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Effect of *Trichoderma* sp. and *Streptomyces* sp. on the growth and production of True Seed Shallots (TSS)

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Abstract. A study aimed to determine the effect of *Trichoderma* sp. and *Streptomyces* sp. on the growth and production of True Seed Shallots (TSS) was conducted from April to October 2018 at the Teaching Farm, Faculty of Agriculture, Universitas Hasanuddin, Makassar, South Sulawesi. The experiment was set using a Randomized Group Design (RBD) replicated three times. The first factor was the application of *Trichoderma* sp. which consisted of three levels, namely control; 2 g per plant; 4 g per plant, while the second factor was the application of *Streptomyces* sp. consisted of three levels, namely: control; 10^4 cfu L^{-1}; 10^8 cfu L^{-1}. The results show that the treatment of *Trichoderma* sp. at a dose of 4 g per plant resulted in better growth and production of shallots on the observed parameters ie. fresh weight of the bulbs per plot (686.60 g), dry weight of the bulbs per plot (532.48 g), production per plot (0.56 kg) and production per hectare (5.61 tons ha^{-1}). No significant effect of *Streptomyces* sp. observed. Application of *Trichoderma* sp. and *Streptomyces* sp. on TSS tended to resulted in better growth and yield was the combination treatment of *Trichoderma* sp. 4 g per plant and *Streptomyces* sp. 10^4 cfu L^{-1} with an average production of 5.84 tons ha^{-1}.

1. Introduction

Shallot (*Allium ascalonicum* L.) is a vegetable plant that is widely cultivated in Indonesia. The plant has many benefits such as for seasoning as well as traditional medicinal ingredients. Shallot also has various content of vitamin. These benefits cause increasing consumer demand in the market for shallots. Consequently, the increase in demand must be followed by an increase in production [1]. On the other hand, there has been a decline in the national shallots productivity in Indonesia caused by a number of factors including declining in soil fertility, high levels of plant pests, micro-climate change, and the use of low-quality seeds.

One of problems faced by shallot farming in Indonesia is the lack of availability of quality planting material. Until now farmers still use bulbs as planting material. The high demand for seed bulbs and the relatively high price of the bulbs makes farmers use their own seed bulbs from previous harvests. Some weaknesses found when using bulbs as planting materials include deterioration of the bulbs during storage indicated by sprouting before the planting season arrives and infectious diseases on the seed bulbs. These problems can reduce production in subsequent cropping season so that alternative planting materials such as true seeds are necessary to support the availability of planting material at the farm level [2]. The use of botanical seeds as seeds for propagation material onion plants is one alternative that needs to be developed. The use of botanical seeds in the production of onions is more beneficial than the use of seed bulbs because it can increase plant productivity up to 100% compared to using seed bulbs [3]. Another advantage is the need for less TSS seeds (± 3-6 kg Ha^{-1}) compared to seeds originating from bulbs (± 1.0-1.2 tons Ha^{-1}), more practical handling and longer shelf life of TSS (1-2 years) compared to bulbs (only 4 months) [4].

In addition to low quality of the planting materials, poor soil fertility is also one of the problems that is often experienced by shallot farmers. The use of inorganic fertilizers in large quantities and over a long period of time results in suboptimal cultivation land conditions which can reduce the production of shallots. This will cause a decline in soil fertility due to nutrient imbalance or other nutrient deficiencies, and the decline in the content of organic matter in each layer in the soil [5]. One way to increase soil fertility is to enrich the growing media using microbes. *Trichoderma* sp. is one of the microorganisms that has the ability to accelerate the process of decomposition of organic matter and is a symbiotic fungus that is harmless, even mutually beneficial between soil infectious fungi with plant roots [6].

The use of microbes in shallot plants can reduce the need for inorganic fertilizers, able to increase the number of bulbs of shallots [7]. According to Kristanto [8], the use of microbes in corn plants can reduce the need for fertilizer N up to a dose of 20,000 L Ha^{-1}. Biofertilizers can increase plant growth and production in a number of ways, including by providing nutrients, fixation of nitrogen from the air by several types of fungi [9]. One of microbes widely used was *Trichoderma* sp. *Trichoderma* sp. has several advantages such as easily isolated, broad adaptability, can grow quickly on various substrates, this fungus also has a wide range of microparasitism and is not pathogenic in plants [10].

Some research results are known that biological agents such as *Trichoderma* sp. can also function as a decomposer. *Trichoderma* sp. acts as a decomposer in the composting process to break down organic materials such as cellulose into glucose compounds. Another advantage of *Trichoderma* sp. which can be used as an environmentally friendly biofungicide. *Trichoderma* sp. as decomposer help degrade organic matter so that more nutrients are available for plant growth [6].

Based on research on endophytic associations of *Trichoderma asperellum* in *Theobroma cacao,* the best dose of *Trichoderma* resulted in better plant growth was 4 g per plant [11]. In addition, based on research conducted by Sepwanti et al. [12] suggested that the best dose of compost enriched with *Trichoderma harzianum* is at a maximum dose of 20 g per plant for chili. The results of Dermawan et al. [13](2018) showed that the treatment of *Trichoderma asperellum* 4 g per plant significantly gave the highest chili fruit length per plant at 110 days after planting (DAP).

Besides the wide use of *Trichoderma* in improving plant growth and production, *Streptomyces* sp., a group of filamentous bacteria that are abundant in the soil, is also received more attention recently. *Streptomyces* sp. is the largest genus of *Actinomycetes* sp. Genus *Streptomyces* sp. has characteristics including filamentation with a diameter of 0.5-1 µm, aerobes, gram-positive bacteria and sexual production with spores produced by aerial mycelium. Vegetative mycelium is a collection of hyphae that grow in the substrate. Aerial mycelium is a collection of hyphae that grow vertically through the substrate and are permanently in contact with air [14].

These aerobic microorganisms are able to degrade compounds that are difficult to degrade such as chitin. Chitin is a polymer commonly found in the cell wall of the fungus class Basidiomesetes, Ascomisetes, and several types of Deuteromycetes. Therefore *Streptomyces* sp. can be used as an alternative biological control agent for plant diseases caused by fungi. *Streptomyces* sp. known as an antibiotic-producing bacteria, because among more than 10,000 antibiotics have been found, two-thirds are produced by the bacteria *Streptomyces* sp. [15]. Based on research conducted by Sahur [16], it is known that several types of *Actinomycetes* sp. such as *Streptomyces* could influence root nodulation in soybean plants by increasing the frequency of root nodulation in areas infected by *Rhizobium* sp. *Actinomycetes* sp. also form colonies that are useful in the cell surface layer of the nodules and produce spores. Based on the description above, this study was conducted to determine the growth and production of shallots from seeds applied by *Trichoderma* sp. and *Streptomyces* sp.

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2. Methodology

This research was carried out in the Teaching Farm, Faculty of Agriculture, Universitas Hasanuddin, Makassar from April to October 2018. The trial employed a Randomized Group Design (RBD) as an environmental design with the application of fungus *Trichoderma* sp. (T) as the first factor consisted of three levels, namely: t0 = control; t1 = 2 grams per plant; t2 = 4 grams per plant, while the second factor was the inoculation of *Streptomyces* sp. (A) consisted of three levels, namely: a0 = control; a1 = 10^4 cfu L^{-1}; a2 = 10^8 cfu L^{-1}.

2.1. Isolation of Streptomyces sp.

The bacteria *Streptomyces* sp. were propagated on Nutrient Agar (NA) media, a solid shaped medium which is a blend of agar, Nutrient broth and aquades. The process of propagation was carried out in a sterilized laminar air flow.

2.2. Planting and plant maintenance

Prior to planting in the field, seeds of TSS variety of Tuktuk were sown in nursery tray filled with a mixture of soil, sand and chicken manure media with a ratio of 1: 1: 1. Seedlings were transplanted into 7.5 x 15 cm polybags at three weeks after sowing and maintained for another two weeks. Seedlings were then planted into 1 x 1 m beds with a height of 40 cm. A distance of 50 cm was set between plots and a distance of 70 cm was set between replications. Each hole was planted as much as 1 shallot seedlings with a spacing of 10 cm x 10 cm resulted in a total of 64 plants per plot. Ridging was conducted to avoid exposure of the bulbs to direct sunlight. Watering was carried out in the morning and evening every day until the age of 60 DAP then the frequency of watering was reduced to one time a day until harvest. The TSS were harvested at the age of 80 DAP marked with yellowed leaves, softened stem necks and collapsed, bulbs were visible on the surface of the ground with a deep red color.

2.3. Data collection and analysis

Parameters observed were vegetative and production components. The component of growth were indicated by parameters of plant height and number of leaves, while the production component observed consisted of fresh and dry weight of the bulbs formed, production per plot and production per hectare. Bulb fresh weight parameter was measured by weighing all parts of the plant including the leaves, roots and bulbs at harvest using analytical scales. Bulb dry weight parameter was measured by weighing all parts of the plant (total bulbs weight, leaves and roots) dried for one week after harvest. Drying was carried out outdoors and not exposed to direct sunlight. The data obtained were analyzed using analysis of variance and if there was a real effect of the treatment, further tests were performed using the Least Significance Difference (LSD) test (p = 0.1).

3. Results

3.1. Effect of Trichoderma sp and Streptomyces sp. on the vegetative growth of TSS plant

Analysis of variance conducted on observation data of the plant height and the number of leaves of the TSS show no significant effect of both treatments either the application of *Trichoderma* sp. and *Streptomyces* sp. Figure 1 shows that in the treatment of *Trichoderma* sp. 4 g per plant combined with *Streptomyces* sp. 10^4 cfu L^{-1} tended to produce the highest average plant height of 40.56 cm, while the treatment of *Trichoderma* sp. 4 g per plant without *Streptomyces* sp. tended to produce the lowest average plant height of 37.67 cm.

Figure 2 shows that in the treatment of *Trichoderma* sp. 4 g per without *Streptomyces* sp. tended to produce the highest average number of leaves, 6.94 leaves, while the *Streptomyces* sp. of 10^8 cfu L^{-1} without *Trichoderma* sp. tended to produce the lowest average number of leaves, 5.72 leaves.

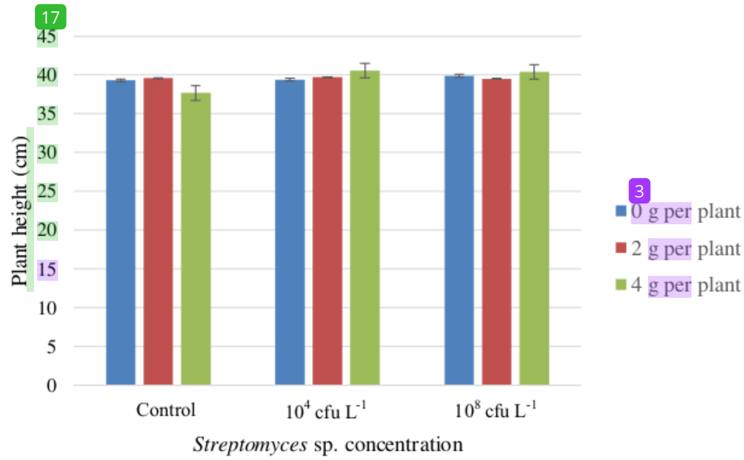

Figure 1. Average of True Seed Shallots (TSS) plant height due to application of *Trichoderma* sp. and *Streptomyces* sp. Bars represents of *Trichoderma* sp. treatments

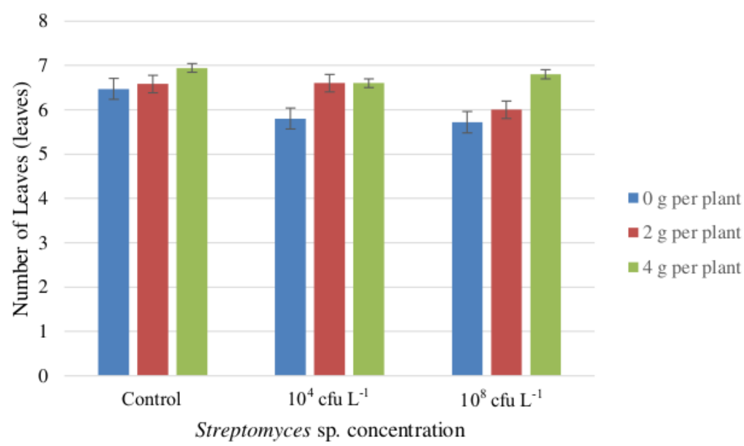

Figure 2. Average of True Seed Shallots (TSS) leaves number due to application of *Trichoderma* sp. and *Streptomyces* sp. Bars represents of *Trichoderma* sp. treatments.

3.2. Effect of Trichoderma sp and Streptomyces sp. on the production of TSS plant

The variance analysis show that the treatment of *Trichoderma* sp. significantly affected some production component of TSS observed in this tria i.e. fresh and dry weight bulbs per plot, yield per plot and the productivity of TSS. On the contrary, no significant effect of the inoculation of

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Streptomyces sp. The average fresh and dry weight per plot of the TSS bulbs formed (g) and the yield per plot and the productivity of TSS shown in table 1.

Table 1. Average of Bulbs Fresh and Dry Weight, Yield per plot, and Productivity of TSS plants applied with *Trichoderma* sp. and *Streptomyces* sp.

Trichoderma sp. dose	Bulbs Fresh Weight per plot (g)		Bulbs Dry Weight per plot (g)		Yield per plot (kg)		Productivity ton.Ha^{-1}	
0 g per plant	899.90	a	716.04	a	0.73	a	7.26	a
2 g per plant	660.09	b	457.02	b	0.5	b	4.97	b
4 g per plant	686.60	ab	532.48	ab	0.56	ab	5.61	ab
LSD $_{p = 0.1}$	197.09		193.71		0.18		1.78	

Numbers followed by different letters in the each column were significantly different at the level of LSD, p=0.1.

LSD p = 0.1 in table 1 shows that treatment of *Trichoderma* sp. of 2 g per plant produced the lowest average of fresh bulbs weight of TSS (660.09 g per plot) and was not significantly different from the treatment of *Trichoderma* sp. 4 g per plant but significantly different from the control that produced the highest wet tuber weight (899.90 g per plot). This results were similar in the dry bulb weight per plot parameter, yield per plot and the productivity parameter of the TSS. The treatment of *Trichoderma* sp. 2 g per plant resulted in the lowest average yield of bulbs per plot of 0.50 kg per plot and was not significantly different from the treatment of *Trichoderma* sp. 4 g per plant. The highest yield per plot of 0.73 kg per plot was obtained in control treatment. The control treatment also resulted in the highest productivity of the TSS. The value was not significantly different with the application of 4 g per plant *Trichoderma* sp.

4. Discussion

The experimental results showed that the treatment of *Trichoderma* sp. significantly affected the fresh and dry bulbs weight per plot of the TSS, bulbs production per plot, and the productivity of the TSS. However, no significant effect on plant height, number of leaves were found.

On the plant height parameter, the treatment of *Trichoderma* sp. 2 g per plant resulted in higher plant compared to control plants. While in the leaves number parameter, plants applied with *Trichoderma* sp. 4 g per plant showed higher number of leaves compared to the control plants. This might due to the function of *Trichoderma* sp. as one of soil microorganisms that has the ability to absorb nutrients and water for plant needs and help to protect plants from pests and diseases. Nasaruddin [17] suggested that *Trichoderma* sp. has the ability to improve plant growth and production either when used single or combined with other microorganism.

In the recent study, control plants showed the highest results on the production parameters of the TSS. The values of these parameters were not significantly different with treatment of *Trichoderma* sp. 4 g per plant. Application of the fungi gave good influence on production TSS bulbs (5.84 tons Ha^{-1}) but still below the potential productivity of shallots in general which is 9 tons Ha^{-1}. The contributing factor to the low productivity of the TSS in this study probably due to the lack of plant response to the application of *Trichoderma* sp. Lack of interaction between *Trichoderma* sp. and plants in the field could be caused by climatic conditions which are also very influential. Unfavourable environmental conditions, such as uncertain rainfall conditions, low humidity and temperature can affected the interaction of *Trichoderma* sp. with the plant.

The results of statistical analysis show that the treatment of *Streptomyces* sp. had no significant effect on all parameters observed. This is allegedly due to acidity of the soil which is not suitable for supporting the breeding of *Streptomyces* sp. which was applied to each experiment bed. Soil acidity is very influential on the distribution of *Streptomyces* sp. in the field, soils with neutral to basic (alkaline)

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pH are very good for the growth of the body with a range of 6.5 to 8.0. Conversely at acidic pH these organisms are less able to survive, and at pH below 5.0 in the soil it is generally rare to find *Streptomyces* sp. [18]. Continuous fertilization using ammonium without the addition of lime will suppress the growth of *Streptomyces* sp. This condition happens because the ammonium will be oxidized by microbes to nitric acid thereby reducing the pH of the soil which makes the environment unsuitable for the pathogen. Instead, liming will help the vegetative growth of *Streptomyces* sp. because the pH becomes neutral or basic. Besides the pH of the soil, moisture is very important for the growth of *Streptomyces* sp. Bacteria *Streptomyces* sp. generally are aerobic so that it will grow well on well aerated soils. Thus, soil immersion that causes humidity reaches 80-100 percent will inhibit its growth, thus it is necessary to properly manage irrigation in the management of utilization of *Streptomyces* sp. Percentage of *Streptomyces* sp. in the total soil microbial population will increase along with the depth of the soil, however that does not mean that the surface of the soil is not found *Streptomyces* sp.

Nutrients contained in the planting media cannot be optimally absorbed by plants at the beginning of growth. This is consistent with the statement of Sutedjo [19] which stated that the ability of plants to absorb nutrients during their growth and development (especially in the case of uptake or absorption) is not the same. Plants need different amounts of time and nutrients. According to Wedhastri [20], which states the difference between biological fertilizers and chemical fertilizers is the response of slow plants, the supply of indirect nutrients, the environmental impact does not exist so that slow biological fertilizers are available to plants, therefore plants have not given a response that has a real influence, and the bacteria present in biological fertilizers have less influence on plant growth because the application of biological fertilizers in the soil can be washed away due to extreme weather.

5. Conclusion

Based on the results of research that has been done, it can be concluded that treatment of *Trichoderma* sp. 4 g per plant had a better effect on the growth and production of shallots on the observed parameters ie. wet tuber weight per plot, dry tuber weight per plot, tuber production per plot, and onion tuber production was 5.84 tons Ha^{-1}.

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