Enhancing Potato Crop cv. Granola Kembang – G2 Resilience Agaist *Phytophthora infestans* with Bamboo Rhizobacteria

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Abstract. . This study aimed to assess the ability of bacteria living in bamboo roots to suppress the pathogen *Phytophthora infestans* in *Solanum tuberosum* L. cv. Granola Kembang—G2. The research was conducted in Pujon Village, Malang Regency, East Java, from March to June 2022, using a factorial experiment arranged at random groups. The first factor is Plant Grow Promoting Rhizobacteria (PGPR), *i.e*. P1, PGPR derived from bamboo (*Bambusa vulgaris* Schrad. ex J.C.Wendl) roots; and P2, PGPR from Biopharma. The second factor is the concentration of PGPR, namely 10 mg L^{-1} (C1), 20 mg L^{-1} (C2), and 30 mg L^{-1} (C3). There was no significant interaction between the PGPR source and the PGPR concentration treatment on the observed variables except tuber weight ha^{-1}. The PGPR source did not show significant differences in the intensity of disease attacks, the number of tubers $plant^{-1}$, and the percentage of tuber damage. PGPR concentrations showed significant differences in these three variables. The concentration of PGPR, which effectively and efficiently suppresses disease, is 20 mg L^{-1} (C2), which is suitable for potato tuber production is 30 mg L^{-1} (C3).

Keywords: *Bambusa vulgaris,* biological agent, plant disease control, plant growth promoting rhizobacteria*, Solanum tuberosum.*

1 Introduction

Potato (*Solanum tuberosum* L.) is a staple in the world besides wheat, corn, rice, and wheat. European countries and the United States are countries that use potatoes as a staple food. In Indonesia, the potato commodity has priority to be developed because it is one of the sources of non-rice carbohydrates and has the potential for food diversification programs [1]. Potato production of Indonesia since 2016 has decreased by 3.9 % [2]. The problem is due to the attack of the fungus *Phytophthora infestans* [Heinrich Anton de Bary, 1888] which attacks the leaves, stems, and tubers [3]. Potato plants that were attacked by *P. infestans* showed

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symptoms of small, wet patches of pale green to dark green color from the leaf margins. At a temperature of 18 ℃ to 20 ℃ and humidity > 80 %, the spots widen, and necrosis is brown or black. On the underside of the leaves appear white sporangia and soon all the leaves will wither and die [4].

Plant Growth Promoting Rhizobacteria (PGPR) is a group of soil microbes that inhabit around the root surface and are directly or indirectly involved in promoting plant growth and development [5–7]. These bacteria actively colonize the root area of plants and have a major role for plants, namely as a biofertilizer that is able to accelerate the process of plant growth by accelerating the absorption of nutrients [8–11]. PGPR can produce IAA, Cytokinins, Gibberellins, and Ethylene [12–14]; and as a bioprotectant that protects plants from pathogens [15–17].

The results of research by Rachma *et al.* [18] show that the application of PGPR can reduce the intensity of anthracnose attacks on Hiyung Chili plants. There are several groups of microbes contained in PGPR including: *Azotobacter* sp. [Beijerinck, 1901], *Azospirillum* sp. [Tarrand, 1979], *Bacillus* sp. [Cohn, 1872], and *Pseudomonas* sp. [Migula, 1894] [19]. The manufacture of PGPR can be obtained from several plant roots such as bamboo (*Bambusa vulgaris* Schrad. ex J.C. Wendl.) roots, corn (*Zea mays* L.), beans (*Phaseolus*), weeds, and elephant grass [*Cenchrus purpureus* (Schumach.) Morrone], among some of these materials PGPR from bamboo roots gives the best effect on plant growth [20–22]. The bacteria found in bamboo roots are able to dissolve minerals so that they can remodel organic matter into nutrients needed by plants.

Control of potato plant diseases using chemical pesticides will produce waste and has caused various side effects such as environmental pollution. If it enters the food chain, the toxic nature of chemical pesticides can cause various diseases. For this reason, the use of Plant Growth Promoting Rhizobacteria (PGPR) is an alternative technology to increase safer agricultural production [23, 24, 9, 14]. This study aimed to assess the ability of bacteria living in bamboo roots to suppress the pathogen *P. infestans* in *Solanum tuberosum* L. cv. Granola Kembang —G2.

2 Material and methods

2.1 Field research

The research was carried out on farmers' land in Pujon, Malang, East Java, Indonesia from March 2022 to June 2022 and an altitude of 1 100 m asl to 1 200 m asl, coordinate S 7°57'27.1332" and E 112°36'50.7312". Using a factorial experiment arranged randomly in groups, the first factor is the source of PGPR, namely: PGPR derived of bamboo root (P1) and PGPR form Biopharma (P2). The second factor are the concentration of PGPR, namely 10 mg L^{-1} (C1), 20 mg L^{-1} (C2), 30 mg L^{-1} (C3). Each combination treatment was repeated three times. Concentration treatment based on research results by Elfina [25], namely effective concentrations of controlling anthracnose disease by the pathogen *Colletotricum capsica* [Bulter and Bisby, 1931]. PGPR was given flush starting 2 wk after planting until the vegetative period of potato plants ended with a dose of 50 mL per plant, and once a week at intervals. The potato cultivar using Granola Kembang—G2. The mother plant (plantlet) was 3 wk old after acclimation [26]. The size of the cuttings is two segments, part from the leaves were removed, leaving three shoots and two strands below. Potato cuttings were planted out a single row system on beds with a spacing of 25 cm × 80 cm, which had been installed with silver-black plastic mulch.

2.2 Laboratory test

PGPR is made from bamboo roots using the combination method of Syamsiah and Royani [27]; Lisa *et al.* [28], namely: 200 g of bamboo roots were soaked in 1 000 mL of water for 7 d, then filtered and the filtrate was stored as a bacterial starter. Bacterial growth media is: 100 g of shrimp paste as a source of protein [29], 1 kg of rice (*Oryza sativa* L) bran as a provider of fiber and carbohydrates, 500 g of sugar as a provider of glucose and fructose [29], 5 g of lime as a pH neutralizer and 10 L of clean water. All media ingredients are mixed and cooked until boiling then cool about room temperature. Furthermore, 4 L of media mixed with 500 mL of bacterial starter were fermented for 7 d until it smelled sour and there was foam on the surface [30, 31]. During the fermentation process, stirring is done every day.

2.3 Observation variable

Observation variables include: (i) The intensity of disease attacked calculated using the formula quoted from [25] as follows Equation (1):

$$I = \frac{\Sigma(n.v)}{N.Z} \times 100\ \% \quad (1)$$

Where,
I = Attack intensity
Z = Scale value
N = Number of plants observed
n = Number of plants in each attack category
v = Scale value of each attack category

(ii) Number of tubers per plant, by calculating the number of tubers produced in each plant. (iii) Weight of tubers per plant, how to weigh the weight of tubers produced in each plant. (iv) Tuber weights per hectare, by converting tuber weight per plot or bed. (v) Grading (classification) of tuber potato based on size using the method of Diwa *et al.* [3], that is A (large: 120 g tuber^{-1} to 200 g tuber^{-1}); B (medium: 80 g tuber^{-1} to 120 g tuber^{-1}); C (small: 50 g tuber^{-1} to 80 g tuber^{-1}); D (very small: < 50 g tuber^{-1}). The percentage of tuber damage is calculated using on Equation (2):

$$P = \frac{A}{B} \times 100\ \% \quad (2)$$

Where,
P = Percentage of tuber damage
A = Number of damaged tubers
B = Total number of tubers.

2.4 Analysis data

Data analysis performed was analysis of variance and 5 % Duncan's test if the results of the analysis of variance showed significant differences. Data analysis was carried out using statistical software SPSS version 29 [32, 33].

3 Result and discussion

3.1 Result

The results showed that there was no significant interaction between source of the PGPR treatment and the PGPR concentration treatment of all observed variables except tuber weights per hectare.

3.1.1 Intensity of disease attacked

There was no significant interaction between source of the PGPR treatment and the PGPR concentration treatment of disease attack intensity. The treatment of PGPR sources showed no significant differences in the intensity of disease attacks, while the treatment of PGPR concentrations showed significant differences in the intensity of disease attacks. The results of observations of each disease attack intensity are presented in Figure 1(a) and Figure 1(b).

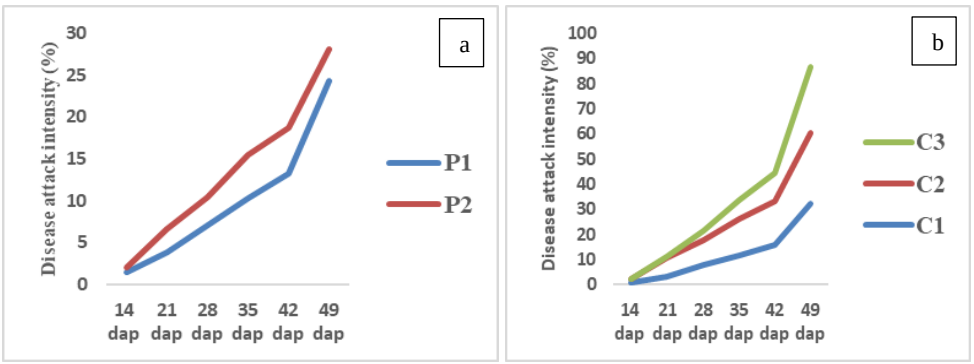

Fig. 1. (a) Effect of PGPR source treatment on intensity of disease attacted. (b) Effect of PGPR concentration treatment on intensity of disease attacted.

Notes: P1: derived from bamboo root; P2: Biopharma; dap (days after planting); C_1: 10 mg L^{-1}; C_2: 20 mg L^{-1}; C_3: 30 mg L^{-1}; dap (days after planting).

Figure 1(a) shows that the PGPR derived from bamboo root (P1) and PGPR form Biopharma (P2) treatments were not significantly different from the intensity of the disease attack. Meanwhile, the PGPR concentration treatment showed a significant difference [Figure 1(b)]. The best PGPR concentration was 30 mg L^{-1} (C3) which were able to reduce the intensity of disease attacks by up to 73.9 % at the age of 49 d after planting, whereas at the same age the PGPR concentration of 20 mg L^{-1} (C2) suppressed the intensity of disease attacked by 71.9 % and the PGPR concentration of 10 mg L^{-1} (C1) suppressed the intensity of disease attacked by only 67.8 %. The recommended concentration is 20 mg L^{-1} because it is more efficient than a concentration of 30 mg L^{-1}.

3.1.2 The number of tubers

There was significant difference between the treatment of PGPR derived from bamboo root (P1) and PGPR form Biopharma (P2) on the number of tubers variable. The concentration treatment showed significant difference on the number of tubers variable. The results of observations of the number of potato tubers per plant are presented in Figure 2A and Figure 2B.

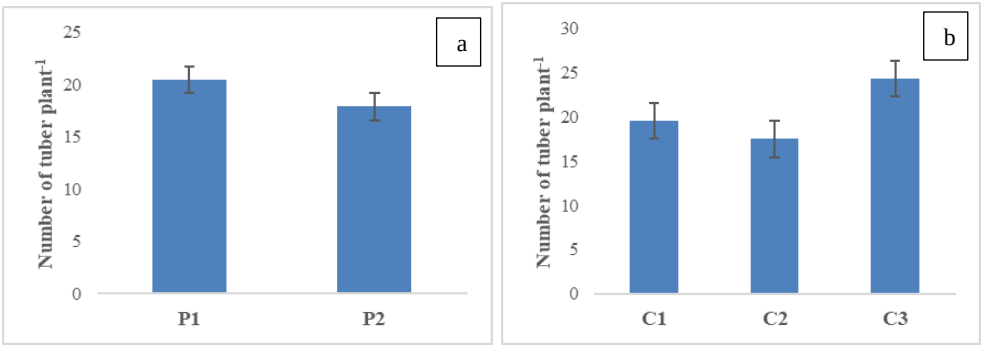

Fig. 2. (a) Effect of PGPR source treatment on number of tubers. (b) Effect of PGPR concentration treatment on number of tubers.

Notes: P_1: derived bamboo root (Standard deviation -STD 0.96); P_2: Biopharma (STD 0.86); C1: 10 mg L^{-1} (STD 0.80), C2: 20 mg L^{-1} (STD 0.80), C3: 30 mg L^{-1} (STD 0.99).

In Figure 2(a) it appears that the PGPR derived from bamboo root (P1) showed more potato tuber yields per plant than the PGPR Biopharma treatment. The best concentration treatment was 30 mg L^{-1} (C3), which produced 24.5 tubers $plant^{-1}$, followed by a 20 mg L^{-1} (C2) concentration treatment that produced 19.5 potato tubers $plant^{-1}$ then a concentration of 10 mg L^{-1} (C1) produced 17.5 tubers $plant^{-1}$. This is as presented in Figure 2(b).

3.1.3 Percentage of tuber damage

There was significant difference between the treatment of PGPR derived from bamboo root (P1) and PGPR Biopharma (P2) on percentage of tuber damage variable. The concentration treatment showed significant difference on percentage of tuber damage variable. The results of observations of damage to potato tubers are presented in Figure 3(a) and Figure 3(b).

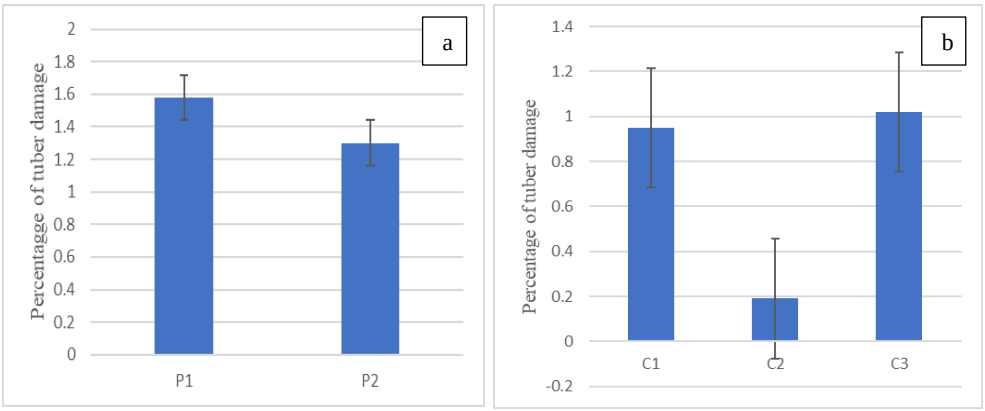

Fig. 3. (a) Effect of PGPR source treatment on percentage of tuber damage. (b) Effect of PGPR concentration treatment on percentage of tuber damage.

Notes: P_1: bamboo root (STD 0.73); P_2: Biopharma (STD 0.74); C1: 10 mg L^{-1} (STD 0.79); C2: 20 mg L^{-1} (STD 0.81); C3: 30 mg L^{-1} (STD 0.60).

Figure 3(a) shows that the PGPR derived from bamboo roots treatment showed a higher percentage of potato tuber damage per plant than the PGPR of Biopharma. A good concentration treatment for reducing the percentage of damage to potato tubers per plant was

20 mg L^{-1} (C2 = 0.19 %) and followed by a concentration of 10 mg L^{-1} (C1 = 0.95 %) then a concentration of 30 mg L^{-1} (C3 = 1.01 %). This is as presented in Figure 3(b).

3.1.4 Tuber weights (t ha^{-1})

The results showed that there was significant interaction between the PGPR treatment and the concentration treatment of tuber weights per hectare. To find out the difference between each combination treatment, a 5 % DMRT test was carried out, the results of which are presented in Figure 4.

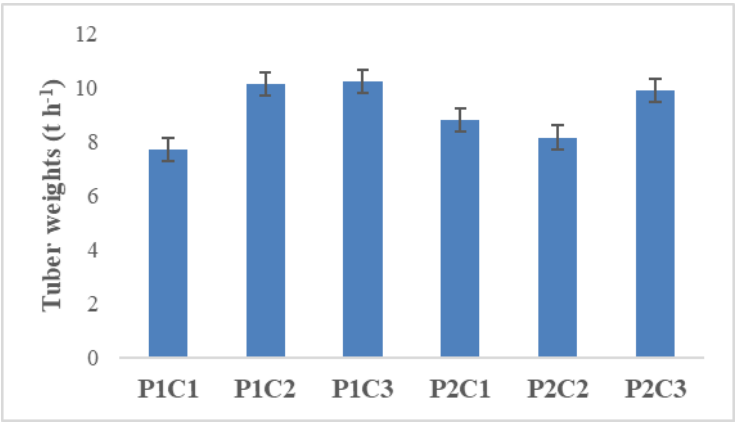

Fig 4. Effect of PGPR combination treatment and concentration on potato tuber weight (t ha^{-1}).

Notes: P_1: bamboo root, P_2: Biopharma, C_1: 10 mg L^{-1}, C_2: 20 mg L^{-1}, C_3: 30 mg L^{-1}. P_1C_1 (STD 1.01), P_1C_2 (STD 1.07), P_1C_3 (STD 1.00), P_2C_1 (STD 0.90), P_2C_2 (STD 1.05), P_2C_3 (STD 1.20).

Figure 4 shows that the combination treatment of PGPR derived from bamboo root with a concentration of 20 mg L^{-1} was not significantly different from the combination treatment of 30 mg L^{-1} concentration of PGPR derived from bamboo root on potato tuber weight variables ha^{-1}. However, it is suggested that to produce potato tubers it is better to use a concentration of 20 mg L^{-1} rather than 30 mg L^{-1} because it is more efficient at PGPR.

3.1.5 Grading (classification) of tuber potato

The results of observations of potato tuber grading showed that there was no significant interaction between the PGPR source treatment and the PGPR concentration. The PGPR source treatment did not show a significant difference ($P < 0.05$) to the potato tuber grading, while the concentration treatment showed a significant difference ($P > 0.05$). The results of observations of potato tuber grading are presented in Figure 5(a) and Figure 5(b).

Figure 5(a) and Figure 5(b) shows each treatment of PGPR sources and PGPR concentrations only produced a few class A and class B tubers. On average, they only had class C tubers and produced more class D tubers. The PGPR source treatment did not show a significant difference in grading potato tubers (Figure 5A), while the PGPR concentration treatment showed a significant difference in grading potato tubers [Figure 5(b)]. The best concentration is 30 mg L^{-1}.

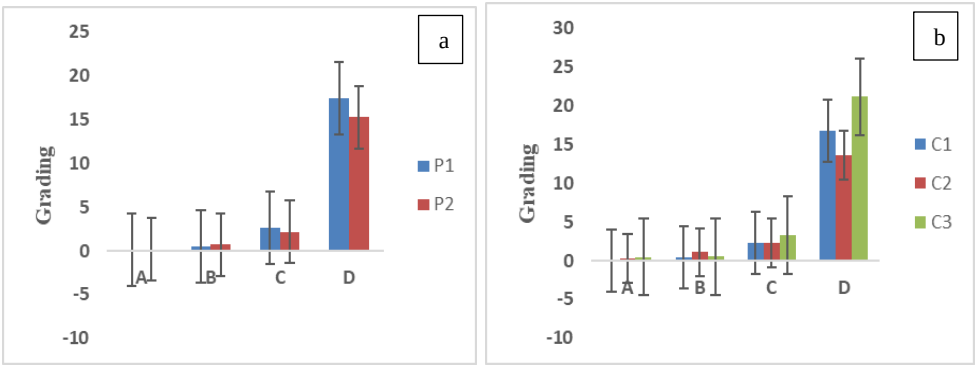

Fig. 5. (a) Effect of PGPR source treatment on tuber potato grading. (b) Effect of PGPR concentration treatment on tuber potato grading.

Notes: P_1: bamboo root, P_2: Biopharma. A (large: 120 g tuber^{-1} to 200 g tuber^{-1}): P_1 (STD = 0.01), P_2 (STD = 0.03); B (medium: 80 g tuber^{-1} to 120 g tuber^{-1}): P_1 (STD = 0.11), P_2 (STD = 0.17); C (small: 50 g tuber^{-1} to 80 g tuber^{-1}): P_1 (STD = 0.50), P_2 (STD = 0.54); D (very small: < 50 g tuber^{-1}): P_1 (STD = 0.81), P_2 (STD = 0.98).
C_1: 10 mg L^{-1}, C_2: 20 mg L^{-1}, C_3: 30 mg L^{-1}. A (large: 120 g tuber^{-1} to 200 g tuber^{-1}): C_1 (STD = 0.00), C_2 (STD = 0.06); C_3 (STD = 0.05); B (medium: 80 g tuber^{-1} to 120 g tuber^{-1}): C_1 (STD = 0.25), C_2 (STD= 0.36), C_3 (STD= 0.35); C (small: 50 g tuber^{-1} to 80 g tuber^{-1}): C_1 (STD = 0.61), C_2 (STD = 0.70), C_3 (STD = 0.66); D (very small: < 50 g tuber^{-1}): C_1 (STD = 0.78), C_2 (STD = 0.75), C_3 (STD= 0.74).

3.2 Discussion

Utilization (PGPR) is an alternative technology to increase agricultural production which is safer because there are soil bacteria that live in plant root areas and are able to stimulate plant growth [8, 9, 11, 19]. These bacteria colonize or grows aggressively in the plant rhizosphere due to the presence of plant exudates [34, 13]. The presence of these microorganisms is beneficial to physiological processes and plant growth [35, 9, 11, 13]. These microbes mobilize or facilitate the absorption of various nutrients in the soil, synthesize and change the concentration of growth-promoting phytohormones [36, 37, 9, 11, 22]. PGPR bacteria are able to fix free nitrogen, this nitrogen is converted into ammonia and then distributed among plants [38, 13]. These bacteria are also able to provide some of the minerals needed by plants, including iron, phosphorus, and sulfur [39, 10, 25].

The results showed that the PGPR of bamboo roots did not show a significant difference compared to the PGPR of Biopharma in being able to suppress the intensity of disease attacks caused by the potato plant *P. infestans* pathogen. The recommended concentration is 20 mg L^{-1} because it is more efficient at reducing attack intensity by up to 71.9 % at 49 dap. While a concentration of 30 mg L^{-1} at the same age can reduce attack intensity by up to 73.9 %, as shown in Figure 1(b). Likewise, the observed variable percentage of tuber damage is presented in Figure 3(b), which in the PGPR treatment with a concentration of 20 mg L^{-1}, damage to potato tubers was only 0.19 %, while in the PGPR treatment with a concentration of 30 mg L^{-1}, damage to potato tubers was 1.01 % and PGPR and a concentration of 10 mg L^{-1} potato tuber damage 0.95 %.

The highest tuber yield ha^{-1} was the combination of bamboo root PGPR treatment for a concentration of 20 mg L^{-1} although it was not significantly different to a concentration of 30 mg L^{-1}. This is due to the role of PGPR which is beneficial to plant growth and production. As stated by several researchers [4, 5, 15–17, 20–22, 31, 34] that PGPR can prevent and control disease and can stimulate plant growth [40]. Furthermore, it was also stated that

PGPR can be used as a biological pesticide due to the presence of several antagonistic microbes that live around the roots [12, 18, 27–29]. Another opinion states that PGPR can increase growth because of the role of several phytohormones contained in it [12].

PGPR is useful in suppressing pathogenic activity by producing antibiotic compounds, spurring plant growth, namely stimulating the formation of hormones or plant growth regulators, auxins, cytokinins and gibberellins so that plants are more fertile. PGPR can be used as an additive to speed up the composting process [40, 41, 13, 15, 36]. The biological control agents that have been widely studied are the genera *Bacillus* [Cohn, 1872], *Streptomyces* [Waksman and Henrici, 1943], *Pseudomonas fluorescens* [Flugge, 1886], *Burkholderia* [Yabuuchi, 1993] and *Agrobacterium* [Conn, 1942] [42]. These microbes suppress disease growth through the following mechanisms: induction of systemic resistance from plants; production of siderophores that chelate (bind) iron so that iron is not available to pathogens (*P. fluorescens*); synthesis of antifungal metabolites such as antibiotics, enzymes that degrade fungal cell walls, or hydrogen cyanide that suppresses the growth of pathogenic fungi; able to compete with pathogens for nutrients or growth space in the rhizosphere [27, 35–37]. Some PGPR bacteria are capable of producing toxins for plant pathogens, for example *Bacillus subtilis* [Ehrenberg, 1835] bacteria are able to fight pathogenic fungi [43, 44]. PGPR bacteria can reduce the severity of many diseases, for example *Ganoderma boninense* in plants by bacteriocin produced [45, 46].

The mechanism of PGPR microbial action in stimulating plant growth involves the availability of nutrients derived from genetic processes [47], such as biological nitrogen fixation and phosphate dissolution, coping with plant stress through modulation of ACC deaminase expression, and production of phytohormones and siderophores [48, 49]. Soil microorganisms that produce the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase promote plant growth by removing and breaking down plant-produced ACC, thereby lowering plant ethylene levels [50]. Reducing ethylene levels allows plants to be more resistant to various environmental stresses [51]. PGPR microbes can be commercialized and have prospects of overcoming the problem of plant pathogens that are environmentally friendly.

4 Conclusion

Based on the results of research and discussion, it can be concluded that there was no significant interaction between the PGPR source treatment and the PGPR concentration treatment for all observed variables except tuber weight per hectare. The treatment of PGPR sources did not show significant differences in the intensity of disease attack, the number of tubers per plant and the percentage of tuber damage. Meanwhile, the PGPR concentration treatment had a significant difference to the three variables. The concentration of PGPR which effectively and efficiently suppresses disease is 20 mg L^{-1}, which is good for potato tuber production is 30 mg L^{-1}. To suppress the pathogen *P. infestan*, it is recommended to using PGPR derived from bamboo roots with a concentration of 20 mg L^{-1}.

The authors would like to thank the University of Muhammadiyah Malang for providing financial assistance and experimental facilities (E.2.b/130/FPP-UMM/II/2022).

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