

## The effect of organic fertilizers on the leaf morphology and stomatal density of *Pogostemon cablin* Benth

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**Abstract.** *Pogostemon cablin* Benth is one of the most important crops grown in Indonesia, especially in Aceh province. It is well known as Patchouli belongs to *Lamiaceae* family and shows a great demand for perfume, luxury products, food and beverage industry. This study conducted to determine the effect of organic fertilizers application on the leaf morphology and stomatal density on the *Pogostemon cablin* Benth. The seedlings were transplanted for three months on the soil mixed either with manure or compost. The results obtained that was no significant different in affecting the leaf morphology of *Pogostemon cablin* Benth. The treatment with manure application showed the highest leaf length (15.23 cm) and the highest leaf width (10.5 cm), the leaf color is green. While the stomatal density obtained the significant difference among the treatments, the highest stomatal density in both side of the leaf surface showed in the application of manure, 663.5 mm<sup>-2</sup> for adaxial part and 486.38 mm<sup>-2</sup> for abaxial part. Stomatal type is anisocytic and on the leaf surface was found glandular and non-glandular trichomes.

**Keywords:** Stomatal density, organic fertilizer, leaf morphology, *Pogostemon cablin* Benth.

### INTRODUCTION

*Pogostemon cablin* Benth or well known as Patchouli belongs to the *Lamiaceae* family and native to south Asia. This plant shows a great demanded on flavor, perfumery, cosmetic, luxury products, food industry and beverage, and pharmaceutical [1]. The plants that belonging to *Lamiaceae* family mostly contains a lot of essential oils and widely used for food and pharmaceutical industries such as; Basil (*Ocimum basilicum*), mint leaves (*Mentha piperitha*) and Patchouli (*Pogostemon cablin* Benth) [2]. *Pogostemon* consists of more than 80 species spread over South Asia, Southeast Asia, China, Japan and one species found in Australia [3], Indonesia is one of the biggest exporters of patchouli oil especially in Aceh Province. There are three species cultivated in Indonesia; *Pogostemon cablin*, *Pogostemon*

*hortensis* and *Pogostemon heyneatus*, while *Pogostemon cablin* is the most cultivated [2].

Indonesia as the major producer and exporter of patchouli oil in the world market contributes about 90% of the oil supply. This plant grows well at altitudes up to 1.200 m above the sea level with 70-90% of humidity, rainfall 1500-3000 mm/year, and the temperature around 24-28°C [4]. Some amount of nutrients needed and must be given at the right time as one way to let it grow fertile and high production of leaves. With an appropriate dose of fertilizer could stimulate the vegetative growth and improve the assimilation process and the oil production [5].

The production of patchouli oil in Aceh province, Indonesia is declining during these few years due to some problems. The quality of patchouli produced by the farmers is still low and under the ISO standard, the cultivation and the oil refinery are still in traditional way [19]. One of important problem faced by the farmers during the *Pogostemon cablin* Benth's cultivation is the reduction in soil fertility level which will affect the plant growth and yield

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[20]. Land as growth support for plant provided nutrients, water, air and soil organism in an optimum condition [21]. Providing organic matter and fertilizer could optimize the soil function, loose the good soil, high porosity, aerated, able to hold and provide water for plant growth, and consist a lot of nutrients for plant [20].

Plants need some nutrition for growth such as nitrogen, phosphorus and sulfur which can be absorbed from soil. The application of chemical fertilizer will be affected on the environmental pollution, soil structure and nutrition quality of plant [7]. This kind of chemical proved increases the soil compaction and degradation, the size of soil pore spaces among the soil particles reduce, reducing the soil volume as well as the total porosity and then will increase the soil bulk density and resistance [8]. While the application of organic fertilizer proved that it can improved the soil fertility, provides the nutrition form long term in plants, increases the soil physical and chemical properties, the water storage in the soil, and the net photosynthetic rate and plants quality [7,9,10]. This condition will also improve the growth of *Pogostemon cablin* Benth, this study conducted to determine the effect of organic fertilizer (compost and manure) and its impact to the leaf morphology and stomatal density of *Pogostemon cablin* Benth. The information regarding to the purposes of this study is still limited.

## METHODOLOGY

### Plant materials and organic fertilizers

The study was conducted from September to December 2020 in Meunasah papeun village, Aceh Besar, Aceh Province, Indonesia. Two months old *Pogostemon cablin* Benth's seedling from 'lhokseumawe' variety were collected from Atsiri Research Center (ARC) Universitas Syiah Kuala, Banda Aceh, Indonesia. The organic fertilizers used were compost that was collected from Waste Bank Syiah Kuala University, and manure was collected from Samahami, Aceh Besar, Aceh Province, Indonesia.

### Field experiments

The field experiments were conducted for three months. The seedlings were transplanted to the soil which was mixed with organic fertilizer either compost or manure for five replications.

A total of 10 kg of mixed soil and fertilizer were placed in 18 x 18 cm polybag (700 g top soil + 300 g either compost or manure).

### Leaf morphology measurement

The third leaf of shoot was selected for the leaf morphology measurement and stomatal density calculation. The leaf length and leaf width were measured using the Vernier caliper, while the leaf color was analyzed visually.

### Stomatal density calculation

Stomatal density was calculated after the total number of stomata with three views as the repetition counted with the following formulas:

$$\text{Stomatal density} = \frac{\text{Number of stomata}}{\text{Field of view under microscope}}$$

The number of stomata was calculated under a microscope with 10x magnifications, while the field of view calculated with the same formula with circle area formulas. Field of view under microscope =  $\pi \times r^2$ . Value of  $r = 0.25 \text{ mm}^2$  for 10x magnifications [6].

### Statistical analysis

The experiments were laid out in a single factor of completely randomized block design with five replications. The results evaluated after three months of cultivation. The parameters recorded were leaf length, leaf width, leaf color, stomatal density on adaxial and abaxial part. The data were subjected to analysis of variance (ANOVA) and significant differences among the treatments were tested using two-way ANOVA and means separated by Duncan multiple range test (DMRT) at  $p \leq 0.05$  using SPSS

## RESULTS AND DISCUSSION

### Leaf morphology

The results showed that the difference of organic fertilizers was not significantly difference in affected the leaf morphology of *Pogostemon cablin* Benth (Table 1.). After three months of transplantation, the treatment with manure obtained higher results in leaf length (15.23 cm) and as well as in leaf width (10.5 cm) with green color compared to the plants that transplanted to soil mixed with compost with green yellowish leaf color (Fig.1).

**Table 1.** The effect of organic fertilizers on the leaf morphology of *Pogostemon cablin* Benth.

No	Treatment	Leaf morphology		
		Leaf length (cm)	Leaf width (cm)	Leaf color
1	Compost (P1)	12.16 a	9.68 a	Green yellowish
2	Manure (P2)	15.23 a	10.5 a	Green
3	Control (P0)	10.33 a	8.1 a	Green yellowish
P0-P1		F = NS	F = NS	
P0-P2		F = NS	F = NS	
P1-P2		F = NS	F = NS	

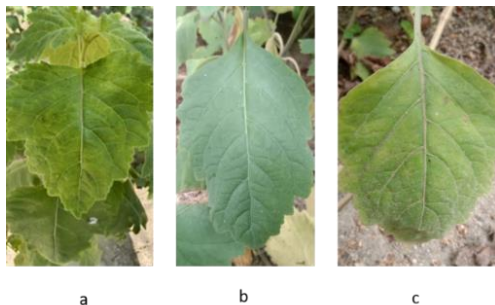
Data are means of five replicates. Means followed by the same letter in each column do not differ by Duncan's multiple range test at  $p \leq 0.05$ . \* and \*\*\* indicate significance at  $p \leq 0.05$  and  $p \leq 0.001$ , respectively while NS are non-significantly difference.

Leaf is the most important part of the plants, as it is produced carbohydrate from water and carbon dioxide combination and the process called photosynthesis. The application of organic fertilizer proved significantly affected the net photosynthetic rate and stomatal conductance in some plants, such as pear jujube [7]. Another research reported that the application of manure and liquid organic fertilizer significantly affected the leaf growth and plant height of this plant [13]. Leaf length, leaf width and leaf color indicated good or poor was the leaf growth of plants, as the photosynthetic rate of the entire plant depends on the photosynthesis of individual leaf, so that a sufficient supply of Nitrogen and other nutrients must be available during the leaf growth and development [14]. The use of organic fertilizer for growth of *Pogostemon cablin* Benth proved in increasing the soil environment which is encourage the root proliferous and better in absorbing the moisture, nutrients, and higher biomass [11,12]. In this study higher leaf properties obtained in the application of manure compare to the compost application.

### Stomatal density

After three months of transplantation, the stomatal density was calculated as well as for the leaf morphology measurements, and the results showed that the application of organic fertilizers in this study showed the significantly difference among the treatments (Table 2). The highest stomatal density obtained in manure application for both side of the leaf,  $663.5 \text{ mm}^{-2}$  for adaxial part (AD) and  $486.38 \text{ mm}^{-2}$  for abaxial part (AB). As mentioned before that the application of organic fertilizer proved significantly affected the net photosynthetic rate and stomatal conductance in some plants [7]. The stomatal density is one of the characteristics of the stomata that involved in stomatal conductance and as well as in transpiration rate. A research conducted in rice crop proved that low in stomatal density could conserved more water, maintain the stomatal conductance, drought tolerance and survive in high temperature ( $40^{\circ}\text{C}$ ) for long period. Low stomatal density also gave equivalent and improved yield, despite reducing the photosynthetic rate in some conditions [15].

The stomatal type of *Pogostemon cablin* Benth is anisocytic that also known as unequal celled where the guard cells surrounding with unequal size of neighboring cells, which is two larger neighboring cells and one distinctly smaller cell. This type of stomata commonly found in *Cruciferae*, *Solanaceae* and etc. [16]. There are glandular (Figure 2) and non-glandular trichome performs on the leaf surface, this condition is appearing in several genera from *Lamiaceae* family [18].



**Figure 1.** Leaf color, (a) Compost treatment, (b) Manure treatment, and (c) Control

**Table 2.** The effect of organic fertilizers on the stomatal density of *Pogostemon cablin* Benth.

No	Treatment	Stomatal density	
		Adaxial (AD) (mm <sup>-2</sup> )	Abaxial (AB) (mm <sup>-2</sup> )
1	Compost (P1)	579.3 b	244.89 c
2	Manure (P2)	663.5 a	486.38 a
3	Control (P0)	369.3 c	318.02 b
P0-P1		F = *	F = *
P0-P2		F = *	F = *
P1-P2		F = *	F = *

Data are means of five replicates. Means followed by the same letter in each column do not differ by Duncan's multiple range test at  $p \leq 0.05$ . \* and \*\*\* indicate significance at  $p \leq 0.05$  and  $p \leq 0.001$ , respectively



**Figure 2.** Stomatal type and glandular trichome of *Pogostemon cablin* Benth.

Trichomes are derived from leaf epidermal cells with small hair structures. The morphology, function, and density of the trichomes vary among plant species. There are two types of trichomes; glandular, which is able to excrete secondary metabolites such as patchouli oil, and non-glandular, which is not able to do the mentioned function [17]. The essential oils produced from glandular trichomes have a function in protecting plants from herbivores and pathogens [18].

## CONCLUSION

This study was conducted for 3 months. The seedling was transplanted into soil mixed with manure or compost. The results showed that manure obtained a better result in both treatments. The soil mixed with manure obtained higher results in leaf length (15.23 cm) and as well as in leaf width (10.5 cm) with green color. The highest stomatal density

was obtained in manure application for both sides of the leaf, 663.5 mm<sup>-2</sup> for the adaxial part (AD) and 486.38 mm<sup>-2</sup> for the abaxial part (AB). Based on the results obtained, it is proven that the application of manure for soil fertility improvement could be applied for *Pogostemon cablin* Benth. growth as well as in oil production.

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