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IMPACT OF BIOFERTILIZERS AND CHEMICAL FERTILIZERS ON NODULATION, N UPTAKE AND GROWTH OF SOYBEAN (*Glycine max* L.)

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ABSTRACT

Soybean is one of the important food crops as a source of protein. Nowadays, soybean production should be increased due to higher demand in certain region in Indonesia. Biofertilizer inoculation combine with chemical fertilizer is suggested to increase the soil fertility to support soybean cultivation and decrease the use of chemical fertilizer. The pot experiment was conducted to get the information concerning nodulation, nitrogen uptake and growth of soybeans (*Glycine max* L.) after biofertilizer and Nitrogen (N), Phosphorous (P) as well as potassium (K) single fertilizer application. The experiment was set up in a randomized block design with seven treatment and four replications. The treatments consisted of two doses of biofertilizer (3 L ha^{-1} and 5 L ha^{-1}) combined with three doses of N, P, K fertilizer (50%, 75% and 100% recommended dosage). The pot control received no biofertilizer. Consortium biofertilizers contained N-fixing bacteria (*Azotobacter chroococcum, A. vinelandii, Azospirillum* sp. and endophytic *Acinetobacter* sp.) and phosphate solubilizing microbes *(Pseudomonas cepaceae* and *Penicillium* sp.). The results of experiment showed that the application of biofertilizer 5 L ha^{-1} combined with 75% chemical fertilizer increased the nodules number, nitrogen uptake and dry weight of plant at the end of vegetative stage. This study suggested that biofertilizer might be used to increase growth of soybean and chemical fertilizer efficiency.

Keywords: Biofertilizer, Inceptisols, N-uptake, Nodule, Soybean (*Glycine max* L.).

INTRODUCTION

Soybean is known as a plant that requires large amounts of nitrogen. If these needs by inorganic N fertilizer, it requires a large cost and long-term use has a negative impact on the environment.

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Soybean plants to get N through symbiosis with N-fixing bacteria called rhizobia. Therefore it can be overcome with the application of biological fertilizers that contain bacteria that can fix N_2 from the air (Htwe et al., 2019). Biofertilizers are inoculants made from active living organisms in liquid or solid forms that have the ability to mobilize, facilitate and increase the availability of nutrients not available into available forms through biological processes.

One way to increase soybean production is to improve the root area. Rooting areas are important to note because roots have a major role in transporting water and nutrients to the leaves which are related to plant survival. In addition, good root development will also support the process of nitrogenase, absorption of other nutrients and adaptation and acclimatization of plants more quickly (Kleinert et al. 2018). Soybean roots will symbiosis with Rhizobium bacteria to form root nodules. Nodule development in legumes directly affects nitrogen fixation. The effects of exogenous factors affecting nodulation in soybeans as well as in other larger legumes (Choudhury et al., 2019). N-fixing bacteria increased significantly plant growth, nodulation, nitrogen fixation, NPK uptake, and yield of mung beans and soybeans (Htwe et al., 2019). Phosphate solubilizing bacteria increase soil P-available due to organic acids and phosphatases produced by PSB that can release fixed P and P mineralization (Kalayu, 2019).

The use of a biofertilizer can help the growth of soybean plants and increase crop yields because it can fixed free nitrogen from the air, help provide phosphate for plants (Fitriatin et al. 2014) and can produce growth-stimulating hormones such as IAA, cytokinins, gibberellins, auxins (Olanrewaju et al., 2017) and exopolysaccharide (Hindersah et al., 2017). The added consortium of biofertilizers containing phosphate solubilizing bacteria (PSB) such as Pseudomonas cepaceae; phosphate solubiizing fungi (PSF) such as Penicillium sp; and nitrogen-fixing bacteria such as Azospirillum, Azotobacter vinelandii, and Azotobacter chroococcum; and endopytic bacteria (Acinetobacter). These microbes are used for the consortium of biofertilizers because it can provide benefits to plants. Some research results showed that consortium isolates are better than single isolates. According to Olumwambe and Kofoworola (2016) that tomato seed treated with the consortium of several effective strains for growth enhancement performed better than their individual culture.

Utilization of the consortium of biofertilizers on soybean is expected to improve plant growth and productivity and reduce chemical residues caused by inorganic fertilization. Therefore, research is needed the application of biofertilizers to find out treatment gives the best response to nodulation, nitrogen uptake and growth of soybean.

MATERIALS AND METHODS

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The pot experiment was conducted in field station, Faculty of Agriculture, Universitas Padjadjaran, West Java, Indonesia. The experimental site was located in the tropics at 756 m above the sea lavel. The soybean cv Anjasmoro were sown in soil of Inceptisols order collected from Jatinangor District, West Java, Indonesia (clay texture; pH 5,58; C-org 1,89%; N 0,24%; P_2O_5 Bray 30,25 ppm; CEC 20,76 cmol.kg^{-1}). Consortium biofertilizer which contained N-fixing bacteria *Azotobacter chroococcum, A. vinelandii,Azospirillum* sp, and *Acinetobacter* sp., as well as Phosphate solubilizing microbes *Pseudomonas cepaceae* and *Penicillium* sp. has been developed by Laboratory of Soil Biology in said Institution. The density of bacteria and fungi in liquid biofertilizers were 10^7 CFU mL^{-1} and 10^5 CFU mL^{-1} respectively. Urea, TSP and KCl single Chemical fertilizers were produced by national fertilizer industry. The recommended dosage of said fertilizer for soybean cultivation in Indonesia were Urea (46% N) 50 kg ha^{-1}, triple super phosphate (36% P_2O_5) 100 kg ha^{-1}, KCl (60% K_2O)100 kg ha^{-1} and cow manure 2 t ha^{-1}. The compost of cow manure was prepared by Faculty of Husbandry Universitas Padjadjaran; manure was neutral in acidity 7.50 , N 0.94%, P_2O_5 0,37% and K_2O 0.29% and contained 25.38% water.

The experiment arranged in a randomized block design with seven combinations and four replications. The treatments consisted of two doses of biofertilizer/BF (0, 3 L ha^{-1} and 5 L ha^{-1}), combined with three recommended doses of chemical fertilizer/CF (50%, 75% and 100%). All the treatments were replicated three times. N uptake, nodulation and growth of Soybean (plant height, shoot and root dry weight) were measured at the end of vegetative period.

Soil was collected from top soil prior to separating plant debris from the bulk soil. A total of 15 kg soil was placed in polybag, mixed with 15 g organic matter (equal to 2 t ha^{-1}) and incubated for 5 days in field without shade.

Soybean seeds were inoculated with liquid *Bradyrhizobium* inoculant mixed with gum arabic; two soybean seeds were placed in seperated hole, and covered with soil. Biofertilizer was diluted 5% by using ground water and inoculated 20 mL per polybag by pouring around 5-days old transplant. Chemical fertilizers were applied twice at 7 days and 14 days after planting. The experimental units were maintain for 42 days after planting when the plants were at the end of vegetative period.

At the end of experiment, shoots were separated from roots and heated in the oven of 70^{o} C prior to nitrogen content analysis and dry weight measurement. Roots were washing gently using tap water; all the nodule were collected and dried using filter paper and weighed. Nitrogen uptake were calculted based on Nitrogen content which determined by Kjeldahl Method. All the data were subjected to variance analysis using F test (p 0.05%). If the effect of treatment on said plant parameter was significant then Duncan's Multiple Range Test (p 0.05%) was conducted.

RESULTS AND DISCUSSIONS

Nodule number

The effect of biofertilizers and chemical fertilizers increase nodule number. Figure 1 shows that application of 5 L ha^{-1} biofertilizers + 75% dosage of chemical fertilizers are better treatment to increase the nodule number. This is supported that *Azotobacter* in the biofertilizers able to produce exopolysaccharides needed for *Rhizobium* sp. in inducing the formation of nodules (Gauri et al. 2012). This exopolysaccharide is used as a signal by the bacterium *Rhizobium* so that it can stick to the ends of the roots of soybean hair and then infect the soybean plant root cells (Ibanez et al. 2017).

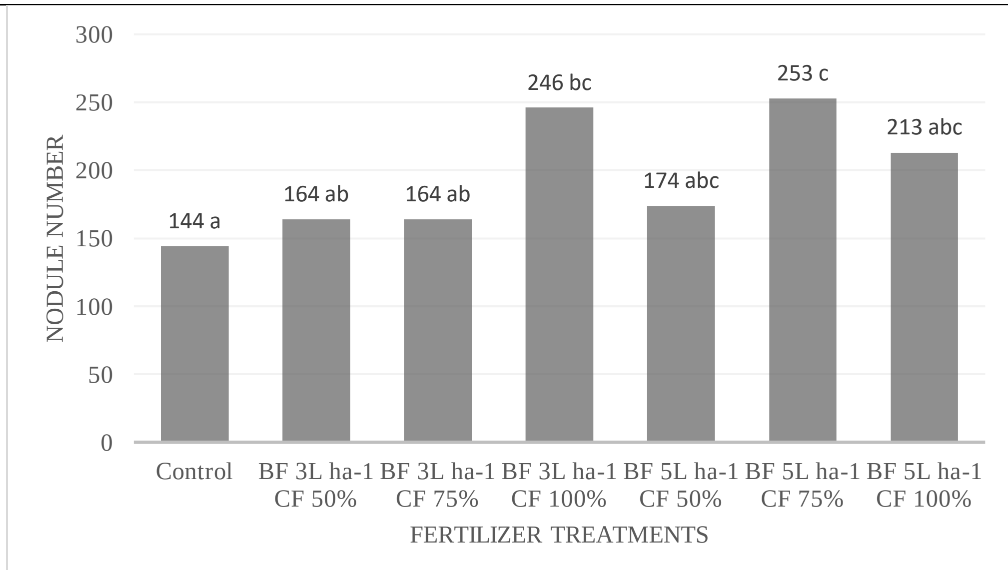

Fig 1. Nodule number of soybean grown with different biofertilizer and chemical fertilizer treatments at the end of vegetative period; BF biofertilizer, CF chemical fertilizer

Treatment of 100% dosage of chemical fertilizer (control) had roots with the lowest nodule number. This is caused by the treatment was not combined with biofertilizers so that the infection of *Rhizobium* sp. into the root is not as effective as other treatments. Bekere et al. (2013) reported that higher amount of inorganic fertilizer inhibits the nitrogen fixation in early stage of plant.

Phosphate solubilizing microorganisms in the consortium of biofertilizers affect the formation of nodules because it can increase phosphate availability (Zaidi et al. 2010). This is because phosphate is one of the energy sources by plants to compile adenosine triphosphate (ATP) where ATP is used as an energy source by *Rhizobium* sp. (Malhotra et al. 2018).

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The addition of sufficient chemical fertilizer also plays a role in increasing the effectiveness of *Rhizobium* sp. in the formation of nodules. Application of sufficient amount of N fertilizer stimulate growth of root hair more quickly and cause N inhibition by Rhizobium sp. to the maximum. P fertilizer plays an important role in the formation of root nodules because it helps the synthesis of ATP and nicotinamide adenine dinucleotide phosphate (NADPH) as a source of energy for microbes (Malhotra et al. 2018). K Fertilizer plays a role in increasing the translocation of photosynthesis to the roots used by *Rhizobium* sp.

Plant N uptake

Table 1 shows that the combination of biofertilizers and chemical fertilizers increase the N uptake of soybean significantly. This shows that N-fixing bacteria contained in biofertilizers increased soil N so that N absorbed by plants increases. The treatment of 5 L ha^{-1} biofertilizer and 75% dosage of chemical fertilizer are able to absorb N higher than other treatments. Increased N uptake of soybean plants is thought to be due to an increase in the nodules number caused by N fixation activity by Azotobacter sp., Acinetobacter sp., and Azospirillum sp. contained in the consortium's biological fertilizer. According to Ohyama et al. (2017) which states that soybean plants that have enough N due to N_2 fixation by effective nodules can increase nutrient uptake and reduce the dose of chemical fertilizer so that plants will grow better. With the increase in the number of root nodules, the absorption of nitrogen by plants will also increase.

Table 1: Plant N-uptake at the end of vegetative period

Treatments	N uptake g $plant^{-1}$
Control (CF 100%)	0.17 a*
BF 3L ha^{-1} + CF 50%	0.27 ab
BF 3L ha^{-1} + CF 75%	0.28 ab
BF 3L ha^{-1} + CF 100%	0.37 bc
BF 5L ha^{-1} + CF 50%	0.30 b
BF 5L ha^{-1} + CF 75%	0.42 c
BF 5L ha^{-1} + CF 100%	0.31 bc

**Note: Numerics followed by the same letters were non-significant on 95 % Duncan's New Multiple Range Test.*

Increasing of chemical fertilizers did not improve N-uptake with application biofertilizers 5 L ha^{-1}. However, application 100% dosage of chemical fertilizers were too high, and biofertilizers could have partially replaced the NPK fertilizer inputs. According to Solanki et al. (2018) that nutrient uptake decreases with increasing NPK fertilizer dosage.

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Growth of soybean

The application of biofertilizers and chemical fertilizers did not affect significantly on plant height at the end of vegetative period. This was thought to be due to humidity reaching 92% during the planting period making soybean growth less than optimal. According to An et al. (2001), that humidity influence to growth of soybean. The optimal air humidity for soybean growth is 75-90%.

Table 2: Growth of soybean (plant height, shoot dry weight and root dry weight) at the end of vegetative period.

Treatments	Plant height (cm)	Shoot dry weight (g)	Root dry weight (g)
Control (CF 100%)	28,6 a	5,31 a	1,69 a
BF 3L ha^{-1} + CF50%	31,1 a	7,68 ab	2,52 ab
BF 3L ha^{-1} + CF 75%	32,9 a	8,69 bcd	2,39 ab
BF 3L ha^{-1} + CF 100%	34,7 a	11,10 cd	3,47 cd
BF 5L ha^{-1} + CF 50%	31,6 a	8,31 bc	2,54 ab
BF 5L ha^{-1} + CF 75%	35,3 a	11,38 d	3,61 d
BF 5L ha^{-1} + CF 100%	34,5 a	8,95 bcd	2,72 bc

**Note: Numerics followed by the same letters were non-significant on 95 % Duncan's New Multiple Range Test*

Table 2 shows that the plant height in the treatment of 100% dosage of chemical fertilizers were 28.6 cm while 5 L ha^{-1} of biofertizer and 75% dosage of chemical fertilizers was 35.31 cm. This indicates that the application of biofertilizers has the potential to increase plant height of soybean. Azotobacter sp., Azospirillum sp., and Acinetobacter sp. in biofertilizers can produce growth regulators such as IAA, cytokinins and gibberellins which promote cell elongation and division (Olanrewaju et al. 2017). Pseudomonas cepaceae and Penicillium sp. which is also present in biofertilizers dissolve P into available so that it can stimulate cell division and cell differentiation which can increase plant height. According to Fitriatin et al. (2018), that biofertilizers contain Pseudomonas cepaceae and Penicillium sp. as phosphate solubilizing microorganisms increase plant height.

The results of experiment showed that the combination of biofertilizers and chemical fertilizers significantly affected on the plant dry weight. Table 2 shows that the application of biofertilizers and chemical fertilizers increased the shoot and root dry weight. The application 5 L ha^{-1} of biofertilizer and 75% dosage of chemical fertilizers increased shoot dry weight up to 114,31%

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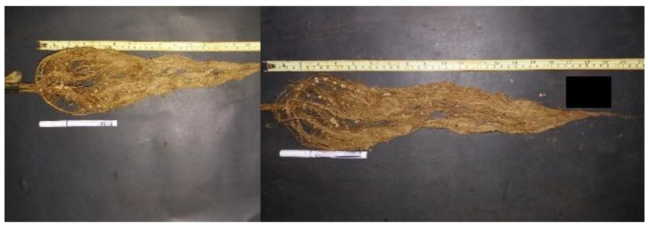
compare with control (Fig.2). This increase is higher than other treatments. The increase in plant growth due to this treatment is in line with the data on plant N uptake as has been shown in Table 1. The same effect of this treatment on the increase to root dry weight reached 81.18% was higher than other treatments.



Control Biofert 5L ha^{-1} + chemical fertilizers 75%

Fig. 2: Growth of root at the end of vegetative period (comparison of control with BF 5L ha^{-1} + CF 75%)

CONCLUSIONS

This study indicate that biofertilizers consortium of N-fixing bacteria (*Azotobacter chroococcum, Azotobacter vinelandii, Azospirillum)*, phosphate solubilizing microbes *(Pseudomonas cepaceae, Penicillium* sp.) and endopytic bacteria (*Acinetobacter* sp.) increased nodulation, plant N uptake and growth of soybean. The application of biofertilizers 5 L ha^{-1} and 75% N, P, K fertilizer increased the nitrogen uptake, nodules number, dry weight of plant. This study implied that biofertilizer might be used to increase growth of soybean and NPK fertilizer efficiency.

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