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## Utilization of *Rhizobium* spp as substitution agent of nitrogen chemical fertilizer on soybean cultivation

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**Abstract.** *Rhizobium* is one of the important microorganisms in free nitrogen fixation. The study aims to determine the use of *Rhizobium* spp as substitute for chemical fertilizer Nitrogen on soybean cultivation. The experiment design was a randomized complete block with four replications, using *Rhizobium* isolate in granule and powder media with spraying volume 600cc/kg granules and 400cc/kg of powder. The treatment dosage consisted of 3 g; 4 g; 5g and 6 g/plant, while the control was Phonska fertilizer 1,5 g/plant. The results showed that the use of rhizobium granules and powder as much as 4 g / plant was not significantly different in the formation of root nodules compared with control; the use of *Rhizobium* in granules and powder 6 g/plant for the number of pods did not show a significant difference; the use of 4 g - 6 g/plant for seed weights compared to controls also did not show a significant difference. The use of *Rhizobium* isolate 400cc/kg granules and 600cc/kg of powder can be used to replace the use of Phonska in soybean cultivation.

### 1. Introduction

Nitrogen is an essential nutrient that is needed in large quantities by plants. This element is abundantly present (78%) in the air in the form of N<sub>2</sub> and cannot be absorbed directly by plants. Air N<sub>2</sub> can be transformed into a form available to plants through the symbiosis of mutualism between legume plants (soybeans) and *Rhizobium* microbes in the form of nodules of plant roots. Formation of nodules can cause free N<sub>2</sub> in the air to become available to plants. At present the fulfillment of Nitrogen for soybean plants is very dependent on chemical fertilizers [1]. Many studies have been carried out showing that the use of *Rhizobium* has been able to reduce or even replace the use of chemical fertilizers of nitrogen sources so that their abundant presence can be available and absorbed by soy plants naturally.

The mutualism relationship between soybean plants and *Rhizobium* and certain types of microbes has been able to utilize the nitrogen element in the air and is able to support growth and production optimally. The availability of free nitrogen can take place naturally because they both benefit from each other. Soybean plants will get nitrogen nutrients derived from free air and *Rhizobium* bacteria



will get photosintat from their host plants so that both will be able to carry out life together in a mutually beneficial symbiosis [2].

The addition of Rhizobium spp as a natural fertilizer using carrier media on soybean cultivation land turned out to have been able to increase the number of bacteria that can make a symbiosis with soybean plants as its host, the addition of Rhizobium bacteria in cultivated soybeans can optimize plant growth and production optimally through tethering nitrogen from the air thereby increasing the availability of nitrogen in the soil [3]. Rhizobium is one of the bacteria with the ability to tether the element of nitrogen from the air that can be developed in solid media in the form of granules or in the form of powder so that it can be applied as biological fertilizer and it is able to replace the use of inorganic chemical fertilizers with a positive impact in the form of reducing environmental pollution and residue free products excessive amounts that can interfere with health.

## 2. Research Methods

The study was conducted in February to August 2019 in the Jember State Polytechnic Protection Laboratory for the making of Rhizobium Izolat and the Rhizobium application field research using solid and liquid media was carried out in the Sukorejo village, Bangsalsari District, Jember Regency at an altitude of 89 m asl.

Tools used are sprayers, buckets, rulers, cameras, petri dishes, refrigerators, incubators, tweezers, Erlenmeyers, autoclaves, pipettes, balance sheets, plastic containers, vortexes, LAFC, blenders, besides that required isolation of Rhizobium spp bacteria from Polije protection laboratory, litter of soybean plants, Detam 4 soybean seed varieties, N starter fertilizer, natural phosphate, agricultural lime, tapioca, aquades.

Field research using a non-factorial randomized block design of 4 replications by treating Rhizobium isolates in granule carrier media and sprayed rhizobium isolates of 600 cc / kg granules and 400 cc / kg powders with treatment dosages consisted of: control, 3 g/ plant , 4 g/ plant, 5 g/ plant and 6 g / plant. Each treatment was applied in granules and powder media. The total treatment was 8 treatments and 1 control. The composition of the carrier media granules and powder consisted of sterile edamame soybean waste, zeolite, natural phosphate, agricultural lime and tapioca, Rhizobium isolates obtained from the Jember Polytechnic protection laboratory. The dosage of each treatment consisted of 3 g/ plant, 4 g/ plant, 5g/ plant and 6 g/ plant.

The treatment was compared with the control (using 1.5 g/ plant of chemical fertilizer phonska (equivalent to 180 kg Phonska / ha)). The parameters of the study include the number of root nodules aged 56 and 70 DAP, the number of leaves aged 70 DAP, stover weight, number of pods and seed weight per sample. Data of the results of the analysis of diversity (Anova) and to compare the treatment and control used Dunnet further tests.

### 2.1. Preparation of pure Rhizobium spp

Rhizobium bacterial suspension was inoculated on YEMA + Congo Red media on a petri dish, then incubated for 24 hours at 37 °C. Rhizobium bacterial colonies are marked in white, then the bacterial colonies are stored in a physiological solution in the refrigerator to get pure culture, developed again on YEMA media incubated for 24 hours at 37 °C, after that it is inoculated 1-2 ose on YMB media and put in seker incubator ± 9 days, then taking 0.1 ml of YMB solution to be inoculated on YEMA media.

### 2.2. Making Carrier Media

Carrier media is made from soybean litter which is first washed and dried, then smoothing using a blender until smooth, then the media is sterilized in an autoclave at 121 ° C with a pressure of 17.5 psi for 60 minutes. The media formulation of both granules and powder consists of a mixture of 50% sterile edamame powder, 10% natural phosphate, 20% zeolite, 10% agricultural lime, 10% tapioca, enter the material that has been mixed into the plastic, for the powder form media and granulated for the form media granule.

### 2.3. *Mixing of Rhizobium Bacteria Isolate in Carrier Media*

Rhizobium isolates were obtained from pure culture by bacterial culture method then mixed with a carrier medium as much as 10 ml / 25 g (powder) and 15 ml / 25 g (granules). Each mixture is put into a different plastic container weighing 25 g / container. Then the media is air dried and inoculated and then stored at room temperature before it is applied. As a control, fertilization was carried out using Phonska according to the recommended dosage, ie a dose of 1.5 g / plant as a supplementary fertilizer after the starter fertilizer (equivalent to 180 kg / ha). K and P fertilizers are treated equally for control and all treatments.

### 2.4. *Treatment Application*

Rhizobium biological fertilizer granules and powder with treatment-adjusted doses, carried out at 21 and 49 DAP. Fertilization is done by digging, then watering the beds. Additional fertilization using SP-36 and KCL fertilizers at a dose of 80 kg / ha was carried out at 30 DAP and 45 DAP. In the control treatment, the application of first supplementary fertilizer with a dose of 80 kg / ha at 30 DAP and second fertilizer with a dose of 100 kg / ha at 45 days after using Ponska NPK fertilizer.

## 3. Result and Discussion

Data for the next research variable was analyzed by analysis of the results with all the variables having a very significant effect on all research variables, then further tests were carried out using the Dunnet Test as shown in the following table.

**Table 1.** Recapitulation of the results of diversity analysis on all study variables

Parameters	F calculate	F table 0,01
Number of root nodules	4,95 **	
Number of pods	16,66 **	
Seed weight per sample	8,04 **	4,72
Number of leaves	12,21 **	
Stover weight	16,93 *	

Table 1 shows that the treatment significantly affected all variables observed. Furthermore, to find out the difference between control and each treatment of rhizobium, further tests were carried out using the 0.01 Dunnet Test as the following table.

The results of analysis of variance in table 1 show that all research variables produce calculated F values greater than F table 0.01. Thus it can be explained that the treatment has a very significant effect on the growth and production of soybean plants which is described from all observed variables. To find out the extent of the ability of substitution from the use of Rhizobium as a nitrogen fixation agent free of air carried out in replacing the role of chemical fertilizers phonska tested using Dunnet Test at a level of confidence 0.01 as shown in Table 2.

The results of further tests using the Dunnet Test as in Table 2 (Test to compare the treatment of rhizobium and control) showed that all observed variables showed the result that the treatment of Rhizobium had a very significant effect compared to control. Complete and detailed results from the further tests are presented as follows:

#### 3.1. *Variable number of nodules, number of pods and seed weight*

Dunnet 0.01 test results showed that all treatments differed very significantly from controls. Treat Rhizobium with a dose of more than 4 g / plant is very distinct and better than control. Meanwhile, the treatment of Rhizobium 6 g / plant showed no significant difference with the control in producing the number of pods. Likewise, the treatment of rhizobium 4 g / plant has produced the same seed weight as control.

**Table 2.** Dunnet test results on various research variables of the production components (Treat Rhizobium vs Control)

Treatments	Number of root nodules	Number of pods	Seed weight per sample (g)	Number of leaves	Stover weight (g)
3 g granule	24,15 B	71,40 B	12,75 B	27,70 B	15,80 B
4 g granule	23,70 B	77,15 B	13,80 A	28,80 B	16,85 B
5 g granule	24,25 B	75,70 B	13,75 A	29,20 B	17,45 B
6 g granule	24,75 B	80,40 A	14,15 A	30,55 A	18,30 A
3 g powder	23,45 A	68,45 B	12,65 B	25,40 B	15,70 B
4 g powder	23,75 B	73,50 B	13,45 A	28,75 B	16,40 B
5 g powder	24,10 B	75,45 B	13,45 A	28,75 B	16,95 B
6 g powder	24,55 B	77,60 A	13,40 A	30,10 A	17,70 B
Control	22,40 A	84,20 A	13,95 A	30,60 A	18,95 A
Dunnet	1,12	4,10	0,64	1,82	0,95

### 3.2. Variable number of leaves and stover weight

Dunnet test for both variables shows that the treatment of rhizobium 6 g/ plant did not differ significantly from the control in producing the number of leaves and weight of plant trimming.

Rhizobium sp bacteria are one example of bacteria that have the ability to provide nutrients for plants [4]. When symbiotic with legume plants including soy plants, this group of bacteria will infect the roots of plants and form root nodules in them. Rhizobium sp bacteria can only fix nitrogen in the atmosphere when it is in the root nodules of its legume partners [5]. The role of the Rhizobium sp bacteria on plant growth is particularly related to the problem of nitrogen availability for the host plant.

The process of root nodules formation begins with the production of tryptophan and other compounds that cause an increase in the number of Rhizobium sp around the roots of plants [6]. Tryptophan is used by bacteria and is converted to Indo Acetic Acid (IAA). This acid causes the root hairs to swell before bacteria enter it. Other researchers argue that changes in triptfan and IAA are influenced by ketoglutaric acids and glutamic acid which act as substrates [5]. Rhizobium sp bacteria surrounds the root hairs, rolled root hairs and Rhizobium sp infects the root hairs entering the threads. Infection threads enter the cortex cells and sometimes enter the pericycle cell [7]. These bacteria are in the cytoplasm which results in stimulants causing cells to split or pericycle to divide. This cleavage causes tissue swelling, which then forms nodules containing bacteria, protruding beyond the roots [8].

Rhizobium's ability to replace nitrogen chemical fertilizers in supporting plant growth and production is illustrated by the number of root nodules formed and active root nodules because of the symbiosis between the host plant and Rhizobium [9]. Root nodules have red-brown characteristics that indicate the presence of leghemoglobin. Leghemoglobin functions as an electron carrier (carrier electron), supplying oxygen to bacteroids for the production of ATP and at the same time protecting the nitrogenase system against oxygen [10].

Rhizobium sp bacteria when applied to legume plants, the percentage of nodules effectively can reach 85% so that the nitrogen fixation process can take place and is able to meet the nitrogen needs of plants. Theoretically, legume plants that have effective nodules are able to carry out nitrogen fixation processes and become available to plants.

### 4. Conclusion

Rhizobium spp application can replace the use of chemical fertilizers phonska. Giving Rhizobium 4 g/ plant can replace phonska fertilizer at a dose of 1.5 g / plant with the result of the number of root nodules and seed weight equal to the control treatment (equivalent to 180 kg phonska / ha). Giving Rhizobium 6 g/ plant can replace phonska fertilizer 1.5 g / plant in producing the same number of leaves and stover weight.

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