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Research Article

Effectiveness Test of Local Arbuscular Mycorrhizal Fungi (AMF) and Cocoa Waste Compost on the Growth of Cocoa Seedlings (*Theobroma cacao.* L) in Former Mining Sites

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Abstract

There is ample opportunity for cocoa (*Theobroma cacao* L.) plantations because the world demand for cocoa increases every year. Cocoa plants bear fruit every year without knowing the season so cocoa cultivation provides promising prospects. The demand for cocoa must be balanced with increased in production from the cultivation scale from providing seeds to expanding cocoa planting land. The experimental design used in this study was a two-factor Randomised Group Design, namely the provision of AMF F0 (without AMF) and F1 (with AMF). The second factor is cocoa waste compost in planting media (v/v) with five levels, namely k0 (0%), k1 (5%), k2 (10%), k3 (15%),and k4 (20%). The aim of the research was to see the effectiveness of AMF and cocoa waste compost on the growth of cocoa seedling. Based on the research results, it can be concluded that the treatment of FMA can increase plant height, root volume, root dry weight, and percentage of colonized roots. Cocoa seedlings gave the best response with a dose of 0% cocoa waste compost with AMF treatment, with an average height of 60.19 cm . The percentage of AMF colonization is high at 44.6%. The final soil analysis showed an increase in P-available pH and soil Ald with mycorrhiza and cocoa shell compost treatment at a dose of 10%

Keywords: AMF, cultivation, nutrient uptake, plant, post-mining soil

1. Introduction

One of Indonesia's mainstay plantation commodities essential for foreign exchange is Cocoa (*Theobroma Cacao.* L). Environmental aspects and cultivation techniques influence the productivity of cocoa plants in their management. One of the environmental aspects that affect cocoa growth is land availability. Ideally, land that is suitable for the development of a commodity is land that has a match between land characteristics and the growing requirements of the commodity Hae *et al.*, 2021. One of the lands that can be utilized is the critical land of the mining industry. Ex-mining land is land left over from the mining process. On active mining land, tailings and post-mining sand often face extreme physical, chemical, and biological problems (Suharno and Sancayaningsih, 2013).To restore the condition of unutilized post-mining land into active land that can be used in agricultural cultivation by utilizing the potential of microorganisms living in the soil using arbuscular mycorrhizal fungi (AMF) and cocoa waste compost.

Soil quality factors influence cocoa production on ex-mining land, so it is necessary to improve soil quality to optimize productivity. AMF is reported to be symbiotic with 97% of terrestrial plant families (Smith and Read, 2008) and plays a role in increasing plant growth and production in post-mining land conditions, including in gold post-mining land soil media (Tuheteru *et al*., 2017). According to Asmarahman *et al.* 2018, Several limiting factors are highly considered in cocoa cultivation on mine lands, such as low pH, low levels of organic matter and nutrients, high levels of P fixation, and aluminum saturation. Providing organic materials such as compost is another alternative to increasing plant growth. Compost serves as a nutrient contributor, improves soil physical properties through its role as a soil aggregate stabilizer, and can increase the activity of soil microorganisms. Several studies have shown that applying compost and AMF positively affects plants (Ermansyah, 2012; Rahmayanti et al., 2013). The effect of compost on soil and plant productivity is influenced by the type and quality of compost (Widodo and Zaenal, 2018). The research aimed to see the effectiveness of AMF and cocoa waste compost on the growth of cocoa seedlings.

2. Materials and Methods

a. Time and Places

The research was conducted in Limau Manih Padang Pauh sub-district Padang West Sumatra. Soil analysis was conducted at the soil laboratory, Department of Soils, University of Andalas Padang. The research will be conducted from February to August 2022

b. Materials and Tools

The materials used in this study are F1 hybrid cocoa seeds (TSH858, ICS60), location-specific Arbuscular Mycorrhizal Fungi (AMF) Glomus Sp1, Gigaspora, and Acaulospora (multi spore) obtained from the rhizosphere of cocoa from the district of Fifty cities in West Sumatra the results of exploration and identification of AMF in previous studies and have been observed and propagated in the central laboratory of Andalas University and the Plant Physiology laboratory of the Faculty of Agriculture, Andalas University. Soil from the former quarry land of Semen Padang Limestone initial analysis to determine the initial fertilization of the planting media, cocoa fruit peel compost collected from smallholder farmers in West Sumatra EM4, trypan blue, cow dung SP-36 fertilizer, Urea, and KCl. The tools used were polybags, a paranet, an oven, a sieve, analytical scales, a microscope, a hoe, a machete, a meter, a shovel, gloves, a tub for making compost, stakes, other stationery, a digital camera, raffia rope, an analytical scales, a vernier scale, plastic samples, trash bag, and spectrophotometer.

c. Research Method

The experimental design used in this study was a 2 x 5 factorial Randomised Group Design (RAK). There were two factors studied, namely AMF Application (F) consisting of F0= (without AMF) 0 g/plant, F1= (given AMF) 50 g/plant and cocoa waste compost in planting media (v/v) with five levels, namely k0 (0%), k1 (5%), k2 (10%), k3 (15%), k4 (20%). Analysis of variance (ANOVA) at a level of 5%. If the treatment shows an actual effect, it will be continued with the Least Significant Difference (BNT) test at an actual level of 5%. The separation of the middle value on the factor of AMF applications using the Least Real Difference Test (BNT) at an actual level of 5%.

d. Research Implementation

Composting. In the stage of making cocoa pod compost, cocoa waste was cut into homogeneous pieces measuring approximately 2-5 cm2. Next, 300 kg of cocoa waste was poured with 80 ml of EM4 and dissolved with 300 grams of brown sugar in 20 liters of water. Then, the mixture was covered with plastic. The mixture is stored in an open space but should not be exposed to sunlight. The compost matured after two months with 0.61% N-total, 2.96% P-total, 10.03 C-organic, 7.22 pH, and 16.61 C/N ratio. 4.3.2 Initial soil analysis. Ex-Mine Soil was taken from the field and analyzed on the initial soil before treatment, including pH (pH H2O and pH KCl 1:5), total N (Kjeldahl method), organic C (Walkley and Black method), potential P and K (25% HCl extract), available P (Bray I extract), exchangeable K, Ca, Na, Mg

(NH4OAc 1 N pH seven extract), CEC, Al-dd, Hdd, Al saturation, and soil texture. Seedling preparation Cocoa seeds The planting medium used in the nursery is sand. Before use, the sand needs to be sterilized first, and then planting holes are made using a finger, and then the cocoa seeds are sown in the seedbed.

After the seedlings were 14 days old, they were transferred to the prepared polybags. A soil mixture is first made before the media is put into the polybags. Ex-Mine

(1:1) and compost in a large tub according to the treatment. The media was mixed evenly (homogeneous). After the media is homogeneous, it is put into polybags measuring 12 x 19 cm).

Furthermore, planting holes were made with a length, width, and height of about 10 cm. Maintenance includes watering, weeding, and fertilizing. Watering was done twice a day. Weeding was done manually, while fertilization was done when the plants were one month old by giving NPK fertilizer at a dose of 1 g per seedling, which was done every month after transplanting.

Observations include growth factors and analysis of mined soil. Growth factors are Height measurement, leaf count, root volume, crown dry weight, and root dry weight. The percentage of root infection by AMF was measured using the Kormanik and McGraw method (Brundett, Bougher, Dell, Grove, and Malajczuk, 1996). The formula used to calculate the percentage of root infection by FMA is: The percentage of AMF colonization was then classified based on O'Connor *et al.* (2001). The level of AMF colonization is based on O'Connor et al. (2001) in Table 1.

Table 1. AMF colonization level categories (O'Connor et al., 2001)

Percentage colonisation %	Category
0	Not colonised
< 10	Low
10-30	Medium
>30	High

3. Results and Discussion

a. Growth Factors

Table 2 shows that AMF application showed significantly different results on plant height, root volume, root infection percentage, root dry weight, and cocoa peel compost treatment, which had no significant effect on cocoa seedling growth. The results showed that applying cocoa peel compost had no significant effect on cocoa growth. There is another possibility that the cause of the absence of the effect of cocoa peel compost on plant growth is tannin compounds contained in the cocoa peel that can inhibit plant growth, as described by Fapohunda and Afolayan (2012). Cocoa peel is rich in phenolic compounds, such as cinnamic acid, tannins, pyrogallol, epicatechin-3-gallate, quercetin, and retinol. The results of research by Suryadi *et al.* (2018) show that cocoa fruit skin contains tannins of 4.981%. Tannins inhibit enzyme work and substrate removal (protein), bind to lipids and proteins, and bind protease enzymes that catalyze proteins into amino acids needed for growth. Amino acids are the raw material for the formation of plant growth hormones. If hormone formation is disrupted, growth will be inhibited.

Processing is needed before making cocoa.

Fruit peels into compost to reduce tannin levels; according to research by Sartini *et al.* (2017), drying cocoa fruit peels under direct sunlight can reduce tannin levels in cocoa fruit peels to 0.174%. However, this method only reduces tannin levels and does not eliminate it. In line with this, a higher dose of cocoa pod husk compost can increase the tannin content, thereby inhibiting growth. Root volume and dry weight were affected by AMF, indicating that the presence of AMF in the plant growth system affects root morphology. Plant root morphology is essential to maximize nutrient absorption because root systems with a high surface area and volume ratio will explore large soil volumes more efficiently. Therefore, mycorrhizae are essential in plants, especially in terms of P uptake, because they increase the ability of roots to explore the soil more widely. Root hairs are a standard root structure, and increased root length is a plant adaptation to increase P uptake and plant competition when plant P is limited for growth. Increased P uptake in plants is obtained from association with AMF (Brundrett, 1996).

Cocoa seedlings gave the best response with a dose of 0% cocoa waste compost with AMF treatment, with an average height of 60.19 cm, as seen in Table 3. This study shows that the average plant height is 60.19 cm and the average number of leaves is 18 strands by the criteria of ready-to-plant seedlings proposed by Rahardjo (2011), plant height of more than 60 cm and the number of leaves more than 12 strands can be said to be cocoa seedlings with good quality

The results of the study in the form of AMF structure in the form of internal hyphae (HI) and external hyphae (HE) showed that cocoa roots were colonized by AMF (Figure 1). The structure of *tai ling* through internal hyphae, external hyphae, and glomalin production in the form of adhesive glands from AMF. In addition, the presence of AMF structure can increase the growth ability of cocoa seedlings due to an increase in nutrient uptake. This study is in line with the statement of Gaur and Andholeya (2004) that AMF is widely able to increase plant growth even on degraded land.

Furthermore, Budi et al. (2015) stated that AMF can change the morphology and physiology of roots to increase the rate of photosynthate from leaves to roots, followed by an increase in overall plant growth. Research related to plant growth with AMF application is also reported to be able to increase the growth of *Myristica fragrans* Houtt (Mahdi, 2015), kuku wood (Husna, 2015), and *Albizia saponaria* (Lour.) Miq (Tuheteru & Husna, 2011). The association of AMF with cocoa seedlings can be known by the formation of typical structures from root colonization by AMF. AMF structures found from observations of cocoa seedling roots are hyphae, vesicles, and spores. Each structure was found in hyphae, vesicles, and spores. The presence of root infection by AMF in this study is ex-mining soil, namely acidic soil pH and very low P content.

Table 2. Recapitulation of the results of the analysis of variance

Observation variable	Significance		
	AMF	Cocoa waste compost	FxK
Plant height	*	tn	tn
Number of leaves	tn	tn	tn
Crown dry weight	tn	tn	tn
Root dry weight	*	tn	tn
Root volume	*	tn	tn
Percent Root infection	*	tn	tn

*Notes: tn = not significant, * = significant at α0.05, ** = significant at α0.01*

Table 3 Average height of cocoa seedlings treated with cocoa husk compost and AMF.

Treatment		Cocoa waste compost					Average
		..................cm..................					
		K0	K1	K2	K3	K4	
AMF	F0	59.20	57.90	56.47	56.70	60.33	58.12 a
	F1	61.31	60.33	60.00	58.00	61.33	60.19 b
Average		60.25	59.11	58.23	57.35	60.83	
KK : 4.49							

Description: The numbers in the columns followed by the same lowercase letters are not significantly different according to the 5% DMRT test.

Figure 1. Internal and external hyphae of cacao seedlings

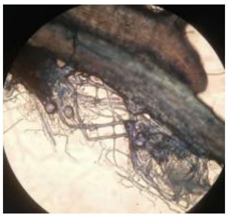

Figure 2. AMF colonization of cocoa seedling roots

in Figure2. AMF colonization, the amount of root colonization, is thought to be due to the P nutrients available in the soil. Nurmasyitah and Khairuna (2017) stated that the increase in the percentage of root colonization is influenced by nutrient levels in the soil, especially the availability of P. Wicaksono *et al.* (2014) also argued that the association between AMF and plant roots is influenced by several factors, namely the type of AMF, plant characteristics, soil pH, and nutrient content of P. AMF application does not only aim to improve the physical and chemical quality of planting media. However, the AMF application is also expected to improve the biological quality of planting media soil (Elfianti and Siregar, 2010). In the end, it is expected that the growth of cocoa seedlings will increase, compared to when only cocoa husk compost is given in the planting media. However, this study showed that the higher level of AMF colonization did not follow the higher percentage of compost mixed in the planting media. This can be seen in Table 4.

Table 4. Average number of spores and percentage of root colonization and AMF colonization level categories

Treatment	Spore count/ 20 g soil	Colonization Percentage (%)	Category*
Without AMF	15	10.7	Low
AMF	50	44.6	High

**Category of FMA colonization level (O'Connor et al., 2001)*

Table 4 above shows that the highest percentage of root colonization of cocoa seedling root samples is 44.6%, and without AMF, it is 10.7%. This data shows that mycorrhiza has a different infectivity from plant roots. 2001 cocoa is classified as high. The development of AMF is influenced by the colonization of AMF on the roots because the ex-mining soil is infertile soil. AMF can develop rapidly to colonize and multiply hyphae and extend the roots to expand the roots to attract minerals and soil organic matter.

The study showed that the AMF infecting cocoa roots in the roots of cocoa seedlings formed a typical structure of AMF colonizers found vesicle structure and arbuscules (hyphae that are branched). Arbuscules play an important role as a place for nutrient and carbon exchange between AMF and host plants and a temporary storage place for minerals, nutrients, and sugars. At the same time, vesicles act as a storage organ for food reserves such as lipids and, at certain times, act as spores, which are the life-defense organs of AMF. Spores are the self-propagating organs of AMF, formed from extradical hyphae with single or colonized forms (sporocarps) (Simanungkalit et al., 2006).

b. Analysis of mined soil

Planting media in the form of ex-mining soil Ex-mining soil is taken in the area around P.T Semen Padang. Limestone mining aimed at meeting the needs of cement raw materials has left environmental problems, especially on ex-mining lands. Mining activities cause damage to structure, texture, porosity, and density, which are physical characteristics of soil that are important for plant growth. Compact soil conditions due to compaction cause poor water and aeration systems, which can have a negative impact on plant growth. The loss of topsoil leads to the loss of essential nutrients, such as nitrogen and phosphorus, a significant problem on post-mining land. This situation also causes poor soil microbial conditions, indirectly affecting plant growth on the land (Soewandita, 2010). The chemical properties of the growth media analyzed included organic C content using the Walkey and Black method, total N using the Kjeldhal method, and available P and K using the 25% HCl extract method. Table 5 shows the differences in the initial soil analyses.

Table 5. Preliminary analysis results of cocoa seedling growing media

Parameters	Initial Analysis	*Criteria
pH H O_2	5.5	sour
C-Organic	0.69	Very low
N-Total (%)	0.05	Very low
C/N (%)	13.8	Medium
Total (ppm)	10.4	Very low
P-Available (ppm)	5.83	Very low
Add (me $100g^{-1)}$	0.75	-

**Hardjowigeno, S. 1995. Soil Science*

P availability is related to the soil's acidity (pH) and Al-dd content. P elements in the soil are immobile because most P elements are unavailable to plants in acid soils. Low levels of P-availability in the soil are caused by high levels of Al-dd, which fix inorganic P in the soil so that it becomes an unavailable form and cannot be absorbed by plants. Munawar (2011) in Rahmi and Biantary (2014) stated that in acidic soil (low pH), P is bound by Al and forms Al-P and Fe-P compounds that are relatively insoluble, so P cannot be absorbed by plants. The results of the initial soil analysis showed that the P-total was low and the P-available was very low. It is because, generally, ex-mining soils have P sources derived from the weathering of mineral P sources that are already low. This statement was made by Syahputra et al. (2015), who stated that P deficiency on the margins can be caused by the weathering of soil parent material / P source minerals, which are generally low. Effect of AMF and cocoa waste compost on P-availability, Al-dd, and soil pH.

Table 6. Final soil analysis results with AMF and cocoa waste compost treatments

Treatment	P-available	Al-dd	pH
F0K0	6.67	0.35	5.5
F0K1	6.79	0.49	5.69
F0K2	6.62	0.40	5.65
F0K3	6.78	0.40	5.60
F0K4	6.77	0.43	5.66
F1K0	8.69	0.67	5.79
F1K2	8.99	0.60	5.83
F1K3	8.56	0.41	5.80
F1K4	8.45	0.56	5.8

The effect of AMF and cocoa husk compost on P-available levels in the research results also increased from the P-available level of 5.83 ppm before treatment doubled after AMF and the highest cocoa husk compost with a value of 8.99 in the F1K2 treatment (AMF application with a dose of 10% cocoa husk compost). This is because AMF colonization will produce organic acids that can release Al-P bonds and bonds with soil clay minerals to make P available to plants. It is reinforced by the results of Nurmasyitah's research (2013) that the presence of AMF colonization is able to release P elements that are fixed by heavy metals to become available to plants with p-available levels before treatment of 1.01 ppm after being given AMF, p-available increased to 2.12 ppm. Based on the results of the analysis of Al-dd and soil pH, it shows that each treatment gives an increase that is almost not much different due to dissolved Al or Fe binding P sourced from chemical fertilizers so that insoluble Al-P or Fe-P bonds are formed and make Al levels very low in the soil. However, the high levels of P bound by Al and Fe resulted in more P becoming unavailable to plants.

Increased soil pH will also increase the availability of P in the soil. This is because the activity of phosphatase enzymes and organic acids from AMF metabolism that fix Al becomes insoluble or precipitates so that the soil will contain more basic cations, which will then increase soil pH and decrease Al-dd levels in the soil. Furthermore, the activity of AMF can also release Al-P fixation, which causes P-availability to increase. Furthermore, the excretion of AMF in the form of phosphatase enzymes can also convert organic P into primary orthophosphate form in acidic soils that can be directly absorbed by plants. Maria (2015) explained that organic acids and phosphatase enzymes produced by Arbuscular Mycorrhizal secretions could spur the mineralization process of organic P by catalyzing the release of P from organic complexes into inorganic complexes to make it available to plants. In addition, the role of AMF colonization in increasing P-availability is to form external hyphae that function to reach nutrients outside the root area of the host plant and can increase nutrient absorption.

4. Conclusions

Based on the research results, it can be concluded that the treatment of AMF can increase plant height, root volume, root dry weight, percentage of colonized roots, and percentage of AMF colonization. Cocoa seedlings gave the best response with a dose of 0% cocoa waste compost with AMF treatment, with an average height o f 60.19 cm and a percentage of AMF colonization with a high category of 44.6%. Cocoa waste compost treatment has not shown significant results. The final soil analysis showed an increase in P-available pH and soil Ald with mycorrhiza and Cocoa waste compost treatment at a dose of 10%.

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