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EFFECTIVENESS TEST OF ARBUSCULAR MYCORRHIZAL BIOFERTILIZER ON THE GROWTH OF MATOA SEEDLINGS (*Pometia pinnata* J.R.FORST. & G.FORST.) IN POST-GOLD MINING SOIL.

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ABSTRACT

Arbuscular Mycorrhiza Fungi (AMF) can be used as a biofertilizer or biological fertilizer so that it increases the absorption of nutrients needed by plants such as P, K, Ca, Mg, Fe. The purpose of this study was to test the effectiveness of the use of arbuscular mycorrhizal fungi fertilizer on the growth of matoa seedlings in post-gold mining soil media. This study used a Randomized Block Design (RAK) with arbuscular mycorrhizal fungi treatment on each plant, including: Control (A), 5 g AMF (B), 10 g AMF (C), 15 g AMF (D). The results showed that the inoculation treatment of 15 g AMF was able to increase the average percentage of root colonization (72.21%), plant height (5.19 cm), diameter (0.80 mm), dry weight of shoots (1.93 g), and seedling quality index (0.26). Therefore, AMF can play a role in increasing the growth of matoa seedlings in nutrient-poor soil, especially post-gold mining soil on a plastic house scale. Therefore, FMA can play a role in increasing the growth of matoa seedlings in nutrient-poor soil, especially post-plastic house-scale gold mining soil.

Keywords: *Arbuscular_Mycorrhizal_Fungi; Biofertilizer; Seedling_Growth; Post-Gold_Mining_Soil.*

INTRODUCTION

Mining can lead to pollution of the environment and land, and traditional or artisanal mining often lacks post-mining land rehabilitation. The utilization of waste soil from gold mining to grow plants that have potential and are beneficial to the community is important. To enhance plant growth in soil that is poor in nutrients and contaminated with heavy metals, the application of fertilizers is necessary. One type of microorganism that has the potential to improve plant growth is arbuscular mycorrhizal fungi (AMF).

AMF is a group of microorganisms that form symbiotic relationships with plant roots (Schüßler and Walker, 2010). Arbuscular Mycorrhizal Fungi are among the types of fungi that have recently gained attention from environmental and biological experts

to be developed as biofertilizers (Mansur, 2003) and for use in soil contaminated with heavy metals (Husna, 2015). Arbuscular Mycorrhizal Fungi is a type of fungi that forms mycorrhizae, symbiotic with 90% of higher plant roots and is known to have beneficial effects on plant growth (Schüßler and Walker, 2010).

AMF can serve as an alternative to support plant growth, productivity, and quality of forest plants, especially in marginal lands that are less fertile (Setiadi, 1999). Previous research has shown that AMF is effective in increasing nutrient levels of P, K, Ca, Mg, and Fe in media poor in nutrients (Husna et al. 2021), including post-gold-mining soil (Tuheteru et al. 2022). Research conducted so far has been limited to wood and fruit-producing plants, such as the matoa tree (*P. pinnata*) in post-gold-mining media. Generally, post-gold-mining media tends to have acidic soil, P deficiency, aluminum and iron toxicity, low microbial activity, and experiences water stress. There has been no research on the utilization of local AMF on matoa plants in post-gold-mining soil.

Matoa belongs to the Sapindaceae family, characterized by its unique flavor and texture of the fruit flesh; the taste of matoa fruit is similar to that of durian, while the texture of its flesh is comparable to that of rambutan. Matoa wood has a strength class of II-III and durability class of III-IV (Nugroho, 2009), making it suitable for use in furniture making, home furnishings, wall panels, and other construction materials. According to the IUCN conservation status (Thaman and Thomson, 2019), several regional species have gone extinct. Therefore, conservation efforts must be made, as large-scale exploitation without conservation efforts could lead to genetic diversity degradation of *Pometia* before this wealth can be explored, preserved, and utilized appropriately for domestication purposes (Nugroho, 2009). Based on the explanation above, the research objectives are established to test the effectiveness of using arbuscular mycorrhizal fungi as fertilizer on the growth of matoa seedlings in post-gold-mining soil.

RESEARCH METHODOLOGY

Time and Location

This research was conducted over a period of 3 months, from October to December 2024. It took place at the permanent seedling house of the Bone Bolango Watershed Management Hall (BPDAS) and the Agro-Technology Department Laboratory at Gorontalo State University.

Tools and Materials

The materials used in this research include matoa seeds, sterilized post-gold mining soil, sterilized sand, water, bleach (active ingredient NaOCl), polybags, seedling trays (30cm x 20cm x 10cm), label paper, envelopes, 10% KOH, 2% HCl, 0.05% Trypan blue, 50% glycerol, aquades, and Fungal Mycorrhizae (*Glomus claroideum*, *Glomus coronatum*, *Claroideoglomus delicata*, *Acaulospora constrictum*).

The equipment used in this research includes an analytical balance, ruler (30 cm), digital caliper, camera, oven, root scissors, marker pens, OHP pens, microscope slides, cover glasses, tweezers, autoclave, thermohygrometer, and binocular microscope.

Experimental Design

This research used a Randomized Group Design (RAK) with treatments of arbuscular mycorrhizal fungi on each plant, namely: Control (A), FMA 5 g (B), FMA 10 g (C), FMA

15 g (D). Each treatment was repeated 3 times, with each repetition consisting of 5 units, resulting in a total of 60 plant units.

Research Stages

1. Seedling Matoa Seeds: Matoa seeds were germinated in plastic trays after seed extraction. Before germination, the seeds were washed using bleach diluted to 20 ml/liter of water. The seeds were soaked in Atonik at a ratio of 1/1000 ml of water for 12 hours, then germinated in plastic trays measuring 30 x 20 x 10 cm filled with sterilized sand media.
2. FMA Inoculum: The FMA inoculum was sourced from the Indonesian Mycorrhiza Association, Southeast Sulawesi branch, which had been cultured using zeolite. Inoculation was performed on seedlings filled with sterilized post-gold mining soil. The seedlings were maintained and watered daily for three months.

Measurement Parameters

Observation of Seedling Growth

Seedling height (cm) is measured with a ruler from the base of the stem to the highest growth point. The diameter of the seedling (mm) is measured 1 cm above the surface of the medium using a caliper. Both height and diameter are measured every two weeks. The leaf count (number of blades) tracks the increase in the number of leaves. Observations of seedling growth provide data for analysis based on the differences calculated from week 0.

Dry Weight of Seedlings

Seedling parts are dried in an oven at 70°C for two 24-hour periods before being weighed. The shoot-to-root ratio, classified as NPA 1–3 and NPA 2–5, is deemed favorable and is calculated based on the ratio of dry weight of the shoot to that of the root at the conclusion of the study.

Seedling Quality Index is calculated using the formula:

$IMB = [\text{dry weight of shoot} + \text{dry weight of root}] / [(\text{height/diameter}) + (\text{dry weight of shoot/dry weight of root})]$. A seedling is considered of good quality if the IMB value is ≥ 0.09 (Dickson et al. 1960).

Observation of Fungal Mycorrhizae Colonization and Mycorrhizae Inoculation Effect (MIE)

Fungal Mycorrhizae (FMA) colonization is calculated using the formula: $FMA \text{ Colonization} = [\Sigma \text{ view area with mycorrhizae} / \Sigma \text{ total view area observed}] \times 100\%$ (Husna et al. 2021). The criteria for FMA colonization are outlined in Table 1, and the formula for MIE is: $MIE = [\text{dry weight of mycorrhizal plants} - \text{dry weight of non-mycorrhizal plants} / \text{dry weight of mycorrhizal plants}] \times 100\%$.

Table 1. Criteria for root colonization by mycorrhiza

Class	Criteria
1	0-5% (very low)
2	6-26% (low)
3	26-50% (medium)
4	51-75% (high)
5	76-100% (very high)

Source : Rajapakse dan Miller (1992)

Data Analysis

Data analysis will be conducted using analysis of variance (ANOVA) with an F-test, and if significant, it will be followed by Duncan's Multiple Range Test (DMRT) at a 95% confidence level.

RESULTS AND DISCUSSION**FMA Colonization and Mycorrhizae Inoculation Effect (MIE)**

This study reveals remarkable insights into the colonization of Fungal Mycorrhizal Associations (FMA) in the roots of matoa seedlings, demonstrating a colonization range of 3.33-72.21% (Table 2). Notably, the treatment with 15 g of FMA achieved an impressive colonization level of 72.21%, categorizing it within the very high range. This colonization showcases a fascinating network comprising internal hyphae, external hyphae, and vesicles (Figure 1). The control group's FMA inoculation is believed to have occurred through airborne spores or irrigation (Husna, 2015). Matoa seedlings exhibited a low dependency on mycorrhizae (MIE) at levels greater than 75%, falling within the range of 9.49-17.72% (Table 2). The strikingly high percentage of FMA colonization observed at the 15 g dosage is likely attributable to both the specific plant species and the quality of the FMA used. Interestingly, while FMA colonization was high, it did not correspond with an increased dependency on mycorrhizae. Research by Muleta (2010) and Husna (2015) suggests that the low dependency on FMA among matoa seedlings may be due to variations in root morphology as well as favorable climatic conditions, particularly adequate humidity. The symbiotic relationship between FMA and optimal plant fertility amplifies plant health, illustrating the profound benefits of FMA for host plants (Prihantoro et al., 2023).

In post-mining environments, FMA plays a crucial role in maintaining soil stability and constructing intricate networks of hyphae that create both macro and micro aggregates in the soil. The contribution of FMA to soil structure is based on: i) Hyphal growth penetrating the soil matrix to form a robust framework that binds soil particles together through physical interactions, and (ii) The integration of hyphae and micro-aggregate root networks into macro-aggregate formations, which enhances nutrient and carbon storage capacity while simultaneously providing a microhabitat for beneficial soil microbes (Suharno and Sanchayaningsih, 2013).

Table 2. Effects of Treatments on FMA Colonization and Mycorrhizae Inoculation Effect (MIE) of Matoa Seedlings (*P. pinnata*) Aged 3 Months.

Treatment	Colonization (%)	MIE (%)
Kontrol	3.33 c	0 d
FMA 5 g	59.29 ab	9.49 c
FMA 10 g	42.35 b	17.72 a
FMA 15 g	72.21 a	14.78 b
Pr>f	0.0021	<.0001

Note: Average values followed by different letters in the same column indicate significant differences at the DMRT test level of 0.05.

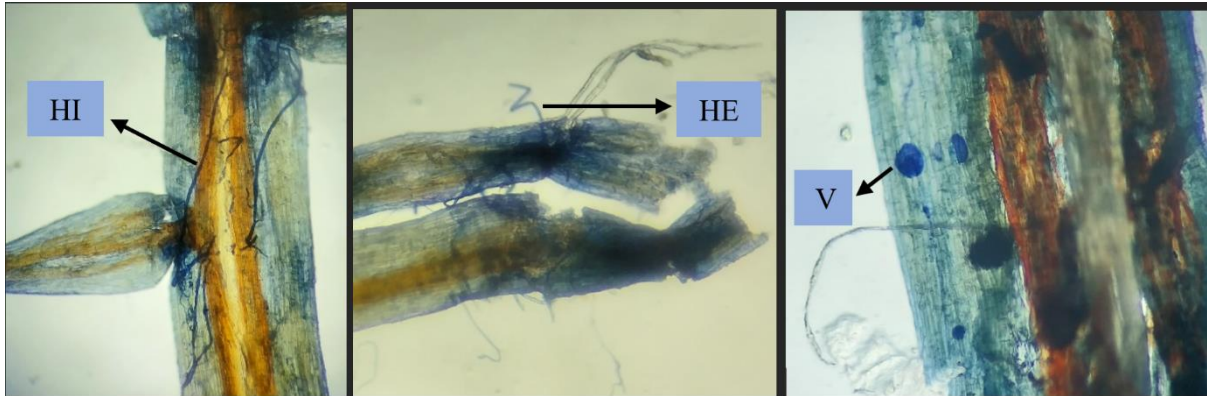


Figure 1. FMA Colonization on the Roots of Matoa Seedlings; HI (Internal Hypha), HE (External Hypha), V (Vesicle)

Seedling Growth

Analysis of variance revealed that FMA inoculation exerted a significant influence on the diameter and number of leaves, although it did not impact the height of the 3-month-old matoa seedlings (Table 3). While the height did not yield significant differences, there was a noticeable trend indicating that the mixed FMA inoculation treatment of 15 g had the highest average height at 5.19 cm (Figure 1). In terms of seedling diameter, the 15 g FMA inoculation treatment exhibited a significantly larger average than the control, reflecting a remarkable 40% increase and demonstrating distinct differences from other treatments. Furthermore, the 5 g FMA inoculation treatment produced a greater average number of leaves (32.33 leaves), showing a significant difference from the control, though not from other FMA inoculation treatments.

The effectiveness of local FMA in conjunction with matoa plants in soil contaminated by gold mining may be attributed to several factors: 1) Local FMA is well-suited to the prevailing environmental conditions, 2) Local FMA is compatible with the root exudates produced by matoa seedlings, and 3) Genetically, local FMA exhibits a remarkable ability to absorb water and nutrients beneficial for host plants (Muleta, 2010). The increase in leaf number aligns beautifully with the role of FMA in enhancing the uptake of crucial nutrients such as P, K, and Mn (Tuheteru et al. 2022) and nitrogen in post-gold mining soils (Husna et al. 2021). Nitrogen is a fundamental component of chlorophyll, integral to the processes of photosynthesis, thus underscoring the critical importance of these findings (Silva and Uchida, 2000). In examining the plant height parameter, the findings revealed no significant effects. This lack of impact may stem from the plants' inability to fully exploit nutrient absorption from the arbuscular mycorrhizal fungi (AMF), which could potentially stimulate growth in height among seedlings. Adequate nutrient levels are crucial as they enhance the photosynthesis process, leading to vital cellular elongation, division, and differentiation, ultimately propelling the growth and development of the plants (Sarief, 1986; Sari et al., 2016).

Table 3. Influence of Treatments on the Growth of Matoa Seedlings (*P. pinnata*) at 3 Months of Age

Treatment	Height (cm)	Diameter (mm)	Number of Leaves (sheets)
Kontrol	4.48	0.47 b	24.80 b
FMA 5 g	5.10	0.52 b	32.33 a
FMA 10 g	4.82	0.36 c	29.46 a
FMA 15 g	5.19	0.80 a	32.06 a
Pr>f	0.1481	0.0002	0.0216

Note: Average values accompanied by different letters in the same column indicate a significant difference at the 0.05 level of DMRT.



Figure 2. Performance of Matoa Seedlings

Seedling Biomass

The analysis of variance exhibited that the dry weight of roots did not significantly affect the outcomes, while both the dry weight of shoots and total dry weight revealed noteworthy results (Table 4). Although the dry root weight did not demonstrate significant variation, there was a notable trend where the treatment without AMF inoculation at a dosage of 10 g achieved the highest average of 0.75 g, whereas the lowest treatment, receiving 10 g of AMF inoculation, resulted in an average of 0.60 g. The dry weight of shoots highlighted that the AMF inoculation at 15 g garnered the highest value at 1.93 g, showing no significant difference from other AMF inoculation treatments but differing significantly from the control group. When it came to total dry weight, the 10 g AMF inoculation treatment triumphed with an average of 2.71 g, standing above the control's average of 2.23 g.

Biomass serves as a testament to a plant's ability to effectively absorb nutrients from its growing medium, thereby supporting its growth (Karepesina, 2007). An increase in plant biomass is intrinsically linked with plant metabolism and the presence of favorable growth conditions that facilitate essential metabolic processes, including photosynthesis. The reported increase in total dry weight at the 10 g AMF dosage corroborates findings from earlier research by Maulidi and Zulfita (2024) on rubber

plants. The absence of a significant impact on root dry weight may be attributed to the low moisture content in plant samples (Rilian et al., 2020), which can hinder the photosynthetic process until the close of the vegetative phase (Audi, 2016).

Table 4. Impact of Treatments on Biomass, Shoot-Root Ratio (SRR), and Seedling Quality Index (SQI) for Matoa (*P. pinnata*) at 3 Months of Age.

Treatment	Berat Kering (g)		
	Root	Shoot	Total
Kontrol	0.75	1.47 b	2.23 c
FMA 5 g	0.63	1.83 a	2.24 b
FMA 10 g	0.60	1.83 a	2.71 a
FMA 15 g	0.68	1.93 a	2.61 a
Pr>f	0.0729	0.0553	0.0001

Note: Average values accompanied by different letters in the same column indicate a significant difference at the 0.05 level of DMRT.

The shoot-root ratio and seedling quality index are vital indicators of seedling excellence. SRR reflects the relative abundance of above-ground resources compared to those in the root zone. This crucial ratio is calculated based on the dry weight of shoots relative to the dry weight of roots. The results yielded an SRR ranging from 5.47 to 2.13 (Table 5), a value that does not yet meet the established SRR standard of 2-5 (Husna, 2015).

Regarding the seedling quality index, the treatment with 15 g of AMF inoculation achieved the highest average of 0.26, while the treatment with 10 g of AMF inoculation recorded the lowest average of 0.13. According to the established standard of $SQI \geq 0.09$ (Dickson et al., 1960), all treatments surpassed the SQI benchmark, except the 10 g AMF inoculation treatment (Table 3). The low SQI value can be attributed to seedlings under stress conditions, which often redirect a portion of their photosynthetic energy toward underground organs (Husna, 2015).

Table 5. The Impact of Treatment on Root Shoot Ratio (RSR) and Seedling Quality Index (SQI) of Matoa (*P. pinnata*) at Three Months of Age

Treatment	Shoot to Root Ratio	Seedling Quality Index
Kontrol	2.13 a	0.21 b
FMA 5 g	2.88 b	0.20 b
FMA 10 g	5.47 a	0.13 c
FMA 15 g	2.78 b	0.26 a
Pr>f	0.0015	<.0001

Note: Average values accompanied by different letters in the same column indicate a significant difference at the 0.05 level of DMRT.

CONCLUSION

In summary, this research highlights the remarkable ability of Fungal Mycorrhizal Inoculation (FMA) to significantly enhance the growth of Matoa (*P. pinnata*) seedlings at three months of age, particularly when cultivated in soil derived from post-gold mining activities. Specifically, the application of a 15 g dosage of FMA not only boosts

the colonization rates of these beneficial fungi but also promotes substantial improvements in seedling height, diameter, dry weight of shoots, and overall seedling quality index. This research underscores the potential of FMA as a powerful tool in fostering the growth and vitality of Matoa seedlings in challenging environments.

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