Response of Soybean (*Glycine max* L.) to Arbuscular Mycorrhizal Fungi (AMF) applied with organic liquid fertilizer

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**Abstract.** This study aimed to obtain the best dose of Arbuscular Mycorrhizal Fungi (AMF) and the best concentration of liquid organic fertilizer made from overripe fruits for the growth and production of soybean plants. The trial was conducted at the Experimental Farm, Faculty of Agriculture, Universitas Hasanuddin, Makassar, South Sulawesi, from June to August 2017. The research was carried out in the form of a 2-factor factorial experiment using a Randomized Blocked Design (RBD) as environmental design. The first factor was the AMF dose with three levels, namely control (0 g polybag⁻¹), 10 g polybag⁻¹, and 15 g polybag⁻¹. The second factor was the concentration of liquid organic fertilizer with three levels, namely control (0 ml L⁻¹), 15 ml L⁻¹, and 20 ml L⁻¹. The experimental results show that the application of the mycorrhizal fungi on the soybean plants did not have a significant effect on several growth and production components, but it has a very significant effect on the number of branches parameter. Application of AMF of 10 g polybag⁻¹ gave the best result on parameters of flowering age, number of pods, dry seed production and weight of 100 seeds. The concentration of liquid fertilizer of 15 ml L⁻¹ resulted in the best result on parameters of plant height, number of leaves, and flowering age.

1. **Introduction**

Every year soybeans have increased in demand. The gap between soybean production and soybean demand in Indonesia for decades has triggered dependence on imported soybeans [1]. Although soybeans are native to Asia, ironically, countries in Asia are importing soybeans from outside the region. Indonesia is a major producer of soybeans, but still imports soybean seeds, meal, and oil [2].

Along with the increase in domestic soybean demand to meet the needs of industry and food, including for tempeh [3], soybean production needs to be increased. Until now, Indonesia still imports soybeans because domestic production is inadequate and the quality of soybean seeds is still low. This is why imported soybeans are still in high demand, especially as a raw material for the food industry. Needs and production of soybeans are spread throughout Indonesia as a food crop that is cultivated especially in South Sulawesi [4]. Application of Arbuscular Mycorrhizal Fungi (AMF) in plants is an effort to overcome stunted growth due to drought stress. AMF is a symbiotic form of mutualism between fungi and higher plant root systems. AMF can increase the absorption of macronutrients and
some micro nutrients. Mycorrhizal plant roots can absorb nutrients in the form of bound and not available for plants. In addition, more nutrients increase in absorption from the presence of AMF. Nutrients that increase their absorption are N, P, K, Ca, Mg, Fe, Cu, Mn, and Zn. AMF can serve as a biological protector for root pathogen infections [5]. AMF is an endomycorrhizal group which is an obligate symbiotic soil fungus with plant roots that can increase nutrient uptake and has the ability to increase water and nutrient input from the soil into plant tissues so as to improve plant growth, productivity, and quality without reducing the quality of soil ecosystems [6]. The fungi also can help rehabilitate critical land [7] and increase the productivity of agricultural crops, plantations, forestry on marginal lands [7].

The mutualistic symbiosis of AMF that forms between the most common plant roots is the Glomus type. AMF helps plants absorb mineral nutrients from very low available soils such as phosphorus (P), molybdenum (Mo) and cobalt (Co). This fungus is also reported to consistently stimulate the absorption of nutrients by plants such as zinc (Zn) copper (Cu) and also increase plant resistance to various pressures such as water, salt, dryness and heavy metal toxicity [5]. An important role of AMF in plant growth is its ability to absorb nutrients both macro and micro. Besides that, roots infected by AMF can absorb nutrients in the form of bound and which are not available for plants [9].

In addition to AMF, liquid organic fertilizer can function in stimulating plant growth and contains macronutrients (N, P, K, Ca, Mg and S) micronutrients (Bo, Fe, Zn, Cu, Cl, Co, and Mo). Organic fertilizers play a role in increasing the chemical, physical and biological soil content and sources of plant nutrients [10]. Other than that, the benefits of the organic fertilizer are to stimulate leaf formation, cytokinesis in connection with plant growth and development, increase fertilizer efficiency, and stimulate shoot growth or lateral growth [11–13]. One of the uses of AMF and liquid organic fertilizer is to maintain land sustainability, as nutrition to overcome the problem of macro and micronutrient deficiencies in cropping, improving soil quality and soil health (soil remediator), as well as practical and environmentally friendly applications [13,14]. In general, the influence of organic fertilizer in the soil includes three ways, namely through the physical, chemical, and biological properties of the soil. Through physical function, organic fertilizer with parts of the fibers plays an important role in improving the physical properties of the soil. Its constituent components are smooth, and high carbon content can increase the growth of mycelial mycelia, and increase soil aggregates [15].

Based on the description above, this research was conducted regarding the response of soybean plants (Glycine max L.) to the application of Arbuscular Mycorrhizal Fungi (AMF) and liquid organic fertilizer.

2. Methodology

2.1. Experimental design
The research was conducted from June to August 2017. The experiment was conducted at the Experimental Farm, Faculty of Agriculture, Universitas Hasanuddin, Makassar, South Sulawesi. The research was conducted in the form of a 2-factor factorial experiment using a Randomized Blocked Design (RBD) as environmental design. The first factor was the AMF dose with three levels, namely control (0 g polybag\(^{-1}\)) (m0), 10 g polybag\(^{-1}\) (m1) and 15 g polybag\(^{-1}\) (m2). The second factor was the concentration of liquid organic fertilizer with three levels, namely control (0 ml L\(^{-1}\)) (b0), 15 ml L\(^{-1}\) (b1), and 20 ml L\(^{-1}\) (b2). There are 9 combinations of treatments, each treatment consisted of two experimental units replicated three times resulted in a total of 54 experimental units.

2.2. Preparation of liquid organic fertilizer
Liquid organic fertilizer was made by preparing the material consisted of coconut fiber soaking water (Solution A) and mix of blended overripe fruits, rice washing water, and old coconut water (Solution B). Composition of the material used for the liquid organic fertilizer is shown in table 1. The coconut fiber soaking water was made by soaking approximately 1 kg of fiber in 5 litres of water then closed
and left for two weeks. After two weeks the solution then filtered. Pineapple, banana, papaya, maja and tomato fruits used were selected with the criteria of 50% blackened fruit skin. The fruits were crushed using a food processor and mixed with rice washing water and coconut water. The solution was fermented with the aerobes system and stirred every 2 days. After 15 days the solution is filtered and ready to use. Prior to the application, the two solutions were mixed and poured into the polybag according to the treatment.

| Table 1. The composition of the ingredients for making liquid organic fertilizer |
|---------------------------------|-------------------------------|
| **Materials**                   | **Volume (Litres)**           |
| **Solution A**                 |                               |
| Coconut Fiber Soaking Water     | 2                             |
| **Solution B**                 |                               |
| Overripe fruit mix (pineapple, banana, tomato, bael fruit (Aegle marmelos (L.) Correa) and papaya) | 2                          |
| Rice washing water (first wash) | 2                             |
| Coconut water                   | 2                             |
| Water                           | 2                             |
| **Total**                       | 10                            |

2.3. **Planting**

Prior to planting, soybean seeds variety of Anjasmoro were soaked in water for about 1 hour. Two seeds were planted into 2 cm depth hole on each polybag filled with planting media that previously added with chicken manure with a ratio of 1:1 as basic fertilization. The application of AMF was carried out at planting by adding the fungi into the planting hole according to the treatment dose, ie control or without AMF, 10 g and 15 g polybags\(^{-1}\). The application of the liquid organic fertilizer was started at two weeks after planting and applied every 10 days until the plants entered the generative phase. The application of organic fertilizer was done by pouring it directly into the plants.

2.4. **Data analysis**

Observation data were analyzed using analysis of variance (ANOVA). The treatment that showed significant effect is carried out further tests with Tukey’s Honestly Significant Difference (HSD) analysis at the 0.05 level.

3. **Results and discussion**

3.1. **Number of branches**

The analysis variance shows that the AMF treatment had a significant effect on the number of branches of soybean plants (p≤0.05), but the liquid organic fertilizer treatment and its interactions with AMF had no significant effect.

| Table 2. Average number of branches (stems) of soybean at 42 days after planting (DAP) on different dose of Arbuscular Mycorrhizal Fungi (AMF) and liquid organic fertilizer |
|---------------------------------|-----------------|-----------------|-----------------|
| Liquid organic fertilizer dose  | AMF dose         |                 |                 |
| Control                         | 10 g polybag\(^{-1}\) | 15 g polybag\(^{-1}\) |                 |
| Control                         | 3.42            | 3.71            | 3.88            |
| 15 ml L\(^{-1}\)               | 3.46            | 3.54            | 3.71            |
| 20 ml L\(^{-1}\)               | 3.50            | 3.54            | 3.96            |
3.2. Number of pods

The analysis of variance results shows that interaction between the application of AMF and liquid organic fertilizer treatments had a significant effect ($p \leq 0.05$) on the number of soybean pods. Table 3 shows the application of 15 g AMF per polybag without liquid organic fertilizer resulted in the highest number of pods ie 79.33 pods followed by the use of 15 mL$^{-1}$ liquid fertilizer without application of AMF and application of 10 g AMF per polybag and the use of 10 g AMF per polybag combined with 20 mL$^{-1}$ liquid fertilizer.

**Table 3.** Average number of pods (pods) of soybean on different dose of Arbuscular Mycorrhizal Fungi (AMF) and liquid organic fertilizer

| Liquid organic fertilizer dose | AMF dose               | Mean | Tukey’s 0.05 |
|-------------------------------|------------------------|------|--------------|
| Control                       | Control                | 59.17 b | 70.28        |
| 15 g polybag$^{-1}$ AMF       | 10 g polybag$^{-1}$    | 72.33 ab | 79.33 a      |
| 20 mL L$^{-1}$ liquid fertilizer | 15 g polybag$^{-1}$    | 66.67 b | 65.78        |
| Mean                          | Control                | 65.78 | 69.44        |
|                               | 10 g polybag$^{-1}$    | 69.44 | 64.44        |

Numbers followed by the different letters in the same row (x, y) and column (a, b) mean that the results are significantly different based on the Tukey’s test ($\alpha=0.05$).

**Figure 1.** Average weight of 100 grains (g) of soybean on different dose of Arbuscular Mycorrhizal Fungi (AMF) and liquid organic fertilizer

M0B0: 0 g polybag$^{-1}$ AMF + 0 mL L$^{-1}$ liquid organic fertilizer; M1B0: 10 g polybag$^{-1}$ AMF + 0 mL L$^{-1}$ liquid organic fertilizer; M2B0: 15 g polybag$^{-1}$ AMF + 0 mL L$^{-1}$ liquid organic fertilizer;
3.3. Weight of 100 grain
The analysis of variance show no significant effect of all treatments on the parameter of the weights of 100 grains of soybean plant. Figure 1 shows that the treatment of 10 g AMF per polybag and liquid organic fertilizer of 15 ml L⁻¹ (M1B1) resulted in the highest average value of 13.37 grams, and the lowest average of 9.57 grams was found in the treatment of without AMF combined with 15 ml L⁻¹ liquid organic fertilizer (M0B1).

3.4. Dry weight of grain per plant
The analysis of variance show no significant effect of all treatments on the parameter of the dry weight of grain per plant of soybean. Figure 2 shows that the treatment of 10 g AMF per polybag without liquid organic fertilizer (M1B0) resulted in the highest average value of 19.27 g plant⁻¹ and the lowest average of 12.18 g plant⁻¹ was found in the treatment of without AMF combined with 15 ml L⁻¹ liquid organic fertilizer (M0B1).

![Figure 2. Average dry weight of grain per plant (g plant⁻¹) of soybean on different dose of Arbuscular Mycorrhizal Fungi (AMF) and liquid organic fertilizer](image)

4. Discussion
Application of AMF on the soybean found to have an effect in increasing the growth of soybean plants. Application of AMF as much as 10 to 15 grams per plant resulted in better growth compared to the control treatment may due to availability of nutrients P and K. Soybeans require P, a macronutrient that plays a role in the formation of proteins and carbohydrates, in relatively large amounts and phosphate nutrients are absorbed by plants throughout their growing period [16]. AMF can produce the hormone phosphatase which can release P bound in the soil [17]. Phosphorus can accelerate and
strengthen the growth of young plants into mature plants [18]. Research on the identification of mycorrhizae in Melina plants in South Sulawesi shows that the types of spores observed in root samples were Glomus, Gigaspora, and Scutellospora. The percentage of colonization on both sites is medium [19].

The association between plant roots and AMF provided very good benefits for the soil and host plants where the fungus grows and multiply. The working principle of this AMF is to infect the host root system, producing intensive hyphae braiding so that plants containing the AMF will be able to increase capacity in absorbing nutrients [20]. This is in line with research conducted by Muddar [21], that that the rate of root infection by AMF increased with the increase in AMF dose.

The results of the statistical analysis show that the liquid organic fertilizer treatment had a very significant effect on the growth of soybean plants indicated by the number of branches parameter. This is presumably because the availability of organic matter in the soil becomes an available element that can be absorbed by plant roots to be transplanted to active plants which is not optimal. According to Novizan [22], the use of decomposer bacteria in the soils that are very poor in organic matter will not be effective. AMF which is spread into the soil will look for food in the form of organic material that has not been decomposed. If organic matter is reduced the bacterial population will decrease. Furthermore, the high and low levels of nutrients in plants are strongly influenced by the content of organic matter in the soil and the ability of plants to absorb nutrients [12].

5. Conclusions
Based on the results obtained, it can be concluded as follows:

a) Interaction of Arbuscular Mycorrhizal Fungi (AMF) dosage of 15 g polybag$^1$ and liquid organic fertilizer control gives the best results on the number of soybean pods.

b) Application of AMF at a dose of 10 g polybag$^1$ resulted in the best results on the parameters of the number of branches of soybean plants and tend to increase the weight of 100 grains and production of dry grain per plant of the soybean.

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