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The Effect of Arbuscular Mycorrhizal Fungi and Bio-Hara Liquid Organic Fertilizer on the Growth of Siompu Tangerines in Wabula, Buton Regency

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ABSTRACT. Siompu tangerines are one of the mainstay horticultural crops in Southeast Sulawesi. Siompu tangerines are naturally distributed on Siompu Island, South Buton Regency. Siompu tangerines have great market potential. The purpose of this study was to examine the effect of the interaction between FMA and Biohara Plus IPB liquid organic fertilizer on the growth of Siompu tangerines in the field. This research was conducted at the BPP Wabula office yard in Wabula District, Buton Regency, the Indonesian Mycorrhiza Association (AMI) Southeast Sulawesi Branch plastic house in Kendari, the Agrotechnology Laboratory of the Faculty of Agriculture, Halu Oleo University, and the Biomolecular Laboratory of the Faculty of Mathematics and Natural Sciences, Halu Oleo University, Kendari. This research was conducted from June to September 2024. This study used a randomized block design (RBD) with a factorial pattern consisting of 2 factors. The variables observed were plant height, stem diameter, number of leaves, root length, root colonization, and N and P content in the leaves. The results showed that the interaction between the FMA inoculum from Kaimbulawa and the Biohara liquid organic fertilizer at 5 ml/liter of water was the best treatment, significantly enhancing the growth of 4-month-old Siompu orange seedlings.

Keywords : *FMA, Siompu Tangerine, Liquid Organic Fertilizer*

INTRODUCTION

Siompu tangerines are one of the mainstay horticultural crops in Southeast Sulawesi. Siompu tangerines are naturally distributed on Siompu Island in South Buton Regency (Husna et al., 2023) and are woody plants from the Rutaceae family (Husna et al., 2022). Siompu tangerines currently have very low fruit production and quality due to various soil and climate factors that pose obstacles (Zainun and Sri, 2021). Siompu tangerines are grown in karst (rocky) areas (Husna et al., 2022) and are very susceptible to drought stress during the dry season (Bahrun et al., 2014). One effort that can be made to overcome this drought stress is to utilize beneficial microbes such as arbuscular mycorrhizal fungi (AMF), which can help absorb nutrients in marginal lands (Mirshad and Jos, 2016).

Arbuscular mycorrhizal fungi (AMF) are root symbionts that form symbiotic relationships with most plants and are commonly found in terrestrial ecosystems (Smith and Read, 2008). Arbuscular mycorrhizal fungi can form mutualistic symbiosis with plant roots, thereby helping plants to grow better, where both parties benefit. Among other things, AMF obtains a source of carbon from photosynthesis, while plants obtain a supply of nutrients from AMF (Ansiga et al., 2017). AMF play an important role in phosphorus uptake, nitrogen circulation in ecosystems, tolerance to heavy metals, increased protection from nematodes and root diseases in the rhizosphere, and soil contamination (Cheng et al., 2021). In addition, AMF can absorb minerals and nutrients such as N, P, K, Ca, Cu, Mn, and Mg for plants (Yawan et al., 2017).

Arbuscular mycorrhizal fungi in symbiosis have a very wide host range, but their effectiveness varies. Certain types of AMF show specificity in selecting and symbiotically associating with a particular host plant species. The effectiveness of nutrient uptake by host plants depends on complex interactions between soil capacity, nutrient requirements, the ability of AMF to infect and provide nutrients to plants (Nuridayanti et al., 2019), and effectiveness in plant improvement and cultivation (Finmeta et al., 2018). FMA in plants have been widely reported to effectively increase plant growth and biomass compared to plants without mycorrhiza, both in insentisol soil and post-mining soil with high heavy metal content (Margaretha et al., 2017; Husna et al., 2018).

Based on the results of research by Zou et al (2020), it was revealed that citrus fruits form associations with AMF. Arbuscular mycorrhizal fungi effectively enhance growth in citrus plants, significantly increasing root dry weight and shoot P uptake (Zarei and Paymaneh, 2014). AMF also increases the number of flowers, number of fruits, and weight per citrus fruit (Nahak et al., 2018). The results of exploration and identification of AMF have been carried out at 5 locations in Siompu, showing that 9 types of AMF from 3 families (Acaulosporaceae, Claroideoglomeraceae, and Glomeraceae) were found. The Glomeraceae family has the most types, with 7 types (Husna et al., 2022). In addition to FMA, the use of liquid organic fertilizer (POC) is also necessary because it has many benefits, including improving soil fertility. One type of POC that can be used is POC Biohara plus IPB.

Biohara liquid organic fertilizer is a fertilizer made from natural organic materials and processed in liquid form. Biohara plus organic fertilizer contains organic C (15.22%), N (56.70 ppm), P (0.26%), K (122.50), Na (67.00), Ca (418.50), Mg (23.00), Fe (2 ppm), Cu (3 ppm), Zn (20 ppm), Mn (5 pmm), B (2 ppm), pH (7.1), Azadirachtine, Pikoretin, Morindine, Azotobacter sp, and Lactobacillus sp. Laboratory test results from the Department of Soil Science and Land Resources, Faculty of Agriculture, IPB: Biohara Plus IPB liquid organic fertilizer can enhance growth and productivity through mechanisms that enrich the soil, increase soil microorganism activity, improve resistance to pest and disease attacks, and have high water retention capacity. (Husna et al., 2024).

The combination of FMA and biohara liquid organic fertilizer is used to supply the nutrients needed by siompu orange plants so that they can grow well. In addition, the combination of FMA and POC allows plants to photosynthesize optimally (Halim et al. ., 2020), thus FMA and POC interact with each other in influencing plant growth. Furthermore, the application of liquid organic fertilizer with mycorrhizal fungi at various doses can enhance plant growth and nutrient uptake in marginal soils (Rahmawati et al., 2020). Therefore, the use of FMA and POC biohara can be an alternative in stimulating the growth of siompu orange seedlings, so this study is needed to determine the effect of FMA and the application of biohara liquid organic fertilizer plus IPB on the growth of siompu orange seedlings.

MATERIALS AND METHODS

Research Location and Time

This research was conducted at the BPP Wabula Office in Wabula District, Buton Regency, the Indonesian Mycorrhiza Association (AMI) Branch Laboratory in Southeast Sulawesi, Kendari, the Agrotechnology Laboratory of the Faculty of Agriculture, Halu Oleo University, Kendari, and the Biomolecular and Environmental Laboratory of the Faculty of Mathematics and Natural Sciences, Halu Oleo University, Kendari, from June to September 2024.

Materials and Equipment

The materials used in this study were siompu orange seedlings, label paper, taly sheets, envelopes, 10% KOH, 2% HCl, staining solution (glycerol, lactic acid, and distilled water in a 2:2:1 ratio, plus 0.05 grams of trypan blue powder), 50% glycerol, and Biohara.

The tools used in this study were a hoe, bucket, pen, OH pen, marker (*Snowman White Marker*), digital *caliper* (Nankai 0-150 mm), ruler (100 cm), scissors, root scissors, tweezers, film bottles, glass slides, microscope slides, light microscope (Yazumi), oven (*Memmert)*, and analytical scale (AC adapter DC 12V 0.3A HR-200), *software* Ms. Word, Ms. Excel, SAS 9.1.3 Portable, and writing instruments.

Research Design

This study used a Randomized Block Design (RBD) with a factorial pattern consisting of 2 factors:

a. FMA (A) consisting of
   A0: Without FMA
   A1: FMA from Nggula-Nggula (*Acaulospora scrobiculata*, *Claroideoglomus clarodeum*, *Funneliformis dimorphicus*, *Glomus* sp.1, and *Glomus* sp.2)
   A2: FMA from Lapara (*Claroideoglomus clarodeum*, *Sclerocystis sinousa*, and *Glomus* sp.2)
   A3: FMA from Kaimbulawa (*Claroideoglomus clarodeum*, *Funneliformis dimorphicus*, *Glomus* sp.1, *Glomus* sp.2, *Glomus* sp.3, and *Glomus* sp.4)
b. Biohara POC (B) consisting of
   B0: Without Biohara POC
   B1: 2.5 ml/L water
   B2: 5 ml/L water

Research Procedure

Seed germination

The germination process for siompu tangerine seeds involves washing the seeds using Bayclin at a dosage of 1 ml/liter of water. After that, they are washed in running water 3 times. Then, they are soaked in atonic solution at a dosage of 1 ml/L water for 24 hours. After that, they are drained and the siompu mandarin orange seeds ( ) are germinated in plastic trays containing a mixture of sand and charcoal ash that has been sterilized at a weight ratio of 1:1.

Seedling Preparation for Planting Location

The ready-to-plant siompu orange seedlings were transported from the Indonesian Mycorrhiza Association nursery to the planting site at the Wabula BPP Office, Wabula District, Buton Regency, using a vehicle.

Land Preparation

Before planting, the land was cleared, planting distances (3 x 3 m) were measured, stakes were installed, and planting holes (40 x 40 x 40 cm) were made.

Planting

The seedlings were planted simultaneously. Before planting them in the planting holes, the polybags around the seedlings were removed to prevent slow root growth in penetrating the soil. The plants were placed in the planting holes until the root collars were level with the soil surface, then soil was sprinkled to cover the planting holes, and finally watered.

Application of Biohara Liquid Organic Fertilizer

Biohara liquid organic fertilizer is applied to Siompu orange seedlings according to the treatment by watering around the plant roots with 250 ml per plant.

Maintenance

After planting, the plants are watered daily, and weed, pest, and disease control is carried out.

Research Variables

Height and diameter are measured at a position 1 cm from the surface of the medium. The number of leaves is counted based on leaf growth. FMA Colonization, Orange roots are washed in running water. Roots are cleaned in 10% KOH for 24 hours, acidified with 2% HCl for 30 minutes, and stained with trypan blue. The percentage of colonized roots can be calculated using the formula:

$$Kolonisasi\ Akar = \frac{\sum sampel\ akar\ \ terkolonisasi\ mikoriza}{\sum sampel\ yang\ diamati} x\ 100\ \%$$

Tissue analysis of leaves (N and P) using the Kjeldahl method and spectrophotometric method.

Data Analysis

Observation data were analyzed using analysis of variance (ANOVA). Results showing calculated F > table F were followed by Duncan's Multiple Range Test (DMRT) at a 95% confidence level.

RESULTS AND DISCUSSION

Plant Growth

The results of the Duncan Multiple Range Test analysis of the height variable of Siompu tangerine plants in Wabula at four months of age are presented in Table 1. Table 1 shows that the combination of FMA from kaimbulawa and biohara liquid organic fertilizer significantly increased plant height, with the highest value in treatment A3B2 (36.75) for four-month-old siompu oranges. There was no significant difference between this treatment and the others, except for treatments A0B0, A0B1, A1B1, and A2B1. The combination of FMA from kaimbulawa and 5 ml/L biohara liquid organic fertilizer (A3B2) with a value of 10.21 significantly increased the diameter of four-month-old siompu orange plants and was significantly different from treatment A3B1. The increase in the number of leaves showed that the interaction treatment of FMA from kaimbulawa and 5 ml/L biohara liquid organic fertilizer (A3B2) significantly increased the number of leaves of four-month-old siompu orange plants with a value of 107.66 and was significantly different from the other treatments.

Table 1. Averages of Siompu tangerine Plant Growth

Treatment		Plant Growth		
FMA	Biohara POC	Plant Height (cm)	Plant Diameter (mm)	Number of Leaves (pieces)
A0	B0	21.68 c	3.75 f	40.16 d
	B1	28.03 b	6.23 e	49.83 d
	B2	32.66 ab	6.81 de	54.33 cd
A1	B0	36.16 a	8.01 cd	66.16 bc
	B1	27.66 b	8.55 bc	70.00 bc
	B2	32.83 ab	7.30 cde	68.83 bc
A2	B0	32.20 ab	8.56 bc	77.50 b
	B1	31.83 ab	8.51 bc	71.00 bc
	B2	36.16 a	7.85 cd	69.16 bc
A3	B0	35.66 a	9.66 ab	76.50 b
	B1	36.50 a	10.03 a	94.66 a
	B2	36.75 a	10.21 a	107.66 a

Note: Numbers followed by the same letter are not significantly different according to Duncan's test. A0 (Control), A1 (FMA from Nggula-Nggula), A2 (FMA from Lapara), A3 (FMA from Kaimbulawa), B0 (without fertilizer), B1 (fertilizer dose of 2.5 ml/l water), and B2 (fertilizer dose of 5 ml/l water).

Root Colonization

The results of Duncan's multiple range test analysis of root colonization variables in four-month-old siompu wabula are presented in Table 2. Table 2 shows that the highest interaction between FMA and biohara POC was in combination A3B2 (FMA from Kaimbulawa with biohara POC at a dose of 5 ml/L of water), which significantly increased root colonization at four months of age with a value of 60.00 and was significantly different from treatment A2B0 (FMA from Lapara without biohara POC) and treatment A0B0 (without FMA and without biohara POC), but not significantly different from (A2B2) FMA from Lapara with a biohara POC concentration of 5 ml/L water.

The research results show that the roots of Siompu mandarin oranges were colonized by FMA, as indicated by the presence of FMA structures in the form of internal hyphae (HI), external hyphae (HE), and vesicles (V), as shown in Figure 1.

Table 2. Root colonization

Treatment		Root Colonization (%)
FMA (A)	Biohara Liquid Organic Fertilizer (B)	
A0	B0	15.83 e
	B1	26.66 de
	B2	19.16 e
A1	B0	50.00 abc
	B1	53.33 abc
	B2	42.50 bc
A2	B0	39.16 cd
	B1	50.00 abc
	B2	58.33 a
A3	B0	56.66 ab
	B1	51.66 abc
	B2	60.00 a

Note: Numbers followed by the same letter are not significantly different according to Duncan's test. A0 (Control), A1 (FMA from Nggula-Nggula), A2 (FMA from Lapara), A3 (FMA from Kaimbulawa), B0 (no fertilizer), B1 (fertilizer dose of 2.5 ml/l water), and B2 (fertilizer dose of 5 ml/l water).

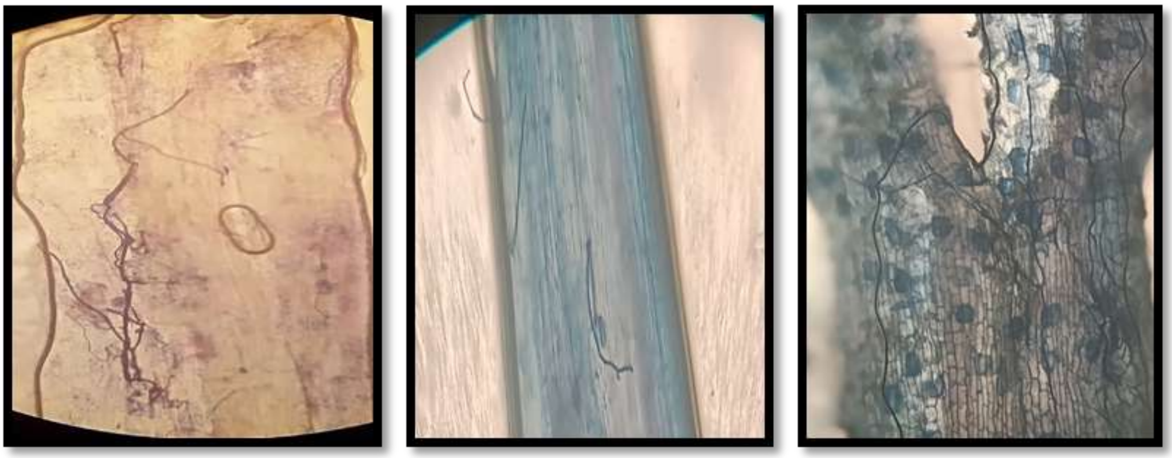


Figure 1. FMA colonization of three-month-old siompu orange seedlings. [FMA structure: internal hyphae (HI), external hyphae (HE), and vesicles (V)].

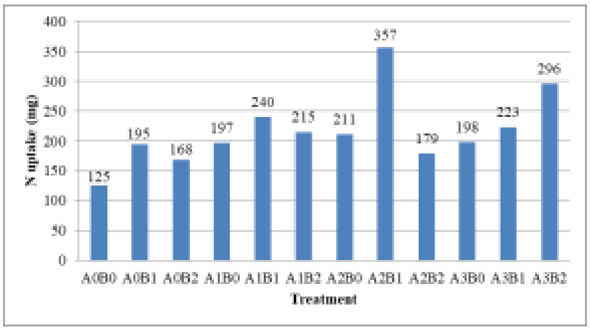
Analysis of N and P Nutrient Levels and Uptake in Siompu Orange Plant Tissue

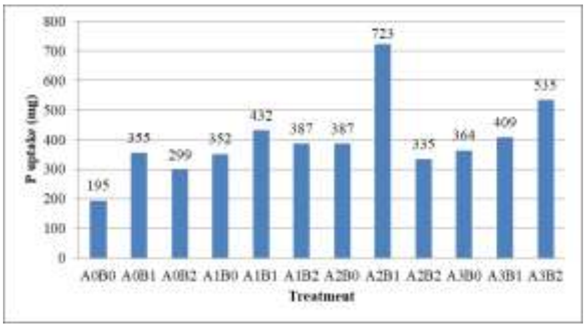
The results of the analysis of N and P nutrient uptake in the leaf tissue of four-month-old siompu orange plants are presented in Table 3, Figures 2 and 3.

Table 3, Figures 2 and 3. Show the results of tissue analysis of N and P content and uptake in Siompu orange plants that had been treated with FMA and POC. The highest nutrient content was obtained in treatment A2B1 with a total N content of-total of 0.42 ppm and P-available of 0.85 ppm, while the lowest was obtained in treatment A0B2 with N-total content of 0.23 ppm and treatment A0B0 with P-available content of 0.39 ppm. The highest nutrient uptake analysis in siompu orange leaves was obtained in treatment A2B1 with total N uptake of 357 mg and available P of 723 mg, and the lowest nutrient uptake was obtained in treatment A0B0 with total N uptake of 125 mg and available P of 195 mg.

Table 3. Analysis of N and P levels

Treatment	Level (ppm)	
	Total N	Available P
A0B0	0.25	0.39
A0B1	0.26	0.48
A0B2	0.23	0.41
A1B0	0.24	0.43
A1B1	0.25	0.45
A1B2	0.25	0.45
A2B0	0.24	0.44
A2B1	0.42	0.85
A2B2	0.23	0.43
A3B0	0.26	0.48
A3B1	0.24	0.44
A3B2	0.26	0.47





Figures 2 and 3. show the analysis of N and P uptake in citrus plants.

Based on the research results, FMA and POC biohara were generally effective in increasing the growth of four-month-old siompu orange plants. Treatment with FMA from Kaimbulawa and 5 ml of biohara POC effectively increased the average height, number of leaves, and diameter of siompu orange plants compared to the control treatment. This is thought to be due to the important role of FMA in increasing water content, nutrients, and nutrient absorption, especially phosphorus (Smith and Read, 2008). This is in line with the research by Syah et al. (2005), which states that FMA effectively increases plant growth in 3-month-old citrus plants. The presence of mycorrhiza can increase nutrient absorption, especially P. Increased P content in plant tissues can accelerate cell division, especially in the development of plant meristem tissues (Muzakir, 2015). The addition of 5 ml/l of biohara POC is expected to maximize the effect of FMA inoculation from Kaimbulawa village on the growth of siompu citrus plants. Biohara liquid organic fertilizer is a fertilizer that can assist in the absorption of nutrients needed by FMA. The results of this study indicate that the addition of POC up to 5 ml/l of water can support the performance of FMA in enhancing the growth of siompu orange plants. FMA originating from different locations have different properties and abilities in responding to the addition of POC and provide benefits to their host plants by maximizing their own benefits to infect the root area of these plants (Husna et al., 2021).

The results of the research on siompu orange seedlings inoculated with FMA showed FMA colonization, as evidenced by the discovery of FMA structures in the form of internal hyphae, external hyphae, and vesicles, as shown in Figure 2. The percentage of FMA colonization ranged from 15.83% to 60.00%. The highest colonization percentage was from Kaimbulawa and POC Biohara 5 ml/l water (60.00%). According to O'Connor et al. (2001), colonization is classified based on the following categories: 0 is uncolonized, <10 is low, 10-30 is moderate, and >30 is high. This indicates that the colonization percentage has a high category with a value of 60.00. In addition to internal factors, external factors are also important variables in this study, such as temperature, humidity, and light intensity. FMA colonization is high under drought stress conditions because the plants are in unsuitable conditions. According to Lakitan (2012), FMA can infect plant roots in nutrient-deficient or dry conditions. Therefore, under drought conditions, plants will experience stress and come into contact with FMA, resulting in high colonization.

Based on the results of the analysis of N and P nutrient levels and absorption in the leaves of siompu citrus plants that had been treated with FMA and POC biohara, the highest nutrient levels were obtained in treatment A2B1 with a total N of 0.42 ppm and an available P level of 0.85. The highest nutrient uptake analysis in siompu orange leaves was obtained in treatment A2B1 with N uptake of 357 mg and P uptake of 723 mg, while the lowest nutrient uptake was obtained in treatment A0B0 with N uptake of 125 and P uptake of 195. The decrease in soil organic matter content also causes a reduction in plant nutrients, especially N and P, and inhibits soil microbial activity. In addition, low soil organic matter content is often closely related to a decline in soil physical properties such as: massive or loose soil structure, low water holding capacity and water infiltration rate, and high soil erodibility. Generally, the concentration of nitrogen in leaf tissue is about 1-5% higher than the nitrogen content in stem tissue (Nuryani et al., 2010).

The potential for nitrogen absorption into plant tissues increases if the concentration of nitrogen available in the soil is also high (Walunguru et al., 2009). Nitrogen stimulates plant growth and the formation of chlorophyll, fats, proteins, and other compounds (Kurnia et al. 2021). The nitrogen content is generally around 1-5% per unit of dry plant weight. The amount of nitrogen in young tissues is higher than in mature tissues. In general, the concentration of nitrogen in plant tissue tends to decrease with the age of the plant (Kusumawati, 2021). Nitrogen is needed as a component of cell structure, namely chlorophyll, which plays a role in plant photosynthesis. In addition, nitrogen is needed by plants as a component of protein for the formation of growth hormones, especially auxin and gibberellin.

Phosphorus (P) is a very important and essential macro nutrient for plant growth. Phosphorus makes up about 0.2% of the dry weight of plants, and plants will not grow if this element is not sufficient. Phosphorus plays a role in energy circulation in plants, which is obtained from photosynthesis, respiration, and carbohydrate metabolism in the form of ATP and ADP. Organic matter content is positively correlated with phosphorus uptake by plant tissues (Nuryani et al., 2010). Even if the phosphorus available in the soil is insignificant, phosphorus uptake can still proceed well if the plant roots develop properly, thereby increasing the phosphorus content in plant tissues. Phosphorus stimulates root development, making plants more tolerant to drought, accelerating fruit ripening, and increasing nutritional content (Kurnia et al. 2021). Phosphorus is the second most essential element after nitrogen that plants need. Increased P content in plants will increase the rate of photosynthesis and stimulate the formation of new leaves.

CONCLUSION

The interaction between the FMA inoculum treatment from Kaimbulawa and POC biohara 5 ml/L water was the best treatment that significantly increased the growth of four-month-old siompu orange seedlings in terms of plant height, number of leaves, diameter, and root colonization. Analysis of N and P levels and uptake for the best treatment, which is FMA from Lapara and POC Biohara 2.5 ml/L.

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