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Research Article

Antagonistic Activity of *Paenibacillus polymyxa* against *Xanthomonas oryzae*: An *In Vitro* Dual Culture Study

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ABSTRACT

Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* is a major constraint to rice production in Tulungagung, especially in the District of Gondang. This study aimed to evaluate the antagonistic effect of *Paenibacillus polymyxa* on *Xanthomonas oryzae* using an *in vitro* dual culture assay. The experiment was conducted as a randomized complete design with four treatments: P0 (*X. oryzae*, P1 (*X. oryzae* + bactericide), P2 (*X. oryzae* + sterile water), and P3 (*X. oryzae* + *P. polymyxa*), each replicated five times. Observations were made for seven days by measuring the diameter of *X. oryzae* colonies. Data were analyzed using one-way ANOVA and a Duncan Multiple Range Test post-hoc test was done if a significant difference were detected. The analysis showed significant difference among treatments. The application of *P. polymyxa* (P3) resulted in significantly smaller colony diameters compared to P0 and P2, and was statistically similar to bactericide treatment (P1). These results showed that *P. polymyxa* is effective in inhibiting the growth of *X. oryzae* and has strong potential as a biological control agent for sustainable rice cultivation.

Keywords: antagonist; dual culture; *Paenibacillus polymyxa*; rice disease; *Xanthomonas oryzae*

INTRODUCTION

The Regency of Tulungagung, especially District of Gondang, is a major rice producing region due to its large rice field area and high fertility. There areas are important in supporting food needs of surrounding communities. District of Gondang has 1,329.3 ha of rice fields with annual productivity reaching 13,189 ton (*Programa Kecamatan Gondang*, 2024). The Village of Bendungan is one area within the District of Gondang with large production area and high yield. However, annual yield may fluctuate due to external factors, such as weather, soil quality, pest and disease infections (Aldi *et al.*, 2021). Bacterial leaf blight (BLB), caused by *Xanthomonas oryzae* pv. *oryzae*, is an important disease (Yogaswara *et al.*, 2022). BLB infection can cause unoptimal growth or even plant mortality and this has been reported to cause yield loss to up to 50% (Chukwu *et al.*, 2019). According to Nuryanto (2017), BLB infection in Indonesia may cause between 15−24% of yield loss.

BLB symptoms include wet leaf spots that develop necrotic symptoms, leaf drying, and later reduced photosynthetic ability. BLB does not only reduce yield quantity but also quality by causing empty grains (Sari & Ilmiah, 2021).

BLB is a disease that constantly occur every planting season in the District of Gondang, Village of Bendungan, making it an endemic disease. This can be cause due to farmers monoculture and continues rice planting practices (Nuryanto, 2018). In addition, growers commonly plant varieties that are resistant to *X. oryzae*, such as Ciherang and Inpari (Marlina *et al.*, 2020). Neutral to acid soil pH (5.5−6.5) combined with high soil moisture duet to inefficient irrigation exacerbates the spread of this disease (Leiwakabessy *et al.*, 2018). Farmers in the Village of Bendungan still heavily rely on chemical pesticides. Overuse of pesticides can result in resistant pathogen, environmental contamination, and residue in yield (Nurkartika *et al.*, 2018).

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One management alternative that is safer for the environemnt is the use of biological control agent, such as antagonistic bacteria. *Paenibacillus polymyxa* is a bacteria that has been shown to produce antibiotic compounds, such as polymyxin and fusaricidin which can effectively suppress pathogenic bacteria, including *X. oryzae* (Gunawan *et al*., 2020). Therefore, this study aimed to evaluate the effectiveness of *P. polymyxa* to inhibit *X. oryzae* growth using a dual culture method. Results from this research will support whether these bacterial species may inhabit similar microclimates and if *P. polymyxa* effectiveness was similar to chemical bactericides.

MATERIAL AND METHODS

This research was done at the *Unit Pelaksana Teknis Proteksi Tanaman Pangan dan Hortikultura* (UPT PTPH) Regency of Tulungagung. This research was done in vitro using a Complete Randomized Design (CRD) with one factor consisting of four treatments and five replicates resulting in 20 experimental units. Antagonisic bacteria were obtained from UPT PTPH isolate collection whcih is *P. polymyxa* BP18 while *X. oryzae* were isolated from rice fields from the Village of Kiping, District of Gondang, Regency of Tulungagung. *X. oryzae* were sampled from susceptible rice plants showing BLB symptoms. Treatments used the following design: P0 = *X. oryzae* (negative control); P1 = *X. oryzae* + chemical bactericide (positif control); P2=*X. oryzae* + sterile water; P3=*X. oryzae* + *P. polymyxa*.

Bioassays

X. oryzae and *P. polymyxa* were grown on Nutrient Agar (NA). Both cultures were rejuvenated on new NA media before experiments for 48 h. Colonies with the size of 1−2 mm were sampled and then placed on fresh NA for dual culture testing. Inoculation were done on two spots by inoculating *P. polymyxa* on one side of the petridish and *X. oryzae* was inoculated on the opposite side. Inoculation were placed 2.5 cm from the side of Petri dish. Inoculated dishes were incubated in room temperature and checked daily for seven days. *X. oryzae* colony diameter growth were measured from *P. polymyxa* colony.

Data Analysis

Data were analyzed using ANOVA and a DMRT post-hoc test was done if significant effects were detected. All test were done at $a = 0.05$.

RESULTS AND DISCUSSIONS

Treatments affected *X. oryzae* colony growth (Table 1). The negative control (P0) resulted in the largets colony diameter over the seven day observation period of 1.40 cm on the seventh day. Results from P0 was not significantly different to P2 where colony diameter were slightly larger of 1.43 cm. This indicated that sterile water did not have the ability to inhibit pathogen growth. In contrast, P1 and P3 were able to decrease the growth rate of *X. oryzae* colonies compared to P0 and P2 over the observation period (Figure 1). *X. oryzae* colony from P1 and P3 were smaller and denser (Table 2,

Table 1. Diameter of *Xanthomonas oryzae* colony

Observation (day)	Diameter of *Xanthomonas oryzae* colony (cm)			
	P0 (negative control)	P1 (bactericide)	P2 sterile water	P3 *Paenibacillus polymyxa*
1	1.08	0.93	1.09	0.88
2	1.12	1.00	1.17	1.01
3	1.18	1.09	1.22	1.08
4	1.23	1.11	1.31	1.10
5	1.26	1.17	1.34	1.14
6	1.40	1.20	1.40	1.22
7	1.40	1.21	1.45	1.24

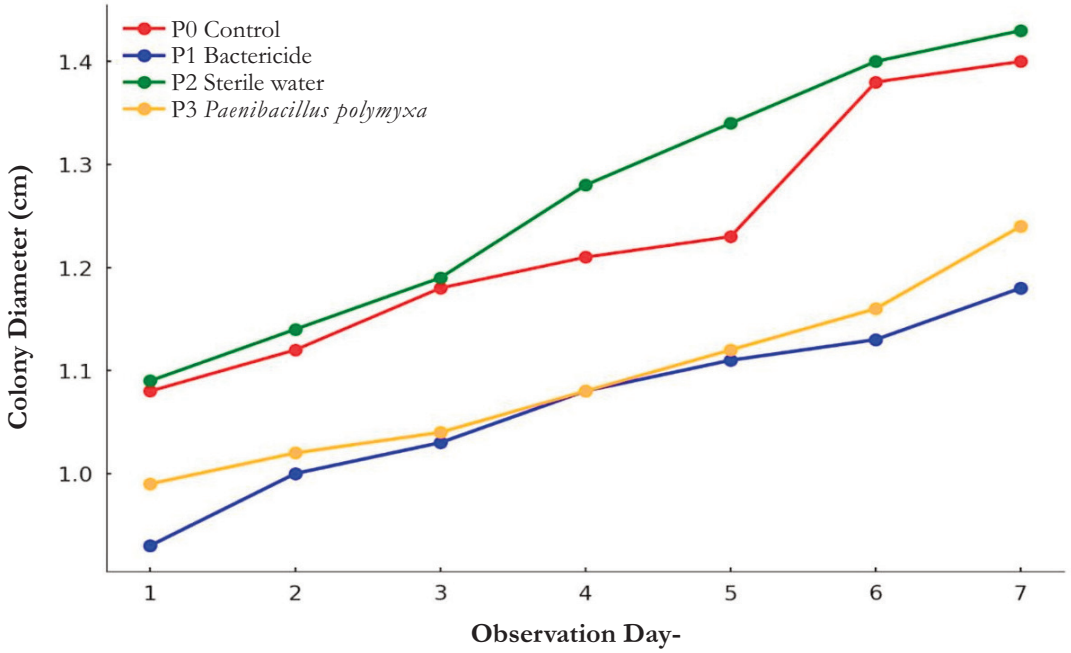


Figure 1. *Xanthomonas oryzae* colony growth over over 7 observation days

Figure 2). Research from Yogaswara *et al.* (2023) demonstrated that application of *P. polymyxa* was able to decrease BLB to up to 40%. Two major groups occured after post-hoc test. *X. oryzae* colony diameter from P1 and P3 were not significantly different between each other but significantly different to P0 and P2. Therefore, *P. polymyxa* can inihibit *X. oryzae* comparably to chemical bactericides and has potential to be used as a biological control agent against *X. oryzae*. Inhibition mechanisms of *P. polymyxa* is related to the antibacterial compounds it produces, such as polymyxin, fusaricidin, and other volatile compounds. Polymyxin can disrupt the integrity of bacterial cell membrane while fusaricidin inhibit protein synthesis and pathogen metabolisms (Goswami *et al.*, 2016). In addition, *P. polymyxa* produce lisozim and protease that are able to hydrolyze bacteria cell walls while siderophores that competively bind Fe^{3+} and cause nutrient defficiency for *X. oryzae* (Raza *et al.*, 2015).

Table 2. Treatment effects on diameter of *Xanthomonas oryzae* colony

Treatment	Colony diameter (cm)
P0 (control)	1.23 ± 0.12 b
P1 bactericide	1.07 ± 0.85 a
P2 sterile water	1.27 ± 0.13 b
P3 *Paenibacillus polymyxa*	1.09 ± 0.87 a

These results were consistent with findings from Gunawan *et al.* (2020) that demonstrated that *P. polymyxa* can inhibit plant pathogens by producing antibacterial secondary metabolites. Research from Kantikowati *et al.* (2018) also demonstrated that *P. polymyxa* local isolates from healthy plants could inhibit pathogens by colonizing rizosphere and inducing systemic resistance. This support Plant Growth Promoting Rhizobacteria (PGPR) property possessed by *P. polymyxa* (Abdallah *et al.*, 2020). Besides on rice plant, research of Syiami *et al.* (2025) demonstrated that *P. polymyxa* was effective against Fusarium wilt disease on chili pepper.

Although *P. polymyxa* effectiveness on *X. oryzae* in short periods were lower than chemical bactericides, it still provides ecological benefits and support sustainable agricultural practices. *P. polymyxa* does not promote resistance among *X. oryzae* population by using several control mechanisms, such as antibiosis, competition, or induced resistance (Chen *et al.*, 2022). Therefore, *P. polymyxa* integration into Integrated Pest Management (IPM) pro-

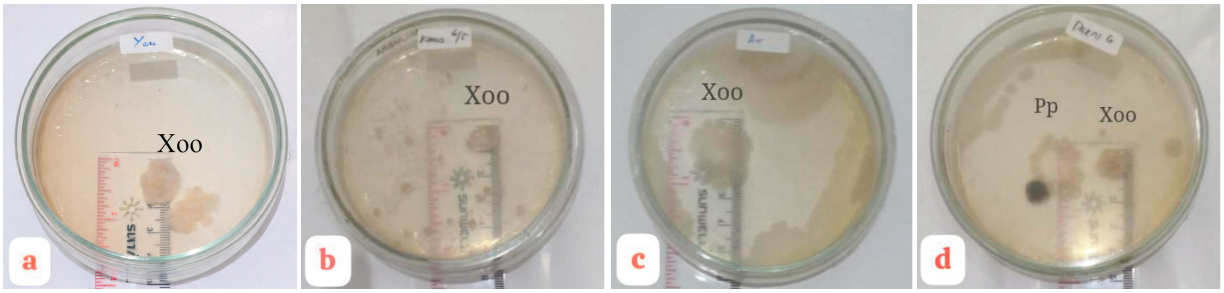


Figure 2. *Xanthomonas oryzae* colonies after each treatment: (a) P0 control, (b) P1 bactericide, (c) P2 sterile water, and (d) P3 *Paenibacillus polymyxa* (Xoo: *Xanthomonas oryzae*, Pp: *Paenibacillus polymyxa*)

grams can provide BLB management while supporting sustainable agricultural practices that are safe for the enviroment and consumers.

CONCLUSION

Paenibacillus polymyxa could effective inhibit *Xanthomonas oryzae* colony growth in vitro comparably to bactericide with the active ingredient of copper sulfate. These result support *P. polymyxa* use as environmentally safer biological control agent to manage BLB on rice while also reducing pesticide residue in tropical agroecosystems.

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