Figure 2. Seed fresh weight per panicle in various treatments. Note: The value followed by each different letter shows a significant difference in each bar based on the DMRT test results with a 95% confidence level.

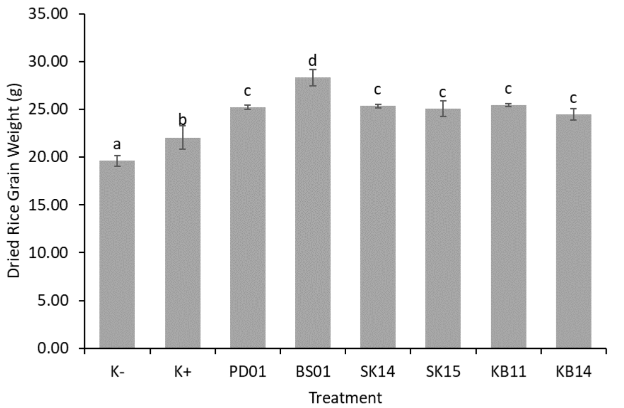

Figure 3. Seed dry weight per panicle in various treatments. Note: The value followed by each different letter shows a significant difference in each bar based on the DMRT test results with a 95% confidence level

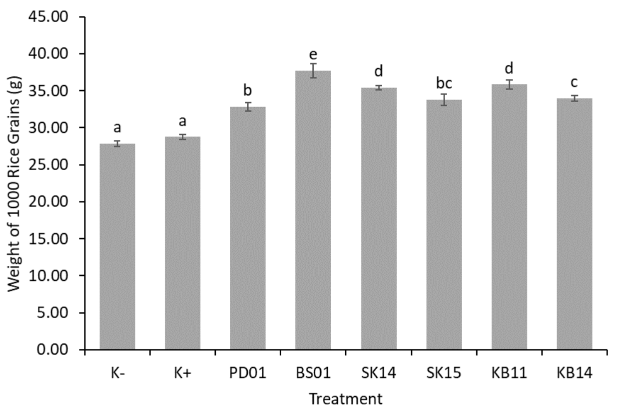

Figure 4. Weight of 1000 rice seeds in various treatments. Note: The value followed by each different letter shows a significant difference in each bar based on the DMRT test results with a 95% confidence level

The effect of combination of plant growth-promoting bacteria and botanical pesticides on rice earhead bug populations

Based on the results of observations on the rice earhead bug population, it is shown that the rice earhead bug population from the first observation to the sixth observation tends to increase. The increase in population is in line with the growing stages of the rice plants. From the first observation to the fourth observation, the rice earhead bug population has decreased after the treatment application. The decline in the rice earhead bug population was quite high in plants treated with K+, BS01, and PD01. In contrast to the K-treatment, the 2nd and 4th observations actually showed the increase of the rice earhead bug population after treatments were given. This result indicates that treatments using botanical pesticides either singly (K+) or in combination with bacteria positively affect the rice earhead bug population. In the fifth observation, neither K- nor K+ suppressed the rice earhead bug population, but the combination formula suppressed the rice earhead bug population after treatments were given. Furthermore, in the last observation (6th observation), all treatments containing botanical pesticides could significantly suppress the rice earhead bug population. The data on the rice earhead bug population is presented in Figure 5.

Discussion

Plant growth-promoting bacteria as agents to promote plant growth and control pests and plant diseases have been widely reported. In previous studies, it was reported that the combination of bacteria with other substances needed to be tested for compatibility first (Panpatte et al. 2021). The compatibility tests can be carried out either among organisms or organisms and their carrying materials (Chandra et al. 2016). In this study, it was reported that all tested bacteria were compatible when combined with botanical pesticides. Compatibility means that bacteria are able to survive or not be affected at all by the presence of external substances. Several factors affecting the compatibility of a bacterium with other substances are the species of bacteria, the ability to tolerate antibiotic compounds, and the ability to produce cell-protective compounds such as polysaccharides (Eveno et al. 2020; Fitriatin et al. 2020).

Plant growth-promoting bacteria's effectiveness in promoting plant growth and controlling pests and plant diseases is closely related to their cell density. The denser number of cells that touch the surface of plants or soil, the higher effectiveness of the biological agents (Jha et al. 2013; Afzal et al. 2019). In this study, it is known that the density of bacterial cells has decreased insignificantly at 2 months of storage and began to look significant at the 3rd month of storage. Many factors affect the viability of bacterial cells on a carrier medium. The most influencing factor is the availability of nutrients for bacteria to survive on the carrier media (Herrmann and Lesueur 2013). The nutrients needed by bacteria to survive during the storage period include water, carbon sources, nitrogen sources, sugar sources, non-metal elements (NA ++, K ++, CA ++, Mg ++, Mn ++, Fe ++, Zn ++, Cu ++, Co ++), and vitamins (Stewart 2012). In previous reports, the combination of bacteria with some organic matter was able to last between 3 and 6 months (Pradana et al. 2020). Putri et al. (2016) reported that bacteria from the genus *Bacillus sp.* stored in the talk carrier media can last up to 3 months. Furthermore, in a separate study, bacteria from the genus *Pseudomonas* sp were able to survive in organic liquid carrier media for up to 6 months (Gopi et al. 2019).

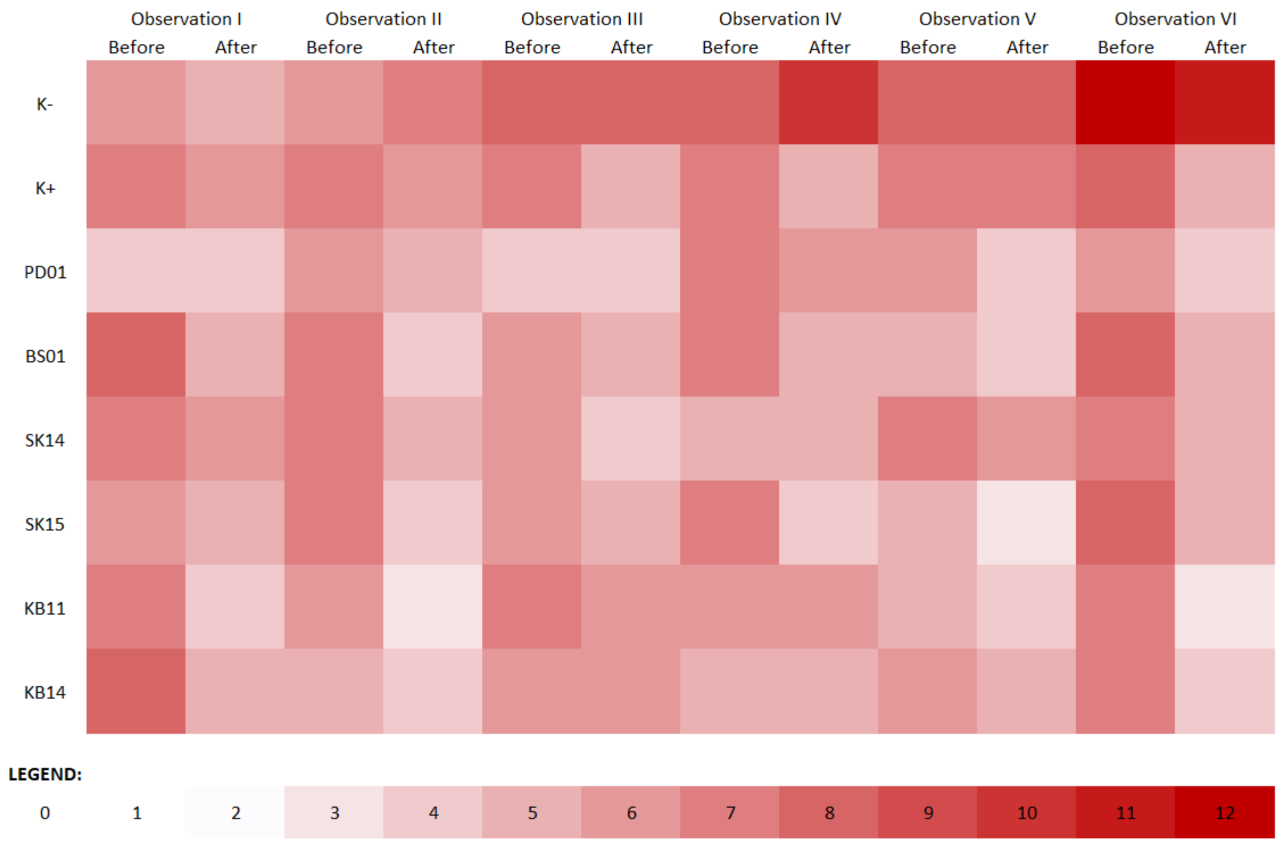

Figure 5. The population of rice earhead bugs in various treatments

Plant growth variable showed that all combinations of bacteria and botanical pesticides have a significant positive effect. This result indicates plant growth-promoting bacteria has a significant role in the growth of rice plants. In a separate study, Ashrafuzzaman et al. (2009) reported that the application of rhizobacteria could increase rice plants' growth. Similar results were also reported by Etesami et al. (2014), the application of endophytic bacteria was able to increase rice plant growth while increasing rice yields. Plant growth-promoting bacteria's ability to promote the growth of rice plants cannot be separated from its ability to produce various secondary metabolites needed by plants (Jha and Saraf 2015).

In addition, the physiological activity of bacteria can also help plants obtain nutrients (Singh and Singh 2014). Maheswar and Sathiyavani (2012) reported that bacteria from the genus *Bacillus* sp. was able to dissolve P and bind N. Ahmad et al. (2005) also reported that bacteria from the genus *Pseudomonas* sp. was able to produce Indole Acetic Acid (IAA) compounds needed for plant growth. The availability of nutrients needed by plants is an important factor in plant growth. For example, nitrogen is needed by plants for protein biosynthesis. Elemental N also plays an essential role in plants' vegetative growth, such as the formation of leaves, roots, stems, and tillers (Wang et al. 2014). This element is also known to play a role in the formation of chlorophyll for leaf photosynthesis, the production of amino acids and proteins, and other metabolites that play a role in forming plant cell walls (Krapp 2015). The increase in plant growth obtained in this study is thought to have occurred due to the influence of bacterial physiological activity and the presence of secondary metabolites produced by bacteria.

Through this study, it can be concluded that the combination of bacteria with botanical pesticides was effective in increasing the yield variables of organic red rice. The ability of plant growth-promoting bacteria to increase crop yield has been previously reported. Singh et al. (2016) reported that the application of rhizobacteria in rice effectively increased rice yields. In a separate report, Hastuti et al. (2012) report that the application of endophytic bacteria in rice plants is also effective in increasing rice yields while reducing disease incidence due to *Xanthomonas oryzae* infection in rice plants. The ability of bacteria to increase rice yield variables cannot be separated from their physiological activities. For example, *Bacillus* sp. and *Pseudomonas* sp. are two genera of bacteria that are reported to have the ability to dissolve P in soil (Etesami et al. 2017). The element P is one of the elements with an essential role in determining rice plants' production. Element P is known to play a role in producing several types of protein, helps assimilation in plants, accelerates flowering, and accelerates the ripening of seeds and fruit (Raghothama 2000).

Observation variable of the rice earhead bug population indicated that all treatments mixed with botanical pesticides had a good performance in suppressing the rice earhead bug population. The botanical materials used in this study, namely the leaves of *A. indica*, *A. odorata*, and *A. conyzoides*, were reported to contain insecticidal compounds (Grdiša and Gršić 2013; Isman 2020). *A. indica* leaves are reported to contain several active ingredients such as β-sitosterol, hyperoside, nimbolide, quercetin, quercitrin, routine, azadirachtin, and nimbine. Some of these active ingredients are reported to be insecticidal (Benelli et al. 2017). Another study reported that *A. odorata* leaves contain alkaloids, saponins, flavonoids, tannins, and essential oils. *A. odorata* leaves not only contains rocaglamide but also found contain three derivative compounds, namely desmethylrocaglamide, methyl rocaglamide and rocaglamide (Zhang et al. 2012). *A. conyzoides* leaves were reported to contain several insecticidal compounds, namely saponins, flavonoids, polyphenols, coumarin, 5% eugenol, HCN and essential oils (Rioba and Stevenson 2017).

Based on the results obtained from this study, it was concluded that the combination of plant growth-promoting bacteria with botanical pesticides from *A. indica*, *A. odorata*, and *A. conyzoides* leaf extracts was effective in increasing the growth and yield of organic red rice plants. In addition, the combined application of the two is also effective in suppressing the rice earhead bug population.

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