Effectiveness formulation made from PGPR Bacillus spp. in the protection of shallot plants from fusarium wilt

Suyono¹ and Baharuddin²

¹Agroekoteknologi Program Study, Faculty of Agriculture and Forestry, Universitas Sulawesi Barat, Majene, Indonesia
²Department of Pests and Plant Diseases, Faculty of Agriculture, Universitas Hasanuddin, Makassar, Indonesia

Email: suyono@unsulbar.ac.id

Abstract. Fusarium wilt disease (Fusarium oxysporum f. Sp. Cepae) is one of the critical and dominant diseases in shallots in South Sulawesi, which can reduce production by up to 60 percent. This study aims to determine the ability of the PGPR Bacillus spp. in protecting the onion plants from fusarium wilt (F. o. cepae) in the onion plants produced. The implementation method consists of; preparation of planting media, preparation of onion planting, supply and propagation of microbes (fungi F. o. cepae and PGPR bacteria Bacillus spp.), manufacture of PGPR bacteria Bacillus spp. formulations, and application of PGPR bacteria Bacillus spp. on planting. The study was conducted using a Completely Randomized Design (CRD) consisting of 5 treatments and three replications. Data were analyzed with ANOVA at the level of 5% and 1%. If it shows any treatment effect, then it is continued with BNJ test at 1% level. The results showed that treatment C showed the results of the emphasis on the intensity of the attack F. o. the best cepae with an average suppression of 83.44% compared to other treatments at the last observation.

1. Introduction
Onion production in Indonesia is still seasonal, like agricultural products in general. While the need for onion is almost used every day, especially on religious holidays. This causes the needs of Indonesian onion outside the harvest season cannot be fulfilled. So that the national onion production is still far below the requirements and import measures are needed to fulfill it. Imports shallots by government need to maintain the availability of domestic shallots and the stability of market prices. This import action made Indonesia a net importer of onions. Every year Indonesia carries out exports and imports of shallots, but the amount of exports is much smaller than the number of onions imported to Indonesia [1].

The large volume of imported shallots shows that there are still enormous opportunities for the domestic market. Shallot cultivation business has excellent prospects and business opportunities in the future. In terms of productivity, in the last seven years (2007-2013) the national average onion productivity was only around 9.46 tons / ha, far below the production potential which is above 20 tons/ha [2].

The national shallot productivity in 2015 decreased compared to the previous year. Based on the projections of shallots in 2014 and 2015, there was a decline of around 15.8 percent [1]. Meanwhile, according to the Director General of Horticulture, the Ministry of Agriculture each month, the need for shallots in the country is around 90 thousand tons. In 2014 it reached 1.23 million tons with a harvest
area of 119,966 hectares, while in 2015 the production of shallots reached 1.14 million tons or around 120 thousand tons per month [3].

The problem of low productivity is caused by the intensity of the attack of plant pests (OPT) and the difficulty of getting seed tubers that are free of disease for planting material. One of the pathogens of disease that is often carried on seed bulbs is fusarium wilt disease many people call as moler disease [4]. Fusarium wilt caused by Fusarium oxysporum F. sp. cepae Schecht (Hanz.). This fungus is a soil-borne pathogen that can survive for a long time in the form of chlamydospora, although no host plants are available [5]. This disease in 1997 is not significant in shallots, but in the past five years, there has been an increase in disease attacks. So it has become a major disease in various regions of onion production centers in Indonesia [6].

Fusarium wilt (moler) is commonly found in fields, where the onion is planted without rotation. It is an important and dominant disease for shallots in South Sulawesi that can produce up to 60 percent [7]. Fusarium wilt is an economic disease that can be detrimental to farmers because until now, there has been no effective chemical control. Whereas in reality, farmers rely more on synthetic pesticides in dealing with pest problems, because it is easier to obtain them and apply them. However, the use of synthetic pesticides is not the only choice in dealing with the problem. Some diseases cannot be overcome only with synthetic pesticides, such as soil infectious and systemic diseases.

Biological control and fertility management are efficient choices for controlling this disease. In addition to biological control, it is safer, cheaper, and environmentally friendly. Biological control is one of the appropriate control options and needs to be pursued. Utilize soil microorganisms such as beneficial rhizosphere fungi are antagonistic to soil-borne pathogens is one of biological control and fertility plant management [8].

Biological agents that can be used are rhizobacteria in the root region which can support plant health through the release of secondary metabolites and are also able to increase growth with the production of auxin hormone [9]. Based on the definition, rhizobacteria are a group of bacteria that can occupy the rhizosphere aggressively, and rhizobacteria that benefit plants are known as PGPR (Plant Growth Promoting Rhizobacteria) [10].

PGPR is a plant growth booster bacteria that has received attention in recent decades as an option in controlling plant diseases that are effective and more environmentally friendly. These bacteria in the future can have good prospects for overcoming plant diseases because several mechanisms are owned, in addition to the production of antibiotics that directly suppress pathogens, can also produce phytohormones and induce plant resistance [11].

Kafrawi [12] has successfully isolated PGPR potential bacteria Bacillus spp. from the rhizosphere of onion plants, in several centers of onion cultivation on the island of Sulawesi. Based on testing the PGPR selection stage showed an increase in most parameters compared to controls such as the isolate GR25 capable of producing auxin ZPT (IAA) of 2.33 ppm and nitrogen binding of 2.935 ppm. LB10 isolates were able to dissolve phosphate (P) by 504.04% and synthesize siderophore by 0.174 ppm. MG14 isolates can produce gibberellin (GA3) of 1.516 ppm and GR7 isolates can inhibit the growth of F. o. cepae in vitro by 87.41%. To be more efficient, the microbial formulation of PGPR bacteria Bacillus spp. This needs to be tested in the field and enriched microbes that can stimulate plant growth and increase tuber production. This study aims to determine the ability of the bacterium PGPR Bacillus spp. in protecting the onion plants from fusarium wilt (F. o. cepae) onion plants produced.

2. Methods

2.1. Time and Place
This research was conducted at the Laboratory of Agricultural Biotechnology, Research and Development Center (PUSLITBANG) Biotechnology, Research Center Activities Building (PKP), Hasanuddin University and the Practice Field of the Makassar Community Training Center. Which runs from June to October 2016.
2.2. Planting media preparation

Planting media used include a combination of soil, charcoal husk, manure with a ratio of 1:1:1. Before being put into a polybag, the soil is first air-dried for three weeks. The lumpy soil is destroyed. After that it is sifted with a rough sieve (0.5 cm), then all the media is combined and put into a polybag. Polybags used to have a size of 30 cm x 40 cm. First, the media is watered before the onion seeds are planted in a polybag.

2.3. Preparation for shallot planting

Onion seedlings tubers are chosen with the same size (homogeneous), with medium size weighing between (2.5-5.0 g / tuber). Next, the seed tubers to be planted are cut off by the edges + 1/3 parts. Cutting the tuber seedlings is done one day before planting. Then the onion seeds are planted in a polybag amount of 3 tubers per polybag and arranged about 15 cm between the bulbs. Bulbs are planted into the planting medium until only the tubers stem.

2.4. Provision and propagation of f.o. cepae fungi

Shallot clove samples that showed symptoms of tuber rot were successively dipped in sterile distilled water, 70% alcohol, and sterile distilled water twice. Then put in a petri dish containing filter paper. After a few days, the fungus F. o. cepae growing is taken with a needle and then placed and incubated on the Potato Dextrose Agar (PDA) media. The isolate was determined based on its microscopic morphology.

Pathogenic fungal F. o. cepae isolates were isolated from diseased tubers on the PDA medium. Then the fungus was dissolved into sterile water with a concentration of 106 spores/ml. Then it is shaken to separate the spores from the media that the application will later do.

2.5. Propagation of Bacteria PGPR Bacillus spp.

Bacteria PGPR Bacillus spp. used in this study is the result of previous research by Kafrawi [12], namely four isolates: MG14, LB10, GR7, and GR25. The four isolates were propagated on Nutrient Glokusa Agar (NGA) media.

2.6. Making bacteria formulations PGPR Bacillus spp

MG14, LB10, GR7, and GR25 bacterial isolates that have been propagated on nutrient agar (NA) were diluted in 0.1 mol/L MgSO47H2O solution. So that the bacterial cell wall did not undergo lysis, then population density was calculated using a spectrophotometer at wavelength 660 nm, with an optical density of 0.06 (108 cfu / ml). Then add the formulation material in the form of Carboxyl Methyl Cellulose (CMC) solution, glycerin, tween 80, Na alginate and sorbitol which function as adhesives, protectors, fillers, grading and emulsifiers. Furthermore, the isolates were mixed according to treatment in sterile containers (bottles) with a volume of 1 liter, then the containers (bottles) were tightly closed. All procedures were incubated for three days on a shaker.

2.7. Effectiveness test of PGPR bacteria isolate formulation bacillus spp.

Suspension of PGPR Bacillus spp. with a volume of 60 ml/polybag applied to the onion plant directly to the root surface of the plant. Application of PGPR Bacillus spp. bacterial formula was carried out on shallot plants 14 days after each plant medium (polybag). The administration of pathogenic fungi to the media was carried out seven days after application of the bacterial formula PGPR Bacillus spp. with a population density of 106 cfu/ml and a suspension volume of the pathogenic fungus 60 ml/polybag. This study used a Completely Randomized Design (CRD) consisting of 5 treatments with three replications. The treatments are as follows:

- Treatment of isolate A: Isolat PGPR Bacillus spp isolat MG14 + F. o. cepae
- Treatment of isolate B: PGPR isolate Bacillus spp LB10 + F. isolate o. Cepae
- Treatment of isolate C: PGPR isolate Bacillus spp isolate GR7 + F. o. Cepae
- Treatment of isolating D: PGPR isolate Bacillus spp isolate GR25 + F. o. Cepae
• Control Treatment K (-): Treatment without bacterial isolates
• Control Treatment K (+): Treatment with + F. o. Cepae

2.8. Data analysis
The experiment was conducted with a Completely Randomized Design (CRD), here were seventeen treatments with three replications. Data were analyzed with ANOVA at the level of 5% and 1%. If it shows any treatment effect, then it is continued with BNJ test at 1% level.

3. Results and discussion
Table 1 shows that the effectiveness of inhibition of Bacteria PGPR Bacillus spp. against F. o. Cepae attacks in all treatments ranged from 53.78% to 83.44% at the observation 49 days after inoculation. In treatment C (PGPR Bacillus sp Isolate GR7 bacterial formulation), it was seen to be more effective in suppressing the intensity of the F. o. Cepae attack which is 83.44% compared to other treatments.

| Treatment | Attack intensity (%) on the day after inoculation (hsi) |
|-----------|-------------------------------------------------------|
|           | 7          | 14         | 21         | 28          | 35         | 42         | 49         |
| A         | 100        | 63.54      | 59.53      | 67.97       | 70.45      | 66.15      | 62.96      |
| B         | 100        | 80.97      | 75.93      | 82.56       | 82.78      | 78.31      | 62.21      |
| C         | 100        | 90.48      | 87.37      | 89.77       | 89.42      | 88.47      | 83.44      |
| D         | 100        | 86.05      | 82.61      | 86.81       | 86.96      | 86.20      | 75.83      |
| K(+)      | 99.47      | 81.49      | 76.92      | 57.64       | 46.92      | 40.17      | 27.99      |

In this study showed that treatment C (formulation of PGPR bacteria Bacillus sp Isolate GR7) showed the best results of suppression of the intensity of the attack F. o. Cepae with an average pressure of 83.44% at the age of the plant observation from 14 hsi to 49 hsi. Based on the results of the PGPR Bacillus spp. in vitro. Kafrawi [12] showed that the PGPR bacteria Bacillus sp. GR7 isolate code has a powerful inhibitory ability (++++) with an inhibition index of 87.41% compared to controls. The low intensity of attack F. o. Cepae caused by antagonistic activity in the form of antibiotic compounds produced by the bacterium PGPR Bacillus spp. The ability of plant pathogens to colonize rooting or rhizosphere regions can be effectively suppressed.

Bacteria Bacillus spp is a gram-positive bacteria that can be used as a potential biological control agent. These bacteria in controlling plant diseases through several mechanisms such as competition, inducing systemic resistance in plants and producing antibiotics [13]. Anti-microbial compounds produced by bacteria, among others, iturin compounds, is a group of lipopeptides that are excreted from most strains of B. subtilis when grown in liquid media. Also, certain strains of B. subtilis are capable of producing the antibiotic bacilysosin, a new phospholipid antibiotic that can inhibit the growth of various bacteria and fungi [14].

According to Klement et al. [15] that the intensity of plant-pathogen attack is strongly influenced by the virulence of the pathogen, the level of resistance of a plant variety, the time of occurrence of infection and the environmental conditions in which the plant is growing.

4. Conclusion
Treatment C shows the results of the emphasis on the intensity of the attack F. o. Cepae the best with an average suppression of 83.44% compared to other treatments at the last observation. It is necessary to test the application formulation of PGPR Bacillus spp. in field conditions, to find the most effective biological control agent in controlling wilting F. o. Cepae in shallot plants.
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