



# Response of Citronella Grass on Several Phosphate Levels Application at Andosol

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## ABSTRACT

Currently, the cultivation of citronella grass (*Cymbopogon nardus* L.) is widely developed in highland areas dominated by Andosol soil. Andosol possesses low phosphorous availability, strongly bound to Al and Fe. This research was aimed to examine the effect of P fertilizer on *C. nardus* L. at Andosol. The research was conducted from June 2014 to August 2015. Phosphate fertilization stimulated plant growth, yield and oil quality of citronella grass on Andosol. The most suitable Phosphorus dosage at 1.08 g plant<sup>-1</sup> (10.8 kg ha<sup>-1</sup>) produced high total fresh herb yield and oil quality of citronella grass. Total fresh herb yield increased 21.2% (3.4 kg plant<sup>-1</sup> year<sup>-1</sup>) compared to control. Whereas citronella content, oil content and oil yield were 35.2%, 1.64% and 1.27% respectively. The phosphate residue in Andosol can be potentially utilized for the next planting season.

**Key words:** Andosol, Citronella, *Cymbopogon nardus* L., Phosphate, Yield.

## INTRODUCTION

Citronella grass (*Cymbopogon nardus* L.) is one of the plants producing essential oil. The main essential oil from citronella grass is citronella oil. It has used in many industries including cosmetic, pharmaceuticals, food and agriculture (Hanaa *et al.* 2012), as antifungal (Nakahara *et al.*, 2013) and antibacteria (Wei and Wee, 2013).

Currently, citronella grass plantations developed rapidly in the highlands which was dominated by Andosol soil. Andosol is fertile soil, but low in P availability. It is a high P retention because these were formed from acidic parent materials containing high Al and or Fe, which strongly bind P (Cordova *et al.*, 1996 and Sukarman and Dariah, 2014). This affects the effectiveness of P fertilization because the added P might be bound by Al or Fe. The phosphate amount bound by Al or Fe depended on Al and Fe content in the soil. The bond will be stronger following the increase of Al and Fe content.

Citronella grass oil was extracted from the leaves through distillation process. Its quality is determined by the secondary metabolites content (citronella), belongs to terpenoid, especially monoterpenes. The terpenoid group was formed through mevalonic acid pathways, which required P in each phase (Ganjewala, 2009), especially in the formation of Isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), precursors in mevalonic acid biosynthesis (Rehman *et al.*, 2016). Therefore, P availability in the soil can affect the secondary metabolites content in the essential oils of citronella grass.

Fertilizer application is essential to promote plant growth, but mostly performed without considering soil characteristics. It is often applied in high dose which is inefficient and causes high production cost. Moreover, the continuous P fertilization was reported causing P accumulation in soil ground level (Fei *et al.*, 2011).

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Phosphate residue in the Andosol was quite high (94-96%) (Mudjiharjati *et al.*, 2012), hence P fertilization was unnecessary in the next planting season.

The examination of P nutrient required by citronella grass on Andosol is important, considering the current development of citronella grass in this soil. This study aimed to obtain the appropriate dosage of P fertilizer to enhance plant growth, yield, oil quality and citronella content of citronella grass at Andosol.

## MATERIALS AND METHODS

The trial was conducted at Manoko Experimental Garden (1200 m asl), Lembang West Java, Indonesia from June 2014 to August 2015. The plant material used was citronella seedlings of the G3 accession, cow manure, urea, SP-36 (36% P<sub>2</sub>O<sub>5</sub>), KCl, chemical substances to measure citronella and oil content. The trial was arranged in a complete randomized block designed, repeated five times. Treatments tested were five dosage of P (P<sub>2</sub>O<sub>5</sub>); 0 (P<sub>0</sub>), 1.08 (P<sub>1</sub>), 2.16 (P<sub>2</sub>), 3.24 (P<sub>3</sub>) and 4.32 g plant<sup>-1</sup> (P<sub>4</sub>) equivalent to 0; 10.8; 21.6, 34.2 and 43.4 kg p ha<sup>-1</sup>year<sup>-1</sup>.

**Soil sampling for soil chemical properties analysis**

Soil sampling was conducted prior to the trial to analyze its chemical properties. The analysis to measure C-organic, total N and P followed Walworth (2006).

**Seed preparation**

Citronella grass was propagated using tiller from 1 year old plant. The tillers were separated from the clumps, its leaves were cut 3-5 cm from the stem and were planted in the field.

**Land preparation**

Soil tillage was performed once to obtain good soil structure and aeration for plant growth, divided into 25 plots (400 cm x 500 cm) with 75 cm between plots in the same block and 100 cm between blocks, with plant spacing at 100 cm x 100 cm.

**Fertilization**

Cow manure, urea and KCl were used as basic fertilizers. Manure was given one week before planting 10 tons ha<sup>-1</sup> (1kg plant<sup>-1</sup>). Urea was applied 1 and 2 months after planting (MAP), 5 g plant<sup>-1</sup>, whereas KCl 10 g plant<sup>-1</sup> was given at the time of the planting. Phosphate fertilizer (36% P<sub>2</sub>O<sub>5</sub>) was applied at the time of planting. No fertilizers applied between the 1<sup>st</sup> harvest and the 3<sup>rd</sup> harvest.

**Observation**

Plant growth (plant height and the number of tillers<sup>-1</sup> clumps) were observed at 1-6 MAP. Fresh herb yield, oil yield and oil contents were observed at harvesting time (6, 10 and 14 MAP). Citronella content was measured at 6 MAP (the first harvest). The yield of citronella oil from distillation process was calculated following the formula (SNI, 1995):

$$\text{Density} = \frac{\text{Oil mass (g)}}{\text{Oil volume (ml)}} = \text{g/ml}$$

Oil mass = density x oil volume

$$\text{Yield} = \frac{\text{Oil mass (g)}}{\text{Leaf dry weight (g)}} \times 100\%$$

Citronella content was calculated by measuring the content of carbonyl compound (in the form of aldehyde or ketone) using formula as follows (SNI, 1995):

$$\frac{M (V_o - V_r)}{20 \text{ m}}$$

Note :

M = mass of oil sample.

V<sub>r</sub> = the volume of hydrochloric acid used in the sample solution analysis.

V<sub>o</sub> = the volume of hydrochloric acid used in the blank solution analysis.

M = relative molar mass of the aldehyde or ketone added into the citronella oil standard.

**Data analysis**

The data were analyzed using analysis of variant and tested further with Duncan's Multiple Range Test (DMRT) at 5%, if there were significant differences.

**RESULTS AND DISCUSSION****Soil chemical properties**

Soil fertility level was high indicated by slightly acidic pH; high content of C-organic, N, P and Ca; but low in K and Mg content (Table 1).

**Plant height and tiller number of citronella grass**

At 6 MAP, P dosage at 1.08 g plant<sup>-1</sup> was able to enhance plant height and tillers number significantly. On the contrast, the higher dosage showed no significant effects on plant growth compared to control. The P fertilizer application enhanced plant height and tiller number from 2.2 to 17.1% and 0.61 to 12.76% respectively (Table 2).

The application of 1.08 g P plant<sup>-1</sup> was already able to stimulate plant growth. Thus, a higher dosage would have no significant effect on plant growth. Nutrient uptake can be affected by nutrient content in the soil and plant species. High dosage fertilization would be ineffective for fast-growing type crops planted in soil with high nutrient content, since the nutrient uptake was limited in this particular condition (van Wijk *et al.*, 2003). Based on the soil analysis, the trial plots were high on P content (29.92 ppm P<sub>2</sub>O<sub>5</sub>) (Table 1).

The low impact of higher P dosage to plant growth also related to Andosol characteristic which has high retention capacity of P. The high frequency of P fertilization and excess P accumulation in the soil would also inhibit nutrient uptake by plants (Sugito and Shinano, 2013). Applying high P dosage on Andosol did not ensure high P availability for plants, due to its low absorption efficiency (10-20%) (Christine *et al.*, 2007). Thus, the frequency was more important than dosage for P fertilization in Andosol soil.

**Fresh herbs yield of citronella grass**

Phosphates fertilization increased fresh herb yield at the 1<sup>st</sup> harvest (26-37%) and the 3<sup>rd</sup> harvest (18-26%). The highest fresh herb yield was at 1.16 g P plant<sup>-1</sup> treatment (3.5 kg plant<sup>-1</sup> year<sup>-1</sup>), was significantly different from control but not significantly different with 1.08 g P plant<sup>-1</sup> (3.4 kg ha<sup>-1</sup> year<sup>-1</sup>). In contrast, 3.24 to 4.32 g P plant<sup>-1</sup> treatment indicated no

**Table 1:** Soil chemical properties at experiment plot.

Analysis Type	Test Results	Analysis Type	Test Results	Analysis Type	Test Results
pH H <sub>2</sub> O	6.25	N-Total (%)	0.55	K (cmol(+) kg <sup>-1</sup> )	0.20
pH KCl	5.51	C/N ratio	8.64	Na (cmol(+) kg <sup>-1</sup> )	0.33
C-organic (%)	4.75	Ca (cmol(+) kg <sup>-1</sup> )	12.34	Al (cmol(+) kg <sup>-1</sup> )	no detected
		Mg (cmol(+) kg <sup>-1</sup> )	1.19	CEC (cmol(+) kg)	46.65

significant increase in total fresh herb yield. Thus, the most suitable dosage was 1.08 g P plant<sup>-1</sup> which can increase yield up to 21.2% (Table 3). Murthy *et al.*, (2015) stated that P improved yield of grain, rice (Debnath *et al.* 2015) and onion (Kamboj *et al.*, 2017).

At low P dosage, the citronella grass showed more rapid response in growth and production. Thus, applying higher P amounts will be inefficient in citronella grass cultivation in Andosol. Utami *et al.*, (2012) stated that it was not necessary to add high P dosage on Andosol, however, its availability can be increased through the provision of organic materials and natural silica. Phosphate in high quantities has no significant effect on plant growth and yield (Chairunnisa and Hanum, 2013). Low absorption efficiency of P fertilizer in Andosol, high frequency of P fertilization and excessive P accumulation in soil would inhibit nutrient uptake by plants (Sugito and Shinano, 2013).

Fresh herb yield at the 2<sup>nd</sup> harvest was lower than the 1<sup>st</sup> and 3<sup>rd</sup> harvest and fertilizer application indicated no significant effect on yield (Table 3). Beside soil fertility, yield was also influenced by climatic conditions. The climatic condition during plant growth until the 1<sup>st</sup> harvest (June-Dec 2014) and the 3<sup>rd</sup> harvest (April-August 2015) were different from the 2<sup>nd</sup> harvest (Dec 2014-April 2015). Climatic conditions observed were temperature, humidity, rainfall and rainy days (Fig 1A).

Extreme differences were shown in rainfall and number of rainy days during the 1<sup>st</sup> and the 2<sup>nd</sup> harvest. The rainfall

**Table 2:** The effect of P fertilizer on plant growth and yield at 6 month after planting (MAP).

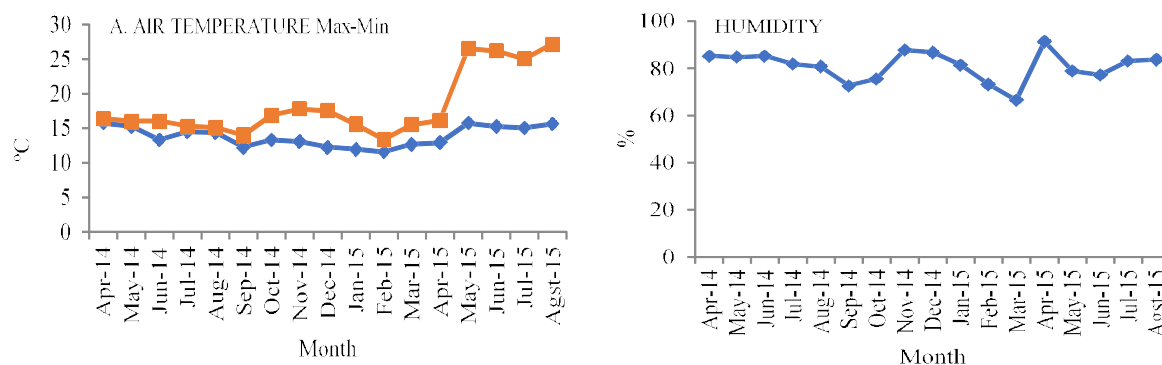
Treatments P dosage (g <sup>-1</sup> plant)	Plant height (cm)	Tiller number
P0= 0	57.73 b	79.78 b
P1= 1.08	67.72 a	89.96 a
P2= 2.16	59.00 ab	81.71 ab
P3= 3.24	61.93 ab	80.27 ab
P4= 4.32	67.63 ab	75.62 b
CV (%)	15.70	18.50

Note: Numbers followed by the same letter in the same column were not significantly different at 5% DMRT.

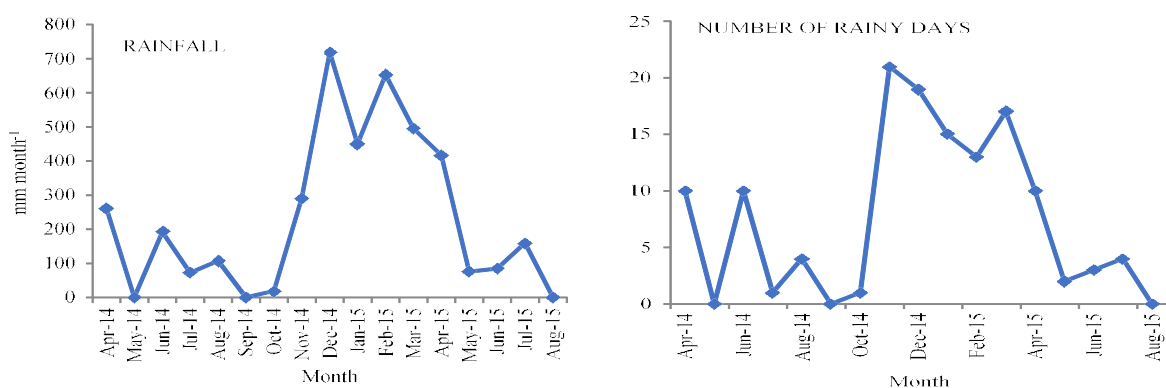
**Table 3:** The effect of P on fresh herb yield of Citronella grass.

Treatments P Dossages (g/plant <sup>-1</sup> )	First harvest	Second harvest	Third harvest	Total Yield Year <sup>-1</sup>
Kg plant <sup>-1</sup>				
P0= 0	0.95 b	0.89 a	1.04 b	2.88 b
P1= 1.08	1.20 a	0.90 a	1.30 a	3.40 a
P2= 2.16	1.20 a	1.00 a	1.31 a	3.51 a
P3= 3.24	1.30 a	0.87 a	1.23 ab	3.23 ab
P4= 4.32	1.20 a	0.86 a	1.30 a	3.36 ab
CV (%)	17.80	19.70	18.20	18.90

Note: Numbers followed by the same letter in the same column were not significantly different at 5% DMRT.



**Fig 1A:** The average of max-min temperature and humidity in the trial location.



**Fig 1B:** The average of rainfall and number of rainy days in the trial location.

and number of rainy days was quite high, with the monthly average being 400-700 mm and 10-20 days, respectively (Fig 1B). The environmental requirement for citronella grass, especially for rainfall, were 1800-2500 mm year<sup>-1</sup> or 150-180 mm month<sup>-1</sup> (National Science Foundation Grant, 1999). Thus, the higher rainfall and number of rainy days inhibited plant growth, resulted in lessening yield than from the 1<sup>st</sup> and the 3<sup>rd</sup> harvest.

#### P uptake on citronella grass and nutrient content in soil

At 6 MAP, optimal P uptake at 2.16 g/plant application and tend to decrease if being given in high dosage (Fig 2). On the contrast, P level in the soil was higher following the treatments (Fig 3). This indicated the plants inability to absorb nutrients in high quantities, as available P was low at Andosol. Nuryani *et al.*, (2006) stated that P was bound by Al and Fe. This implied that in Andosol, high dosage of P fertilization would be useless since it will be bound by Al or Fe caused P was unavailable for plant. It was also indicated by the presence of high P levels in the soil after the trial. The P residue in the soil can be utilized for the next planting season, but requires efforts to make P available for plants.

Phosphate fertilization increased nutrient uptake compared to control at 6 MAP (the 1<sup>st</sup> harvest), although P content in plant tissue were not different in all treatments. Nutrient uptake of P by citronella grass increased at 1.08 to 3.24 g P plant<sup>-1</sup> and decreased at 4.32 g P plant<sup>-1</sup> (Fig 2). Nutrient uptake rate was determined by nutrient availability in the soil. Low nutrient availability will cause low nutrient uptake, despite high nutrient content in the soil.

#### Citronella content and oil yield of citronella grass

Phosphate application increased citronella content from 35.2 to 35.6% higher than control (34.4%) (Fig 4) and fulfilled the Indonesian National Standard (35%) (SNI, 1995). Phosphate application enhanced oil yield of citronella grass at all harvest period. At the 1<sup>st</sup> harvest, 4.32 g P plant<sup>-1</sup> dosage produced the highest oil yield (0.91%). However, at the 2<sup>nd</sup> and the 3<sup>rd</sup> harvests, 1.08 g P plant<sup>-1</sup> application indicated the highest oil yield (1.27%) (Fig 5). Phosphate is required in the formation of primary and secondary metabolites especially in terpenoid groups. Phosphate affected terpenoid biosynthesis, particularly in the formation of terpenoid precursor (IPP: isopentenyl diphosphate and DMAPP: Dimethylallyl pyrophosphate) which contained high-energy phosphate bonds (Sharifi-Rad *et al.*, 2017) and also a key component of ATP and NADPH (Johnson *et al.*, 2017). Phosphate requirement depended on plant species, P content and its availability in the soil. In this research, the dosage of 1.08 g P plant<sup>-1</sup> was able to increase citronella content of citronella grass. Gusmaini *et al.*, (2016) also reported that P application enhanced andrographolide content of *Andrographis paniculata*.

#### Oil content of citronella grass

At the 1<sup>st</sup> harvest, the oil content ranged from 0.9-1.3% and the highest oil content was at 4.32 g P plant<sup>-1</sup> treatment

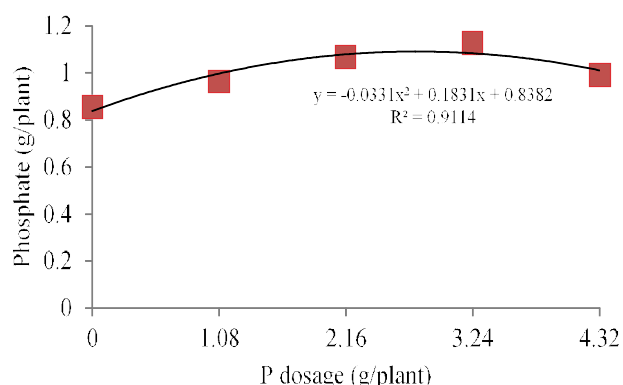


Fig 2: Effect of P fertilizer on P nutrient uptake at 6 MAP.

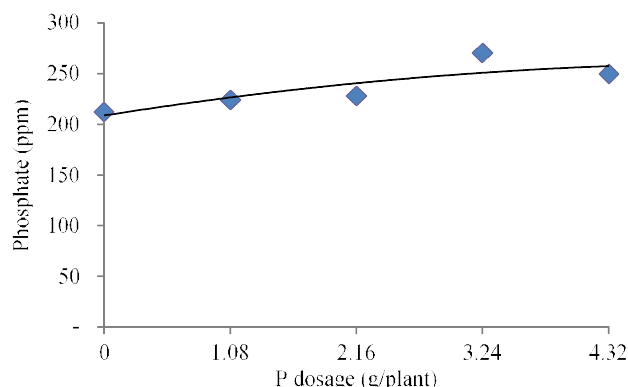


Fig 3: Effect of P fertilizer on P content in the soil at 6 MAP.

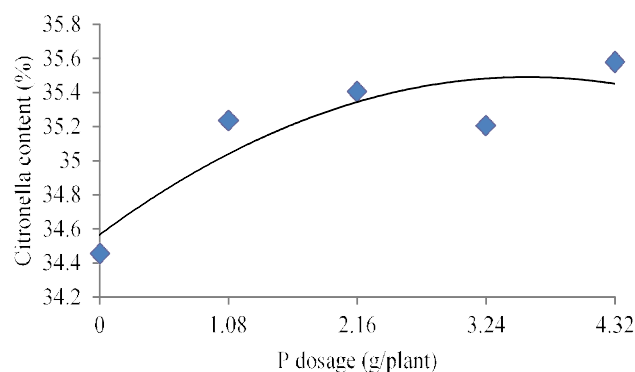


Fig 4: The effect of P on citronella content of citronella grass at 6 MAP.

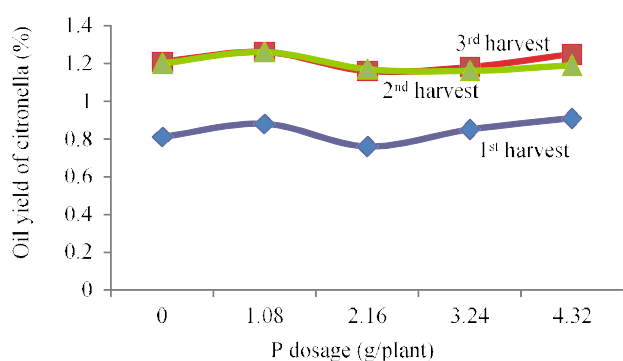
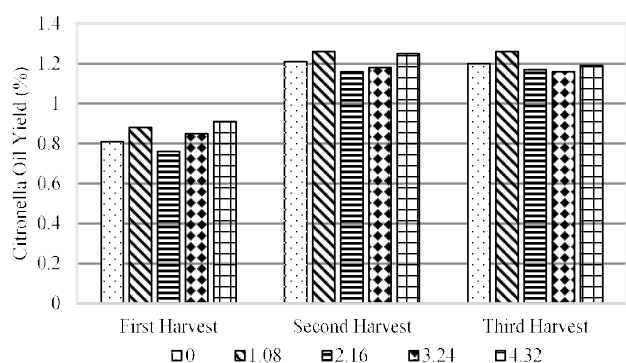


Fig 5: The effect of P on oil yield of citronella grass at three times harvest.



**Fig 6:** Effect of P fertilizer on Oil content of citronella grass at 3 times harvest.

(1.3%). At the 2<sup>nd</sup> and the 3<sup>rd</sup> harvest, the oil content increased (1.59 to 1.64%) and the highest content was at 1.08 g P plant<sup>-1</sup> (1.64%) treatment. Moreover, the oil production from the 2<sup>nd</sup> harvest was as high as the 3<sup>rd</sup> harvest (Fig 6). The same result was also found on *Mentha* sp. At the 1<sup>st</sup> year, 100 kg P ha<sup>-1</sup> produced the highest essential oil content, but in the 2<sup>nd</sup> year the lower dose at 50 kg P ha<sup>-1</sup> gave the highest result (Yesil and Kara, 2016). This suggested that for the next harvest, high-P dosage fertilization was not necessary because of the P residue from previous fertilization.

## CONCLUSION

Phosphate affected positively on plant growth, fresh herb yield and oil quality of citronella grass at Andosol. The most suitable dosage of P<sub>2</sub>O<sub>5</sub> to enhance yield and oil quality of citronella grass in Andosol was 1.08 g P plant<sup>-1</sup> (10.8 kg P ha<sup>-1</sup>). Total fresh herb yield increased 21.2% (3.4 kg plant<sup>-1</sup>year<sup>-1</sup>) compared to control. Whereas citronella content, oil content and oil yield were 35.2%, 1.64% and 1.27% respectively. The phosphate residue in Andosol can be potentially utilized for the next planting season.

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