

# Effectivity of Biopriming Pre-Planting Seed Based Mixed Indigenous Rhizobakteria to Improve Plant Growth and Yield of Soybean

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## ABSTRACT

Research aims to assess the effectiveness of bio-priming seeds with a mixture of indigenous biological agents on the growth and yield of soybean. Research conducted at the Laboratory of Agronomy, Faculty of Agriculture, Halu Oleo University. Research design using randomized completely block design consisting of eight treatments, namely: Treatment without bio-priming (B<sub>0</sub>), Bio-priming *Bacillus* sp. CKD061 (B<sub>1</sub>), Bio-priming *Pseudomonas fluorescens* PG01 (B<sub>2</sub>), Bio-priming *Serratia* sp. CMN175 (B<sub>3</sub>), Bio-priming *Bacillus* sp. CKD061 + *P. fluorescens* PG01 (B<sub>4</sub>), Bio-priming *Bacillus* sp. CKD061 + *Serratia* sp. CMN175 (B<sub>5</sub>), Bio-priming *P. fluorescens* TBT214 + *Serratia* sp. CMN175 (B<sub>6</sub>), Bio-priming *Bacillus* sp. CKD061 + *P. fluorescens* PG01 + *Serratia* sp. CMN175 (B<sub>7</sub>). Each treatment was repeated three times, so overall there are 24 experimental units. Observations were made of the variables plant height, stem diameter, days to flowering, number of productive branches, number of pods pithy, 1000 grain weight and production. The results showed that the Bio-priming seeds with a mixture of indigenous biological agents capable of increasing the growth and yield of soybean. Bio-priming seeds using a mixture of biological agents B<sub>4</sub> (Bio-priming campuran *Bacillus* sp. CKD061 + *P. fluorescens* PG01) gives better results on the growth and yield of soybean compared to the untreated bio-priming and other treatments.

**KEYWORDS:** *Bacillus* sp. CKD061, Bio-priming, Mixed biological agents, kedelai, *Pseudomonas fluorescens* PG01, *Serratia* sp. CMN175

## INTRODUCTION

Soybean (*Glycine max* L. Merrill) is an important commodity and component of the national food supply. It is not only source a protein, but also a source of minerals, vitamins and fat. Soybean is an industrial raw material for processed products such as tofu, Tempe, and soy sauce that have increased significantly. Soy grains are rich in protein and fat as well as several other important nutrients, such as vitamins (*phytic acid*) and *lecithin* [1]. This will certainly have an impact both on society and nutrition can improve of the body. It will have good impact both on public health and nutrition supply.

The growth of soybeans demand has been very significant for recent years especially for consumption and raw material for industry, in line with the increase in population, so it is necessary to increase soybean production in Indonesia. Although demand for soybeans is increasing every year, but the rate of production of soybeans every year tends to decrease. Soybean demand in Indonesia in 2013 as much as 10.20 kg capita<sup>-1</sup> yr<sup>-1</sup> with the total soybean consumption is reached 2.47 million tons. Mean while, soybean production in 2012 only 843,153 tonnes of dry grain with the harvested area 567.624 ha (productivity of 1.48 tonnes ha<sup>-1</sup>). In 2013,

soybean production reached 779.992 tons and harvested area was reached 550.796 ha (productivity of 1.42 tonnes ha<sup>-1</sup>). The soybean production in 2013 has decreased by 63.161 tonnes (7.49%) if compared to that in 2012. The decline of soybean production may occur due to decrease in lower harvested area 16.828 ha (2.97%) and a decrease in productivity of 0.07 tonnes ha<sup>-1</sup> (4.65%) [2].

An effort has been done to increase soybean production; either through extensification and intensification, but soybean production has not yet reached the planned target. The high of demand is not comparable with the production capacity and to meet the needs of national soybean must be import from other country especially from USA that reached 70% total need soybean in Indonesia. The amount absorbed for food or tempeh craftsmen 83.7%; soy industry, and other tauco 14.7%; seed 1.2% and 0.4% for feed. The decline in soybean production partly due to the use of seeds that are not qualified as the low viability and vigor Scarcity of quality seeds in the market lead to farmers using inferior seed, derived from the crop itself or from fellow farmers. The use of quality seeds is the key to success in cultivation. The use of high vigor seed is expected to produce a plant that can produce at maximum. The low crop production is exacerbated due to the seed-borne pathogens. The yield losses due to seed-borne diseases could reach 60% and even up to 100% [3]. Alternatives are being made to increase soybean production is by seed priming techniques are integrated with rizobakteri known as bio-priming seeds. Bio-priming is a technique to hydrate of seed by using biological compounds or rhizobacteria [4]. Rhizobacteria is a microorganism competitor that can act as plant growth promoters, nutrient cycling, and disease control and at the same time capable of increasing its viability and vigor. The results showed that the use rizobakteri can promote the growth, yield and plant resistance against plant diseases [5,6]. The results showed that the bio-priming seeds with rizobakteri able to increase the growth and yield of pepper plants [7].

The use of biological agents it can promote the growth and yield of pepper plants [8]. The use of biological agents from the class of *Bacillus* sp., *Pseudomonas* sp. and *Serratia* sp. has been done and proven to give better effect in enhancing the growth and yield [8,9,10,11,12]. Further explained that the use rizobakteri as Plant Growth Promoting Rhizobacteria (PGPR), in addition to acting as plant growth promoters, also play a role in improving plant resistance to disease [13].

Based on the background, the technique of bio-priming seeds with a mixture of indigenous biological agents are an alternative bio-priming technology that can be used to improve the growth and yield of soybean. The aim of this research was to examine the effectiveness of bio-priming seeds with a mixture of indigenous biological agents to increase the growth and yield of soybean.

## MATERIAL AND METHOD

### *Time and Place:*

Research conducted at the Laboratory of Agronomy and Agriculture Experimental Farm of Halu Oleo University, starting in November 2014 until January 2015.

### *Land Preparation:*

Land cultivated two times using tractor and followed by plotting the experimental unit of 50m x 3m. Fertilizer given consisting of organic fertilizers and inorganic fertilizers. The organic fertilizer is applied one week before planting at adose of 2 tonnes ha<sup>-1</sup> and inorganic fertilizer is given two weeks after planting with a dose of Urea 50 kg ha<sup>-1</sup>, SP<sub>36</sub> 100 kg ha<sup>-1</sup> and KCl 50 kg ha<sup>-1</sup>.

### *Multiplying of Isolate and Seed Treatment:*

The medium used for the multiplication of bacteria that TSA and TSA King's B. Media TSA made from a mixture to be 20 g and 30 g TSB. King's B media consists of 20 g jelly, protease peptone 20 g, and glycerol 15 ml, 2.5 g K<sub>2</sub>HPO<sub>4</sub>, and MgSO<sub>4</sub>.7H<sub>2</sub>O 6 g. The mixture of materials for the manufacture of TSA media and King's B were dissolved in 1000 ml of distilled water and boiled for ± 20 minutes. A mixture of materials that have been simmering *scott bottle* put into bottles and sterilized using an autoclave (T 121°C, p 1 atm, t 20 minutes).

After that, the mixture is poured into a petri dish 0.5 cm thick aseptically in a laminar air flow cabinet then cooled and ready for use. Isolates of *Bacillus* sp. CKD061 and *Serratia* sp. CMN175 grown on TSA media while *P. fluorescens* PG01 in King's B media dense and incubated for 48 hours. Bacterial colonies growing suspended in sterile distilled water until it reaches a population density of 10<sup>9</sup> cfu / ml. Furthermore soybean seed is inserted into a bacterial suspension in accordance with the treatment. The process of bio-priming seed (seed priming) is done for ± 4 hours. After treatment, seed re-aired dried in a laminar air flow cabinet. In addition to the application on the seed, treatment applications mixture of indigenous biological agent is given on crops in the field as much as 5 ml plant-1 is applied at the time of 3 weeks after planting (WAPt). Application of rhizobacteria in plants is by spraying on the the plants and surface of the soil.

*Experimental Design:*

Research design using randomized complete block design consisting of eight treatments, namely: Treatment without bio-priming (B<sub>0</sub>), Bio-priming *Bacillus* sp. CKD061 (B<sub>1</sub>), Bio-priming *Pseudomonas fluorescens* PG01 (B<sub>2</sub>), Bio-priming *Serratia* sp. CMN175 (B<sub>3</sub>), Bio-priming *Bacillus* sp. CKD061 + *P. fluorescens* PG01 (B<sub>4</sub>), Bio-priming *Bacillus* sp. CKD061 + *Serratia* sp. CMN175 (B<sub>5</sub>), Bio-priming *P. fluorescens* TBT214 + *Serratia* sp. CMN175 (B<sub>6</sub>), Bio-priming *Bacillus* sp. CKD061 + *P. fluorescens* PG01 + *Serratia* sp. CMN175 (B<sub>7</sub>). Each treatment was repeated three times, so overall there are 24 experimental units.

*Observation:*

Observations were made to variable of plant height, stem diameter, days to flowering, and number of productive branches, pods, pods pithy, 1000 grain weight and production (tonnes ha<sup>-1</sup>).

*Data Analyses:*

The data were analyzed using analysis of variance (ANOVA). Results of analysis of variance which shows the significant, followed by a Duncan Multiple Range Test (Duncan Multiple Range Test) on the real level  $\alpha = 0.05$ .

**RESULTS AND DISCUSSION***Plant Height:*

Treatment of seed bioprimer using a mixture of indigenous biological agents has very significant effect on soybean plant height at 15 and 45 days after planting (DAP) and has significant effect on the age of 30 DAP. Result of mean separation with DMRT to the height plant showed in Table 2. Table 1 indicates that the highest average plant height at 15, 30 and 45 DAP found at treatment of B<sub>4</sub> and has different effect if compare to control.

**Table 1:** The effect of bioprimer on the plant height at various day after planting.

Treatment	Soybean Plant Height (cm) on Various Day After Planting (DAP)					
	15		30		45	
B <sub>0</sub>	7.58	c	22.49	c	37.10	c
B <sub>1</sub>	8.38	ab	23.81	bc	41.67	ab
B <sub>2</sub>	8.32	ab	23.69	bc	41.39	b
B <sub>3</sub>	8.13	bc	24.18	bc	41.71	ab
B <sub>4</sub>	8.88	a	27.91	a	46.02	a
B <sub>5</sub>	8.78	ab	26.35	ab	43.61	ab
B <sub>6</sub>	8.79	ab	26.93	ab	43.25	ab
B <sub>7</sub>	8.81	ab	27.18	ab	45.02	ab

Remarks: Number followed by the same index in the same row, are not significantly different at Duncan's multiple Range Test (DMRT). Control (B<sub>0</sub>), *Bacillus* sp. CKD061 (B<sub>1</sub>), *P. fluorescens* PG01 (B<sub>2</sub>), *Serratia* sp. CMN175 (B<sub>3</sub>), *Bacillus* sp. CKD061 + *P. fluorescens* PG01 (B<sub>4</sub>), *Bacillus* sp. CKD061 + *Serratia* sp. CMN175 (B<sub>5</sub>), *P. fluorescens* TBT214 + *Serratia* sp. CMN175 (B<sub>6</sub>) and *Bacillus* sp. CKD061 + *P. fluorescens* PG01 + *Serratia* sp. CMN175 (B<sub>7</sub>)

*Stem Diameter:*

Variance analyses showed that bioprimer seed treatment has significant effect to stem diameter at 15 and 45 DAP, but no significant effect on 30 DAP. Result of mean separation with DMRT to stem diameter showed in Table 2. It indicates that the highest average stem diameter at 15 DAP at treatment of B<sub>4</sub> and has no different effect compare to other treatment. The highest average stem diameter at 30 DAP found at treatment B<sub>7</sub> and no significant different to treatment of B<sub>4</sub>, B<sub>5</sub>, B<sub>6</sub> and B<sub>7</sub>. But has different effect to control. The highest average stem diameter at 45 DAP found at treatment B<sub>4</sub> and has a different effect to the control (B<sub>0</sub>)

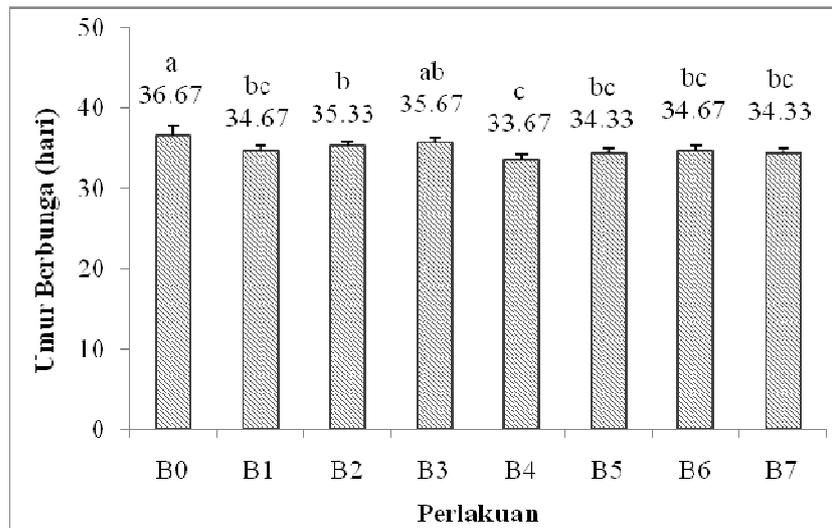
**Table 2:** The effect of bioprimer on average stem diameter at various day after planting

Perlakuan	Average Stem Diameter (cm) on Various Day After Planting (DAP)					
	15		30		45	
B <sub>0</sub>	0.2321	b	0.3940	d	0.4812	b
B <sub>1</sub>	0.2442	ab	0.4704	bc	0.5510	ab
B <sub>2</sub>	0.2467	ab	0.4631	c	0.5495	ab
B <sub>3</sub>	0.2491	a	0.4738	bc	0.5455	ab
B <sub>4</sub>	0.2596	a	0.5107	ab	0.6447	a
B <sub>5</sub>	0.2493	a	0.5045	abc	0.6005	a
B <sub>6</sub>	0.2563	a	0.5129	ab	0.6250	a
B <sub>7</sub>	0.2518	a	0.5197	a	0.6069	a

Remarks: Number followed by the same index in the same row, are not significantly different at Duncan's multiple Range Test (DMRT). Control (B<sub>0</sub>), *Bacillus* sp. CKD061 (B<sub>1</sub>), *P. fluorescens* PG01 (B<sub>2</sub>), *Serratia* sp. CMN175 (B<sub>3</sub>), *Bacillus* sp. CKD061 + *P. fluorescens* PG01 (B<sub>4</sub>), *Bacillus* sp. CKD061 + *Serratia* sp. CMN175 (B<sub>5</sub>), *P. fluorescens* TBT214 + *Serratia* sp. CMN175 (B<sub>6</sub>) and *Bacillus* sp. CKD061 + *P. fluorescens* PG01 + *Serratia* sp. CMN175 (B<sub>7</sub>)

*Time of Flowering:*

Variance analyses showed that biopriming seed treatment has significant effect on the time of flowering of soybean. Result of mean separation with DMRT to time of flowering shown in Figure 1.

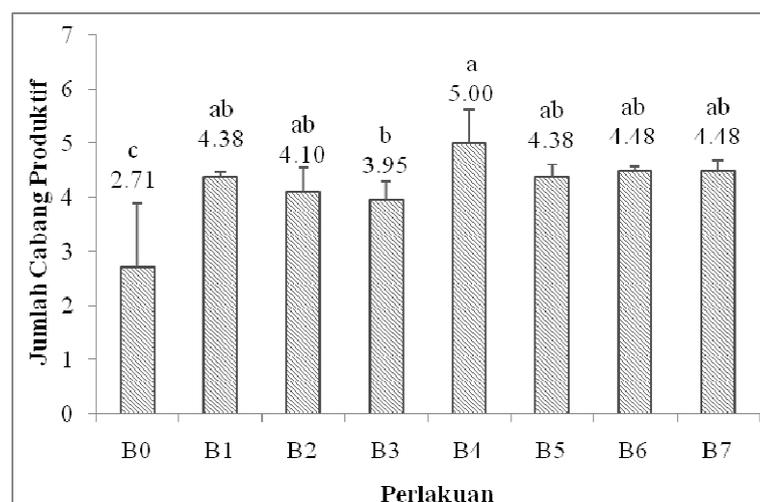


**Fig. 1:** Effect of biopriming on average time of flowering. Number followed by the same index, are not significantly different at Duncan's multiple Range Test (DMRT). Control (B<sub>0</sub>), *Bacillus* sp. CKD061 (B<sub>1</sub>), *P. fluorescens* PG01 (B<sub>2</sub>), *Serratia* sp. CMN175 (B<sub>3</sub>), *Bacillus* sp. CKD061 + *P. fluorescens* PG01 (B<sub>4</sub>), *Bacillus* sp. CKD061 + *Serratia* sp. CMN175 (B<sub>5</sub>), *P. fluorescens* TBT214 + *Serratia* sp. CMN175 (B<sub>6</sub>) and *Bacillus* sp. CKD061 + *P. fluorescens* PG01 + *Serratia* sp. CMN175 (B<sub>7</sub>)

Figure 1 shown that the application of biopriming on seed of soybean has affect on delaying of flowering. Result indicated that the time flowering on treatment B<sub>4</sub> was 37 days. Those 3 days behind if compare to the control that flowering in 34 days (B<sub>0</sub>).

*Number of Productive Stem:*

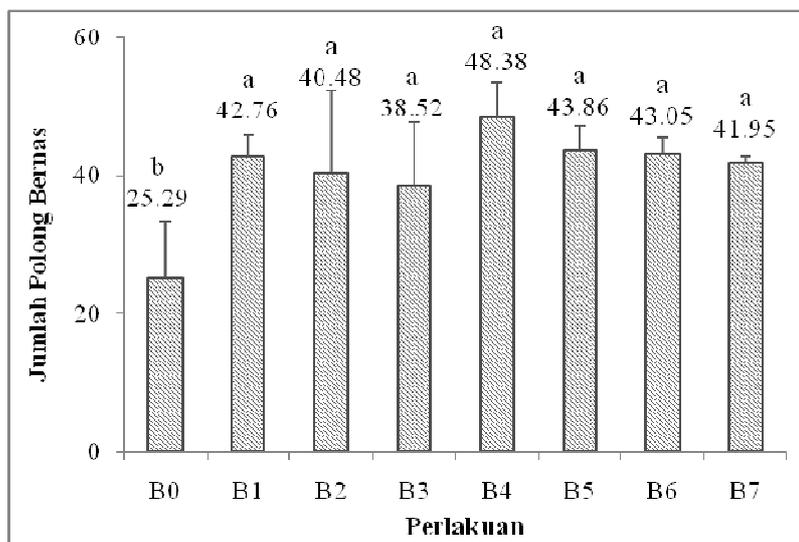
Variance analyses showed that application of biopriming on seed of soybean has significant effect on number of productiv stem. Result of mean separation with DMRT to number of flowering shown in Figure 2. Figure 2 shown that the highest number of productive stem has found in treatment B<sub>4</sub> (5 number of productive stem) and no significant different with other treatment, even has significant different if compared to control.



**Fig. 2:** Effect of biopriming on productive stems. Number followed by the same index, are not significantly different at Duncan's multiple Range Test (DMRT). Control (B<sub>0</sub>), *Bacillus* sp. CKD061 (B<sub>1</sub>), *P. fluorescens* PG01 (B<sub>2</sub>), *Serratia* sp. CMN175 (B<sub>3</sub>), *Bacillus* sp. CKD061 + *P. fluorescens* PG01 (B<sub>4</sub>), *Bacillus* sp. CKD061 + *Serratia* sp. CMN175 (B<sub>5</sub>), *P. fluorescens* TBT214 + *Serratia* sp. CMN175 (B<sub>6</sub>) and *Bacillus* sp. CKD061 + *P. fluorescens* PG01 + *Serratia* sp. CMN175 (B<sub>7</sub>)

### The Number of Productive Pod:

Variance analyses showed that application of biopriming on seed of soybean has very significant effect on number of productive pod. Result of mean separation with DMRT to number of productive bean shown in Figure 3.

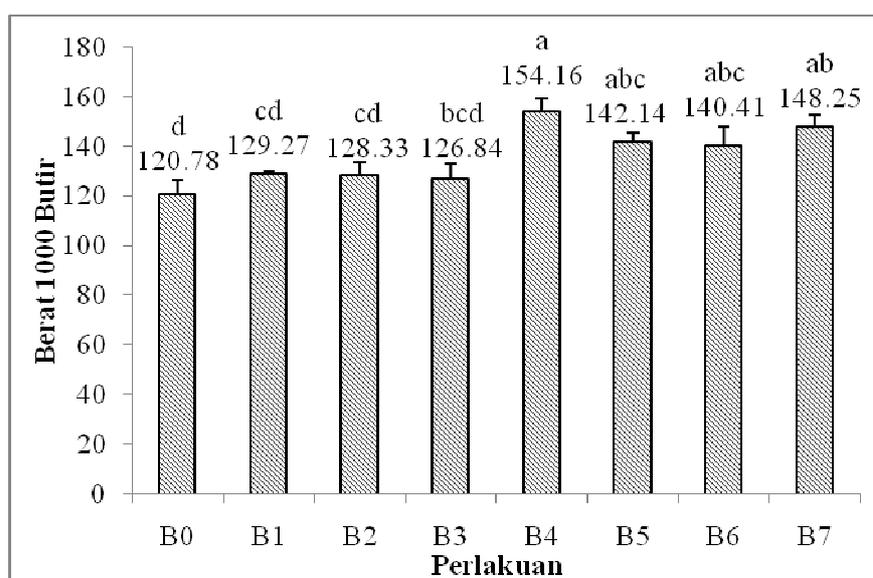


**Fig. 3:** Effect of biopriming on productive pods. Number followed by the same index, are not significantly different at Duncan's multiple Range Test (DMRT). Control (B<sub>0</sub>), *Bacillus* sp. CKD061 (B<sub>1</sub>), *P. fluorescens* PG01 (B<sub>2</sub>), *Serratia* sp. CMN175 (B<sub>3</sub>), *Bacillus* sp. CKD061 + *P. fluorescens* PG01 (B<sub>4</sub>), *Bacillus* sp. CKD061 + *Serratia* sp. CMN175 (B<sub>5</sub>), *P. fluorescens* TBT214 + *Serratia* sp. CMN175 (B<sub>6</sub>) and *Bacillus* sp. CKD061 + *P. fluorescens* PG01 + *Serratia* sp. CMN175 (B<sub>7</sub>)

Figure 3 shown that the highest number of productive pod has founded in treatment of B<sub>4</sub> with 48.48 pods per plant. There is no significant different the treatment of B<sub>4</sub> if compare with other treatmen but has significant different if compare to control.

### Weight of 1000 Grain:

Variance analyses showed that application of biopriming on seed of soybean has very significant effect on weight of grain. Result of mean separation with DMRT to weight of 1000 grain shown in Figure 4.



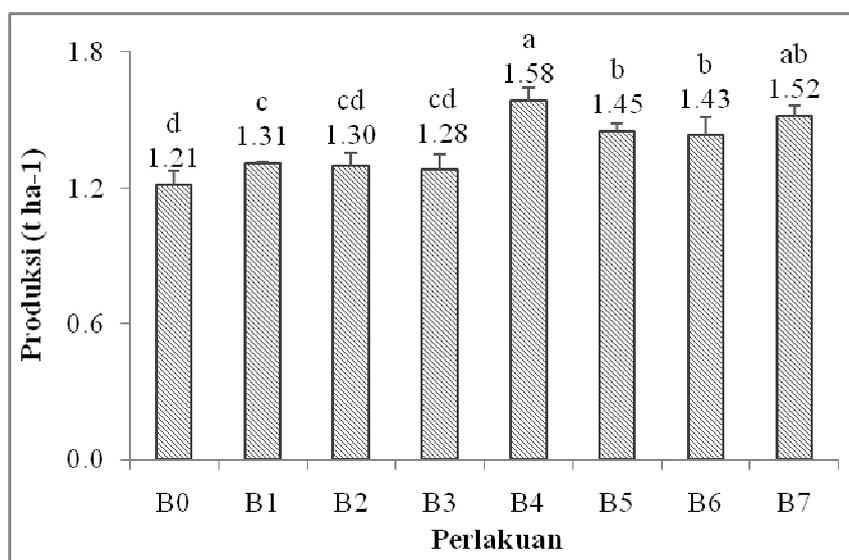
**Fig. 4:** Effect of biopriming on weight of 1000 grains. Number followed by the same index, are not significantly different at Duncan's multiple Range Test (DMRT). Control (B<sub>0</sub>), *Bacillus* sp. CKD061 (B<sub>1</sub>), *P. fluorescens* PG01 (B<sub>2</sub>), *Serratia* sp. CMN175 (B<sub>3</sub>), *Bacillus* sp. CKD061 + *P. fluorescens* PG01 (B<sub>4</sub>), *Bacillus* sp. CKD061 + *Serratia* sp. CMN175 (B<sub>5</sub>), *P. fluorescens* TBT214 + *Serratia* sp. CMN175 (B<sub>6</sub>) and *Bacillus* sp. CKD061 + *P. fluorescens* PG01 + *Serratia* sp. CMN175 (B<sub>7</sub>)

Figure 4 shown that the highest weight of 1000 grain has founded in the treatment of B4. The weight of 1000 grain on treatment B4 is 154.16 gram. Treatment of B4 has no significant different compare to other treatment but has different significant compare to control, with the weight of 154.16 for 1000 grain of soybean. Number of weightproductive pod has found in treatment B4 with 48.48 pod per plant. There is no significant different the treatment of B4 if compare with other treatment but has significant different if compare to control. Figure 4 indicate that the application of biopriming can increase the weight of 1000 grain or in other has effect on the increase of weigh and size of grain.

#### Estimated Production ( $\text{ton ha}^{-1}$ ):

Variance analyses showed that application of biopriming on seed of soybean has very significant effect on the yield of soybean. Result of mean separation with DMRT to yield shown in Figure 5.

Figure 5 shown that the application of biopriming on B4 treatment give the highest production and reached  $1.58 \text{ ton ha}^{-1}$ . There is no significant different the production on treatment B4 compare to the treatment B7 and get reached of  $1.52 \text{ ton ha}^{-1}$ , but has significant different if compare to control that only get production  $1.21 \text{ ton ha}^{-1}$ . Figure 5 also indicated that the application of biopriming affect on the increase of soybean production.



**Fig. 5:** Effect of biopriming on estimated soybean production. Number followed by the same index, are not significantly different at Duncan's multiple Range Test (DMRT). Control (B<sub>0</sub>), *Bacillus* sp. CKD061 (B<sub>1</sub>), *P. fluorescens* PG01 (B<sub>2</sub>), *Serratia* sp. CMN175 (B<sub>3</sub>), *Bacillus* sp. CKD061 + *P. fluorescens* PG01 (B<sub>4</sub>), *Bacillus* sp. CKD061 + *Serratia* sp. CMN175 (B<sub>5</sub>), *P. fluorescens* TBT214 + *Serratia* sp. CMN175 (B<sub>6</sub>) and *Bacillus* sp. CKD061 + *P. fluorescens* PG01 + *Serratia* sp. CMN175 (B<sub>7</sub>)

#### Discussion:

Result showed that application of biopriming on seed has increase of growth and production soybean. It also indicated that the application of biopriming has given double effect on growth and production of soybean. Results of this study are consistent with previous result that the use rhizobacteria either single or double able to increase soybean seed physiological quality. The consortium rhizobacteria endofitic bacteria, *Azotobacter*, *Azospirillum* and bacteri posfat solubile has ability to increase growth and production of rice [14].

Application of seed biopriming with the combination of indigenous rhizobacteria (*bio-priming* combine with *Bacillus* sp. CKD061 + *P. fluorescens* PG01), has ability to increase growth and production of soybean. Sundaramoorthy *et al.* (2013), reported that PGPR consortium (*P. fluorescens* Pf1, *Pseudomonas* spp. TDK1, *Pseudomonas* spp. TV5 and *Bacillus* spp. TH10) has ability to increase of growth and production of rice, if compare to single formulation of rhizobacteria.

The application of rhizobacteria on seed, followed by application of rhizobacteria on root 2-4 week after, can increase the production of tomato in ultisol soil [16]. Further [17] reported also that application of rhizobacterial formulation (*B. subtilis* ST21b, *B. cereus* ST21e and *Serratia* strain SS29a) on seed has effective to control rhizotocnia, increases of plant height and number of leaves respectively 119% and 170% and also increase the weight of soybean grain on ultisol soil reached 187% compare to control.

Improved growth and yield of soybean may due to the ability of rhizobacteria to made colonise on soybean rhizosphere that began when the seeds germinate. It can indirectly lead to a mutually beneficial relationship between rhizobacteria and soybeans. The results of this study are consistent with results from [18], that dual-

inoculation had a positive effect on the growth increasing of mung bean grown under natural conditions. It is caused that the dual inoculation PGPR has effective for improving root growth and shoot growth better. The consortium of rhizobacteria with combination endophytic bacteria [14], Azotobacter, Azospirillum and bacterial phosphate solubilization has effect on the increase of growth and rice production. The treatment rhizobacteri using mixing of *B. polymixa* BG25 and *P. fluorescence* PG01 enabled to increase number of fruit, germination rate, vigor index and reduce the disease appearance on chili seeds [9].

Plants produce organic compounds such as root exudates that can be utilized by rizobakteri as a source of nutrients, so that the rizobakteri can provide carrying capacity to stimulate plant growth. It is also caused by the ability of the soybean crop in association with *Rhizobium* to fixing nitrogen and increasing plant growth when compared to controls. This is in line with the results of [19], that the combination of inoculation *Sinorhizobium meliloti* and PGPR (*P. putida*) were able to increase the number of nodules and biomass root nodules per plant on alfalfa at 60 and 120 days after inoculation of *S. meliloti* in when compared to the control treatment.

Giving the species *Rhizobium* and inoculum dose able to increase plant biomass, the weight of nodules per plant and dry weight of grains per plant compared to control [20]). *Rhizobium* bacteria have ability to fixation nitrogen simbioticly. Nitrogen is the most important element in the growth of plants because nitrogen is one of the essential nutrients. In plant tissues, nitrogen is an essential nutrient and building blocks of amino acids, proteins and enzymes [21]. In addition, nitrogen is also contained in chlorophyll, hormone cytokinin and auxin, so as to increase growth and yield.

Increasing plant growth due to the biopriming seed treatment with a mixture of indigenous biological agents indirectly affect on crop yield. The study of the variable component to crop flowering time observations indicate that treatment of bio-priming seeds with a mixture of indigenous biological agents able to accelerate the time of flowering soybean plants from two to three days sooner compare with controls (Figure 1). R also showed that the increase of growth spurred the formation of a branch of the plant. Indirectly this may increase the number of branches of plants which resulted in increased number of pods can be produced at each plant (Figure 2 and Figure 3). Increasing the number of productive branches thought to be caused by the ability of plants to optimize the photosintate.

The results also showed that the treatment of bio-priming seeds with a mixture of biological agents indigenous was also able to increase the weight of 1000 grains (Figure 4) which in turn will impact on increased production of soybeans (Figure 5). This is may caused the ability of rizobakteri as biofertilizer. The results also consistent with the results [22], which states that the bacterial consortium applications can significantly increase grain weight per panicle and yield of rice paddy fields to reach 8.7%- 12.2% compared with no bacterial consortium in the SRI system. [23] reported that the strain *Pseudomonas* sp. either singly or mixed significantly increased the percentage of seeds that live in the greenhouse and field. However, a mixture of strains of *Pseudomonas* sp. far is more effective than either alone. In addition, the mixture is more effective in improving results. Furthermore, [24] reported that the inoculation of *Rhizobium* and PGPR singly or in combination and both were able to increase the weight of the grain respectively 14% and 30%, compared with non-inoculated (control). Biopriming on sunflower seeds using *P. fluorescens* UTPf76 and UTPf86 can significantly increase the stem length, root length and seedling dry weight compared to other treatments and control [25].

#### Conclusion:

A Biopriming seeds with a mixture of indigenous biological agents has capable to increase growth and yield of soybean. Bio-priming seeds with a mixture of biological agents B4 (bio-priming mixture of *Bacillus* sp. CKD061 + *P. fluorescens* PG01) gives better results on the growth and yield of soybean compared to control and other treatments.

Need further research by using bio-priming mixture of *Bacillus* sp. CKD061 + *P. fluorescens* PG01 to increase quality of soybeans crop in the field on a wide scale.

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