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The Effect of Addition of AMF (Arbuscular Mycorrhizal Fungi) and Yomari Liquid Organic Fertilizer Concentration on the Growth of Agarwood Production Plants (*Aquilaria malacensis* Lamk.) on Ex-Lime Mining Soil

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Abstract— *This plant has a very large opportunity to be developed on ex-mining land, considering that this plant is an adaptive non-timber forest product plant that grows and produces naturally without inoculation (injection) on ex-mining land: limestone, nickel, rocky sand, tin and coal. and added witharbuscular mycorrhizal fungi (AMF) and liquid organic fertilizer such as Yomari on Aquilaria malacensis Lamk. AMF is able to increase root growth, root range, so that it can facilitate roots to absorb water and nutrients throughexternal hyphae and later obtained quality seeds which have many and strong roots. Yomariliquid organic fertilizer has high organic and nutrient content, can increase soil organisms and improve soil pH and. This study aimed to obtain the best interaction between the doseof AMF and the concentration of Yomari liquid organic fertilizer on the growth of Aquilaria malacensis Lamk seedlings. in the ex-limestone mine soil, the best dose of AMFwas obtained on the growth of Aquilaria malacensis Lamk seedlings. in limestone ex- mining soil and the best concentration of Yomari organic fertilizer on the growth of Aquilaria malacensis Lamk. in the former limestone quarry. This research was conducted from June to November 2022 in the nursery of the Faculty of Agriculture, Andalas University. This study used a factorial experimental method in a Completely Randomized Design (CRD) with 2 factors, namely the first factorwas the administration of the AMF dose which consisted of 5 levels: 0.0 grams (C0); 15 grams (C2); 30 grams (C3) and 45 grams (C4) and the second factor concentration of Yomari organic fertilizer consisted of 5 levels: 0.00 ppm (Y0); 25.00 ppm (Y1); 50.00 (Y2); 75.00 ppm (Y3) and 100.00 ppm (Y4), each treatment level consisted of 4 replications. Observational data in the form of qualitative and quantitative data were analyzed using the F-test at a significance level of 5%. The different effects on the treatmentwere analyzed by further test of LSD at 5% level. Observations were made including: percentage of live seedlings, increase in seedling height, increase in number of leaves, widest leaf width, root weight, and percentage of roots infected by AMF on lime-mined soil media. There is an interaction between the dose of AMF and the concentration of liquidorganic fertilizer on the growth of agarwood-producing plant seeds (Aquilaria malacensisLamk.)) on the ex-limestone mining soil. Giving a dose of AMF 45 grams with a concentration of 100 ppm liquid organic fertilizer was the best in increasing the percentage of life, increasing the number leaves, increase in plant seed height, widest leaf width, percentage of agarwood-producing plant seeds infected with AMF.*

Keywords— ***Seedlings, agarwood, Yomari liquid organic fertilizer, AMF, limestonemining soil***

I. INTRODUCTION

Limestone is a solid rock that contains a lot of calcium carbonate (Lukmanet al., 2012 and Sucipto et al., 2007). Calcium carbonate is an inorganic mineral that is known to be commercially available cheaply. Physical properties of calcium carbonate such as morphology, phase, size, and size distribution must be modified according to the field of application (Kirboga and Oner, 2013; Lailiyah et al., 2012). Ex-lime mining land at PT. Holcim Indonesia Tbk West Java, precisely in the Cirebon area, obtained the results of chemical analysis of lime soil including the pH value of H2O 8.4, KCL 7.8 while organic matter C was 0.98%, N 0.7% and C/N 14% and for available P it was obtained at 25 ppm (Wildasari, 2016 and Wahdi, 2016).

Rehabilitation activities of ex-lime mining land generally require quality seeds, however, the seedlings die in large numbers after being planted in the field because the availability of nutrients and water is not met for growth and development, as a result of the plant roots being few, shallow, not strong and damaged (Sari, 2018 and Kimi, Sutoyo and Satria, 2021). This root problem can be overcome by using liquid organic fertilizers such as Yomari fertilizer and Arbuscula mycorrhizal fungi (AMF) at a certain dose.

Yomari liquid organic fertilizer is a fertilizer that functions to increase the development of soil organisms, increases soil pH and stimulates vegetative growth of plants, plays a role in the formation of leaf green matter and stimulates plant roots to become more abundant and stronger. Soaking the coffee cuttings in 1 ml/l of Yomari liquid organic fertilizer and spraying the cuttings 3 times at a dose of 1ml/l within 10 days will produce many cutting shoots, have many roots with strong roots (Satria *et al*, 2021). The presence of AMF can increase the availability of nutrients, especially Phosphate (P) which is very low in the soil of ex-lime mining, improve soil structure, increase water absorption and protect plants from root pathogens and toxic elements. Inoculation of 40 grams of AMF on ultisols and in planting media from limestone ex-mining soil can increase the growth of

plant seeds Aquilaria malacensis Lamk .) and Satria, Fadli, Herawati and Aprisal,2021).

This plant has a very large opportunity to be developed on ex-mining land, considering that this plant includes adaptive non-timber forest products that grow and produce naturally on ex-mining land: nickel, rocky sand, tin and coal (Sari, 201 and Kimi *et al. al*, 2021). In connection with the explanation above, the researchers have conducted a study entitled "**The Effect of Dosage of AMF (*Fungi*) and Yomary Liquid Organic Fertilizer Concentration on the Growth of Agarwood-Producing Plant Seeds (*Aquilaria malacensis Lamk.)*) on Ex-Limestone Mining Soil".**

This research was conducted to answer some of the problems formulated in the following questions: 1. Is there an interaction between the dose of AMF and the concentration of Yomari organic fertilizer on the growth of gaharu-producing plant seeds (*Aquilaria malacensis* Lamk.) in the former lime-mining soil? 2. What is the effect of the dose of AMF? on the growth of these seedlings in the former limestone quarry soil? And 3. How is the effect of Yomari organic fertilizer concentration on the growth of the seeds in the former lime-mining soil?

The specific objectives of this research are: 1. To obtain the best interaction between the dose of AMF and the concentration of Yomari organic fertilizer on the growth of gaharu-producing plant seeds (*Aquilaria malacensis* Lamk.) in the former limestone quarry; 2. Obtaining the best dose of AMF for the growth of these seedlings in the former lime-mining soil; 3. Obtaining the best concentration of

Yomari organic fertilizer on the growth of these seeds in the former lime-miningsoil.

This research can provide information about the seeds of gaharu-producing plants (*Aquilaria malacensis* Lamk.) which are able to associate well with the dose of AMF and Yomari organic fertilizer concentration and are compatible to be planted on ex-lime mining land.

The results of this study are expected to contribute to the development of plant science, especially plant breeding. These contributions include: Providing information on the standard method of AMF Dosage and concentration of Yomari Liquid Organic Fertilizer and Types of AMF) on the Growth of Agarwood- Producing Plant Seeds (*Aquilaria malacensis Lamk.)*in Ex-Limestone Mining Soils Provide a positive contribution to the development of plant cultivation science and technology gaharu (*Aquilaria malacensis*) and become a reference in adaptability and germplasm preservation.

II. METHODS

The research was conducted in the experimental garden of the Faculty of Agriculture, Andalas University. The research is planned for 8 months starting in June 2022 until November 2022.

The tools used in this research are hoe, bucket, polybag, scissors, hotplate, ruler, digital, meter, stationery, tweezers, scissors, cover glass, hose, tea filter, object glass, camera, and microscope. The materials used

were gaharu-producing plant seeds of Aquilaria malaccensis Lamk species, limestone ex-mining soil, ultisol, compost, Yomari liquid organic fertilizer, Arbuscula mycorrhizal fungi (Acaulospora sp., Gigaspora sp., and a mixture of Acaulospora sp. and Gigaspora sp.), NPK fertilizer, Aquadest, KOH 10%, HCL 2%, Trypan blue which is useful for root coloring, as well as clean water.

This study used a factorial experimental method in a Completely Randomized Design (CRD) with 2 factors, namely the first factor was the administration of the AMF dose which consisted of 5 levels: 0.0 grams (C0); 15 grams (C2); 30 grams (C3) and 45 grams (C4) and the second factor concentration of Yomari organic fertilizer consisted of 5 levels: 0.00 ppm (Y0); 25.00 ppm (Y1); 50.00 (Y2); 75.00 ppm (Y3) and 100.00 ppm (Y4), each treatment level consisted of 4 replications. Observational data in the form of qualitative and quantitative data were analyzed using the F-test at a significance level of 5%. The different effects on the treatment were analyzed by further test of BNT at 5% level.

The seedlings of agarwood plant species Aquilaria malaccensis Lamk came from the Gaharu Kanagarian farmer group Muaro Linggae, Sijunjung Regency. The seeds were prepared in each polybag measuring 8 cm X 9 cm and when the research was going to be carried out, the plants were transferred to polybags measuring 12 cm X 17 cm. Seedlings with seed criteria were not attacked by pests and diseases, had a height ranging from 5- 15 cm with 2-5 leaves. The soil used is the former limestone mining soil of PT Semen Padang mixed with Ultisol obtained from the experimental garden area of the Faculty of Agriculture, Andalas University. The soil is treated evenly and the planting media is put into a polybag measuring 12 cmx 17 cm.

The doses of AMF used included: 0 grams/polibaq, 15 grams/polibaq, 30 grams/polibaq and 45 grams/polibaq with multispora AMF types (a mixture of Acaulospora sp and Gigaspora sp) to plants by sprinkling them in the planting holes and then seedlings, then covered again with soil in the polybag . Furthermore, seedlings aged 4 and 8 weeks after planting were watered with Yomari liquid organic fertilizer concentration according to treatment. Observations included: percentage of viable seedlings, number of leaves, increase in seedling height, widest leaf width, weight of seedling roots and percentage of plant roots infected with AMF.

III. RESULTS AND DISCUSSIONS

A. Persentase Hidup Bibit Tanaman Pnghasil Gaharu

The results of the analysis of variance showed that there was an interaction between the AMF treatment and LOF treatment on the percentage of live seedlings (Appendix 4 and Table 1). The best response was found at a dose of 45 g of AMF with a concentration of 100 ppm LOF which showed a significant difference with other treatments at the age of 3 months after planting (DAT). This is because the higher the dose of AMF and the higher the concentration of LOF given to agarwood-producing plants, the higher the percentage of live agarwood seedlings.

Table 1. Percentage of Life of Agarwood-Producing Plant Seeds due to AMF and LOFtreatment at 3 months of age

age	Concentration LOF (ppm)				
	0	25	50	75	100
	%				
0	55.00 aD	60.00 bCD	65.00 c BC	67.50 cAB	72.50 dA
15	50.00 a D	62.50 b C	70.00 bc B	75.00 b B	82.50 c A
30	52.50 a E	65.00 ab D	72.50 b C	80.00 b B	92.50 b A
45	55.00 a D	70.00 a C	82.50 a B	87.50 a B	100.00 a A
CV = 6.24%					

Note: The numbers followed by the same uppercase letter in the same row and the numbers followed by the same lowercase letter in the same column are not significantly different according to theLSD

That can increase economic value. The existence of AMF that has been inoculated into the agarwoodseedling media, makes the agarwood seeds have high economic value and better productivity. This is, in accordance with the opinion of Kimi *et al*,. 2020 that AMF can help plant growth, increase crop productivity and quality through increasing theavailability and absorption of nutrients in the soil, especially plants grown on less fertile lands such as infertile lands such as ex-lime mining lands. Mosse *et al*. (1981)also added that the seedling phase is a phase that is highly dependent on mycorrhizae, plants associated with ACM can adapt better to critical lands that have limited nutrient conditions.

Yomari liquid organic fertilizer is a fertilizer that functions to increase soil organisms, increase soil pH and stimulate plant vegetative growth, helps formation of cell division albumin for leaves, fruit, flower seeds and plays a role in the formation of leaf green matter and improvement of plant nutrients to stimulate plantroots to grow. more and stronger. This fertilizer contains several nutrients needed by plants and has aftif ingredients. The composition of nutrients contained in this fertilizer includes: Methyl Purine is a substance that can make plants resistant to allweather, Potassium 2,4 Dinitrophenol is a substance that synthesizes amino acids and proteins that make plants grow optimally, Potassium 5 Nitroguailacol and Potassium Paranitrophenol. Besides, this fertilizer contains organic C, organic N, elements P, K, Fe, MN, Zn and (Satria *et al*, 2021)

B. Increase in the number of leaves of agarwood-producing plant seeds

The results of the analysis of variance showed that there was an interaction between AMF treatment and LOF treatment on the increase in the number of leaves of agarwood-producing plant seeds (Appendix 4 and Table 2). The best response was found at a dose of 45 g of AMF with a concentration of 100 ppm LOF which showed a significant difference with other treatments at the age of 3 months after planting (DAT). This was because the higher the dose of AMF and the higher the concentration of LOF given to agarwood-producing plants, the higher the number of leaves of agarwood-producing plant seeds increased.

Table 2. Increase in the number of leaves of agarwood-producing plant seedsdue to AMF and LOF treatment at the age of 3 months of seedlings

Dosage AMF (g)	Concentration LOF (ppm)				
	0	25	50	75	100
	helai				
0	7.75 aC	8.25 bC	9.25 bB	9.50 bAB	10.25 bA
15	7.75 aD	8.75 abC	9.50 bBC	9.75 bB	10.75 bA
30	7.25 aC	9.25 aB	9.75 abB	10.00 bB	11.00 bA
45	7.50 a	9.50 a	10.50 a	11.25a	12.50 a
	D	C	B	B	A
CV = 5.6%					

The percentage of live seeds is influenced by various biotic and abiotic factors. Biotic factors such as seed quality, plant seeds used from both species came from healthy seeds. The characteristics of healthy plants are that they have green leaves and stems, the seeds are not diseased, the stems are straight, which is in accordance with the Indonesian national standard (SNI) 01-5006.1-2006 regarding seed quality which states that healthy seeds are fresh seeds. that are not attacked bypests and diseases, and do not show symptoms of nutrient deficiency (stems are notstraight and pale yellow in color).

The high percentage value of live seedlings *Aquilaria malacensis* seedlings had the best growth response to the AMF dose treatment with the highest LOF concentration.

Thewhich has a good impact, and can produce productsNote: The numbers followed by the same uppercase letter in the same row and the numbers followed by the same lowercase letter in the same column are not significantly different according to theLSD

This is because the higher the dose of LOF given and the higher the dose of AMF given to agarwood-producing plants, the higher the number of leaves of agarwood-producing plant seeds. LOF contains optimum nutrients for plant D.growth heterophyllum (Satria and Raesi, 2021).

C. Agarwood-producing Plant Seed Height Increase

The results of the analysis of variance showed that there was an interaction between AMF treatment and LOF

treatment on the increase in agarwood-producing plant seed height (Appendix 4 and Table 3). The best response was shown in the 45 g AMF treatment with 100 ppm LOF treatment, which was significantly different from the other treatments at 3 months after planting (DAT).

Table 3. Plant Seed Height Increase due to AMF and LOF treatment atseedling age of 3 months

Dosage AMF (g)	Concentration LOF (ppm) 0	25	50	75	100
	cm				
0	7.1250 cD	7.8750 dC	8.0625 dBC	8.4375 dB	8.6875 dA
15	7.8125 bD	8.3750 cC	8.5000 cB	9.5000 cB	10.6250 cA
30	8.2500 aE	9.5000 bD	10.8125 bC	11.8750 bB	12.5000 bA
45	8.4375 aE	10.3125 aD	11.5625 aC	12.9375 aB	14.4375 aA

Note: The numbers followed by the same uppercase letter in the same row and the numbers followed by the same lowercase letter in the same column are not significantly differentaccording to the LSD test

This is because this is because the higher the dose of LOF given and the higher the dose of AMF given to agarwood-producing plants, the higher the growth of agarwood-producing plant seeds. In addition, this plant is able to utilize N2 in the air, and the organic matter produced by this plant is rich in N nutrients (Kimi et al., 2020).

The results of the analysis of variance showed that there was an interaction between AMF treatment and LOF treatment on the widest leaf width of agarwood-producing plant seeds (Appendix 4 and Table 3). The best response was shown in the 45 g AMF treatment with 100 ppm LOF treatment, which was significantly different from the other treatments at 3 months after planting (DAT).

D. Widest Leaf Width of Agarwood Seedlings

Table 4. Widest Leaf Width of Agarwood-Producing Plant Seeds due to AMFand LOF treatment at 3 months of seedling

Dosage AMF (g)	Concentration LOF (ppm) 0	25	50	75	100
	cm				
0	2.0250 bD	2.1350 cCD	2.2750 cBC	2.3925 cB	2.5975 cA
15	2.1100 bC	2.3550 bB	2.4250 bcB	2.4725 cB	2.6950 cA
30	2.3675 aC	2.4100 bC	2.5400 bC	2.8125 bB	3.4825 bA
45	2.4500 a E	2.6500 a D	2.8425 a C	3.5400 a B	4.3550 a A
KK = 5.11%					

Note: The numbers followed by the same uppercase letter in the same row and the numbers followed by the same lowercase letter in the same column are not significantly different according to theBNT test

This is because the higher the dose of POC given and the higher the dose of AMF given to the producing plant, the wider the width of the widest leaf of agarwood-producing plant seeds. In addition, this plant is able to utilize N2 in the air, and the organic matter produced by this plant is rich in N nutrients (Kimi et al.,2020).

E. Root Weight of Agarwood Seedlings

The results of the analysis of variance showed that there was an interaction between AMF treatment and POC treatment on root weight of agarwood-producing plant seeds (Appendix 4 and Table 3). The best response was shown in the 45 g AMF treatment with 100 ppm POC treatment, which was significantly different from the other treatments at 3 months after planting (DAT).

Table 5. Root Weight of Agarwood-Producing Plant Seeds due to AMF andPOC treatment at 3 months of seedling age

Dosage AMF (g)	Concentration LOF (ppm)				
	0	25	50	75	100
	g				
0	11.3000 cC	12.3175 bB	12.9925 cB	13.1600 cAB	13.8675 cA
15	12.2325 bC	13.0750 bBC	13.5550 cAB	13.7150 cAB	13.9900 cA
30	13.0275 bD	14.1000 aC	15.1225 bB	15.8875 bB	17.2300 bA
45	14.3925 a C	14.8600 a C	16.8500 a B	17.5800 a B	20.6975 a A
CV = 4.12%					

Note: The numbers followed by the same uppercase letter in the same row and the numbers followed by the same lowercase letter in the same column are not significantly different according to theLSD test at the 5% level.

This is because the higher the dose of AMF given and the higher the concentrationof LOF given to agarwood-producing plants, the higher the root weight of agarwood-producing plant seeds, nitrogen is needed for the formation and growth of vegetative plant parts such as leaves, stems and roots. Meanwhile, phosphorus can help increase plant growth, produce chlorophyll, increase protein levels and accelerate leaf growth (*Satria et al.*, 2021).

F. Percentage of Roots of Agarwood-Producing Plant Seedlings infected with AMF

The results of the analysis of variance showed that there was an interaction between AMF treatment and LOF treatment on the widest leaf width of agarwood-producing plant seeds (Appendix 4 and Table 3). The best response was shown in the 45 g AMF treatment with 100 ppm LOF treatment, which was significantly different from the other treatments at 3 months after planting (DAT).

This could be due to the fact that mycorrhizal fungi with a dose of AMF and LOF concentration were able to develop AMF on a planting medium which was dominated by sandy soil and had larger soil pores compared to clay soil and this condition was thought to be suitable for the development of AMF spores which tend to larger and capable of infecting plant roots (Asmarahman, 2018).

Table 6. Percentage of Roots of Agarwood-Producing Plant Seedlings infectedwith AMF due to AMF and LOF treatment at 3 months of age

Dosage AMF(g)	**Concentration LOF (ppm)**				
	0	25	50	75	100
	%				
0	0.0000 aA	0.0000 aA	0.0000 aA	0.0000 aA	0.0000 aA
15	32.7500 bE	34.5000 bD	36.2500 bC	38.2500 bB	44.2500 bA
30	36.0000 bE	43.5000 cD	46.5000 cC	51.5000 cB	58.0000 cA
45	57.5000 c E	62.5000 d D	65.7500 d C	85.7500 d B	94.5000 d A
CV = 1.85%					

Note: The numbers followed by the same uppercase letter in the same row and the numbers followed by the same lowercase letter in the same column are not significantly different according to the LSD test

According to Brundrett (1996) mycorrhizae is a form of mutualistic symbiotic relationship between fungi and plant roots, both symbionts benefit. AMF includes obligate symbionts, which means that AMF can work after infecting the host plant. AMF is able to infect the root system of the host plant which can be seenin Figure 4, then it will produce an intensive network of hyphae so that mycorrhizal plants are able to increase their capacity to

absorb nutrients from LOF and water.

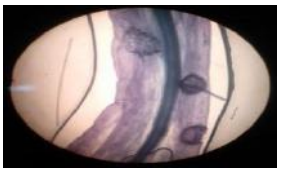

Fig.4. The shape of the roots infected by AMF as seen from themicroscope with 400X magnification

The additional colonization of AMF begins with the formation of an appresorium. Appresorium is an important structure that is in the AMF life cycle, is a key event for successful interaction with potential host plants, then the contactphase will be followed by a symbiotic phase. Since that phase, fungi complete the complex morphological process by producing intercellular and intracellular hyphae, vesicles, and arbuscules, the main structures of AMF are arbuscules, vesicles, external hyphae, and spores (Dewi, 2007). The opinion of Sufaati *et al.* (2011) arbuscula is a hyphal structure derived from branching hyphae in the corticalcells of the root of the host plant, the shape of the arbuscule resembles a small treethat functions as a place for the exchange of primary metabolites (especially glucoseand phosphorus) between fungi and plant roots. The distribution of external hyphaeis influenced by biotic and abiotic factors such as chemical properties, soil physics, organic matter, microflora and microfauna around the soil as a planting medium (Trisilawati *et al.*, 2012).

IV. CONCLUSIONS

Based on the results of research on the effect of giving doses of AMF and yomari liquid organic fertilizer on the growth of agarwood-producing plant seeds (Aquilaria malacensis Lamk.)) on limestone ex-mining soils, it can be concluded that: 1. There is an interaction between the dose of AMF and the concentration of liquid organic fertilizer on the growth of agarwood-producing plant seedlings (Aquilaria malacensis Lamk.)) on the ex-lime mining soil, and 2. Administration of 45 grams of AMF with a concentration of 100 ppm liquid organic fertilizer is the best in increasing the percentage of life. , increase in number of leaves, increase inplant seed height, widest leaf width, percentage of agarwood-producing plant seedsinfected with AMF.

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