The efficacy of biofertilizer contains *Bradyrhizobium japonicum* isolates on soybean yields grown in Inceptisols, Bogor, West Java, Indonesia

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Abstract. The use of biofertilizers is growing rapidly and promising for food crops and horticulture. They are capable to improve soil fertility, increase crop growth and yield, and environment friendly. Soybean is a strategic commodity in Indonesia. Various ways of cultural practice to improve soybean yield have been reported. The biofertilizer containing six *Bradyrhizobium japonicum* isolates with husk charcoal powder carrier material has been tested on soybean in Cibungbulang Bogor. Our objective was to evaluate the efficacy of *Bradyrhizobium japonicum* biofertilizer on soybean growth and the yield on Inceptisols Cibungbulang Bogor. The experiment was a randomized block design consisted of seven treatments with four replications. The treatments were (1) Control, no fertilizer (2) 100% NPK (3) Biofertilizer, (4) Biofertilizer + 25% NPK, (5) Biofertilizer + 50% NPK, (6) Biofertilizer + 75% NPK and (7) Biofertilizer + 100% NPK. The results showed that the promising treatment to improve soybean yield was Biofertilizer + 50% NPK and Biofertilizer + 75% NPK as indicated by significantly increase of soybean yield from 18.56 to 25.77% compared to the treatments of 100% NPK, reduced NPK fertilizer 25 to 50%. The values of Relative Agronomic Effectiveness were 191% and 165%. This study implies that the application of biofertilizer gave a positive result both on the growth and yield of soybean.

1. Introduction

Soybean (*Glycine max* L. Merrill) contains a very high nutritional value, which is useful for food crops for human nutrition, source of protein for animals, medicinal plant, and as an industrial crop [1]. The protein content of soybean seeds is around 36 to 40%, oil (18 to 20%) which has a great potential as a source of protein to overcome malnutrition [2-5], soybeans are the only legume which provides insufficient of the essential omega-3 fatty acid, α-linolenic acid (ALA) [6]. A by-product from the production of soybean oil (soybean meal) is used as a high protein animal feed in many countries [7].

Indonesia has not been able to meet domestic soybean needs, because the domestic soybean production is still low. Central Bureau of Statistics Republic of Indonesia [8] reported that the total domestic soybean production is only 982,598 t, even though the national soybean demand reaches 3.36 million t has resulted in a deficit of 2.38 million t. To overcome this problem, the government imports soybeans from abroad. Efforts to increase national soybean production are focused on increasing land productivity and expanding cultivated land to suboptimal land. Continuous use of inorganic fertilizers (N, P and K) in the long term causes a decrease in soil organic matter, damage soil physical properties,
and environmental pollution, supposing if this situation continuing it will reduce the soil quality and fertility [9]. Excessive use of inorganic fertilizers has an impact on increasing the dosage of fertilizer use per unit of land with an insignificant increase in yield. The increasing price of fertilizer and its impact on the environment forces people to look for other sources of nutrients needed by plants.

The use of biofertilizers is growing rapidly and promising for food crops and horticulture. Several studies have reported that fertilization using inorganic fertilizer 50 to 75% NPK and the use of biological fertilizers provide results equivalent to 100% NPK [10-12]. The benefit and profit of using bio-fertilizers were improving soil fertility, increasing crop growth and yield, and reduce the growth of parasite microbes [13]. The population and growth of this bacterium are highly influenced by pH, humidity, climate, soil physics, and chemistry properties [14]. Therefore, the effectivity test of bio-fertilizer toward crop production is needed.

Atmospheric nitrogen is converted into the form available to plants through biological nitrogen fixation (BNF), both symbiotic and non-symbiotic. Nitrogen in gaseous form is reduced to N available to the host plant, while the host plant supplies photosynthate to rhizobia as an energy source. BNF has many advantages compared to the use of inorganic nitrogen fertilizers, including not causing environmental pollution, even controlling pollution, increasing fertility and soil productivity, and making efficient use of inorganic N fertilizers [15]. The utilization of BNF is not dangerous for the environment or do not have the side effect, relatively low cost, and easily applied technology.

Biofertilizer containing single or multi-microbial formulas that can provide nutrients for plants is an alternative to increase crop production and reduce environmental pollution. Nitrogen is one of the essential nutrients for plants. The need for nitrogen in soybeans can also be met by symbiotic nitrogen fixation involving rhizobium bacteria. BNF of Bradyrhizobium japonicum produces mucus from the cell surface carbohydrates that are mostly in the form of extracellular polysaccharides (EPS) and serves as tolerance to acid [16]. B. japonicum is a rhizobium strain that is symbiotic with soybean roots which play a role in the nitrogen-fixing activity. B. japonicum symbiosis with soybean plants by infecting the roots of soybean plants, then forming special root structures called nodules or nodules. In these nodules, bacteria differentiate into bacteroids and convert N₂ to ammonia by using a nitrogenase enzyme complex, a process called nitrogen fixation [17]. Soybean contributes to a greater symbiosis of N fixation than other legume crops [18]. The ability to fix N₂ in the symbiotic atmosphere with the rhizobia strain made legume plants able to grow in soils with low N levels [19].

The objective of this study was to evaluate the efficacy of Bradyrhizobium japonicum biofertilizer on the soybean growth and yield on Inceptisols Cibungbulang Bogor.

2. Materials and methods

2.1. The experimental site

Bogor Regency has a wet tropical climate with an average annual rainfall of 2,500 to 5,000 mm year⁻¹ with an average temperature of 20° to 30°C, with an average annual temperature of 25°C. Humidity is 70% and wind speed is quite low, with an average of 1.2 m s⁻¹ with evaporation in open areas averaging 146.2 mm month⁻¹ [20]. Soil types are dominated by Latosol, Alluvial, Regosol, Podzolic and Andosols. The soil types at the experimental site categorized as Inceptisols.

Characteristics of Inceptisols from Cibungbulang, Bogor has slightly acid soil reaction (pH 5.6), low organic C content (1.17%), very low total-N (0.14%), and moderate C/N ratio. The potential-P (extracted with 25% HCl) is classified as very high but the available P was very low. Phosphate deficiency often limits N₂ fixation by legumes, especially those grown in tropical and subtropical areas under acidic soil conditions [21]. To improve the use efficiency of P fixed in the soil, it is required biofertilizer containing P-solubilizing bacteria. Cation exchange capacity (CEC) is classified as low, but the level of base saturation is very high. The high base saturation is caused by the free cations in the soil solution other than those contained in the exchange complex. The results show that the main problems of Inceptisols from Cibungbulang, Bogor are acid soil reaction, low organic-C content, very low total N, available P and exchangeable K, and low CEC. Although the soil P (P-potential) content is relatively high, however,
its availability level is relatively low. Therefore, the Inceptisol from Cibungbulang, Bogor have a relatively low fertility level.

2.2. Description and sources of experimental treatments and materials

This research was carried out on a farmer's field of Cibungbulang, Bogor Regency from April to August 2017. The field study was arranged using a randomized block design, which consists of seven treatments with four replications, and tested on soybean Anjasmoro variety. The treatments included were (1) Control, no fertilizer (2) 100% NPK (3) Biofertilizer, (4) Biofertilizer + 25% NPK, (5) Biofertilizer + 50% NPK, (6) Biofertilizer + 75% NPK, (7) Biofertilizer + 100% NPK. NPK dose based on the upland soil test kit which shows the recommendation fertilizer dose (100%NPK) of Urea: 50 kg ha⁻¹, SP-36: 100 kg ha⁻¹, KCl:100 kg ha⁻¹).

The soybean seed of Anjasmoro variety and biofertilizer contains six B. japonicum isolates (B. japonicum KDL176, B. japonicum KDL92A, B. KDL92B, B. japonicum KDL196, B. japonicum KDL182, B. japonicum KDL92) with husk charcoal powder carrier obtained from Indonesian Legumes and Tuber Crops Research Institute, Malang, Indonesia.

2.3. Land preparation, inoculation and planting

Land preparation was done in conventional practice to make the land suitable for planting. Dolomite and manure of 500 kg ha⁻¹ and 1,000 kg ha⁻¹ were given along with tillage. The experimental plots were made and incubated for 2 weeks before planting was carried out. Each plot is 4 m x 5 m (20 m²), the plots are separated by 0.5 m spacing, while the distance between blocks is 1 m. Land preparation was carried out in conventional practice to make the field suitable for planting.

The biofertilizer as much as 200 g inoculated in soybean seeds is approximately 40 kg of seeds per hectare to the seeds that are layered by this biofertilizer. To ensure that the biofertilizer that is applied sticks to the seeds, the seeds are moistened until all the surface of the seeds has been wetted with water. Subsequently, the biofertilizer was applied is slowly mixed so that all the seeds receive a thin layer of inoculant. All inoculations were carried out just before transplanting under the shade to maintain the viability of the bacterial cells. The seeds are allowed to dry for a few minutes and then planted in the planting hole (drilled) that has been provided. Plots with uninoculated seeds were planted first to avoid contamination. The seeds are covered with soil immediately after planting to avoid cell death from solar radiation.

2.4. Data collection and statistical analysis

Five hills of plants from each plot were randomly sampled at the time of flowering (7 weeks after planting/WAP) and maturity. The data collected of flowering stage i.e to observe plant height, number of leaves, and number of root nodules. The number of filled and empty pods collected at the maturity stage. The mean values were reported as plant height, the number of leaves, and the number of root nodules. Meanwhile, the observation of biomass and seed yields was carried out by harvesting with a plot area of 1 m x 2 m (2m²), then the data was converted to ton ha⁻¹.

The data were subjected to analysis of variance (ANOVA) following the standard procedure given by Gomez and Gomez [22] using DSTATAT computer software and the means separated using Duncan Multiple Range Test at 5% level of probability. Harvest Index (HI) is the ability of plants to transmit assimilates. These observations were made during the harvest period and were calculated by Sitompul and Guritno [23] using formula:

\[ HI = \frac{(Y/W)}{1} \]  

HI= Harvest Index; Y= Grain yield; W= Dry weight of plant biomass
The Relative Agronomic Effectiveness (RAE) is calculated with the formula of [24].

\[
RAE = \frac{(\text{Yield of tested bio fertilizer} - \text{Control})}{(\text{Yield of standard recommendation} - \text{Control})} \times 100\%
\]

(2)

3. Results and discussion

3.1. Plant growth and the number of root nodules

Biofertilizer inoculation at various levels of NPK fertilizer doses had a significant effect on the growth of soybean plants, namely plant height and number of nodules observed at 7 weeks after planting. Application of Biofertilizer + 25% NPK resulted in more plant height compared to the application of Biofertilizer only or control, however, the highest plant height was obtained by Biofertilizer + 75% NPK indicates that the plant height is not significantly different from Biofertilizer + 100% NPK (table 1). Regarding the effect of the treatment on the number of leaves does not show a significant difference, but visually it appears that the leaves area in the biofertilizer and control treatments appear smaller (narrower) and thinner than the treatments were given NPK and Biofertilizer. This is in line with the results of research by Daramola et al. [25] reported that the availability of adequate nutrition can improve growth and yield parameters. The need for nitrogen from the soil or fertilizers with sufficient doses is needed for legumes (soybeans) for plant vigor during the first weeks [26].

The highest number of effective root nodules achieved by Biofertilizer + 50% NPK (60.7 nodules hill\(^{-1}\)), which was not significantly different from Biofertilizer + 75% NPK and Biofertilizer + 100% NPK. This treatment showed that the number of nodules increased significantly compared to 100% NPK. This shows that effective root nodules are influenced by biofertilizer and NPK fertilizer at certain doses. The hurt of increasing NPK fertilizer on nodule formation in soybean plants is thought to be due to the influence of N nutrients at different stages of the nodule formation process, so the increase in N availability in soil solution resulted in inhibition of nodule formation and growth [27,28]. However, these results indicate that the increase in N fertilization (NPK) did not interfere with the formation of soybean root nodules, where the use of N fertilizers in soybean plants did not hurt the number of root nodules.

However, the increasing number of root nodules is not in line with the increase in plant height and number of leaves. Research conducted by Appunu et al. [29] reported that B. japonicum can increase seed yield per plant, accumulation of plant dry matter, and root nodules effectively. The percentage increase in number and dry weight of nodules of soybean cultivars inoculated with Bradyrhizobia strains ranged from 71 to 486% and 0 to 200%, respectively [30]. In the results of this study, the ranging increasing number of nodules was treated with B. japonicum about 60.25 to 214%.

Research result by Saraswati [31] showed that the application of more mineral fertilizer able to negatively impact the microbial ecology, particularly in the area covered with fertilizer particles. The mineral fertilizer will balance the salt concentration in the soil, nutrient imbalance, low or high pH, and high nitrates. Michael et al [32] reported that P nutrients affect the growth of B. japonicum cells and can significantly affect parameters such as the number and weight of nodules. Rhizobia requires sufficient nutrients for the metabolic activities involved in invading and successfully colonizing the host root system to build an effective legume plant host symbiosis [33-35].

3.2. Number of filled and empty pods

The application of NPK combined biofertilizer showed a significantly different on the number of filled and empty pods. The highest number of filled pods was found in the treatment of Biofertilizer + 50% NPK (39 pods hill\(^{-1}\)). However, the results do not show significant differences with the treatment of Biofertilizer + 75% NPK, Biofertilizer + 100% NPK, 100% NPK (table 2). Table 2 recorded that the application of Biofertilizer would save NPK fertilizer as much as 25 to 50% to obtain a similar yield of standard NPK (100% NPK). Also, the lowest number of empty pods obtain by Biofertilizer + 100% NPK as many as 6.67 pods hill\(^{-1}\).
3.3. Plant biomass, grain yield and harvest index

The treatment of Biofertilizer + 50% NPK and Biofertilizer + 75% NPK gave significantly different increases to dry weight plant biomass, grain yield per hill, harvest index, and RAE compared to the 100% NPK (table 3). The combination of NPK fertilizer and B. japonicum had a significant effect on the weight of plant biomass. The highest results were achieved in the Biofertilizer + 100% NPK. Plants need N for growth, this is shown in the treatments tested, the increasing dose of N (NPK) gave plant biomass weight was higher. However, the increase in the amount of biomass plant was not matched by an increase in grain yield and harvest index, the highest grain yield and harvest index were achieved in the Biofertilizer + 100% NPK.

Table 1. Plant height, number of leaves and number of root nodules under different level of NPK fertilizer combined with biofertilizer on Inceptisols Bogor at 7 WAP.

| Treatment           | Plant Height (cm) | Number of leaves (sheets) | Number of root nodules (nodules hill⁻¹) |
|---------------------|-------------------|---------------------------|----------------------------------------|
| Control (no fertilizer) | 58.19 a           | 10.39 a                   | 15.6 a                                 |
| 100% NPK            | 74.44 c           | 9.81 a                    | 20.0 a                                 |
| Biofertilizer       | 63.14 a           | 10.42 a                   | 25.0 ab                                |
| Biofertilizer + 25% NPK | 65.17 b         | 9.83 a                    | 40.0 b                                 |
| Biofertilizer + 50% NPK | 69.67 bc       | 9.75 a                    | 60.7 c                                 |
| Biofertilizer + 75% NPK | 74.92 c         | 9.83 a                    | 48.5 bc                                |
| Biofertilizer + 100% NPK | 67.92 b         | 9.14 a                    | 49.6 bc                                |
| C V (%)             | 16.39             | 18.78                     | 22.3                                   |

Remarks: The numbers in the same column and followed by the same letters is not significantly different at 5% DMRT

Table 2. The average of the number of filled and empty pods at the level of NPK fertilizer combined with biofertilizer at Inceptisols Bogor.

| Treatment           | Number of filled pods | Number of empty pods pods hill⁻¹ |
|---------------------|-----------------------|----------------------------------|
| Control (no fertilizer) | 25.13 a               | 18.07 c                          |
| 100% NPK            | 38.73 b               | 9.27 ab                          |
| Biofertilizer       | 25.17 a               | 9.77 ab                          |
| Biofertilizer + 25% NPK | 26.47 a             | 10.10 ab                         |
| Biofertilizer + 50% NPK | 39.00 b             | 10.23 b                          |
| Biofertilizer + 75% NPK | 35.90 b             | 10.07 ab                         |
| Biofertilizer + 100% NPK | 33.07 b           | 6.67 a                           |
| C V (%)             | 16.05                 | 17.90                            |

Remarks: The numbers in the same column and followed by the same letters is not significantly different at 5% DMRT

Therefore, the yield of soybean of Biofertilizer + 50% NPK and Biofertilizer + 75% NPK are higher than 100% NPK, grain yields were 1.15 t ha⁻¹, 1.22 ha⁻¹ and 0.97 t ha⁻¹ consecutively. The treatment of Biofertilizer + 100% NPK was not significantly affecting the 100% NPK, it matters the application of Biofertilizer can save inorganic fertilizer (NPK). While the inoculation of Biofertilizer alone had no significant effect compared to the 0%NPK. Chiezey and Odunze [36] reported that soybean requires between 30 to 60 kg N ha⁻¹ for optimum performance. Daramola et al. [25] and Adeyeye et al. [37] reported that the availability of adequate nutrients could improve crop growth and yield parameters. The planted soybean in Inceptisols Bogor, the nutritional needs are sufficient to be fulfilled by fertilizing 50% NPK or 75% NPK and combined with Biofertilizer. Thies et al. [38] and Palmar and Young [39] reported the efficiency of BNF by legumes is affected by various factors such as soil moisture,
temperature, available soil nutrients, biotic and abiotic stresses, the presence of efficient, competitive rhizobia strains, cropping systems, and field management practices.

**Table 3.** The average of dry weight plant biomass, grain yield, harvest index of soybean and RAE at the level of NPK fertilizer combined with biofertilizer in Inceptisols Bogor, West Java.

| Treatment                     | Plant biomass | Grain yield | Harvest Index (HI) | RAE (%) |
|-------------------------------|---------------|-------------|--------------------|---------|
| Control (no fertilizer)       | 0.9 a         | 0.69 a      | 0.77 a             | -       |
| 100% NPK                      | 1.07 b        | 0.97 b      | 0.91 b             | 100 b   |
| Biofertilizer                 | 1.04 a        | 0.78 a      | 0.75 a             | 32 a    |
| Biofertilizer + 25% NPK       | 1.11 bc       | 0.99 b      | 0.89 b             | 108 b   |
| Biofertilizer + 50% NPK       | 1.09 bc       | 1.15 c      | 1.05 c             | 165 c   |
| Biofertilizer + 75% NPK       | 1.14 c        | 1.22 c      | 1.07 c             | 191 c   |
| Biofertilizer + 100% NPK      | 1.21 c        | 1.01 bc     | 0.83 ab            | 116 b   |
| CV (%)                        | 16.87         | 15.98       | -                  | -       |

Remarks: The numbers in the same column and followed by the same letters are not significantly different at 5% DMRT. RAE=relative agronomic effectiveness

The harvest index explained the ability of plants to distribute assimilate for an economical yield for seed formation. The highest harvest index of Biofertilizer + 75% NPK showed no significant difference with Biofertilizer + 50% NPK. The harvest index of both treatments was significantly different from the 100% NPK. Also, this indicates that the application of biofertilizer contains *B. japonicum* to conserving inorganic fertilizers also increases grain yield and harvest index.

The relationship between levels of NPK fertilization (50 kg Urea ha$^{-1}$, 100 kg SP36 ha$^{-1}$, and 100 kg KCl ha$^{-1}$, and the weight of soybean seeds is presented in figure 1. The application of NPK fertilizer combined with biological fertilizers can increase the dry weight of soybean seeds. Figure 1 shows the increase in fertilization above 75% NPK in combination with biological fertilizer the weight of soybean seeds decreases and tends to decrease, this is shown by the regression equation the equation $y = -1.0629x^2 + 1.3389x + 0.7591$ where $R^2$ was $= 0.9512$. Based on this equation, the optimal dose of NPK fertilizer combined with biofertilizer can be calculated as a differential function of the equation $Y= -1.0629x^2 + 1.3389x + 0.7591$.

![Figure 1](attachment:image.png)

**Figure 1.** Relationship between the rate of NPK fertilizers combined with biofertilizer on soybean grain yield.
3.4. Effect of NPK and biofertilizer on relative agronomic effectiveness (RAE) of soybean

The highest relative agronomic effectiveness (RAE) was achieved by the treatment of Biofertilizer + 75% NPK, followed by Biofertilizer + 50% NPK. Both treatments did not show a significant difference in RAE values. The RAE values at Biofertilizer + 75% NPK was 191%, and the RAE of Biofertilizer + 50% NPK was 165%. The RAE value at Biofertilizer + 75% NPK and Biofertilizer + 50% NPK gave a higher value than the RAE of 100% NPK (table 3). This value indicates that the use of NPK fertilizers that are accompanied by biofertilizer causes the use of chemical fertilizers efficient, increasing soybean yields effectively. This shows the synergistic effect of chemical and biofertilizers.

4. Conclusions

NPK fertilizer combined with biofertilizer significantly different increased plant height, filled pods, dry weight of biomass, and grain yield of soybean compared to the control or solely inoculation biofertilizer. The highest yield (1.22 t ha⁻¹) and harvest index were obtained from the treatment of Biofertilizer + 75% NPK, and the relative agronomic effectiveness (RAE) was 191%, while the harvest index was 1.07.

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