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Control of Bacterial Leaf Blight *Xanthomonas* by Application of *Bacillus subtilis* Biobactericide on Rices

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ABSTRACT

Indonesia's agricultural problems in rice cultivation are increasingly complex, threatening national food security. The attack of *Xanthomonas oryzae* pv. *oryzae (Xoo)* can cause decreased production of bacteria, resulting in rice disease bacterial leaf blight (BLB). The biological agent *Bacillus subtilis* has the potential to control BLB disease. The study aimed to determine the effect of *B. subtilis* bacteria in reducing the intensity of *Xoo* attacks by comparing the use of pesticides. Xoo attacks by comparing the use of citronella vegetable pesticide and its effect on rice production. This experiment was designed as a comparison of 2 plots with different treatments, namely the control treatment of citronella vegetable pesticide with a concentration of 75% and the biobactericide treatment of *B. subtilis* with an antagonistic bacterial suspension density of 33 x 10^4 cfu.mL^{-1}. The variables observed were inhibition, disease intensity, weight of harvested dry grain (HDG)/ per clump, and weight of HDG per plant. The results stated that the biobactericide *B. subtilis* had a real effect on the development of BLB disease up to an intensity of 14.72% and the development of BLB with citronella vegetable pesticide treatment decreased to 28.97%, the weight of HDG per clump and did not give a real effect on the weight of HDG per panicle.

Keywords: Cropping index; overlaying; self-sufficiency; SPOT image; visual interpretation.

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INTRODUCTION

Rice (*Oryza sativa* L.) is the main food in Indonesia, because the majority of the population uses rice as their staple food (Donggulo et al. 2017). According to data from BPS (2023), shows that rice production in Jember Regency from January to December 2022 reached approximately 607.37 thousand tons of dry paddy (GKG), with a predicted decrease of approximately 8.33 thousand tons of GKG, or 1.35%, compared to 615.70 thousand tons of GKG in 2021. With the high level of Indonesian people's reliance on rice as a staple food, the community may eventually shift to a non-rice diet, so the government needs to achieve rice self-sufficiency (Moningka et al. 2020). The complexity of the problems faced by the agricultural industry, especially rice crops, places this country's food security at the forefront of its problems.

Examples of factors that threaten food security include agricultural land conversion, inappropriate use of chemical pesticides, labor force decline, climate change, and pest attacks (Molotoks et al. 2021; Wakil et al. 2025). One important disease affecting rice plants is leaf blight (LBB), caused by the bacterium *X. oryzae pv. oryzae*. Loss of rice production due to bacterial blight disease is around 15-24% (Sutarman 2017). Plant symptoms include yellowing, drying, and straw-colored leaves, commonly known as leaf spots. Several disease control methods have been implemented, including land sanitation, the use of resistant cultivars, synthetic chemical pesticides, biopesticides, and biological agents.

The use of chemical pesticides can be reduced by using biopesticides made from natural and environmentally friendly ingredients. Plants such as citronella (*Cymbopogon nardus*) can act as natural antimicrobials. The use of biological agents, such as those from the *Bacillus subtilis* group, can also be an alternative solution, which has been tested in vitro to suppress the development of *Xoo* in rice seeds (Djatmiko et al. 2022; Srinivas et al. 2024). Many scientists have reported how biological agents prevent disease and increase production.

Increasing production and preventing diseases in rice plants using biological agents of the *Bacillus subtilis* group has not been widely reported, especially in Indonesia (Agustiansyah et al. 2013). Based on the

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explanation above, it is known that *B. subtilis* bacteria can act as a biological agent. If the antagonist produced by *B. subtilis* is high, *Xoo* disease attacks can be controlled. Therefore, research on *B. subtilis* bacteria was conducted to determine the effect of the biotoxin *B. subtilis* in controlling the intensity of *Xoo* disease in rice plants.

MATERIALS AND METHODS

This research was conducted in rice fields in Tlogo Wetan Village, Antirogo, Sumbersari District, Jember Regency, East Java and the Bioscience Laboratory, Jember State Polytechnic in August-December 2023. The tools and materials used in this research are, the tools used in the first stage of research include air flow cabinet (LAFC), sterile petri dishes, loop needles, laminar autoclaves, bunsen, test tubes, cell phone cameras, sprayers, measuring cups, hot plates, pH meters, thermoshakers, vortex, incubators, micropipettes, Ependorf tubes, vernier calipers. While the tools used in the second stage of research include, sample boards, hoes, banners, meters, scales, measuring cups, and sprayers, cameras. The first phase of the research used materials such as Bacillus sp. bacterial isolate, *Xanthomonas oryzae* pv. *oryzae* bacterial isolate, 96% alcohol, 70% alcohol, fine rice bran, distilled water, aluminum foil, chloroform, filter paper (Whatman paper no. 1), sterile tissue, label paper, plastic, camera. In the second phase, among others, were media resulting from the multiplication of *Bacillus subtilis* bacteria, *Xoo* bacterial isolate, topsoil, Ciherang variety rice seeds, manure, botanical pesticides, NPK fertilizers and sample labels. The research design compared two treatment plots on different rice fields, where each field was sprayed with a biological agent and botanical pesticide. Plot 1 will be sprayed using botanical pesticides used when controlling diseases on organic fields with a concentration of 5 ml of citronella leaf extract plus 5 ml of water (Barimbing 2022). Plot 2 was sprayed with the biological agent *B. subtilis* at a concentration of 20 $mL.L^{-1}$. The field research layout was 10 m x 10 m for the plot and 25 cm x 25 cm for the planting distance, with 2-3 seedlings per hole (Erdiansyah et al. 2021). The random sampling method used was a zigzag pattern with a sample of 50 plants.

Bacillus subtilis propagation in the laboratory uses rice bran as a medium. The mechanism for making rice bran media is to weigh 30 grams of processed, fine rice bran and sterilize it using an autoclave for 30 minutes at a temperature of 121°C and a pressure of 1 atm.

Antagonistic tests were conducted between *Bacillus subtilis* bacteria and the pathogenic bacteria that cause bacterial leaf blight, namely *X. oryzae* pv. *oryzae,* to determine the potential of pure bacteria as biological agents. The method used was the modified kawaghuci (fogging) method in NA media. Adding 1 mL.100 mL of starter and incubating for 3 days, then it was ready to be applied. The media resulting from the incubated bacterial multiplication was applied to the plants. Application to rice seedlings was carried out when they were about to be transplanted, by dipping them in a *Bacillus subtilis* biobactericide solution for 10 minutes and ready to be planted.

Spraying is carried out on the seedbed approximately 7 days before transplanting. Biobactericide spraying is carried out after planting at 2, 3, 4, and 5 weeks after planting. The data obtained were analyzed using non-parametric statistical tests in SPSS with the following stages: 1) Data normality test; 2) Data homogeneity test; 3) If the data is not normal and not homogeneous, then it is continued with a non-parametric test using the Mann-Whitney Test; 4) If the data is normal and homogeneous, then it is continued with the Paired-Samples T Test.

RESULTS AND DISCUSSION

Controlling disease with chemical bactericides is very harmful to the environment. Natural bactericides are needed as an alternative to minimize the harmful impact on the environment. Biobactericides are substances or components that can inhibit bacterial growth. Soil bacteria called *Bacillus* spp. are often found in the rhizosphere of plants (Pandango et al. 2018). Because *Bacillus* rhizobacteria can control plant diseases and increase the amount of nutrients in the soil, its use as a biological agent has been shown to increase yields (Nurkartika et al. 2018).

One bacterial capable of addressing root problems is Bacillus. This bacterium is safe to use as a biobactericidal agent and grows rapidly even in liquid media. As a plant growth-promoting rhizobacterium (PGPR) and able to survive harsh climatic conditions, *Bacillus subtilis* has an advantage over other similar bacteria, namely its ability to reduce various plant diseases (Wartono et al. 2015; Serrão et al. 2024; Ali et al. 2025). Based on the results of the research data, the following results were obtained.

Inhibitory Power

To determine the potential of *Bacillus subtilis* bacteria, an inhibitory power test was conducted using the spray method. The results were measured in millimeters (mm). The inhibitory power test results are as follows. The pathogenic bacteria *xoo* was suppressed in its development by the antagonistic bacteria *Bacillus subtilis*, based on the findings of an antagonist test conducted in vitro using NA media in a petri dish. An indication of the ability of the pathogen *xoo* to be inhibited by a biological agent is the formation of an inhibition zone around the antagonist colony. The width of the inhibition zone, or bacterial inhibition zone, indicates how well the microorganism can inhibit the growth of the disease (Azman et al. 2017; Srinivas et al. 2024b).

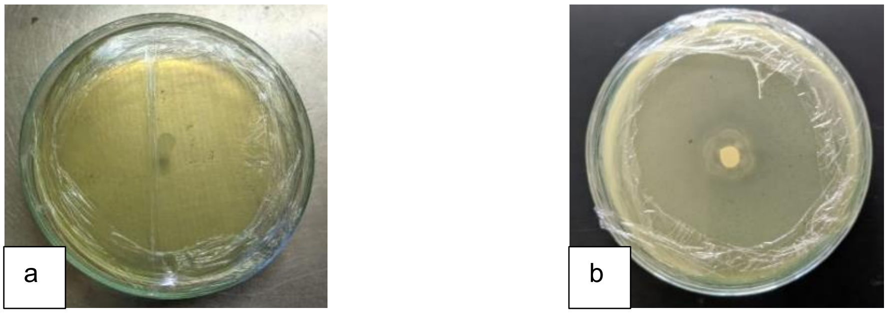

Figure 1. (a) Inhibitory power test of *Bacillus subtilis* bacteria and *Xanthomonas oryzae* pv. *oryzae* bacteria, (b) Results of inhibitory power test of *B. subtilis* bacteria and *X. oryzae* pv. *oryzae* bacteria

Table 1. Results of Inhibitory Power Test Calculations.

Resistance Diameter (mm)	Inhibition Zone Category
5.5 mm	Strong Category

Note: Weak category (≤5 mm), moderate category (6-10 mm), strong category (19-20 mm), and very strong category (≥20 mm).

The antibacterial effect is divided into four levels: weak, moderate, strong, and very strong. If the inhibition zone diameter is <5 mm, bacterial activity is considered weak, moderate if 5-10 mm, strong if 10-20 mm, and very strong if more than 20 mm (Barimbing 2022). Based on the results of measuring the diameter of the clear zone in the *Bacillus subtilis* bacterial isolate against the *Xoo* bacteria. Both replications had an inhibitory power in the moderate inhibition category, namely 5.5 mm in the first replication and 5.5 mm in the second replication. Based on the antagonist test, it can be seen that the *Bacillus subtilis* isolate can inhibit the development of the *Xoo*. The antagonist bacteria *B. subtilis* has an inhibitory substance so that the growth of the antagonist pathogenic bacteria can be inhibited.

The resulting inhibitory compounds act as pathogen antagonists and work by degrading the cell walls of pathogenic bacteria. Previous research has shown that citronella contains essential oils with potent antibacterial properties. Citronella has an inhibition zone of 10.00 mm at a 50% concentration, which is classified as moderate inhibition (Barimbing 2022). Citronella essential oils include sintral, citronella, geraniol, and myrcene, which can be used as natural antimicrobials.

Disease Intensity

Observations showed that inoculation of *X. oryzae* pv. *oryzae* isolates can cause symptoms on leaves 3 weeks after inoculation. Environmental factors such as temperature can influence the development of diseases such as bacterial leaf blight. The average daily temperature in the area where this experiment was conducted ranged from 23°C to 33°C, which supports the development of *Xoo* bacteria in rice plants because 26-30 °C is the ideal temperature for the development of these bacteria.

Based on the data in Table 2 regarding the results of the intensity of bacterial leaf blight disease attacks (*X. oryzae* pv *oryzae*) between the treatment of the biological agent *Bacillus subtilis* and the control treatment of the citronella leaf botanical pesticide provided significant results. The results of the disease intensity in the *Bacillus subtilis* biobactericide treatment were lower compared to the disease intensity with the citronella botanical pesticide treatment. This can occur because the density of the formulation can suppress the rate of infection according to (Wartono et al. 2015), the reduction in bacterial leaf blight disease is influenced by the density of the formulation spraying. The density of the antagonistic bacterial suspension used was 33x cfu/ml. The level of bacterial leaf blight disease intensity is in the moderate category because it is below 25%.

Table 2. Intensity of Bacterial Leaf Blight Disease

Treatment	Minggu Setelah Tanam (MST)		
	3	4	5
Citronella Botanical Pesticide	15.22a	20.22a	28.97a
Bacillus subtilis	16.73a	19.68b	14.72b

Description: Numbers followed by different letters in the same column indicate a significant difference according to the Paired samples T test (*P*<0.05). MST; weeks after planting

According to the Kementerian Pertanian, (2018) the disease intensity levels follow the following provisions: (1) 0% No attacks/healthy plants, (2) 1-11% mild attacks, (3) 12-25% moderate attacks, (4) 26-85% severe attacks, and (5) >85% puso attacks. Therefore, control of bacterial leaf blight disease must be controlled. The effectiveness of these two bactericides is influenced by the active ingredients of the bactericide. This is in accordance with the statement (Meimaharani and Listyorini 2013) that the use of *Bacillus subtilis* plays a very important role in suppressing the population and intensity of disease attacks due to damage to plants. Botanical pesticides contain active ingredients of essential oils as antibacterials which can disrupt the process of causing pathogenic bacteria in leaves (Bota et al. 2015).

Observations were conducted before and after biobactericide application. Observations before application were conducted at 3 WAP with the aim of determining the rate of disease infection within the control threshold after inoculation of the pathogen *Xanthomonas oryzae pv oryzae*. The decrease in the intensity of bacterial leaf blight disease is suspected to be due to the influence of compounds and toxins produced by the bacteria *Bacillus subtilis* that infect bacterial leaf blight. At 4 WAP, the disease population increased again. Plant age influences the development of bacterial leaf blight disease; seedlings transplanted at a younger age often have a higher percentage of disease (Khaeruni et al. 2014).

Based on observations of disease severity, bacterial leaf blight disease progression in each treatment was determined on sample leaves. The disease severity showed that the earlier the rice plant was infected, the slower the disease spread; conversely, the earlier the rice plant was infected, the faster the disease spread. A decrease in disease severity was seen in the percentage of bacterial leaf blight disease at different periods after biobactericide application. Compared to the previous growth phase, disease progression occurred more rapidly during the vegetative growth phase, which lasted between 3 and 4 weeks after planting. The average disease progression reached 18-33%. The level of resistance of plant varieties is also influenced by the morphological structure of the leaf surface (Khaeruni et al. 2014). The slow rate of development in the reproductive phase is likely due to the complete formation of the plant's resistance structure. Plant epidermal cells have a waxy layer and a thick cuticle that are ideal for increasing plant resistance to diseases that directly penetrate the epidermal layer.

Dry Rice Harvest Results

Test results showed that the application of the biobactericide *Bacillus subtilis* significantly affected the weight of dry grain per clump in rice plants. The test results are presented in Table 3. The results of the *Bacillus subtilis* treatment were higher than those of plants treated with the citronella leaf pesticide, reaching an average weight of 27.26 grams. The percentage of dry paddy weight in the *Bacillus subtilis* biobactericide treatment was 11.54% higher than that in the citronella plant pesticide treatment. This is due to the lower disease intensity in the biobactericide treatment. Production results and disease severity correlated consistently. Based on the research findings, population influences yield within a certain area

Table 3. Weight of Dry Paddy (GKS**)**

Treatment	Weight of Dry Paddy per Clump (g)
Citronella Botanical Pesticide	24.44a
Bacillus subtilis	27.26b

Note: Numbers followed by different letters in the same column indicate significant differences according to the Mann-Whitney test (*P*<0.05)

The severity of the disease attack also affects the weight per sample; the greater the intensity of the attack caused by bacterial leaf blight, the greater the risk of rice yield loss (Listianti et al. 2019). However, the yield per hectare from the biobactericide application treatment was not higher than the description of the Ciherang variety, which yields 5-7 tons per hectare. Meanwhile, the production yield in the P1 treatment resulted in a yield of 4.5 tons per hectare. This indicates that disease intensity affects production yield, because bacterial leaf blight attacks the leaves of the plant. Infected leaves cannot produce panicles, resulting in low productivity.

Biological agents have the ability to bind nitrogen, produce plant growth hormones, and dissolve phosphate, among other methods to stimulate growth and increase crop yields. The number of panicles in rice plants is influenced by the nutrient phosphate (Mindari et al. 2018). The panicle is where the grain is attached, and its length determines how many panicles or grains are produced on each panicle. The longer the panicle, the more grains are produced on each panicle.

Weight of Dry Paddy (GKS) Per Panicle

The test results showed that the *Bacillus subtilis* bactericide treatment had no significant effect on the weight of dry rice grains per panicle. The test results are presented in Table 4. The weight of dry rice grains per panicle has a difference of only about 3.54%. The attack of the bacteria *Xanthomonas oryzae* pv. *oryzae* which causes bacterial leaf blight is thought to inhibit the development of rice plants. By reducing the number of growing panicles or preventing rice grains from filling, the fewer leaves have an indirect effect in limiting crop yields (Khaeruni et al. 2014; Kone et al. 2025). The amount of assimilates spread from the source to the hole is indicated by the overall grain content. According to Pranata and Kurniasih (2019), the absence of a percentage of filled grains indicates that the assimilates distributed during filling are rather low

Table 4. Weight of Dry Paddy Grain (GKS) per Panicle

Treatment	Weight of Dry Paddy Grain (g)
Citronella Botanical Pesticide	2.26a
Bacillus subtilis	2.34a

Description: Numbers followed by the same letter in the same column show no significant difference according to the Mann-Whitney test (*P*>0.05).

Grain Weight Per 1000 Grains

Paddy quality is determined by weighing 1.000 grains; the heavier the grain, the higher the quality and the more substantial the grain. The results of the average dry paddy weight test per 1,000 grains are presented in Table 5. *Bacillus subtilis* biobactericide treatment, which significantly affected the weight of dry paddy rice per 1000 grains. The weight of 1000 grains of full paddy in the *Bacillus subtilis* treatment was 32.56 grams, this weight is in accordance with the description of the Ciherang variety. This condition is caused by the use of *Bacillus subtilis* bacteria, which produce growth regulators that have the ability to stimulate root development (Agustiansyah et al. 2020).

Table 5. Weight of 1000 grains of dry paddy from rice fields

Treatment	Weight of 1000 Grains (g)
Citronella Botanical Pesticide	29.47a
Bacillus subtilis	32.56b

Description: Numbers followed by different letters in the same column indicate a significant difference according to the Paired Samples T Test (P<0.05).

Bacillus subtilis treatment had a significant effect on the weight of paddy per 1000 grains. This indicates that the photosynthesis process in plants is functioning well, resulting in the production of assimilates which are the final product (Pranata and Kurniasih 2019). In addition to producing plant hormones, vitamins, and amino acids, *Bacillus subtilis* also functions as N binding and absorbing P and K (Sitepu et al. 2017). Bacteria take nutrients from available nutrients for their own growth and development, thereby slowing plant growth and development and reducing fruit production. Apart from that, the concentration given is low and the application time is too long, making it difficult to synthesize growth regulators (Nusyirwan and Syahadah 2020).

This is suspected to be due to the attack of *X. oryzae* pv. *oryzae*, which causes bacterial leaf blight, which will inhibit growth. This research is supported by Pranata and Kurniasih (2019), who stated that reducing the number of leaves definitely reduces production by reducing the number of leaves formed, thus reducing grain filling. The extent of assimilates distribution from the source to the hole is indicated by the overall grain content. The absence of a total percentage of filled grains indicates that the assimilates dispersed during filling are quite low.

CONCLUSIONS AND SUGGESTIONS

The biological agents *B. subtilis* was able to inhibit the spread of bacterial leaf blight disease. The spread of bacterial leaf blight disease attacks will be inhibited because *B. subtilis* bacteria are effective as antagonists of the pathogen *X. oryzae pv oryzae* with a medium inhibitory power category with a suspension density of antagonistic bacteria 33x10^4 cfu/ml. The treatment of *B. subtilis* biobactericide and citronella plant pesticide showed a significant effect on the intensity of bacterial leaf blight disease namely in the *B. subtilis* treatment 14.72% and citronella plant pesticide 28.97%. The dry weight of rice fields per clump in the *B. subtilis* biobactericide treatment with an average of 27.26 grams was significantly different compared to the citronella plant pesticide treatment with an average of 24.44 g.

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