



Original Paper

Application of Rhizobacteria to Control *Phytophthora capsici* Disease Causes Stem Base Rot and Increase Growth of Pepper Seedlings In-Vivo

La Mudi^{1*}, Zainal Abidin¹, Riama Rita Manullang¹, Sopian Agus¹, Helmi Ahmad Gyn'nandi¹, Tasya Valentina Putri¹, Gusti Ayu Kade Sutariati²

1) State Agricultural Polytechnic of Samarinda, Samarinda, 75131, Indonesia

2) Department of Agrotechnology, University of Halu Oleo, Kendari, 93231, Indonesia

*) Corresponding Author: lamudi89@gmail.com

Received: 13 November 2023; Revised: 24 January 2024; Accepted: 27 February 2024

DOI: <https://doi.org/10.46676/ij-fanres.v5i1.245>

Abstract— One obstacle to pepper production is the high intensity of plant disease attacks. One of these is stem rot disease caused by the fungus *Phytophthora capsici*. Therefore, appropriate technology is needed to control disease and simultaneously increase plant growth by using rhizobacteria that are environmentally friendly and can produce growth hormones, dissolve phosphate, and fix nitrogen. This study aimed to obtain rhizobacterial isolates that can control the pathogen *P. capsici*, which causes root rot disease, while also increasing plant growth. This study was conducted at the Agronomy Laboratory of the Plant Cultivation Study Program, Samarinda State Agricultural Polytechnic. The study used a completely randomized design consisting of four treatments with four replicates to obtain 16 experimental units. The variables observed included the latent period, disease incidence and severity, increase in plant height, number of leaves, and stem diameter. The resulting data were analyzed using analysis of variance (ANOVA). The results of the analysis showing a real effect were followed by the Least Significant Difference test (BNT) _{$\alpha=0.05$} . The results of this study showed that the application of a combination of rhizobacteria was able to overcome stem rot disease and simultaneously increase the growth of pepper plant seedlings. The combination of rhizobacteria isolate P01 + TA2 treatment can reduce the latent period of disease to 12.00 days compared to 5.50 days without rhizobacteria, the disease incidence rate was 6.25% with a severity level of 2.50%, lower than of treatment (control) at 37.50 % (disease incidence) and 47.50% (disease severity). In addition, the combination of rhizobacterial isolates P01 + TA2 was able to increase the growth of pepper plant seeds, as shown by the increase in plant height, number of leaves, and stem diameter.

Keywords—Rhizobacteria combination, disease incidence, *P. capsici*, latent period.

I. INTRODUCTION

Pepper is a traditional commodity in East Kalimantan (Kaltim) and has been widely developed because it is an export crop. Pepper in East Kalimantan is widely developed in Loa Janan, Muara Badak, Kutai Kartanegara District, Sepaku District, North Penajam Paser District, and Berau District [1]. Pepper contains 3.13% - 4.46% essential oil, and 5.25% - 6.63%

piperine [2]. Pepper production in 2019 was 5,799 tons, and the demand for pepper increased every year [1]. However, pepper production has decreased due to high rainfall, and many pepper plants are attacked by stem rot.

Stem rot disease is caused by the fungus *Phytophthora capsici*. The losses caused start from seeding until just before the harvest. The intensities of *P. capsici* attacks reached 14.58% [3], 55.66% [4], and 61.2% [5]. *P. capsici* is a soilborne disease microbe and can survive on infected plant parts. In addition, the spread of stem rot disease can begin in nurseries. It has been reported that the development of pathogens through seeds/cuttings can transmit stem rot disease [4].

Therefore, disease control techniques using rhizobacterial inoculations are required. Rhizobacteria are beneficial microbes in the rhizosphere of plants that can act as biofertilizers and bioprotectants [6]. Microbes can suppress the growth of *P. capsici* [7]. It was further reported that the use of biological agents could reduce the incidence of disease by up to 54-76% [8]. Furthermore, the use of microbes can also act as a bioprotectant to control *P. capsici*, *P. infestans*, and *P. nicotianae* diseases [9].

The use of rhizobacteria alone is quite effective in controlling disease [10-11] and can also increase plant growth [12]. Based on the advantages of rhizobacteria alone, it is hoped that the combined application of rhizobacteria can provide significant results in increasing plant resistance to disease and plant growth. The combination of endophytic microbes and rhizobacteria can increase salicylic acid production and peroxidase enzyme activity in plants, which can reduce the incidence and severity of blast disease when compared to controls [13]. The application of salicylic acid is more resistant to *P. capsici* [14]. The use of a consortium of biological agents can reduce the incidence of *P. capsici* and *Colletotrichum* sp., while also increasing the growth and yield of paprika plants [15]. Furthermore, it has been reported that a mixture of rhizobacteria can increase plant yields [12]. Research related to rhizobacteria single is reported to be effective in controlling growth and pepper diseases. However, the combination of rhizobacteria for growth and disease control in pepper plants has not yet been widely reported.

The specific aim of this study was to obtain rhizobacterial isolates that can control the *P. capsici* pathogen that causes stem base rot, while also increasing the growth of pepper plant seedlings.

II. MATERIAL AND METHODS

A. Description of Study Area

This study was conducted at the Agronomy Laboratory of the Plant Cultivation Study Program. Pathogen samples were obtained from community gardens in Batuah Village, Samboja District. This study was conducted between July and September 2023.

B. Experimental Material

Rhizobacteria have been isolated from the rhizosphere of palm oil plants [16]. Pepper seeds were obtained from Samboja District, Batuah Village.

C. Experimental Design

An experimental design is used in this study. The study was structured using a completely randomized design consisting of four treatments with four replications to obtain 16 experimental units. Each experimental unit consisted of four polybags, resulting in 64 experimental polybags. Details of the rhizobacteria application treatment are presented in TABLE I.

TABLE I. DETAIL OF RHIZOBACTERIA APPLICATION TREATMENTS

Treatments	Treatment Details
A0	Without rhizobacteria (control)
A1	Rhizobacteria isolate P01
A2	Rhizobacteria isolate TA2
A3	Rhizobacteria isolates P01 + TA2

D. Method

Phase 1 of the experiment began by collecting the *P. capsici* pathogen from sick pepper plants (plant roots). The plant roots were then transported to the laboratory. The surfaces of the plant roots were then cleaned using 5% NaOCl for 3 min, followed by 70% alcohol for 3 min, and rinsed with sterile distilled water three times. The root samples were then cut horizontally into small pieces and grown in solid Vjuice medium. The V juice is made by weighing tomatoes, carrots, pears, green spinach, celery leaves, baby corn, and bean sprouts (mashed by blending in 200 ml water and then filtering). The extract is then centrifuged at rpm speed, then the supernatant is taken and mixed with 20 g of agar and 5 g of CaCO₃ (boiled until boiling on a hot plate and sterilized in an autoclave at 121°C, 2 atm pressure for 15 minutes and then poured into a petri dish with a thickness of 0,5 ml aseptically in a Laminar Air Flow Cabinet (LAFC). The pathogen that grew was then purified and observed under a microscope and characterized to confirm the test pathogen. Next, the pathogen was multiplied for 7 days, suspended in sterile distilled water (1 Petri dish of 50 ml), and shaken for 24 h at a speed of 150 rpm. Next, the pathogen was applied to the plants (15 ml per plant). Rhizobacterial isolates were grown on solid Tryptic Soy Agar media (30 g Tryptic Soy Broth + 20 g agar + 1 l distilled water boiled until boiling and sterilized in an autoclave at 121oC, pressure 2 atm for 15

minutes and then poured into a petri dish with a thickness of 0.5 ml aseptically in the LAFC). The rhizobacteria were then incubated for 24 h, a suspension was made (1 Petri dish of 50 ml of sterile distilled water), and then shaken for 24 h at a speed of 150 rpm. The application of rhizobacteria was carried out according to the treatment, namely 15 ml of rhizobacterial suspension (for single application) and 30 ml (for combination application of rhizobacteria). The rhizobacterial application was carried out first, followed by pathogen application 5 days after rhizobacterial application. Rhizobacteria were applied 2 times, namely 7 days and 30 days after moving the plants.

E. Observations

The variables observed in the study included the latent period of the disease, disease incidence [17], disease severity [18], increase in plant height, number of leaves, and stem diameter of pepper seedlings. The disease resistance test formula is as follows:

$$li = \sum \left(\frac{ni}{Ni} \right) \times 100\% \quad (1)$$

Where li = incidence of diseased seedlings; ni = number of diseased seedlings at time i ; and Ni = total population of inoculated seedlings.

$$DS = \sum \left(\frac{ni \times vi}{(N \times Z)} \right) \times 100\% \quad (2)$$

Where DS = disease severity; y = number of infected plants; N = number of plants observed; ni = number of diseased plant parts with a certain score; v = numeric scale (score); Z = highest score

F. Data Analysis

The observation data were analyzed using analysis of variance. The analysis results showing a real effect were followed by the Least Significant Difference test (BNT) $\alpha=0.05$. Data were analyzed using the STAR (Statistical Tool for Agricultural Research) program version 2.0.1.

III. RESULTS AND DISCUSSION

Research on the application of rhizobacteria has shown that it has a significant effect on controlling disease and increasing the growth of pepper seedlings.

A. Control of *P. capsici* disease

The results of the research on latent period observations are presented in Figure 1, while disease incidence and disease severity are presented in Figure 2.

The results in Figure 1 show that the fastest latent period for *P. capsici* pathogen infection was obtained without treatment for 5.50 days. In a single treatment with rhizobacteria, the latent period for pathogen infection reached 8.50 days (isolate P01) and 7.50 days (isolate TA2). The latent period of pathogen infection in the combination treatment of rhizobacterial isolates P01 and TA2 reached 12 days. This ability is thought to be caused by the ability of rhizobacteria to compete with pathogens to obtain nutrients and living space, so that pathogens will be slow to develop [19]. In addition, the ability of rhizobacteria to produce antimicrobial compounds can increase plant resistance to pathogens [20-21]. In this study, the

dual use of biological agents was more effective in controlling pathogens due to the dual ability of rhizobacteria. This is in line with research reporting that microbial consortia can increase plant resistance to pathogens [11, 22].

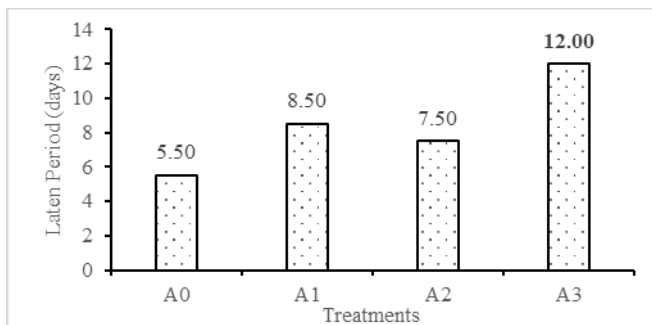


Fig. 1. Latent period of *P. capsici* disease in various rhizobacteria treatments

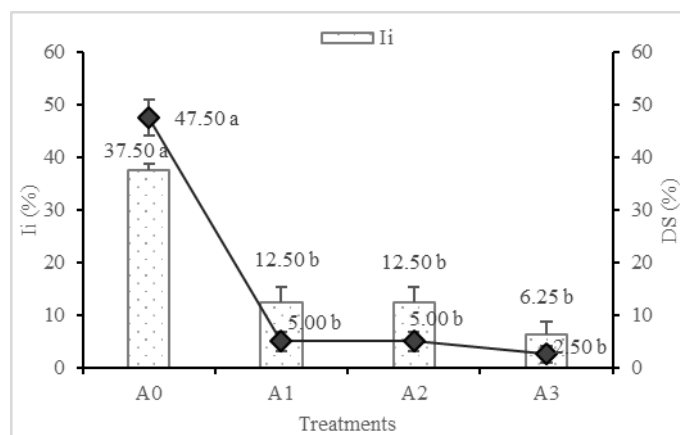


Fig. 2. Occurrence and severity. Note: Numbers followed by the same letter in the image are not significantly different in the BNT $\alpha=0.05$ test.

The results in Figure 2 show that rhizobacterial treatment had the highest incidence of disease without treatment (control) at 37.50% with a disease severity level of 47.50%, which was significantly different from rhizobacterial treatment either alone or in combination with rhizobacterial treatment. The lowest disease incidence was observed in the treatment with the combination of rhizobacterial isolates P01 + TA2 at 6.25%, with a disease severity level of 2.50%. This shows that the use of rhizobacteria, whether using single isolates or combinations of rhizobacteria, can control *P. capsici*, which causes stem base rot in pepper plants.

Seed bio-priming treatment increases the resistance of local cayenne pepper plants [23]. Bio-priming seeds with biological agents can also increase plant resistance to pathogens [243]. Other studies have reported that a combination of microbes can suppress the incidence of disease [25-26]. The combined application of *B. subtilis* and *P. fluorescens* increased plant resistance to the pathogen *R. solanacearum* by up to 44.75% and 67.30%, respectively, compared to the control [27]. The consortium of biological agents was able to suppress *P. colocasiae* disease in the greenhouse by up to 88.75-99.37% [28]. Other studies have also reported that the combination of

rhizobacteria and mycorrhiza can increase the absorption of soil nutrients, which play a role in plant growth, infection of root pathogens, and increased plant resistance to drought [29].

B. The Growth of pepper seedlings

The results of the research on the increase in plant height, number of leaves, and stem diameter of pepper seedlings are presented in TABLE II, III, and IV, respectively.

TABLE II. INCREASING HEIGHT OF PEPPER PLANT WITH RHIZOBACTERIA TREATMENTS

Treatments	Plant Height (cm)			
	2 WAP	4 WAP	6 WAP	8 WAP
A0	5.78 \pm 1.05 ^c	7.61 \pm 1.32 ^c	9.59 \pm 1.69 ^c	11.46 \pm 2.23 ^c
A1	8.40 \pm 0.00 ^{ab}	12.23 \pm 0.20 ^b	14.43 \pm 1.71 ^b	16.25 \pm 2.99 ^b
A2	7.88 \pm 1.05 ^b	12.23 \pm 1.19 ^b	14.71 \pm 0.89 ^b	16.76 \pm 1.40 ^b
A3	9.50 \pm 0.90 ^a	15.96 \pm 1.82 ^a	21.99 \pm 2.22 ^a	26.76 \pm 3.02 ^a

Note: Numbers followed by the same letter in different columns are not significant in the BNT $\alpha=0.05$ test (\pm SE)

The results in TABLE II regarding the highest increase in plant height at an observation age of 2 MSP were obtained in the combination treatment of rhizobacteria isolate P01 + TA2 (9.50 cm which was not significantly different from the single treatment of isolate P01 (8.40 cm but was significantly different from the single treatment at isolate TA2 (7.88 cm, especially without rhizobacteria treatment, it was 5.78 cm. The results of the study on the highest increase in plant height at the ages of 4, 6, and 8 WAP were obtained in the combination treatment of rhizobacteria isolate P01 + TA2, which was significantly different from other treatments, especially without rhizobacterial treatment (control). The use of rhizobacteria, either single or in combination, can increase plant height. increase in the number of pepper plant leaves in the rhizobacterial treatments.

TABLE III. INCREASING THE NUMBER OF PEPPER LEAVES IN RHIZOBACTERIA TREATMENTS

Treatments	Number of Leaves (Strands)			
	2 WAP	4 WAP	6 WAP	8 WAP
A0	1.38 \pm 0.25 ^c	3.25 \pm 0.65 ^c	4.25 \pm 0.50 ^c	5.13 \pm 0.48 ^c
A1	2.00 \pm 0.00 ^b	4.13 \pm 0.48 ^b	5.50 \pm 0.41 ^{ab}	6.50 \pm 0.91 ^b
A2	1.88 \pm 0.25 ^b	3.75 \pm 0.65 ^{bc}	5.13 \pm 0.48 ^b	6.25 \pm 0.50 ^{bc}
A3	2.50 \pm 0.41 ^a	5.00 \pm 0.41 ^a	6.13 \pm 0.48 ^a	8.75 \pm 0.96 ^a

Note: Numbers followed by the same letter in different columns are not significant in the BNT $\alpha=0.05$ test (\pm SE)

The results of the research in TABLE III show that rhizobacterial treatment using rhizobacteria isolates increased the number of leaves of pepper plants at the ages of 2, 4, and 6 WAP obtained in the combination treatment of rhizobacteria isolates P01 + TA2, which was not significantly different from the single rhizobacteria treatment, but was different. real without treatment. Meanwhile, at the observation age of 6 WAP, the highest number of leaves was obtained in the P01 + TA2 isolate treatment which was not significantly different from the P01 isolate but was significantly different from the other treatments, especially when compared with no treatment.

The results in TABLE IV show that the stem diameter increased with each observation time. The highest treatment was obtained in the P01 + TA2 treatment, which was not significantly different from the TA2 isolate and the P01 isolate at the observation ages of 4 and 6 WAP, but was different from that without treatment. Meanwhile, the highest observation results for stem diameter at the observation age of 8 WAP were obtained in the P01 + TA2 isolate treatment, which was not significantly different from the TA2 isolate treatment but was significantly different from other treatments, especially without treatment.

TABLE IV. INCREASE IN STEM DIAMETER OF PEPPER PLANTS IN RHIZOBACTERIA TREATMENTS

Treatments	Stem Diameter (cm)			
	2 WAP	4 WAP	6 WAP	8 WAP
A0	0.053 ±0.009	0.100 ±0.008 ^b	0.127 ±0.009 ^b	0.200 ±0.013 ^c
A1	0.070 ±0.025	0.116 ±0.024 ^{ab}	0.142 ±0.023 ^{ab}	0.218 ±0.026 ^{bc}
A2	0.079 ±0.005	0.126 ±0.004 ^a	0.156 ±0.005 ^a	0.236 ±0.011 ^{ab}
A3	0.084 ±0.003	0.133 ±0.006 ^a	0.159 ±0.005 ^a	0.244 ±0.017 ^a

Note: Numbers followed by the same letter in different columns are not significant in the BNT α =0.05 test (\pm SE)

The increase in plant growth is caused by the ability of rhizobacterial isolates to produce growth hormones in the form of IAA, dissolve phosphate, and fix nitrogen [30]. The research results in TABLE I, II, and III show that the rhizobacteria combination treatment gave the best results when compared with other treatments, especially the controls. This is due to the ability of rhizobacteria to produce growth hormones in the form of IAA, dissolve phosphate, and fix nitrogen [30], which, when combined, can have a double effect on increasing the growth of plant height, number, and diameter of pepper plant stems. This is in line with previous research, which reported that a mixture of rhizobacterial isolates PKLK7 + *Bacillus* sp. CKD0061 increased the growth of peanut plants [12]. A combination of biological agents was also reported to increase the IAA concentration in tobacco roots [31].

by the ability of rhizobacteria to solubilize phosphate [32]. Phosphate nutrients can store and transfer energy in plants [33]. Phosphorus also plays an important role in photosynthesis, carbohydrate metabolism, nucleus formation, cell division, and multiplication [34]. This can increase the plant growth [35]. Apart from that, rhizobacteria are also reported to be able to fix nitrogen. Nutrient N functions as a building block for proteins, chlorophyll, amino acids, and many other organic compounds, and the functions and roles of most of these compounds support and complement each other [36-37]. It was further reported that rhizobacteria can produce IAA. IAA synthesis by PGPR influences cell division, cell enlargement, phototropism, root initiation, growth rate, apical dominance, and geotropism in plants [38].

Application of rhizobacteria has been reported to increase plant growth [39-40]. It was further reported that the application of a combination of rhizobacteria increased the growth of chili plants and chili plant production by 90 % when compared to the control [41]. Application of the endophytic bacterial consortium *Bacillus* sp. SJI + *S. marcescens* JB1E3 increased plant height growth by up to 38% and increased the number of chili leaves by 70% compared to the control [42].

IV. CONCLUSION

The application of a combination of rhizobacteria can overcome stem rot disease and increase the growth of pepper seeds. The combination treatment of rhizobacteria isolates P01 + TA2 suppresses the latent period of disease for 12.00 days when compared without rhizobacteria (control) for 5.50 days, the disease incidence rate is 6.25% with a severity level of 2.50%, with lower when compared without treatment (control) by 37.50% (disease incidence) and 47.50% (disease severity). In addition, it can also increase the growth of pepper plant seeds, as shown by the increase in plant height, number of leaves, and stem diameter.

ACKNOWLEDGMENT

We Thank the State Agriculture Polytechnic of Samarinda for the experimental budget through the Politani DIPA Fund for Fiscal Year 2023 (number 023.18.2.677611/2023).

CONFLICT OF INTEREST

We state that there are no conflicts of interest for this experiment.

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