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The Effect of Rhizobium sp Inoculation and Nitrogen Fertilizer Application on the Growth, Yield, and Protein Content of Jack Bean (*Canavalia Ensiformis L*) Seeds

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Abstract:
Indonesia has a wealth of leguminous plants, one of which is Jack Bean (*Canavalia ensiformis L*). Jack Bean has great potential as an alternative food source to replace Soybeans and as an easily accessible source of protein at an affordable price. The objective of this study was to determine the effect of rhizobium inoculation and nitrogen fertilizer application on the growth, yield, and protein content of Jack Bean seeds. This study was conducted from June to November 2025 in Kaliaren Village, Kuningan, West Java, Indonesia. The study employed a Randomized Block Design (RBD) experimental method, consisting of 6 treatment combinations of nitrogen fertilizer dosage and rhizobium inoculation, repeated 4 times. Observation parameters included plant height, stem diameter, number of branches, RGR, NAR, weight of root nodules and weight of active root nodules (growth), number of pods, pod weight, dry seed weight (yield), and seed protein content. The results showed that there was no significant difference between treatments in terms of the protein content of Jack Beans, but the treatment of 200 kg N/ha and 0 g Rhizobium sp/kg seeds. had a significant effect on the growth and yield of Jack Beans.

Keywords: Nitrogen, Protein, Growth, Yield, Jack Bean

INTRODUCTION

Jack Beans (*Canavalia ensiformis L*) are tropical plants rich in protein and carbohydrates. These plants are found and cultivated in various regions, including Africa, Asia, India, and Latin America (Arnanto et al., 2024). Jack Beans are quite adaptable and can grow well in suboptimal and marginal soils, including dry or acidic soils (Sakinah, 2023). Jack Beans are a source of vitamin B1, various minerals, and dietary fibre, which are important for maintaining health. (Shabrina, 2017), Applicationally, Jack Beans contain 30.36% protein. When dry, Jack Beans contain 66% carbohydrates and 2.6% fat

(Prasetyowati et al., 2019). Jack Beans have great potential as an alternative food source and protein source.

According to data from BBPP Lembang in 2021, Jack Beans are not yet widely cultivated. This commodity is only widely grown in Central Java, such as in the districts of Blora, Temanggung, Grobogan, Banjarnegara, Wonogiri, Kebumen, Boyolali, and Batang. Applicationally, the production of Jack Beans in 2016 reached 12 tons per hectare (Food and Agriculture Research and Development Agency, 2016). Given this opportunity, efforts to increase Jack Beans production are necessary to meet domestic market demand.

Efforts to increase Jack Bean production must be supported by appropriate cultivation techniques. Some cultivation techniques that can be implemented include the use of plant nutrients as soil fertility enhancers or fertilization (Rom & IP, 2021). Based on their role, fertilizers are classified as essential and non-essential nutrients. Essential nutrients are those whose role cannot be replaced by other elements (Hidayat & Hidayat, 2020). One of the essential nutrients for plants is nitrogen. Nitrogen is very important in plant growth because it is one of the components of plant cells (Rahmadani et al., 2020). In application, nitrogen is also a component of protoplasm, chlorophyll, nucleic acids, and amino acids, which are the main components of proteins (Zega & Lase, 2025). This element enters the soil in various ways, such as in the form of ammonia and nitrate (NH_3) carried by rainwater (Cassim et al., 2024), the result of nitrogen fixation by microorganisms (Mendrofa et al., 2024), or through the application of artificial fertilizers. The presence and availability of nitrogen compounds in the soil are very limited due to the nature of nitrogen compounds, which are easily lost (leaching) (Suparto, 2018). Therefore, the utilization of free N_2 from the air through fixation is important to increase the availability of nitrogen for plants. Bacteria capable of binding free N_2 are of the genus Rhizobium (Meitasari & Wicaksono, 2017). Rhizobium bacteria are a group of bacteria that have the ability to provide nutrients for plants. When associated with legume plants, these bacteria infect the plant roots and form structures called root nodules. Rhizobium can only fix nitrogen from the atmosphere when it is inside the root nodules of its host plant (Sari & Prayudyaningsih, 2015).

Previous research on the use of Rhizobium sp and the application of nitrogen fertilizer in Jack Bean cultivation explains that the application of NPK at a dose of 200 kg/ha and a rhizosphere microbial concentration of 4% increases the pod weight per plant, and produces the highest seed weight per hectare of Jack Bean (Kurniawati et al., 2021), as well as the report (Kurniawan et al., 2020) states that NPK fertilizer treatment at a dose of 400 kg/ha accompanied by rhizobium treatment resulted in a higher total number of root nodules and active roots in both grumusal and sandy soils. Then, (Velhal et al., 2014) show that the application of Rhizobium inoculant significantly increases the growth and yield of *Lablab purpureus* plants. (Darini & Kusdiarti, 2017) explain that applying 75 kg of urea per hectare can increase the growth and yield of White Jack Bean by 42.64%. The report (Safira et al., 2019) states that applying 200 kg/ha of urea fertilizer can produce 31.31% crude protein from Jack Bean's plant.

The combination of Rhizobium inoculation doses with nitrogen fertilizer used in Jack Bean cultivation needs further research to achieve maximum productivity and quality. This study aims to determine the optimal combination of Rhizobium inoculation with nitrogen fertilizer used in producing the yield, quality, and protein content of Jack Bean seeds.

LITERATURE

One way to improve Jack Bean plant growth and crop quality is through fertilization. Fertilization is the process of adding nutrients to plants. There are several types of fertilizers, namely organic, inorganic, and biofertilizers, all of which serve to provide nutrients for plants. The combination of two treatments, urea fertilizer (inorganic) at the right dose and biofertilizer (*Rhizobium sp*), where these bacteria can be found in the soil and form a symbiotic relationship with the roots of leguminous plants, is expected to support growth and improve the quality of Jack Bean crop yields. (Suryono & Sudadi, 2015) states that the best treatment to increase the fresh weight of the plant is nitrogen fertilizer at a dose of 150 kg/ha. This is because the nitrogen requirement for peanut plant growth is available and sufficient in this treatment. Applicationally, the study (Km, 2017) mentions that urea fertilizer at a dose of 100 kg/ha can increase the weight of 100 legume seeds by 10.17 g. Furthermore, the report (Laili, 2019) shows that the application of 100 kg/ha of N and K fertilizer on Mung Bean plants resulted in a dry weight per plant of 5.34 g/plant, which is 22.48% higher than the application of 25 kg/ha of N and K fertilizer.

METHOD

This study was conducted from June 2025 to November 2025 on land in Kaliaren Village, Kuningan, West Java, Indonesia. The tools used during the study were hoes, shovels, mini hoes, wood, scissors, rope, sacks, measuring tapes, callipers, digital scales, ovens, books, pens, Labels, etc. The materials used in the research were nitrogen fertilizer, *Rhizobium sp* inoculant, Jack Bean seeds, and pesticides.

This study used a randomized block design (RBD) with one factor, consisting of six treatments combining N fertilizer doses and Rhizobium inoculation, namely A (0 g Rhizobium sp/kg seeds and 0 kg N/ha), B (0 g Rhizobium sp/kg seeds and 100 kg N/ha), C (0 g Rhizobium sp/kg seeds and 200 kg N/ha), D (10 g Rhizobium sp/kg seeds and 0 kg N/ha), E (10 g Rhizobium sp/kg seeds and 100 kg N/ha), F (10 g Rhizobium sp/kg seeds and 200 kg N/ha). The experiment was repeated 4 times, resulting in 24 experimental units. Each unit had an area of 6 m (2 x 3 m), with a population of 24 plants using a planting distance of 50 cm x 50 cm.

Growth observation parameters were measured at four time points: when the plants were 4, 6, 8, and 12 weeks after planting (WAP). The parameters observed were: plant height, stem diameter, number of branches, RGR calculated using the formula $\frac{InW2 - InW1}{t2-t1}$, NAR calculated using the formula $\frac{(w2-w1)}{(A2-A1)}$ X $\frac{(InA2-InA1)}{(T2-T1)}$, weight of root nodules and weight of active root nodules, and yield observation parameters conducted at 16, 17, and 18 WAP, including: number of pods, pod weight, and dry seed weight. Protein content in seeds was also observed.

The observation data were analyzed using the analysis of variance (ANOVA) at a 5% level, and if there was a significant effect between treatments, a multiple range test or Duncan's Multiple Range Test (DMRT) was performed at a 5% level using SPSS version 25.

DISCUSSION

Soil Analysis Results of the Experimental Plot

The results of soil structure and nutrient analysis in Kaliaren Village, Cilimus District, Kuningan Regency, West Java, Indonesia, are presented below (Table 1). Soil analysis shows a low total nitrogen concentration, thus requiring the use of nitrogen fertilizer. Meanwhile, the soil pH is suitable for cultivating Jack Bean (*Canavalia ensiformis L*), which can grow optimally within a pH range of 5.5–6.5 (Sakinah, 2023).

Table 1. Soil Analysis Results.

No.	Parameters	Results	Units
1	Moisture Content	7,87	%
2	pH H_2O	6,2	-
3	C Organic	0,85	%
4	N Total	0,13	%
5	C/N	6,5	-
6	P_2O_5 HCI 25%	96	mg/100g
7	K_2O HCI 25%	16	mg/100g
8	P (Available Olsen)	35,37	mg/kg
9	Cation Exchange Capacity	18,38	cmol[+]/kg
10	Zn	1,24	mg/kg

Plan Height

In this study, there was a significant difference in the use of N fertilizer at different doses, with a tendency that the higher the dose of N fertilizer applied, the taller the plants grew. These differences were observed at 4, 6, 8, and 12 weeks after planting, with the tallest plants achieved by the treatment using 200 kg of N fertilizer without the application of Rhizobium. The results of Duncan's test for the average plant height between treatments are presented in Table 2.

Table 2. Table of Average Plant Height of Jack Bean at 4, 6, 8, 12 Weeks After Planting.

Treatment	**Plant Height (cm)**			
	4 WAP	**6 WAP**	**8 WAP**	**12 WAP**
A (0 g Rhizobium sp/kg seeds and 0 kg N/ha)	39 ab	51 ab	63 ab	67 abc
B (0 g Rhizobium sp/kg seeds and 100 kg N/ha)	50 bc	62 bc	68 abc	70 bc
C (0 g Rhizobium sp/kg seeds and 200 kg N/ha)	54 c	68 c	76 c	78 c
D (10 g Rhizobium sp/kg seeds and 0 kg N/ha)	36 a	49 a	56 a	57 a
E (10 g Rhizobium sp/kg seeds and 100 kg N/ha)	44 abc	58 abc	64 abc	65 ab
F (10 g Rhizobium sp/kg seeds and 200 kg N/ha)	47 abc	62 c	69 bc	71 bc

Note : Means followed by the same letter in the same column indicate no significant difference based on Duncan's Multiple Range Test (DMRT) at the 5% level.

The most optimal plant height growth was achieved by treatment C (0 g Rhizobium sp/kg seed and 200 kg N/ha) and had a significant effect compared to treatment A (0 g

Rhizobium sp/kg seed and 0 kg N/ha) and treatment D (10 g Rhizobium sp/kg seed and 0 kg N/ha) at almost every observation time. The application of higher doses of N fertilizer can increase plant height growth, presumably because nitrogen nutrients are able to fertilize plants in the vegetative phase. Nitrogen is an element that has a rapid impact on plant growth. When nitrogen is sufficient, the vegetative parts of the plant are bright green to dark green. Nitrogen also acts as a regulator to control the use of fertilizer elements such as potassium and phosphorus (Supandji et al., 2021). This is further supported by the report (Lewar et al., 2020), which states that treatment with a combination of NPK plus fertilizer at a dose of 250 kg/ha can produce the best plant height at 2 WAP for the Inerie variety of Red Beans.

Stem Diameter

Stem diameter is one of the important parameters that can be an indicator of the success of the plant growth phase. Table 3 shows that there are significant differences between Rhizobium inoculation and N fertilizer application treatments in terms of stem diameter observed at 4, 6, 8, and 12 weeks after planting.

Table 3.Table of Average Stem Diameter of Jack Bean at 4, 6, 8, and 12 Weeks After Planting.

Treatment	Stem Diameter (mm)			
	4 WAP	**6 WAP**	**8 WAP**	**12 WAP**
A (0 g Rhizobium sp/kg seeds and 0 kg N/ha)	6,8 a	8,5 a	9,8 a	11,5 a
B (0 g Rhizobium sp/kg seeds and 100 kg N/ha)	8,6 bc	10,6 ab	12,9 b	14,1 bc
C (0 g Rhizobium sp/kg seeds and 200 kg N/ha)	9,2 c	11,9 ab	15,8 c	17,7 d
D (10 g Rhizobium sp/kg seeds and 0 kg N/ha)	7,7 ab	9 ab	10,4 a	11,9 ab
E (10 g Rhizobium sp/kg seeds and 100 kg N/ha)	8,7 bc	11,1 ab	13,7 bc	14,8 c
F (10 g Rhizobium sp/kg seeds and 200 kg N/ha)	8,3 bc	12,6 b	13,2 b	14,5 bc

Note : Means followed by the same letter in the same column indicate no significant difference based on Duncan's Multiple Range Test (DMRT) at the 5% level.

The largest stem diameter was achieved by plants treated with treatment C (0 g Rhizobium sp/kg seed and 200 kg N/ha), namely 17.7 cm, and showed a significant difference compared to treatment A (0 g Rhizobium sp/kg seed and 0 kg N/ha) and treatment D (10 g Rhizobium sp/kg seed and 0 kg N/ha) at almost every observation time. Rhizobium inoculation had a more significant effect on stem diameter growth when combined with N fertilizer at a dose of 100 kg/ha, and without N fertilizer. Meanwhile, when combined with

N fertilizer at a dose of 200 kg/ha, Rhizobium application showed lower results. The report (Yusdian et al., 2018) states that nitrogen is an important nutrient for increasing plant growth, promoting healthy leaf growth, broad leaves with a greener colour, and is generally essential for the formation or growth of vegetative parts of plants, such as leaves, stems, and roots. This is supported by the report (Lewar et al., 2020), which states that treatment with a combination of NPK fertilizer at a dose of 250 kg/ha can produce the best stem diameter at observation ages of 2 and 4 WAP for the Inerie variety of Red Beans.

Number of Branches

Due to its influence on plant physiological processes, the number of branches is an important parameter in plant growth. In this study, treatment C (0 g Rhizobium sp/kg seed and 200 kg N/ha) showed a significant difference compared to treatment D (10 g Rhizobium sp/kg seed and 0 kg N/ha), as measured at 4, 6, 8, and 12 weeks after planting.

Table 4. Table of Average Number of Branches of Jack Beans at 4, 6, 8, and 12 Weeks After Planting.

Treatment	**Number Of Branches**			
	4 WAP	**6 WAP**	**8 WAP**	**12 WAP**
A (0 g Rhizobium sp/kg seeds and 0 kg N/ha)	9a	18 a	24 ab	26 ab
B (0 g Rhizobium sp/kg seeds and 100 kg N/ha)	12 b	20 ab	25 abc	25 ab
C (0 g Rhizobium sp/kg seeds and 200 kg N/ha)	14 b	25 c	28 c	29 b
D (10 g Rhizobium sp/kg seeds and 0 kg N/ha)	9a	17 a	21 a	22 a
E (10 g Rhizobium sp/kg seeds and 100 kg N/ha)	12 ab	22 bc	25 bc	26 ab
F (10 g Rhizobium sp/kg seeds and 200 kg N/ha)	12 b	22 bc	27 bc	27 b

Note : Means followed by the same letter in the same column indicate no significant difference based on Duncan's Multiple Range Test (DMRT) at the 5% level.

From the data presented in the table above, treatment C (0 g Rhizobium sp/kg seed and 200 kg N/ha) had the highest number of branches consistently in each week of observation. Meanwhile, treatment D (10 g Rhizobium sp/kg seed and 0 kg N/ha) always had the lowest results compared to other treatments. The number of branches can be attributed to the sufficient nitrogen content in the process of branch growth in Jack Beans. According to (Wahyudin et al., 2015), nitrogen is one of the essential macronutrients for plants, which is needed in the formation and vegetative growth of plants and as a basic material for protein synthesis and chlorophyll formation. This is reinforced by the report

(Supandji et al., 2020) stating that treatment with a fertilizer dose of N 200 kg/ha can have a significant effect on the growth of bean plant branches.

Weight of Root Nodules

The weight of root nodules is an important parameter to observe in order to understand how Jack Bean plants can form a mutualistic symbiosis with Rhizobium sp. by forming root nodules that can then help meet nitrogen requirements by fixing free nitrogen from the air.

Table 5. Table of Average Weight of Root Nodules in Jack Bean Plants at 4, 6, 8, and 12 WAP.

Treatment	**Weight of Root Nodules (g)**			
	4 WAP	**6WAP**	**8 WAP**	**12 WAP**
A (0 g Rhizobium sp/kg seeds and 0 kg N/ha)	3,75 a	7,38 a	11,14 ab	14,14 ab
B (0 g Rhizobium sp/kg seeds and 100 kg N/ha)	5,75 a	9,28 a	13,88 ab	16,94 ab
C (0 g Rhizobium sp/kg seeds and 200 kg N/ha)	4,73 a	4,95 a	14,42 ab	20,06 b
D (10 g Rhizobium sp/kg seeds and 0 kg N/ha)	2,55 a	9,63 a	16,72 b	21,47 b
E (10 g Rhizobium sp/kg seeds and 100 kg N/ha)	1,20 a	6,15 a	15,08 ab	20,52 b
F (10 g Rhizobium sp/kg seeds and 200 kg N/ha)	1,28 a	7,25 a	3,48 a	5,63 a

Note : Means followed by the same letter in the same column indicate no significant difference based on Duncan's Multiple Range Test (DMRT) at the 5% level.

Table 5 shows that at 4 and 8 WAP, treatment D (10 g Rhizobium sp/kg seed and 0 kg N/ha) had the highest weight of root nodules and had a significant effect compared to treatment F (10 g Rhizobium sp/kg seed and 200 kg N/ha), which had the lowest weight of root nodules. This is thought to occur because the N fertilizer dose of 200 kg actually inhibits the activity of Rhizobium to form a symbiotic relationship with the plant, as the N requirement is already met by urea fertilizer, (Idiyah, 1997) states that urea fertilizer applied at sufficiently high rates will cause biological fixation activity to become less effective, and the report (Hodiyah & Suhardjadinata, 2020) states that the combination of Rhizobium 10 g and 0 kg urea treatment on Green Bean plants has a significant effect on the number of root nodules formed.

Weight of Active Root Nodules

This parameter serves to determine how effective the root nodules formed are in absorbing N nutrients in the air, because the weight of active root nodules determines the N fixation process by Rhizobium bacteria.

Table 6. Table of Average Weight of Active Root Nodules in Jack Bean at 4, 6, 8, and 12 WAP.

Treatment	**Weight Of Active Root Nodules (g)**			
	4 WAP	**6 WAP**	**8 WAP**	**12 WAP**
A (0 g Rhizobium sp/kg seeds and 0 kg N/ha)	3,6 a	7,23 a	10,25 a	12,71 ab
B (0 g Rhizobium sp/kg seeds and 100 kg N/ha)	5,45 a	9,13 a	10,91 a	13,93 ab
C (0 g Rhizobium sp/kg seeds and 200 kg N/ha)	4,53 a	4,83 a	11,43 a	16,70 ab
D (10 g Rhizobium sp/kg seeds and 0 kg N/ha)	2,28 a	9,43 a	11,74 a	15,62 ab
E (10 g Rhizobium sp/kg seeds and 100 kg N/ha)	1,08 a	5,98 a	12,72 a	18 b
F (10 g Rhizobium sp/kg seeds and 200 kg N/ha)	1,15 a	7,05 a	3,41 a	5,45 a

Note : Means followed by the same letter in the same column indicate no significant difference based on Duncan's Multiple Range Test (DMRT) at the 5% level.

Table 6 shows the results at 12 WAP. Treatment E (10 g Rhizobium sp/kg seed and 100 kg N/ha) obtained the highest average and also had a significant effect compared to treatment F (10 g Rhizobium sp/kg seed and 200 kg N/ha) on the parameter of effective root nodules. This is thought to be due to the combination of treatment E (10 g Rhizobium sp/kg seed and 100 kg N/ha) being the appropriate dose for the formation of effective root nodules, so that the plants obtain a balanced supply of N nutrients from both urea fertilizer and N fixation by Rhizobium bacteria. This finding is supported by the results of the study (Saputra, 2022), which showed that the application of urea, TSP, and KCL at 8.1 g/plot (100 kg/ha) each had a significant effect on the average percentage of effective root nodules of 75.99% in Soybean plants, as well as the report (Hodiyah & Milati, 2022) that the application of Rhizobium sp inoculum at a dose of 10 g/kg of seeds had a significant effect on the number of effective root nodules in peanut plants.

Relative Growth Rate

Relative growth rate observation is an effort to determine the increase in dry weight of a plant within a certain time interval. Observations were conducted at two-week intervals when the plants were 4-6 WAP and 6-8 WAP old.

Table 7. Table of Average Relative Growth Rates of Jack Bean Plants.

Treatment	**Relatif Growth Rate (g/g/day)**	
	4 - 6 WAP	**6 - 8 WAP**
A (0 g Rhizobium sp/kg seeds and 0 kg N/ha)	0,079 a	0,020 a
B (0 g Rhizobium sp/kg seeds and 100 kg N/ha)	0,084 a	0,017 a
C (0 g Rhizobium sp/kg seeds and 200 kg N/ha)	0,066 a	0,030 a
D (10 g Rhizobium sp/kg seeds and 0 kg N/ha)	0,062 a	0,018 a
E (10 g Rhizobium sp/kg seeds and 100 kg N/ha)	0,077 a	0,021 a
F (10 g Rhizobium sp/kg seeds and 200 kg N/ha)	0,079 a	0,024 a

Note : Means followed by the same letter in the same column indicate no significant difference based on Duncan's Multiple Range Test (DMRT) at the 5% level.

Table 7 shows the results. During the 4-6 WAP period, all treatment combinations showed relatively uniform values, ranging from 0.062 to 0.084. There was no apparent effect from the application of Rhizobium or nitrogen fertilizer.

A significant decrease in RGR in all treatments entering the 6-8 WAP phase indicates a shift in the plant growth phase. Annual plants such as Jack Bean will shift the allocation of carbon and nitrogen resources from leaf and stem formation (vegetative growth) to seed formation and filling (generative growth) after reaching a certain phase.

Net Assimilation Rate

Net assimilation rate (NAR) is the ability of plants to produce dry matter from assimilation per unit leaf area per unit time (g/cm²/week). NAR is highest when the plants are still small, and most of their leaves are exposed to direct sunlight. NAR is likely to decrease as the leaf area increases, preventing optimal photosynthesis.

Table 8. Table of Average Net Assimilation Rates of Jack Bean Plants.

Treatment	**Net Assimilation Rate (g/cm^2/day)**	
	4 - 6 WAP	**6 - 8 WAP**
A (0 g Rhizobium sp/kg seeds and 0 kg N/ha)	0,0028 a	0,0009 a
B (0 g Rhizobium sp/kg seeds and 100 kg N/ha)	0,0025 a	0,0007 a
C (0 g Rhizobium sp/kg seeds and 200 kg N/ha)	0,0022 a	0,0012 a
D (10 g Rhizobium sp/kg seeds and 0 kg N/ha)	0,0016 a	0,0005 a
E (10 g Rhizobium sp/kg seeds and 100 kg N/ha)	0,0016 a	0,0006 a
F (10 g Rhizobium sp/kg seeds and 200 kg N/ha)	0,0028 a	0,0009 a

Note : Means followed by the same letter in the same column indicate no significant difference based on Duncan's Multiple Range Test (DMRT) at the 5% level.

Table 8 shows the results of the analysis of net assimilation rate tested with a single factor Randomized Block Design (RBD), which shows an interesting pattern, even though statistically there are no significant differences between treatments in the same observation period. In the 4-6 WAP phase, the net assimilation rate tends to be higher than in the 6-8 WAP phase, indicating a decrease in net photosynthetic activity as the plant enters the next phase of development. The net assimilation rate was highest when the plants were still small, and most of their leaves were exposed to direct sunlight. Maximum light reception by plant leaves can increase the net assimilation rate in plants.

Number of Pods per Plant

The number of pods parameter falls into the category of observation parameters for Jack Bean crop yield. The combination of Rhizobium inoculation and N fertilizer treatments had a significant effect on the number of Jack Bean pods in each experimental treatment.

Table 9. Table of Average Number Pod Yield per Plant.

Treatment	**Number of Pods**
A (0 g Rhizobium sp/kg seeds and 0 kg N/ha)	6a
B (0 g Rhizobium sp/kg seeds and 100 kg N/ha)	8 ab
C (0 g Rhizobium sp/kg seeds and 200 kg N/ha)	10 bc

D (10 g Rhizobium sp/kg seeds and 0 kg N/ha)	6a
E (10 g Rhizobium sp/kg seeds and 100 kg N/ha)	11 c
F (10 g Rhizobium sp/kg seeds and 200 kg N/ha)	9 abc

Note : Means followed by the same letter in the same column indicate no significant difference based on Duncan's Multiple Range Test (DMRT) at the 5% level.

Table 9 shows that treatment E (10 g Rhizobium sp/kg seed and 100 kg N/ha) had the highest average yield and had a significant effect on the number of pods compared to treatment B (0 g Rhizobium sp/kg seed and 100 kg N/ha), A (0 g Rhizobium sp/kg seed and 0 kg N/ha) and D (10 g Rhizobium sp/kg seed and 0 kg N/ha), which had the lowest average yield of only 6 pods. This increase in yield is thought to be related to the synergy between biological nitrogen fixation by rhizobia and the availability of applicational nitrogen from fertilizer. This phenomenon is supported by the study (Oladosu et al., 2018), which found that the combination of Rhizobium inoculant and the right dose of nitrogen in peanuts produced the highest number of pods because the applicational nitrogen supported early plant growth before symbiotic fixation became effective, while Rhizobium supplied nitrogen continuously during the generative phase. Applicationally, the report (Meitasari & Wicaksono, 2017) states that rhizobium inoculation plays a role in stimulating nodule formation, and nodules help supply nitrogen, which in turn promotes the formation of proteins, protoplasm, and chlorophyll, ultimately aiding the pod formation process.

Pod Weight per Plant

The weight of pods per plant is a direct indicator of total crop yield. Optimal seed weight indicates physiological maturity and good seed quality for replanting or further processing. The results of Duncan's test on the weight of pods per plant in this study are presented in Table 10.

Table 10. Table of Average Pod Weight per Plant.

Treatment	Pod Weight (g)
A (0 g Rhizobium sp/kg seeds and 0 kg N/ha)	261 ab
B (0 g Rhizobium sp/kg seeds and 100 kg N/ha)	260 ab
C (0 g Rhizobium sp/kg seeds and 200 kg N/ha)	370 b
D (10 g Rhizobium sp/kg seeds and 0 kg N/ha)	233 a
E (10 g Rhizobium sp/kg seeds and 100 kg N/ha)	245 ab
F (10 g Rhizobium sp/kg seeds and 200 kg N/ha)	292 ab

Note : Means followed by the same letter in the same column indicate no significant difference based on Duncan's Multiple Range Test (DMRT) at the 5% level.

Table 10 shows that Treatment C (0 g Rhizobium sp/kg seeds and 200 kg N/ha) had a significant effect compared to Treatment D (10 g Rhizobium sp/kg seeds and 0 kg N/ha) and produced the highest pod weight of 370 g. This indicates that in the absence of symbiotic N-fixing bacteria, Jack Beans are completely dependent on the availability of inorganic N from the soil and fertilizers. This positive response to high doses of N fertilizer is consistent with findings in many legumes that have not been inoculated or are grown in soils poor in specific Rhizobium, where growth is severely limited by nitrogen (Thilakarathna et al., 2016). External N application is crucial to meet the metabolic needs of plants, including pod development. This is also similar to the report (Rahayu et al., 2020), which shows that the highest peanut pod weight per plot was obtained in the 150 kg/ha urea fertilizer treatment, which was significantly different from the Biocar treatment.

The lowest result was found in treatment D (10 g Rhizobium sp/kg seeds and 0 kg N/ha) at 233 g. This phenomenon can be explained by the concept of the "lag phase" or temporary nitrogen starvation in newly inoculated plants. In the early stages of symbiosis, the plant allocates a lot of energy to forming root nodules and maintaining symbiotic bacteria, before the N_2 fixation process runs optimally. During this phase, the plant may experience N deficiency because it has not yet benefited from fixation, while also not receiving any external N supply. This can suppress early vegetative growth, which affects the final yield, including pod weight.

Dry Seed Weight per Plant

This dry seed weight data seems to continue the previous narrative, but with a focus on the more essential final yield, namely the contents of the pod itself. The combination treatment of N fertilizer dose and Rhizobium inoculation had a significant effect on the dry seed weight per Jack Bean plant.

Table 11. Table of Average Dry Seed Weight per Plant.

Treatment	Dry Seed Weight (g)
A (0 g Rhizobium sp/kg seeds and 0 kg N/ha)	156 ab
B (0 g Rhizobium sp/kg seeds and 100 kg N/ha)	152 ab
C (0 g Rhizobium sp/kg seeds and 200 kg N/ha)	203 b

D (10 g Rhizobium sp/kg seeds and 0 kg N/ha)	130 a
E (10 g Rhizobium sp/kg seeds and 100 kg N/ha)	168 ab
F (10 g Rhizobium sp/kg seeds and 200 kg N/ha)	170 ab

Note : Means followed by the same letter in the same column indicate no significant difference based on Duncan's Multiple Range Test (DMRT) at the 5% level.

Table 11 shows that treatment C (0 g Rhizobium sp/kg seeds and 200 kg N/ha) had a significant effect compared to treatment D (10 g Rhizobium sp/kg seed and 0 kg N/ha) and emerged as the winner with the highest seed weight of 203 g. This is direct evidence of the reliability of inorganic nitrogen in promoting quantitative yields. Under conditions without natural symbionts, plants allocate resources obtained from fertilizers maximally for seed filling. In many production systems, the response of legumes to N fertilizer is often more consistent and directly measurable than the response to inoculation, especially if soil and other environmental factors are not optimal. Applicationally, the report (Supandji et al., 2021) shows that the 200 kg NPK/ha treatment yielded the highest average and had a significant effect on the production of Long Beans.

Seed Protein Content

Based on the seed protein content data presented, a different narrative emerges when compared to previous yield data. However, statistically, there are no significant differences between treatments.

Table 12. Table of Average Seed Protein Content per Plant.

Treatment	**Seed Protein Content (%)**
A (0 g Rhizobium sp/kg seeds and 0 kg N/ha)	27,86 a
B (0 g Rhizobium sp/kg seeds and 100 kg N/ha)	27,92 a
C (0 g Rhizobium sp/kg seeds and 200 kg N/ha)	28,44 a
D (10 g Rhizobium sp/kg seeds and 0 kg N/ha)	26,18 a
E (10 g Rhizobium sp/kg seeds and 100 kg N/ha)	27,87 a
F (10 g Rhizobium sp/kg seeds and 200 kg N/ha)	28,93 a

Note : Means followed by the same letter in the same column indicate no significant difference based on Duncan's Multiple Range Test (DMRT) at the 5% level.

Table 12 shows that treatment C (0 g Rhizobium sp/kg seed and 200 kg N/ha) produced the highest pod weight and dry seed weight, and also yielded a fairly good protein content (28.44%). This indicates that the abundant supply of inorganic nitrogen was successfully converted not only into biomass but also partially into protein quality.

Furthermore, a consistent pattern was observed in the inoculated groups (treatments 0 g Rhizobium and 0 kg N, 10 g Rhizobium and 100 kg N, and 10 g and 200 kg N): seed protein content increased with increasing N fertilizer dose. This trend suggests something fundamental. When Jack Beans have Rhizobium partners, the nitrogen they obtain may be allocated with a slightly different strategy. Although in the early growth phase (as seen from the lower pod and dry seed weights in the 0 g Rhizobium sp/kg seeds and 0 kg N/ha, and 10 g Rhizobium sp/kg seeds and 100 kg N/ha treatments), the presence of continuous biological fixation, especially when supported by applicational N supply from fertilizer, can create a more stable and possibly more efficient "nitrogen flow" towards protein synthesis in the seeds during the filling phase. Nitrogen from symbiotic fixation is often transported in the form of certain compounds (such as amides or ureides) that may have a direct metabolic pathway to amino acid synthesis, compared to nitrate nitrogen from fertilizers, which requires prior reduction.

Thus, the protein content data of Jack Bean seeds can provide insight. It shows that the benefits of Rhizobium inoculation may not always be seen in "how much" is harvested, but in "how good" the quality of the yield is, especially in terms of protein content.

CONCLUSION

During the growth phase, treatment C (200 kg N/ha and 0 g Rhizobium sp/kg seeds) was the most optimal dose, consistently obtaining the highest values for plant height, stem diameter, and number of branches. In root nodule observations, the application of Rhizobium had a better effect, as indicated by the highest results in the observations of root nodules and active root nodules weight obtained from the treatment using Rhizobium. In terms of yield, treatment C (200 kg N/ha and 0 g Rhizobium sp/kg seeds) was the most optimal, as evidenced by the highest pod weight and dry seed weight values achieved by treatment C (200 kg N/ha and 0 g Rhizobium sp/kg seeds). Except for the seed protein content parameter, the combination of Rhizobium inoculation dose and N fertilizer did not have a significant effect on any of the experimental treatments.

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