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Monoterpenes accumulation inducing by nutrient status under rhizobacteria and organic manure supply in *Cymbopogon nardus*

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Abstract. *Larasati F, Sudiarso, Barunawati N, Aini N. 2022. Monoterpenes accumulation inducing by nutrient status under rhizobacteria and organic manure supply in* Cymbopogon nardus. *Biodiversitas 23: 4055-4063.* The quality type of *Cymbopogon nardus* in Indonesia is called as Java Citronella Oil. One of the oil components determining the quality of *C. nardus* is citronellal content as well-known monoterpenes. The citronellal component in *C. nardus* is obtained through the redistillation process in leaves. This study aims to obtain citronellal content, organic matter, and carbon which are affected by dosage of fertilizer on *C. nardus*. In the overall, the citronellal component of plants without PGPR (P0) treatment at all levels of organic manure supply is 10% higher than those treated rhizobacteria (P20). The final content of carbon and organic matter in respectively (2,25%) and (3,08%) leaves of plant (P0) at K10, K20, K25, K30 is 20% higher than those treated with P20. This might be caused by the carbon that is distributed to the vegetative part during the fast-growth phase, by contrast, the component of citronellal was reduced. The growth rate of *C. nardus* depends on the accumulation of dry weight at 5 and 6 MAP, which increases significantly with organic manure treatment of K30 and supply of rhizobacteria (P20), which had nutrient status 1,362% nitrogen, 0,194% phosphorus, 0,882 % potassium in leaf following by citronellal and citronellol.

Keywords: Biomass, carbon, *Cymbopogon*, organic matter, rhizobacteria

INTRODUCTION

One of the determinants of the quality of Citronella (*Cymbopogon nardus* (L.) Rendle) oil is its citronellal oil, a type of volatile compound. In Indonesia, Citronella as well-known as monoterpenes is categorized into one specific quality type called the Java Citronella Oil. The quality of the oil is determined by the citronellal content, i.e. 35% at the minimum according to the Indonesian National Standard No. 06-3953-1995. Citronellal component of essential oils determine the quality of essential oils based on the purity, which in turn determines its commercial value. The low citronellal component means a lower commercial value and vice versa (Alighiri et al. 2018).

The growth and yield of *C. nardus* are expected to increase with higher availability of nutrients through soil biological community. That nutrient availability could be increased by applying organic manure and PGPR. The management of organic matter application supports the availability of soil nutrients and nutrients status in plant. One of the organic materials from animal, which contain 2.10% of nitrogen, 2.09% of phosphorus, 0.82% of potassium, 28.20% of carbon organic, and 49.07% of organic matter (Laboratory Analysis Data 2021). The effectiveness of nutrient supply by organic manure is influenced by microorganisms, including PGPR which contains bacteria and fungi. One of the roles of soil microorganisms is to assist the availability of plant nutrients through the overhaul of organic matter by microorganisms in the soil. Manure is a source of organic matter that contains nutrients such as nitrogen, phosphorus, and organic matter (Drozdz et al. 2020). Supply of organic manure can provide nutrients from the decomposition and mineralization process to become available nutrients that can be absorbed by plants, which provide essential macronutrients N, P, and K as well as micronutrients such as Ca faster than other animal manure fertilizers (Atmaja et al. 2017). These chemical reactions are interactions between organic matter and the supply of rhizobacteria that supply plant nutrients.

The yield of biomass and the quality of *C. nardus* are influenced by environmental factors such as the availability of soil organic matter and rhizobacteria that accumulate as that nutrient status for the photosynthetic products. PGPR protects plants from pathogens that can administer the limiting factors originating from the environment (Lehman et al. 2015). The mechanism in the rhizosphere is the interaction between plant roots that release exudates such as ions, oxygen, amino acids, and organic compounds containing carbon. Plant roots and microorganisms interact through exudates released by plant roots, most of which are secondary metabolites, including amino acids, sugars, vitamins, organic acids, and nucleotides (Sasse et al. 2018). Hence, the bacteria interact through organic compounds from the root hairs to form a symbiotic relationship with soil microbes. The population and types of bacteria in the soil formed in abundance supported by the availability of organic matter; one of which is organic manure, whose carbon and nitrogen are to be converted into complex compounds useful for plant growth (Liao et al. 2019). Root exudation is a communication medium between plants and microorganisms in the soil through root exudates released by plant roots to establish a beneficial relationship in this case the acquisition of nutrients (Meier et al. 2017).

The abundance of root exudates rich in organic carbon is a communication tool to invite various soil microbes to colonize the area. Root exudates are the main source of food and energy for organisms living in the rhizosphere (Zhuang et al. 2013). The production of *C. nardus* secondary product from the increase of carbon and accumulation of organic matter is the determinant for the quality of the essential oil content. The formation of citronellal oil component in *C. nardus* depends on the supply of organic matter such as organic manure and PGPR. Moreover, this treatment as well as increase of carbon content and organic matter on leaf slightly. Meanwhile, citronellal precursor is well-known isopentenyl diphosphate (IPP), produced through carbon fixation and reduction on leaf. The precursor of all types of terpenoid compound is IPP (Mukarram et al. 2021). It expect that the carbon support secondary metabolite synthesis. According to Romagni (2009), secondary metabolite production occurs when energy and carbon condition are high. In supply, the time of rhizobacteria application affects the contribution of root exudates in absorbing nutrients to maximize primary metabolite formation. The aim of the research is to obtain the carbon and organic matter in leaf as well as citronellal content which is affected by dosage of fertilizer on *C. nardus*. The hypothesis of the research is that the application of organic manure can increase accumulation of carbon and organic matter in leaf and elevate citronellal content.

MATERIALS AND METHODS

This research material is *C. nardus* seed, i.e. Citronella 1 variety, chicken manure, PGPR, Urea, SP36, and KCl fertilizer. This research was conducted at the Experimental Garden of Griya Santa, Faculty of Agriculture, University of Brawijaya, Malang, from August 2021 to March 2022. The site is located at an altitude of 460 meters above sea level with a minimum temperature of 20°C and a maximum temperature of 28°C. This factorial experiment using the randomized block design was repeated three times. This experiment consists of two factors. The first is two doses of PGPR: P0 (without PGPR) and P20 (20 mL plant^{-1} of PGPR). The second factor is five levels of chicken manure addition: K10 (10 t ha^{-1}), K15 (15 t ha^{-1}), K20 (20 t ha^{-1}), K25 (25 t ha^{-1}), and K30 (30 t ha^{-1}).

Non-destructive growth observations were performed in the first, second, third, fourth, fifth, and sixth months after planting. Leaf number was obtained by counting the fully expanded leaves of *C. nardus* which are active in photosynthesis. The destructive growth observations were carried out by the first, second, third, fourth, fifth, and sixth MAP. The leaf area of *C. nardus* is measured using a *Leaf Area Meter* (LAM), namely by placing the leaves one by one on the surface of the LAM until the total leaf area is obtained. The fresh and dry weight observations were carried out destructively at harvest, i.e. 6 MAP, by weighing the cleaned plants on a digital scale, dry them out in an oven at 80°C until a stable dry weight was obtained. The leaves and roots of the harvested plants were separated, then each organ part was put into a paper envelope.

There are 3 quality indicator parameters observed in 6 MAP: oil, carbon, and organic matter. The citronellal content was observed by distilling the oil from leaves. The refined oil was diluted with n-hexane as solvent and the components of the oil were analyzed using GC-MS (QP-2010 Ultra/Shimadzu). Carbon was analyzed by the *Walkley and Black* method: 1 g of *C. nardus* leaves were placed into an Erlenmeyer tube, adding 5 ml of 1 N $K_2Cr_2O_7$, and shaked. Then, after the supply of 5 ml of concentrated H_2SO_4, the shaking was carried out again, and the solution was allowed to stand for 30 minutes. Next, the samples were added with 15 ml of aquadest, 5 ml of H_3PO_4, and 1 drop of diphenylamine indicator. They were titrated with 1 N $FeSO_4$ until the color changed to light blue. The volume of the added titrant was written, and the organic C content was calculated by the formula below.

$$\textbf{C-Organic} = \frac{(N\ K2Cr2O7\ x\ V\ K2Cr2O7) - (N\ FeSO4\ x\ V\ FeSO4)}{sample\ weight\ x\ 0{,}77}\ \textbf{x 0,33}$$

Where:
N $K_2Cr_2O_7$ = $K_2Cr_2O_7$ concentration
V $K_2Cr_2O_7$ = $K_2Cr_2O_7$ volume
N $FeSO_4$ = $FeSO_4$ concentration
V $FeSO_4$ = $FeSO_4$ volume

The plant growth rate was measured according to Sitompul and Guritno (1995) by weighing the dry biomass of the leaves every month. The growth rate was calculated using the formula below.

$$\textbf{CGR} = \frac{1}{GA}\ \textbf{x}\ \frac{W2-W1}{T2-T1}$$

Where:
GA : area covered by the plant (m^2)/ distance planting
W : Dry weight (g per plant)
T : time

Data Analysis

The results of the observations were analyzed using variance analysis (the F Test) at 5%. If a significant difference between treatments is found, 5% DMRT shall follow.

RESULTS AND DISCUSSION

Leaf number

The chart of leaf number is presented in Figure 1. In all treatments, with and without rhizobacteria, the leaf number tends to be stable at 1 to 4 MAP and increased 2 to 4.5 times at 5 and 6 MAP respectively in all doses of organic manure. At 6 MAP, the leaf number on P20 treatment increased 20% in the average, as compared to P0. The supply of rhizobacteria increased the average leaf number until 5 MAP, and the number started to decrease after 6 MAP.

Leaf area

The average leaf area of *C. nardus* is presented in Figure 2. In the overall, P20 treatment increased the average leaf number by 40%, as compared to P0 treatment. The leaf area increased significantly at K30 treatment of 20% at 6 MAP. Meanwhile, P0 treatment at 15, 25, and 30 t ha^{-1} of organic manure and P20 treatment at 10, 15, and 20 t ha^{-1} of organic manure produced in that the leaf number increased from 1 MAP to 5 MAP and then decreased at 6 MAP. This is different from P0 treatment with of 10 and 20 t ha^{-1} organic manure additions and P20 treatment with 25 and 30 t ha^{-1} of organic manure addition, which shows an upward trend in the chart at 1 to 6 MAP. At 6 MAP, the P20K30 treatment significantly increased leaf area by 15% compared to other treatments.

Fresh weight (g)

The fresh weight chart of *C. nardus* at 6 MAP is presented in Figure 3. P20 treatment with 25 and 30 t ha^{-1} of organic manure gave a significant increase of 40% as compared to P0. However, it was different from P20 treatment with the supply of 10, 15, and 20 t ha^{-1} of organic manure. The fresh weight is almost the same as the P0 treatment for all organic manure treatments. Organic manure fertilization-only, there is no effect on fresh biomass accumulation, while the supply of P20 increases the biomass yield only at K20 and K25 combined treatment. Overall dry weight shows the same trend.

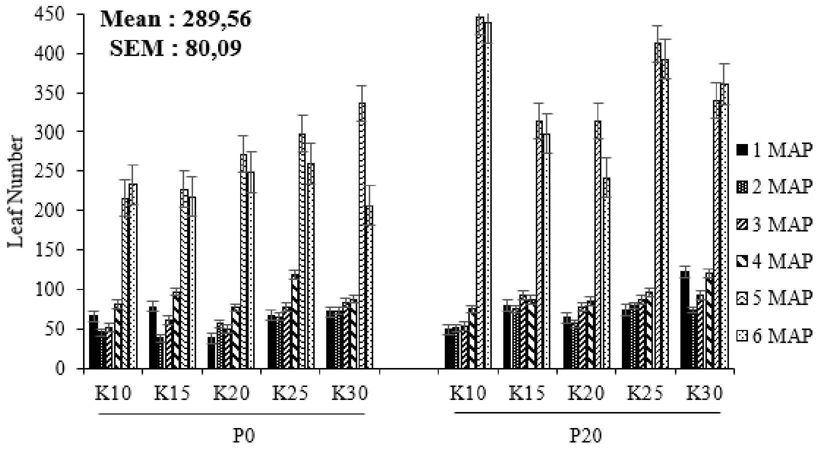

Figure 1. *Cymbopogon nardus*' leaf number

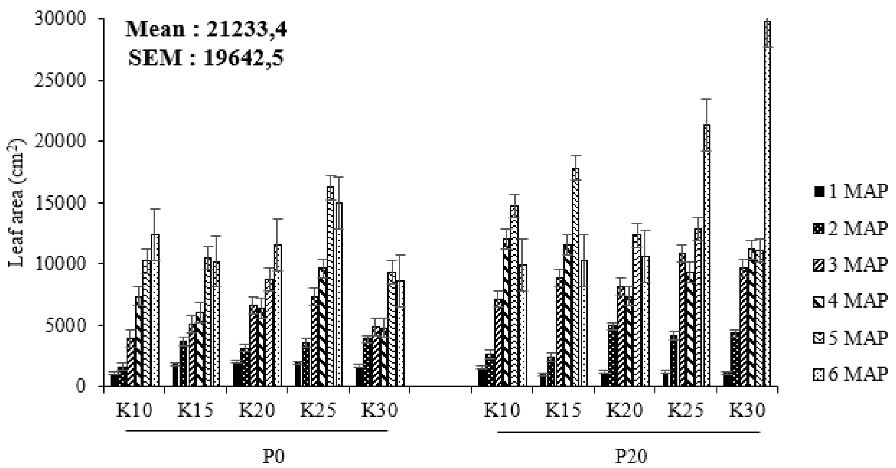

Figure 2. *Cymbopogon nardus*' leaf area

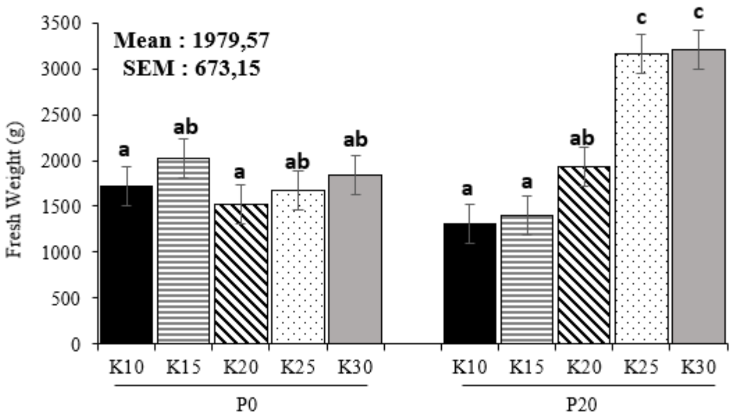

Figure 3. Fresh weight of *Cymbopogon nardus* at 6 MAP

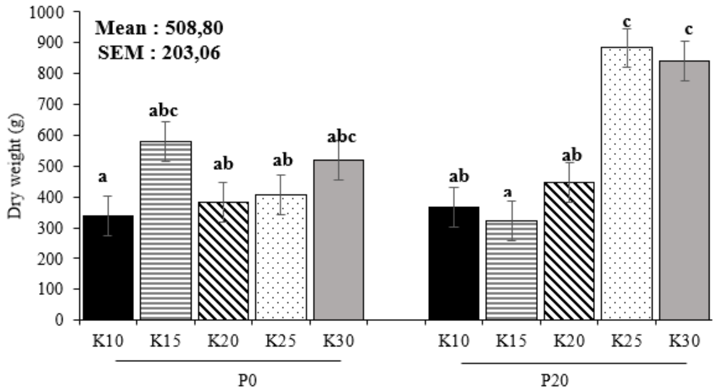

Figure 4. *Cymbopogon nardus*' dry weight at 6 MAP

Dry weight (g)

The data of the dry weight observations, which is the accumulation of organic compounds synthesized by plants, are presented in Figure 4. The dry weight of *C. nardus* at 6 MAP follows its fresh weight at 6 MAP. P20 treatment with 25 t ha^{-1} and 30 t ha^{-1} of organic manure produces a higher average dry weight of 30% compared to P0 with 30 t ha^{-1} of organic manure. At P20 with organic manure treatments of 25 and 30 t ha^{-1} at 6 MAP, the dry weight increases significantly by 40% as compared to P0 treatment. The dry weight for P20 treatment with 10, 15, and 20 t ha^{-1} of organic manure had a relatively stable trend: an average of 400 g per plant. At P0 treatment, the average dry weight of *C. nardus* with organic manure of 10, 20, and 25 t ha^{-1} increased at the similar rate: 400 g per plant. However, P0 treatment with organic manure of 15 and 30 t ha^{-1} increased its dry weight by 20% than plants with other treatments.

Plant growth rate

Figure 8 shows that the plant growth rate without rhizobacteria treatment and several levels of organic manure supply at K10 treatment had the same relative growth rate pattern as K15. At K10, the growth rate was at the highest at 3 MAP, while for K15, the highest rate was at 4 MAP. The relative growth rates of both decreased drastically until 5 MAP. The plant's relative growth rate at K25 increased slowly and reached its peak at 3 MAP and tended to decrease slowly at 5 MAP. The pattern of the plant's relative growth rate for treatments K20 and K30 tended to slope at 1 MAP to 4 MAP, and it increased sharply until 5 MAP (K30).

The chart for growth rate, which is the converted photosynthetic yield in dry weight gain on the supply of rhizobacteria (P20), is presented in Figure 9. Organic manure treatment of 10 t ha^{-1} (K10) produced the highest relative growth rate at 3 MAP, which decreased in 2 months afterwards, i.e. 5 and 6 MAP. The pattern of the plant's relative growth rate for treatments P15 and P20 was the same: it increased slowly, reaching the peak at 3 MAP for organic manure treatment of 20 t ha^{-1} (K20). The organic manure treatment of 15 t ha^{-1} (K15) produced the peak growth at 4 MAP. The growth pattern of plants with K25 treatment was the same as that of K30 treatment, reaching the highest rate at 2 MAP, sloping at 3 MAP, and increasing sharply at 5 MAP.

Citronellal components

Regarding the citronellal oil content, Figure 5 shows that the effect of organic manure treatment at various doses (P0) except for K10 resulted in a 10% higher citronellal component as compared to P20 treatments. Citronellal content at P0 treatment with the supply of 15, 20, 25, and 30 t ha^{-1} of organic manure resulted in 20% higher citronellal as compared to P20 in all organic manure additions.

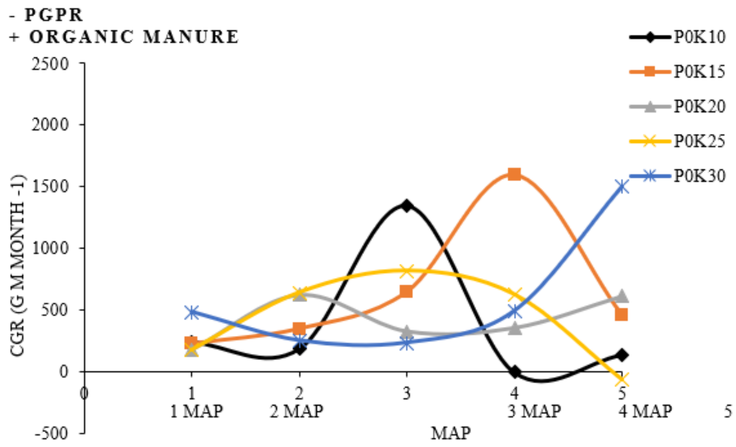

Figure 8. Plant growth rate with no-rhizobacteria treatment and several levels of organic manure addition

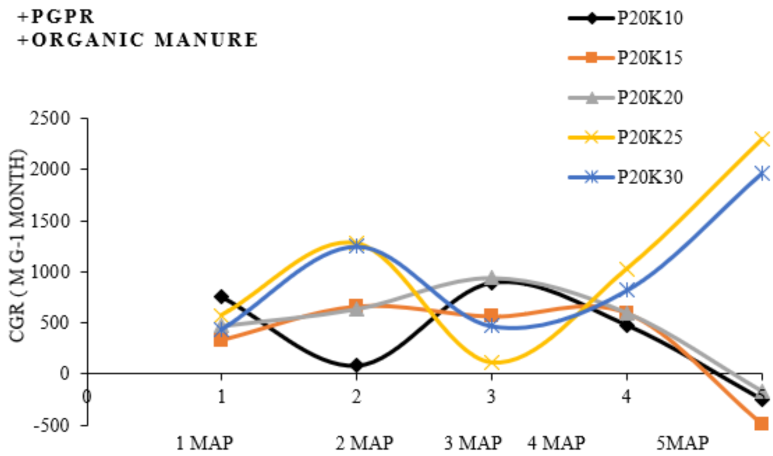

Figure 9. Plant growth rate for rhizobacteria treatment and several levels of organic manure addition

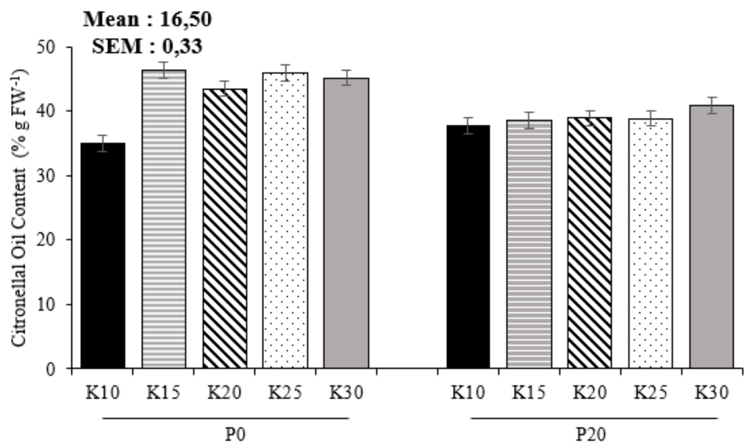

Figure 5. Citronellal oil content in *Cymbopogon nardus*

Carbon

The carbon and the organic matter of *C. nardus* are presented in Figure 6 and Figure 7. The increase in the plant's organic matter was followed by an increase in its carbon. The carbon content for P0 at various levels of organic manure supply increased the number of carbon in the leaf by 30% as compared to P20 at all organic manure supply levels. The carbon of plants treated with P0 with 10 and 30 t ha^{-1} of organic manure increased significantly, as compared to P20. Meanwhile, P20 treatment at all doses of organic manure treatment produced a stable percentage of carbon. The carbon content of *C. nardus*' leaves is inversely proportional to the average accumulation of its produced organic compounds. This was assured by the percentage of leaf carbon at P0 treatment which reached 3% with an accumulation of plant organic compounds of approximately 600 g. This was also found in the supply of rhizobacteria (P20), i.e. 2.25% with accumulated organic compounds of 900 g. This means, at P0 treatment, 18 g of leaf carbon can convert dry weight, while at P20 treatment, it can convert dry weight to 20, 25 g of leaf carbon.

Nutrient status in leaf

Nutrient status in *C. nardus*' leaf at 6 MAP is presented in Figure 8. Nitrogen and phosphorus content in leaf is slightly higher when supply by rhizobacteria (P20). In contract, potassium is slightly higher compare to rhizobacteria treatment.

Discussion

In general, the combination treatment of rhizobacteria 20 mL (P20) with several levels of organic manure supply on *C. nardus* increased the leaf number, leaf area, fresh weight, and dry weight by 10% compared to without rhizobacteria supply. In contrast, without-rhizobacteria treatment (P0) with several levels of organic manure decline the leaf number, leaf area, fresh weight, and dry weight but incline the citronellal content at about 20%, as compared to supply of 20 mL of rhizobacteria (P20). Citronellal content in leaves had opposite pattern with fresh weight total at the end of observations at 6 MAP, presented on the fresh weight figure for without-rhizobacteria treatment (P0). Here 2000 g of fresh weight yielded 48% of citronellal component. As for rhizobacteria treatment (P20), 3250 g of fresh weight yielded 40% of oil component. PGPR supply in all organic manure additions had relatively the same citronellal content, i.e. 35-40%.

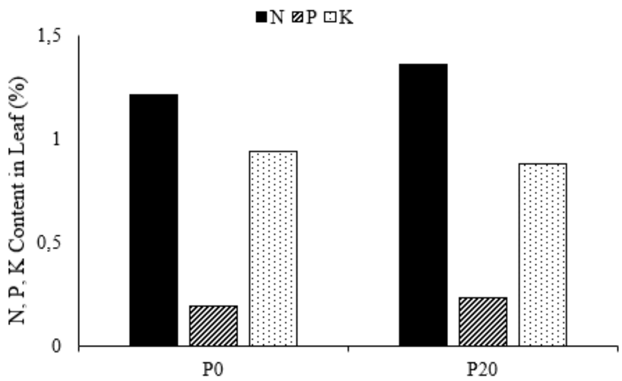

Figure 8. Nutrient Status in *Cymbopogon nardus*'s Leaf at 6 MAP

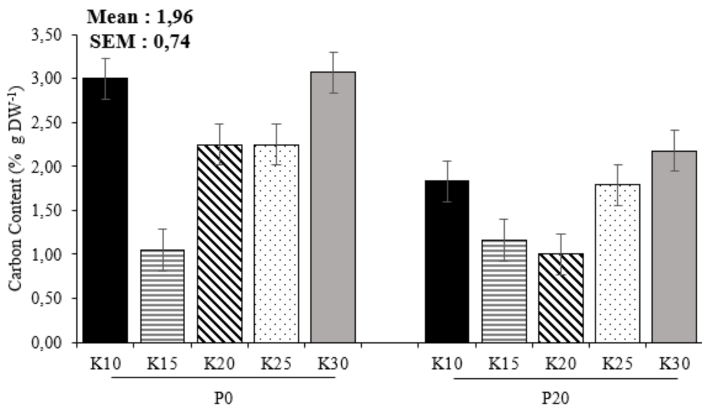

Figure 6. Carbon content in *Cymbopogon nardus'* leaf

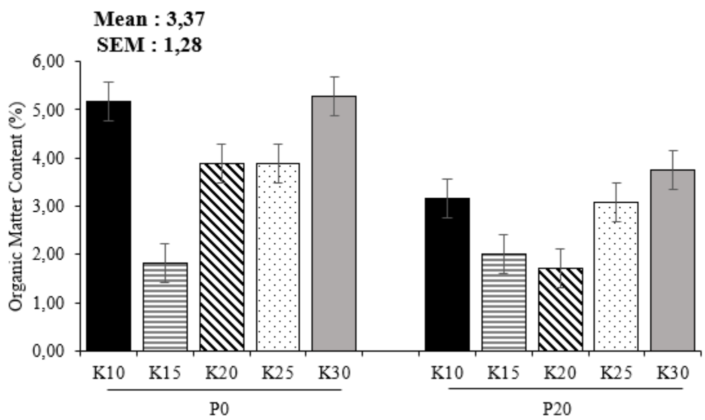

Figure 7. Organic matter content in *Cymbopogon nardus*' leaf

Therefore, it can be assumed that, P0 treatment requires 41.6 g of fresh weight to produce 1% of citronellal component of *C. nardus*, while P20 treatment requires 81.2 g of fresh weight to produce 1% of citronellal component**.** Citronellal content was relatively stable and increased with each additional dose of organic manure 5 tons ha-1 on plants treated with rhizobacteria 20 mL plant^{-1} (P20), but it was different from the treatment without rhizobacteria (P0), which tends to fluctuate with each supply of organic manure. In the research conducted by Cappellari et al. (2015) showed that the increase in essential oil yield was influenced by inoculation of growth-promoting bacteria associated with an increase in chlorophyll content, trichome gland density, fresh weight, and leaf area. However, from the results of this study, the treatment without rhizobacteria (P0) found a higher citronellal content than the rhizobacteria treatment. Rhizobacteria help reorganize organic matter which then nutrients are available faster than without rhizobacteria (P0). According to Ningsih et al. (2016), the decomposition of organic matter in the soil will be faster when rhizobacteria are added. In addition, the supply of soil organic matter affects the diversity and composition of rhizobacteria (Ling et al. 2022).

This means that plants treated with rhizobacteria (P20) can provide nutrients availability more quickly thus the primary and secondary metabolic are processed as well as formed earlier. Regards to the results of the study that plant growth at 1 MAP to 3 MAP had slowly increased due to the primary metabolites being converted quickly. to secondary metabolism. It is supported by Santoro (2011) that the essential oil content is not due to an increase in biomass, but because rhizobacteria induce secondary metabolism. from defense mechanisms. A higher accumulation of secondary metabolite such as monoterpenes was associated with an increase in the number of trichomes in the leaves (Santoro et al. 2015). Lange and Turner (2013) added that the total count and distribution of trichomes, which are glands for the biosynthesis of oil components, correlated with the production of essential oils in aromatic plants. This means that the high production of *C. nardus* biomass in the treatment of rhizobacteria (P20) might have less trichomes so that the oil component produced is lower. While the treatment without (P0) showed lower biomass production but higher volatile oil components, which might have more total trichomes on leaves.

Citronellal content in *C. nardus* was slightly higher without rhizobacteria treatment compared to its content under rhizobacteria supply. Moreover, in *C. nardus* without-rhizobacteria treatment (P0) and several levels of organic manure supply indicates an increasing citronellal content, as compared to rhizobacteria supply (P20). This is because the availability of carbon and nutrients is converted more quickly by rhizobacteria - due to rhizobacteria being applied at early vegetative phase - thus nutrients are readily available, absorbed, and translocated at 3 and 4 MAP. This is evidenced by the significant effect of adding rhizobacteria 20 mL (P20) in the third and fourth months. It was confirmed by Irving (2015) that the average leaf area and number of leaf affect plant carbon gain which is correlated with plant growth. Moreover, Poorter et al. (2012) stated that the carbon partition for the formation of plant biomass is influenced by the availability of nutrients, temperature, light intensity, and the age of the plant. The dynamics of fresh and dry weight by rhizobacteria treatment (P20) in Figure 9 are represented by the growth rate, which increased sharply at 5 and 6 MAP, particularly for K25 and K30 treatments. N and P were slightly lower accumulated in leaf based on the organic manure application without rhizobacteria (P0) there are at 1,21% Nitrogen and 0,194% Phosphor since lack of the soil microbes compared to their content at organic manure and rhizobacteria (P20) supply. This is evidenced by the fact that, in the rhizobacteria treatment (P20) of 3 and 4 MAP, the primary cycle was more allocated to the formation of the monoterpene cycle, which was followed by the formation of the vegetative parts at 6 MAP. It can be due to the pull of carbon and organic matter in leaf of *C. nardus* which correlate to partitioning of primary and secondary metabolites.

Ganjewala and Luthra (2013) stated that the availability of sucrose or the photosynthesis products directly affect the synthesis of essential oils which will then be determined by the distribution of photosynthetic products and their utilization. Indeed, secondary metabolic response is also based on carbon resource availability (Larbat et al. 2016). The distribution of carbon in plants is followed by a balance between supply (source) and demand (sink) (Fatichi et al. 2014) such as the distribution of carbon to be channeled into primary metabolism and secondary metabolism (Heinrich et al. 2015). While organic manure with various dose levels affects citronellal levels because the results of the carbon synthesis have been channeled to secondary metabolism, namely monoterpene synthesis. The results of photosynthesis, i.e. photosynthates, are used by plants for growth or tissue differentiation and for the production of secondary metabolism, in this case terpenoids. The precursors for biosynthesis of secondary metabolism in plants are supplied from carbon metabolism or referred to as primary metabolism such as photosynthesis (Delphine et al. 2019). Then it is supported by the statement of Chen et al. (2018) that the rate of photosynthesis affects the biosynthesis of carbon and C-based compounds such as terpenoids and phenols.

Hence, the low component of citronellal oil in plants is assumed to be caused by the interaction of plants with rhizobacteria through root exudates, in which rhizobacteria use carbon as energy for nutrient availability. Moreover, reported by Ranade (2015), that the production of essential oil in citronella is found in young leaves which are then accumulated in oil cells evidenced by (Mukarram et al. 2021) with a total oil ranging from 1-2% of the total dry weight. In particular of *Cymbopogon* species, the main constituent is higher in younger tissues, and it decreases along the leaf senescence (Dubey et al. 2000). The main component of *C. nardus*, namely citronellal, belongs to the terpenoid group whose main components are cyclic and acyclic monoterpenes (Mukarram et al. 2021). Formation of oil components in lemongrass through the MEP/MVA pathway (Mukkaram et al. 2021) in the MEP pathway, the biosynthesis of secondary metabolites is determined by the availability of carbon (Banerjee and Sharkey 2015). In the results of the study, the content of monoterpene, accumulated 6 months after planting reached 43,20% without rhizobacteria supply. By contrast with Banchio (2010), that the concentration of monoterpene increases in inoculation with rhizobacteria is possible due to the presence of plant growth-promoting substances produced by microorganisms. Compare to application of 20 mL rhizobacteria treatment, addressed to increasing fresh weight, dry weight, leaf number, and leaf, followed by an increase in plant age. Moreover, more substrate availability will be used for monoterpene biosynthesis (Harrewijin et al. 2001).

The increase in monoterpene concentrations in plants inoculated by PGPR can be attributed to the role of plant growth-promoting substances produced by microorganisms inoculated by plants that affect their metabolic processes. Meanwhile, the essential oil metabolism is regulated by the utilization of photosynthesis products (Srivastava and Luthra 1994). The synthesis of other compounds by plants requires energy, and carbon is the main ingredient. Giri (2003) stated that net photosynthesis of a plant inoculated with PGPR increased due to the availability of nutrients that can be utilized directly by plants. Factors that increase the production of dry matters can affect the relationship between primary and secondary metabolism, which is related to an increase in secondary biosynthesis. In the study of Backer et al. (2018), rhizomicrobiome microbes play an important role in modulating plant growth through the production of hormones, secondary metabolites, and antibiotics as well as various compounds in nutrient acquisition so that the commercial use of rhizobacteria can minimize the use of synthetic fertilizers. Further studies show that PGPR is more effective in increasing the production of essential oils, compared to such production without PGPR, so oils from aromatic plants can be used commercially.

The significant increase in essential oil content at the beginning of plant growth was explained by the ability of bacterial strains to improve mineral nutrient status (Kutlu et al. 2019). The evidence from the former research result that increase in mineral nutrients, particularly N, P, and K, can increase the content of essential oils and modulate the composition of essential oils (Nurzynska 2013). The availability of nutrients in plants inoculated with growth-promoting bacteria accumulate Nitrogen and Phosphorus adsorption is 1,36 % and 0,23% respectively. Meanwhile, without rhizobacteria supply, potassium accumulates at about 0,94% in leaf. Indeed, the energy distribution for increased concentrations of secondary metabolites, including essential oils (Santoro et al. 2016). Irving (2015) states that the allocation of biomass in plants, both in the leaf and root organs, is influenced by nitrogen availability. In grass species, nitrogen assimilation has a significant effect on biomass partitioning (Bloom 2015). In brief that the citronellal component is induced slightly by rhizobacteria through earlier availability of nutrients in the soil due to the bacteria are responsible for inducing the accumulation of various mechanisms of bioactive secondary metabolites (Asghari et al. 2020). Hence it can be concluded, *C. nardus* with only organic manure fertilizer treatment, the content of citronellal could increase 10% at all fertilizer doses except 10 ton ha^{-1} doses. On the other hand, to the application of rhizobacteria which showed an increase citronellal component was relatively the same at all doses of organic manure fertilizer. However, if we look at the average of nutrient uptake, the application of rhizobacteria can elevate the nitrogen and phosphorus nutrient status in leaves. This thing happen due to the material for the formation of oil component, namely carbon is partitioned into the vegetative part which is proven that the organic C content is lower in rhizobacteria treatment.

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