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APPROPRIATE DOSE OF PGPR ANTIFUNGAL TO INHIBIT FUNGI THAT CAUSE ROTTEN ON THE RHIZOME OF RED GINGER (*Zingiber officinale* var. Rubra)

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ABSTRACT

Red ginger (Zingiber officinale var. Rubra) is one of the leading commodities producing essential oils and oleoresins. In red ginger cultivation, several obstacles were encountered, including the fungus attack, which caused rotten rhizome, so that the nursery process failed. Generally, fungicides are given to control the fungus. However, chemical fungicides can harm health and the environment. Therefore natural environment fungicides are needed. PGPR has antifungal activity. It can use as a natural fungicide. This study aims to determine the antifungal activity of PGPR against fungi that cause rotten red ginger rhizomes in the nursery process using doses of 0%, 25%, 50%, 75%, and 100%. The results, the 75% PGPR dose, give the highest average inhibitory zone to fungi causing rhizome in red ginger but not significantly different from the 100% dose. Furthermore, at a dose of 75%, the resistance of fungal growth amounted to 26.7%. The use of PGPR at appropriate dosages will induce the growth of fungi that cause rot in the rhizome of Red Ginger.

Keywords: Antifungal, Red Ginger (*Zingiber officinale* var. Rubra), Rotten Rhizome Fungi, PGPR.

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Introduction

Red Ginger (*Zingiber officinale* var. Rubra) is one of the essential oil-producing and oleoresin-producing plants, which is a superior commodity in Indonesia (Suharti et al., 2011). Demand for red ginger from year to year is increasing along with the development of treatment with herbal plants. However, the high demand has not been able to offset the increase in production. Ginger production figures, including red ginger since 2015 has decreased. BPS data shows that ginger production from 2016-2017 decreased by 20.94% (Ministry of Agriculture, 2017).

The decline in production was due to several obstacles faced by farmers, including the presence of crop pest organisms (Retno, 2012). One of the pests that can cause a decrease in red ginger production is *Fusarium* sp., which is a group of soil-borne fungi that cause rhizome rot in ginger. This fungus attack can cause stunted growth and crop failure. The symptoms caused by the fungus attack are characterized by the body of the plant becoming yellowing.

On the other hand, in the nursery process, this group of fungi will produce unhealthy seeds. The fungus develops well at temperatures of 15-38 ^{o}C and humidity of 87-95%. In 2003 and 2005, the fungus group attack was found in 8 districts of ginger growing centers in Indonesia, and the attack rate was low to high (Retno, 2012).

Control of fungi that cause rot rhizomes is done using chemical fungicides (Harmono & Andoko, 2005) — in general, soaking ginger in fungicide for 0.5-1 hours before sowing (Hapsoh et al., 2010). The use of chemical fungicides can harm the environment and health. Therefore, the use of natural fungicides that are environmentally friendly and able to control the attack of fungi that cause rhizome rot in red ginger is essential. The use of natural fungicide used in this study was PGPR.

PGPR (Plant Growth Promoting Rhizobacteria) is a group of bacteria that colonizes the rhizosphere region of plants that provides benefits for plants. PGPR can play a role as a biofertilizer, biostimulant, and bioprotectant (Rai, 2006). Besides, the role of PGPR produces antagonistic compounds to control plant pathogens, including fungi and can induce plant resistance to pathogens (Beneduzi et al., 2012; Glick, 1995).

In the study of Zainudin et al. (2014) showed that PGPR could control downy mildew on maize plants caused by fungal pathogens (*Peronosclerospora maydis*). In the study of Laili and Agustiyani (2016) also showed that seven isolates of endophytic bacteria from Lombok could inhibit the growth of pathogenic fungus *Fusarium oxysporum* f. sp. *lycopersici*.

Based on the description, it is expected that the provision of PGPR in this study can inhibit the growth of fungi that cause red ginger rhizome rot so that it can be used as a substitute for chemical fungicides. The purpose of this study was to determine the antifungal power PGPR against fungi that cause red ginger rhizome rot in the process of seeding.

Research Method

Research Design

This research is experimental research with a completely randomized design. In this study, PGPR was used at a dose of 0%, 25%, 50%, 75%, and 100%.

Tools and Materials

The tools used in this study were Petri dishes, volume pipettes, Erlenmeyer, glass beaker, balance sheets, measuring cups, paper holes, bunsen, triangles and gauze, slide calipers, chopper, mortal and pistil, gloves, masks, polybags, and cameras. The materials used are PDA (Potato Dextrose Agar) media, 70% alcohol, distilled water, 0.01M phosphate buffer pH 7, rooting soil (reeds, corn, bamboo, elephant grass, and sugar cane), filter paper, red soil, and red ginger rhizome.

Research Procedure

PGPR seedlings are made by taking soil around the roots of Imperata, corn, bamboo, elephant grass, and sugar cane as much as 50 grams each. Then the sample was macerated and added a solution of a concentration of 0.01 M phosphate buffer (pH 7) of 500 ml and incubated for one week. The PGPR seedlings were then diluted according to the dose in this study (0%, 25%, 50%, 75%, and 100%).

PGPR seedlings that have been made according to the dosage are used to soak the red ginger root before planting. This immersion is done for 1 hour. Furthermore, the rhizome is broken based on the presence of buds and planted on red soil media. After 11 Weeks After Planting, red ginger is harvested, and an observation is made of the presence of fungi and the condition of the red ginger rhizome to determine the antifungal ability of PGPR in vivo.

Furthermore, in vitro antifungal testing by isolating fungi that cause rotting red ginger rhizomes on infected red ginger rhizomes. Red ginger rhizomes that show signs of rhizome are cut 1x1 cm in size and dipped in 70% alcohol for 5 minutes. The pieces were then isolated using PDA media and incubated for 3-5 days at room temperature (Agrios, 1999).

Fungal cultures obtained from the isolation process were taken with a size of 9 mm to test PGPR antifungal activity against fungi that cause rhizome rot in vitro. The isolate was inoculated on PDA media and incubated for two days. On the second day, wells were made on all three sides of the fungal colony and filled with 0.1 ml of PGPR according to the dose and incubated for nine days (Fratiwi et al., 2018).

The antifungal activity of PGPR bacteria is calculated based on the following formula (Nourozian et al., 2007).

$$\%\ inhibition = \frac{C - T}{C}\ x\ 100\%$$

Information:
C = diameter of control mushroom
T = diameter of the fungus by treatment

Result and Discussion

The results of the in vivo antifungal PGPR activity test on fungi that cause rhizome rot in red ginger were observed by calculating the percentage of rhizomes of red ginger that suffered rot due to fungal attacks harvested after 11 MST (see Figure 1).

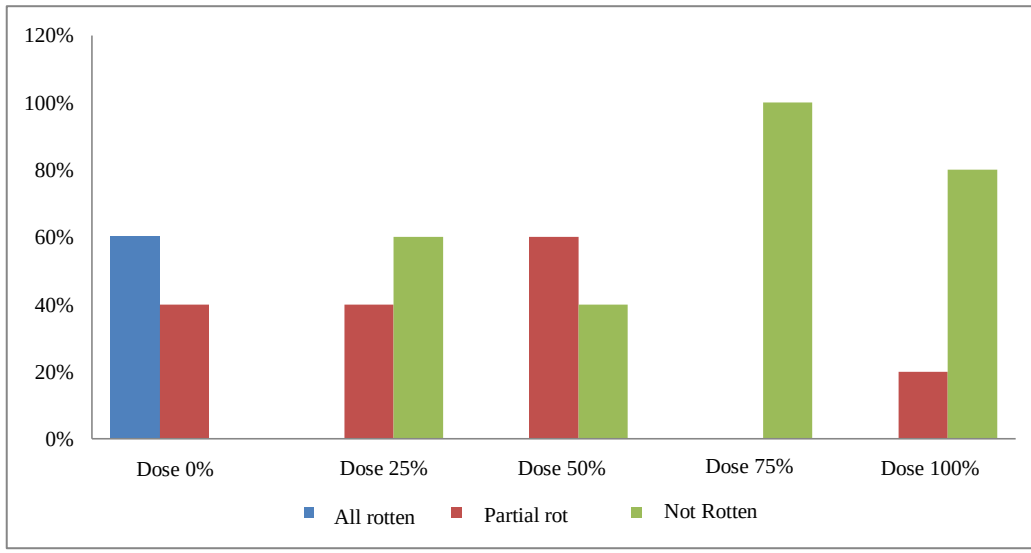

Figure 1. Rotten Red Ginger Rhizome 11 weeks after planting

Figure 1 shows that in control (0% PGPR), all rhizomes were rotten due to fungal attack, both partially and totally rot. However, the PGPR immersion treatment (doses of 25%, 50%, 75%, and 100%) showed that there were rhizomes that did not rot. It shows that the treatment of ginger rhizome immersion by using PGPR before sowing can reduce the level of the rotten rhizome.

At a dose of 75%, all rhizomes do not rot. However, at 100% doses of rhizomes before seeding is rotten in some rhizomes. It shows the right dosage to produce good quality rhizomes before sowing.

The results of rhizome immersion in PGPR are also supported by in vitro testing. This test aims to determine the percentage of fungi that cause rot in the rhizome. The highest percentage of inhibition of PGPR on fungi that causes red ginger rot is at a dose of 75% (see Table 1).

Table 1. Test of PGPR Antifungal Activity Against Fungi that Causes Red Ginger Rhizome Rot

Treatment	Percentage inhibition (%)	Antifungal Activity Level
0%	0	Inactive
25%	8,0	Weak
50%	16,7	Weak
75%	26,7	Medium
100%	25,3	Medium

Note: Classification of antifungal activity levels based on Mori et al. (1997)

Table 1 shows that PGPR treatment at 25%, 50%, 75%, and 100% doses showed antifungal activity. In Table 1, it can also be seen that the highest level of inhibitory activity of PGPR treatment is the 75% dose with a percentage of inhibition of 26.7% and belongs to the medium category. The 100% treatment also shows a moderate level of antifungal activity, but the percentage of inhibition is lower than the 75% treatment, which is 25.3%. However, 25% and 50% of treatment showed a weak level of antifungal activity.

Several things cause the ability of PGPR to inhibit the growth of fungi that cause rhizome rot in red ginger. Tan et al.

(2015) state that PGPR can induce systemic plant resistance to plant pathogenic agents. On the other hand, Febriyanti et al. (2015) state that the antifungal activity of PGPR is possible because of the presence of competitors. One of the competitors is Rhizobacter bacteria. The bacterium is the most efficient competitor of microbes that can compete with pathogenic microbes in the environment of the plant rhizosphere.

Furthermore, the research results of Prihatiningsih, et al. (2017) showed that the ability of PGPR bacteria to inhibit the growth of pathogens is partly because PGPR bacteria can produce siderophores. This Siderofor will bind Fe^{3+} that is around the roots into siderophore-iron. By binding Fe^{3+} by siderophore will cause pathogens deficient in Fe^{3+} (Sharma & Johri, 2003). That will inhibit the proliferation of pathogenic cells.

In addition, Joko et al. (2015) also mentioned that PGPR has the ability as a fungicide. PGPR bacteria can produce hydrolytic enzymes, such as chitinase, protease, β-1-3 glucanase, lipase, and amylase (Prihatiningsih et al., 2017). Furthermore, Laili & Agustiyani (2016) stated that the hydrolytic enzymes (protease, β-1-3 glucanase, and chitinase) could inhibit the growth of pathogenic fungi. The enzymes chitinase and β-1-3 glucanase will lyse cell wall fungal pathogens that contain chitin and β-glucan (Vejan et al., 2016).

The antifungal activity of PGPR can also occur due to the ability of PGPR bacteria to produce HCN, which is a toxic compound for fungal pathogens (Salamiah & Wahdah, 2015). The process of blocking the growth of the fungus is expected to help the process of red ginger cultivation. That can increase the yield of red ginger.

Conclusion

Soaking the red ginger rhizome with PGPR solution has been proven to prevent rotting red ginger rhizome (*Zingiber officinale* var. Rubra), where a 75% dose gives the best results. The 75% dose also has the highest percentage of inhibition of fungi that cause red ginger rotten rhizome but belongs to the medium category, such as the 100% dose. Suggestions for future research are the need to identify PGPR bacteria and test the antifungal activity of each bacterial isolate obtained. It will increase the effectiveness of the removal of fungal growth in red ginger.

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