Control of Fusarium Disease in Onion with Plant Growth Promoting Rhizobacteria (PGPR) and Mycorrhizae and Its Effect on Growth and Yield

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ABSTRACT

The productivity of onion in Indonesia is generally low due to fusarium wilt disease. Biological controls can be applied using PGPR and mycorrhizae. The purpose of this research was to understand the interaction between PGPR and mycorrhizal inoculation against fusarium wilt intensity as well as the growth and yield of onions. The isolation of *Fusarium oxysporum* f. sp. *cepae* and PGPR, followed by the tests of PGPR inhibition ability, phosphate solvent, and HCN compound productivity. The field research was set in a completely randomized design (CRD) with two replications. The results showed that the combination of PGPR and mycorrhizae was unable to suppress Fusarium wilt disease, but had a significant effect to postpone the incubation period (26,19 days after inoculation) and increased the growth and yield of onion compared to plants infected with Fusarium but without the combined treatment of PGPR and mycorrhizae and the PGPR treatment and mycorrhizal treatment as single treatments; the application of mycorrhizae as the single factor had a very significant effect on the number of bulbs, but had no significant effect on the inhibition of fusarium wilt intensity as well as the growth and yield of onions.

Keywords: PGPR, gigaspora, glomus, mycorrhizae, onion

INTRODUCTION

The production of onions in South Kalimantan Province reached only 9.8 t ha⁻¹ (Distan Prop Kal-Sel, 2014), while the productivity potential could reach 17 t ha⁻¹ (Pitojo, 2003). Onions are usually planted seasonally from April to October, given the dry season in Indonesia occurring in these months. It causes fluctuations in prices and increase in losses for farmers (Pitojo, 2003). To anticipate them, the onion cultivation needs to be carried out in the other months as well.

The main problem of onion cultivation in the off-season is that the high risk of crop failure due to the intensity of pest and disease attacks in harvest time which is higher than in the growing season. Sumarni and Hidayat (2005) revealed that low crop productivity and pest attacks generally increase in onion

cultivation outside the season.

Fusarium wilt disease is a disease that attacks the onion plants either in growing season or out of season (the rainy season) and can reduce crop yields to 27 – 75% (Adiyoga *et al.*, 2000) and in general farmers use fungicides as the control and even as the prevention with the intensity and doses that exceed the the recommended limit.

Ditlin Horti (2014) stated that some controls embracing the concepts of IPM are the uses of biological agents and mycorrhizal fungi because they are environmentally friendly and even improve the health of the soil in order to develop the sustainable cultivation. PGPR (Plant Growth Promoting Rhizobacteria) is one of the biological agents beneficial for plants.

Onion plants have roots that are relatively short, so it is suspected that the colonization by PGPR is also less. It is necessary to provide a treatment to lengthen and strengthen the

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roots. A mycorrhiza is an interaction between a fungus and plant roots that affect the widespread uptake of nutrients and simultaneously lengthen and strengthen the plant roots; the more lengthy and sturdy the roots, the more the colonizations by bacteria. Mycorrhizae are proven to protect plants against various diseases such as *Ganoderma boninense*. Pat (Nildayanti, 2011), *Phytophthora megaspermae* (Ross, 1972), *Meloidogyne incognita* (Schenk, Kinloh and Dickson, 1975), *Rhizoctonia solani* (Kasiamdari *et al.*, 2000), *Tobacco Mosaic Virus* and *Fusarium oxysporum* (SchÖnbeck, 1980).

However, the use of PGPR -especially the site-specific PGPR and mycorrhizal fungi for biological control of fusarium wilt in onions- in the province of South Kalimantan has not been carried out.

Therefore, it is necessary to study the specific PGPR inoculation and mycorrhizal fungi against the intensity of fusarium wilt and towards the productivity of onions in South Kalimantan Province.

The purposes of this study were to find out the interaction between PGPR and mycorrhizae against the intensity of fusarium wilt and towards growth and yield of onions, and to obtain the best combination of PGPR isolate and mycorrhizal type to lower the intensity of fusarium wilt and improve the growth and yield of onions.

METHODOLOGY

The research was conducted at the Laboratory of Phytopathology, Department of Plant Pests and Diseases, Faculty of Agriculture, Lambung Mangkurat University and in Batang Kulur Kanan Village, Sungai Raya District, South Kalimantan Province.

Experimental designs

The design used in this research was a completely randomized design (CRD) in a factorial experiment consisting of two treatments, namely the inoculation of PGPR with three levels consisting of PGPR Isolate 1 (P₁), PGPR Isolate 2 (P₂) and PGPR Isolate 3 (P₃) which were interacted to mycorrhiza with two levels consisting of two species, glomus (M₁) and gigaspora sp (M₂).

Coupled with seven units of control experiments, 13 treatments were obtained. Each treatment was repeated four replications and each replication consisted of four plants, so the total experimental units were 208.

Laboratory Testing (*In Vitro*)

1. Isolation of *Fusarium oxysporum* f.sp *cepae*

F. oxysporum f. sp. *cepae* was isolated from soil, roots, bulbs and stems of onion plants that indicated the symptoms of fusarium wilt, in Batang Kulur Kanan Village, Sungai Raya District, Hulu Sungai Selatan Regency, South Kalimantan Province, Indonesia. The isolates were purified and identified by examining the color, colony formation and morphology microscopically (Domsc and Anderson, 1980; Barnett and Hunter, 1972). The Koch's postulate was then carried out.

2 Isolation of rizobacteria

Rizobacteria were isolated from the rhizosphere of onion, bamboo and sensitive plant, three sample location for each, and were then taken to the laboratory to be isolated as prospective antagonist agents.

The methods of isolation were performed like what Agustiansyah, Satriyas, Sudarsono and Machmud (2013) had applied. The isolation was carried out to obtain nonpathogenic bacterial isolates from various

species as the potential biological agents for biopesticides and biofertilizers.

3. Test for PGPR Inhibition ability against *F. oxysporum f. sp. cepae*

Test for PGPR inhibition ability against the growth of *F. oxysporum f. sp. cepae* was carried out using the method by Yuliana *et al.* (1987) in Khalimi and Wirya (2009). The in vitro test for PGPR inhibition ability against *F. oxysporum f. sp. cepae* was performed using a completely randomized design (CRD) with five treatments on all rizobacteria obtained with three replications.

Three isolates with the highest inhibition ability against *F. oxysporum f.sp cepae* would be tested in the field and the development of the interaction of mycorrhizal biofertilizer againsts the intensity of fusarium wilt and towards the growth and yield of onion was observed.

4. Test for phosphate dissolving ability of PGPR isolates

The test for the PGPR isolate ability to dissolve phosphate was evaluated using Pikovskaya's agar test with the addition of tri-calcium phosphate (TCP) as a source of phosphate. The phosphate dissolving ability of three isolates was tested qualitatively by ¹⁸ formation of a halo around the hole containing PGPR suspension (Thakuria *et al.*, 2004).

5. Test for HCN (Hydrogen Cyanide)

The production of qualitative HCN compounds was analyzed using the methods developed by Bekker and Schipper in Munif (2001). The rhizobacteria isolates tested were grown on glycine media in a petri dish.

The color of filter paper that remained yellow indicated that the isolates tested did not produce HCN while the light brown, dark brown and brick red indicated the increasing production of HCN.

6. Preparation of PGPR liquid Formulation

PGPR treatment in the field was applied in a liquid formulation. The preparation of PGPR liquid formulation for the volume of 1: 1 with a type of bacteria used the ingredients as follows: 984 mL of sterile water, 5 mL of liquid medium of Potato Dextrose Broth (PDB), 10 mL of Tween 80%, 10 mL of molasses and one mL of rhizobacteria that had been rejuvenated in a liquid medium of GDP. After all the ingredients were mixed, each bacteria formulation was incubated for one week in room temperature. After one week, each formulation was ready to use.

Field Testing (*in Vivo*)

The objective of this test was to find out the resistance of onion plants inoculated with PGPR and mycorrhizal biofertilizer against the attacks of pathogen *F. oxysporum f. sp. cepae*. The process of conducting this field research was as follows:

1. Inoculation of *F. oxysporum f.sp cepae*

After the preparation of the planting media that included tillage, the inoculation of fusarium wilt cause, namely *F. oxysporum f. sp. cepae* was done by pouring a suspension with a density of $2.0 \times 10^7 \text{ mL}^{-1}$ as much as 10 mL bulb⁻¹ in the planting hole in a polybag. The pathogen inoculation was performed a week before the onion planting.

2. Inoculation of PGPR

The provision of three PGPR isolates, the results of the selection, was done on a regular basis by three times of application. The first was carried out by soaking the bulbs (seed treatment), and after 10 days and 21 days the seed bulbs were planted, applied directly to the soil media in the polybags.

The PGPR inoculation in the second and third application was at a dose of 10 mL for each plant sample (Widiawati *et al.*, 2015).

3. Inoculation of Mycorrhizal Biofertilizer

Inoculation of mycorrhizae was performed by sprinkling mycorrhizal biofertilizer as much as 15 g planting hole⁻¹, 5 cm below the seed bulbs when they were about to be planted (Halis and Fitria. 2008).

4. Harvest

In this experiment, the harvest was carried out when 60 to 70% onion leaves was turning yellow, the stems was limp and bulbs started to swell the surface of the soil, *i.e.* approximately 60 days old. Harvesting was done when the terrain was dry. The onions were harvested by pulling out the bulbs and letting them for a couple of hours.

Observations and Data Collection

Observations conducted to find out the robustness of onion plants due to inoculation of PGPR and mycorrhizal biofertilizer against *F. oxysporum* f. sp. *cepae* were as follows:

1. Inhibition Ability of PGPR

The purpose of this observation was to determine the PGPR isolates with the most excellent inhibition againsts the growth of *F. oxysporum* f. sp. *cepae*. The determination of the inhibition of antagonists was performed by the formula (Fokkema, 1976):

$$I = \frac{R1 - R2}{R1} \times 100 \%$$

Where I is the percentage of inhibition, R1 the radius of pathogen colony moving away from the antagonistic bacteria, and R2 the radius of pathogen colony approaching the antagonistic bacteria. The test for this inhibition was also useful for selecting three PGPR isolates that inhibited the development

of *F. oxysporum* f. sp. *cepae* in vitro the most, which at a later stage were going to be tested in a greenhouse (*in vivo*).

2. Incubation Period

This observation was to determine the development rate of *F. oxysporum* f.sp. *cepae* on each treatment in order to find out the most effective PGPR isolates and mycorrhizal species in suppressing the development rate of the disease. The observation started from four days after inoculation until the appearance of the first symptom in each treatment tested.

3. Symptoms of Fusarium Wilt

The observation of the symptoms on the plant began 4 days after inoculation of *F. oxysporum* f. sp. *cepae* up to 50 days afterwards. The observation was carried out by recording all phenotype symptoms that occurred in the plants, such as changes in leaf color, leaf shape, and bulb shape as well as plant condition in each sample.

4. Intensity of Disease

The intensity of disease was observed every week from the initial appearance of the symptoms until harvest time. Based on the characteristics of systemic disease, the intensity of disease was counted using the formula developed by Wiyatiningsih (2007):

$$I = \frac{a}{b} \times 100\%$$

Where I: Intensity of disease

a: Number of sick plants

b: Number of total plants

To assess the intensity of the disease, the following scales were used:

- No attack: when the intensity of disease 0.00% - 5.00%
- Light attack: when the intensity of disease >5.00% - <10.00%
- Medium attack: when the intensity of disease ≥10.00% - <30.00%

- d. Heavy attack: when the intensity of disease $\geq 30.00\%$ - $< 75.00\%$
- e. Puso attack: when the intensity of disease $\geq 75.00\%$

The observations were conducted 26 to determine the crop growth and yield after each treatment to all crops. The variables observed consisted of:

a. **Number of Leaves**

The number of leaves was calculated every week, starting the first week after planting until one week before harvest manually.

b. **Diameter of Bulbs**

This is a destructive observation by dismantling the entire parts of plant. After the parts of plant were cleaned from residual soil and dirt, the diameter of the bulb was measured at harvesting time. Diameter of the bulbs was measured using a caliper in mm by shifting the measuring field and entering the calliper at the center of the bulb. The diameter was determined by reading the scales on *nonius* (shear field).

c. **Number of Bulbs**

This was also a destructive observation by dismantling the entire parts of plants at the harvest time. The plants were cleaned of residual soil or dirt, and the number of bulbs was calculated in unit of fruit.

d. **Wet Weight of Plant**

This is a destructive observation by dismantling the whole plant sample, and after

the dirt and soil were cleaned, the plant parts were then weighed and calculated in g.

e. **Dry Weight of Plant**

Measurements were taken at the harvest time. The measurements included all parts of plants that were on the surface of the soil or inside the soil (roots, bulbs and leaves) of the plant samples by dismantling the entire parts of plants. After parts of the plants were cleaned from residual soil or dirt, they were then weighed. After that, they were put into an oven at 65 °C for 72 hours or until it reached a constant weight (Abdel and Al-Jubori, 2006). The unit of measurement was g.

RESULTS

The rhizobacteria isolation carried out on the cultivation land of onions in Batang Kulur Kanan Village, Sungai Raya District, Hulu Sungai Selatan Regency, resulted in 27 isolates. The results of PGPR inhibition ability can be seen in Table 1.

Of all rhizobacteria obtained, three bacteria with the greatest inhibition ability against *Fusarium oxysporum* f. sp. *cepa* were taken. The three bacteria were tested again for the ability of bacteria to produce HCN and dissolve phosphate (Figure 1).

Table 1. Observation data of average PGPR inhibition ability

No.	Isolate	Inhibition (%)	No.	Isolate	Inhibition (%)
1.	P ₁	1.3 a	16.	P ₁₆	1.4 b
2.	P ₂	2.0 b	17.	P ₁₇	0.0 a
3.	P ₃	2.1 b	18.	P ₁₈	1.1 a
4.	P ₄	9.6 d	19.	P ₁₉	8.6 c
5.	P ₅	0.0 a	20.	P ₂₀	4.6 b
6.	P ₆	1.8 b	21.	P ₂₁	3.9 b
7.	P ₇	3.2 b	22.	P ₂₂	4.7 b
8.	P ₈	0.0 a	23.	P ₂₃	3.1 b
9.	P ₉	2.7 b	24.	P ₂₄	2.3 b
10.	P ₁₀	9.9 d	25.	P ₂₅	0.3 a
11.	P ₁₁	0.0 a	26.	P ₂₆	4.7 c
12.	P ₁₂	34.2 e	27.	P ₂₇	4.2 b
13.	P ₁₃	9.0 c			
14.	P ₁₄	5.4 c			
15.	P ₁₅	4.2 b			

Note: Means with the same letters indicate no significant difference between means according to DMRT test at the 5% significance level, P = PGPR isolates.

Figure 1 The ability of bacteria to produce hydrogen cyanide (HCN) and dissolve phosphate

The results of the analysis variance showed that the combined treatments had no significant effect on the number of leaves at week 1, 2, 3, 5, 6 and 7, bulb diameter, plant dry weight, incubation period of the pathogen, and intensity of the disease, but had the significant effect on the number of leaves at the observation week four and wet weight of bulbs per hill. It also had the very significant

effect on the number of onion bulbs. In addition, the results of the analysis of variance showed that the PGPR treatment as the sole factor provided a significant effect on the PGPR inhibition ability, number of leaves at the observation weeks 4, 5, 6, 7, number of bulbs, wet weight of bulbs per hill, plant dry weight, and intensity of the disease. The treatment of mycorrhizae as the single factor

also provided a significant effect on the observation results of the number of bulbs and wet weight of bulbs per hill (Tabel 4).

Inhibition Ability of PGPR

The results of inhibition ability test showed that the P₄ isolate was able to inhibit the growth of pathogen *Fusarium oxysporum* f. sp. *cepae*. However, P₄ did not produce HCN and did not have the ability to dissolve phosphate, thereby isolate P₄ was replaced with P₂₆ isolates that produced HCN and had the ability to dissolve phosphate. Meanwhile, P₁₀ and P₁₂ isolates were visually tested to be able to inhibit the growth of *Fusarium oxysporum* f. sp. *cepae* on a petri dish and had the ability in terms of generating HCN and dissolving phosphate.

Table 4 presented the observation data of inhibition ability test of all PGPR isolates

obtained from the isolation of the pathogen *Fusarium oxysporum* f. sp. *cepae*.

The naming of PGPR isolates for the field tests was modified for the ease of study; P₁₀ turning into P₁, P₂₆ into P₂, and P₁₂ into P₃.

Incubation Period

The results of the analysis of variance showed that the combined treatments as well as treatments of PGPR and mycorrhizae as the single factors did not give a significant effect on the incubation period of the pathogen.

The comparison of the incubation period between the control treatment, PGPR treatment and mycorrhizal treatment as the single factors, and combined treatments of PGPR and mycorrhizae are presented in Figure 2.

Figure 2. Comparison of incubation periods of control treatment, PGPR treatment, mycorrhizal treatment, and the combination of PGPR and mycorrhizae

Descriptions: K1 = healthy control, K2 = sick control, M = mycorrhizae, P = PGPR, PM = combination of PGPR and mycorrhizae

It can be concluded that the combined treatment of PGPR and mycorrhizae (PM) made the pathogen incubation period tend to be longer than other treatments, while the shortest pathogen incubation period was in sick control treatment (K₂).

Symptoms of Fusarium Wilt Disease

Plants infected with spp. showed early symptom of yellow leaves starting from the base of the leaves and then spread to the middle. The leaves of onion plants were easily twisted and torn from the soil, caused by rotting bulbs and roots of plants, so the holding ability to the planting medium became weaker

Symptoms of infected plants were in accordance with the symptoms of fusarium wilt, namely twisted leaves, damaged stems to the tips of the leaves and unpleasant odor in bulbs caused by rotting onion bulbs.

In this study, the symptoms gene 22 arose on day 20 and 21 after planting. it

indicated that the pathogen was not derived from bulbs but from the ground that had been inoculated on the planting medium.

Death due to the attack of *Fusarium oxysporum* f. sp. *cepae* in this study occurred to the onions aged between five and six weeks after planting.

Intensity of Disease

The test results of variance analysis showed that the combined treatments did not give significant effect on the intensity of the disease, but the application of PGPR as the single factor was capable of providing a significant effect on the intensity of the disease (Table 2).

Table 2 presents the results of Least Significant Difference (LSD) test of the treatments of isolates of PGPR 1 (P₁), isolates of PGPR 2 (P₂) and isolates of PGPR 3 (P₃) to the intensity of the *Fusarium oxysporum* f. sp. *epae* disease in the onion plants.

Table 2. LSD test of PGPR treatments to the intensity of disease

Treatment	Intensity of Disease (%)
P ₁	14.06 b
P ₂	3.13 a
P ₃	2.34 a

Note: Means with the same letters indicate no significant difference between means according to the LSD at 5% significance level. P = PGPR Isolates.

The treatment of PGPR isolate P₃ showed that it was able to suppress more *Fusarium* wilt disease than the treatment of PGPR isolate P₁, but the treatment of PGPR isolate P₃ was not significantly different from the treatment of PGPR isolate P₂.

Comparison of the disease intensity between the control treatment, PGPR

treatment and mycorrhizal treatment as the single factors, and combined treatments of PGPR and mycorrhizae is presented in Figure 3.

Figure 3. Comparison of the disease intensity in the treatments of control, PGPR, mycorrhizae, and combination of PGPR and mycorrhizae.

Description: K1 = healthy control, K2 = sick control, M = mycorrhizae, P = PGPR, PM = combination of PGPR and mycorrhizae.

The treatment with the lowest disease intensity was the treatment of healthy control (K1), while the one with the highest disease intensity was the treatment of sick control (K2).

Number of leaves

The test results of the analysis of variance showed that the combined treatments did not

provide a significant effect on the number of leaves at the observation week 1, 2, 3, 5, 6, and 7, but showed a significant effect on the number of leaves at the observation week 4 (Table 5).

Table 3 presents data of average variables of leaf number in onion plants at the fourth observation week of each combined treatment of PGPR and mycorrhizae.

Table 3. Data of number of onion leaves at observation week 4

Treatment	Number of Leaves
P ₁ M ₁	22.94 b
P ₁ M ₂	11.06 a
P ₂ M ₁	25.19 b
P ₂ M ₂	25.81 bc
P ₃ M ₁	30.06 cd
P ₃ M ₂	33.63 d

Note: Means with the same letters indicate no significant difference between means based on DMRT test at 5% significance level. P = PGPR Isolate, M1 = mycorrhizal fungus *Glomus*, M2 = mycorrhizal fungus *Gigaspora*.

The greater number of leaves was in the combined treatment of third PGPR isolate and mycorrhizal fungus gigaspora (P_3M_2), but it was not significantly different from the number of leaves found in the combined treatment of third PGPR isolate and mycorrhizal fungus glomus (P_3M_1).

The table also shows that the combined treatment of first PGPR isolate and mycorrhizal fungus gigaspora (P_1M_2) produced the least number of leaves compared with the other combined treatments.

In addition, the application of PGPR as the single factor was also able to influence the

results of observations at week 4, 5, 6, and 7 (Table 6). Based on the further test, PGPR isolate P_3 was able to stimulate more number of leaves than PGPR isolate P_1 , but the number of leaves in treatment P_3 was not significantly different from the number of leaves found in the treatment of PGPR isolate P_2 .

The following table, Table 4 presents the results of the Least Significant Difference (LSD) test of the treatments of PGPR, PGPR isolate 1 (P_1), 2 (P_2) and 3 (P_3) to the number of onion leaves in the observations at week 4, 5, 6 and 7.

Table 4. LSD test for PGPR treatment on the number of onion leaves at observation week 4, 5, 6, and 7

PGPR	Number of Leaves			
	4	5	6	7
P_1	17.00 a	17.56 a	18.09 a	18.19 a
P_2	25.50 ab	30.03 b	32.41 b	38.69 b
P_3	31.84 b	38.22 b	42.66 b	54.91 b

Note: Means with the same letters indicate no significant difference between means according to the LSD at 5% significance level. P = PGPR Isolates.

Comparison of the number of leaves between the control treatment, the application of PGPR and mycorrhizae as the single factors, and combined treatment of PGPR and mycorrhizae is presented in Figure 4.

Note: K_1 = healthy control, K_2 = sick control, M = mycorrhizae, P = PGPR, PM = combination of PGPR and mycorrhizae.

Number of Bulbs

Figure 4 shows that the greatest number of leaves at observation week 1, 2, 4, 5 and 6 was in the combined treatment of PGPR and mycorrhizae (PM), while at the observation week 3 and 7 the greatest number of leaves was in the treatment of PGPR (P) and healthy control (K_1). In addition, the smallest number of leaves was in observation of the application of infected control treatment (K_2).

The results of ANOVA test showed that the combined treatment of PGPR and Mycorrhizae provided a significant effect on the number of bulbs. The PGPR treatment and mycorrhizal treatment as the single factors each also had very significant and significant effects on the number of onion bulbs (Table 5).

Figure 4. Comparison of the number of leaves in control treatment, PGPR treatment, mycorrhizal treatment, and the combined treatment of PGPR and mycorrhizae.

Table 5. Data of observation on the average number of onion bulbs

Treatment	Number of Leaves
P ₁ M ₁	4.94 a
P ₁ M ₂	4.75 a
P ₂ M ₁	12.38 b
P ₂ M ₂	12.63 b
P ₃ M ₁	12.19 b
P ₃ M ₂	21.81 c

Note: Means with the same letters indicate no significant difference between means according to DMRT test at 5% significance level. P_nM_n = combination of PGPR isolat n and mycorrhizal type.

Table 4 showed that the highest number of bulbs was in the combined treatment of third PGPR isolate and mycorrhizal fungus gigaspora (P₃M₂), while the smallest number of bulbs was in the combined treatment of first PGPR isolate and mycorrhizal fungus gigaspora (P₁M₂), but it was not significantly different from the combined treatment of first PGPR isolate and mycorrhizal fungus glomus (P₁M₁).

Diameter of Bulb

The results of ANOVA test showed that the combined treatment did not give any significant effect on the diameter of bulbs. However, the application of PGPR and mycorrhizae as the single factors respectively gave very significant and significant effects towards the number of onion bulbs (Table 6).

Table 6. LSD Test for PGPR treatment on onion bulb diameter

Treatment	Diameter of Bulb (cm)	
	Original Data	Transformed Data
P ₁	10.70	0.79 a
P ₂	10.80	1.40 b
P ₃	13.07	1.71 b

Note: Means with the same letters indicate no significant difference between means based on the LSD test at 5% significance level. P_n = the-n PGPR Isolate

Table 6 shows that the treatment of PGPR isolate P₃ was able to stimulate root diameter greater than the treatment of PGPR isolate P₁, but the treatment of PGPR isolate P₃ was not significantly different from the treatment of PGPR isolate P₂.

Table 7. Least significant difference (LSD) test for mycorrhizal treatment on diameter of onion bulb

Treatment	Diameter of Bulb (cm)	
	Original Data	Transformed Data
M ₁	10.16	1.10 a
M ₂	10.50	1.51 ab

Note: Means with the same letters indicate no significant difference between means based on the LSD test at 5 % difference level. M₁ = Mycorrhizal fungus Glomus, M₂ = Mycorrhizal fungus Gigaspora

Figure 5. Comparasion of bulb diameter between control treatment, PGPR treatment, mycorrhizal treatment, and combined treatment of PGPR and mycorrhizae

Note: K₁ = healthy control, K₂ = sick control, M = mycorrhizae, P = PGPR, PM = combination of PGPR and mycorrhizae

Table 7 presents the results of least significant difference (LSD) test for the treatment of mycorrhizal fungi both glomus (M_1) and gigaspora (M_2) towards the diameter of onion bulbs (Figure 5).

The treatment with the smallest bulb diameter was the sick control (K_2) treatment, while the treatment with the largest bulb diameter was the PGPR treatment (P).

Wet Weight of Plant

The results of ANOVA test showed that the combined treatment of PGPR and mycorrhizae gave a significant effect on the wet weight of plant (Table 8).

Table 8. Observational data of average wet weight of onion plants

Treatment	Wet Weight of Plant (g)
P_1M_1	38.72 a
P_1M_2	30.71 a
P_2M_1	82.38 b
P_2M_2	90.16 bc
P_3M_1	103.47 c
P_3M_2	163.75 d

Note: Means with the same letters indicate no significant difference between means based on the DMRT test at 5% significance level.

Table 8 shows the observational data on average wet weight of onion plants in the combined treatment of PGPR isolates 1, 2 and 3 with mycorrhizal fungi glomus and gigaspora.

Table 8 shows that the largest wet weight was in the treatment combination of third PGPR isolate and mycorrhizal fungus gigaspora (P_3M_2) while the smallest wet weight of plant was in the combined treatment of first PGPR isolate and mycorrhizal fungus gigaspora (P_1M_2), but it was not significantly different from the combined treatment of first PGPR isolate and mycorrhizal fungus glomus (P_1M_1).

The comparison of wet weight of onion plant between the control treatment, PGPR treatment and mycorrhizal treatment as the single factors, and combined treatment of PGPR and mycorrhizal fungi is presented in Figure 6.

It can be concluded that the treatment with the smallest plant wet weight was the treatment of sick control (K_2), while the treatment with the highest plant wet weight was the treatment of PGPR (P).

Dry weight of Plants

The results of ANOVA test showed that the combined treatment did not give any significant effect on the dry weight of plants, but the treatment of PGPR as the single factor was capable of providing a significant effect on the dry weight of onion plants (Table 9).

The third PGPR isolate treatment (P_3) was able to stimulate the dry weight of plants greater than the first PGPR isolate treatment (P_1), but the third PGPR isolate treatment (P_3) was not significantly different from the second PGPR isolate treatment (P_2) (Figure 7).

Figure 6. Comparison of wet weight of onion plant between control treatment, PGPR treatment, mycorrhizal treatment, and combined treatment of PGPR and mycorrhizae.
Note: K₁ = health control, K₂ = sick control, M = mycorrhizae, P = PGPR, PM = combination of PGPR and mycorrhizae.

Table 9. LSD test for PGPR treatment on dry weight of onion plants

Treatment	Dry Weight of Plants (g)
P ₁	5.84 a
P ₂	17.40 b
P ₃	24.07 b

Note: Means with the same letters indicate no significant difference between means based on the LSD test at 5 % significance level. P_n=the-n PGPR isolate.

Figure 7. Comparison of plant dry weight between control treatment, PGPR treatment and mycorrhizal treatment as single factors, and combined treatment of PGPR and mycorrhizae.

Note: K₁ = healthy control, K₂ = sick control, M = mycorrhizae, P = PGPR, PM = combination of PGPR and mycorrhizae.

The treatment with the smallest dry weight was the sick control treatment (K₂), while treatment with the greatest dry weight was the combined treatment of PGPR and mycorrhizae (PM).

DISCUSSION

The combination of PGPR and mycorrhizae as a whole was unable to suppress fusarium wilt disease, but could postpone the incubation period (26,19 days after inoculation) and increase the growth and yield of onion compared to the onion pl²⁸ infected with fusarium but without the combined treatment of PGPR and mycorrhizae and the PGPR treatment and mycorrhizal treatment as single treatments.

According to Susanto (2008), one of PGPR mechanisms to support the plant growth is as the driver of mycorrhizae. However, in

this study the combination of PGPR and mycorrhizae was not only able to support the plant growth, but also capable of stimulating the yield of onion plants and suppress Fusarium wilt. It supports the results of the research by Linderman and Meyer (1986) and Von *et al.* (1993) reporting that PGPR can improve mycorrhizal infection, stimulate the formation of spores and mycorrhizal mycelia, and engage in mycorrhizal associations in the soil (Barea *et al.*, 1998).

The results of research by Irnayuli *et al.*, (2010) also stated that there was a positive teraction between PGPR and mycorrhizae in which there was an increased percentage of successful infection of mycorrhizae on seedlings of jelutung (*Dyera polyphylla* Miq. Steenis) applied with PGPR.

PGPR mechanism in stimulating the growth and yield has not yet been fully investigated, but it was expected to include the

abilities to produce phytohormones, fixate N₂, and dissolve phosphate minerals and other nutrients. Meanwhile, the mycorrhizal mechanism in stimulating the growth and yield, by the presence of phosphatase enzyme, was capable of hydrolyzing phytat compound, the complex P compound. Phytat is buried in the ground by 20 to 50% of total organic-P and a strong binder for cations such as Ca²⁺, Mg²⁺, Zn²⁺, Fe²⁺, and protein. With the help of the phosphatase enzyme, phytat can be hydrolyzed to myoinositol, free-P, and minerals, so the availability of P and minerals in the soil can be supplied (Fuady, 2013). Additionally, Pacovsky (1986) through his research stated that the plants inoculated with mycorrhizal fungi have higher concentrations of Cu and Zn but lower Fe and Mn than the control plants.

Not only did mycorrhizae provide phosphate and minerals but it was also able to increase the surface area of root uptake by 80% (Rousseau *et al.*, 1994). Smith and Read (1997) add that mycorrhizal hyphae can spread up to 25 cm from the roots resulting in an increased soil exploration to obtain nutrients. Mycorrhizae can also increase soil fertility by fixing mechanisms of soil aggregates (Koske, 1975) and stabilize the soil structure and contribute positively to the biological processes in the soil (Oades, 1993).

The mechanism of PGPR in suppressing the disease is by producing siderophores, synthesize antibiotic and fungicide component (Gholami *et al.*, 2009). According to Susanto (2008), each microbial antagonist including PGPR has its own mechanism and can have more than one mechanism of inhibition in controlling plant pathogens. The way this agent work is by using the results of secondary metabolism, either in the form of antibiotics, toxins, enzymes, or hormones, or without involving the results of the secondary metabolism, for example by parasitism.

According to Compants *et al.*, (2005) in Yulianti (2013) a number of antibiotics are produced by bacteria of pseudomonas group, namely amphisin, 2,4 diasetilfloroglucinol, hydrogen cyanide, oomisin, A. phenazine, pioluteorin, pirrolnitrin, tensin, tropolone, and cyclic lipopeptide; whereas those produced by *Bacillus*, *Streptomyces*, and *Stenotrophomonas* include oligomisin A, kanosamine, zwittermicin A, and xanthobasin.

At the beginning and end of the study, the application of the combined treatment of PGPR and mycorrhizae did not contribute to the number of leaves, number of bulbs, wet weight of bulbs per hill, and intensity of disease. It is because PGPR and mycorrhizae are living creatures which require adaptation to the environment at the beginning of inoculation. When PGPR and mycorrhizae are able to adapt, the combination of the two is capable of triggering the growth and yield of onion. However, such good condition did not take place during the study because the other nature of PGPR and mycorrhizae as the living organisms is that they need nutrients to grow and develop, so there was competition between PGPR and mycorrhizae in the condition of lack of nutrients in the end of the study. This was in line with the research by Sitepu *et al.*, (2010) stating that the interaction of mycorrhizae and rhizobacteria was not always beneficial for plants because of the awful condition of food competition among the microorganisms themselves.

PGPR and mycorrhizal interaction is discussed in detail by Artursson (2005); the specific interaction between the two is open by their root exudates formed from the composition of chemical compounds. Changes in chemical composition occur because mycorrhizal colonization will select and determine the bacterial communities that will colonize the roots and mycorrhizal hyphae. It

is indicated by the majority of bacteria groups that interact synergistically with mycorrhizae, namely the positive gram bacteria. Several *pseudomonas* spp as the bacterial colonizers of the roots are often found attached to the surface of the hyphae.

Associated with organic compounds exudated by plant roots and mycorrhizal hyphae, Toljander *et al.*, (2007) found that organic compounds exudated by mycorrhizae can stimulate the selection of specific bacterial groups, thus imposing the specific interactions between bacteria and mycorrhizae.

The results of research by Barrea *et al.*, (1998) showed that *Pseudomonas* strain F113G22 producing anti fungal compound 2.4 and its association with *Glomus mosseae* still can support the development of hyphae by stimulating the germination of spores in the soil as well as improving *G. mosseae* on the roots of tomato plants.

PGPR isolate P₃ in combination with mycorrhizae either *Glomus* or *Gigaspora* was able to suppress the disease and to improve the growth and yield of onions. In addition, PGPR isolate P₃ given as a single factor was also better than PGPR isolates P₁ and P₂. The ability of PGPR isolates P₁ and P₃ in suppressing the disease compared to other isolates was likely related to their better abilities in producing antimicrobial HCN and competing with pathogens for nutrients compared with the other isolates shown in the *in vitro* testing.

In *in vivo* testing, the ability of PGPR isolate P₃ in enhancing the growth and yield of onions is most likely related to its ability to increase the availability of nutrients to the plants by removing the chemical bonds from bound phosphate to become soluble phosphate and absorbed by the plants. On acidic soils, P element is bound by Al (Al-P) and Fe (Fe-P). The presence of organic acids produced by PGPR directly can dissolve phosphate and

bind Fe and Al previously binding P (Rodrique and Fraga, 1999).

Mycorrhizae as the single factor could also increase the yield of onions, especially mycorrhizal fungus *Gigaspora*. According to Fuady (2013), there are differences in the length of hyphae on the same host plant between mycorrhizal fungus *Glomus* and mycorrhizal fungus *Gigaspora*. On the host plants such as onions, the length of mycorrhizal hyphae *Glomus* ranges from 0.71 to 2.5 mcm⁻¹ roots. The length of mycorrhizal hyphae *Gigaspora* can reach 12.3 mcm⁻¹ roots on the same host plant. Mycorrhizal hyphae are the main functional organs in uptaking nutrients, so the size of hyphae also greatly affects the efficiency of mycorrhizal fungus in uptaking nutrients for host. The lengthier the hyphae are, the greater it is likely for the hyphae to get and absorb nutrients, and conversely the shorter the hyphae, the smaller it is likely for the hyphae to get and absorb nutrients.

It is reinforced by O'Dell *et al.* (1993) that the length of hyphae determines the level of nutrient uptake in the soil as a result of the increased extent of the exploitation due to the length of mycorrhizal hyphae. In addition to the absorption of nutrients, the hyphae also increase the water absorption and reduce toxic metal absorption from soil.

According to Mosee (1986), the relative dependence on mycorrhizae may vary among plant species or even among varieties (cultivars) within a species. The plants have very different needs and responses to phosphate and dependence on mycorrhizae. The different dependence on mycorrhizae among various types of plants can be classified into high, moderate, and low. According to the list below the plants with high dependence on mycorrhizae have large roots and or limited root hairs like cassava and citrus.

It is clear that onion has a great degree of dependence on mycorrhizae, so the growth rate of onion plants is better when applied with mycorrhizae rather than without it. However, the response of each variety to mycorrhizae is different to one another, and further research on it has not yet conducted.

CONCLUSIONS

1. There was no significant effect of inoculation interaction between PGPR and mycorrhizae on the number of leaves at the observation week 1, 2, 3, 5, 6 and 7; diameter of bulb; dry weight of plant, incubation period of pathogen and intensity of disease. However, it had a significant effect on the number of leaves at the observation week 4, wet weight of bulbs per hill, and number of onion bulbs. In addition, the application of PGPR as the single factor provided very significant effects on the inhibition ability of PGPR, number of leaves at the observation week 4, 5, 6 and 7, number of bulbs, wet weight of bulbs per hill, dry weight of plants and intensity of the disease. Meanwhile, the application of Mycorrhizae as the single factor provided very significant effects on the number of bulbs and wet weight of bulbs per hill.
2. The best interaction was between the PGPR isolate 3 and mycorrhizal fungus *Gigaspora* in terms of the intensity of the disease, incubation period of the disease, number of leaves, wet weight of plants, number of bulbs and diameter of bulb.

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