West Science Social and Humanities Studies
Vol. 4, No. 04, April 2026, pp. 427-435

Rhizobium Inoculation Experiment to Increase Soybean (*Glycine max*) Production in Horticulture Learning

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Article Info

Article history:

Received Apr, 2026
Revised Apr, 2026
Accepted Apr, 2026

Keywords:

Rhizobium
Soybean Production
Horticulture Learning
Experimental Learning Method
Glycine max

ABSTRACT

The purpose of this study was to determine: (1) whether Rhizobium inoculation has an effect on soybean production; (2) whether the use of experimental methods in horticulture learning can improve learning outcomes? This experimental research used a simple posttest only control group design. The data obtained in the form of soybean production (wet seed weight) were tested for normality and homogeneity. The normality test was carried out using Kolmogorov-Smirnov and Shapiro-Wilk statistics. If the normality requirements have been met, a parametric analysis using the t-test was conducted. To test whether the experimental method in horticulture learning can improve learning outcomes, Classroom Action Research (CAR) was used. The data collection technique used a learning outcome test instrument in each cycle. The collected data were then processed using a quantitative descriptive method. From the analysis, it can be concluded that: (1) Rhizobium inoculation on soybean seeds can increase soybean production compared to without Rhizobium inoculation. (2) Students learning outcome after using the experimental method continued to increase from the sufficient category (60.30) in the initial reflection, to the good category (72.60) in cycle I and excellent (87.60) in cycle II.

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1. INTRODUCTION

Soybeans are a plant that has been cultivated by humans since 2500 BC and originally originated in China. In Indonesia, soybeans began to be cultivated on the island of Java in the 16th century and continued to spread to other islands. Soybeans are known by several names, including Glycine soja and Soja max. In 1984, the scientific name for soybean was agreed upon as *Glycine max (L.) Merril* [1].

Soybeans (*Glycine max*) are commonly used as a raw material for tofu, tempeh, soy sauce, soy milk, and animal feed. However, today, soybeans are used not only as a source of protein but also as a functional food that can prevent degenerative diseases, such as coronary heart disease and hypertension. The isoflavones found in soybeans function as antioxidants. The diverse uses of soybeans have fueled the increasing demand for this commodity.

Indonesia's increasing population has significantly impacted the demand for food, including soybeans. Soybean shortages often occur in the market, leading to price spikes. The Ministry of Agriculture, Republic of Indonesia projects that the national soybean harvest area will continue to decline until 2024. In 2021, the projected soybean harvest area was 362,612 hectares, then decreased 5% to 344,612 hectares in 2022.

The harvested area is estimated to decrease by another 5.1% to 326,861 hectares in 2023, and a further 5.2% to 309,849 hectares in 2024. The decrease in harvested area will have a direct impact on reduced soybean production. National soybean production is projected at 594,600 tons in 2022, which is a 3.05% decrease from 2021. Soybean production is also expected to continue to decline at around 3% per year, reaching 558,290 tons in 2024 [2].

One environmentally friendly solution to increase soybean production is to utilize *Rhizobium* bacteria, which form a symbiotic relationship with legumes to bind atmospheric nitrogen and provide it to the plant. Inoculating soybean seeds with *Rhizobium* can promote root nodule formation, improve nitrogen availability, and potentially increase soybean growth and yield. The soybean root system is characterized by a symbiotic relationship with the root bacteria *Rhizobium japanicum*, which leads to the formation of root nodules. Root nodules play a crucial role in nitrogen fixation. The resulting nitrogen is essential for soybean growth and development [3].

Nitrogen assimilation is achieved through N fixation and N supplementation. Legumes differ from other plants in that they can assimilate N in root nodules due to a symbiotic relationship between soybean root cells and *Rhizobium japonicum*. N2 is absorbed from the atmosphere and reduced in the nodules using the energy of photosynthesis transported from the leaves. The reduced N is transported to the sink (storage area) [4].

Rhizobium bacteria are a type of microorganism that lives in symbiosis with leguminous plants and functions to fix nitrogen biologically. They were first introduced in 1888 by Hellriegel and Wilfarth. *Rhizobium* bacteria are a group of bacteria capable of providing nutrients for plants. *Rhizobium* are heterotrophic microbes and grow well at temperatures of 25°C to 30°C. This group of bacteria will infect plant roots and form root nodules. Rhizobium inoculation is the addition of bacteria that can increase N from the air and form a symbiosis with legume plants [5].

Root nodules can form on young soybean plants after root hairs appear on the main root or branch roots. Root nodules are formed by *Rhizobium*. The roots secrete tryptophan and other substances that promote the rapid growth of bacteria and other soil microbes around the roots. Tryptophan is utilized by the bacteria and converted into IAA (indole acetic acid), which causes the root hairs to curl before the bacteria invade them. This curling phenomenon occurs when the root infection occurs during root hair growth, but is not seen when the infection occurs late in root hair growth. It is suspected that the bacteria stimulate the formation of polygalacturonase or other pectic enzymes at the site of infection, causing the cell wall to soften as some of the cell wall material dissolves. This allows the tailed *Rhizobium* cells to slip through the microfibrillar network early in the infection process. When the bacteria enter the root hair cells, their cytoplasm forms infection threads that fuse with the cell wall.

Infection threads containing bacteria grow toward the base of the epidermal cells, taking about two days. Through these infection threads, the bacterial cells penetrate two to five cell layers into the cortex. The resulting infection threads are a series of interconnected segments. The penetration of the infection threads never reaches the endodermis. The cells penetrated by these infection threads can be stimulated to become nodule primordia. As the infection threads penetrate the nodule primordia, small spots appear on the cellulose walls of the infection threads, where one or two bacteria enter the root cells. Both bacteria and root cells multiply rapidly. The nodule primordia cells and the surrounding uninfected cells divide, differentiate, and develop to form root nodules. In this process, the xylem and phloem tissues of the nodule fuse with the corresponding elements of the root.

By the end of the fourth week after bacterial infection, the nodules stop enlarging. Mature nodules contain a pink mass composed of bacteroid cells mixed with uninfected cells. The red color is due to leghemoglobin. These red nodules are considered active in nitrogen fixation, while nodules with a green mass are inactive in nitrogen fixation. Although many factors influence the duration of nodule activity, generally by the sixth or seventh week, nodules begin to decay [2].

The Horticulture course is an elective course in the Biology Education study program, Faculty of Mathematics and Natural Sciences Education, IKIP Saraswati. One of the learning outcomes of horticulture is that students are able to understand, plan, implement, evaluate, and communicate basic horticultural concepts and principles as well as apply basic horticultural knowledge in horticultural plant cultivation and gardening. IKIP Saraswati, as an educational institution, needs to improve the quality of its graduates from year to year. To improve the quality of graduates, the quality of the learning process must also be continuously improved. Horticulture learning should be delivered by emphasizing student involvement in the active learning process and training students to think critically and objectively [6].

To measure the achievement of learning objectives can be measured through the improvement of student learning outcomes. Learning outcomes in this case include three aspects, namely cognitive, affective and psychomotor aspects. Cognitive aspects, cognitive abilities that include knowledge, understanding, application, analysis, synthesis and evaluation. Effective aspects, effective abilities include acceptance of participation, assessment and determination of attitudes, organization and formation of lifestyle patterns. Psychomotor aspects, psychomotor abilities include perception, readiness, guided movement, habitual movement, compact movement, adjustment movement and creativity.

Learning outcomes are the most important part of learning. Quality learning is student-centered learning. According to the constructivist view, learning currently implemented must be oriented towards building students' knowledge independently. Students are trained to find information for independent learning and actively create cognitive structures in interaction with their environment, thus realizing student-centered learning. One good learning strategy and in line with the nature of constructivism is the application of an experimental-based learning model. In experimental-based learning, students are more directed towards experimental learning (learning based on concrete experiences), discussions with friends, which will then obtain new ideas and concepts. Therefore, learning is seen as a process of compiling knowledge from concrete experiences, collaborative activities, and reflection and interpretation [7].

The advantages of the Experimental Method are: (1) improving conceptual understanding (students do not only memorize, but also understand through real experience); (2) fostering curiosity (experiments encourage students to ask questions, investigate, and draw their own conclusions); (3) developing science process skills (such as observing, measuring, classifying, concluding, and predicting); (4) increasing active involvement (learning becomes more interactive, not just one-way from teacher to student); (5) motivating students (practical activities make students more interested and motivated in learning). Experimental learning is very suitable for science learning, because it is able to provide learning conditions that develop thinking skills and creativity optimally. Students are given the opportunity to construct their own concepts in their cognitive structure, which can then be applied in their lives. The use of this experimental method has the aim of making students able and finding their own answers or problems faced by conducting their own experiments. In addition, students can be trained in scientific thinking, with experiments students find evidence of the truth and theory of something being studied [8].

The learning process that uses the experimental method will provide students with the opportunity to experience/conduct themselves, analyze, prove, and draw their own conclusions. The learning process using the experimental method can help students develop their own way of thinking, because by using this experimental method, students will work independently and experience what they will learn for themselves. In other words, students will have a better understanding of the material being discussed.

From the description above, the focus of discussion in this research is twofold, namely: (1) how does *Rhizobium* inoculation affect soybean production? (2) Does the involvement of students taking horticulture courses in *Rhizobium* inoculation experiments improve their learning outcomes?

2. METHODS

This experimental research used a simple experimental design, posttest only control group design. The population was soybean plants (*Glycin max*) of the Willis variety which were planted in 240 plant pots. The soybean plants used as samples were taken randomly as many as 180 plant pots from 240 plant pots that had been prepared. This sample of 180 plant pots was grouped into two, namely the first group as a control group (90 plant pots) and the second group as an experimental group (90 plant pots). Plants in the control group were divided into 3 sub-groups because there were three repetitions (testing), so the number of plants in each sub-group was 30 potted plants. Plants in the experimental group were also divided into 3 sub-groups because there were three replications, so the number of plants in each sub-group was 30 potted plants.

The control group's seeds were soaked in 50 cc of well water, while the experimental group's seeds were soaked in well water that had been inoculated with Rhizobium. Rhizobium inoculation was carried out by soaking 25 grams of root nodules in 50 cc of well water. The process of soaking seeds is carried out in the shade, not exposed to direct sunlight because Rhizobium bacteria cannot tolerate direct sunlight.

The data obtained in the form of soybean production (wet seed weight) were tested for normality and homogeneity. The normality test was conducted using the Kolmogorov-Smirnov and Shapiro-Wilk statistics. The significance level (α) was set at 0.05. The normality test criteria used were if the significance number (sig.) is greater than the significance level (α), then the statistical number obtained is insignificant, meaning the sample data comes from a normally distributed population. Vice versa. If the normality requirements have been met, then parametric analysis is conducted using the t-test.

To test whether the experimental method in horticulture learning can improve learning outcomes, Classroom Action Research was used. The subjects were 15 sixth-semester students of the Biology Education study program, consisting of 8 women and 5 men. The location of this research was at IKIP Saraswati, Tabanan Regency, Bali Province. The data collection technique used a learning outcome test instrument, in each cycle. The collected data were then processed using a quantitative descriptive method. Before the instrument in the form of a learning outcome test was used, its reliability, normality, and validity were first tested using the Winstep application.

3. RESULTS AND DISCUSSION

To get a clear picture of the effect of Rhizobium bacteria inoculation on soybean production (wet seed weight) is presented in Table 1.

Table 1. Soybean production data for the experimental group and control group in 3 cycles

Cycle	Experimental group production (gram)		Control group production (gram)	
	Total	Average	Total	Average

I	458,2	15,27	363,1	12,10
II	577	19,23	474,2	15,81
III	437,6	14,59	335,8	11,19
Total	1472,8	49,09	1173,1	39,10
Average	490,93	16,36	391,03	13,03

Based on the average soybean production in table 1, it can be described as in figure 2.

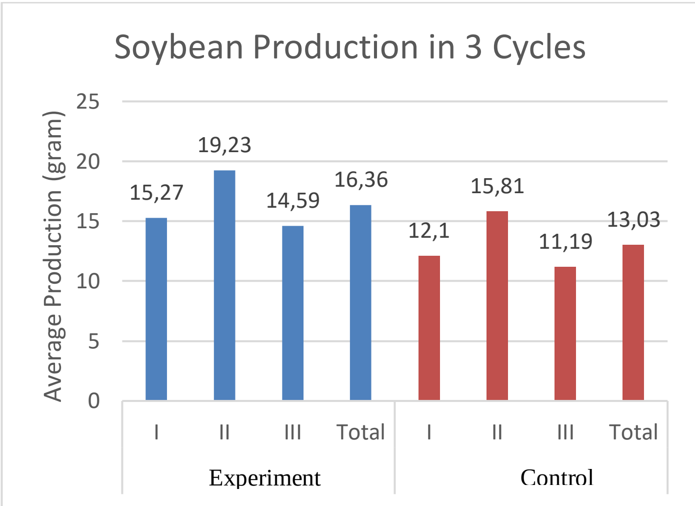

Figure 1. Comparison graph of the average soybean production of the experimental group and the control group in 3 cycles

3.1 Inferential Statistical Analysis through T-Test

A t-test was conducted to determine whether the average soybean yields between the experimental and control groups differed significantly. A pre-requisite normality test was first performed. The results of the data normality test are shown in Table 2. All data obtained a significance value (sig.) above 0.05, concluding that all data from the experimental and control groups were normally distributed. Therefore, the t-test could proceed.

Table 2. Summary of normality tests

Tests of Normality							
	Group	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistics	df	Sig.	Statistics	df	Sig.
Production	Experimental Group	.051	90	.200*	.989	90	.687
	Control Group	.060	90	.200*	.992	90	.880

Table 3. Summary of t-test results for the experimental group and control group

		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
Soybean Production	Equal variances assumed	.134	.715	7.572	178	.000	3.33044	.43986

	Equal variances not assumed			7.572	177.313	.000	3.33044	.43986

Based on the results of the t-test, it can be explained as follows. The average difference test between the experimental group and the control group with the assumption of equal variance obtained a calculated t value of 7.572 and a significance value (sig.) of 0.000. The sig. value is smaller than the specified significance level of 0.05. Therefore, it can be concluded that there is a significant difference between the average production of the experimental group and the control group where the experimental group is superior with an average soybean production of 16.36 grams/tree.

The average learning outcomes of students who took the horticulture course before and after using the experimental method are presented in Table 4, Table 5, and Table 6.

Table 4. Student learning outcomes in initial reflection

No	Learning Outcomes	Average	Percentage	Category
1.	Individual Completion			
	Complete (3 students)	-	20 %	-
	Incomplete (12 students)	-	80 %	-
2.	Average Score ($\overline{X}$)	60.30	-	Fair
3.	Absorption Capacity	0.79	79%	Complete
4.	Classical Completion	0.79	79%	Incomplete

Before using the experimental method, student learning outcomes had not yet reached a level considered successful or optimal. This is evident from the average student learning outcome score, which only reached the adequate category of 60.30.

Table 5. Student learning outcomes in cycle I

No	Learning Outcomes	Average	Percentage	Category
1.	Individual Completion			
	Complete (8 students)	-	53.33 %	-
	Incomplete (7 students)	-	46.67%	-
2.	Average Score ($\overline{X}$)	72.60	-	Good
3.	Absorption Capacity	0.84	84%	Complete
4.	Classical Completion	0.84	84%	Complete

After using the experimental method in cycle I, student learning outcomes increased to the good category with an average of 72.60.

Table 6. Student Learning Outcomes in Cycle II

No	Learning Outcomes	Average	Percentage	Category
1.	Individual Completion			
	Complete (15 students)	-	100 %	-
	Incomplete (none)	-	0 %	-
2.	Average Score ($\overline{X}$)	87.60	-	Very Good
3.	Absorption Capacity	0.92	92%	Complete
4.	Classical Completion	0.92	92%	Complete

In cycle II, students' learning outcomes were in the very good category 87.60.

The recapitulation of the improvement in learning outcomes of students taking the horticulture course using the experimental method in table 4, table 5, and table 6 can be described in figure 3.

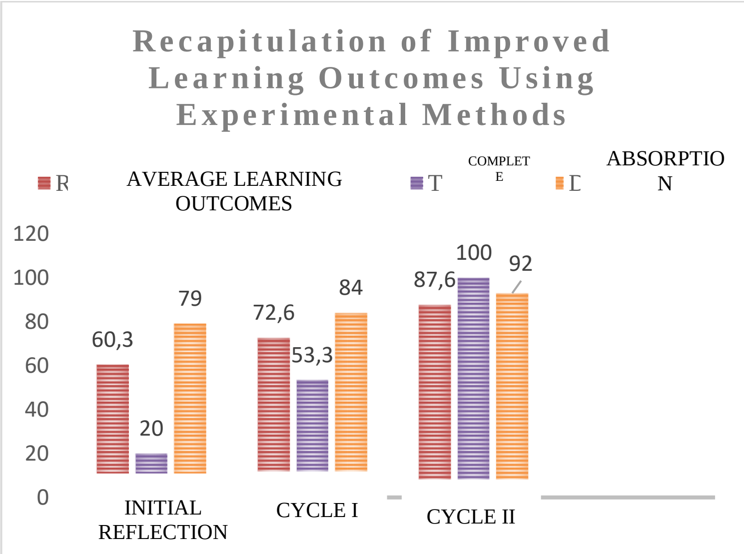

Figure 2. Recapitulation of learning outcomes of students taking horticulture courses using the experimental method

From the recapitulation results above, it is clear that student learning outcomes before and after using the experimental method continued to increase from the sufficient category of 60.30 to the good category of 72.60 and very good 87.60. The increase in learning outcomes was followed by an increase in student completeness and absorption in horticulture learning.

3.2 Discussion

Based on the results of the t-test, it can be concluded that there is a significant difference between the average soybean plant production of the experimental group (the group inoculated with Rhizobium) and the control group (the group without Rhizobium inoculation) where the experimental group is superior with an average soybean production of 16.36 grams/plant. Rhizobium bacteria are generally heterotrophic, meaning their energy source comes from the oxidation of organic compounds such as sucrose and glucose. Thus, to obtain these organic compounds, bacteria need a host plant. The form of symbiosis between soybean plants and Rhizobium is a mutualistic symbiosis, because the bacteria in symbiosis infect the plant and the plant responds by forming nodules. Rhizobium bacteria obtain food in the form of minerals, sugar/carbohydrates and water from their host plant, while the bacteria provide a reward in the form of nitrogen that they fix from the atmosphere [9].

Rhizobium inoculation of soybean seeds can promote root nodule formation, improve nitrogen availability, and potentially increase soybean growth and yield [10]. The soybean root system is characterized by a symbiotic relationship with the root bacterium Rhizobium japanicum, which leads to the formation of root nodules. Root nodules play a crucial role in nitrogen fixation. The resulting nitrogen is essential for soybean plants' growth and development [3].

Rhizobium is a heterotrophic microbe and grows well at temperatures of 25 °C to 30 °C. This group of bacteria will infect plant roots and form root nodules. Rhizobium inoculation is the addition of bacteria that can increase N from the air and form a symbiosis with legume plants [5]. Rhizobium that successfully interacts with soybean roots will increase the number of root nodules which will affect the amount of Nitrogen levels for plants so that the N element that is fulfilled in plants will stimulate vegetative and generative growth such as increasing the number of plant pods. Rhizobium and soybean plants have a beneficial relationship between the two plants. Research by Imam et al. [11] that Rhizobium bacteria can overcome the excessive use of synthetic N fertilizers because Rhizobium that successfully interacts with roots can meet 75% of the N needs of soybean plants.

This is in line with the research results of Purwaningsih [12] and Augusta et al. [13] which showed that Rhizobium inoculation can increase the growth of soybean plants compared to soybean plants without Rhizobium inoculation. The higher production of soybean plants in the Rhizobium inoculation treatment is due to the presence of root nodules that are effective in binding N nutrients to support plant growth. The results of research by Jumrawati [14] showed that Rhizobium inoculation can increase N_2 fixation.

Student learning outcomes before and after using the experimental method continued to improve from the sufficient category (60.30) in the initial reflection, to the good category (72.60) in cycle I and very good (87.60) in cycle II. The improvement in learning outcomes was followed by an increase in student completeness and absorption in horticulture learning. This improvement in learning outcomes occurred because students were provided with learning conditions that could optimally develop thinking and creativity skills. In addition, students were given the opportunity to conduct experiments, observe the process, write down the results of the experiments, then the results of these observations were presented in front of the class and evaluated by the teacher. The opportunities experienced by students to conduct experiments enabled students to develop concepts in their cognitive structure and then students could apply them in everyday life. This is in line with the statement of Nurhasanah et al. [15], who stated that the experimental method can increase students' activeness in scientific thinking and problem solving, which in turn strengthens their understanding of the material being taught.

The improvement in learning outcomes using the experimental method in learning indicates that the experimental method is an appropriate and suitable method for application in horticulture learning. The application of the experimental method can make learning more meaningful because students can develop their creativity in experimental activities. Furthermore, the learning process is more enjoyable and interesting for students because they are directly involved in the learning process. This confirms the research of Nisa and Efendi [16], which revealed that the experimental method is effective in helping students build deeper knowledge through direct exploration and discovery of scientific concepts. This is also supported by the results of previous research, such as that expressed by Permatasari et al. [17], which showed that the experimental method can improve learning outcomes. Therefore, the experimental method can be a very effective alternative to improve student understanding, especially in materials that require direct scientific exploration, such as the Rhizobium inoculation experiment on soybean seeds. Based on these findings, it is recommended that the experimental method be implemented more widely in science learning in schools to improve the quality of education and the overall student learning process.

4. CONCLUSION

Rhizobium inoculation of soybean seeds can increase soybean yield compared to non-inoculated soybeans. This is because Rhizobium inoculation of soybean seeds can increase root nodule formation and improve nitrogen availability, potentially increasing soybean growth and yield. Student learning outcomes after using the experimental method continued to increase from the sufficient category (60.30) in the initial reflection, to the good category (72.60) in cycle

I and very good (87.60) in cycle II. This is because the experimental method can develop students' activeness and creativity in scientific thinking and problem solving, which in turn strengthens their understanding of the material being taught so that learning becomes more meaningful.

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