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Effectiveness of Indigenous Rhizobacteria Formulations in Increasing the Growth and Yield of Shallots (*Allium ascalonicum* L.)

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ABSTRACT

Using beneficial microbes to increase plant growth and yield is an appropriate choice in order to reduce synthetic chemicals that may cause negative impacts on the environment. The objective of this study was to evaluate the effectiveness of post-save formulations of Wakatobi indigenous rhizobacteria in increasing the growth and yield of shallots. The study was conducted in Jati Bali Village, South Konawe District, using a Randomized Block Design consisting of 10 rhizobacterial formulation treatments, a combination of three types of biological agents, namely Pseudomonas sp. LP03, Pseudomonas sp. TWB02 and Pseudomonas sp. TWB11, and three types of formulation materials: ground brick powder, ground burned rice husk powder, and bentonite; one control using NaCl 0.85%. The experiment was repeated three times so that there were 30 treatment units. The results showed that of the three types of rhizobacteria, only Pseudomonas sp. TWB02 and Pseudomonas sp. LP03 displayed better performance to increase the growth and yield of shallots. These rhizobacteria were more compatible with the ground burned rice-husk powder formulation. The treatment of Pseudomonas sp. TWB02 and Pseudomonas sp. LP03 in the formulation of ground burned rice-husk powder increased shallot yield (tuber fresh weight) of 121% and 117%. Further research is needed on different environmental conditions to see the stability of these biological agents' effects on the growth and yield of shallots.

INTRODUCTION

Various efforts to meet the community's needs for healthy food products that free from hazardous chemical residues are inseparable from the use of beneficial microbes, which will continue to be a research trend in all parts of the world, especially those oriented to environmentally

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friendly technology in plant cultivation. As a tropical country with rich in microbial biodiversity, Indonesia has a massive opportunity to produce superior microbes, which could be utilized as alternative technological solutions in the cultivation of organic plants free of chemical pesticides.

Potential biological agents resulting from the exploration and isolation of microbial indigenous onion plants in marginal land are expected to have advantages and can be applied to various land conditions and can even adapt to various environmental stress conditions that always experience of fluctuations each year. Biological agents derived from the onion plant rhizosphere are mainly dominated by groups of bacteria (*Bacillus* spp., *Pseudomonas fluorescents, Serratia* spp.). This microbial group has the dual ability to act as a biological agent to promote plant growth and at the same time be able to increase plant resistance to pests and diseases. The superiority of microbes as a growth promoter is due to their ability to produce growth hormones (IAA, gibberellins, or cytokines), which are naturally needed by plants to enhance their growth and development (Vacheron *et al*., 2013; Ilyas *et al*., 2015). In addition, these microbes can chelate essential elements from the area around the plant's roots to be utilized by plants (Ahemad and Kibret, 2014; Suman *et al*., 2016). Microbial applications in plants also produce healthier and toxin-free products (Gupta *et al.,* 2015; Sutariati *et al*., 2016; Zahid *et al*., 2015). Meanwhile, the ability of these microbes as biological control of pathogens (Sivasakthi *et al.,* 2014) is related to their ability to control plant diseases through competition mechanisms, antibiosis, siderophore, hydrogen cyanide and excretion of hydrolytic enzymes that function as anti-microbial compounds (Dinesh *et al*., 2015; Gupta *et al.* 2015).

Until now, the materials used in various tests on the effectiveness of biological agents (bacteria) in plants are still in the form of bacterial cell suspensions, which are generally applied to seeds as a seed treatment. However, the use of cell suspensions is not practical when used in large scale applications. This study aimed to evaluate the effectiveness of post-save biological agent formulations on the growth and yield of shallots.

MATERIALS AND METHODS

This research was conducted at the Agronomy Unit of Agrotechnology Laboratory, Faculty of Agriculture, University of Halu Oleo and on the plantation land of Jati Bali Village, West Ranomeeto District, South Konawe Regency, Southeast Sulawesi. This research was carried out from October 2019 until March 2020.

Research Design

The experiment was organized in a randomized block design (RBD), consisting of 10 treatments of rhizobacterial formulations, namely: (1) Control (F0); (2) Formulation of ground brick powder + *Pseudomonas* sp. LP03 (F1); (3) Formulation of ground burned-rice husk powder + *Pseudomonas* sp. LP03 (F2); (4) Formulation of Bentonite + *Pseudomonas* sp. LP03 (F3); (5) Formulation of ground brick powder + *Pseudomonas* sp. TWB02 (F4); (6) Formulation of ground burned-rice husk powder + *Pseudomonas* sp. TWB02 (F5); (7) Formulation of Bentonite + *Pseudomonas* sp. TWB02 (F6); (8) Formulation of ground brick powder + *Pseudomonas* sp. TWB11 (F7); (9) Formulation of ground burned-rice husk powder + *Pseudomonas* sp. TWB11 (F8); (10) Formulation of Bentonite + *Pseudomonas* sp. TWB11 (F9). Each treatment was repeated three times so that, in total, there were 30 treatment units.

Rhizobacterial Preparation and Formulation

The biological agent (rhizobacteria) was made in the formulation of *Pseudomonas* sp. LP03, *Pseudomonas* sp. TWB02 and *Pseudomonas* sp. TWB11. Isolated bacteria were propagated by scraping a full circle of needles into the Tryptic Soy Agar (TSA), then incubated for 28 hours at 28 ^{o}C - 30 ^{o}C. After incubation, as many as 150 bacterial loops were suspended in 1000 ml of sterile waterand homogenized with a magnetic stirrer. The rhizobacteria formulation was prepared by adding carrier material (red brick powder, husk charcoal powder and bentonite) each of 50 g to 50 ml of bacterial suspension with a density of 109 ml^{-1}. The control was made by adding 0.85% NaCl solution, then mixed for 10 minutes. All bacterial formulations were put into a Schott bottle, covered with clear plastic that had been perforated with three puncture needles, then stored at room temperature (28 °C-30 °C) for three months.

Effectiveness Test of Rhizobacteria Formulation in the Field

Wakatobi local shallot seeds were used in this test. The seeds were washed with water and dried with aerated air. Application of seed treatment with rhizobacteria formulation using seed biomatriconditioning techniques. Each 20 g of rhizobacteria formulation was dissolved in 250 ml of water; then, the onion seeds were soaked in the solution for 5 minutes. The seeds were drained back from the formulation material and added 20 g of the husk charcoal powder as a biomatriconditioning plant. The seeds and media are mixed evenly so that the entire surface of the seeds was covered with media, then incubated for 12 hours. The effectiveness of post-storage rhizobacteria formulations was observed on the variables of the number of tillers, dry biomass weight, tuber number and tuber weight yield.

The data were analyzed using ANOVA, and when it showed a significant effect, it was furtherly tested with Duncan's Multiple Range Test (DMRT) at α=0.05. Data analysis was conducted using SAS program.

RESULTS AND DISCUSSION

Tillers Number of Shallots

Seed treatment using formulated rhizobacteria significantly increased tillers number of shallots. Among the treatments tested, the formulation of ground burned rice-husk powder + *Pseudomonas* sp. TWB02 showed a better increase in the number of tillers, except the treatment of ground brick powder formulation + *Pseudomonas* sp. TWB02 (Figure 1).

Biomass Dry Weight of Shallots

The seeds treatment using formulated rhizobacteria was also able to increase the dry biomass weight of shallots. Formulation of ground brick powder + *Pseudomonas* sp. TWB02 and formulation of ground burned rice-husk powder + *Pseudomonas* sp. TWB02

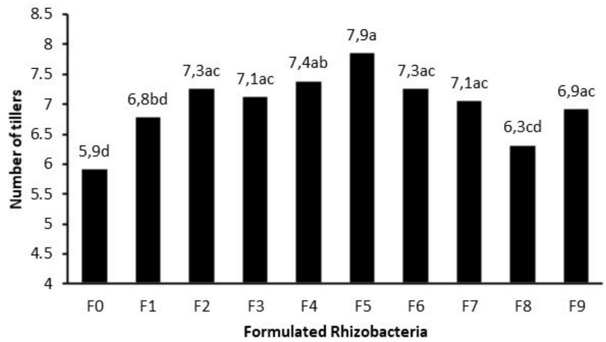

Figure 1. The effect of formulated rhizobacteria on number of shallot tillers
Notes: Means in the same bar chart suffixed with different letters are different at 5% levels of significance according to DMRT .

showed a better performance in increasing dry biomass weight of shallots. Increased biomass dry weight of shallot in this treatment of ground brick powder formulation + *Pseudomonas* sp. TWB02 and ground burned rice-husk powder + *Pseudomonas* sp. TWB02 reached 130% and 132% compared to controls (Figure 2).

Tuber Number of Shallots

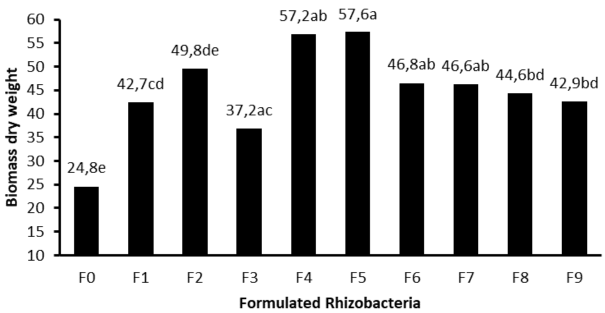

Figure 2. The effect of formulated rhizobacteria on biomass dry weight of shallot
Notes: Means in the same bar chart suffixed with different letters are different at 5% levels of significance according to DMRT

Seeds treatment using formulated rhizobacteria significantly increased the tuber number of shallots. Among the treatments tested, the formulation of ground burned rice-husk powder + *Pseudomonas* sp. TWB02 showed a highest increase in the tubers number of shallots. Increasing the number of tubers, reaching 33% compared to the control (Figure 3).

Tuber Fresh Weight of Shallots

Seed treatment using formulated rhizobacteria significantly increased the fresh tuber weight of shallots. Among the treatments tested, the formulation of ground burned rice-husk powder + *Pseudomonas* sp. LP03 and ground burned rice-husk powder formulations

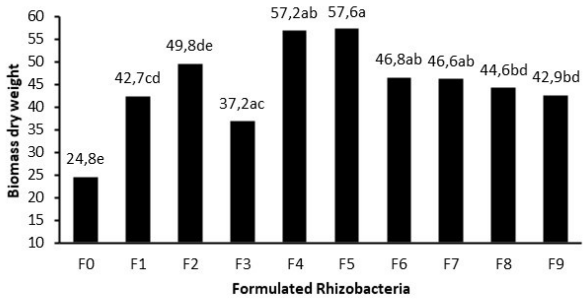

Figure 3. The effect of formulated rhizobacteria on biomass dry weight of shallot
Notes: Means in the same bar chart suffixed with different letters are different at 5% levels of significance according to DMRT.

+ *Pseudomonas* sp. TWB02 showed the same performance in increasing the fresh tuber weight, and the effect of both was better than other controls and treatments. Increased fresh weight in the treatment of ground burned rice-husk powder formulations + *Pseudomonas* sp. LP03 and ground burned rice-husk powder + *Pseudomonas* sp. TWB02 reached 117% and 121%, respectively, compared to controls (Figure 4).

In general, all variables observed in testing the effectiveness of post-storage biological agent formulations on the growth and yield of shallots showed that the formulation material of husk charcoal powder is better than that of red brick powder and bentonite. The husk powder formulation ingredients are able to maintain the viability of biological agents for three months of storage without reducing their effectiveness as a growth booster onion plant. On the other hand, of the three biological agents used, rhizobacteria *Pseudomonas* sp. TWB02 showed the best performance in improving the growth and yield of shallots, followed by *Pseudomonas* sp. LP03. The three rhizobacteria used in this study had the advantage of boosting the growth of

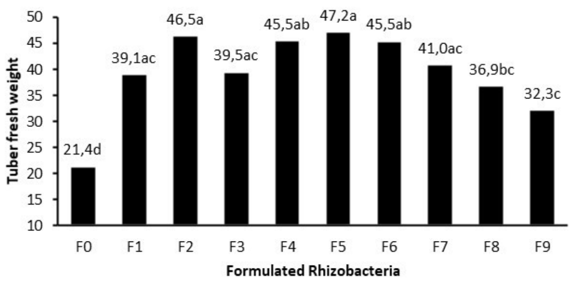

Figure 4. The effect of formulated rhizobacteria on tuber fresh weight of shallots
Notes: Means in the same bar chart suffixed with different letters are different at 5% levels of significance according to DMRT

shallots based were reported in the previous studies (Afa *et al*., 2020).

As explained earlier, rhizobacteria that belong to the PGPR (Plant Growth Promoting Rhizobacteria) group were proven effective in increasing plant growth and yield. The roles of rhizobacteria of the group *Pseudomonas* sp. as PGPR were able to dissolve phosphate, fix nitrogen, and produce auxin growth hormone (Paul *et al.,* 2017; Sutariati *et al*., 2018; Sutariati *et al*., 2019; Afa *et al*., 2020). This is also in line with the results of several studies reported that biological agents from the rhizosphere bacterial group improved the growth and yield of various plant commodities (Tuhuteru *et al*., 2016; Guyasa *et al*., 2018; Wati and Despita, 2018; Gamez *et al*., 2019).

The ability of this biological agent to survive in the formulation material without losing its role as a growth-promoting agent was shown by its effectiveness during the testing process through indicators of parameters of plant growth and yield. Increased in the number of tillers, dry weight of biomass were observed compared with the control. There has also been an increase in the number and the fresh weight of tubers treated with biological agents, especially *Pseudomonas* sp. TWB02 and *Pseudomonas* sp. LP03 in the formulation of husk charcoal powder. Husk charcoal powder is a type of organic material derived from the combustion of rice husk that was processed imperfectly (burning with limited oxygen). There were no much research results exposed the benefits of using this husk charcoal powder in agricultural practices. So far, what had been studied is the potential of husk charcoal as a planting material because it has a structure that can maintain aeration balance, the crumbly structure so that air, water, and roots easily enter the soil fraction and bind the water (Irawan and Kafiar, 2015; Kolo and Tri, 2016).

In relation with the potential of various organic materials to be used optimally, it is inseparable from the comparative advantage possessed by these materials. It is well known that the main content of husk charcoal powder is activated carbon and silica. The silica content and carbon in the husk charcoal powder can reach 52 and 31%, respectively. Besides that, it also contains

Fe_2O_3, K_2O, MgO, CaO, MnO and Cu in small amounts (Septiani, 2012). The composition of chaff charcoal macronutrients are N, P_2O, and K_2O of 50.3, 15, and 31%, respectively, with a pH of 6.8 (Fahmi, 2013). The nutrient content of the husk charcoal powder, which is quite complex and the pH close to neutral, thought to be one of the reasons of the ability of rhizobacteria to maintain its viability during storage.

CONCLUSION

This study concluded that *Pseudomonas* sp. TWB02 and *Pseudomonas* sp. LP03 were able to display better performance in increasing the growth and yield of shallots. Both of these biological agents were more compatible using the ground burned rice-husk powder formulation. The increased shallot yield (tuber fresh weight) in seed treatment using *Pseudomonas* sp. TWB02 and *Pseudomonas* sp. LP03 in the formulation of ground burned rice-husk powder reached 121 and 117%, respectively.

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