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Combination of Biofertilizers in Soybean Cultivation (*Glicyne max* L. Merril) in Acidic Soil

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ABSTRACT
The utilization of acidic soil is often not maximized due to various problems, especially those related to the physical and chemical properties of the soil. Efforts to increase productivity in acidic soil can be done through the use of biological fertilizers such as Arbuscular Mycorrhizal Fungi (AMF) and Rhizobium, which play a role in increasing phosphorus and nitrogen absorption. This study aims to determine the most effective combination of biological fertilizers for soybean cultivation. The study was conducted in Sirnabaya Village, East Telukjambe District, Karawang Regency, West Java. The experiment was conducted over a period of 3 months, from March 2025 to May 2025. The method used was an experimental method using a single-factor Randomized Block Design (RBD). There were a total of 9 treatments repeated 4 times, resulting in 36 experimental units. The treatments consisted of A (control), B (AMF 15 g/plant), C (AMF 20 g/plant), D (Rhizobium 15 ml/kg of seeds), E (AMF 15 g/plant + Rhizobium 15 ml/kg of seeds), F (AMF 20 g/plant + Rhizobium 15 ml/kg seed), G (Rhizobium 20 ml/plant), H (AMF 15 g/plant + Rhizobium 20 ml/kg seed), I (AMF 20 g/plant + Rhizobium 20 ml/kg seed). The observation data were tested using the F test, and if there was a significant effect, it was further tested using the Duncan Multiple Range Test (DMRT) at a 5% level. The results showed that treatment H had the best effect on root infection parameters, root length per plant, root dry weight, number of pods per plant, seed weight per 100 seeds, plant height, and number of leaves.

KEYWORDS: Arbuscular Mycorrhizal Fungi, Nutrient uptake, Rhizobia, Soil Microorganism

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INTRODUCTION

Acidic soil is soil with a low pH value and low nutrient concentration. Acidic soil in Indonesia is estimated to cover 18.5 million hectares (Muzaiyanah and Subandi 2016). Low pH levels can affect crop productivity. Acidic soils can influence the physical and chemical characteristics of the soil, hinder plant growth, alter nutrient availability, disturb microbial cycles and biochemical processes in the soil, and negatively affect plant health (Nazir *et al.,* 2017).. According to Tambanung *et al.,* (2019), soil conditions in some parts of Indonesia have an acidic pH with an average pH of less than 6. Soils with a pH lower than 6 are highly prone to Zn, Mn, and Al toxicity, which results in a reduced availability of essential nutrients, including K, P, Mg, and S, due to the acidic conditions (Siswanto 2019).

Plants grown in acidic soil experience stunted growth compared to plants grown in soil with a neutral pH. This condition can hinder root development and impair the plant's capacity to take up essential nutrients. An elevation in soil phosphorus levels will subsequently lead to enhanced phosphorus absorption by plants, which can be achieved by using biofertilizers containing phosphate-solubilizing bacteria. Phosphate-solubilizing bacteria can degrade the connections between phosphorus and micronutrients, making the phosphorus available in a form that can be absorbed by plants (Setiawati, *et al.,* 2014). Phosphatesolubilizing bacteria possess the ability to degrade phosphorus bonds with microelements in soils contaminated with heavy metals such as lead (Pb) (Susilowati, 2014).

Arbuscular Mycorrhizal Fungi (AMF) play a crucial role in enhancing soil fertility, particularly in improving the absorption of phosphorus nutrients available in the soil. AMF is a biological agent that helps plants increase nutrient absorption (Abdillah, *et al.,* 2022). Another microbe that plays a role in nutrient absorption is Rhizobium, which can help increase nitrogen availability for plants. This process is very important because nitrogen is an essential nutrient for plant growth, especially in acidic soils (Pamungkas and Irfan, 2018). Sari and Prayudyaningsih, (2015) explain that Rhizobium can only effectively fix nitrogen when living in the root nodules of its host plants. The success of the nitrogen fixation process by Rhizobium is greatly influenced by a number of factors, such as environmental conditions and nutrient availability.

Under extreme weather conditions such as very high rainfall, the growth of these bacteria can be inhibited, consequently, there is a reduction in the formation of root nodules and a decline in nitrogen fixation efficiency (Liem et al., 2019). Therefore, simultaneous inoculation with phosphorus-solubilizing microbes is essential. The combination of AMF and Rhizobium can have a significant effect on nitrogen fixation in nutrient-deficient soils (Suryatini, 2015). AMF can convert organic phosphorus into primary orthophosphate, which is a form of phosphorus that is available to plants (Rajmi et al., 2018).

Soybeans are one of the food crops with increasing demand, but production has not yet been able to meet this need. According to (Kementan, 2021), the soybean harvest area experienced a 5.1% decline, reaching 326,861 hectares in 2023, and is projected to further decrease by 5.2%, totaling 309,849 hectares in 2024. The decrease in land area directly affects soybean output. In 2022, soybean production is projected to be 594,600 tons, reflecting a 3.05% decline compared to 2021. Production is anticipated to keep decreasing by 3% annually, reaching 558,290 tons in 2024. Therefore, the application of a combination of AMF and Rhizobium can help soybean cultivation in acidic soils. Research by Wen et al., (2023) shows that the combination of AMF and Rhizobium significantly increases soybean shoot and root biomass in acidic soils.

MATERIAL AND METHODS

Time and Place of Research

This research was conducted on experimental land in Sirnabaya Village, East Telukjambe Subdistrict, Karawang Regency, West Java. The experiment was conducted over a period of 3 months, from March 2025 to May 2025.

Materials and Equipment

The materials used were: Arbuscular Mycorrhizal Fungi produced by the Bogor Agricultural University Laboratory, Rhizobium bacteria produced by the Padjajaran University Soil Chemistry and Plant Nutrition Laboratory, Grobogan variety soybean seeds, NPK 16-16-16 fertilizer, 2% HCl, 10% KOH, 0.05% methylene blue, and distilled water.

The tools used were: hoses, measuring cups, hoes, 60 cm x 60 cm polybags, measuring tapes, digital scales, calipers, thermohygrometers, plant labels, scissors, cameras, microscopes, beaker glasses, object glasses, test tubes, microscope slides, pipettes, tweezers, and writing instruments for observation.

Method

The research employed an experimental approach utilizing a single-factor Randomized Block Design (RBD), consisting of a combination of Arbuscular Mycorrhizal Fungi (AMF) and Rhizobium at different doses. A total of 9 treatments were applied, each repeated 4 times, yielding 36 experimental units. The treatments were A (Control/No Treatment), B (AMF 15 g/plant), C (AMF 20 g/plant), D (Rhizobium 15 ml/kg seed), E (AMF 15 g/plant + Rhizobium 15 ml/kg seed), F AMF 20 g/plant + Rhizobium 15 ml/kg seed), G (Rhizobium 20 ml/kg seed), H (AMF 15 g/plant + Rhizobium 20 ml/kg seed), I (AMF 20 g/plant + Rhizobium 20 ml/kg seed).

Data analysis

The observation data were statistically analyzed using analysis of variance (ANOVA) based on a single-factor randomized block design. The F test was performed at a 5% level. If the F test analysis showed significant differences (calculated F value > F value in the 5% table), it was followed by a Duncan Multiple Range Test (DMRT) to distinguish the effects between treatments more specifically.

Experiment Implementation

The soil was sieved with a mesh size of 1-2 cm and then placed in polybags weighing 37 kg. AMF was then inoculated into the planting medium according to the treatment and incubated for 7 days. Rhizobium was applied to the soybean seeds according to the treatment. The seeds were soaked, drained, and then inoculated with Rhizobium before planting according to the treatment dosage. Three seeds were planted in each planting hole provided, at a depth of 2 cm into the soil medium, and then the holes were covered with soil. At 7 days after planting, 1 plant per polybag was left. Maintenance during planting consisted of watering, weeding, fertilizing, and controlling plant pests mechanically and chemically. The variables observed were root infection (%), root length per plant (cm), dry root weight per plant (g), number of pods per plant (fruits), seed weight per 100 seeds (g), plant height (cm), and number of leaves (pieces).

DISCUSSION

Supporting Observations Soil Test Analysis

Based on the results of soil tests conducted at the Padjajaran University Laboratory, it was found that the soil in the experimental field had a pH (H_2O) of 5.6, which is classified as slightly acidic. Soil pH greatly affects the absorption of nutrients by plants in agricultural cultivation. According to Siswanto, (2019), in soil with low pH, phosphorus nutrients will react with iron and aluminum ions to form insoluble compounds that are not available to plants. According to Anwar and Rover, (2023), the ideal soil pH for soybean growth is between 6.6 and 7.5 for optimal growth and development.

The results of the organic carbon analysis of the soil were classified as high at 3.63%, with a C/N ratio of 13 (moderate). Macro nutrients include nitrogen at 0.28% (moderate), available P_2O_5 at 52.12 ppm (very high), potential P_2O_5 at 98.15 mg/100 g (very high), and K_2O at 8.12 mg/100 g (very low). Based on soil texture analysis, the soil fraction composition is 11% sandy, 63% silty, and 26% clayey, thus falling under the silty loam soil texture criteria.

Key Observations Root Infection

The analysis of variance revealed that administering varying doses of AMF significantly influenced the level of AMF infection, as presented in Table 1.

Table 1. Average percentage of AMF infection degree in soybean roots (*Glycine max* L. Merril)

Code	Treatment	Percentage of mycorrhizal infection (%)
A	Control	11,66d
B	AMF 15 g/plant	46,75c
C	AMF 20 g/plant	56,75bc
D	Rhizobium 15 ml/kg seed	16,08d
E	AMF 15 g/plant + Rhizobium 15 ml/kg seed	57,50bc
F	AMF 20 g/plant + Rhizobium 15 ml/kg seed	66,75b
G	Rhizobium 20 ml/kg seed	18,50d
H	AMF 15 g/plant + Rhizobium 20 ml/kg seed	81,50a
I	AMF 20 g/plant + Rhizobium 20 ml/kg seed	59,25b
	KK (%)	16,43

Note: Values followed by the same letter in each column indicate no significant difference at the 5% DMRT level.

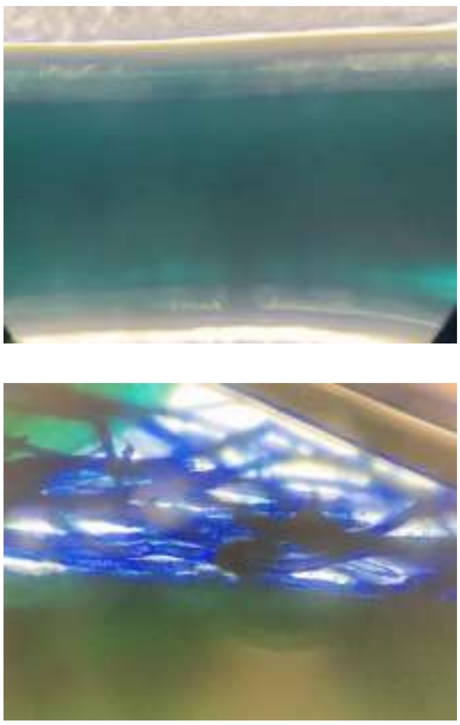

Figure 1. Results of soybean root infection observations identified roots without infection (top) and roots with infection (bottom).

The application of AMF at a dose of 15 g/plant + Rhizobium at 20 ml/kg of seeds had the best effect on root infection parameters. It is suspected that the AMF dose of 15 g/plant used can grow well under the conditions of the experimental field. This growth then produced a greater number of hyphae, which served to extend the reach of the roots, thereby increasing the capacity to absorb phosphorus available in the soil. This is consistent with the study by Matondang *et al.,* (2020) that a AMF dose of 15 g/plant resulted in the highest AMF infection with a yield of 82.22% in chili plant roots.

Observations showed symptoms of root infection in soybean plants with the discovery of hyphae, vesicles, arbuscules, and spores. The presence of this infection can be determined through microscopic observation after the roots undergo a staining process (Sulfiah et al., 2021). Root infection can be assessed through microscopic analysis of root tissue to detect mycorrhizal structures, including hyphae, vesicles, and arbuscules (Pulungan, 2013). The presence of one of the three mycorrhizal fungal structures on plant roots indicates that the roots have been infected by arbuscular mycorrhizal fungi. According to Asriani, (2015), AMF can form symbiotic relationships with 97% of plant species. This is supported by the statement by Loo *et al.,* (2022) that legumes form close symbiotic relationships with AMF, resulting in improved nutrition, resistance to environmental stress, and optimal crop yields.

Root Length per Plant

The analysis of variance indicated that the application of the AMF and Rhizobium combination significantly influenced the average root length of soybean plants, as shown in Table 2.

Table 2. Average Root Length of Soybean Plants (*Glicyne max* L. Merril)

Code	Treatment	Average Root Length Per Plant (cm)
A	Control	15,08e
B	AMF 15 g/plant	17,33cde
C	AMF 20 g/plant	18,58cd
D	Rhizobium 15 ml/kg seed	16,83cde
E	AMF 15 g/plant + Rhizobium 15 ml/kg seed	18,99cd
F	AMF 20 g/plant + Rhizobium 15 ml/kg seed	23,83b
G	Rhizobium 20 ml/kg seed	19,83c
H	AMF 15 g/plant + Rhizobium 20 ml/kg seed	28,58a
I	AMF 20 g/plant + Rhizobium 20 ml/kg seed	15,99de
	KK (%)	9,64

Note: Values followed by the same letter in each column indicate no significant difference at the 5% DMRT level.

The application of AMF 15 g/plant + Rhizobium 20 ml/kg seed showed the best effect on increasing root length per plant. It is suspected that AMF helps extend the root range, with AMF hyphae spreading outside the roots, causing soybean roots to become long and extensive. This increases the efficiency of water absorption and phosphorus nutrients available in the soil. The results of Yuliana, (2024) found that the combination of AMF and Rhizobium resulted in a significant increase in soybean plant root length, reaching 28.6 cm. Compared to the application of AMF doses of more than 15 g/plant, which resulted in the shortest root length, this is because too high a dose of AMF can create competition between microorganisms in the root zone and trigger an imbalance in soil microbes and disrupt effective colonization, resulting in suboptimal root growth. The results of the study by Watoni *et al.,* (2020) showed that the application of 20 g/plant of AMF resulted in the shortest average root length.

AMF that is capable of mutualistic symbiosis through infection of soybean plant roots helps increase the absorption of available phosphorus in the soil so that it can be absorbed by plants. Basri, (2018) reports that AMF helps increase phosphorus absorption for plant growth. This is done through external mycorrhizal hyphae that grow on soybean roots, which then channel phosphorus to the roots (Susanti et al*.,* 2018). Root development is influenced by soil structure and the nutrients contained in AMF, which cause corn roots to grow longer (Widodo and Kusuma, 2018). The application of 15 g/plant of AMF in this study was able to absorb the available phosphorus in the soil, resulting in longer root growth. This result is in line with the research by Silitong and Nasution, (2020), which found that mycorrhiza treatment at a dose of 15 g resulted in longer corn roots.

Rhizobium is a genus of soil bacteria that also has the ability to form a symbiotic relationship with plant roots. These bacteria, through the process of nitrogen fixation, have a positive effect on the growth and production of soybean plants (Yusran et al. 2022). This is thought to be due to sufficient nitrogen as a result of Rhizobium bacterial activity, which improves the root system and thus increases the absorption of plant nutrients. Prasetyowati and Yuliani, (2018) reported that Rhizobium can stimulate root hair and root branching growth to expand the root system's reach in searching for nutrients in the soil. Additionally, an increase in nitrogen nutrients in the soil can also trigger root growth, allowing roots to grow longer (Kumara and Jumadi, 2022).

Dry Weight of Roots

The analysis of variance indicated that the combination of AMF and Rhizobium significantly affected the average dry weight of soybean roots, as shown in Table 3.

Table 3. Average dry weight of soybean roots *(Glicyne max* L. Merril)

Code	Treatment	Dry Weight of Roots (g)
A	Control	1,54c
B	AMF 15 g/plant	1,63c
C	AMF 20 g/plant	1,73bc
D	Rhizobium 15 ml/kg seed	1,65c
E	AMF 15 g/plant + Rhizobium 15 ml/kg seed	1,80bc
F	AMF 20 g/plant + Rhizobium 15 ml/kg seed	2,11ab
G	Rhizobium 20 ml/kg seed	1,66c
H	AMF 15 g/plant + Rhizobium 20 ml/kg seed	2,43a
I	AMF 20 g/plant + Rhizobium 20 ml/kg seed	1,73bc
	KK (%)	14,37

Note: Values followed by the same letter in each column are not significantly different at the 5% DMRT level.

AMF applied at 15 g/plant combined with Rhizobium at 20 ml/kg of seeds showed the most significant effect on enhancing root dry weight. It is suspected that AMF extends the root range of plants so that phosphorus available in the soil is easily absorbed by plants, while Rhizobium produces growth hormones that stimulate root elongation. Root elongation increases the efficiency of water and nutrient absorption, thereby increasing root biomass and dry root weight. Duan et al., (2024) found that the combination of AMF and Rhizobium increases the dry weight of legume roots and strengthens nitrogen and phosphorus absorption.

The application of a AMF dose of 15 g/plant is thought to be able to expand the root range through hyphae, thereby increasing phosphorus uptake, which is important in root formation and growth. This is in line with the research by Silitonga and Nasution, (2020), which found that a dose of 15 g/plant gave the best results in white corn root weight. Hyphae act as a medium for the exchange of photosynthates from plants and nutrients, especially phosphorus, from the environment (Parapasan et al., 2014). Phosphorus plays a very important role in plants because it has a significant effect on cell division, root development, and stem strengthening in cereal crops (Sudiarti 2018).

The application of Rhizobium at a dose of 20 ml per kilogram of seeds stimulates root growth by increasing enzyme activity and the production of growth hormones such as auxin and cytokinin. This process triggers cell division and root elongation, allowing the roots to grow larger and heavier. Jumiatun et al., (2022) found that applying Rhizobium at a dose of 20 ml/kg of seeds resulted in the highest average root dry weight. Rhizobium helps improve soil balance and structure, which consequently promotes plant growth (Sari and Prayudyaningsih, 2015).

Number of Pods per Plant

The analysis of variance indicated that the combination of AMF and Rhizobium significantly influenced the average number of pods per soybean plant, as shown in Table 4.

Table 4. Average Number of Pods per Soybean Plant (*Glicyne max* L. Merril)

Code	Treatment	Number of Pods Per Plant (Fruit)
A	Control	13,58d
B	AMF 15 g/plant	18,33bcd
C	AMF 20 g/plant	23,08b
D	Rhizobium 15 ml/kg seed	16,58cd
E	CMA 15 g/plant + Rhizobium 15 ml/kg seed	22,25b
F	CMA 20 g/plant + Rhizobium 15 ml/kg seed	29,66a

G	Rhizobium 20 ml/kg seed	20.75cb
H	AMF 15 g/plant + Rhizobium 20 ml/kg seed	33,41a
I	AMF 20 g/plant + Rhizobium 20 ml/kg seed	20,33bc
	KK (%)	15,72

Note: Values followed by the same letter in each column are not significantly different at the 5% DMRT level.

The results showed that the combination of AMF 15 g/plant + Rhizobium 20 ml/kg seed produced the best number of pods. It is thought that AMF extends the root range so that phosphorus available in the soil is easily absorbed by plants through external hyphae, while Rhizobium produces growth hormones that support flower and pod formation. Root elongation increases nutrient absorption efficiency, resulting in more optimal photosynthesis and more yield allocated to generative organs, thereby increasing pod yield. This is in line with the research by Igiehon and Babalola, (2021) that the application of a combination of AMF and Rhizobium produced significant results in terms of soybean pod yield.

AMF aids in the absorption of phosphorus, which is very important in pod formation. According to Adi Pratama et al., (2019), plants with sufficient phosphorus promote more flower formation and will result in a maximum number of pods. The amount of nutrients available to plants also affects the number of fruits formed, such as phosphorus nutrients required during the generative phase for flower and fruit formation (Roni and Hartatika, 2021).

The application of Rhizobium to soybean plants has been proven to have a significant effect on increasing the number of pods per plant. This is due to the increased availability of nitrogen around the plant roots, which affects the generative growth of the plant. The rise in pod number following Rhizobium inoculation indicates that nitrogen fixation is crucial for enhancing soybean yields. This is supported by the statement of Sitorus and Tyasmoro, (2021) that Rhizobium inoculation can increase nitrogen fixation, thereby affecting the formation of pods. Research by Koesriharti, (2022) shows that Rhizobium inoculation results in a greater number of pods.

Seed Weight per 100 Beans

The results of the analysis of variance showed that the application of a combination of AMF and Rhizobium had a significant effect on the average seed weight per 100 soybean beans (Table 5).

Table 5. Average seed weight per 100 grains of soybean *(Glycine max* L. Merril) at 14, 21, 28, 35, and 42 days after sowing.

Code	Treatment	Weight per 100 Seeds (g)
A	Control	18,48d
B	AMF 15 g/plant	20,18cd
C	AMF 20 g/plant	21,60bcd
D	Rhizobium 15 ml/kg seed	22,50bc
E	AMF 15 g/plant + Rhizobium 15 ml/kg seed	22,38bc
F	AMF 20 g/plant + Rhizobium 15 ml/kg seed	24,30b
G	Rhizobium 20 ml/kg seed	22,18bc
H	AMF 15 g/plant + Rhizobium 20 ml/kg seed	28,13a
I	AMF 20 g/plant + Rhizobium 20 ml/kg seed	22,45bc
Code	Treatment	Weight per 100 Seeds (g)
	KK (%)	9,8

Note: Values followed by the same letter in each column are not significantly different at the 5% DMRT level.

The application of AMF at a dose of 15 g/plant + Rhizobium at 20 ml/kg of seeds had a significant effect on the weight of 100 soybean seeds. It is believed that AMF and Rhizobium strengthen root absorption so that phosphorus available in the soil becomes easily absorbed and supports the photosynthesis process. Both improve nutrient and water absorption, so that the photosynthesis and seed filling processes are more optimal, resulting in an increase in seed weight per 100 grains. This is supported by the statement by Kusumastuti, (2021) that the application of AMF and Rhizobium increases the seed weight per 100 grains in soybean plants.

AMF is able to support the absorption of available phosphorus in the soil. The availability of phosphorus in the soil can affect ATP formation in plants (Permadi and Haryati 2015). Plants treated with AMF exhibit an increased seed count per plant and greater seed weight (Permanasari et al., 2016). Research results indicate that applying AMF at a dose of 15 g/plant produces good growth and yield in terms of 100-seed weight in peanut plants (Mayasin et al., 2021).

The role of Rhizobium in increasing seed weight per 100 grains has a significant effect, which is influenced by increased plant growth due to high nitrogen absorption. Rhizobium's ability to form a symbiotic relationship with plants to fix nitrogen increases crop seed production. This is consistent with research findings that Rhizobium inoculation increases seed weight per 100 grains in soybean plants (Dina and Koesriharti 2022).

Plant Height

The results of the analysis of variance showed that the application of the combination of AMF and Rhizobium had a significant effect on the average height of soybean plants at 14, 21, 28, 35, and 42 days after plantin (Table 6).

Table 6. Average Height of Soybean Plants (*Glicyne max* L. Merril) at 14, 21, 28, 35, and 42 Days After Planting

Code	Treatment	Plant Height (cm)				
		14 hst	21 hst	28 hst	35 hst	42 hst
A	Control	16,87bc	21,91d	27,25d	35,49c	40,16c
B	AMF 15 g/plant	18,10ab	24,37bc	32,08b	37,66bc	43,08bc
C	AMF 20 g/plant	18,58ab	24,71bc	31,16cd	40,10bc	45,41bc
D	Rhizobium 15 ml/kg seed	16,25c	22,25cd	30,83cd	39,25bc	45,99bc
E	AMF 15 g/plant + Rhizobium 15 ml/kg seed	18,83ab	24,50bc	30,04cd	40,50bc	46,66bc
F	AMF 20 g/plant + Rhizobium 15 ml/kg seed	18,62ab	24,25bcd	32,33abc	41,92bc	49,08b
G	Rhizobium 20 ml/kg seed	19,83a	26,04ab	36,25ab	45,58ab	49,25b
H	AMF 15 g/plant + Rhizobium 20 ml/kg seed	20,92a	27,71a	36,91a	50,83a	59,17a
I	AMF 20 g/plant + Rhizobium 20 ml/kg seed	19,58a	25,04b	32,91abc	39,08bc	44,49bc
	KK (%)	7,9	6,2	9,2	11,9	9,7

Note: Values followed by the same letter in each column are not significantly different at the 5% DMRT level.

Applying 15 g/plant of AMF combined with 20 ml/kg of Rhizobium to seeds notably influenced the height of soybean plants. It is suspected that AMF expands the root system, making phosphorus in the soil more readily available for absorption, while Rhizobium provides nitrogen from fixation that can be directly utilized by the plants. The combination of the two produces an optimal balance of nutrient and energy availability, thereby supporting the process of cell division and elongation in the stem, which leads to increased plant height. Root elongation plays an important role in supporting nutrient uptake, which affects plant height. Longer roots allow plants to explore a wider volume of soil, making nutrient uptake more efficient. This is in line with the research by Mpongwana *et al.,* (2024) that the combination of AMF and Rhizobium has a significant effect on plant height.

AMF at a dose of 15 g/plant can expand the hyphal network, thereby increasing the ability of soybean plants to absorb available nutrients. The results of Missdiani, (2022) research show that the application of 15 g/plant of AMF has a significant effect on plant height. Applying AMF at a dose of more than 15 g/plant can trigger competition between microorganisms, thereby reducing the efficiency of root colonization.

The application of Rhizobium at a dose of 20 ml/kg of seeds can form a symbiotic relationship with soybean plants through infection of the root system, where the bacteria play a role in the process of fixing nitrogen from the air. With sufficient nitrogen available due to Rhizobium activity, plant growth becomes faster and taller than plants without inoculation. This is in line with the results of research by Shumet *et al.,* (2022) that Rhizobium inoculation treatment increases soybean plant height by up to 12%. Rhizobium is able to form a symbiotic relationship with soybean plants through infection of the root system, where the bacteria play a role in the process of nitrogen fixation from the atmosphere (Pabela et al*.,* 2024).

Number of Leaves

The analysis of variance results showed that the application of the combination of CMA and Rhizobium had a significant effect on the average number of soybean leaves at 21, 28, 35, and 42 days after sowing (Table 7).

Table 7. Average number of soybean leaves (Glicyne max L. Merril) at 14, 21, 28, 35, and 42 days after planting

Code	Treatment	Number of Leaves (pieces)				
		14 hst	21 hst	28 hst	35 hst	42 hst
A	Control	7,25bcd	9,49c	15,83bcd	22,42c	30,91bc
B	AMF 15 g/plant	8,16bc	9,58c	14,75bcd	25,66bc	34,41abc
C	AMF 20 g/plant	5,75d	10,42bc	15,58bcd	29,49abc	36,08abc
D	Rhizobium 15 ml/kg seed	6,16cd	9,58c	13,75cd	23,25c	33,08abc
E	AMF 15 g/plant + Rhizobium 15 ml/kg seed	7,75bcd	8,99c	12,25d	22,33c	27,99c
F	AMF 20 g/plant + Rhizobium 15 ml/kg seed	7,58bcd	9,40c	14,08cd	24,66bc	29,10bc
G	Rhizobium 20 ml/kg seed	8,41abc	11,92ab	18,33ab	30,83ab	39,75ab
H	AMF 15 g/plant + Rhizobium 20 ml/kg seed	10,49a	13,16a	20,25a	35,16a	43,50a
I	AMF 20 g/plant + Rhizobium 20 ml/kg seed	8,58ab	11,83ab	17,33abc	28,91abc	37,33abc
	KK (%)	18,23	10,86	14,76	16,97	19,22

Note: Values followed by the same letter in each column indicate no significant difference at the 5% DMRT level.

The application of a combination dose of 15 g/plant of AMF and 20 kg/seed of Rhizobium showed the best effect on increasing the number of leaves. It is thought that AMF is able to expand the hyphal network to increase the absorption capacity of phosphorus available in the soil. AMF can release bound phosphorus so that it is more easily absorbed by plants, thereby producing auxin and cytokinin which stimulate leaf formation. Rhizobium, through nitrogen fixation, accelerates root elongation, thereby increasing nutrient uptake and supporting the allocation of photosynthates for leaf growth. The combination of the two makes nutrient uptake more efficient, resulting in an increase in the number of plant leaves. This is supported by the statement by Duan et al*.,* (2024) that the combination of AMF and Rhizobium significantly increases plant vegetative biomass.

Higher doses of AMF result in suboptimal plant growth. This is because higher doses exceed the plant's requirements, preventing some mycorrhizae from developing properly and causing competition with Rhizobium bacteria. The results of a study by Watoni et al*.,* (2020) show that AMF doses of more than 15 g/plant result in lower leaf count parameters.

The application of AMF can form a mutualistic symbiosis with plant roots, creating external and internal hyphal structures that expand the root absorption area. This directly increases the efficiency of phosphorus nutrient absorption, which plays an important role in leaf formation and development. Phosphorus greatly influences the processes of growth, photosynthesis, respiration, and nitrogen fixation. The photosynthesis process will run efficiently if phosphorus is sufficient because one of the roles of phosphorus is to form energy compounds in the form of ATP, which is useful for producing photosynthesis results that will then be used for plant growth in terms of increasing the number of leaves. (Lestari et al. 2019). The results of research by Yuliyati et al*.,* (2023) show that the use of a AMF dose of 15 g/plant is proven to be optimal in supporting the growth of soybean leaves with the best results.

The application of Rhizobium plays an important role in increasing the number of leaves. This is because Rhizobium is a nitrogen-fixing bacterium that forms a symbiotic relationship with soybean roots. Nitrogen is a major nutrient that is essential for plant growth, particularly leaf growth, as it is directly involved in the formation of chlorophyll, amino acids, proteins, and enzymes that support cell division and photosynthesis. Sufficient nitrogen availability through biological fixation leads to an increase in the rate of photosynthesis, resulting in faster leaf growth and a significant increase in the number of leaves. This is in line with the statement by Khan et al*.,* (2019), who explained that nitrogen significantly accelerates the formation of new leaf structures and increases the number of leaves. The results of Rahmatullah, (2018) study showed that the application of Rhizobium at a dose of 20 ml/kg of seeds produced the highest number of leaves in peanut plants.

CONCLUSION

The combination of AMF 15 g/plant with Rhizobium 20 ml/kg seed (treatment H) yielded the highest results in terms of root infection at 81.50%, root length per plant of 28.58 cm, dry root weight of 2.43 g, number of pods per plant of 33.41, seed weight per 100 seeds 28.13 g, plant height of 59.17 cm, stem, number of leaves of 43.50.

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