

Efficacy Of Indigenous Arbuscular Mycorrhizal Fungi And Liquid Organic Fertilizer For Promoting The Vegetative Growth Of Soybean Plants (*Glycine max* L.) On Ultisols

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Abstract.

Utilization of ultisols in Southeast Sulawesi may be approached by inoculating viable arbuscular mycorrhizal fungi (AMF) exposing plant growth-promoting activities to the growth of native economical plants. This study investigated a combination of AMF and liquid organic fertilizers from coconut husks to improve the growth of soybean plants grown on ultisols as one of the potential marginal soils in the region. The coconut husk served as an abundant, cheap, yet potential source for the preparation of liquid organic fertilizers. This study used two factorial design with four treatment levels. The first factor was designed for different AMF species (10 g/pots): control (M_0), *Acaulospora delicata* (M_1), *Septoglomus constrictum* (M_2), and *Claroideoglossum etunicatum* (M_3) while the second factor was designed for different concentration of liquid organic fertilizer (mL/pots): control (P_0), 150 mL/pots (P_1), 200 mL/pots (P_2), and 250 mL/pots (P_3). Based on ANOVA results, the independent application of AMF species and liquid organic fertilizers gave significant results in regards to the experimental variables such as plant height, number of leaves, leaf area, and stem diameter. The best combination of treatment was observed in the treatment using *Septoglomus constrictum* with the concentration of liquid organic fertilizer of 250 mL (M_2P_3) to yield the highest growth performance of soybean plants on ultisols.

Keywords: Arbuscular mycorrhizal fungi, coconut husk, growth performance, soybean, ultisol.

1. INTRODUCTION

Soybean (*Glycine max* L. Merrill) belongs to the legume group as one of the important agro-commodities in Indonesia after rice and maize [1]. Soybeans are high in proteinaceous (40%) and decent sources of other nutritional components such as 9% water, 18% lipid, 3.5% fiber, 7% sugar and about 18% other minor components. The high protein content in soybeans have promoted its consumption as raw material for various staple food industries such as tempeh, tofu, milk, soy sauce, animal feed and other industrial materials [2].

Along with the increasing population growth rate and public perception of the protein need and nutrients, soybean demand rises every year [3]. Based on data from the Southeast Sulawesi Food Crops and Animal Husbandry Service (2018), soybean production reached 9,853 tons of dry beans in 2018 and 16,136 tons of dry beans in

2016. Soybean production in Southeast Sulawesi has decreased by 6,283 tons in 2018 as opposed to soybean production in 2016.

The low production of soybean is thought to be due to low soil fertility and lack of technical mastery. In fact, Southeast Sulawesi is one of the areas with very extensive agricultural land potential, with a total area of 3,814,000 hectares, 69% of which is dryland [4]. However, more than 70% of the Southeast area of Sulawesi is dominated by Ultisol soils, which have the potential to be utilized for soybean planting. Conversely, this land is low in fertility and poor in organic matter. Ultisols have a high content of clay, high pH or acidity, poor nutrient and unstable water balance in quality [5].

Attempts to improve crop yields and soil conditions while retaining environmental balance is achieved through the application of arbuscular mycorrhizal fungi (AMF). Husna et al. [6,7], reported that AMF is a group of soil microorganism capable of initiating a mutualistic symbiosis with plants. The inoculation of AMF will help plants to absorb the unavailable nutrients in soil environment. In addition, Sadaghiani et al. [8] reported that the mycelium of certain mycorrhizal fungi effectively absorbed N, P, K, Ca and Mg nutrients for plants.

Some species of indigenous AMF from the rhizosphere are rarely tested on agricultural crops, one of which is soybeans. Meanwhile, application of liquid organic fertilizers to soybean plants will increase the concentration of amino acids and proteins in soils, thereby promoting the plant growth. This research utilized brown coconut husk as the abundant source for the preparation of liquid organic fertilizers. Waryanti et al. [9] stated that coconut husk contains macronutrients such as organic C, nitrogen, phosphorus, and potassium, with the percentage of 11.69%, 2.251%, 0.71%, and 0.029% respectively on the 14th day of application and 11.28%, 2.366%, 0.70%, and 0.041% on the 28th day of application. On the basis of these findings, it is necessary to conduct a preliminary investigation regarding the efficacy of indigenous AMF isolates and the addition of coconut husk liquid organic fertilizer to the vegetative growth of soybean plants (*Glycine max* L. Merrill.) on ultisols.

2. METHODS

Experimental design

This study was conducted at Field Laboratory of Faculty of Agriculture, Laboratory of Agrotechnology, and Integrated Laboratory of Universitas Halu Oleo. This study used two factorial designs with four treatment levels. The first factor was designed for different AMF species (10 g/pots): control (M₀), *Acaulospora delicata* (M₁), *Septoglomus constrictum* (M₂), and *Claroideoglomus etunicatum* (M₃) while the second factor was designed for different concentration of liquid organic fertilizer (mL/pots): control (P₀), 150 mL/pots (P₁), 200 mL/pots (P₂), and 250 mL/pots (P₃). The final experiments were designed in 16 combinations with each three replicates, resulting in 48 experimental units.

Preparation of planting media

The planting media were ultisols which were sterilized in a furnace with a drum container. The sterilized soils were then air-dried prior experimentation. The sterilized soils were sorted from debris such as twigs, roots, and foliar remnants, loosened and put into polybags measuring 30 × 40 cm weighing ± 10 kg.

Preparation of liquid organic fertilizer

Brown coconut husks were used as the raw materials for preparing the liquid organic fertilizers. Coconut husks were cut into small fragments, then subjected to submerged fermentation in water for 14 days. The fermentation step was stopped when there was a presence of pungent odor or alcoholic scent with reddish fluid indicating that the liquid organic fertilizers were ready to use for further experimentation.

Source of indigenous arbuscular mycorrhizal fungi (AMF)

The AMF strains used in this study were provided by Prof. Dr. Ir. Hj. Husna Faad, M.P maintained in the soils at the Laboratory of Forestry, Faculty of Forestry, Universitas Halu Oleo, Kendari. Inoculation of AMF into soybean plants were adjusted to certain concentration levels. The soybean seeds were firstly soaked in water for 60 min. Three seeds were planted in a polybag containing 10 g of AMF soils.

Application of liquid organic fertilizers

The liquid organic fertilizers were applied at the rhizospheric region of soybean plants. The application started after 7 day after sowing (DAS), followed consecutively at 14, 21, 28, and 35 DAS. The sequential application were meant to maximize the growth promoting effect from the fertilizers.

Maintenance

Plant maintenance includes watering, replanting, weeding and thinning. Watering was given 2 times a day (morning and evening) or according to the field conditions. Replanting was done when the plant died or displaying suboptimal growth at 7 DAS. Weeding was done manually by removing the weeds in the polybags. Thinning was done at 14 DAS by pulling out the plants with suboptimal growth, leaving other plants to be maintained until harvesting period.

Harvesting

The harvesting was done by cutting the base of the soybean stem which have undergone physiological ripening. The signs of harvest-ready soybean plants were having the most of leaves (90–95%) turning brownish yellow and then shed, the stems dried out and were slightly brownish yellow in color.

Experimental variables

The variables observed in this study were plant height (cm), observed by measuring the soybean plants from the stem base until the longest plant tips. Number of leaves was observed by counting the number of blades that have opened perfectly. Leaf area (cm²) was measured from the length of the leaves in proportion to the width on the upper, middle, and lower side of leaves using the following formula:

$$LA = L \times W \times c$$

Where:

LA = Leaf area (cm²)

L = Leaf length (cm)

W = Leaf width (cm)

C = Constant (0.74) [10]

The stem diameter (cm) was measured using calipers. All variables were measured at 14, 28, 42, and 56 DAS.

Data analysis

Experimental data were analyzed using an analysis of variance (ANOVA). If the analysis resulted in which $F_{\text{count}} > F_{\text{table}}$, then multiple comparisons were performed using Duncan's multiple range test at 95% level of significance.

3. RESULT AND DISCUSSION

Ultisols have a high degree of acidity (pH) and low availability of N, P and K nutrients. The high Al concentration in ultisols naturally fixed the phosphate elements yet limiting the phosphate availability for root absorption and low N cycling in the soil environment. This is due to the intensive leaching of Ultisols, supported by the use of inorganic fertilizers in agricultural systems resulting in low productivity. This condition then requires an effective solution, therefore by utilizing the indigenous AMF and liquid organic fertilizers from coconut husks to facilitate the natural nutrient cycling in soils.

The results showed that the interaction between the application of AMF and liquid organic fertilizer at various concentrations had no significant effect in promoting the growth of soybean plants based on plant height, number of leaves, leaf area, and stem diameter. The results showed that the response of soybean plants to each factor in growth and productivity affected independently (Table 1).

1. Results of ANOVA of soybean plants growth performance

The growth performance of soybean plants after application of AMF and liquid organic fertilizers were analyzed using ANOVA as presented in Table 1.

Table 1. Analysis of variance (ANOVA) of growth performance of soybean plants after application of indigenous AMF and liquid organic fertilizers

Observational Variables	ANOVA (<i>F</i>)		
	AMF (M)	Liquid Organic Fertilizers (P)	Interaction (M*P)
Plant height (cm)			
14 DAS	ns	*	Ns
28 DAS	**	*	Ns
42 DAS	**	*	Ns
56 HST	**	**	Ns
Number of leaves			
14 DAS	ns	Ns	Ns
28 DAS	ns	Ns	Ns
42 DAS	**	Ns	Ns
56 DAS	**	Ns	Ns
Leaf area (cm²)			
14 DAS	**	Ns	ns
28 DAS	*	Ns	Ns
42 DAS	ns	Ns	ns

56 DAS	ns	Ns	Ns
Stem diameter (cm)			
14 DAS	ns	Ns	Ns
28 DAS	**	Ns	Ns
42 DAS	**	*	Ns
56 DAS	**	Ns	Ns

Notes: ns = non significant, * = significant, ** = highly significant. DAS = day after sowing, AMF = arbuscular mycorrhizal fungi, cm = centimeter.

The efficacy of indigenous AMF in promoting plant growth in suboptimal lands have also been reported by other studies [11,7,12,13] in which the nutrient absorption by the roots of nandu wood (*Pericopsis mooniana*) were increased when grown on soils in mine tailings of nickel, gold, and coals. The results then indicated that the AMF played roles in supporting the growth of plants under harsh conditions by providing the nutrients supplies for vegetative growth (Figure 1) which also increased the biomass of plants. The successful root colonization of AMF in soybean plants initiated the optimum absorption of water and nutrients through hyphal penetration in soils.



Fig.1. Comparison between control treatment w/o AMF (M_0) + liquid organic fertilizer (P_0) and experimental treatment with AMF (M_2) + liquid organic fertilizer (P_3)

2. Plant height

The growth dynamics of the average height of soybean plants treated with indigenous AMF and liquid organic fertilizer from coconut husk at the ages of 14, 28, 42 and 56 DAS are presented in Figure 1. Independent effects of indigenous AMF and liquid organic fertilizer treatment at the ages of 14, 28, 42 and 56 DAS are presented in Tables 2 and 3.

Figure 1 shows that the dynamics of the height increase of soybean plants treated with indigenous AMF and the optimum concentration of liquid organic fertilizer were obtained in the combination treatment of the *S. constrictum* and 250 mL of liquid organic fertilizer (M_2P_3).

Nitrogen plays an important role in increasing plant height and biomass. The inoculation of AMF and applied liquid organic fertilizers provided additional nutrients

which were more likely available in the soils and can be absorbed by plants therefore increase the cell division as a consequence to plant growth and development. The result was in accordance with the statement by Nasrudin [14], who stated that nitrogen is the main macronutrient for plants that can accelerate plant vegetative growth. According to Hidayati [15], N, P, K fertilizers are needed for plant growth, especially in stimulating the plant height and enlarging the stem diameter. Apart from N and P K nutrients, organic fertilizers also have a role in supporting plant vegetative growth.

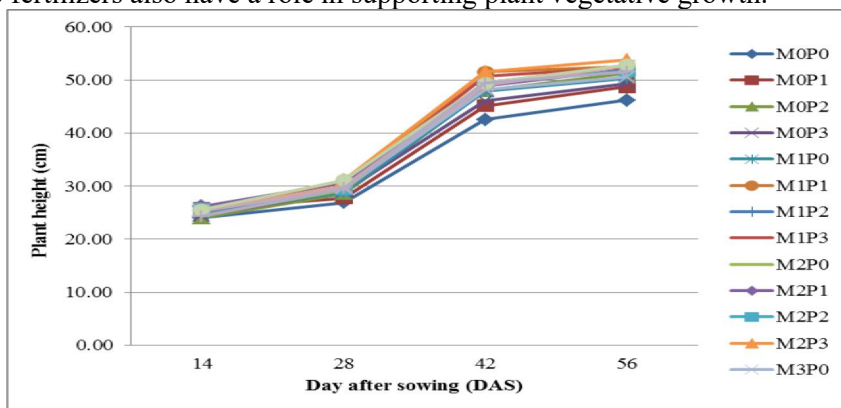


Fig.2. Dynamics of the height increase of soybean plants treated with AMF and liquid organic fertilizer from coconut husk.

Table 2. The effect of AMP application to the plant height of soybean plants at 14, 28, 42, and 56 DAS

FMA (M)	Age of plants			
	14 DAS	28 DAS	42 DAS	56 DAS
Control (M ₀)	24.56b	28.05b	45.43b	48.88b
<i>A. delicata</i> (M ₁)	25.32 ab	29.73 a	49.89 a	51.98 a
<i>S. constrictum</i> (M ₂)	25.44 a	30.15 a	49.93 a	52.31 a
<i>C. etunicatum</i> (M ₃)	25.15 ab	30.02 a	49.02 a	51.92 a
DMRT 95 %		2=0.8439	2=1.450	2=1.404
		3=0.8869	3=1.524	3=1.475
		4=0.9147	4=1.572	4=1.521

Notes: Values followed by the same letter in the same column are not significantly different based on the DMRT at the 95% level of significance.

Table 2 shows that the best indigenous AMF treatment at age of 14 DAS and 56 DAS was obtained in the treatment using *S. constrictum* (M₂) which was higher than control (M₀), but not significantly different from *A. delicata* (M₁) and *C. etunicatum* (M₃). Meanwhile, the application of *A. delicata* (M₁) and *C. etunicatum* (M₃) were not significantly different without AMF treatment on the average plant height at 14 DAS.

Table 3. The effect of liquid organic fertilizer application to the plant height of soybean plants at 14, 28, 42, and 56 DAS

LOF (P)	Age of plants			
	14 DAS	28 DAS	42 DAS	56 DAS
LOF 0 ml (P ₀)	24.68 b	28.62 b	47.08 b	49.69 b
LOF 150 mL/polybag (P ₁)	25.83 a	29.75 a	48.63 a	51.47 a
LOF 200 mL/polybag (P ₂)	25.07 b	29.71 a	49.14 a	52.14 a
LOF 250 mL/polybag (P ₃)	24.89 b	29.88 a	49.42 a	51.78 a

DMRT 95 %	2= 0.7580	2= 0.8439	2= 1.450	2= 1.477
	3= 0.7966	3= 0.8869	3= 1.524	3= 1.552
	4= 0.8216	4= 0.9147	4= 1.572	4= 1.601

Notes: LOF = liquid organic fertilizer. Values followed by the same letter in the same column are not significantly different based on the DMRT at the 95% level of significance.

Table 3 shows that the treatment of liquid organic fertilizer (LOF) from coconut husk at the age of 14 DAS with the best results were obtained in the treatment of 150 mL/polybag (P₁) which was higher than the 200 mL (P₂), 250 mL (P₃), and control w/o fertilizer (P₀). The average height of soybean plants at the age of 28 DAS and 42 DAS with the highest increment was obtained in the treatment of 250 mL (P₃) compared to control treatment (P₀), although not significantly different from P₁ and P₂. The highest average height of soybean plants at the age of 56 DAS was obtained in the treatment of 200 mL (P₂) and not significantly different from P₃ and P₁, but significantly different from control (P₀).

3. Number of leaves

The results of the application of AMF and liquid organic fertilizer to the number of leaves of soybean plants are presented in Figure 3. The independent application of AMF species to the number of leaves of soybean plants at 14, 28, 42, and 56 DAS is presented in Table 4.

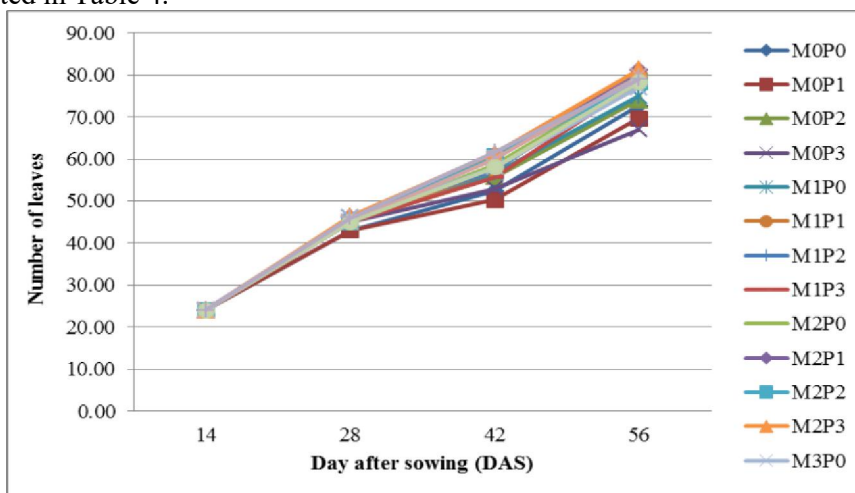


Fig.3. Dynamics of the number of leaves of soybean plants treated with AMF and liquid organic fertilizer from coconut husk.

Figure 3 shows the dynamic increase in the number of leaves of soybean plants treated with indigenous AMF species and the optimum concentration of liquid organic fertilizer from coconut husk. The best results were obtained in the combination treatment of *S. constrictum* and liquid organic fertilizer in the concentration of 250 mL (M₂P₃) compared with other treatments, especially without the inoculation of AMF and liquid organic fertilizer (M₀P₀). Proborini (2011) stated that plants colonized with AMF species may display a more active photosynthetic activity which will yield a higher number of leaves, plant biomass, root biomass and plant canopy than the plants without AMF colonization.

Table 4. The effect of AMP application to the number of leaves of soybean plants at 14, 28, 42, and 56 DAS

AMF (M)	Age of plants			
	14 DAS	28 DAS	42 DAS	56 DAS
Control (M ₀)	24.00 a	44.00 b	52.83 c	70.83 b
<i>A. delicata</i> (M ₁)	24.00 a	45.50 a	56.75 b	78.33 a
<i>S. constrictum</i> (M ₂)	24.00 a	45.83 a	60.16 a	79.91 a
<i>C. etunicatum</i> (M ₃)	24.00 a	45.50 a	59.41 ab	78.50 a
			2= 2.957	2= 5.070
DMRT 95 %			3= 3.107	3= 5.328
			4= 3.205	4= 5.495

Notes: Values followed by the same letter in the same column are not significantly different based on the DMRT at the 95% level of significance.

Table 4 shows that the treatment of indigenous AMF species at the age of 14 DAS has the similar number of leaves as the control treatment (M₀) without any AMF. Observation of the average number of leaves of soybean plants at the age of 28, 42, and 56 DAS yielded the highest number as obtained in the treatment of *S. constrictum* (M₂) compared with the control (M₀), but not significantly different from the treatment of *A. delicata* (M₁) and *C. etunicatum* (M₃). Observation of the average number of leaves of soybean plants at the age of 42 DAS in the treatment of *S. constrictum* (M₂) was not significantly different from *C. etunicatum* (M₃), but significantly different from *A. delicate* (M₁) and control treatment (M₀).

4. Leaf area

The results of the application of AMF and liquid organic fertilizer to the leaf area of soybean plants are presented in Figure 4. The independent application of AMF species to the leaf area of soybean plants at the age of 14, 28, 42, and 56 DAS is presented in Table 5. Figure 3 shows that the highest leaf area was obtained in the combination of *S. constrictum* liquid organic fertilizer in the concentration of 250 mL (M₂P₃) and was higher than control (M₀P₀).

Table 5 shows that the treatment of indigenous AMF at the age of 14 and 28 DAS was considered the best treatment as obtained in the treatment of *S. constrictum* (M₂) compared to control (M₀), but it was not significantly different from *A. delicata* (M₁) and *C. etunicatum* (M₃). Observation of the average leaf area of soybean plants at the age of 42 and 56 DAS tended to be the highest as seen from the treatment of *S. constrictum* (M₂), although not significantly different among other AMF species tested in this study.

The administration of the optimum concentration of liquid organic fertilizer from coconut husk gave the best results on plant vegetative growth. This means that all parts of the plant, not just leaves, act as photosynthate and cooperate to generate new plant parts [16]. Liquid organic fertilizers have several benefits, including being able to improve the synthesis of leaf chlorophyll leading to accelerated photosynthesis under optimum conditions [17].

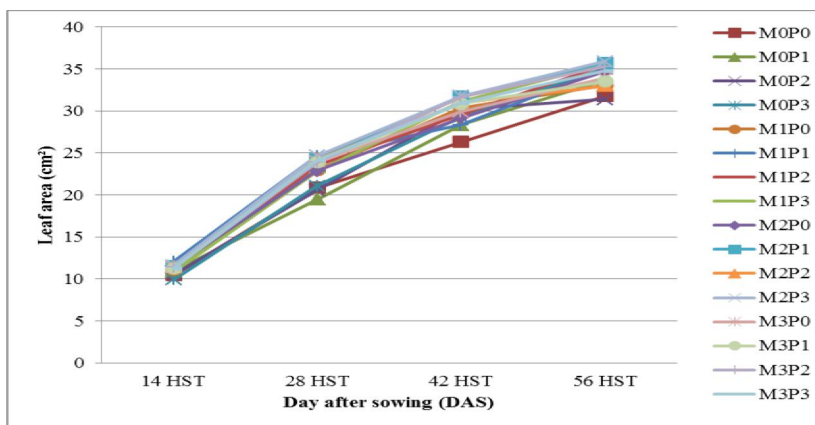


Fig.4. Dynamics of the leaf area of soybean plants treated with AMF and liquid organic fertilizer from coconut husk.

Table 5. The effect of AMP application to the leaf area of soybean plants at 14, 28, 42, and 56 DAS

AMF (M)	Age of plants			
	14 DAS	28 DAS	42 DAS	56 DAS
Control (M ₀)	10.45 b	20.49 b	28.53 a	32.97 a
<i>A. delicata</i> (M ₁)	11.21 a	23.48 a	29.86 a	34.75 a
<i>S. constrictum</i> (M ₂)	11.50 a	24.01 a	30.92 a	34.83 a
<i>C. etunicatum</i> (M ₃)	11.34 a	23.98 a	30.84 a	34.36 a
DMRT 95%	2=0.5880	2= 2.006		
	3=0.6179	3= 2.108		
	4=0.6373	4= 2.174		

Notes: Values followed by the same letter in the same column are not significantly different based on the DMRT at the 95% level of significance.

5. Stem diameter

The growth dynamics of the average stem diameter of soybean plants treated with indigenous AMF and liquid organic fertilizer from coconut husk at the ages of 14, 28, 42 and 56 DAS are presented in Figure 5.

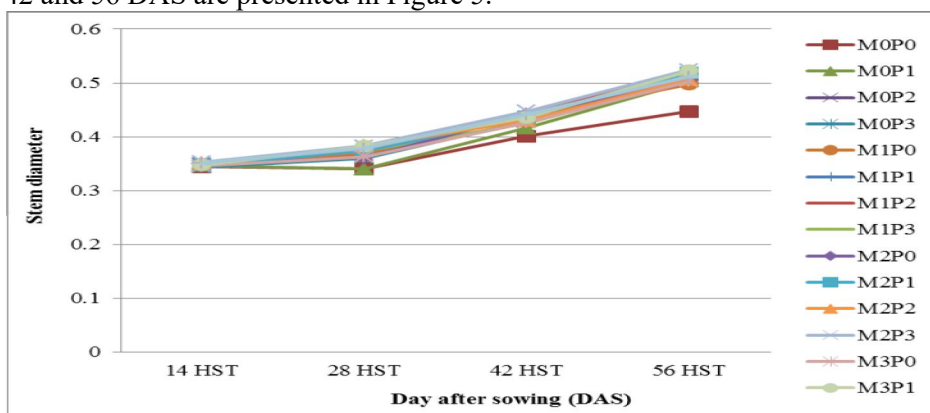


Fig.5. Dynamics of the stem diameter of soybean plants treated with AMF and liquid organic fertilizer from coconut husk.

Figure 5 shows the dynamic of stem diameter of soybean plants with the higher results obtained in the combination of *S. constrictum* and liquid organic fertilizer in the concentration of 250 mL (M_2P_3) compared to other AMF species and control (M_0P_0). Table 6 shows the inoculation of AMF to soybean plants at the age of 14 DAS gave the highest result in *S. constrictum* (M_2) but not significantly different from *A. delicata* (M_1), *C. etunicatum* (M_3) and control (M_0). The average stem diameter of soybean plants at the age of 28, 42, and 56 DAS showed that the treatment of *S. constrictum* (M_3) was not significantly different with *C. etunicatum* (M_3) and *A. delicata* (M_1), but significantly different with control (M_0). Independent effects of indigenous AMF and liquid organic fertilizer treatment at the ages of 14, 28, 42 and 56 DAS are presented in Tables 6 and 7.

Table 6. The effect of AMP application to the stem diameter of soybean plants at 14, 28, 42, and 56 DAS

AMF (M)	Age of plants			
	14 DAS	28 DAS	42 DAS	56 DAS
Control (M_0)	0.34 a	0.35 b	0.41 b	0.49 b
<i>A. delicata</i> (M_1)	0.34 a	0.37 a	0.43 a	0.51 a
<i>S. constrictum</i> (M_2)	0.35 a	0.37 a	0.43 a	0.51 a
<i>C. etunicatum</i> (M_3)	0.34 a	0.37 a	0.43 a	0.51 a
		2= 0.0147	2=0.0137	2=0.0148
DMRT 95%		3= 0.0155	3=0.0144	3=0.0155
		4= 0.0160	4=0.0149	4=0.0160

Notes: Values followed by the same letter in the same column are not significantly different based on the DMRT at the 95% level of significance.

Table 7. The effect of liquid organic fertilizer application to the stem diameter of soybean plants at 14, 28, 42, and 56 DAS

LOF (P)	Age of plants			
	14 DAS	28 DAS	42 DAS	56 DAS
LOF 0 ml (P_0)	0.34 a	0.35 b	0.42 a	0.48 b
LOF 150 mL/polybag (P_1)	0.34 a	0.36 ab	0.43 a	0.51 a
LOF 200 mL/polybag (P_2)	0.34 a	0.37 a	0.43 a	0.51 a
LOF 250 mL/polybag (P_3)	0.34 a	0.37 a	0.43 a	0.51 a
		2 = 0.0137		
DMRT 95%		3 = 0.0144		
		4 = 0.0149		

Notes: LOF = liquid organic fertilizer. Values followed by the same letter in the same column are not significantly different based on the DMRT at the 95% level of significance.

Table 7 shows that the application of liquid organic fertilizer from coconut husk to soybean plants at the age of 14 DAS gave the similar results among treatments (Control, P_1 , P_2 , P_3). The highest result in stem diameter of soybean plants at the age of 28 DAS were obtained in the concentration of 200 mL (P_2) and 250 mL (P_3) compared to 150 mL (P_1) and control (P_0). While the average stem diameter of soybean plants at the age of 42 DAS was obtained in the concentration of 150 mL (P_1), 200 mL (P_2), and 250 mL (P_3) as showed from its same value of 0.43 cm, but not significantly different

from control (P_0). In the end of the observation period, the highest stem diameter at the age of 56 DAS was also obtained from the three treatments (P_1 , P_2 , P_3) and was significantly different from control (P_0).

4. CONCLUSION

Independent application of indigenous AMF species and liquid organic fertilizers from coconut husk with various concentrations increased the growth of soybean plants on ultisols. The highest result was obtained in the treatment of *S. constrictum* and the concentration of liquid organic fertilizer of 250 mL/ polybag (M_2P_3) to yield the best growth performance of soybean plants.

5. ACKNOWLEDGMENTS

The authors would like to express the highest gratitude to Graduate Program of Agronomy, Universitas Halu Oleo for the opportunity in majoring the master program in the university.

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