Australian Journal of Crop Science

Article | https://doi.org/10.21475/ajcs.26.20.09.pne209
Submitted: 08 December 2025 | Revised: 28 March 2026 | Accepted: 28 March 2026
Pages 719-725

Dosage optimization of arbuscular mycorrhizal fungi for improving soybean (*Glycine max* (L.) Merrill) yield components in tropical ultisol: implications for marginal land agriculture

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Abstract: Soybean is a strategic food commodity in Indonesia; however, national productivity remains inadequate, particularly in marginal soils such as Ultisols, which are highly acidic, nutrient-poor, and chemically constrained. Arbuscular Mycorrhizal Fungi (AMF) represents a promising biofertilizer to overcome these limitations. This study assessed the effect and optimal dosage of AMF on soybean growth and yield in Ultisol soil. A Completely Randomized Design (CRD) with five AMF dosages (0, 0.4, 0.8, 1.2, and 1.6 g kg^{-1} soil, each applied to polybags containing 10 kg of Ultisol, with four replications was applied. Data were analyzed using ANOVA followed by Duncan's New Multiple Range Test (DNMRT) at $\alpha = 0.05$. The AMF significantly enhanced key yield attributes, including the percentage of filled pods, seed number, seed weight per plant, and harvest index, whereas seed size and protein content were unaffected. The optimal dosage was 1.2 g kg^{-1} soil. Although AMF inoculation at the optimal dosage of 1.2 g kg^{-1} soil markedly improved plant performance, the yield obtained remained below the documented yield potential of the Anjasmoro variety, indicating that AMF alone is insufficient for full yield expression in Ultisol and that complementary soil amendments or integrated nutrient management strategies are warranted.

Keywords: Arbuscular mycorrhizal fungi, Ultisol, soil fertility enhancement
Abbreviations: AMF_Arbuscular mycorrhizal fungi; ANOVA_Analysis of variance; CEC_Cation exchange capacity; CRD_Completely randomized design; DNMRT_Duncan's new multiple range test; g_Gram; ha_Hectare; K_Potassium; kg_Kilogram; mL_Milliliter; N_Nitrogen; P_Phosphorus; RP_Rock phosphate; Zn_Zinc; Cu_Copper; OM_Organic matter; pH_Soil acidity; BD_Bulk density; EC_Electrical conductivity; cm_Centimeter; mg_Milligram; SP_Soluble powder (fungicide formulation); %_Percentage.

Introduction

Soybean (*Glycine max* (L.) Merrill) is a globally important leguminous crop valued for its high protein content, essential amino acids, and diverse industrial applications. In Indonesia, soybean remains a strategic commodity for food security, supporting the national supply chain of tofu, tempeh, animal feed, and edible oils. Despite this importance, Indonesia's domestic soybean production has continued to decline over the past decade, with current yields meeting less than 20% of national consumption. As a result, more than 60% of soybean demand is fulfilled through imports, indicating persistent constraints in land availability, soil fertility, and crop productivity. Increasing domestic soybean production therefore remains a national priority to ensure long-term food sovereignty.

One major barrier to soybean expansion in Indonesia is the limited availability of fertile agricultural land. Consequently, cultivation has shifted increasingly toward marginal soils, particularly Ultisols, which occupy approximately 45.8 million hectares, roughly one-quarter of Indonesia's land area. Ultisols are highly weathered tropical soils characterized by strong acidity (pH < 5.5), low cation exchange capacity, low base saturation, and high concentrations of exchangeable aluminum. These properties contribute to poor nutrient availability, especially phosphorus (P), which is often rendered insoluble through fixation by Fe and Al oxides. Ultisol soils therefore create suboptimal conditions for soybean growth, reducing root development, biological nitrogen fixation, and yield formation.

Biological amendments, especially Arbuscular Mycorrhizal Fungi (AMF), have emerged as promising biotechnological tools to improve soil fertility and enhance crop performance in degraded soils. AMF form mutualistic associations with plant roots, extending the absorptive surface area through extensive hyphal networks. These symbioses enhance nutrient acquisition, particularly for P, N, and micronutrients, improve water uptake, increase drought tolerance, stimulate plant defense mechanisms, and ultimately improve crop growth and productivity. Numerous studies have demonstrated the ability of AMF to increase soybean biomass, nutrient uptake, and yield components under nutrient-limited conditions, including marginal soils. Recent reports also suggest that AMF inoculation can reduce P fertilizer requirements, enhance rhizosphere functioning, and improve soil physical and biological properties.

Despite extensive research on AMF, legume interactions, studies specifically quantifying the dose-response relationship of AMF inoculation under Ultisol conditions, characterized by high acidity, Al toxicity, and P fixation, remain limited. Furthermore, the differential effects of AMF on yield quantity versus nutritional quality attributes in soybean under such edaphic constraints have not been systematically examined. In particular, optimal AMF dosage in highly acidic Ultisol soils has not been well established, and previous studies often report inconsistent results. Some studies have used very low dosages (e.g., <5 g/plant), while others use high dosages (>20 g/plant), yet few have systematically evaluated the dosage–response relationship for soybean in Ultisol. Moreover, most existing studies focus on vegetative responses, while comparatively fewer have quantified key yield components such as filled pods, seed number, seed weight, harvest index, and seed quality indicators under Ultisol conditions. This gap limits the development of precise, science-based recommendations for AMF use in Indonesian soybean production systems.

Results and discussion

Percentage of filled pods

ANOVA results showed that AMF application significantly influenced the percentage of filled pods. The mean comparison using Duncan's New Multiple Range Test (DNMRT) at 5% is presented in Table 1. It shows that the percentage of filled pods increased with the application of AMF at a dose of 0.4 g kg^{-1} soil compared with no treatment, and the subsequent dose increases of 0.8, 1.2, and 1.6 g kg^{-1} soil did not show differences compared with the application of AMF at a dose of 0.4 g kg^{-1} soil. This indicates that the application of AMF affected the increase in the percentage of filled pods. The highest percentage was achieved in the AMF treatment with a dose of 1.6 g kg^{-1} soil, namely 98.38%, while the lowest percentage was found in plants without AMF application, namely 94.81%. These results show that the generative growth phase of the plant requires the availability of nutrients, particularly nitrogen (N), phosphorus (P), and potassium (K). In the formation of filled pods in soybean plants, phosphorus plays an important role, which is also supplied by AMF. According to Asril et al. (2023), the P element functions in stimulating the formation of flowers, fruits, and seeds, and accelerating the fruit ripening process. With the application of AMF, the availability of phosphorus increases, which affects the increase in fruit formation and ripening, thereby increasing the percentage of filled pods. Sirisuntornlak et al. (2024) showed that seed priming treatment with phosphorus on land with low P content can increase the number of flowers, number of pods, and antioxidant compound content in lentil seeds.

In addition to phosphorus (P), potassium (K) is an important macronutrient required for plant growth, especially in the formation and filling of pods. According to Pakpahan (2018), the application of AMF can increase the absorption of various other nutrients, such as nitrogen (N), potassium (K), as well as micronutrients such as zinc (Zn) and copper (Cu). Potassium plays an important role in the translocation of photosynthates from the leaves through the phloem tissue to the reproductive organs and storage sites, including in the process of pod formation.

The pods formed originate from flowers that have been successfully fertilized and then develop into seeds. The higher the number of flowers that undergo fertilization, the greater the chance of pod formation. However, not all pods that are formed will develop perfectly into filled pods, because only some of them successfully pass through the seed formation phase properly.

Table 1. Mean percentage of filled pods under AMF application.

AMF Treatment (g kg^{-1} soil)	Filled Pods (%)
0	94.81 b
0.4	98.28 a
0.8	98.12 a
1.2	97.70 a
1.6	98.38 a

Note: Numbers in the same row and column followed by the same letter are not significantly different according to the DNMRT test at the 5% level.

Number of seeds per plant

The results of the analysis of variance showed that the application of AMF had a significant effect on the percentage of filled pods. Further testing using Duncan's New Multiple Range Test (DNMRT) at the 5% significance level for the percentage of filled soybean pods is presented in Table 2.

Based on Table 2, the number of seeds per plant increased with the application of AMF at a dose of 0.8 g kg^{-1} soil, although the difference was not significant compared with a dose of 0.4 g kg^{-1} soil and plants without treatment. The increase in the number of seeds became more significant at doses of 1.2 and 1.6 g kg^{-1} soil , namely 1.48 and 1.59 times higher, respectively, compared with the control. The application of AMF at a dose of 1.6 g kg^{-1} soil produced the highest number of seeds per plant compared with the other treatments. These results indicate that the increase in AMF dosage tends to increase the number of seeds produced by each plant. This increase is thought to be related to the increased ability of plants to absorb nutrients along with the increased AMF dosage, which has a positive effect on metabolic processes and yield formation.

Table 2. Mean number of seeds per plant under AMF application.

AMF Treatment (g kg^{-1} soil)	Number of Seeds (seeds/plant)
0	22.50 b
0.4	26.00 b
0.8	31.50 a
1.2	33.38 a
1.6	35.75 a

Note: Numbers in the same row and column followed by the same letter are not significantly different according to the DNMRT test at the 5% level.

According to Herlina et al. (2016), the presence of AMF is able to increase the rate of nutrient uptake by roots up to almost four times higher compared with roots without AMF and expand the root absorption area up to 80 times wider.

The application of AMF to plants can increase nutrient uptake, particularly phosphorus (P). Plants that form symbiosis with AMF tend to have a greater ability to absorb phosphorus compared with plants not infected with AMF. Sanou et al. (2024) reported that the application of phosphorus at 100 kg/ha significantly increased seed production and viability in forage legumes such as *Aeschynomene histrix* and *Stylosanthes hamata*. This shows that P not only promotes flowering but also seed formation and seed quality. Phosphorus deficiency can inhibit the formation of pods and seeds. Ishaq et al. (2023) reported that dual inoculation of Rhizobium and AMF increased P availability in the soil, number of pods, and seed weight in soybeans grown on vertisol soils; AMF helped meet phosphorus needs during the seed formation and filling phase. In addition, Wu et al. (2025) showed that AMF inoculation significantly increased P uptake by up to 105% in general, and legume crops (including soybean) showed a greater response compared with non-legumes. The phosphorus (P) element plays a major role in the seed-filling process, fruit or grain ripening, and increasing grain yield. In addition, phosphorus also functions in photosynthesis, carbohydrate metabolism, and the translocation of photosynthates from the leaves to other plant organs, including seed formation. The higher the phosphorus uptake by plants, the greater the number of seeds produced by soybean plants. Siregar et al. (2017) found that increasing phosphorus application can increase the number of seeds in soybean plants.

Seed weight per plant

The results of the analysis of variance showed that the application of AMF had a significant effect on the percentage of filled pods. Further testing using Duncan's New Multiple Range Test (DNMRT) at the 5% significance level for the percentage of filled soybean pods is presented in Table 3.

Seed weight per plant increased significantly with AMF application (Table 3). A dose of 0.8 g kg^{-1} soil enhanced seed weight by 1.53-fold relative to the control, with further increases observed at 1.2 and 1.6 g kg^{-1} soil (1.71- and 1.89-fold, respectively). This improvement aligns with the hypothesis that AMF boosts P acquisition, thereby supporting photosynthetic activity and carbon allocation.

P serves as a critical component of nucleic acids, ATP, and phospholipids, making it essential for biomass accumulation and grain filling. Santosa et al. (2016) confirmed that AMF increases P absorption, while Ghulamahdi et al. (2025) demonstrated that AMF application in saturated acid-sulfate soils increased P efficiency, flowering rate, 100-seed weight, and total seed production.

Higher AMF dosages increase root colonization intensity, expanding the hyphal network and enhancing nutrient scavenging. This improves assimilate flow to developing seeds, increasing overall seed biomass per plant. Therefore, the improvement in seed weight is a direct consequence of increased seed number coupled with improved sink strength during the grain-filling stage.

Table 3. Mean seed weight per plant under AMF application.

AMF Treatment (g kg^{-1} soil)	Seed Weight (g/plant)
0	2.16 c
0.4	2.54 bc
0.8	3.31 ab
1.2	3.69 a
1.6	4.08 a

Note: Numbers in the same row and column followed by the same letter are not significantly different according to the DNMRT test at the 5% level.

Seed size

The results of the analysis of variance showed that the application of AMF had a significant effect on the percentage of filled pods. Further testing using Duncan's New Multiple Range Test (DNMRT) at the 5% significance level for the percentage of filled soybean pods is presented in Table 4.

Table 4 shows that the application of AMF at doses of 0.4, 0.8, 1.2, and 1.6 g g kg^{-1} soil did not increase soybean seed size compared with plants without treatment. This indicates that AMF has a limited effect on increasing soybean seed size. The reason is the use of the same variety, namely Anjasmoro, so that soybean seed size tends to be uniform.

Table 4. Mean seed size under AMF application.

AMF Treatment (g kg^{-1} soil)	Seed Size (mg/seed)
0	0.10 a
0.4	0.10 a
0.8	0.11 a
1.2	0.11 a
1.6	0.11 a

Note: Numbers in the same row and column followed by the same letter are not significantly different according to the DNMRT test at the 5% level.

Seed size in soybean plants is one of the important indicators in assessing soybean seeds. Genetic factors of the plant have a major influence on seed size, so the same variety tends to produce uniform seed size. According to Yang et al. (2023), genetic traits of the plant strongly determine seed size in soybeans. The genes that control seed size are usually stable and strongly regulate phenotype, so even though there are different treatments, such as fertilizer application or mycorrhizal inoculation, seed size tends not to change significantly.

Plants have mechanisms that regulate resource allocation (assimilates) to seeds in a balanced manner. If the number of seeds per plant changes, the plant tends to adjust nutrient allocation so that seed size remains consistent (Fang et al., 2024). Soybean seed size is influenced by the ability of the plant to transfer assimilates to the seeds, where this trait is more influenced by genetic factors. Seed weight and shape are strongly determined by specific genes present in the plant.

Harvest index

The results of the analysis of variance showed that the application of AMF had a significant effect on the percentage of filled pods. Further testing using Duncan's New Multiple Range Test (DNMRT) at the 5% significance level for the percentage of filled soybean pods is presented in Table 5.

Table 5 shows that the harvest index of soybean increased with the application of AMF at a dose of 1.6 g g kg^{-1} soil and tended to increase with the application of AMF at doses of 0.4, 0.8, and 1.2 g kg^{-1} soil compared with no treatment. The application of AMF has been proven to increase soybean production. Faryabi et al. (2015) reported that the harvest index of mung bean plants given AMF increased by 49.83% compared with plants that did not receive AMF. This increase was also triggered by the symbiosis between AMF and soybean roots, which helps plant growth become more optimal.

AMF inoculation significantly increases the ability of soybean to absorb water and nutrients. Wu et al. (2024) found that AMF inoculation increased phosphorus uptake by up to 105% and nitrogen uptake by up to 67% and supported biomass growth by up to 47% overall. In addition, research by Al-Karaki et al. (2021) on soybeans under drought stress conditions showed that inoculation with mixed AMF improved photosynthetic capacity, stomatal conductance, and leaf water retention compared with the control without inoculation. AMF plays a role in increasing the plant's capacity to absorb nutrients and water. In addition, AMF can also expand the area of nutrient and CO_2 absorption in infertile soils and convert bound phosphorus (P) into forms more easily absorbed by plants. Thus, AMF can increase the harvest index of soybean plants.

Table 5. Mean harvest index under AMF application.

AMF Treatment (g kg^{-1} soil)	Harvest Index (%)
0	34.59 b
0.4	52.02 ab
0.8	51.58 ab
1.2	50.39 ab
1.6	63.44 a

Note: Numbers in the same row and column followed by the same letter are not significantly different according to the DNMRT test at the 5% level.

Protein content

The results of the analysis of variance showed that the application of AMF had a significant effect on the percentage of filled pods. Further testing using Duncan's New Multiple Range Test (DNMRT) at the 5% significance level for the percentage of filled soybean pods is presented in Table 6.

Table 6 shows that the protein content of soybeans with the application of AMF at doses of 0.4, 0.8, 1.2, and 1.6 g kg^{-1} soil did not increase protein content compared with no treatment. This is expected to be due to the use of the same variety, namely Anjasmoro, so that soybean protein content is relatively similar.

Soybean seed protein is significantly influenced by genes and their interaction with the environment. A study in Canada on 23 genotypes showed that genotype and environment explained most of the variation in protein content, although there were interactions, with genetics being the dominant factor. This means that without genotype changes, variations in environment or treatment do not always result in large changes in protein content (Zhang et al., 2023). Protein and fat content in soybean seeds are influenced by the plant's genetic characteristics. This shows that genetic factors play an important role in determining protein content in soybean seeds.

Based on Table 6, the protein content of soybean plants was still far below the description, which ranged from 41.78–42.05%. This may occur because the planting medium used was Ultisol soil, which contains low levels of nutrients, thus not

Table 6. Mean protein content under AMF application.

AMF Treatment (g kg^{-1} soil)	Protein Content (%)
0	11.25 a
0.4	16.47 a
0.8	17.64 a
1.2	14.80 a
1.6	17.42 a

Note: Numbers in the same row and column followed by the same letter are not significantly different according to the DNMRT test at the 5% level.

supporting optimal soybean growth and yield. The application of AMF also has not been able to increase soybean protein content, because P is not a major component of protein but affects the absorption of other nutrients including N.

Hati (2023) stated that Ultisol soil has high acidity levels and low macro- and micronutrient content. In addition, Ultisol soil tends to dry out easily during the dry season, so plants grown on it are at risk of experiencing drought stress. These Ultisol soil conditions can affect the compatibility of soybean plants with AMF.

The results of the study showed that all observed parameters in soybean plants had lower growth and yield compared with the standard for the Anjasmoro soybean variety. This condition is thought to be due to soybean plants being grown in polybags using Ultisol soil. Planting in polybags limits root growth, so plants cannot absorb nutrients optimally due to limited planting media, resulting in less than optimal growth and yield. Soybean roots can grow well if the soil is suitable to support plant development. In addition, Ultisol soil, which is poor in nutrients, is also a factor causing suboptimal growth and yield of soybean plants. According to Rifki et al. (2022), marginal land has physical, chemical, and biological soil characteristics that are less supportive, so plant growth in such soils is not optimal.

Materials and methods

Place and time

The experiment was conducted at the Experimental Garden of the Faculty of Agriculture, Universitas Riau, located at the Bina Widya Campus, Km 12.5, Simpang Baru, Tampan District, Pekanbaru, Riau Province, Indonesia. The study area is situated in a humid tropical region with high annual rainfall (>2,000 mm $year^{-1}$) and an average temperature ranging from 26–28°C. The soil type is classified as Ultisol based on USDA Soil Taxonomy, characterized by high acidity, low nutrient availability, and strong leaching. The experiment was carried out from Januari–May 2025.

Experimental materials and soil characterization

The materials used in this research were Anjasmoro soybean seeds, Ultisol soil originating from Batu Belah Village, Kampar District, Kampar Regency, Riau Province, arbuscular mycorrhizal fungi (AMF) biofertilizer sourced from Universitas Andalas, Padang, West Sumatra with sand as the carrier medium, *Rhizobium*, Urea fertilizer, Rock Phosphate (RP) fertilizer, KCl fertilizer, dolomite ($CaMg(CO_3)_2$), water, distilled water, 10% KOH, 3% H_2O_2, soybean seed samples, H_2SO_4, selenium, 1% H_3BO_3, Conway indicator, 40% NaOH, Dupont Lannete 40 SP, Dithane M-45 fungicide, and polybags measuring 35 cm × 40 cm.

The equipment used in this research consisted of a hoe, 20-mesh sieve, sickle, machete, knife, analytical balance, oven, measuring tape, hand sprayer, watering can, paranet, ruler, wooden stakes, buckets, plant stakes, labels, film bottles, measuring cylinders, drop pipettes, stirring rods, scissors, mortar and pestle, Erlenmeyer flasks, Kjeldahl apparatus set, Kjeldahl flasks, boiling flasks, pipettes, burette, stand, weighing bottles, oven, beakers, distillation apparatus, digestion apparatus, desiccator, stationery, and documentation tools.

Experimental design

This research was an experimental study using a Completely Randomized Design (CRD) with treatments consisting of AMF application. The treatments consisted of five levels, namely:

M0 = Without AMF

M1 = AMF 0.4 g kg^{-1} soil

M2 = AMF 0.8 g kg^{-1} soil

M3 = AMF 1.2 g kg^{-1} soil

M4 = AMF 1.6 g kg^{-1} soil

These treatments were repeated 4 times, resulting in 20 experimental units. Each experimental unit consisted of 3 polybags, so this research used 60 polybags.

Cultivar selection and soil sampling procedure

Soybean seeds (*Glycine max* (L.) Merrill) of the Anjasmoro variety were sourced from a certified seed supplier. Seeds were manually selected to ensure uniform size and physiological quality.

Ultisol soil was collected from Batu Belah Village, Kampar District, Riau Province. The soil was air-dried, crushed, and sieved using a 20-mesh sieve before use. A composite sample of the soil was analyzed prior to planting to determine its physical–chemical properties, including pH (H_2O), organic C (Walkley–Black), available P (Bray-1), total N (Kjeldahl), exchangeable cations (NH_4OAc extraction), and texture (hydrometer method).

Mycorrhizal inoculum and other inputs

The Arbuscular Mycorrhizal Fungi (AMF) inoculum originated from the Mycorrhiza Laboratory of Universitas Andalas (West Sumatra). Although the specific fungal species were not designated, the inoculum consisted of a typical consortium of Glomeromycotan spores, hyphae, and colonized root fragments commonly found in AMF formulations used in tropical soil research. The inoculum was mixed with sterilized sand as the carrier medium with an approximate density of 80–120 spores g^{-1}.

Rhizobium inoculant, Urea fertilizer, Rock Phosphate (RP), KCl, and dolomite ($CaMg(CO_3)_2$) were utilized to support plant establishment. Chemical reagents including H_2SO_4, H_3BO_4, H_2O_2, and KOH were prepared according to standard laboratory procedures for use in nutrient and protein analysis.

Planting management

Each polybag (35 × 40 cm) was filled with 10 kg of processed Ultisol soil. Dolomite was applied at 2 t ha^{-1} equivalent (5 g $polybag^{-1}$) to partially neutralize soil acidity and improve nutrient availability. Basal fertilization followed local soybean cultivation recommendations: Urea at 25 kg ha^{-1} equivalent, Rock Phosphate at 100 kg ha^{-1} equivalent, and KCl at 50 kg ha^{-1} equivalent. Fertilizers were incorporated uniformly into the planting media.

Soybean seeds were surface-sterilized using 1% sodium hypochlorite for 3 minutes, rinsed with distilled water, and inoculated with *Rhizobium* prior to planting. Two seeds were sown per polybag at a depth of 2–3 cm, and seedlings were later thinned to one healthy plant per polybag.

AMF inoculum was applied directly below the seed at planting time to ensure effective root contact during early growth. Plants were irrigated manually twice daily to maintain near-field capacity moisture and were protected with paranet shading (50%) during the first two weeks to reduce initial transplant stress. Weed control was performed manually, and pest or disease occurrences were managed using Dithane M-45 when required.

Observed variables

The study assessed growth and yield components associated with AMF application. The primary variables observed included:

Percentage of Filled Pods (%) measured at maturity based on the ratio of filled pods to total pods.

Number of Seeds per Plant determined by manual counting after threshing.

Seed Weight per Plant (g) obtained after oven-drying seeds at 70 °C until constant weight.

Seed Size (mg per seed) calculated as total seed weight divided by number of seeds.

Harvest Index (%), calculated as:

$$\text{Harvest Index} = \frac{\text{Economic Yield}}{\text{Total Biomass}} \times 100$$

Seed Protein Content (%), analyzed using the Kjeldahl method with nitrogen-to-protein conversion factor 6.25.

These parameters were selected to provide a comprehensive assessment of AMF influence on reproductive development, assimilate allocation, and final productivity in nutrient-deficient Ultisol conditions.

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) with an F-test at the 5% significance level. If the F-test showed a significant effect of the treatments on the observed variables, the analysis was continued with Duncan's New Multiple Range Test (DNMRT) at the 5% significance level to compare the means among treatments.

Conclusion

The application of AMF can increase soybean yield, especially in the percentage of filled pods, number of seeds per plant, seed weight per plant, and harvest index, but does not affect seed size and protein content. The optimal dose was found in the application of AMF at 1.2 g kg^{-1} soil . However, based on the research conducted, the growth and production of soybean plants were still far from the expected target, so further research is needed.

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