Production of soybean in the application of *Rhizobium* spp. and *Actinomycetes* spp.

D U Zainuddin¹, E Syam’un² and A Dachlan²

¹Agribusiness Study Program, Faculty of Agriculture and Forestry, Universitas Sulawesi Barat, Majene, Indonesia
²Department of Agrotechnology, Faculty of Agriculture, Universitas Hasanuddin, Makassar, Indonesia

Email: dianutamiz@unsulbar.ac.id

Abstract. This study aims at determining the effect of bacteria *Rhizobium* spp. and *Actinomycetes* spp. on the production of soybean crops. The research was conducted at the Laboratory of the Department of Agriculture Cultivation of Hasanuddin University and in Tarowang Village, South Galesong District, Takalar District, South Sulawesi, from July to November 2017. The research was conducted in the form of 2 factor factorial experiment arranged in Split Plot Design (SPT). The first factor is the bacteria *Rhizobium* spp. consists of no *Rhizobium* spp. and *Rhizobium* spp. with a density of $1 \times 10^6$ CFU mL$^{-1}$, and the second factor is *Actinomycetes* spp., consists of no *Actinomycetes* spp., *Actinomycetes* spp. with density of $1 \times 10^3$ CFU mL$^{-1}$ and $1 \times 10^6$ CFU mL$^{-1}$. The results showed that interactions of *Rhizobium* spp. with a density of $1 \times 10^6$ CFU mL$^{-1}$ and *Actinomycetes* spp. with a density of $1 \times 10^3$ CFU mL$^{-1}$ significantly affected the number of root nodules per plant and the number of effective root nodules, but Interactions of *Rhizobium* spp. with a density of $1 \times 10^6$ CFU mL$^{-1}$ and *Actinomycetes* spp. with a density of $1 \times 10^6$ CFU mL$^{-1}$ no significant increased weight of 100 seeds.. *Rhizobium* spp. with a density of $1 \times 10^6$ CFU mL$^{-1}$ significantly increased the number of pods per plant. *Actinomycetes* spp. with a density of $1 \times 10^6$ CFU mL$^{-1}$ significantly increased seed weight per plot and seed weight per hectare.

1. Introduction

Soybean (*Glycine max* (L.) Merril) is one of the strategic food crops besides rice and corn. Every year the demand for soybeans continues to increase so that the government imports to meet the needs. Some efforts are conducted to increase the production of soybeans. One of the initiatives is proper fertilization (intensification). Initially, the provision of inorganic fertilizers increases the productivity of plants. However, in the long term, it has a negative impact on the reduction of soil fertility and it can damage the environment [1].

According to [2] effort to increase soybean production which can reduce farmers' dependence on inorganic fertilizers is by encouraging the development of eco-friendly, cheap and sustainable ways of cultivation. One of them is utilizing several types of potential microbes as a source of nutrients (organic fertilizer).

Biological fertilizer is a fertilizer that contains beneficial microbes to improve soil fertility and plant quality. It is through an increased biological activity which can improve the physical and...
chemical properties of the soil. Microbes that are often used as organic fertilizers include bacteria nitrogen-fixing, bacteria solvent phosphate, and bacteria-producing fitohormon [3].

*Rhizobium* spp. is a group of nitrogen-promoting bacteria that live symbiotically in plants from the Leguminosae family by forming a root nodule. This root is an organ that is active in nitrogen fixation from the air [4]. *Actinomycetes* spp. is an endophytic microbe that has many functions for plants. It is a biocontrol against pathogenic microbes and produces fitohormon that can increase plant growth [5].

The study examined the role of *Rhizobium* spp. with *Actinomycetes* spp. in the field of agriculture especially in soybean is still very rare. Most studies tend to focus on the role of health. Data on the effect of *Rhizobium* spp. with *Actinomycetes* spp. on soybean crop production is very less. Based on the above description this study was conducted in order to determine the effect of *Rhizobium* spp. and *Actinomycetes* spp. as well as the interaction of these two bacteria against soybean crop production.

2. Materials and Methods
The research was conducted at Bio-Science and Biotechnology Plant Reproduction Laboratory, Department of Agriculture Cultivation of Hasanuddin University and in Tarowang Village, South Galesong District, Takalar District, and South Sulawesi, which started from July to November 2017.

The research was conducted in the form of two-factor factorial experiment arranged in the Split Plot Design (SPT). The main plot or first factor is the bacteria *Rhizobium* spp. (R) consists of no *Rhizobium* spp. (r0) and *Rhizobium* spp. with a density of 1x10^6 CFU mL^-1 (r1). The subplot or the second factor is the *Actinomycetes* spp. (A) consists of no *Actinomycetes* spp. (a0), *Actinomycetes* spp. with a density of 1x10^3 CFU mL^-1 (a1), and *Actinomycetes* spp. with a density of 1x10^6 CFU mL^-1 (a2).

The density of *Rhizobium* spp. and *Actinomycetes* spp. is determined by turbidity measurement method using spectrophotometer. Then, 145 g of soybean seed was moistened with 5 mL of *Actinomycetes* spp suspension. for 30 minutes. Soybean seeds that have a combination of *Rhizobium* spp treatment, with a density of 1x10^6 CFU mL^-1 is coated using husk ash and mixed with 20 mL of *Rhizobium* spp. bacterial suspension. Furthermore, seeds are planted in a plot measuring 3 meters x 4 meters in which the space is 40 cm x 20 cm. Harvesting is conducted manually by cutting the base of stems of soybean plants using scissors or sickles. The criteria for harvesting are the leaves have fallen, and the color of soybean pods is yellow.

Research data were obtained through field observations conducted in each experimental plot. The parameters which are measured include the number of root nodules per plant, number of effective root nodules, number of pods per plant, 100 seed weight, seed weight per plot, and weight of seed per hectare.

This analysis aims at knowing the difference of response of each treatment. The analysis of variance for the observed data will be conducted with F test at 5% level. If the treatment showed a real effect, smallest real difference will be continued at the level of 5%.

3. Results and discussion
Recapitulation of analysis of variance showed that the application of bacteria *Rhizobium* spp. on pod number parameter per plant significantly increased. In addition, the treatment of *Actinomycetes* spp. significantly increased on the parameter of seed weight per plot and seed weight per hectare. The interaction of bacteria *Rhizobium* spp. and *Actinomycetes* spp. significantly affected on the parameters of the number of root nodules per plant age 7 MST and the number of active root nodules aged 7 MST. However, there is no significant affected on the weight of 100 seeds.

In table 1 and table 2, it can be seen that the treatment of *Rhizobium* spp. with a density of 1x10^6 CFU mL^-1 and *Actinomycetes* spp. with a density of 1x10^3 CFU mL^-1 at age 7 MST produced the highest number of root nodules per plant (22.23 nodules) and the highest number of root nodules (3.65 nodules). It occurs due to the interaction between the bacteria of *Rhizobium* spp. and the *Actinomycetes* spp. on soybean crops in which the bacteria of *Rhizobium* spp. can increased the
formation of root nodules and *Actinomycetes* spp. can control plant diseases. Therefore, the rooting systems are free of pathogens that make root nodules more effective in fixing nitrogen in the air.

**Table 1. Number of root nodules per soybean plant**

| Actinomycetes spp. (A) | 7 MST | Rhizobium spp. (R) | Average | Np BNT α<sub>0.05</sub> |
|------------------------|-------|-------------------|---------|--------------------------|
| Without (a0) Density x10<sup>3</sup> CFU mL<sup>-1</sup> (a1) | 17.00<sub>x,a</sub> | 11.67<sub>x,b</sub> | 14.33 | 2.31 |
| Density x10<sup>6</sup> CFU mL<sup>-1</sup> (a2) | 15.33<sub>x,ab</sub> | 22.33<sub>y,b</sub> | 18.83 | |
| Average | 15.50 | 15.67 | | |
| Np BNT<sub>α<sub>0.05</sub></sub> | 6.37 |

Description: The numbers followed by unequal letters in rows (a, b) and columns (x, y) mean significant differences (P<0.05)

**Table 2. Number of effective root nodules**

| Actinomycetes spp. (A) | 7 MST | Rhizobium spp. (R) | Average | Np BNT α<sub>0.05</sub> |
|------------------------|-------|-------------------|---------|--------------------------|
| Without (a0) Density 1x10<sup>3</sup> CFU mL<sup>-1</sup> (a1) | 11.33<sub>x,a</sub> | 7.17<sub>y,a</sub> | 9.25 | 2.65 |
| Density 10<sup>6</sup> CFU mL<sup>-1</sup> (a2) | 9.00<sub>x,a</sub> | 13.50<sub>y,b</sub> | 11.25 | |
| Average | 9.83 | 9.78 | | |
| Np BNT<sub>α<sub>0.05</sub></sub> | 2.55 |

Description: The numbers followed by unequal letters in rows (a, b) and columns (x, y) mean significant differences (P<0.05)

According to [6], the interaction between soybean plants (Leguminosae) and the bacteria of *Rhizobium* spp. will form a new organ called a root nodule that can inhibit nitrogen from the atmosphere to be used by soybean plants. With the interaction between soybean roots and *Rhizobium* spp., the roots will be crooked then the bacteria of *Rhizobium* spp. remodel the soil root cell walls of the soybean plant so that there is a contact.

The infection thread is formed due to the progression of the plasma membrane extending from the infected cell. After that *Rhizobium* spp. develops within the thread of infection that radiates through the cells of the cortex to the parenchyma. Inside the cortical cells, *Rhizobium* spp. is released in the cytoplasm to form a bacterium and produce stimulants that stimulate cortical cells to divide. This division leads to the proliferation of tissue that forms the structure of the root nodule protruding to the exit of plant roots containing *Rhizobium* spp. bacteria.

*Actinomycetes* spp. acts as a biological agent with mechanisms for disease control, so that *Actinomycetes* spp. can protect the root zone by inhibiting the growth of pathogenic microbes by producing siderophores. Siderofors are chemicals substance which is produced outside cells that can bind Fe<sup>3+</sup>. The mechanism of siderophores occurs through the rapid development of *Actinomycetes* spp. which colonizes plant roots and removes the iron in the surface area and the creation of suitable conditions of root growth and is not suitable for the growth of pathogenic microbes [7].
Table 3. Weight of 100 seeds of soybean (g)

| Actinomycetes spp. (A) | Rhizobium spp. (R) |     |     |     |
|------------------------|---------------------|-----|-----|-----|
|                        | Without (r0)        | r1  |     |     |
| Without (a0)           | 15.17               |     |     |     |
| Density 1x10^3 CFU mL^-1 (a1) | 15.80            |     |     |     |
| Density 1x10^6 CFU mL^-1 (a2) | 15.45            |     |     |     |
| Average                | 15.48               | 15.46| 15.46| 15.84|

Description: The numbers not followed by letters on rows and columns mean no real difference.

In table 3, it can be seen that in the treatment of *Rhizobium* spp. With a density 1x10^6 CFU mL^-1 and *Actinomycetes* spp. with a density of 1x10^6 CFU mL^-1 (r1a2) has the highest weight of 100 seeds 16.23 g. However, it no significantly affected the treatment of *Rhizobium* spp. with *Actinomycetes* spp. It occurs because there are no different characters determined by genetic factors, so it is still influenced by environmental factors in the form of high rainfall in the generative phase. According to [8], the response to plants is determined by various factors, including genetic characteristics of plants, climate, and soil, in which the factors are not stand alone but one factor is related to other factors.

Table 4. Number of pods per Soybean Crop (Pod)

| Actinomycetes spp. (A) | Rhizobium spp. (R) |     |     |     |
|------------------------|---------------------|-----|-----|-----|
|                        | Without (r0)        | r1  |     |     |
| Without (a0)           | 24.30               |     |     |     |
| Density 10^3 CFU mL^-1 (a1) | 25.87            |     |     |     |
| Density 10^6 CFU mL^-1 (a2) | 27.87            |     |     |     |
| Average                | 26.01_x            | 30.65_y |     |     |

Np BNT α_{0.05} 3.13

Description: The numbers followed by unequal letters columns (x, y) mean significant differences (P<0.05)

In table 4, it can be seen that the bacterial factor of *Rhizobium* spp. shows a significant effect on the treatment of *Rhizobium* spp. with a density of 10^6 CFU mL^-1 and it has the highest number of pods per plant of 30.65 pods, while the lowest 26.01 pods was no *Rhizobium* spp treatment.

The number of pods per plant on *Rhizobium* spp. is more than without *Rhizobium* spp because there is increasing nitrogen tethering of the air. Increased availability of nitrogen in the soil can stimulate photosynthetic activity [9].

The result of photosynthesis will be converted into energy used for generative growth that is forming of soybean pod [10]. The same results are also obtained the research conducted by [11]. They found that the application of *Rhizobium* spp. is able to increase the number of plant pods. Although the process of pod formation in plants starts from plant growth, the productive branches of plants ultimately determine the number of pods that are formed.
Table 5. Seed weight per plot soybean plant (g)

| Actinomycetes spp. (A) | Rhizobium spp. (R) | Average Np BNT \( \alpha_{0.05} \) |
|------------------------|--------------------|--------------------------|
|                        | Without (r0)       | Density 10^6 CFU mL^-1   |                          |
| Without (a0)           | 276.93             | 353.33                   | 315.13_a 45.12          |
| Density 10^3 CFU mL^-1 | (a1)               | 258.07                   | 315.77_a 286.92_a       |
| Density 10^6 CFU mL^-1 | (a2)               | 363.50                   | 366.30_b 364.90_b       |
| Average                | 299.50             | 345.13                   |                          |

Description: The number followed by unequal letters in rows (a, b) mean significant differences (\( P<0.05 \))

Table 6. Seed weight per hectare soybean plant (ton)

| Actinomycetes spp. (A) | Rhizobium spp. (R) | Average Np BNT \( \alpha_{0.05} \) |
|------------------------|--------------------|--------------------------|
|                        | Without (r0)       | Density 10^6 CFU mL^-1   |                          |
| Without (a0)           | 1.15               | 1.47                     | 1.31_a 0.18             |
| Density 10^3 CFU mL^-1 | (a1)               | 1.08                     | 1.32_a 1.20_b           |
| Density 10^6 CFU mL^-1 | (a2)               | 1.51                     | 1.53_b 1.52_b           |
| Average                | 1.25               | 1.44                     |                          |

Description: The number followed by unequal letters in rows (a, b) mean significant differences (\( P<0.05 \))

From table 5 and table 6, it can be seen that factor of Actinomycetes spp., showed a difference significantly in seed weight per plot and seed weight per hectare. Treatment between Actinomycetes spp., with a density 1x10^6 CFU mL^-1 (a2) produced the highest seed weight per hectare (364.90 gram) and weight per hectare (1.52 tons).

The increase in seed weight is due to Actinomycetes spp., which acts as a decomposer that is capable of decomposing organic material so that it can be absorbed by plants. According to [12], organic materials contain bound nutrients that cannot be absorbed by plants, so that it needs to be decomposed by decomposition process by soil microbes such as Actinomycetes spp. The process of decomposition consists of several processes starting from the process of fragmentation, assimilation, catabolism, humidification, and the last is the process of mineralization that will release nutrients such as a phosphate element that can be absorbed by plants [13].

According to [14], the phosphate nutrients play a role in increasing the filling of soybean seeds so that providing adequate phosphate elements which can be absorbed by plants will increase the weight of soybean seeds. The same is also expressed by [15] the growth and ripening of seeds requires more phosphate nutrient.

4. Conclusion

Based on the results obtained, it can be concluded that the interaction between the treatments of Rhizobium spp, in which the density is 1x10^6 CFU mL^-1 and Actinomycetes spp., in which the density of 1x10^3 CFU mL^-1 produces the number of root nodules per plant and the highest effective number of root nodules. In addition, interaction between the treatments of Rhizobium spp., in which the density of 1x10^6 CFU mL^-1 and Actinomycetes spp., in which the density of 1x10^6 CFU mL^-1 produces 100 seeds. The application of Rhizobium spp. bacteria, in which the density of 1x10^6 CFU mL^-1 produces the highest number of pods per plant. The application of Actinomycetes spp., in which the density of 1x10^6 CFU mL^-1 produces the highest seed weight per hectare.
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