Biological control of *Fusarium oxysporum* in shallot wilt disease using arbuscular mycorrhiza combined with *Trichoderma* sp., *Pseudomonas* sp., and *Bacillus* sp.

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Abstract-This study aimed to control *Fusarium* wilt disease in shallots using arbuscular mycorrhizal fungi, *Trichoderma* sp. and *Bacillus* sp. The research was carried out at the Screen House and Plant Protection Laboratory. The research design used a completely randomized (CRD) method with five treatments repeated four times with three experimental units in each treatment. The treatments were arbuscular mycorrhiza (M), soaking seeds with PGPM (So), Spraying with PGPM (S), arbuscular mycorrhiza + soaking seeds (MSo), and arbuscular mycorrhiza + soaking PGPM + spraying PGPM (MSoS), and control (C). Observation variables included the incubation period of *Fusarium* wilt disease, the incidence of *Fusarium* wilt disease, and plant products consisting of the number of leaves, plant height, number of tubers, and tuber weight. The results show that the fastest incubation period was four days after the inoculation of the *Fusarium* in the control treatment. The treatment of seed soaking, spraying and application of arbuscular mycorrhizae (RSM) showed the longest incubation period, 14 days. The RSM treatment able to suppress the *Fusarium* wilts disease more than 50%. The shallot growth performance and production increase accordingly.

Keywords-*shallot, wilt disease, arbuscular mycorrhiza, Trichoderma* sp., *Pseudomonas* sp., *Bacillus* sp.

I. INTRODUCTION

In the Palu Valley, Central Sulawesi, farmers commonly cultivate Wakegi shallots [1]. Wakegi shallot (Allium × wakegi Araki) is one of the commercial onions in several regions in Indonesia, and most people consider Wakegi shallot to be part of the shallot variety due to their similarity in forming the small size of the bulbs and their whitish-red color.

Shallot cultivation efforts in the Palu Valley to increase the yield often encounter obstacles. Several factors hinder shallot cultivation, one of which is a disease caused by a pathogen. Some common diseases that attack shallots include purple

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spots, tuber rot, anthracnose, white rot, and late blight.

Moler's disease or stem rot, or tuber rot, is one of the main problems in shallot cultivation in the Palu Valley. The causal pathogen is *Fusarium oxysphorum*, which infects roots and bulbs. The symptoms include root rot, discoloration, and necrosis. The disease can also cause root rot, or Moler's disease in Indonesia.

Efforts to control the pathogen *Fusarium oxysporum* have been pursued in various ways, using chemical pesticides, technical cultures, and biological control agents. Utilization of Plant Growth Promoting Microorganisms (PGPM) is an environmentally friendly technology to reduce the yield losses caused by plant pathogens. The PGPM uses antagonistic microbes from groups of fungi, bacteria, or a combination of both. The commonly used groups of fungi are Arbuscular Mycorrhiza and Trichoderma spp. The most familiar group of bacteria are Bacillus spp. and Pseudomonas spp.

The two groups of microbes exert antagonistic effects on plant pathogens. Mycorrhiza, as a biological controller, can increase plant resistance to root pathogen attacks by producing root sheaths or antibiotics that can kill pathogens. The use of mycorrhiza as a biological agent has been widely carried out, for example, in citrus, tomato, and cotton plants [2]. It is known that mycorrhizae cover the surface of plant roots so that plant roots can avoid infection, especially infection with soil-borne pathogens [3]. In addition, mycorrhizae maximize carbohydrates and other root exudates to create an unsuitable environment for the growth of pathogens [4].

Mycorrhizae also play an important role by increasing the root absorption area up to 47 times, helping the decomposition of organic wastes, translocating soil nutrients, especially phosphorus, and increasing the ability of plant roots to absorb water [5]–[7]. In addition, mycorrhizae are also able to increase plant resistance to biotic and abiotic stresses [8]. In this role, mycorrhiza can maintain plant stability in polluted conditions. Pseudomonas sp., Trichoderma sp., and Bacillus sp. can be used as a fertilizer to control plant pathogens and increase plant resistance through induced systemic resistance (ISR) [9].

This research exploits the potential of AMF, Trichoderma sp., Pseudomonas sp, and Bacillus sp.. to control *Fusarium* wilt disease. The results of this study will contribute to the discovery of the potential of Central Sulawesi germplasm as a biological control agent for plant diseases.

II. MATERIALS AND METHODS

A. Experimental Design and Application of the treatments

The research design was completely randomized (CRD) with six treatments, four replicates, and three experimental units in the following treatments.

1. Application of arbuscular mycorrhiza (M) = 5 g/polybag
2. Soaking the seeds with PGPM (So) = 100 g L^{-1} PGPM
3. Spraying with PGPM (S) = 100 ml PGPM per L application
4. Arbuscular mycorrhiza + soaking seeds (MSo) = 100 g PGPM + 5 g mycorrhiza
5. Application of Arbuscular Mycorrhiza = soaking with PGPM + Spraying with PGPM (MSoS) = 100 g PGPM + 100 ml PGPM + 5 g mycorrhiza
6. Control (C) = No treatment

Soil preparation was carried out by taking soil from the farmers' onion farms in Solouwe Village using shovels and hoes. Then the soil was transported to the Faculty of Agriculture by car. The next step is that the soil is sifted and then sterilized by roasting for 2 hours for 20 kg of soil. After the soil had been sterilized, 5 kg of soil was added to the pot.

The mycorrhiza used in this study came from the collection of the Laboratory of Plant Pests and Diseases. Apart from mycorrhizae, this study also utilized other beneficial microbes from commercial preparations, Terrabio (PT. Prima Agro Tech, SNI 6729 2013, No. 144/LSPO-033-IDN/07/13), which contains 4.86×10^6 *Trichoderma* sp.; 1.7×10^7 *Pseudomonas* sp.; and 1.93×10^7 *Bacillus* sp.

The preparation of PGPM was carried out by following the method of use, dissolving a powder preparation with a concentration of 100 grams of PGPM in 1 liter of sterile water and then incubating it for 24 hours. Furthermore, this solution is referred to as the PGPM stock solution. At the same time, the preparation of PGPM for soaking seeds is done by diluting 100 ml of PGPM stock solution in 900 ml of water. Soak the seeds for 30 minutes before planting.

After the application of soaking the seeds, proceed with the planting process. Before planting, pots containing sterile soil were added 5g of mycorrhiza per planting hole in the pot. Planting activities were carried out by placing one onion seed in one pot. Preparation for spraying is carried out by diluting 100 ml of PGPM stock solution in 900 ml of water. Spraying is done when the plants are two weeks after planting and spray every two weeks.

Artificial infection with *Fusarium oxysporum* was carried out by inoculation of the fungus *Fusarium oxysporum*. Inoculation was

carried out 30 days after planting. A 10 ml suspension of pathogenic fungi with a conidia density of 10^6 was poured into the plant's root area in the pot according to the treatment design. The *Fusarium* used in this study has been tested as a pathogen throughout Koch's postulate.

B. Observational Variables and Data Analysis

The incubation period was observed every day after the application of *Fusarium oxysporum* until the plants appeared symptomatic. Observations were made on each plant.

The disease intensity was observed every day after artificial infection was carried out until harvest time. [10], observation of disease intensity uses the following formula: $IP = \frac{\sum(n \, x \, v)}{N \, x \, Z} \, x \, 100\%$.

Where IP is disease intensity, n is the number of plants observed to show a particular score, v is the shallot score, N is the highest score, and Z is the total number of plants.

The scores used were: 0 = no symptoms of the attack, 1 = 0-20% of affected leaves, 2 = 21-40% of affected leaves, 3 = 41-60% of affected leaves, 4 = 61-80% of affected leaves, 5 = 81 - 100% of the leaves are attacked.

The growth and production of shallots in the Palu Valley include plant height, measured using a meter from the soil surface to the tip of the tallest leaf. The number of leaves was also measured at the same time. Measurement starts three weeks after planting (WAP) till 7 WAP. The number and fresh weight of bulbs were calculated at the time of harvest.

Data of plant height, number of leaves, number of tubers, and bulb weight were transformed in logarithmic transformation (LOG). Furthermore, the disease incidence data were transformed into the root of x. The data obtained from each observation was analyzed by analysis of variance (ANOVA). If the treatment had a significant effect, it continued with the least significant difference (LSD) test at the 5% level.

III. RESULTS AND DISCUSSIONS

A. Incubation Period

The incubation period of *Fusarium* pathogens in shallots treated with different treatments can be seen in the table below.

Table 1. The average incubation period (days) of *Fusarium oxysporum* in Palu Valley shallots treated with PGPM.

Treatments	**Incubation period (day)**
MSoS (Mycorrhiza + Soaked +Spray)	14 (1.12) a
So (Soaked)	9 (0.97) b
S (Spray)	10 (0.99) b
MSo (Mycorrhiza + Soaked)	9 (0.93) b
M (Mycorrhiza)	8 (0.89) b
C (Control)	4 (0.63) c

Note: Numbers in brackets are the results of the Log transformation. Numbers with the same letters are not significantly different according to the LSD test at the 5% level.

B. Disease Incidence

The incidence of *Fusarium* wilt in shallots treated with PGPM can be seen in the table below.

TABLE 2. AVERAGE INCIDENCE (%) OF *FUSARIUM* WILT IN PALU VALLEY SHALLOTS TREATED WITH VARIOUS PGPMS.

Treatments	**1st week**	**2nd week**	**3rd week**	**4th week**
MSoS	0.00 b	16.67(2.89) b	33.33 (5.77) c	33.33 (5.77) c
So	8.33 (1.44) b	41.67 (6.37) a	58.33 (7.57) b	75.00 (8.62) ab
S	16.67 (2.89) ab	50.00 (6.97) a	66.67 (8.16) ab	66.67 (8.16) b
MSo	8.33(1.44) b	33.33 (5.77) a	50.00 (6.97) bc	58.33 (7.57) b
M	16.67 (2.89) ab	50.00 (6.97) a	58.33 (7.57) b	75.00 (8.62) ab
C	33.33 (5.77) a	66.67 (8.16) a	83.33 (10.00) a	91.67 (9.54) a

Note: the numbers in brackets are the log transformation values. Numbers with the same letters are not significantly different according to the LSD test at the 5% level.

C. Shallot Growth and Production

The growth and production of shallots treated with PGM and infected with *Fusarium* are shown in the table below.

TABLE 3. THE AVERAGE EFFECT OF TREATMENT ON SHALLOT GROWTH AND PRODUCTION

Treatments	Number of leaves	Plant height (cm)	Number of bulbs	Fresh weight of each bulb
MSoS	33.25 (1.52) a	32.53 (1.51) a	6.50 (0.81) a	6.86 (0.83) a
So	31.25 (1.49) ab	30.28 (1.48) ab	5.25 (0.71) b	5.62 (0.75) b
S	27.25 (1.43) cd	30.30 (1.48) ab	5.50 (0.74) ab	5.51 (0.74) b
MSo	31.25 (1.49) ab	30.40 (1.48) ab	5.25 (0.72) b	5.89 (0.77) ab
M	29.00 (1.46) bc	30.00 (1.48) ab	5.00 (0.69) bc	5.67 (0.72) b
C	25.50 (1.41) d	28.08 (1.45) b	4.25 (0.63) c	3.51 (0.54) c

Note: the numbers in brackets are the log transformation values. Numbers with the same letters are not significantly different according to the LSD test at the 5% level.

This study has shown that the application of PGPM can extend the incubation period, reduce the incidence of *Fusarium* wilt in shallots, and increase shallot production. PGPM treatment by applying mycorrhiza before planting, soaking the seeds, and spraying the plants with PGPM was the best treatment compared to other treatments. These results reinforce that beneficial microbes for plants can not only help protect plants from pathogen attacks but can also increase crop production.

Arbuscular mycorrhizal fungi (FMA) are one of the plants' most essential symbiont groups. FMA forms relationships with most plants and is vital in acquiring plant nutrients. When mycorrhizae cover the surface of plant roots, plant roots can avoid infection, especially soil-borne pathogen infections [11]. In addition, mycorrhizae can maximize carbohydrate levels in plant roots and other root exudates to form an environment that does not support the growth process of a pathogen [12].

The suppression of disease intensity by biological agents is found in all treatments, but the value of suppression varies depending on the suitability of the biological agents for environmental conditions. [13], Also, the plant defense system is highly dependent on the interaction of the host, pathogen, and environment.

IV. CONCLUSION

Plant growth promotion rhizomicrobes (PGPM) significantly affected the disease suppression, growth, and production of shallots. PGPM can show more extended incubation periods needed by *Fusarium* to produce disease symptoms. Applying mycorrhiza in the soil, soaking the shallot seeds before planting, and spraying the plant every two weeks after planting save the production to almost 80% shallot yields, while untreated plants are infected by almost 90%.

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