Application of biological control *Paenibacillus polymyxa* toward bacterial leaf blight disease in rice plant

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**Abstract.** Diseases that often attack rice plants are bacterial leaf blight, this disease can reduce rice production by up to 60%. Control of bacterial leaf blight in addition to using chemicals can also use biological agents, one of which is *Paenibacillus polymyxa*. Testing of biological control applications begins with inoculation of *Xanthomonas oryzae* bacteria on ciherang rice seeds 9 days after planting (DAP). Planting rice using Randomized Block Design using biological control *Paenibacillus polymyxa* consists of four treatments, Control (P0), 2.5 ml l⁻¹ (P1), 5 ml l⁻¹ (P2), and 7.5 ml l⁻¹ (P3). The biological control application is carried out on seeds, 10 DAP, 2 week after planting (WAP), and 4 WAP. Observations were made once a week to observe the rice growth and intensity of the disease. Observations were made in the vegetative phase. The results of the *Paenibacillus polymyxa* biological control test can reduce the level of bacterial leaf blight attack even though the difference in each treatment is not significantly different. The recommended concentration of *Paenibacillus polymyxa* biological control is 7.5 ml l⁻¹. This concentration can reduce the level of bacterial leaf blight attack in the vegetative phase.

1. **Introduction**

Rice plants is a plant that is very important for human because more than half of the world's population including Indonesia depends on the plant as a source of food. Rice consumption in Indonesia amounted to 1.55 kg per capita a week [1]. National rice production has not been able to meet the needs of rice in Indonesia so it must import rice from abroad. The cause of low rice production in Indonesia is caused by low seed quality, reduced agricultural land, and plant disease attack.

Leaf blight disease is a disease that is very harmful to the rice plants because it affects the leaf that will produce a grain of rice. The leaf blight disease is caused by bacterial disease *Xanthomonas oryzae* derived from the soil. These pathogens can infect rice plants at all phases of plant growth from seed nursery to the harvest. Causes of pathogenic diseases infect rice plants on the leaves through wound leaves or natural holes in the form of stomata and damaging chlorophyll leaves [2].

Control of leaf blight disease in rice plants mostly using chemical pesticides. The use of chemical pesticides will cause resistance to bacterial or pathogenic causes of disease and damaging the environment when used continuously. One of the PGPR that can be used as a controlling alternative that is widely utilized in some research is *Paenibacillus Polymyxa* [3].

*Paenibacillus polymyxa* bacteria are antagonistic bacteria which can be identified morphologically from the convex elevation with cloudy milky brown color. These bacteria can be used to control...
several types of diseases in both food crops and horticulture. *Paenibacillus polymyxa* has various benefits, including nitrogen fixation, promotion of plant growth, solubilization of soil phosphorus and production of exopolysaccharides, hydrolytic enzymes, antibiotics, cytokinins [4]. According to [5] antibiotics produced by *Paenibacillus polymyxa* are more effective in controlling plant pathogenic bacteria. The purpose of this research is to controlling leaf blight disease by testing biological control using *Paenibacillus polymyxa* in various concentrations.

2. **Materials and methods**

The research is held in Balai Besar Peramalan Organisme Pengganggu Tumbuhan (BBPOPT), Pangulah Baru Village in Kotabaru Sub-District at Karawang District, West Java. The tools used are trays, measuring cups, ruler, markers, Erlenmayer, petri dish, reaction tube, autoclaves, saucepan, stove, stirrer, knife, analytical and analog scales, label, Cardboard, ruler, laminar air flow cabinet, ose needle, triangle grader, Plastic wrap, syringe socorex, injections, sprayer, micro pipette, and aluminum foil. The materials used are potatoes, bacteriological agar, aquades, tryptone soya broth, sterile water, urea fertilizer, ciherang rice seeds, biological agent isolates *Paenibacillus polymyxa* and pathogenic isolates *Xanthomonas oryzae*.

This study used a randomized block design (RBD) of 4 treatments with 3 replications. The treatment used are Control (P0), 2,5 ml l⁻¹ (P1), 5 ml l⁻¹ (P2), and 7,5 ml l⁻¹ (P3). Observations consist of growth observations, disease incidence observation and severity of illness. Observation of the disease severity according to observation and PGPR control observed using the scale value of each category of disease attacks [6]. The observation was observed once a week in the morning. The following scale values are observed:

| Scale | Description |
|-------|-------------|
| 0     | No infections |
| 1     | Mild attack, when symptoms < 10% per plant |
| 2     | Moderate attack, when symptoms 10-25% per plant |
| 3     | Attack rather severe, when symptoms 26-50% per plant |
| 4     | Severe attacks, when symptoms > 50% per plant |

The disease severity of the infected host of the total observed host.

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\text{Disease Severity} = \frac{n \times v \times 100}{N \times V}
\]

**Description:**
- \(n\) = number of crops classified into an attack category
- \(v\) = score on each category of attack
- \(N\) = number of plants observed
- \(V\) = score for the toughest attack category

2.1. **Preparation of PSA**

PSA (Potato Sucrose Agar) is made with 300 grams of potatoes boiled using 1 liter of water. The potatoes are boiled until soft and separate the potatoes from the water, the potato extract is mixed with 15 grams of sugar and 24 grams of bacteriological order, the solution is transferred to the Erlenmeyer and covered with cotton and aluminum foil then sterilized by autoclaving (temperature 121°C for 30 minutes). PSA media is divided into two, namely:

a. In order to tilt it functions for biological control propagation. Before being sterilized, the PSA solution was put into a 10 ml test tube using a Socorex syringe and covered with cotton and aluminum foil then sterilized using an autoclave. The test tube is tilted 45° after being sterilized and allowed to stand until it is consistent.

b. In order for the block to function to calculate the density of the bacterial colony with biological agents. The sterile PSA solution was put into a petri dish as a container. Media pouring is done in a laminar air flow cabinet. The PSA solution is put into a petri dish of approximately 10 ml.
2.2. Propagation of bacterial isolates (Paenibacillus polymyxa and Xanthomonas oryzae)

Propagation of non-pathogenic and pathogenic bacteria using agar slant media. The isolation of non-pathogenic bacteria *Paenibacillus polymyxa* and pathogenic bacteria *Xanthomonas oryzae* were transferred to the media to tilt using ose needles carried out in a laminar air flow cabinet. The removal of bacterial colonies was carried out by zig zag basting then covered again with cotton and stored at room temperature.

2.3. Bacterial liquid propagation on TSB media (Tryptone Soya Broth)

TSB media is used for the propagation of aerobic bacterial isolates. Making TSB media requires 100 ml of distilled water and 3 grams of Tryptone Soya Broth. The media is dissolved in an Erlenmeyer, the Erlenmeyer is covered using cotton and aluminum foil then sterilized using an autoclave. The isolates of non-pathogenic bacteria *Paenibacillus polymyxa* and pathogenic bacteria *Xanthomonas oryzae* were respectively inoculated into Tryptone Soya Broth media using ose needles carried out in a laminar air flow cabinet. TSB media, each containing the bacteria *Paenibacillus polymyxa* and *Xanthomonas oryzae*, were left to stand for 48 hours. Bacterial growth was seen from the turbidity of TSB media on the Erlenmeyer.

Colonies counts can be seen after stratified dilutions. The stratified dilution requires seven test tubes containing 9 ml of sterile water. TSB media containing 1 ml of bacteria was transferred to a test tube containing 9 ml of sterile water as the first solution. The results of the dilution can be seen after 24 hours of silence. Colony density calculation using total plate count method.

2.4. Application of the isolate to plants

The pathogenic bacterial isolate *Xanthomonas oryzae* was given sterile water and stirred until the bacteria were released from the solid media, then the seeds were soaked for 8 hours in isolates, after the seeds were soaked with pathogenic bacteria, the seeds were divided into 4, including 100 seeds for control, 100 Soaking seeds for *Paenibacillus polymyxa* bacteria 2.5 ml l\(^{-1}\), 100 seeds for 5 ml l\(^{-1}\), and 100 seeds for 7.5 ml l\(^{-1}\) for 15 minutes. Spraying of the *Xanthomonas oryzae* pathogenic bacteria was carried out on 9 DAP with a dose of 7.5 ml l-1 and 1 plant got 3 times the spray. Spraying the *Xanthomonas oryzae* bacteria aims to cause symptoms when transplanting. Spraying of *Paenibacillus polymyxa* bacteria was carried out on 10 DAP with doses according to treatment, namely 2.5 ml l\(^{-1}\) (P1), 5 ml l\(^{-1}\) (P2), 7.5 ml l\(^{-1}\) (P3), and for P0 no treatment.

3. Results and discussion

3.1. Growth observations

The High Growth of Rice Plants Results for four times the high observation of the plant experienced an increase. Treatment 7.5 ml L\(^{-1}\) (P3) get a high yield of plants 1 MST 24.45 cm and 4 MST 48.71 cm. The high observation of rice plants gets no distinct results in the individual treatment. High observation results are listed in Figure 1.
3.2. The number of samplings
Results for four times the high observation of the plant experienced an increase. The final observation of the number of saplings in the 5 ml L$^{-1}$ (P2) treatment received the most saplings from other treatments, although not significant. The results of the number of saplings listed in Figure 2.

![Figure 1. High observation plant](image)

![Figure 2.1 Observation of number of saplings](image)

3.3. Incidence and severity of the disease (Xanthomonas oryzae)
The incidence and severity of disease in the observation of bacterial leaf blight disease (Xanthomonas oryzae) is observed visually with the approximate level of attack of each clump in one plot. Final observation on 4 WAP gained the incidence of the highest bacterial leaf blight disease reached 54.16% control treatment and the lowest reached 16.66% of the application treatment Paenibacillus Polymyxa.
7.5 ml L\(^{-1}\). Observation 1 MST and 2 WAP rice plants already in the infection of bacterial leaf blight disease has not secrete symptoms. The following results of disease incidence can be seen in Table 1.

**Table 1. Incidence of bacterial leaf blight disease**

| Treatment | Average (%) disease incidence on the observation |
|-----------|-----------------------------------------------|
|           | 1 WAP | 2 WAP | 3 WAP | 4 WAP |
| Control   | 0     | 0     | 8.33 a | 54.16 a |
| 2.5 ml L\(^{-1}\) | 0 | 0 | 4.16 b | 29.16 ab |
| 5 ml L\(^{-1}\) | 0 | 0 | 0 | 35.41 ab |
| 7.5 ml L\(^{-1}\) | 0 | 0 | 2.08 c | 16.66 c |

Note: Number followed by same lettershow no real difference in DMRT 5% test

The rate of attack of bacterial leaf blight is caused by several factors, namely the pathogen infecting the host, the resistance of the host plant, and the environment. These three factors greatly affect the incidence of disease against the spread of pathogens into several clumps. Bacterial leaf blight can cause stunted growth and reduce plant quality due to tissue damage. According to [8] stated that utilization of the biological agent *Paenibacillus Polymyxa* with colony density was able to suppress the bacterial mass *Xanthomonas oryzae*. *Paenibacillus Polymyxa* is able to inhibit disease in general and inhibit the onset of early symptoms, as well as suppress the spread of attack intensity. Concentration of application treatment *Paenibacillus Polymyxa* 7.5 ml L\(^{-1}\) (P3) is able to suppress the incidence of bacterial leaf blight that attacks rice plants.

The severity of the disease that can be from the scale value of the category of attack get the highest severity reaches 15.62% without treatment or control (P0) According to the occurrence of the highest disease without treatment or control of 54.16% on the observation 4 WAP. Observations of disease severity with disease severity formula are listed in Table 2.

**Table 2. Severity of bacterial leaf blight**

| Treatment | Average (%) disease severity on the observation |
|-----------|-----------------------------------------------|
|           | 1 WAP | 2 WAP | 3 WAP | 4 WAP |
| Control   | 0     | 0     | 2.06 a | 15.62a |
| 2.5 ml L\(^{-1}\) | 0 | 0 | 1.03 b | 8.33ab |
| 5 ml L\(^{-1}\) | 0 | 0 | 0 | 8.33ab |
| 7.5 ml L\(^{-1}\) | 0 | 0 | 0.52 c | 4.68 c |

Note: Number followed by same lettershow no real difference in DMRT 5% test

One of the use of bio-controlling *Paenibacillus Polymyxa* does not provide a chance of pathogens to reach a high population because *Paenibacillus polyymyx* bacteria can suppress the activity of pathogens that infect rice crops. *Paenibacillus Polymyxa* is a non-pathogenic bacterium but has a role as a bacterial antagonist. *Rhizobacteria* that can produce antibiotic substances and also play a role in controlling pathogens are *P. Fluorescens* and *P. Polymyxa* [9]. Concentration of application treatment *Paenibacillus polyymyx* 7.5 ml L\(^{-1}\) (P2) able to suppress the severity of diseases that attack rice plants. Biological Agens which is the biological Control Agens of all insects, nematode, protozoa, fungi, bacteria, viruses, microplasms as well as other organisms that in all stages of development can be used for the use of pest control diseases of plants or destruction organisms in the production process, processing of agricultural products and various purposes [10]. The use of biological agents has advantages and disadvantages. The advantages of biological agents are selective, available in nature, high mobility, no negative impact, and cheap. The shortage of biological agents in small quantities of nature, developments heavily dependent on the weather, his work is slow, and with simple equipment is difficult to develop in the bulk [11]. The use of biological controls is effective in reducing or
overcoming the destruction of ecosystems in the soil and the lifecycle of organisms beneficial to plants.

Biocontrol *Paenibacillus Polymyxa* is one of the *rhizobacteria* that is beneficial to plants as PGPR (*Plant Growth Promoting Rhizobacteria*). The use of *Paenibacillus polymyxa* against bacterial leaf blight disease is essential because the bacterial leaf blight disease caused by *Xanthomonas oryzae* affects the seeds, the vegetative phase, and the generative phase. Administering *Paenibacillus Polymyxa* before the planted seeds can suppress the development of *Xanthomonas oryzae* on the roots and grounds around the roots because *Paenibacillus polymyxa* can thrive in soils and plants as non-pathogenic bacteria.

4. Conclusion

The biological control application of *Paenibacillus polymyxa* to bacterial leaf blight disease (*Xanthomonas oryzae*) affects the incidence and severity of the disease. The concentration of biological control applications of *Paenibacillus polymyxa* 7.5 ml L⁻¹ (P3) can suppress the incidence and severity of the disease, resulting in 16.66% and 4.68% in 4 MST. *Paenibacillus polymyxa* can suppress bacterial leaf blight disease that attacks rice plants, but has no real effect on plant growth.

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