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The response of soybean plants due to inoculation of rhizobium bacteria and different fertilizer application

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Abstract. The research aimed to obtain information on the benefits of Rhizobium bacteria inoculation with various types of fertilizers in increasing soybean growth and production. The study was conducted in the greenhouse of the Ecology and Crop Production Laboratory, Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang. The soybean seeds used were Anjasmoro. The experimental design was a completely randomized design with 3 x 4 factorial patterns with 4 replications. The first factor was Rhizobium bacterial inoculation including: R1 = without inoculation (control), R2 = Inoculation when planting, R3 = Inoculation 14 days after planting. The second factor was the type of fertilizer treatment includes: P1 = urea 75 kg/ha, P2 = cow manure 7.5 tons/ha, P3 = urea 75 kg/ha + zeolite 7.5 tons/ha, P4 = cow manure 7.5 tons/ha + zeolite 7.5 tons/ha. The parameters observed included plant height, number of leaves, number of branches, number of effective nodules, and number of pods. The data obtained were analyzed of variance analysis and continued with Duncan's Multiple Range Test (DMRT). The results showed Rhizobium bacterial inoculation significantly increases the growth and production of Anjasmoro soybeans both given when planting and 14 days after planting. Fertilization with 7.5 tons of manure/ha shows Anjasmoro soybean growth and production which was equivalent to 75 kg/ha of urea fertilizer.

1. Introduction

Soybean (*Glycine max* L) is a nutritious food commodity with a high source of vegetable protein and low cholesterol. Soybean is also an important food commodity after rice and maize. The government's policy of importing soybeans to solve the problem of soybean shortages in Indonesia has raised concerns for all parties. Indonesia as an agricultural country should not lack food. Abundant natural resources should be able to be optimally utilized to solve the food problem. The utilization of millions of hectares of saline land in Indonesia is very potential to support food sufficiency. However, salinity stress is one of the most influential factors in limiting agricultural production [1]. Research by Fuskhah *et al*. [2] showed that high salinity decreased nitrogenase activity of *Caliandra callothyrsus* root nodules. Therefore, it is necessary to look for efforts to overcome the salinity problem.

Soybean plants are legume plants that can have mutualistic symbiosis with Rhizobium sp bacteria that grow in their root areas. The presence of these bacteria causes the formation of nodules that fix free nitrogen from the air so that it can supply the plant's needs for N elements. The results of this symbiosis can increase plant production. Research by Fuskhah *et al*. [3] demonstrated that the use of a Rhizobium inoculum of 20–60 g / kg of seed combined with phosphorus fertilization, increased *Centrosema pubescens* Benth dry matter production. Rhizobium bacteria inoculation combined with sea water nutrient EC 2 mmhos / cm was able to increase the production of Grobogan soybeans [4]. The use of rhizobium in saline fields also requires saline-resistant rhizobium to increase its ability to fix nitrogen. The ability to fix nitrogen can reduce the cost of purchasing artificial N fertilizers, so the application of saline-resistant Rhizobium inoculation in legume plants is very important to promote nitrogen fixation in saline soils.

The application of fertilizers is also important to spur growth and optimal soybean production. The use of excessive inorganic fertilizers to spur the growth and production of soybeans at the farm level is currently very concerning. The use of inorganic fertilizers in excess amounts and in the long term can damage the physical, chemical, and biological properties of the soil which in turn reduces soil fertility and can threaten the sustainability of agricultural land productivity. Therefore, efforts are needed to reduce the use of inorganic fertilizers and switch to organic fertilizers and bio-fertilizers that are environmentally friendly.

The use of zeolite in some studies demonstrates its ability to increase the availability of nitrogen elements in the soil. Zeolite belongs to a group of alumina-silicate minerals that have multifunctional properties. The use of zeolite could efficiently fertilize nitrogen and play a role in regulating the release of nutrients and water [5]. Zeolite affects the absorption and storage capacity of ammonium in fertilizers and soils. The selective properties of zeolite in absorbing nitrogen compounds were utilized to improve the fertilization efficiency of urea. This research aims to examine the use of organic and biological fertilizers in soybean crops that were able to replace the use of inorganic fertilizers.

2. Materials and Methods

The research was carried out in the Ecology and Plant Production Laboratory, and the greenhouse of the Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang. The materials used were saline soil from Tugu Semarang with an Electrical Conductivity (EC) value of 4 dS / m with a pH of 7.89, soybean seeds of Anjasmoro variety obtained from Balitkabi Malang, cow manure, zeolite, urea, SP-36, KCl, Rhizobium inoculum resistant to Saline 10,000 ppm NaCl (Isolate-KK8, 8 x 10^7 CFU/ml), 80% alcohol, YEMA + CR (Yeast Extract Mannitol Agar + Congo Red) medium. The tools used were pots with a diameter of 35 cm, cultivation tools, measuring cups, scissors, mortar, analytical scales, laminar air flow.

The study used a completely randomized design (CRD) with a 3x4 factorial pattern with 3 replications. The first factor was giving rhizobium inoculum R1 = Without giving Rhizobium inoculum (control), R2 = giving Rhizobium inoculum at planting, R3 = giving Rhizobium inoculum at 14 days after planting. The second factor was the type of fertilizer which included P1 = urea, P2 = cow manure, P3 = urea + zeolite, P4 = cow manure + zeolite. The data obtained were analyzed with analysis of variance at the 5% significance level and if it had a real effect, it was continued with the Duncan Multiple Range Test (DMRT).

Rhizobium bacteria isolate KK8 saline resistant up to 10,000 ppm NaCl were prepared, cultured until it reached the exponential phase and given as much as 20 ml/pot. Soil was taken and prepared in pots. Giving cow manure, zeolite according to the treatment mixed together when filling the soil in a pot, then sterilized using hot steam sterilization technique. KCl and SP-36 fertilization were given according to the recommended dosage. Rhizobium bacteria inoculation was carried out according to the treatment. Parameters observed included, 1) number of root nodules, 2) plant height, 3) number of leaves, 4) number of branches, and 5) number of pods.

3. Results and Discussion

3.1 Number of effective root nodules

The results showed that there was an interaction between the time of giving of rhizobium bacteria inoculum and different fertilizers on the number of soybean root nodules (P <0.05). Rhizobium bacteria inoculation treatment itself had a significant effect on the number of soybean root nodules (p <0.05). The treatment of giving various types of fertilizers itself did not have a significant effect on the number of soybean root nodules. The Duncan multiple range test results and the average number of soybean root nodules due to inoculation of Rhizobium bacteria and different fertilizers as shown in Table 1.

Table 1. The average number of root nodules of soybean plants due to inoculation of Rhizobium bacteria and different fertilizers

	Kind of fertilizers				
	P1	P2	P3	P4	
	Urea	Cow Manure	Urea+ Zeolite	Cow Manure+ Zeolite	
Rhizobium inoculum	--------------------(root nodules/plant)			--------------	Average
R1 (without inoculum)	8.75[bcd]	5.88[d]	2.50[d]	4.38[d]	5.38[b]
R2 (Inoc. at planting)	7.00[cd]	8.25[cd]	17.25[abc]	6.13[d]	9.66[b]
R3 (Inoc.14 dap)	16.50[abc]	18.88[ab]	17.25[abc]	20.75[a]	18.34[a]
Average	10.75	11.00	12.33	10.42	

Different superscripts on the interaction matrix, and the same column show significantly different (P <0.05)

Rhizobium inoculation at 14 days after planting resulted in a significantly higher average number of root nodules (P<0.05), namely 18.34 root nodules, compared to 9.66 root nodules on rhizobium inoculations at planting and without inoculation of 5.38 root nodules. *Rhizobium* sp. bacteria need the ability and time to adjust to soybean plants as their hosts and the growing environment. Rhizobium bacteria not only have to live saprophytic but also have to compete with other rhizobium bacteria [6]. Root nodules will function to fix nitrogen elements from the air when the plants are 2 WAP [7]. Rhizobium bacteria are active at fixing nitrogen at 5 - 6 weeks after the infection process, during this time the rhizobium gets energy from the host plant without giving it back [8]. Rhizobium bacteria inoculation at a salinity of 4 dS / m was still able to increase 45% of the number of nodules per plant [9]. Rhizobium cell growth was reported to be tolerant at a salinity level of 4.5-5.2 dS / m but the growth of plant root hair was disrupted [10].

Rhizobium inoculation 14 days after planting using cow manure and zeolite showed the highest yield with an average of 20.75 nodules. Zeolite is a material that has a very high CEC (cation exchange capacity) and can be used as a slow release material. Application of zeolite to soil was reported to reduce plant stress in saline soils [11]. The use of 10% zeolite can reduce the EC value in planting media through adsorption of ammonium ions with other cations [12]. Zeolites can temporarily bind nitrogen so that it slows down the process of biologically changing nitrates [13]. Zeolite was used as a combination of fertilizer with a dose of 7.5 tons / ha given evenly [14].

3.2. Plant height

Plant height is one of the vegetative parameters that can be used to evaluate plant growth. The results showed that there was no interaction between the time of giving of rhizobium inoculums and different fertilizers on the height of soybean plants. The average soybean plant height was due to inoculation of Rhizobium bacteria and different fertilizers, as shown in Table 2.

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Table 2. The average height of soybean plants due to inoculation of Rhizobium bacteria and different fertilizers

	Kind of fertilizers				
	P1	P2	P3	P4	
	Urea	Cow Manure	Urea+ Zeolite	Cow Manure+ Zeolite	
Rhizobium inoculum	--------------------------(cm)---------------------------				Average
R1 (without inoculum)	122.75	130.50	120.75	115.75	122.44
R2 (Inoc. at planting)	118.75	113.50	110.88	118.25	115.34
R3 (Inoc.14 dap)	117.88	121.75	127.38	127.63	123.66
Average	119.79	121.92	119.67	120.54	

Giving various kinds of fertilizers and inoculation of Rhizobium bacteria had not been able to increase plant height significantly. The high number of nodules, especially in rhizobium inoculation 14 days after planting, have not been able to significantly increase the height of soybean plants. Rhizobium bacteria that used have not been able to compete with other microbes in the soil, so the effect on plant height growth has not been seen. Giving exogenous bacteria becomes ineffective, presumably due to competition between endogenous and exogenous bacteria in fighting over nutrients in the roots of host plants [15]. Application of cow manure at 7.5 tons per ha gives an effect equivalent to 75 kg / ha of urea. The cow manure used has a fairly low C / N ratio of 18.45, which indicates that the manure had decomposed completely so that nutrients could be absorbed by plants.

3.3. Number of leaves

The number of leaves is also one of the vegetative parameters that can be used to evaluate plant growth. The results showed that there was an interaction between the time of giving of rhizobium inoculum and different fertilizers on the number of trifoliate leaves of soybean plants ($P<0.05$). Rhizobium bacteria inoculation treatment itself had a significant effect on the number of trifoliate leaves of soybean plants ($p <0.05$). The treatment of giving various kinds of fertilizers itself also had a significant effect on the number of soybean trifoliate leaves ($p <0.05$). Duncan multiple range test results and the mean number of trifoliate leaves of soybean plants due to inoculation of Rhizobium bacteria and different fertilizers, as shown in Table 3.

Table 3. The average number of leaves of soybean plants due to inoculation of Rhizobium bacteria and different fertilizers

	Kind of fertilizers				
	P1	P2	P3	P4	
	Urea	Cow Manure	Urea+ Zeolite	Cow Manure+ Zeolite	
Rhizobium inoculum	---------------------(trifoliate leaves) ------------------				Average
R1 (without inoculum)	19.38^a	23.00^a	4.75^b	8.00^b	13.78^b
R2 (Inoc. at planting)	21.25^a	19.00^a	18.63^a	23.13^a	20.50^a
R3 (Inoc.14 dap)	19.38^a	20.75^a	23.13^a	19.50^a	20.69^a
Average	20.00^a	20.92^a	15.50^b	16.88^{ab}	

Different superscripts on the interaction matrix, the same column and row show significantly different ($P <0.05$)

Rhizobium inoculation either given at planting or 14 days after planting resulted in a higher number of trifoliate leaves than those that were not inoculated. The average number of leaves inoculated by rhizobium at planting was 20.50 trifoliate leaves, while those inoculated with rhizobium 14 days after planting produced 20.69 trifoliate leaves. The number of trifoliate leaves was significantly higher than the average number of leaves without rhizobium inoculation of 13.78 trifoliate leaves.

Legumes have mutualistic symbiotic ability with *Rhizobium sp*. bacteria that grow in the root region. The existence of this bacteria causes the formation of root nodules that are able to fix the free nitrogen from the air so as to supply the plant needs for nitrogen. The symbiotic result is expected to increase the production of forage plants. Fuskhah *et al*.[16] demonstrated that the use of Rhizobium inoculum could increase seed production of Grobogan soybean 36.8% compared with without inoculation.

The average number of leaves of soybean plants given urea fertilizer is 20.00 trifoliate leaves, equivalent to the number of leaves of soybean plants given cow manure of 20.92 trifoliate leaves. This was because cow manure also has P and K nutrients and important micro elements that plants need. Increasing nutrient content causes more leaf growth. Manure contains P and K nutrients which were important in leaf formation. Application of cow manure increased leaf K nutrient content and total N and K absorption of soybean plants [17]. The application of cow manure and zeolite also produced an average number of trifoliate leaves which was not significantly different from fertilization with urea and cow manure, namely 16.88 trifoliate leaves.

3.4. Number of branches

The number of branches is one of the vegetative parameters that can be used to evaluate plant growth. The results showed that there was no interaction between the time of giving of rhizobium inoculum and different fertilizers on the number of productive branches of soybean plants. The average number of branches of soybean plants due to inoculation of Rhizobium bacteria and different fertilizers as shown in Table 4.

Table 4. The average number of branches of soybean plants due to inoculation of Rhizobium bacteria and different fertilizers

	Kind of fertilizers				
	P1	P2	P3	P4	
	Urea	Cow Manure	Urea+ Zeolite	Cow Manure+ Zeolite	
Rhizobium inoculum	--------------------(branches) -----------------				Average
R1 (without inoculum)	6.63	5.88	7.38	6.50	6.59
R2 (Inoc. at planting)	5.38	6.38	5.13	7.38	6.06
R3 (Inoc.14 dap)	7.38	6.50	4.75	5.88	6.13
Average	6.46	6.25	5.75	6.58	

The number of branches determines soybean production. Branching as an assimilate channel will increase the growth of the user organs (sink) and result in greater results as well [18]. The growth and development of the vegetative part of the plant are determined by the activity of the apical meristem that occurs early in the branching of the plant organs. Branching is a plant genotype function that can be calculated by several environmental and biological factors. The process of forming plant pods begins with plant growth, growth of nodes and productive branches which ultimately determine the number of pods formed [19]. In this study, giving inoculum with different times and different types of fertilizers did not show a difference in the number of branches formed. The high number of nodules in the treatment of cow manure plus zeolite was not able to significantly increase the number of branches.

3.5. Number of pods

The results showed that there was an interaction between the time of giving rhizobium inoculums and different fertilizers on the number of soybean pods ($P < 0.05$). Rhizobium bacteria inoculation treatment itself had a significant effect on the number of soybean pods ($p < 0.05$). The treatment of various kinds of fertilizers itself also had a significant effect on the number of soybean pods ($p < 0.05$). The Duncan multiple range test results and the mean number of soybean pods due to inoculation of Rhizobium bacteria and different fertilizers as shown in Table 5.

Table 5. The average number of pods of soybean plants due to inoculation of Rhizobium bacteria and different fertilizers

	Kind of fertilizers				
	P1	P2	P3	P4	
	Urea	Cow Manure	Urea+ Zeolite	Cow Manure+ Zeolite	
Rhizobium inoculum	---------------------(pods)---------------				Average
R1 (without inoculum)	15.50^{ab}	14.25^{ab}	4.75^{b}	5.00^{b}	9.88^{b}
R2 (Inoc. at planting)	12.75^{ab}	19.63^{a}	13.88^{ab}	15.88^{ab}	15.53^{a}
R3 (Inoc.14 dap)	18.38^{a}	17.88^{a}	12.38^{ab}	18.00^{a}	16.66^{a}
Average	15.54^{ab}	17.25^{a}	10.33^{b}	12.96^{ab}	

Different superscripts on the interaction matrix, the same column and row show significantly different ($P < 0.05$)

Application of cow manure can increase the metabolism of pod formation and Rhizobium inoculation can supply elemental N well. The pod formation period and soybean seed yields are highly dependent on the availability of N, both N which was fixed by Rhizobium bacteria from the air and N available in the soil and is also influenced by the availability of the element P [20].

Giving manure caused the available P content and Mg-dd increased [21]. The use of zeolite could be optimal during pod formation because the absorbed nutrients will be released during the generative period. Urea fertilization added with zeolite was able to make fertilization efficient compared to only urea fertilization [22]. However, in this study it seems that the release of nutrients absorbed by zeolite has not been completely excreted, so the results were even low.

Edamame plants given 10 g of zeolite / plant in the vegetative phase showed the lowest yield but actually had more filled pods [23]. Giving of zeolite as an organomineral could increase the available P and K and the dry weight of soybean seeds [24]. Zeolite does not act as fertilizer, therefore zeolite must be given together with fertilizers because zeolite alone can cause natural soil nutrients to be absorbed so that nutrient absorption by plants will interfere. Application of zeolite 5 tons / ha and cow manure 5 tons/ha gave the highest total pod yield [25].

4 Conclusion

Rhizobium bacterial inoculation increased the growth and production of Anjasmoro soybeans both given when planting and 14 days after planting. Fertilization with 7.5 tons/ha of cow manure shows Anjasmoro soybean growth and production which was equivalent to 75 kg/ha of urea fertilizer.

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