Nitrogen Fertilizer and Microbial Inoculations Determined the Nutrient Uptake and Productivity of Soybean

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Abstract. Soybean is an important commodity in Indonesia. This study aimed to identify the application of microbe inoculations with dosage of N in form of urea toward the growth, N uptake and yield in soybean. This research was conducted on October 2017 to January 2018 in Panunggalan village, Pulokulon, Grobogan, Central Java. It used the Randomized Complete Block Design 2 factors: urea dosages (0; 50; 100 kg ha⁻¹) and microbe inoculations (no inoculation; Rhizobium (R); Rhizobium + Trichoderma (R+T); Trichoderma(T)). The result showed that combination of urea dosage and microbe inoculations significantly influenced plant height and number of trifoliate leaves. Urea dosages and microbe inoculations or combination of both factors did not imply nodulation process. Higher N dosage and microbe inoculations increased N uptake of biomass on vegetative period but had no effect on flowering and pod filling periods. The combination of 100 kgHa⁻¹ urea with microbes or without microbes produced the highest productivity. However, treatment without urea and no inoculation also achieved the highest production. This suggested that there was a native population of Rhizobium bacteria strains that effectively supplied N for plants. Besides, application of urea of 100 kg ha⁻¹ or 46 N kg ha⁻¹ was too small to interfere fixation nitrogen by local Rhizobium population. Therefore, N application in form of anorganic fertilizer or microbe for symbiotic N-fixation was not recommended for existing agro climatic conditions of area with soybean history. Thus, it could simultaneously save the cost of agricultural production.

1. Introduction
Soybean is an important commodity in Indonesia and is an important source of protein in the community. Based on data from [1], Indonesia's national soybean productivity in 2015 reached 1.57 t ha⁻¹, while soybean productivity in Central Java Province could reach 1.84 t ha⁻¹. Nevertheless, the productivity is still lower than the potential yield of soybean Grobogan variety which can reach 3.4 t ha⁻¹ [2].

Soybean is known to be able to symbiotic with microbes in the absorption of Nitrogen, so one effort to increase soybean productivity is by Rhizobium inoculation. Rhizobium is a microbial symbiotic with plant roots forming a root nodule to fix nitrogen. Nurhayati [3] stated that Rhizobium inoculation can improve nitrogen fixation and increase seed yield. Rhizobium inoculation also had a significant effect on growth and yield [4]. Thus, inorganic N fertilizers can be reduced because most of the soybean’s N requirement has been met from the results of soya-bradyrhizobium symbiosis [5]. In addition to rhizobium, trichoderma is a symbiotic fungus that is mutually beneficial with plant roots. Trichoderma helps host plants to absorb certain nutrients [6] especially phosphate [7].
Furthermore, trichoderma fungus is known to have the ability to increase the rate of growth and development of plants [8]. N fertilization is necessary if N needs are not fully met from the results of microbial and plant symbiosis [9]. This N fertilization is a starter before the N-fixing microbe is active [10]. Thus appropriate N fertilization dosages should be determined for optimal N absorption and best soybean growth and yield. In addition, the interactions of Rhizobium microbes and Trichoderma fungi have not been studied as well as their effects on the growth and yield of soybean. Therefore, this study aimed to examine the application of microbial inoculation in the form of Rhizobium and Trichoderma and the appropriate dosage of N fertilizer for growth and soybean yield. In addition, this study also investigated the effect of both factors on the level of nitrogen uptake in soybean plant tissue.

2. Materials and Methods

The study was conducted from October 2017 to January 2018, located in Panunggalan Village, Pulokulon Subdistrict, Grobogan District, and Central Java Province. This area was chosen because this area is one of soybean production center in Central Java Province. Based on geographical location, the study sites are located at 7o12’55” LS and 111o07’29” BT. The study area is located at an elevation of 42 meters above sea level. The temperature ranges from 27°C to 39°C. The study area is rainfed lowland with grumusol soil type. Soil fertility level in the study area based on standard of Indonesian Soil Research Institute [11] is high with 4.11% organic material. Organic C is moderate (2.38). This area has a neutral pH and high CEC. The total N level is moderate, available-P is low and exchangeable-K is low. In general, agro climate conditions in the area are suitable for soybean growth. The materials used are soybean Grobogan variety, rhizobium isolates (login from Gadjah Mada University), trichoderma isolates, urea fertilizer, SP-36 fertilizer, and KCl fertilizer. Cultivation of soybeans was carried out with spacing of 18 cm x 35 cm.

This study utilized a complete randomized block design 2 factors with 3 replications. The first factor was the dosages of urea consisting of 3 levels ie 0 kg ha-1, 50 kg ha-1, and 100 kg ha-1. KCl fertilization with a dosage of 45 kg ha-1 and SP36 at a dosage of 60 kg ha-1 was performed on all treatments. The second factor was microbial inoculation consisting of 4 levels ie without inoculation, rhizobium (R), rhizobium + trichoderma (R+T), Trichoderma (T). The dosage of rhizobium was 5 g kg-1 soybean seed. For tricoderma, the dosage was 100 g kg-1 soybean seed. The data were analyzed using Analysis of variance (ANOVA) \( \alpha = 5\% \) and if significant, followed by Duncan's Multiple Range Test (DMRT). The parameters observed were plant height, number of leaves, number of root nodules, root length, root volume, biomass dry weight, number of pods, and productivity.

Soil chemical analysis was conducted at the Soil Chemistry Laboratory of the Faculty of Agriculture, Sebelas Maret University. Total N of plant tissue (%) was analyzed by the Kjeldhal method. The N plant uptake analysis was carried out three times consisting of vegetative phase (20 days after planting (DAP)), flowering phase (35 DAP) and pod filling phase (60 DAP).

3. Results and Discussion

3.1. Plant Growth (Plant Height and Number of Trifoliate Leaves)

Application of urea fertilizers with different dosages and microbial inoculations interacted and affected the height of soybean at 4 and 8 weeks after planting (WAP). At 4 WAP, the highest plant was obtained on the use of a combination of 100 KgHa-1 urea and without inoculation. This suggested that the plants were more likely to utilize the available N elements from inorganic fertilizer. In addition, it also showed that the inoculation process has not been optimal yet to provide nutrients for plants (Table 1). Conversely, at 8 WAP, the combination of urea fertilizer and microbial inoculation influenced the soybean significantly. This was demonstrated by the use of a combination of tricoderma and 0 KgHa-1 urea which produced the highest crop (64.8 cm) (Table 1). [2] stated that Grobogan soybean varieties had plant height 50-60 cm. It could be said that the soybean grew optimally even without N fertilizer and without N-fixing microbial inoculation. Moreover, this indicated that the use of tricoderma has been effective in supporting plant growth even though no inorganic N elements was added. In addition, high plants were also obtained in combination without inoculation and 0 KgHa-1 Urea of 56 cm. Although without the addition of urea and microbial inoculation, the plant achieved the same height as the use of 100 kgHa-1 urea, a combination of urea
100 kgHa-1 and rhizobium, as well as a combination of 100 kgHa-1 urea and rhizobium + tricoderma. This indicated that in the region there have been endemic rhizobium microbes capable of fixing nitrogen to support plant growth.

Table 1. Plant height on the effect of interaction of N dosages and microbial inoculation on 8 WAP

| Urea Dosage (Kg Ha-1) | No Inoculation | R | R+T | T |
|-----------------------|----------------|----|-----|----|
| 0                     | 56.0±7.2       | 53.5±1.8 | 54.3±2.0 | 64.8±2.0 |
| 50                    | 55.8±5.5       | 61.8±2.1 | 59.7±6.3 | 59.5±4.0 |
| 100                   | 58.8±6.6       | 63.7±5.1 | 60.7±4.6 | 55.0±6.1 |

Figures followed by the same letter show no significant difference based on the DMRT test of 5% level. R=rhizobium, T=trichoderma, WAP=week after planting.

In contrast, in 8 WAP, a combination of 100 kg Ha-1 urea and trichoderma inoculation caused the plant to be lower (55 cm). This suggested that trichoderma activity would reduce by high dosages of N. This finding concurred with the results of [12] which stated that Urea and nitrate potassium did not seem to be beneficial to the Trichoderma harzianum fungus at a high rate.

The number of leaves was significantly influenced by the dosage of urea fertilizer and the interaction of fertilizer dosage and microbial inoculation. The highest number of leaves produced by the application of urea fertilizer 50 Kg Ha-1. Conversely, the addition of fertilizer dosages up to 100 KgHa-1 actually decreased the number of soybean leaf (Figure 1).

Table 2. Number of trifoliate leaves on urea dosages and microbi inoculations

| Urea Dosage (Kg Ha-1) | No Inoculation | Number of Trifoliate Leaves |
|-----------------------|----------------|----------------------------|
|                       |                | R  | R+T | T  |
| 0                     | 15.0±1.0       | 15.3±2.8 | 14.7±0.8 | 18.3±1.3 |
| 50                    | 17.8±1.3       | 15.3±0.6 | 18.8±1.9 | 19.8±2.9 |
| 100                   | 16.7±0.8       | 17.7±1.6 | 17.7±1.5 | 13.3±1.5 |

Figures followed by the same letter show no significant difference based on the DMRT test of 5% level. R=rhizobium, T=trichoderma

The combination of urea fertilizer and microbial inoculation interacted significantly and affected the number of leaves (Table 2). This could be seen from the highest number of leaves which produced by the combination of Urea 50 Kg Ha-1 and trichoderma (19.8). The use of a combination of urea 0 Kg Ha-1 and tricoderma also produced the largest number of leaves (18.3). This indicated that without
additional N fertilizer, trichoderma could support plant growth. In addition, it was suspected that there was already an endemic rhizobium capable of providing N for plants. In contrast, in the same pattern with the plant height variables, the combination of urea 100 kg Ha⁻¹ and trichoderma produced the lowest leaf number. This revealed that the excessive use of nitrogen in the use of trichoderma had a negative effect on plants. This finding was in line with [13] that trichoderma application was able to increase the number of banana leaf leaves compared to controls. Furthermore [14] suggested trichoderma had the potential to increase the solubility of phosphate and micronutrients, increase nitrogen fixation and promote plant health at the initial growth.

3.2. Number of Root Nodules
The different dosages of urea fertilizer up to 100 kg ha⁻¹ or 46 N kg ha⁻¹, microbial inoculation or combination of both factors were not induce any visible differences in number of soybean root nodules in vegetative period, flowering period and filling pods period. This statement was as stated by [15] that the application of Nitrogen 56 kg ha⁻¹ or more nitrogen at planting time caused reduction of soybean root nodule. This was also supported by the statement [16] that root nodulation was stimulated by low concentrations of nitrogen and significantly restrained by high concentrations of nitrogen. This illustrated that the addition of inorganic N up to 45 kg ha⁻¹ (100 kg ha⁻¹ urea) still could be a working starter of rhizobium bacteria to form root nodules. Furthermore, there has been indigenous local rhizobia population that were effective at the nodulation process. Moreover, greatest development in nodulation happened when it was compatible and indigenous soil rhizobia were low [17]. If N requirement of soybean could be met from the presence of local rhizobium populations, inoculation with even efficient rhizobia might not show any enhancement SNF or in yield [18], [19], and [20] conveyed that soy required additional dose of N as a starter. However, excessive use of N would interfere the activity of N fixation by bacteria. [21] added that nodule formation was inhibited by applying any dose of N fertilizers. The application of nitrogen, especially nitrate, to soybean was able to strongly suppress nodule formation, growth and nitrogen fixation. Conversely, the growth of lateral roots was improved by addition nitrate [22].

**Table 3.** Number of root nodules, root length and root volume in combination of urea and microbe inoculation

| Treatments                      | Number of Root Nodules | Root Length (cm) | Root Volume (ml) |
|--------------------------------|------------------------|-----------------|-----------------|
|                                | 20 DAP  | 35 DAP  | 60 DAP  |                  |                  |
| Urea 0 Kg Ha⁻¹ + no inoculation| 10.8    | 70.0   | 65.0    | 21.4             | 5.5              |
| Urea 0 Kg Ha⁻¹ + R             | 11.9    | 86.7   | 85.0    | 22.0             | 6.0              |
| Urea 0 Kg Ha⁻¹ + R+T           | 15.0    | 93.3   | 66.8    | 24.5             | 5.8              |
| Urea 0 Kg Ha⁻¹ + T             | 12.7    | 62.5   | 93.8    | 22.6             | 6.0              |
| Urea 50 Kg Ha⁻¹ + no inoculation| 13.2    | 74.2   | 68.0    | 23.2             | 6.5              |
| Urea 50 Kg Ha⁻¹ + R            | 12.6    | 67.5   | 77.3    | 24.7             | 7.2              |
| Urea 50 Kg Ha⁻¹ + R+T          | 10.7    | 73.3   | 70.3    | 22.8             | 6.0              |
| Urea 50 Kg Ha⁻¹ + T            | 10.1    | 58.3   | 94.0    | 22.4             | 6.2              |
| Urea 100 Kg Ha⁻¹ + no inoculation| 16.2    | 74.2   | 69.5    | 21.3             | 6.7              |
| Urea 100 Kg Ha⁻¹ + R           | 14.7    | 75.8   | 74.3    | 20.5             | 6.7              |
| Urea 100 Kg Ha⁻¹ + R+T         | 14.4    | 67.5   | 74.5    | 20.9             | 6.0              |
| Urea 100 Kg Ha⁻¹ + T           | 12.5    | 57.5   | 67.7    | 21.2             | 6.2              |
| Average                        | 12.9    | 71.7   | 75.5    | 22.3             | 6.2              |

DAP=Days After Planting, R=rhizobium, T=trichoderma, 20 DAP= vegetative phase/V stage, 35 DAP= flowering phase/R1 stage, 60 DAP= pod filling phase/R5 stage

The number of root nodules increased with increasing age of soybeans (Table 3). Root nodules reached the highest number in the pod filling phase reaching an average of 75.5 nodules, with maximum of 94.0 nodules (Table 3). The average number of root nodules at age 20 DAP (vegetative phase/V stage) was 12.9 nodules then rose significantly by 456% to 71.7 at age 35 DAP (flowering phase/R1 stage). At the age of 60 DAP (pod filling phase/R5 stage) there was no significant increase
compared to flowering phase. This was in accordance with the statement of [23] that the activity of the root nodules reached the maximum point in the certain phase. However, the number of root nodules has not reached a maximum value of 180 to more than 400 nodules as in the South African soybean genotype PAN809 [24]. The effectiveness level of soy inoculation varied in the rate of nodule formation, yield and nitrogen fixation results [17]. The same nodulation capability in all treatments lead to the length and volume of roots but did not differ significantly at the end of the study (Table 3). Soybean plants were able to form root nodules and utilized atmospheric N2 in association with rhizobia. It was crucial to use both N2 fixation by the root nodules and inorganic nitrogen assimilation by the roots to achieve the optimum yield [25].

3.3. Plant Nitrogen Uptake
In contrast to the number of nodules, plant nitrogen uptake was significantly affected by differences in urea dosage and microbial inoculation especially in the vegetative phase. In the initial phase of soybean growth, the uptake was greatly influenced by the treatment. However, entering the phase of flowering and filling pods, the absorption rate became the same. In the vegetative phase, the greater the N was available (the larger the urea fertilizer), the greater the absorption rate of N in the plant tissue. However, N uptake had the same rate between the use of urea 50 and 100 kg Ha-1 (Figure 2). Nitrogen total was a range of 1.0-3.3% for above ground plant tissues [26].

![Figure 2](image-url)

**Figure 2.** N total of plant tissue in the vegetative phase (20 DAP / V Stage) at dosage of urea 0, 50 and 100 kg Ha-1. The numbers followed by the same letter are not significantly different at the 5%.
The presence of microbial inoculation could increase the absorption of N total of plant tissues. [27] stated that rhizobium bacteria could increase the availability and absorption of nitrogen in the soil. [14] added that trichoderma was able to increase solubility of P and micronutrient and increase N uptake of plant tissue at the beginning of growth. Agronomic advantages from the soil inoculation with several microbes could be determined by the availability of nutrient for the microbe. Addition soil with PGPR and AMF (alone or in combination) were a valuable option to improve nutrient uptake [28].

In contrast to vegetative growth, N absorption in biomass during flowering and pod filling did not show any difference due to differences in combination of fertilizer use and microbial inoculation. In soils with several years of soybean history, N supply from the natural Rhizobium population was adequate [29]. The highest N uptake could be seen on the flowering period reaching 3.27%. After that, it dropped until 2.52% on the pod filling phase. [30] conveyed that the maximum levels of nitrogen fixation were reached at early pod fill when nitrate reductase activity had dropped to 25% of maximum activity. A rapid loss of nitrogen fixation activity occurred shortly after bean fill was initiated, again at a time when the ovules were developing at maximal rates. Leaf N content started to decrease after stage R5 in all soy cultivars, where it indicated the start of nitrogen redistribution [31]. The uptake of Nitrogen was more equally distributed between vegetative phase and pod-filling growth phase [32].

This result was in accordance to [33] which stated that the average of N plant tissue in soybean was 3.16% with combination of NPK and urea without legin. The highest N level of plant tissue was achieved by [34] reaching 3.77% on application of manure 10 tonHa-1. Nitrogen content in vegetative biomass (leaves plus stems) at R7 phase averaged 1.22%, with an overall range of 0.25–3.17% [35]. At the pod filling phase (60 DAP), the N uptake of plant tissue began to decrease (Figure 4). The decrease of N levels of plant tissue in this phase indicated that N of plant tissue has been transferred to seed.

![Graph showing N total of plant biomass (%) in the period of plant growth.](image)

**Figure 4.** N total of plant biomass (%) in the period of plant growth. 20 DAP= vegetative phase/V stage, 35 DAP= flowering phase/R1 stage, 60 DAP= pod filling phase/R5 stage

### 3.4. Number of Pod per Plant and Productivity

As the result of N uptake of plant biomass during the vegetative period, the number of pods was significantly influenced by the urea fertilizer (Figure 5). This showed that the number of soybean pod was strongly affected by nitrogen uptake in the vegetative period (V stage) and also determined by N available on the soil. The highest number of pods was produced on the use of 50 and 100 kg of Ha-1 urea. This indicated that the use of 50 Kg Ha-1 fertilizer was sufficient to form the optimal number of pods in Grobogan soybean varieties. On the other hand, the use of different microbial inoculations did not indicate a different performance in the number of soybean pods.
The combination of urea fertilizer and microbial inoculation also did not interact and did not affect the number of pods per plant. In general, the rate of filling pods in this study was very satisfactory which was marked by the achievement of 96.63% of fully filled pods (Table 4). The combination of urea fertilizer and microbial inoculation also did not interact and did not affect the number of pods per plant. In general, the rate of filling pods in this study was very satisfactory which was marked by the achievement of 96.63% of fully filled pods (Table 4).

**Figure 5.** Total Pods in urea 0, 50, 100 Kg Ha-1

**Table 4.** Total pods, filled pods, percentage of filled pods (%) and productivity (Ton Ha-1) in combination of urea and microbe inoculation

| Treatment                        | Total Pod | Filled Pod | Percentage of Filled Pods (%) | Productivity (Ton Ha-1) |
|----------------------------------|-----------|------------|-------------------------------|------------------------|
| Urea 0 Kg Ha-1 + no inoculation  | 28.43     | 27.75      | 97.60                         | 2.58 bc ± 0.16         |
| Urea 0 Kg Ha-1 + R               | 30.63     | 29.50      | 96.32                         | 2.69 bc ± 0.11         |
| Urea 0 Kg Ha-1 + R+T             | 29.03     | 28.30      | 97.48                         | 2.58 bc ± 0.11         |
| Urea 50 Kg Ha-1 + T              | 26.18     | 25.17      | 96.20                         | 2.21 a ± 0.36          |
| Urea 50 Kg Ha-1 + R              | 29.12     | 28.13      | 96.62                         | 2.49 abc ± 0.23        |
| Urea 50 Kg Ha-1 + R+T            | 31.13     | 30.15      | 96.81                         | 2.73 bc ± 0.08         |
| Urea 50 Kg Ha-1 + T              | 30.30     | 29.15      | 96.29                         | 2.55 bc ± 0.37         |
| Urea 100 Kg Ha-1 + no inoculation| 31.92     | 30.95      | 97.00                         | 2.65 bc ± 0.10         |
| Urea 100 Kg Ha-1 + R             | 31.67     | 30.72      | 96.97                         | 2.75 c ± 0.11          |
| Urea 100 Kg Ha-1 + R+T           | 30.33     | 29.32      | 96.54                         | 2.47 abc ± 0.25        |
| Urea 100 Kg Ha-1 + T             | 31.52     | 30.52      | 96.76                         | 2.55 bc ± 0.13         |
| Average                          | 30.00     | 28.99      | 96.63                         | 2.56±0.26              |

R=rhizobium, T=trichoderm

There was an interaction between urea fertilizer and microbial inoculation and this greatly affected the productivity of soybeans. The combination of 100 kgHa-1 urea with microbes and without microbes produced the highest productivity (Table 6). This indicated that high dosages of inorganic fertilizers remained a major source of N requirement because of its high availability. However, treatment without urea and without microbial inoculation (control) also achieved the highest production. This suggested that although the number of nodules did not differ between treatments, there was a native population of rhizobium bacteria strains that effectively supplied N for plants. The local N-fixing bacteria has been able to meet the needs of the plant [35]. In accordance with the history of the soil, the location of soybean planting was one of soybean production areas in Central Java province which indicated that there has been local rhizobium population. [36] said that
the type of legume that once grew in a field would affect the high population of local rhizobium on the land.

In contrast, the lowest yield was shown on the use of urea 0 kg ha\(^{-1}\) and tricoderma inoculation of 2.21 t ha\(^{-1}\). This suggests that there was a contradiction between plant height and productivity in which the highest crop was produced at the same treatment. Plant height could not be used to estimate crop productivity. The present findings were in conformity with the results obtained by [37] which indicated the height of the plant was negatively correlated with productivity. However, in general, soybean productivity was quite good. As [2] points out, soybean Grobogan variety had a yield potential of 3.4 t ha\(^{-1}\) and an average yield of 2.77 t ha\(^{-1}\).

4. Conclusion
Combination of urea dosage and microbe inoculations significantly influenced plant height and number of trifoliate leaves. Urea dosages and microbe inoculations or combination of both factors did not imply to nodulation process. Higher N dosage and microbe inoculations increased N uptake of biomass on vegetative period but had no effect on flowering and pod filling periods. The combination of 100 kgHa\(^{-1}\) urea with microbes or without microbes produced the highest productivity. However, treatment without urea and no inoculation also achieved the highest production. This suggested that there was a native population of rhizobium bacteria strains that effectively supplied N for plants. Besides, application of urea of 100 kg ha\(^{-1}\) or 46 N kg ha\(^{-1}\) was too small to interfere fixation nitrogen by local rhizobium population. Therefore, N application in form of urea or microbe for symbiotic N-fixation was not recommended for existing agro climatic conditions of area with soybean history.

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