

The Effect of Plant Growth Promoting Rhizobacteria Doses and NASA Liquid Organic Fertilizer on the Growth of Agarwood Seedlings

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ABSTRACT

Agarwood (*Aquilaria malaccensis* Lamk.) is a highly sought-after non-timber forest product due to its valuable resin used in perfumes, medicines, and cosmetics. However, despite its significant commercial potential, agarwood cultivation in Indonesia remains underdeveloped, particularly in Sumatra. Agarwood seedling propagation can be achieved through land extensification, particularly by utilizing marginal lands. In West Sumatra, most marginal lands consist of ultisol soil, which has very low nutrient content. Therefore, the addition of organic matter and microorganisms is necessary to improve soil health and fertility to support plant growth. This study evaluated the effects of Plant Growth-Promoting Rhizobacteria (PGPR) and NASA Liquid Organic Fertilizer (LOF) on the growth of agarwood seedlings in ultisol soil, which is known for its low fertility and high acidity. The experiment employed a Completely Randomized Design (CRD) with two factors: PGPR (0, 10, 20, 30 ml/L) and NASA LOF (0, 20, 30, 40 ml/L). The results showed that the appropriate application of PGPR and NASA LOF significantly enhanced the growth and development of agarwood seedlings, highlighting their potential in improving agarwood cultivation on less productive ultisol soils. The best combination of PGPR and NASA LOF at a dose of 30 ml/L PGPR and 40 ml/L NASA LOF produced the highest average seedling height (82.34 cm) and the greatest number of leaves (38 leaves per seedling), with all seedlings surviving and meeting the quality standards for transplantation.

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INTRODUCTION

Forests in Indonesia produce many products, both timber and non-timber. One of the non-timber forest products is agarwood. Agarwood (*Aquilaria malaccensis* Lamk.) is a highly sought-after non-timber forest commodity. The resin found in agarwood

heartwood can be used as a base material in the production of perfumes, soaps, medicines, and cosmetics, making this product a high-value commercial commodity. The high market value of agarwood encourages excessive exploitation, leading to increased deforestation and more intensive logging of agarwood trees without reforestation efforts

(Anwar & Hartal, 2007; Santoso & Sumarna, 2006). Based on data from the Central Bureau of Statistics, Indonesia's agarwood resin production in 2022 reached 8,123.53 tons per year, produced from Java, Kalimantan, Maluku, and Papua islands (BPS, 2022). It is known that Sumatra has not been a significant agarwood resin producer for years, highlighting the need for further cultivation efforts.

Agarwood cultivation can be carried out both inside and outside forest areas (on agricultural land). However, agricultural land availability is decreasing due to land-use changes, leading to more marginal lands that are less productive for agriculture, such as ultisol soil. In Indonesia, Ultisol soil covers approximately 38.4 million hectares or about 29.7% of the total land area of 190 million hectares. According to Munir & Herman (2019), the distribution of Ultisol in West Sumatra reaches 635,500 hectares, which is intensively used as cultivated land for food crops, horticulture, and plantations. However, Ultisol soil has limitations, such as low fertility due to high acidity, low macronutrient content, and low organic matter content. Despite these challenges, ultisol soil has great potential for agarwood cultivation, provided that appropriate soil and plant management practices are applied (Subagyo et al., 2000). Soil and plant management for agarwood cultivation on ultisol soil can be improved by adding agricultural inputs, such as fertilizers. Fertilization can be carried out using either inorganic or organic fertilizers.

Inorganic fertilizers have the advantage of being easily decomposed and quickly absorbed by plants. However, their use can lead to soil degradation due to residues that cause soil compaction, reduced porosity, decreased soil biota activity, increased soil acidity, and lower organic matter content. Therefore, the use of

organic fertilizers is recommended. Although organic fertilizers take longer to release nutrients because they require time to decompose, they offer significant benefits in improving the soil's physical, chemical, and biological properties. One type of organic fertilizer that can be used is liquid organic fertilizer (LOF). LOF is a liquid fertilizer derived from the fermentation of plant and animal residues. Compared to solid organic fertilizers, LOF has the advantage of being easier to apply, as it can be directly sprayed onto leaves, flowers, stems, and soil. Because it is in liquid form, LOF decomposes more quickly, making nutrients and minerals more readily available to plants. According to Sitanggang et al. (2022), LOF can function as a growth stimulant, especially when plants begin to sprout or transition from the vegetative to the generative phase, stimulating fruit and seed growth. Leaves and stems can directly absorb applied fertilizers through stomata or surface pores.

NASA Liquid Organic Fertilizer (LOF) is a product processed with a special formula made from natural (organic) materials, making it multipurpose for plants, livestock, and fisheries. It is produced by PT Natural Nusantara. The contents of NASA LOF include N 0.12%, K 0.31%, P_2O_5 0.03%, S 0.12%, Ca 60.40 ppm, Cl 0.29%, Mg 16.88 ppm, Mn 2.46 ppm, Fe 12.89 ppm, Cu <0.03 ppm, Zn 4.71 ppm, Na 0.15%, B 60.84 ppm, Si 0.01%, Co <0.05 ppm, Al 6.38 ppm, NaCl 0.98%, Se 0.11 ppm, As 0.11 ppm, Cr <0.06 ppm, Mo <0.2 ppm, V <0.04 ppm, SO_4 0.35%, C/N ratio 0.86%, pH 7.5, Fat 0.44%, and Protein 0.72%. Additionally, NASA LOF contains organic acids (Humate 0.01%, Fulvate, etc.) and plant growth-promoting substances such as Auxin, Gibberellin, and Cytokinin.

Research by Ansoruddin et al. (2017) found that NASA LOF positively affects plant height

and leaf number in agarwood seedlings nine weeks after planting. Besides agarwood, NASA LOF has also been tested on coffee plants. Research by Riyanti (2013) showed that NASA LOF significantly affected plant height, stem diameter, and leaf number in Robusta coffee seedlings after nine weeks, with a concentration of 6 cc/L of water. Research by Muliyani (2010) found that the application of NASA LOF could meet the nutrient needs of coffee seedlings during growth, significantly increasing the height of Robusta coffee seedlings at 60 days after transplanting. Research by Satria et al. (2023) showed that LOF could increase survival rates, leaf number, seedling height, and maximum leaf width in agarwood-producing plants.

In addition to organic fertilizers, another agricultural input for agarwood cultivation on ultisol soil is the application of Plant Growth Promoting Rhizobacteria (PGPR). PGPR are soil microbes found in plant roots that can enhance plant growth and protect against certain pathogens. PGPR can produce plant hormones such as Auxin, Gibberellin, and Cytokinin, as well as solubilize phosphate and fix nitrogen. Rhizosphere microbes, including PGPR, can generate plant growth regulators (PGR), playing a crucial role in nutrient availability, mobilization, and absorption in the soil.

MATERIALS AND METHODS

Time and location of the experiment

The experiment will be conducted in the Faculty of Agriculture, Andalas University greenhouse from May to July 2024.

Equipment and materials used in the experiment

The equipment used in the experiment includes hoes, buckets, scissors, rulers, measuring glasses, digital scales, measuring

tapes, labels, watering cans, calipers, and writing tools. The materials used in the experiment are 2-month-old agarwood seedlings (*Aquilaria malaccensis* Lamk.), 30 cm x 30 cm polybags or 1.5 kg polybags, PGPR (*Pseudomonas fluorescens*, *Bacillus* sp.), NASA LOF, ultisol soil, and cow manure.

Experimental design

The experimental design used is a Completely Randomized Design (CRD) consisting of two factors, namely PGPR and NASA LOF, with three repetitions. Thus, there are 48 experimental units. The data analysis used is ANOVA with a 5% F-test, followed by Duncan's Multiple Range Test (DMRT) and The application used for data analysis is IBM Statistics 27. Each factor has four treatments as follows:

PGPR:	NASA LOF:
- 0 ml/liter (A)	- 0 ml/liter (E)
- 10 ml/liter (B)	- 20 ml/liter (F)
- 20 ml/liter (C)	- 30 ml/liter (G)
- 30 ml/liter (D)	- 40 ml/liter (H)

Implementation of the experiment

1. Soil Analysis

Soil analysis is carried out to determine the nutrient content and obtain data on soil pH, Nitrogen (N), Phosphorus (P), Potassium (K), and Al-dd in the soil. Soil samples are taken randomly from five points in the ultisol area by digging to a depth of 0-20 cm, then placing them in a plastic bag. The soil samples are then analyzed in the Soil Science Laboratory, Faculty of Agriculture, Andalas University.

2. Seedling Preparation

The agarwood seedlings used in this experiment were obtained from a gaharu

seedling provider in Lubuk Minturun, Kec. Koto Tangah, Kota Padang, West Sumatra. The description of the *Aquilaria malaccensis* Lamk. species is as follows: The seedlings used are approximately 2 months old, with a healthy appearance, a height ranging from 20-30 cm, and regular leaves.

3. Preparation of Growing Media

The growing media used in this experiment is ultisol soil from the experimental garden of the Faculty of Agriculture, Andalas University. Before placing it in polybags, the ultisol soil is mixed with cow manure in a 1:1 ratio. The mixture is then placed in polybags and incubated for one week before planting.

4. Planting

The agarwood seedlings are planted in 30 cm x 30 cm polybags filled with the prepared growing media. Each polybag contains one 2-month-old agarwood seedling. The seedlings are transplanted in the evening with care to avoid damaging or breaking the roots.

5. Application of Treatments

Plant Growth Promoting Rhizobacteria (PGPR) and NASA LOF are applied as liquid solutions. The application is done five times, at 0, 3, 6, 9, and 12 weeks after planting (WAP), by watering the area around the plant roots. PGPR and NASA LOF are diluted according to the specified concentration in 1 liter of water. The watering volume starts at 100 ml/plant and increases as the plants grow.

6. Maintenance

Watering

The agarwood seedlings are watered regularly in the morning and evening using a watering can. The purpose of watering is to maintain soil moisture in the polybags.

Replacement

Replacement is done using seedlings of the same age planted in different polybags

according to each treatment. This is done to anticipate any plant deaths.

Weeding

Weeding is done manually by hand pulling out the weeds in the polybags. Weeding is done during each watering session.

Pest and Disease Control

Pest and disease control on agarwood plants is conducted according to the level of infestation. When it poses a threat to the growth of the agarwood plants, chemical control is applied. Mechanical control involves manually removing and disposing of pests from the affected parts of the plant. Pesticides are used depending on the type of pest attacking the seedlings. Agarwood seedlings are commonly attacked by white pests, controlled using systemic insecticides.

5. Observations

Percentage of Surviving Seedlings

The percentage of surviving agarwood seedlings is observed at 12 weeks after planting (WAP). The survival rate is calculated as follows:

$$\% \text{Surviving Seedlings} = (\text{Number of surviving plants} / \text{Total number of dead plants}) \times 100\%$$

Plant Height

Observations are made at 12 weeks after planting (WAP). Plant height is measured from the base of the stem to the top of the stem using a measuring tape.

Leaf Number

Observations are made by counting the number of leaves that emerge on the agarwood plants after each treatment. This observation is made at 12 weeks after planting (WAP). The leaves observed have fully opened

and remain on the plant at the time of observation.

Percentage of Ready-to Transplant Seedlings

The percentage of ready-to-transplant agarwood seedlings refers to high-quality seedlings that meet the seed quality standards. According to the Rimbakita article, ready-to-plant and high-quality, healthy agarwood seedlings have the following characteristics: a stem diameter of about 1 cm, roots that do not penetrate the polybag, fresh leaves that are

not wilted, a height of about 20-30 cm, and a proportional number of leaves and stem.

RESULTS AND DISCUSSION

Soil Analysis

The soil analysis conducted at the Soil Science Laboratory, Faculty of Agriculture, Andalas University, is presented in Table 1. This analysis was carried out to assess the nutrient content in the soil used as a planting medium for seedlings of agarwood-producing plants.

Table 1. Soil analysis

Analysis Parameters	Units	Results	Analysis Criteria
pH H ₂ O		4,23	Acid
N-total	%	0,30	Low
P- available	Ppm	4,15	Very Low
K-dd	Me/100 g	0,35	Low
KTK	Cmol/Kg	12,94	Low
Al-dd	Me/100 g	24,05	Very High

Note: The soil content analysis was conducted by the Soil Science Laboratory, Faculty of Agriculture, Universitas Andalas.

Plant Survival Percentage

The observations indicate that the survival percentage for agarwood-producing plant seedlings shows 100% survival, with all seedlings growing well. This can be seen in Table 2.

Plant Height (cm)

The variance analysis indicates that PGPR and POC NASA significantly affect plant height at 12 months after planting (MST). The results of the comparison of the effects of planting media and POC NASA on the height growth of agarwood seedlings at 12 MST can be seen in Table 3.

Table 3 shows that the best dose for plant height is the combination of PGPR and POC at 30 ml/L and 40 ml/L, with an average height of agarwood seedlings reaching 82.34 cm. Additionally, Table 3 shows that increasing doses of PGPR and POC result in taller agarwood plants. This suggests that the combination of PGPR and POC is highly effective for promoting the growth of agarwood plant height. This effect is likely due to the ability of PGPR and POC to support the nutrient needs and availability for agarwood seedlings in the ultisol soil used.

Table 2. Survival percentage of agarwood-producing plant seedlings

Treatment	Number of Seedlings Observed (Plant)	Number of Seedlings Alive (Plant)
AE	30	30
AF	30	30
AG	30	30
AH	30	30
BE	30	30
BF	30	30
BG	30	30
BH	30	30
CE	30	30
CF	30	30
CG	30	30
CH	30	30
DE	30	30
DF	30	30
DG	30	30
DH	30	30

Note : A = PGPR 0 ml/l, B= PGPR 10 ml/l, C= PGPR 20 ml/l, D= PGPR 30 ml/l, E= NASA LOF 0 ml/l, F= NASA LOF 20ml/l, G=NASA LOF 30 ml/l, H= NASA LOF 40 ml/l

Table 3. Height of Agarwood Seedlings with Various Doses of PGPR and POC NASA at 12 Week After Planting (WAP)

PGPR (ml/L)	NASA LOF (ml/L)				Average
	0 ml/L (E)	20 ml/L (F)	30 ml/L (G)	40 ml/L (H)	
0 ml/L (A)	50,35 c c	56,47 bc c	57,75 b c	62,63 a c	56,80
10 ml/L (B)	63,70 c bc	65,23bc bc	68,55 ab bc	70,05 a b	66,88
20 ml/L (C)	65,86 c b	68,42 bc b	74,50 b ab	78,00 a ab	71,69
30 ml/L (D)	70,34 c a	73,00bc a	76,28 b a	82,34 a a	75,49
Average	62,56	65,78	69,27	73,25	

Note: Numbers followed by the same lowercase letters in rows and the same uppercase letters in columns indicate that differences are insignificant based on the DMRT test ($\alpha=5\%$).

Plant Growth Promoting Rhizobacteria (PGPR) are beneficial bacteria that live around the rhizosphere. PGPR can fix nitrogen, solubilize phosphorus, produce Indole Acetic Acid (IAA), and act as antagonists to

pathogens. According to Ashafuzzaman et al. (2009), PGPR bacteria can colonize plant roots and play a crucial role in plant growth. Examples of PGPR bacteria include Rhizobium, Azotobacter, Azospirillum, Bacillus,

Arthrobacter, Bacterium, Mycobacterium, and Pseudomonas (Situngkir et al., 2021). The association of bacteria with plants stimulates plant growth and contributes to tolerance against biotic and abiotic stresses (Koza et al., 2022).

PGPR in IAA production, as stated by Khiangngam et al. (2023), IAA produced by non-plant sources is known as exogenous IAA. Spaepen & Vanderleyden (2011) indicate that, in addition to regulating physiological functions and adapting to external stresses and microbial interactions, IAA produced by microorganisms often acts as a signaling molecule in interactions between microorganisms and plants, regulating plant growth and development, and causing physiological and pathological changes in plants.

In addition to PGPR, the use of POC NASA also provides essential nutrients for agarwood plants. The nutrient content in POC NASA includes N 0.12%, K 0.31%, P₂O₅ 0.03%, S

0.12%, Ca 60.40 ppm, Cl 0.29%, Mg 16.88 ppm, Mn 2.46 ppm, Fe 12.89 ppm, Cu < 0.03 ppm, Zn 4.71 ppm, Na 0.15%, B 60.84 ppm, Si 0.01%, Co < 0.05 ppm, Al 6.38 ppm, NaCl 0.98%, Se 0.11 ppm, As 0.11 ppm, Cr < 0.06 ppm, Mo < 0.2 ppm, V < 0.04 ppm, SO₄ 0.35%, C/N ratio 0.86%, pH 7.5, Fat 0.44%, Protein 0.72%. Additionally, NASA LOF contains organic acids (Humate 0.01%, Fulvate, etc.) and growth-promoting substances such as Auxin, Gibberellin, and Cytokinin. According to research by Ansoruddin et al. (2017), the application of POC NASA showed an effect on the increase in plant height and the number of leaves in agarwood seedlings at 9 MST.

Leaf Number

Variance analysis shows that PGPR and POC NASA significantly affect the number of leaves at 12 months after planting (MST). The results of comparing the effects of planting media and POC NASA on the number of leaves of agarwood seedlings at 12 MST can be seen in Table 4.

Table 4. Number of Leaves of Agarwood Seedlings with Various Doses of PGPR and NASA LOF at 12 Weeks After Planting (WAP)

PGPR (ml/L)	NASA LOF (ml/L)				Average
	0 ml/L (E)	20 ml/L (F)	30 ml/L (G)	40 ml/L (H)	
0 ml/L (A)	10,00 c	16,00 bc	20,00 b	25,00 a	17,75
	c	c	c	c	
10 ml/L (B)	14,00 d	18,00 c	24,00 ab	28,00 a	21,00
	bc	bc	bc	bc	
20 ml/L (C)	19,00 c	24,00 bc	28,00 b	35,00 a	26,50
	b	b	b	b	
30 ml/L (D)	24,00 c	26,00 bc	30,00 b	38,00 a	29,50
	a	a	a	a	
Average	16,75	21,00	25,50	31,50	

Table 4 shows that the best treatment is the combination of PGPR at 30 ml/L and POC NASA at 40 ml/L, resulting in an average of 38 leaves per agarwood seedling. Additionally, the number of leaves shows a positive correlation with plant height: as the dose of treatment increases, both the plant height and the

number of leaves increase. This is because taller agarwood seedlings tend to develop more branches, resulting in more leaves.

Moreover, it is essential to ensure adequate nutrient availability for agarwood plants to grow and develop well. NASA liquid organic fertilizer application aims to supply

nutrients from outside the soil environment. In contrast, PGPR application is intended to improve soil pH and reduce soil Al-dd levels.

Percentage of Ready-to-Transplant Seedlings

Observations of agarwood seedlings ready for transplanting are conducted to determine their readiness for the next growth phase. This is marked by achieving specific stem height and diameter and optimal root development. Additionally, the physiological condition of the seedlings, such as the number of leaves and

resistance to diseases, is also a critical indicator for their readiness to be moved to the field. These observations aim to ensure that the seedlings have the maximum potential to survive and thrive in a new environment. The criteria observed include a stem diameter of about 1 cm, roots that do not penetrate the polybag, fresh leaves that are not wilted, a height of about 20-30 cm, and a proportional number of leaves to stem height. The number of ready-to-transplant seedlings can be seen in Table 6.

Table 6. Percentage of Ready-to-Transplant Agarwood Seedlings

Treatment	Number of Seedlings Observed (Plant)	Number of Seedlings Ready for Transplanting (Plant)
AE	30	30
AF	30	30
AG	30	30
AH	30	30
BE	30	30
BF	30	30
BG	30	30
BH	30	30
CE	30	30
CF	30	30
CG	30	30
CH	30	30
DE	30	30
DF	30	30
DG	30	30
DH	30	30

Note : A = PGPR 0 ml/l, B= PGPR 10 ml/l, C= PGPR 20 ml/l, D= PGPR 30 ml/l, E= NASA LOF 0 ml/l, F= NASA LOF 20ml/l, G= NASA LOF 30 ml/l, H= NASA LOF 40 ml/l

CONCLUSION

The experiment in this study showed that the application of PGPR and NASA LOF could enhance the growth and development of agarwood seedlings, highlighting its potential to improve agarwood cultivation on less productive ultisol soils. The combination of 30 ml/L PGPR and 40 ml/L NASA LOF was the best dosage for achieving the highest average seedling height (82.34 cm), the greatest number of leaves (38 leaves per seedling), and ensuring that all seedlings survived and met quality standards for transplanting.

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