

## Effect of Mycorrhizae Biofertilizer to Increase the Growth, Production, and Quality of Patchouli Oil (*Pogostemon Cablin* Benth.) on Aceh's Entisols Soil

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**Abstract:** Patchouli (*Pogostemon cablin* Benth.) is a bushy perennial herb that produces a type of essential oil called patchouli oil. The production and quality of patchouli oil is determined by many factors, such as plant cultivation, nutrition, and pest-disease management. In addition, the soil condition of the planting medium determines the oil production and quality of the patchouli. Especially in lowlands, other obstacles to patchouli production include poor soil fertility and biological, chemical, and physical properties of Entisols with low to moderate soil nutrients, and plant growth will not be supported if cultivation is not managed properly. The use of mycorrhizae as biological fertilizer is expected to increase the production and quality of Aceh's organic patchouli. This research aims to determine the effect of mycorrhizae biofertilizer on the growth, production, and quality of Aceh's patchouli cultivated in Entisol soil. This study used a nonfactorial randomized block design with three replications. The factors observed were the application of mycorrhizae biofertilizer, namely *Glomus mosseae*, *Gigaspora cf. gigantea*, and a mixture of *Glomus mosseae* + *Gigaspora cf. gigantea*. The results showed that the mixture of *Glomus mosseae* + *Gigaspora cf. gigantea* provided the best results for growth parameters of plant height (144.65 cm), stem diameter (10.8 cm), number of leaves (337.88), and mycorrhizal root colonization (78.93%) and production parameters of fresh biomass weight (576.90 g) and dry biomass weight (122.74 g). Under normal conditions, Entisol soil has a phosphorus (P) availability of 35.00 mg kgs<sup>-1</sup>, based on Oslen's extractable test, and the application of a mixture of *Glomus mosseae* + *Gigaspora cf. gigantea* increased the P-available uptake to a range of 45.71–47.32 mg kgs<sup>-1</sup> and the oil quality to an oil extraction rate (OER) of patchouli of 2.65 and patchouli alcohol rate of 32.21%. Mycorrhizae biofertilizer in the rhizosphere can promote plant growth, improve nutrient acquisition, and improve the quality of final oil content of patchouli.

**Keywords:** mycorrhizae biofertilizer, Entisols, patchouli, patchouli alcohol, P-available uptake.

## 菌根生物肥料提高广藿香油 (蝮蛇卡布林本斯.) 对亚齐内质土壤生长、产量和质量的影响

**摘要:** 广藿香 (薯草本斯.) 是一种浓密的多年生草本植物, 可产生一种称为广藿香油的

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精油。广藿香油的产量和品质受植物栽培、营养、病虫害管理等诸多因素的影响。此外，种植介质的土壤条件决定了广藿香的产油量和品质。特别是在低地，广藿香生产的其他障碍包括土壤肥力差，土壤养分含量低至中等的内溶胶的生物、化学和物理特性，如果种植管理不当，植物生长将得不到支持。使用菌根作为生物肥料有望提高亚齐有机广藿香的产量和质量。本研究旨在确定菌根生物肥料对在恩蒂索土壤中种植的亚齐广藿香生长、产量和品质的影响。本研究使用具有三个重复的非因子随机区组设计。观察到的因素是菌根生物肥料的应用，即青蒿、巨孢子虫参考巨茶以及青蒿+巨孢子虫。巨茶的混合物。结果表明，青蒿+巨孢子虫。巨茶的混合物在株高 ( 144.65 厘米 )、茎粗 ( 10.8 厘米 )、叶片数 ( 337.88 ) 和菌根定殖 ( 78.93% ) 的生长参数上提供了最好的结果。生物重量 ( 576.90 g ) 和干生物重量 ( 122.74 g ) 的生产参数。在正常条件下，恩蒂索土壤的磷(磷)有效性为 35.00 毫克公斤<sup>-1</sup>，基于奥斯伦的可提取测试，以及应用青蒿+巨孢子虫的混合物参看。巨茶将磷的有效吸收提高到 45.71-47.32 毫克公斤<sup>-1</sup> 的范围，并将油质量提高到广藿香的油提取率(开放式反应堆)为 2.65 和广藿香醇率为 32.21%。根际菌根生物肥可促进植物生长，改善养分获取，提高广藿香最终含油量的品质。

**关键词:** 菌根生物肥料、内溶胶、广藿香、广藿香醇、磷-有效吸收。

## 1. Introduction

Patchouli (*Pogostemon cablin* Benth.) is known as one of the leading plantation commodities driving a community's economy, is widely cultivated by the community [1], and has bright prospects in Aceh, including Aceh Besar, Aceh Jaya, North Aceh, South Aceh, West Aceh, and all other corners of Aceh. Currently, about 90% of the world's patchouli oil produced by Indonesia comes from Aceh. In addition to being used for perfume making, Aceh patchouli is also good for aromatherapy, anti-aging, smoothing the skin, preventing hair loss, and treating diabetic wounds and is used as an anti-bacterial, anti-inflammatory, and disinfectant [2]. Genetic and environmental factors strongly influence the productivity and quality of patchouli. Organic patchouli is indispensable for the medicine, perfume, cosmetic, and biopharmaceutical industries because it is safer and healthier. In addition, the quality of patchouli oil produced from organic cultivation is very high [3].

Especially in Aceh, the community cultivates patchouli generally in Entisol, Inceptisol, Ultisol, and Andisol soils. From the lowlands to highlands, such as in Aceh Besar, South Aceh, West Aceh, and North Aceh, patchouli production is carried out on Entisols and Ultisols. Entisols are commonly found at the site of recently deposited materials (e.g., alluvium) or in parent materials resistant to weathering (e.g., sand). Entisols have a base saturation varying from acidic, neutral to alkaline, a cation exchange capacity < 20, and an N content compared to fine-textured soils, and

the productivity potential of Entisols varies widely from the very productive alluvial soils found on floodplains to the low fertility/productivity of mineral nutrients and organic matter found on steep slopes or in sandy areas [4-5]. Under these conditions, it is necessary to make efforts to improve the physical, chemical, and biological properties of Entisols to support optimal plant growth. Improvement efforts can be made by adding the ameliorants, organic material, and symbionts of mutual microorganisms, such as rhizobacteria and mycorrhizae [6].

Mycorrhizae are widely found in various soils unexcepted for Entisols. Mycorrhizae colonize the roots of about 80% of vascular plants and thus form arbuscular mycorrhizae in host roots of food crops, horticulture, and plantations [7]. Mycorrhizae can promote plant growth (e.g., plant height, leaf growth, biomass), increase mineral element absorption (nutritional acquisition, e.g., N, P, and K elements), and improve plant stress tolerance (e.g., salt stress and drought stress) [8-9]. Mycorrhizae can enhance and absorb more water and nutrients for the host through intraradical and extraradical hyphae [10] and can also secrete glomalin protein into the soil that is related to cement soil aggregates and improve soil moisture-holding capacity and permeability [11]. In addition, mycorrhizae can mitigate the toxicity of heavy metals by competitive uptake of heavy metal ions from the soil [12] and improve the soil environment [13]. Mycorrhizae also plays a role in increasing the number and activity of beneficial soil organisms, such as nitrogen decomposers and phosphate solvents, which

are important for patchouli growth [14-15]. Mycorrhizae could act as a biofertilizer, bioprotector, bioremediator, and bioregulator, which makes them biological agents.

Based on the description above, research is necessary on the effect of mycorrhizae biofertilizer in increasing the growth, production, and quality of organic patchouli oil cultivated in Aceh's Entisols.

## 2. Materials and Methods

### 2.1. Materials and Tools

The materials used in this study were patchouli seedlings of the Lhokseumawe variety; mycorrhizae biofertilizer (*Glomus mosseae*, *Gigaspora cf. gigantea* and a mixture of *Glomus mosseae* + *Gigaspora cf. gigantea*); N, P, and K fertilizer (16:16:16); manure; pesticides; aquadest; KOH solution; and trypan blue solution. The tools used in this study were a hoe, polybag (volume of 25 liters), gas chromatograph mass spectrometer (GC-MS), patchouli distilled flask, grinder, label, analytical scale, watering can, nine-mesh sieve, hand sprayer, filter, object glass, caliper, cover glass, autoclave, ruler, and camera.

### 2.2. Research Design

The experimental design used in this study was a nonfactorial randomized block design with three replications. The factors studied were the mycorrhizae biofertilizer, including *Glomus mosseae*, *Gigaspora cf. gigantea* and the mixture of *Glomus mosseae* with *Gigaspora cf. gigantea*. If the results of the analysis of variance (ANOVA) had a significant effect, then a further test was performed using the honestly significant difference (HSD) at a 0.05 level. The results were evaluated by descriptive and dispersion statistics. The values presented the mean  $\pm$  standard deviation. Parameters observed were plant height, number of leaves, stem diameter, fresh and dry biomass weight, initial and final P-available in the soil, root colonization, patchouli oil extraction rate (OER), and patchouli alcohol content.

### 2.3. Research Implementation

#### 2.3.1. Soil Preparation

The soil used in this research was Entisols soil from Aceh Besar. The soil sample used was topsoil that had been air-dried, then the soil was sieved through a 9-mesh sieve and put into a polybag.

#### 2.3.2. Plant Cultivation and Harvest

The patchouli used in the research was obtained from cuttings with the same performance and growth criteria (e.g., the plant height and number of leaves must be the same). Patchouli was planted in polybags and given 10 grams of mycorrhizae in the planting hole. The basic fertilizer used is NPK (16:16:16), given at

the age of 1 month after planting with a recommended dose of 25 percent (150 kgs ha<sup>-1</sup>) for each plant. Patchouli was placed in a screening house until they were harvested for 90 days with slightly yellowish leaves.

#### 2.3.3. Analysis of Mycorrhizal Colonization of Roots

Analysis of mycorrhizal colonization was conducted at Plant Physiology Laboratory, Syiah Kuala University, Banda Aceh. Samples of the plant's root (100 mg wet mass, length 30 cm of total root length) were used to estimate mycorrhizae colonization. Roots were stained with trypan blue in lactic acid using a modification of the procedure outlined by Kormanik and McGraw [16]. Roots were cleared in 5% KOH for about a minute, stained at room temperature for 2 h, then destained overnight (or longer is fine as well). Root samples were cut into 1 cm long and mounted on microscope slides, and colonization was determined using the slide mount method of McGonigle et al. [17]. If the roots are dark blue, they did not clear long enough in KOH, and further optimization is necessary.

#### 2.3.4. Analysis of Patchouli Oil Extraction Rate

Analysis of patchouli oil extraction rate was conducted at Atsiri Research Center, Syiah Kuala University, Banda Aceh. Patchouli plants had been air-dried for a week to constant weight, then chopped by using wooden scissors to a size of 2 cm. After that, the refining and distillation were carried out using a distillation flask to obtain patchouli oil through evaporation. Distillation was carried out using the steam distillation technique to produce the oil.

Evaporation was carried out through a heating process using an electric stove with a temperature of 95°C. The principle of steam distillation is like the principle in the steaming process. The flask at the bottom, which was directly connected to the stove, contains reverse osmosis (RO) water. Next, the pump at the top that was connected to the bottom pump contains the chopped patchouli [18].

Evaporation will produce water mixed with patchouli oil accommodated in the storage media after going through the cooling in the condenser. Then, the oil was separated from the water through a filtering process using a monel cloth. The filtered oil was accommodated in a 10 ml glass. As a result, patchouli oil was obtained [19]. Then, the percentage of OER was calculated using the following formula:

$$\text{OER (\%)} = \frac{W_1}{W_0} \times 100\% \quad (1)$$

*Theorem 1. OER percentage formula:* OER is the oil extraction rate of patchouli,  $W_1$  is the weight of patchouli oil yield, and  $W_0$  is the weight of patchouli raw material.

#### 2.3.5. Analysis of Patchouli Alcohol

Patchouli's alcohol content was analyzed at the

Laboratory of the Goods Quality Standards Testing Center, Banda Aceh. Patchouli alcohol levels were analyzed using GC-MS. The carrier gas flow rate was set at 3 ml per minute for the packed column and split 100 ml per minute in the capillary column at a pressure of 2 bar or at a rate that provided optimum resolution. In the programmed system, the oven temperature was set to an initial temperature of 100°C and a final temperature of 220°C at 5°C per minute. The detector temperature was set at 250°C. Furthermore, the hydrogen gas flow rate was set at 30 ml per minute or at a flow rate that provides optimum resolution. The injector temperature was set at 200°C. Paper speed is 0.5 cm per minute or according to the state of the tool. The attenuation was set at 128 or according to the recorder's capabilities when set to the minimum area.

The sample was injected with 0.1 microliters for the packaging column and 0.5 microliters for the capillary column. After use, the syringe was washed with acetone and then dried [20].

### 3. Results

#### 3.1. The Character of Aceh's Entisols

The initial soil analysis of the research location can be seen in Table 1. From the results, it could be concluded that the soil fertility status of Aceh's Entisols soil is classified as low. Based on the analysis results, Aceh's Entisols soil has a sandy loam/dust texture and high P content. In detail, the characteristics of Aceh's Entisols soil are listed in Table 1.

Table 1 Results of analysis of soil properties before treatment (initial) (Results of soil analysis in the USK Soil and Plant Research Laboratory, 2021)

No.	Soil Parameters	Value	Unit	Criteria
1.	Soil Texture	56	%	
	Sand, filtering	29	%	
	Silt (Pipette)	15	%	
	Clay (Pipette)			
2.	Texture Class of Soil	I		
3.	Soil reaction			
	pH (H <sub>2</sub> O) (1:25)-Electrometric	8.11		Slightly alkaline
	pH (KCl) (1:25)-Electrometric	7.20		Neutral
4.	C-Organic (Wakley & Black)	0.50	%	Very low
5.	N-total (total N, Kjeldahl)	0.05	%	Very low
6.	(P and K total) :			
	25% HCl-extractable P <sub>2</sub> O <sub>5</sub>	-	%	
	25% HCl-extractable K <sub>2</sub> O	-	%	
7.	P available			
	P extracted Bray II	-	mg kg <sup>-1</sup>	
	P Oslen (Oslen-extractable P)	35.00	mg kg <sup>-1</sup>	High
8.	Cation Exchange (1N NH <sub>4</sub> COOCH <sub>3</sub> pH 7)			
	Ca-exchange (exch, Ca)	12.30	Cmol.Kg <sup>-1</sup>	High
	Mg-exchange (exch, Mg)	0.53	Cmol.Kg <sup>-1</sup>	Low
	K-exchange (exch, K)	0.43	Cmol.Kg <sup>-1</sup>	Medium
	Na-exchange (exch, Na)	0.11	Cmol.Kg <sup>-1</sup>	Low
9.	Cation exchange capacity	15.20	Cmol.Kg <sup>-1</sup>	Low
10.	Degree of alkali saturation	87.96	%	Very high
11.	Potential acidity (1 M KCl) :			
	Al-exchange	tu	Cmol.Kg <sup>-1</sup>	
	H-exchange	0.24	Cmol.Kg <sup>-1</sup>	
12.	Electrical conductivity (EC)	0.18	mS cm <sup>-1</sup>	Very low

Therefore, P absorption is expected to be optimal with the use of mycorrhizal biofertilizers in addition to other nutrients. In addition, Entisol soils hold very little water. Water-holding capacity is expected to be higher through the provision of mycorrhizae through absorption by roots. Besides, the type of mycorrhizal biofertilizer given greatly determines the effectiveness of P uptake by mycorrhizae [21-22].

#### 3.2. Effect of Mycorrhizal Biofertilizers on the Growth, Production, and Quality of Patchouli Oil on Aceh's Entisols

Table 2 shows the average values of the growth parameters and production of patchouli oil with mycorrhizal biofertilizer treatment after being tested with HSD (0.05).

Table 2 Average growth and production of patchouli with mycorrhizae biofertilizer treatment

Parameters		Mycorrhizae biofertilizer				HSD <sub>0.05</sub>
		M <sub>0</sub>	M <sub>1</sub>	M <sub>2</sub>	M <sub>3</sub>	
Plant Height (cm)	30 DAP	30.98 ± 1.49 <sup>a</sup>	33.34 ± 5.49 <sup>a</sup>	37.90 ± 2.84 <sup>b</sup>	42.90 ± 6.13 <sup>c</sup>	3.27
	60 DAP	67.55 ± 7.98 <sup>a</sup>	69.85 ± 5.32 <sup>a</sup>	74.80 ± 9.91 <sup>ab</sup>	82.12 ± 6.42 <sup>b</sup>	7.55
	90 DAP	95.90 ± 9.53 <sup>a</sup>	98.03 ± 12.65 <sup>a</sup>	94.58 ± 9.92 <sup>a</sup>	108.32 ± 4.74 <sup>b</sup>	9.69
	120 DAP	109.96 ± 8.10 <sup>a</sup>	131.41 ± 13.44 <sup>c</sup>	123.61 ± 13.77 <sup>b</sup>	144.65 ± 10.68 <sup>d</sup>	9.20
Stem Diameter (mm)	30 DAP	0.62 ± 0.02 <sup>a</sup>	0.67 ± 0.03 <sup>ab</sup>	0.71 ± 0.03 <sup>b</sup>	0.78 ± 0.02 <sup>c</sup>	0.05

Continuation of Table 2						
	60 DAP	0.82 ± 0.03 <sup>a</sup>	0.84 ± 0.03 <sup>a</sup>	0.88 ± 0.02 <sup>a</sup>	0.94 ± 0.04 <sup>b</sup>	0.04
	90 DAP	0.93 ± 0.00	0.95 ± 0.02	0.97 ± 0.00	0.97 ± 0.00	-
	120 DAP	1.03 ± 0.04	1.08 ± 0.04	1.06 ± 0.03	10.8 ± 0.02	-
Number of Leaves	30 DAP	29.30 ± 4.22 <sup>a</sup>	27.86 ± 8.34 <sup>a</sup>	48.43 ± 8.80 <sup>b</sup>	58.22 ± 7.20 <sup>c</sup>	8.29
	60 DAP	136.17 ± 12.21 <sup>a</sup>	141.70 ± 25.19 <sup>a</sup>	182.23 ± 38.11 <sup>b</sup>	197.93 ± 26.19 <sup>b</sup>	18.35
	90 DAP	256.60 ± 25.63 <sup>a</sup>	278.42 ± 37.70 <sup>ab</sup>	302.62 ± 26.68 <sup>b</sup>	304.31 ± 11.52 <sup>b</sup>	34.46
	120 DAP	307.02 ± 18.25	312.76 ± 26.30	316.77 ± 29.07	337.88 ± 32.94	-
Fresh Biomass Weight (g)		327.34 ± 109.18 <sup>a</sup>	469.14 ± 114.20 <sup>a</sup>	571.05 ± 122.48 <sup>b</sup>	576.90 ± 41.02 <sup>b</sup>	73.55
Dry Biomass Weight (g)		74.32 ± 21.37 <sup>a</sup>	105.19 ± 26.83 <sup>b</sup>	108.12 ± 14.39 <sup>b</sup>	122.74 ± 22.80 <sup>b</sup>	24.96
OER of Patchouli (%)		2.22 ± 0.26 <sup>a</sup>	2.36 ± 0.14 <sup>ab</sup>	2.44 ± 0.18 <sup>b</sup>	2.65 ± 0.07 <sup>c</sup>	0.19
Root Colonization (%)		1.28 ± 0.00 <sup>a</sup>	76.72 ± 5.11 <sup>b</sup>	73.45 ± 10.37 <sup>b</sup>	78.93 ± 8.48 <sup>b</sup>	12.28
Initial of P-available soil		39.86 ± 0.75 <sup>a</sup>	41.69 ± 1.72 <sup>a</sup>	41.70 ± 2.52 <sup>a</sup>	45.71 ± 2.05 <sup>b</sup>	1.88
Final P-available soil		42.82 ± 0.68 <sup>a</sup>	44.03 ± 1.60 <sup>a</sup>	43.08 ± 2.33 <sup>a</sup>	47.32 ± 2.63 <sup>b</sup>	2.33

**Notes:**

- Values are expressed in average ± standard deviation (number of replications = 3). Numbers followed by the same letter are not significantly different at the 0.05 level tested by HSD;

- M - mycorrhizae biofertilizer; M<sub>0</sub> - control (without the mycorrhizae); M<sub>1</sub> - *Glomus mosseae*; M<sub>2</sub> - *Gigaspora cf. gigantea*; M<sub>3</sub> - the mixture of *Glomus mosseae* and *Gigaspora cf. gigantea*; DAP - days after plating.

Table 2 shows that the highest plant heights at 30, 90, and 120 days after planting (DAP) were found in the treatment with mixed mycorrhizae and were significantly different from those in the other treatments. The plant height at 60 DAP is higher in the treatment with mixed mycorrhizae and was significantly different from that in the control treatment and *Glomus mosseae* but not significantly different from that in the treatment with the mycorrhizal biofertilizer *Gigaspora cf. gigantea*. Furthermore, the largest stem diameters at 30 and 60 DAP were also found in the mixed mycorrhizal biofertilizer treatment and were significantly different from those in the other treatments. The highest number of leaves was found in the treatment with mixed mycorrhizal biofertilizer and was significantly different from the number of leaves in the other treatments. Likewise, for the parameters of fresh and dry plant biomass, higher weights were also found in the treatment with mixed mycorrhizal biofertilizer and were significantly different from the weights in the other treatments. Furthermore, the highest extraction rate of patchouli oil, both the initial and the final P-available oil, was also found in the mixed mycorrhizal treatment and was significantly different from the rates in the other treatments. In addition, the standard extraction rate of patchouli oil ranges from 2.00% to 4.14% according to the Research Institute for Spices and Medicinal Plants.

### 3.3. Patchouli Alcohol (%)

The results of patchouli oil refining were then sent to the Laboratory of the Testing and Quality Certification Center for Goods of the Aceh Industry and Trade Department. The results of the analysis of patchouli alcohol levels could be determined by using the values of the parameter test results and then compared with the specification of patchouli alcohol levels issued by the Indonesian Standardization Agency known as SNI 06-3953-1995 at least 31% and ISO 3757: 2002 standard at least 27-35%. Patchouli's alcohol levels can be seen in Fig. 1.

Based on the analysis of patchouli alcohol from the

patchouli oil, the highest patchouli alcohol value was in the mixed mycorrhizae biofertilizer of M<sub>3</sub> (*Glomus mosseae* and *Gigaspora cf. gigantea*), which was 32.21%. In the control treatment, the patchouli alcohol value obtained from GC-MS analysis is below the SNI 06-3953-1995 and ISO 3757: 2002 standard. This is presumably because the Entisols soil cannot provide sufficient mineral nutrients and water supply to support patchouli's growth, production, and quality. While based on the description of the Lhokseumawe patchouli variety released by the Research Institute for Spices and Medicinal Plants, the Lhokseumawe patchouli variety has patchouli alcohol levels ranging from 29.11 to 34.46%. Thus, the patchouli alcohol content of 32.21% reached the standard set by SNI 06-3953-1995 and ISO 3757: 2002 standard.

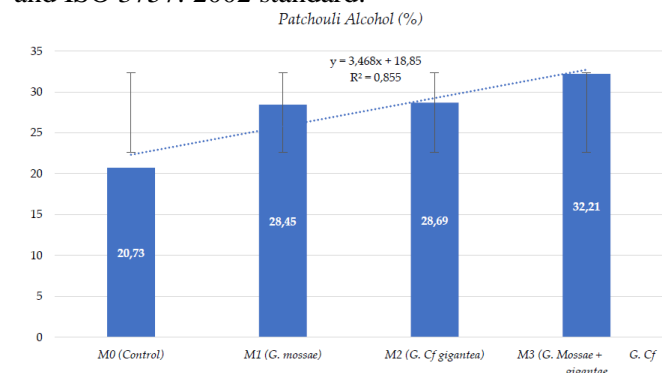


Fig. 1 Patchouli alcohol (%) level analyzed using GC-MS

## 4. Discussion

### 4.1. Effect of Mycorrhizae Biofertilizer on the Growth, Production, and Quality of Patchouli Oil on Aceh's Entisols

Mycorrhizae play an important role in the growth of a plant. Mycorrhizae inoculation in patchouli affects its growth parameters, depending on the mycorrhizae biofertilizer. The mixed mycorrhizae (*Glomus mosseae* + *Gigaspora cf. gigantea*) play an important role in the growth of patchouli with a significant effect on the growth parameters and yield of patchouli. It is

suspected that mixed mycorrhizae biofertilizer is very responsive to the soil conditions. According to Syafruddin et al. [23], *Glomus mossae* could be used on soils with neutral pH, while *Gigaspora cf. gigantea* is suitable for slightly acidic soils. Soil pH value significantly affects the diversity of mycorrhizae and their colonization in patchouli roots. Because mycorrhizae form many spores and hyphae that help provide mineral nutrients for plants (e.g. phosphate) [24], thus the physico-chemical properties of Entisol soils strongly affect the growth and production of patchouli. The increase in soil and plant productivity is determined by the application of the correct mycorrhizae biofertilizer, one of which is mixed mycorrhizae (*Glomus mosseae* and *Gigaspora cf. gigantea*).

In addition, mycorrhizae inoculation can also promote and increase the chlorophyll content, photosynthetic rate, status of gas exchange, and ability of the plant to increase the growth and development of chilli plants and patchouli [25]. Comparison of P-availability in initial and final tests in the Entisol soils (Table 2) showed that mixed mycorrhizae biofertilizer could increase the availability of P, which was significantly different from the control without mycorrhizae treatment. Wang et al. [26] stated that *Glomus mossae* increased P availability, which could be absorbed by plant roots. This was reflected in the mycorrhizae colonization parameters where mixed mycorrhizae biofertilizer had a higher percentage of colonization compared to mycorrhizae inoculation on soils that have low soil fertility, such as Entisols. Mycorrhizae application can increase the percentage of root colonization, plant biomass, number of spores and colonization root. Another study found that the application of mycorrhizae in some plants increased the absorption of P nutrients, and external hyphae in mycorrhizae could maximize water absorption [27].

However, there was no significant effect on the two growth parameters: namely stem diameter at 90 and 120 DAP. It is suspected that the development of mycorrhizae on plant stem growth is generally not very visible because patchouli is a non-woody plant so there is no significant difference in the growth of stem diameter. Several studies from Syafruddin [5] proved that the application of mycorrhizae biofertilizer could increase the growth and yield of patchouli with the use of a mixed mycorrhizae biofertilizer, such as *Glomus mosseae* and *Gigaspora cf. gigantea*, and patchouli yields could increase up to 50 percent. Further research by Syafruddin [14] also concluded that the yield of patchouli – particularly in the Lhoksemawe and Tapak Tuan varieties – could be increased by using various types of mycorrhizae biofertilizer in both Entisols and Ultisols.

The correct mycorrhizae biofertilizer will most significantly determine the growth and production of patchouli. Several mycorrhizae fungi, such as *Glomus*

*etunicatum*, chosen for their symbiotic response, were identified as the best mycorrhizae symbionts to increase growth and P nutrient uptake for patchouli and improve tolerance to drought [28]. This is due to the ability of mycorrhizae roots to absorb nutrients and protect plants from pathogen attack, drought and other extreme conditions [29], and due to the help they provide with the absorption of nutrients [30-31]. The use of mycorrhizae biofertilizer also influences the production of auxin and gibberellin [25] and can help remediate polluted land [26]. Among the mycorrhizae biofertilizers most commonly used are *Glomus mosseae*, *Gigaspora cf. gigantea*, and *Acaulospora manihotis* [24].

#### 4.2. Patchouli Alcohol Content

Patchouli alcohol is the most important natural compound, which is an indicator of the quality of patchouli oil. Based on the analysis, the highest patchouli alcohol value was found in the mixture of *Glomus mosseae* and *Gigaspora cf. gigantea*, which was 32.21%. This highest value still conformed to the minimum patchouli alcohol standard value, 30%, if the appropriate climatic conditions at the time of the study caused the patchouli alcohol content to be classified as high. The results obtained on the treatment of mixed mycorrhizae biofertilizer conformed to SNI and yield criteria for Aceh's patchouli superior varieties. Setiawan and Rosman [32] concluded that patchouli oil's low patchouli alcohol value could be caused by land use, climate, genetics, and microorganisms.

In addition, other allegations of harvest and post-harvest handling could also be the cause for the decrease in the patchouli alcohol levels of patchouli oil. In this case, they are related to time, temperature, and the interaction between these factors during the vacuum redistillation process of patchouli oil. This is in line with the results of research by Aisyah [33] that stated time, temperature, and the interaction between them could be affected in the process of vacuum redistillation of patchouli oil, which could affect the oil's quality.

## 5. Conclusions

Mycorrhizae biofertilizer can colonize the root system and establish a symbiosis, such as promoting plant growth and development, and accelerating nutrient uptake; and improve the quality of patchouli's final oil content (Fig. 1). Mycorrhizae biofertilizer promotes the absorption of nutrients (especially P) from the Entisol soil. It has been researched by Soil and Plant Research Laboratory, Universitas Syiah Kuala, Aceh, that the potential of P uptake in Entisol soil before being given mycorrhizae was in a range of 35.00 mg kgs<sup>-1</sup> (Olsen extraction test) and after mycorrhizae application increased in the range of 45.71–47.32 mg kgs<sup>-1</sup>.

In addition, mycorrhizae also promote plant growth

and development. Based on the researched treatment, the best mycorrhizae biofertilizer is found to be a mixture. The mixed species of *Glomus mosseae* + *Gigaspora cf. gigantea* gave the best results for growth parameters on plant height (144.65 cm), stem diameter (10.8 cm), number of leaves (337.88), mycorrhizal root colonization (78.93%); production parameters on fresh biomass weight (576.90 g) and dry biomass weight (122.74 g); and initial (45.71 mg kgs<sup>-1</sup>) and final (47.32 mg kgs<sup>-1</sup>) P-available uptake.

Earlier studies on mycorrhizae biofertilizer and patchouli plants focused on plant growth (including plant biomass) and soil nutrients. Few studies have addressed the effects of mycorrhizae biofertilizer on the functional constituents of patchouli and the oil yield. The oil yield parameters in this research are the oil extraction rate (OER) of patchouli (2.65%) and the quality of patchouli alcohol (32.21%).

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