AF-904
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RESEARCH ARTICLE Indian Journal of Agricultural Research

Effects of Nitrogen Fertilizer and Rhizobium on Nodule Growth and Yield of Soybean (*Glycine max* L.)

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ABSTRACT

Background: Soybean production in Indonesia needs to be improved to fulfill 9.15% of its demand. This causes the level of soybean imports in Indonesia to be very high. Production enquities and wants encourage the intensification of soybean cultivation. Intensification efforts are carried out with appropriate fertilization. Soybeans are a plant belong to Leguminaceae family so that they have a symbiotic relationship with many microorganisms, including rhizobium. The prime importance of conduct this experiment was to find out the impact of nitrogen fertilizer and rhizobium on nodule growth and soybean yield.

Methods: The experiment was conducted in two- factors in factorial completed randomized design. The first factor was the doses of nitrogen fertilizer (0, 50, 100 and 150 kg ha^{-1}). The second factor was Rhizobium inoculation (control, inoculant and soybean used soil). Data analysis was carried out by analysis of variance, if there is a significant difference, it was analysed with Duncan's Multiple Range Test (DMRT) at the 5% level of significance.

Result: Nitrogen fertilizer reduces the number of effective root nodules. Application of nitrogen resulted significantly increase the growth and yield attributes *viz* plant height, number of leaves, root shoot ratio, plant biomass, number of pods per plant and seed weight per plant. The optimum dose of nitrogen fertilizer to support soybean growth and yield was 95 kg ha^{-1}. Application of rhizobium can increase soybean yield. Application of rhizobium inoculation can increase the number of effective root nodules and seed weight per plant. Combined application of nitrogen and rhizobium can help in intensification of soybean cultivation in Indonesia.

Key words: Legume, Nitrogen fixation, Nodulation, Pods, Symbiotic.

INTRODUCTION

Soybean (*Glycine max* L.) is one of the food crop that Indonesian people demand. Soybean productivity in Indonesia is moderately good, but it still needs to meet the soybean demand of the Indonesian people. Based on data from the Central Bureau of Statistics (2022), the average soybean productivity in Indonesia in 2021 was 1.67 tons/ha (BPS, 2022). According to planting method, soybean productivity reaches add 1.758 tonnes/ha for the monoculture method and 1.275 tonnes/ha for intercropping. This shows that soybean had relatively high yield potential in Indonesia.

Plant cultivation separate connect words from each other providing fertilizer to sustain plant growth. Generally, farmers in soybean cultivation add inorganic fertilizers rather than organic fertilizers. Manik and Sebayar, (2019) stated that soybean yields can increase where organic and inorganic fertilizers was applied at the balance doses. Continuous use of inorganic fertilizers will harm the environment. Substituting inorganic fertilizer with organic fertilizer is one step to reduce the adverse impact on soil. The use of organic fertilizers can have a positive effect, namely improving the soil's physical, chemical and biological indicates. The application of biological fertilizer can also be done to reduce the use of inorganic fertilizer. In addition, organic fertilizers also help leach salts in the soil and improve soil chemical properties, such as reducing acidity (Han *et al.,* 2021). Furthermore, an increased application of organic matter such as compost generates an increase in the humic content of the soils, which also induces changes in biological soil properties, which, in turn, help to increase the growth and reproduction of useful macro- and microorganisms (Akhtar *et al.,* 2019). According to Liu *et al.* (2024), these organic materials also enhance the soil's carbon and nitrogen levels, contributing to enhanced agricultural soil fertility and productivity. Also, the composting process of organic fertilizers can help mitigate unprocessed organic waste and aid in reducing pollution engendered in the ecosystem (Kapila *et. al.,* 2024). The application of organic fertilizers has had beneficial impacts on soybean yields in both the short term and the long term.

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How to cite this article: Budiastuti, M.T.S., Setyaningrum, D., Supriyono and Purnomo, D. (2024). Effects of Nitrogen Fertilizer and Rhizobium on Nodule Growth and Yield of Soybean (*Glycine max* L.). Indian Journal of Agricultural Research. 1-6. doi: 10.18805/IJARe.AF-904.

Submitted: 01-08-2024 **Accepted:** 19-12-2024 **Online:** 07-01-2025

Microorganisms that have a good relationship with legume plants, especially soybeans, are Rhizobium bacteria. The symbiotic legume Rhizobium is essential for soybean and is considered a source of available nitrogen synthesized as a result of the biological fixation of atmospheric N2 by prokaryotic organisms (Krutyakov *et al*., 2022; Zou *et al*., 2019). Rhizobium will work in conditions where low nitrogen is available in the soil. According to Kibido *et al*. (2019), rhizobacteria of the Bradyrhizobium type interact under nitrogen-limiting conditions with soybean roots to develop root nodules. These bacteria fix nitrogen from the air through root nodules formed on soybean roots. Application of organic fertilizers can cause new interactions between legume plants and rhizobium, namely increasing symbiotic functions (Simonsen *et al.,* 2017). Early colonization by efficient N2-fixing rhizobia can also encourage positive feedbacks that increase plant nutrition and growth (Ramoneda *et al.,* 2021).

The element nitrogen has a vital role in plant growth; one additional source of nitrogen that is often used is urea fertilizer. According to Sikka *et al*. (2022), the optimum dose of urea applied when the nitrogen nutrient is met will increase pod formation. This happens because the nitrogen plays a vital role in the pods formation. When increase the availability of nitrogen will reduce the Rhizobium activates. Nitrogen availability is crucial in the legume-rhizobia symbiosis. However, higher levels of nitrogen fertilizer can hinder the establishment of this relationship. When soil nitrogen is high, particularly from seed inoculation to germination, forming an effective symbiosis becomes challenging. Excess nitrogen in the soil decreases the plant's reliance on nitrogen fixation and restricts the development of root nodules (Abd-Alla *et al.,* 2023). Keeping above facts in view this experiment was conducted to find out the effect of nitrogen application in association with rhizobium inoculation on growth and yield of Soybean in Indonesia.

MATERIALS AND METHODS

The research was carried out from February 2023 to February 2024. The experiment was conducted at the Agricultural Laboratory of Universitas Sebelas Maret, Jumantono, Karanganyar Regency, Central Java, Indonesia with an altitude of 148 meters above sea level with coordinates 07°37′831 South Latitude and 110°56′905 East Longitude and alfisol soil type. The research used a two-factor, factorial completed randomized design. The first factor was nitrogen, which consisted of 4 levels *viz*. control (N0) and nitrogen 50 kg ha^{-1} (N1); nitrogen 100 kg ha^{-1} (N2) and nitrogen 150 kg ha^{-1} (N3). The second factor was Rhizobium inoculation, which consisted of 3 levels, namely control (R0), *Rhizobium japonicum* inoculant (R1) and used soybean land (R2). Based on these factors, 12 treatment combinations were spelling obtained and repeated thrice, resulting in 36 experimental combinations. The stages of this research start from soil analysis, preparation of planting media, seed preparation, sowing, gapfilling, plant protection and harvesting.

The materials used in this research were polybags with a height of 26 cm, a length of 17.5 cm and a width of 17.5 cm, soil, soybean seeds of Devon 1 variety, urea fertilizer as a source of nitrogen, several sources of Rhizobium such as rhizobium inoculant and used planting soil: soybeans, SP36 fertilizer, KCL fertilizer, manure and pesticides. The tools used include tillage tools, rulers, Leaf Area Meters (LAM), ovens, analytical scales, cameras and knapsack sprayers. Observed variables were plant height (cm), number of leaves (per plant), number of nodes (per plant), leaf area (cm^2), root shoot ratio, fresh weight (g), plant biomass (g), number of nodules per plant, effective root nodules per plant, chlorophyll content (mg g^{-1}), light interception (%), root volume (ml), number of pods per plant, number of filled pods per plant, weight fresh pods (g), dry weight of pods (g), number of seeds per plant weight of seeds per plant (g) and weight of 100 seeds (g). The observation data obtained was analyzed using analysis of variance (ANOVA); if there were significant differences, it would be continued with Duncan's Multiple Range Test (DMRT) with a level of 5%.

RESULTS AND DISCUSSION

Soybean growth

Neither nitrogen nor rhizobium fertilizers have a significant effect on soybean plant height. Nisak and Supriyadi (2019) stated that soil used for soybean contained a total N of 0.10 g 100g^{-1}. The N content in the soil was very low. This might be due to nitrogen in the soil being available in low amounts due to leaching.

Soybean plant height ranges from 44 to 51 cm when maximum growth means it is lower than the description (±58.1 cm). Referring to the understanding that plant height is related to the addition of cells which are influenced by nitrogen, genetics and physiological factors (Li *et al.,* 2021), soybean growth was not in accordance with this opinion. The plant height of soybean was lower than the description. It can be said that the planting conditions are not ideal for soybean, namely with an average humidity of 80.5% which exceeds the ideal humidity for soybean which requires 65-75% humidity. The application of nitrogen fertilizer and rhizobium did not have a significant effect on the number of leaves. Nitrogen can not be released from the vegetative phase of plants which influences the formation of leaves with wider leaf blades and higher chlorophyll content (Fadlilah *et al.,* 2023). Similarly, in this study the application of nitrogen fertilizer did not increase the number of leaves. This is in line with the research results of Darini *et al*. (2020) which shows that applying 50 and 100 kg ha^{-1} of nitrogen provides almost the same number of leaves.

The research results of Gebremariam and Tesfay, (2022) who found that rhizobium inoculation at the doses of 5, 10, or 20 g did not provide a significant difference in the number of leaves. Rhizobium activity which was not yet optimal because lack of real effect. Borowska and Prusiński (2021) stated that humidity that is too high will result in a decrease in rhizobium activity. Nitrogen fixing activity by rhizobium will decrease if nitrogen levels in the soil are high (Wijayanti *et al.,* 2022). Based on data in Table 1, the application of nitrogen fertilizer and rhizobium did not have a significant effect on the number of nodes. This may occur as a result of the environmental conditions for growing soybean. The number of nodes on soybean plant can be influenced by genetic factors and environmental conditions, also the type of stem growth and length of exposure to light can also influence it (Simbolon *et al*., 2020). Prasetya *et al*. (2023) added that water availability can also influence the number of soybean nodes. Rhizobium inoculation from inoculant and used soil had an effect on the number of soybean nodes but was not significantly different. This might be due to nitrogen fixing activity of the rhizobium not being optimal. This is in line with Yusran *et al*. (2022) who explained that rhizobium activity will decrease when there is excess water, thereby increasing humidity. Nitrogen plays an important role in soybean vegetative growth. The availability of macro nutrients will stimulate cell division and enlargement in stem branch primordia which causes the number of nodes to increase (Hodijah *et al*., 2023).

Application of nitrogen or rhizobium fertilizer did not have a significant effect on plant biomass. Different nitrogen doses also showed relatively the same effect on soybean biomass. When less than optimal nitrogen content in the soil, only 0.06%, is thought to be the cause of the same effect. Many factors can remove nitrogen from the soil. Brar and Lawley (2020) explained that initial nitrogen fertilization has no effect on soybean biomass, but additional fertilization must be given.

Rhizobium inoculation from used soil can increase soybean biomass production. Panjaitan *et al*. (2023) in their research showed that rhizobium can increase soybean biomass at the maximum vegetative phase. The highest biomass was shown by soybeans treated with used soybean soil (16.18 g), while the lowest was shown by inoculant (10.91 g). The increase in biomass due to used soil was around 20.83% compared to the control. Rhizobium that successfully form root nodules can fix the nitrogen that plants need.

Nodulation

The data in Table 2 revealed that application of nitrogen fertilizer or rhizobium did not significantly affect the number of root nodules. Referring to the notion that applying high N fertilizer will inhibit the formation of nodules by rhizobium (Mayani and Hapsoh 2011), the number of soybean root nodules does not match this opinion. Different doses of nitrogen fertilizer showed no significant effect on the number of root nodules. The research of Szpunar-Krok *et al.* (2023) showed that nitrogen fertilizer doses of 30 and 60 kg ha^{-1} had the same effect on the number of nodules because the nitrogen dose used was too low.

Rhizobium inoculation also reflected non significant different on the number of root nodules. Rhizobium activity, which still needs to be optimal, is thought to cause a lack of real impact. Rhizobium activity is closely related to root nodules formed on legume roots (Gebrehana and Dagnaw 2020). This is in line with research by Riviezzi *et al.* (2020), which showed that rhizobium inoculation did not affect the number of root nodules.

Data in Table 2 reveals that, application of nitrogen and rhizobium fertilizer did not significantly affect effective root nodules. The difference in nitrogen fertilizer dosage showed an actual. The lower available nitrogen, the greater effectiveness of root nodules. This aligns with research by Ningsih *et al.* (2020), which shows the effect of nitrogen dose on effective root nodules, with the control treatment having the highest average effective root nodules. Without nitrogen fertilizer, there were 120.22 effective root nodules, the highest average compared to other nitrogen dose treatments. Rhizobium inoculation showed the same effect on effective root nodules of soybean. This lack of real influence is thought to be because the symbiosis between rhizobium and soybean roots is not optimal due to

Tabel 1: Effect of nitrogen and rhizobium inoculation on plant height, number of leaves, number of nodes, leaf area, root shoot ratio, fresh weight and plant biomass.

Treatment	Plant height (cm)	Number of leaves (sheet)	Root shoot ratio	Plant biomass (g)
Nitrogen dosage (kg ha^{-1})				
0	44.31	61.33	2.21	13.02
50	50.15	69.00	2.29	12.38
100	49.77	67.50	2.16	12.47
150	48.38	65.08	2.75	16.09
Rhizobium				
Control	48.63	64.50	2.36	13.39a
Inoculant	47.19	64.75	2.13	10.91a
Land used for soybean planting	48.63	67.94	2.56	16.18b
Interaction	-	-	-	-

Numbers followed by the same notation in the same column group indicate no significant difference in DMRT at the 5% level. Negative notation (-) indicates no interaction.

Table 2: Effect of nitrogen and rhizobium incoulation on number of root nodules, effective root nodules, light interception and root volume.

Treatment	Number of root nodules per plant	Effective root nodules	Root volume (ml)
Nitrogen dosage (kg ha^{-1})			
0	155.44	120.22b	124.44
50	148.56	110.56b	122.16
100	140.44	74.22a	116.67
150	143.44	74.67a	101.67
Rhizobium			
Control	145.92	64.50	106.20
Inoculant	155.58	64.75	121.67
Land used for soybean planting	139.42	67.94	120.83
Interaction	-	-	-

Numbers followed by the same notation in the same column group indicate no significant difference in DMRT at the 5% level. Negative notation (-) indicates no interaction.

Table 3: Effect of nitrogen and rhizobium inoculation on growth and yield attributes of soybean.

Treatment	Number of pods per plant	Number of seeds per plant
Nitrogen dosage (kg ha^{-1})		
0	197.33	329.78
50	256.67	390.67
100	236.67	464.11
150	202.11	381.44
Rhizobium inoculation		
Control	203.75	332.92a
Inoculant	217.42	367.75a
Land used for soybean planting	248.42	473.83b
Interaction	-	-

Numbers followed by the same notation in the same column group indicate no significant difference in DMRT at the 5% level. Negative notation (-) indicates no interaction.

unsuitable conditions. Mutmainah *et al.* (2022) showed that rhizobium inoculation did not really affect root nodules because symbiosis had not occurred optimally due to unsuitable environmental conditions.

Soybean yield

Based on the results in Table 3, the application of nitrogen and rhizobium fertilizer did not significantly influenced the number of pods per plant. This same effect was because nitrogen plays a role in the vegetative phase of soybeans, so in the generative phase, its role is less than optimal. Nget *et al*. (2022) explained that macronutrient plays a critical role and is needed in the formation of soybean pods and seeds by phosphorus application. Rhizobium inoculation showed not significant effect on the number of pods. This is in line with Soverda *et al*. (2021), which shows that there is no significant influence of rhizobium on the number of pods due to environmental conditions and the availability of nutrients in the soil, so the application of rhizobium was not optimal. Kurniawan and Sunaryo (2020) explained that the number and size of nodules influences the ability of rhizobium to fix nitrogen, the bigger the nodules or the more nodules, the greater the nitrogen that is fixed. Application of nitrogen or rhizobium fertilizer did not significantly influenced seed weight per plant. Low nitrogen availability was the main reason of not effect. Shukla *et al.* (2024) explained that formation and ripening phases of soybean seeds require the availability of sufficient quantity of nitrogen. Rhizobium inoculation reflected significantly different effects on soybean seed weight. The average weight of soybean seeds indicated by the used soybean soil was 47.30 g, the highest compared to other treatments, with an increase of around 36.66%. Nitrogen, fixed by the rhizobium through root nodules, plays a crtical role in seed formation. This is in line with research by Htwe *et al*. (2019), which shows that inoculation of rhizobium as a bio-fertilizer can increase seed weight as an illustration of soybean production compared to without rhizobium.

CONCLUSION

Higher dose of nitrogen fertilizer reduces the number of effective root nodules in soybean. Besides, application of nitrogen resulted increase in plant height, number of leaves, root:shoot ratio, biomass, number of pods and seed weight. The optimum dose of nitrogen to support for soybean growth and yield was 95 kg ha^{-1}. Application of rhizobium can increase soybean yield by 11% variable number of seeds per plant. Application of rhizobium inoculation can increase the number of effective root nodules and seed weight. The combination use of nitrogen and rhizobium inoculation can support the intensification of soybean cultivation in Indonesia.

ACKNOWLEDGMENT

Thank you to Sebelas Maret University for providing funding for this research through the Research Grant Group Research with funding sources from Non-State Revenue and Expenditure Budget with contract number: 194.2/ UN27.22/PT.01.03/2024.

Disclaimers

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Conflict of interest

The authors declare no conflict of interest.

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