Arbuscular mycorrhizal fungi promote the growth and production of environmentally friendly grown shallots (*Allium oscalonicum* L.)

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Abstract. This research aimed to study the effect of different formulas of organic liquid fertilizer and Arbuscular Mycorrhizal Fungi (AMF) inoculation level on the growth and production of environmentally friendly grown shallot. The study was carried out at the experimental farm of the Faculty of Agriculture, Universitas Hasanuddin, Makassar, from August to November 2015. Three formula of organic liquid fertilizer used was formula HNI.A (fresh cow feces + rice washing water + *Annona muricata* leaf extract), formula HNI.B (fresh cow feces+ rice washing water + banana fruit extract + banana peel extract), and commercial liquid Bioslurry (fermented cow urine). The inoculation of AMF applied was at the concentration of 0.50 g, 0.70 g, and 1 g per plant. The results show that the interaction between the use of liquid organic fertilizers and AMF inoculation affect some vegetative characters of shallots such as the number of leaves, the number of stems at 30 days after planting (DAP), and 55 DAP, and leaf area at 30 DAP. The highest shallot yield was in 0.7 g AM per plant, which was 3.32 tons per hectare compared to control of 1.68 tons per hectare.

1. Introduction

Recently, people are increasingly aware of healthy food, and there is a change in mindset to consuming more organic products. On the other hand, shallot cultivation carried out by farmers today generally uses inorganic fertilizers. Despite the result of high production when using the inorganic fertilizer, long term use of the fertilizer is generally bad for the environment as it leaves residues on plants and affects soil fertility. Eco-friendly agriculture is successful management of resources for agricultural enterprises. Eco-friendly agriculture can maintain or improve environmental quality and preserve natural resources. Apart from that, it is also economically profitable; profits are not only measured directly based on the results of farming according to economic measures, but also based on the function of sustainability of resources and suppressing the possibility of risks that occur to the environment [1].

Implementation of eco-friendly agriculture in the field sometimes face problems of low yield obtained. Unlike in the use of inorganic fertilizer where nutrients could quickly be available to be used
by the plants, nutrients in the organic fertilizer are slowly released into the soil by the decomposition process. Therefore, the supply of nutrients in the organic fertilizer is slower than chemical fertilizer. One way to overcome the problem is by the use of liquid organic fertilizer sprayed on the plant leaves. Liquid organic fertilizer can be made from various organic sources such as livestock manure or urine, household wastes, and rotten fruits. The benefits of liquid organic fertilizer will be determined by the component materials. The more ingredients combined, the more nutrients that contained following the needs of plant nutrients.

One of the liquid organic fertilizers is bioslurry. Bioslurry is a liquid organic fertilizer derived from the urine of fermented livestock. Fermentation technology is used in processing urine into bio-urine. This process can cause changes in the properties of materials into simpler molecules so that they are easily absorbed by plants. While the use of Bioslurry basically provides the following benefits (1) improving the physical structure of the soil, (2) increasing the ability of the soil to bind or hold water longer which is beneficial, (3) increasing soil fertility, and (4) increasing worm activity and soil microorganisms that are useful for soil and plants [2]. Bioslurry is widely used in vegetable and fruit plants and food crops. The results of the study in Manokwari showed that the administration of liquid Bioslurry to kale plants could increase the yield of the crop [3]. Arinong and Lasiwua [4] in their study suggested that the use of cow manure as a liquid organic fertilizer on growth and production of mustard plants gave a very significant influence on the level of treatment of 75 mL L⁻¹ water on vegetative growth and production of mustard plants.

Besides the nutrient supply problem in the environmentally friendly grown shallot, the limited water in the dry season is also an obstacle that causes a decrease in shallot production. Lack of water in plants can inhibit cell formation and development. The condition causes retarded growth of plant roots so that water absorption and nutrients decrease and eventually the plant becomes dwarfed. Arbuscular mycorrhizal fungi (AMF) can increase nutrient uptake in the soil and increase the efficiency of water use so that plants are more resistant to drought and can fix nutrients in the soil. AMF is associated with various types of plants such as shallots, barley, grass, beans, oranges, grapes, cassava, olives, pineapple, rubber, papaya, coffee, cocoa, tea, oil palm, carrots, oats, wheat and marigolds [5]. Arma dan Rinsawati [6] state that mycorrhiza plays a role in increasing plant capacity to absorb nutrients and water. According to Setiadi [7], mycorrhiza is also able to expand the surface of nutrient uptake areas and CO₂ in infertile soils (marginal soil) and absorb the P-shaped nutrients bound to become available to plants. This ability is because P will form ATP (Adenosine Triphosphate), which is very useful for mineral nutrient absorption. Utami [8] in her study suggested that the working principle of mycorrhizae is to infect the root system of host plants, producing intricate hyphae so that plants containing these mycorrhizae will be able to increase their capacity to absorb nutrients. Mycorrhizae have enormous potential to increase plant growth and improve soil aggregation.

2. Methodology
This research was carried out in the experimental farm of the Faculty of Agriculture, Universitas Hasanuddin, Makassar, from August to November 2015. The geographical location of the study was at coordinates of 5.10650 South Latitude and 119.48680 East Longitude, at an altitude of 16 meters above sea level (asl). The research was carried out in the form of a 2-factor factorial experiment and set based on the Randomized Block Design (RBD) pattern. The treatment given was the formula of liquid organic fertilizer (P) as the first factor and inoculation of Arbuscular Mycorrhizal Fungi (M) as the second factor. The formula of liquid organic fertilizer consisted of 4 levels, namely control (p0), formula HN1A (fresh cow feces + rice washing water + Annona muricata leaf extract) (p1), formula HN1B (fresh cow feces+ rice washing water + banana fruit extract + banana peel extract) (p2), and commercial liquid Bioslurry (fermented cow urine) (p3). The inoculation of AMF zeolite as the second factor consisted of 4 levels namely control (m0), inoculation of AMF 0.50 g per plant (m1), inoculation of AMF 0.70 g per plant (m2), and inoculation of AMF 1 g per plant (m3). The experiment used three replication resulted in 48 treatment plots used. The collected data is processed using
variance analysis (ANOVA) to test the effect of the treatment given. If there is a significant effect, it will be continued by further testing using the Least Significance Difference (LSD) test at the level of \( p \leq 0.05 \).

3. Results

3.1. Number of Leaves

Application of different formula of liquid organic fertilizer and AMF inoculation significantly increased the number of leaves of shallot at 15 and 30 DAP. While at the later plant age of 55 DAP, the number of leaves was only affected by AMF inoculation treatment (table 1). The highest leaves number at 15 DAP resulted from the application of HNI.A formula with 0.5 g AMF per plant (13.57 leaves) and is not different significantly with the number of leaves shown by the application of HNI.A formula with 1 g AMF per plant (12.13 leaves).

Similarly, the highest number of leaves is shown by the application of HNI.A formula with 0.5 g AMF per plant (16.77 leaves) at 30 DAP and is not significantly different with the application of HNI.B formula with 0.5 g per plant AMF. AMF inoculation on environmentally friendly grown shallot increased leaves number at 55 DAP compared to control. Treatment of AMF 0.5 g per plant produced the highest number of leaves with an average of 20.73 leaves but is not significantly different from AMF 1 g per plant (19.99 leaves) and is significantly different to control (18.29 leaves).

Table 1. The average number of shallot leaves at age 15, 30, and 55 days after planting (DAP) on various formula of liquid organic fertilizer and the application of Arbuscular Mycorrhizal Fungi (AMF)

| Formula of Liquid Organic Fertilizer | AMF               |
|-------------------------------------|-------------------|
|                                     | \( m_0 \) (Control) | \( m_1 \) (0.5 g/plant) | \( m_2 \) (0.7 g/plant) | \( m_3 \) (1 g/plant) |
|-------------------------------------|-------------------|
| **Number of Leaves 15 DAP**         |                   |
| \( p_0 \) (Control)                | 11.47 ab x        | 11.87 a x                | 10.77 a x                | 11.80 a x                |
| \( p_1 \) (HNI.A)                  | 10.17 a x         | **13.57 a y**            | 11.57 a x                | 12.13 a xy               |
| \( p_2 \) (HNI.B)                  | 10.77 ab x        | 11.73 a x                | 11.30 a x                | 11.83 a x                |
| \( p_3 \) (Bioslurry)              | 12.47 b x         | 11.83 a x                | 11.30 a x                | 11.20 a x                |
| **LSD** 0.05                       | 1.99              |
| **Number of Leaves 30 DAP**         |                   |
| \( p_0 \) (Control)                | 15.67 a x         | 14.83 a x                | 14.00 a x                | 14.77 a x                |
| \( p_1 \) (HNI.A)                  | 13.73 a x         | **16.77 a y**            | 14.13 a xy               | 14.77 a xy               |
| \( p_2 \) (HNI.B)                  | 13.67 a x         | 16.30 a x                | 15.00 a x                | 13.67 a x                |
| \( p_3 \) (Bioslurry)              | 15.03 a x         | 14.43 a x                | 15.13 a x                | 14.97 a x                |
| **LSD** 0.05                       | 2.67              |
| **Number of Leaves 55 DAP**         |                   |
| \( p_0 \) (Control)                | 18.67             | 21.10                     | 17.53                     | 19.27                     |
| \( p_1 \) (HNI.A)                  | 17.17             | 21.37                     | 18.60                     | 19.57                     |
| \( p_2 \) (HNI.B)                  | 17.30             | 20.43                     | 20.47                     | 19.07                     |
| \( p_3 \) (Bioslurry)              | 20.03             | 20.03                     | 20.03                     | 22.07                     |
| **Mean**                            | 18.29 x           | **20.73 z**               | 19.16 xy                  | 19.99 yz                  |
| **LSD** 0.05                       | 1.14              |

The numbers followed by the same letters in the row (x, y, z) and columns (a, b, c) mean not significantly different at the level of the LSD test of 0.05. DAP = Days After Planting.

3.2. Stem Number
Use of different formula of liquid organic fertilizers and AMF inoculation did not significantly affect the shallot stem number during a couple of weeks of early phase at 15 DAP and no difference found in stem number between all treatments (table 2). However, the treatments were in effect to the plant at 30 to 55 DAP with the application of HNI.A formula with 0.5 g AMF per plant showed the highest number of stem at 30 and 55 DAP (4.97 and 5.37 stems, respectively).

Table 2. The average stem number of shallot at 15, 30, and 55 DAP on various formulas of Liquid Organic Fertilizers and inoculation of Arbuscular Mycorrhizal Fungi (AMF)

| Formula of Liquid Organic Fertilizer | AMF | m₀ (Control) | m₁ (0.5 g/plant) | m₂ (0.7 g/plant) | m₃ (1 g/plant) |
|--------------------------------------|-----|--------------|------------------|------------------|---------------|
| p₀ (Kontrol)                        |     | 3.20         | 3.70             | 3.13             | 3.33          |
| p₁ (HNI.A)                          |     | 3.20         | **3.90**         | 3.33             | 3.37          |
| p₂ (HNL.B)                          |     | 3.30         | 3.10             | 3.43             | 3.50          |
| p₃ (Bioslurry)                      |     | 3.30         | 3.47             | 3.23             | 3.40          |

**Stem Number 15 DAP**

| p₀ (Control)                        | 4.13 bc yz | 4.70 b w | 3.87 a x | 4.27 a z |
| p₁ (HNI.A)                          | 3.90 a x   | **4.97 c w** | 4.17 bcd yz | 4.27 a z |
| p₂ (HNL.B)                          | 4.13 c x   | 4.50 a z  | 4.33 d y  | 4.23 a xz|
| p₃ (Bioslurry)                      | 4.57 d z   | 4.47 a yz | 4.17 cd x | 4.13 a x |

LSD₀.₀₅ = 0.16

**Stem Number 30 DAP**

| p₀ (Control)                        | 4.67 ab x  | 5.10 a y  | 4.40 a x  | 4.80 a x |
| p₁ (HNI.A)                          | 4.50 ab x  | 5.33 a y  | 4.87 ab xy| 4.83 a x y|
| p₂ (HNL.B)                          | 4.40 a x   | **5.37 a z** | 5.03 b yz | 4.57 a x y|
| p₃ (Bioslurry)                      | 5.10 b x   | 5.10 a x  | 4.67 b x  | 5.00 a x |

LSD₀.₀₅ = 0.57

**Stem Number 55 DAP**

The numbers followed by letters that are not the same in the line (x, y, z) and columns (a, b, c) mean not significantly different at the level of the LSD test of 0.05. DAP = Days After Planting.

3.3. Leaf Area

Application of different formula of liquid organic fertilizer and inoculation of AMF significantly affected leaf area of shallot (table 3). The use of bioslurry (fermented cow urine) and 0.7 AMF of 0.7 g per plant resulted in the widest leaf area (15.35 cm²) followed by the combination of HNI.A formula and 0.5 g AMF per plant control.

Table 3. Average leaf area of shallot (cm²) at 30 DAP on various formulas of Liquid Organic Fertilizer and the inoculation of Arbuscular Mycorrhizal Fungi (AMF)

| Formula of Liquid Organic Fertilizer | AMF | m₀ (Control) | m₁ (0.5 g/plant) | m₂ (0.7 g/plant) | m₃ (1 g/plant) |
|--------------------------------------|-----|--------------|------------------|------------------|---------------|
| p₀ (Control)                        |     | 15.00 c w   | 11.80 a x       | 12.84 a y       | 13.85 c z    |
| p₁ (HNI.A)                          |     | 12.00 a x   | 15.35 d w       | 13.22 a yz      | 13.40 bc z |
| p₂ (HNL.B)                          |     | 12.45 ab x  | 13.34 bc y      | 12.45 a x      | 12.18 a x  |
| p₃ (Bioslurry)                      |     | 13.00 b x   | 13.65 c x       | **15.74 b y**   | 12.98 a x   |

LSD₀.₀₅ = 0.84

The numbers followed by letters that are not the same in the line (x, y, z) and columns (a, b, c) that are the same mean that they are significantly different from the level of the LSD test of 0.05. DAP= Days After Planting.
3.4. Production

The application of different formula of liquid organic fertilizer and inoculation of AMF had no significant effect on the production component. No significant difference was found between the treatments either in the parameter of the number of bulb per plant, bulb weight per plant or diameter of bulb per plant. Nevertheless, figure 1 shows that the application of 0.5 g per plant AMF on control treatment or without liquid organic fertilizer resulted in the highest number of bulbs (5.57 bulbs per plant) while the lowest number of bulb per plant was shown by the AMF control treatment (without application of AMF) applied with liquid organic fertilizer of HNI.A formula. Application of Bioslurry with AMF 0.7 g per plant resulted in the highest values of bulb weight per plant and bulb diameter parameter with an average of 3.70 g and 3.81 mm, respectively. The lowest values for the weight and diameter of the bulb produced is shown by plants without application of AMF.

Despite the insignificant effect of all treatments on the production component parameters, shallot production per hectare increased with the concentration of AMF inoculation to an optimum dosage of 0.7 g per plant which beyond this dosage, shallot production decreased slightly (figure 2). Base on the regression analysis, response of the environmentally grown shallot to the inoculation dosage of AMF follow the equation of \( y = -0.9607x^2 + 1.5303x + 1.9269 \) \( (R^2 = 0.89) \). The increase of shallot production due to the inoculation of the fungi to the plant can increase the shallot production up to 42% compared to control. However, the addition of AMF inoculation to 1 g per plant did not increase the production; on the contrary, production per hectare slightly decreased.

![Figure 1](image_url)

**Figure 1.** Average number of bulbs per plant, bulbs weight per plant, bulbs diameter per plant of shallot on different formulas of liquid organic fertilizer and inoculation of Arbuscular Mycorrhizal Fungi (AMF).
Figure 2. Production per hectare of shallot on different inoculation of Arbuscular Mycorrhiza Fungi (AMF).

3.5. Root Infection
Figure 3 shows that the treatment of liquid organic fertilizer of HNI.B formula with AMF 1 g per plant gave the highest value of 93% root infected by the AMF while the lowest value was obtained without liquid organic fertilizer treatment with AMF 0.5 g per plant (60%).

Figure 3. The percentage of shallot roots infection on a different level of Arbuscular Mycorrhizal Fungi (AMF) inoculation

4. Discussion
Use of liquid organic fertilizers formulas in this study significantly interacted with the application of AMF in affecting some vegetative characters of shallot such as leaf numbers, stem numbers, and leaf area. However, no significant effect of these treatments on the yield components. The formula of liquid organic fertilizer that gave the best result on shallot plant growth was HNI.A that consisted of a fermented mix of fresh cow feces, rice washing water, and Annona muricata leaf extract, especially for leaf and stem numbers. While for the development of leaf area, bioslurry which made from fermented cow urine resulted in the highest leaf area.
The interaction between the liquid organic fertilizer formula and AMF in affecting the vegetative growth of the shallot is probably due to the function of the organic fertilizer that acts as a source of nutrition for the plant while AMF can increase nutrient uptake and water in the soil [6]. Application of the organic fertilizer will increase soil fertility, hence increase nutrient content in the soil and help the plant to form the vegetative organ in the surrounding environment. Organic fertilizers contain more organic material from the use of livestock waste, green manure, market waste, and ruminant waste, which are processed naturally and without chemicals. Arinong and Lasiwua [4] in their study suggested that the use of cow manure as a liquid organic fertilizer on the growth and production of mustard plants.

Compared to the use of inorganic fertilizer in the production of shallot, the yield obtained in this study were lower. This indicates that the nutrient absorption by the use of liquid organic fertilizer of shallot is not as fast as when using inorganic fertilizers. Despite this fact, an increase in the inoculation level of AMF tends to improve shallot yield per hectare. Solahuddin [9] stated that plants colonized by mycorrhizae would provide better growth. Mycorrhizae play a role in increasing plant capacity to absorb nutrients and water. Rahayu and Akbar [10] suggested that plant root-AMF symbiotic can formed a complex interplay of interactions. AMF is an obligate soil fungus group that cannot grow and develop if it is not symbiotic with its host plants.

The insignificant effect of the liquid organic fertilizer used and inoculation of AMF treatments on production component may occur due to unfavorable climate during the trial when the shallot plants entered its generative phase. Drought and excessive sunlight caused plant leaves to dry up and died before harvesting. Excessive light has a negative impact on plant growth and development and causes production to decline. It can be assumed that shallot plants experienced heat stress resulted in early senesced leaves with the tip turning yellow and dry. This condition during the generative phase caused stunted bulb development, hence low production.

Even though the result of environmentally grown shallot produced in the trial was low, plants grown using organic fertilizers produce healthy products, free from drug residues and lethal chemicals and higher beta carotene content [11]. This shows that the production of environmentally friendly agriculture is safe for consumption in the long term compared to agricultural production that uses inorganic fertilizers.

5. Conclusion

Based on the results of the research, it can be concluded as follows:

- Liquid organic fertilizer formula that had the best influence on the growth and production of shallots were namely Bioslurry (fermented cow urine).
- The AMF inoculation level that gave the best effect was 0.5 g per plant.
- The highest shallot production obtained with the application of Bioslurry (fermented cow urine) and AMF 0.7 g per plant (3.32 tons per hectare).

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