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Research Article

Determination of Nitrogen dose and PGPR concentration to promote growth and flavonoid content of *Ocimum sanctum* L. (Lamiaceae)

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Abstract: *Ocimum sanctum* L. is a plant that contains flavonoids and is often used as a side crop in cultivation. CBS data (2015) show that the number of *O. sanctum* horticulture business households is 195 with a planting area of 303,134 m^2 and the average planting area cultivated per household is 1554 m^2. The flavonoid content in *O. sanctum* plants has benefits, including counteracting free radicals and antibiotics. The problem of *O. sanctum* cultivation, which is only used as a side crop, causes less intensive maintenance, one of which is the lack of attention to nutrition. The application of nitrogen fertilizer and plant growth-promoting rhizobacteria (PGPR) can help spur growth and flavonoid content. The N fertilizer levels used were N 92 kg/ha, N 138 kg/ha and N 184 kg/ha while the PGPR concentration consisted of 0 mL/L, 20 mL/L and 40 mL/L. There was an interaction between the dose of N fertilizer and PGPR concentration on the number of leaves, leaf area and flower emergence time. The form of interaction between the dose of N fertilizer and PGPR concentration is an antagonistic interaction, where the addition of PGPR concentration can reduce the dose of N fertilizer, which is indicated by the lowest dose of N fertilizer accompanied by a higher concentration of PGPR that can increase the growth of *O. sanctum*. N fertilization produced significant differences in fresh weight consumption and flavonoid levels where the 92 kg/ha N fertilizer dose gave 16.33% higher flavonoid levels than the 184 kg/ha N fertilizer dose.

1. INTRODUCTION

Indonesia is a tropical country with a high probability of herbal plants. Herbal plants are widely used by the community because they are believed to cure diseases without causing side effects that are used as medicines but only about 250 species are used in the traditional medicine and pharmaceutical industries (Widaryanto & Azizah, 2018). One of the contents of herbal plants widely used as medicinal raw materials is flavonoids. Flavonoids are natural phenolic compounds with potential antioxidant properties and bioactivity as drugs. Their benefits include protecting cell structures, enhancing the effectiveness of vitamin C, possessing anti-

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inflammatory properties, and preventing bone loss, anti-virus, cardiovascular protection, antibiotics and improvement immunity (Chen *et al.*, 2022).

O. sanctum is a plant that contains flavonoids and is often used as a side crop in cultivation. This is reinforced by Central Bureau of Statistics Indonesia (CBS, 2015), which states that the number of *O. sanctum* horticulture business households is 195, with a planting area of 303,134 m^2, and the average planting area cultivated per household is 1,554 m^2. Based on these data, it is known that the problem of *O. sanctum* cultivation is that it is only used as a side crop, so maintenance is less intensive, one of which is the lack of attention to nutrition (Ramadani, 2020).

The nutrient that is very influential on the growth of *O. sanctum* plants is nitrogen. This is because *O. sanctum* is widely used for its leaves, where nitrogen is crucial for chlorophyll in the leaves. Damayanti *et al*. (2018), in their research, explained that giving nitrogen to *O. sanctum* in the form of NH_4^+ contained in urea can increase the vegetative phase of *O. sanctum*, especially stems, branches and leaves. In addition to nitrogen, Plant Growth Promoting Rhizobacteria (PGPR) can also increase plant growth, yield, and land fertility (Naihati *et al*., 2018). The use of PGPR in agriculture can increase the percentage of nutrient uptake by plants, one of which can help the absorption of urea which can only be absorbed by plants as much as 30-60% while the rest is wasted into the environment (Beig *et al.*, 2020). Based on the research of Ghaffari *et al*. (2018), there is an interaction between PGPR and nitrogen, where the content of living nitrogen-fixing microbes can cause an increase in nitrogen concentration in plants to reduce the input of chemical N fertilizers while increasing the efficiency of N absorption.

Previous research by Nurfitriyah et al. (2022) only examined the impact of nitrogen fertilizer doses of urea origin on the development and productivity of *O. sanctum*. Next, the research of Tahami *et al*. (2017) only examined the concentration of PGPR on the essential oil content of *O. sanctum*. However, research on the effect of N fertilizer and PGPR on the flavonoid content of *O. sanctum* is still rarely studied. Therefore, research is needed to investigate the impact of PGPR concentration and N fertilizer dose on the growth and flavonoid content of *O. sanctum*.

2. MATERIALS and METHODS

The plant materials used were the result of self-cultivation with the application of nitrogen fertilizer and PGPR using Tidore variety, urea fertilizer (46% N), PGPR Mupus (containing *Bacillus* sp., *Pseudomonas* sp., *Azotobacter* sp., *Azospirillum* sp. each as much as 1×10^8 CFU/mL) which was commercially obtained, manure, 70% ethanol, Whatman no. 42 filter paper, Quercetin Standard, 10% $AlCl_3$, 1 M sodium acetate, and distilled water. The experiments were conducted in two locations, observations of growth variables were carried out at the Screenhouse of the Agricultural Instrument Standardization Agency, East Java (Figure 1) while yield variables were carried out at the Plant Physiology Laboratory, Faculty of Agriculture, Brawijaya University.

Figure 1. Screenhouse environmental conditions.

2.1. Research Design and Data Analysis

This study used a Randomized Group Design (RGD) with 3 replications of 2 factors (Table 1). The first factor was the dose of N fertilizer using urea, consisting of 3 levels which are N1 = N 92 kg/ha; N2 = N 138 kg/ha; N3 = N 184 kg/ha, and the second factor was PGPR concentration consisting of 3 levels which were P0 = without PGPR application; P1 = PGPR 20 mL/L; P2 = PGPR 40 mL/L.

Based on these two factors, 9 treatment combinations were obtained in each replication. The placement of each treatment was randomized and repeated 3 times, resulting in a total of 27 experimental units. Each treatment consisted of 28 plants, resulting in 756 plants in the experiment. The data analysis employed the analysis of variance or F test at the 5% level, and the significantly different results were followed by a comparison test between treatments using the 5% HSD further test.

Table 1. Experimental design.

The Dose of N fertilizer	The concentration of PGPR		
	P0	P1	P2
N1	N1P0	N1P1	N1P2
N2	N2P0	N2P1	N2P2
N3	N3P0	N3P1	N3P2

N1 = N 92 kg/ha; N2 = N 138 kg/ha; N3 = N 184 kg/ha; P0 = without PGPR application; P1 = PGPR 20 mL/L; P2 = PGPR 40 mL/L

2.2. The Preparation on N Fertilizer and PGPR

The preparation of N fertilizer began with the calculation of fertilizer requirements for each plant. Calculation of N fertilizer needed, in this case researchers used urea fertilizer which contained 46% of Nitrogen, it was necessary to convert N needs into urea which needed using the following formula:

a) Conversion of urea fertilizer requirement

$$\frac{100}{46} \times t$$

Whereas: t = treatment

b) Calculation of urea fertilizer requirement per plant

Calculation of fertilizer requirements per plant was carried out in the same way as the calculation of urea fertilizer requirements.

Plant spacing = 20 ×20 cm

Populasi within 1 Ha $= \frac{10.000\ m^2}{20\ cm \times 20\ cm} = 250.000$

Calculation:

$(\frac{100}{46} \times t) \times 2$ times of application

Whereas: t = treatment

The preparation PGPR preparation began with calculating PGPR requirements per plant using the following dilution formula:

$$M1 \times V1 = M2 \times V2,$$

Whereas: M1 = initial concentration; V1 = Initial water volume; M2 = Concentration to be obtained; V2 = Volume of water added.

2.3. The Application of The Treatment

The application of N fertilizer and PGPR explained in the following table:

Table 2. Timing of treatment application.

Treatment type	Timing (DAP)	Application method	Dosage and concentration
PGPR application	-2	Watering at plant roots	1.68 mL/L (from 20 mL/L initial concentration); 3.36 mL/L (from 40 mL/L initial concentration)
PGPR application	23	Watering at plant roots	Repeat the concentration above
N fertilizer application	7	Soil application around roots	1.74 g/plant (N 92 kg/ha); 2.6 g/plant (N 138 kg/ha); 3.5 g/plant (N 184 kg/ha)
N fertilizer application	25	Soil application around roots	Repeat dosage as above

DAP = Days after planting

2.4. Flavonoid Content Analysis

Flavonoid content analysis of basil plant leaves was carried out using the maceration method according to the Indonesian Herbal Pharmacopoeia edition 2 (Ministry of Health Republic of Indonesia, 2017). The analysis of flavonoid levels began with drying *O. sanctum* leaves at 40^{o}C for 24 hours using an oven and then were mashed using a blender. *O. sanctum* powder was then weighed as much as 1 g. 1 g of *O. sanctum* powder was then macerated in 10 mL of 70% ethanol until submerged for 24 hours. The filtrate was then filtered using Whatman paper. Furthermore, quantitative analysis of flavonoid content by making a blank solution by mixing 1.5 mL of 70% ethanol, 0.1 10% $AlCl_3$, 0.1 mL of 1M sodium acetate, and 1M sodium acetate, sodium acetate 1M, and 2.8 mL of distilled water, followed by a 30 -minute incubation at room temperature. Standard solution was then made by mixing 50 mg of Quercetin Standards with 125 mL of distilled water, and then, made a dilution series of 75 ppm, 100 ppm, 125 ppm, 150 ppm, and 175 ppm. The sample solution was prepared by dissolving 1 g of *O. sanctum* leaf extract in 10 mL of ethanol. All solutions were subsequently measured for absorbance at a wavelength of 425 nm. Subsequently, the flavonoid content was analyzed using the following formula:

a) Calculate the flavonoid concentration in the extract solution equivalent to the Quercetin concentration, based on the regression equation from the standard curve of the Quercetin-$AlCl_3$ complex

$y = ax + b$
$ax = y-b$
$x = (y-b)/a$

Whereas: x = Flavonoid concentration in the extract solution; y = Sample absorbance value

b) Calculate FC (mgQE/g) using the formula:

$$FC = \frac{C\ (mL) \times X\ (mg/mL) \times DF}{m}$$

Whereas: FC = Flavonoid content; V= volume of sample (10 mL); X = flavonoid concentration in the extract solution; DF, dilution formula; m = mass of the sample (g).

3. RESULTS

3.1. Growth Variables

The doses of N fertilizer and PGPR concentrations gave significant differences in the number of leaves, leaf area and number of primer branches. The growth pattern of the number of leaves of *O. sanctum* plants increased linearly with increasing plant age (Figure 2). The application of

N fertilizer at a dose of 92 kg/ha accompanied by PGPR at a concentration of 40 mL/L produced a higher number of leaves and significantly differed from PGPR at concentrations of 0 mL/L and 20 mL/L (Figure 2a). On the other hand, the addition of 138 kg/ha of N fertilizer accompanied by PGPR with a concentration of 20 mL/L produced a higher number of leaves and was significantly different compared to PGPR at 0 mL/L and 40 mL/L (Figure 2b). While the addition of 184 kg/ha N fertilizer dose did not give a significant difference in the number of leaves at different PGPR concentrations (Figure 2c).

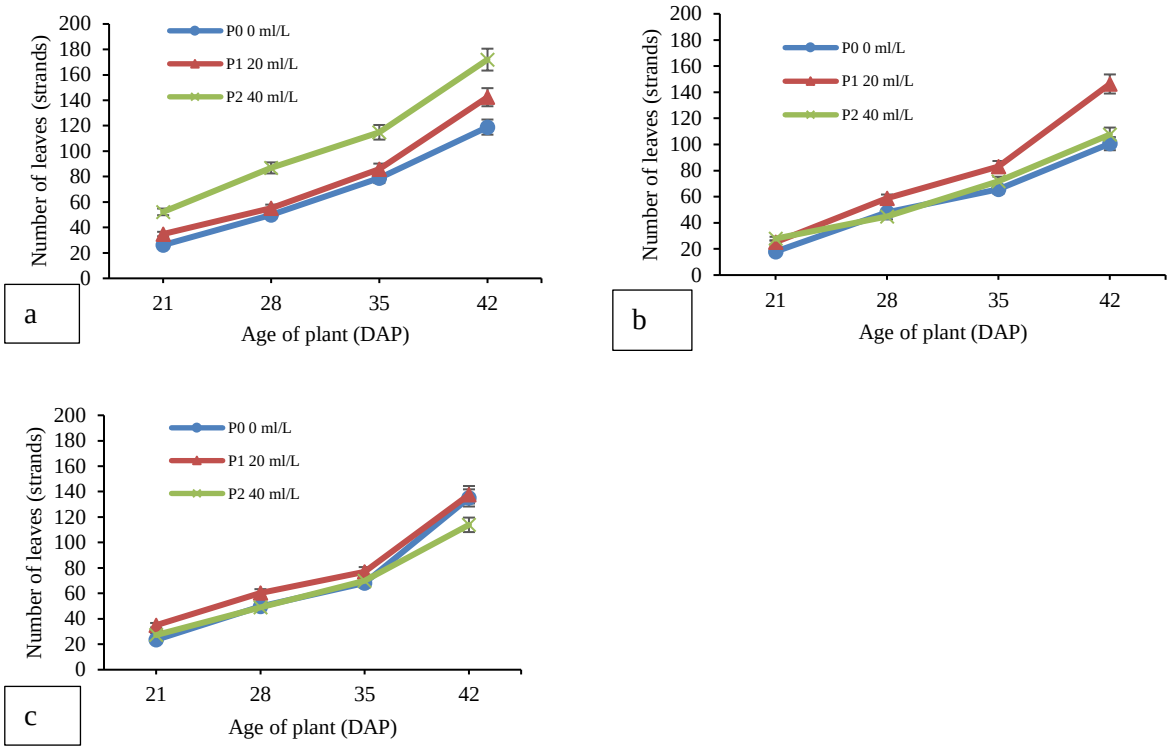

Figure 2. The growth pattern of *O. sanctum* number of leaves at doses of N fertilizer (a) 92 kg/ha; (b) 138 kg/ha; (c) 184 kg/ha with the addition of various concentrations of PGPR

N fertilizer dose of 92 kg/ha accompanied by PGPR concentration of 40 mL/L gave an effect on the number of leaves that was significantly different compared to the application of N fertilizer dose of 138 kg/ha accompanied by PGPR 0 mL/L and 40 mL/L with a percentage increase of 70.92% and 59.91% respectively, and N fertilizer dose of 184 kg/ha + PGPR concentration of 40 mL/L with a percentage increase of 60% but was not significantly different from the other treatments (Table 3).

Table 3. Number of leaves (strands).

Treatment	Number of leaves (strands) at 42 DAP		
	PGPR concentration (mL/L)		
	0	20	40
N fertilizer dosage (kg/ha)			
92	118.82 ab	142.42 ab	171.97 b
138	100.61 a	146.28 ab	107.54 a
184	135.06 ab	137.56 ab	113.89 a
HSD 5%		55.13	
CV (%)		14.55%	

Numbers sharing the same letter within the identical column and treatment do not display significant differences according to the 5% HSD test; DAP = days after planting; HSD = honestly significant difference; CV = coefficient of variation

The growth pattern of leaf area of *O. sanctum* plants increased in line with the increasing age of observation (Figure 3). *O. sanctum* fertilized with N at a dose of 92 kg/ha accompanied by PGPR with a concentration of 40 mL/L produced a higher leaf area and was significantly different from that without PGPR and PGPR 20 mL/L (Figure 3a). *O. sanctum* fertilized with N at a dose of 138 kg/ha accompanied by PGPR with a concentration of 20 mL/L gave a higher leaf area compared with no PGPR and PGPR 40 mL/L (Figure 3b). In addition, the 184 kg/ha dose of N fertilizer without the addition of PGPR produced a higher leaf area and was significantly different compared to the addition of PGPR (Figure 3c).

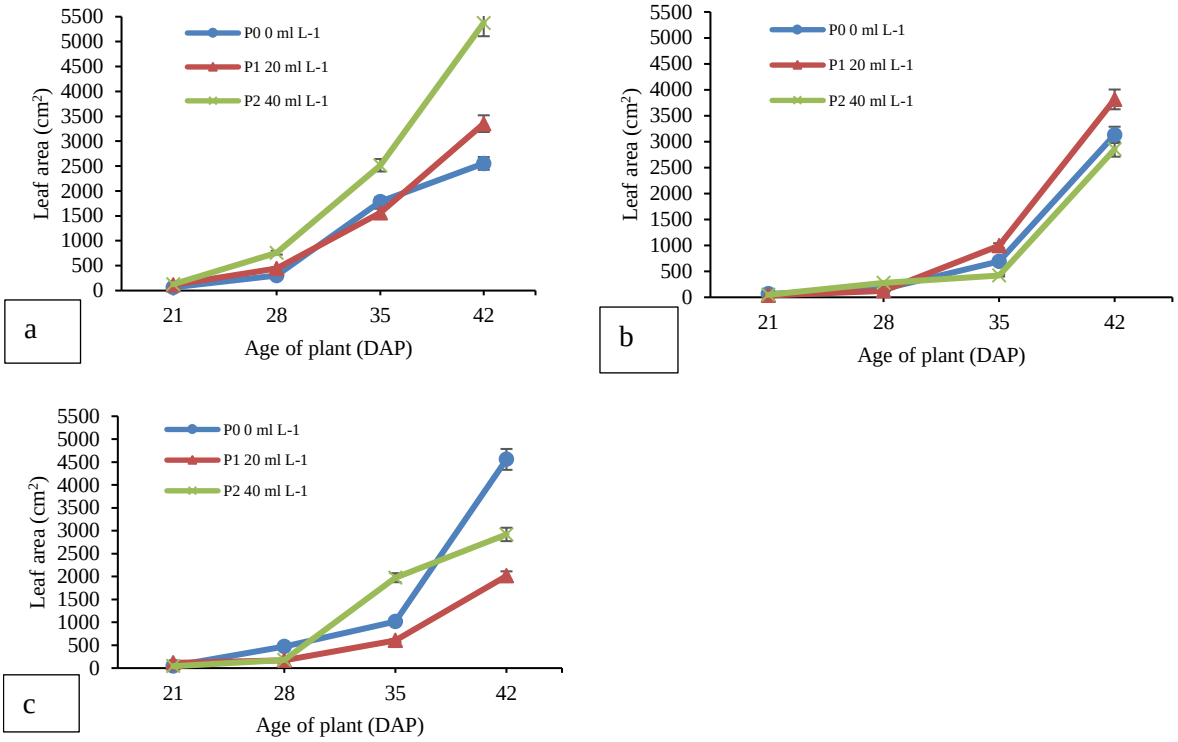

Figure 3. The growth pattern of *O. sanctum* leaf area at doses of N fertilizer (a) 92 kg/ha; (b) 138 kg/ha; (c) 184 kg/ha with the addition of various concentrations of PGPR.

The application of N fertilizer dose of 92 kg/ha accompanied by PGPR concentration of 40 mL/L resulted in higher leaf area compared to other treatments, but not significantly different from the application of N fertilizer dose of 184 kg/ha with PGPR concentration of 0 mL/L. On the other hand, the application of N fertilizer dose of 138 kg/ha with PGPR concentrations of 20 mL/L, 40 mL/L and without PGPR did not have a significant effect on leaf area. Furthermore, the application of 184 kg/ha N fertilizer dose without PGPR produced a larger leaf area than those given PGPR at various concentrations (Table 4). This indicates that *O. sanctum* plants fertilized with higher doses of N are not responsive to PGPR application.

Table 4. Leaf area (cm^2).

Treatment	Leaf area (cm^2) at 42 DAP		
	PGPR concentration (mL/L)		
	0	20	40
N fertilizer dosage (kg/ha)			
92	2553.86 ab	3352.45 bc	5376.77 e
138	3132.48 abc	3814.38 cd	2851.65 abc
184	4557.77 de	2012.51 a	2919.80 abc
HSD 5%	1129.05		
CV (%)	11.44%		

Numbers sharing the same letter within the identical column and treatment do not display significant differences according to the 5% HSD test; DAP = days after planting; HSD = honestly significant difference; CV = coefficient of variation

The number of primary branches of *O. sanctum* in N fertilizer at a dose of 92 kg/ha accompanied by PGPR at a concentration of 40 mL/L produced a higher number of primary branches and was significantly different compared with no PGPR and PGPR 20 mL/L (Figure 4a). *O. sanctum* treated with N fertilizer at a dose of 138 kg/ha accompanied by PGPR 20 mL/L produced more primary branches than without PGPR 0 mL/L and PGPR 40 mL/L (Figure 4b). On the other hand, *O. sanctum* fertilized with N at a dose of 184 kg/ha accompanied by PGPR at a concentration of 20 mL/L produced a higher number of primary branches but not significantly different without PGPR and PGPR 40 mL/L (Figure 4c).

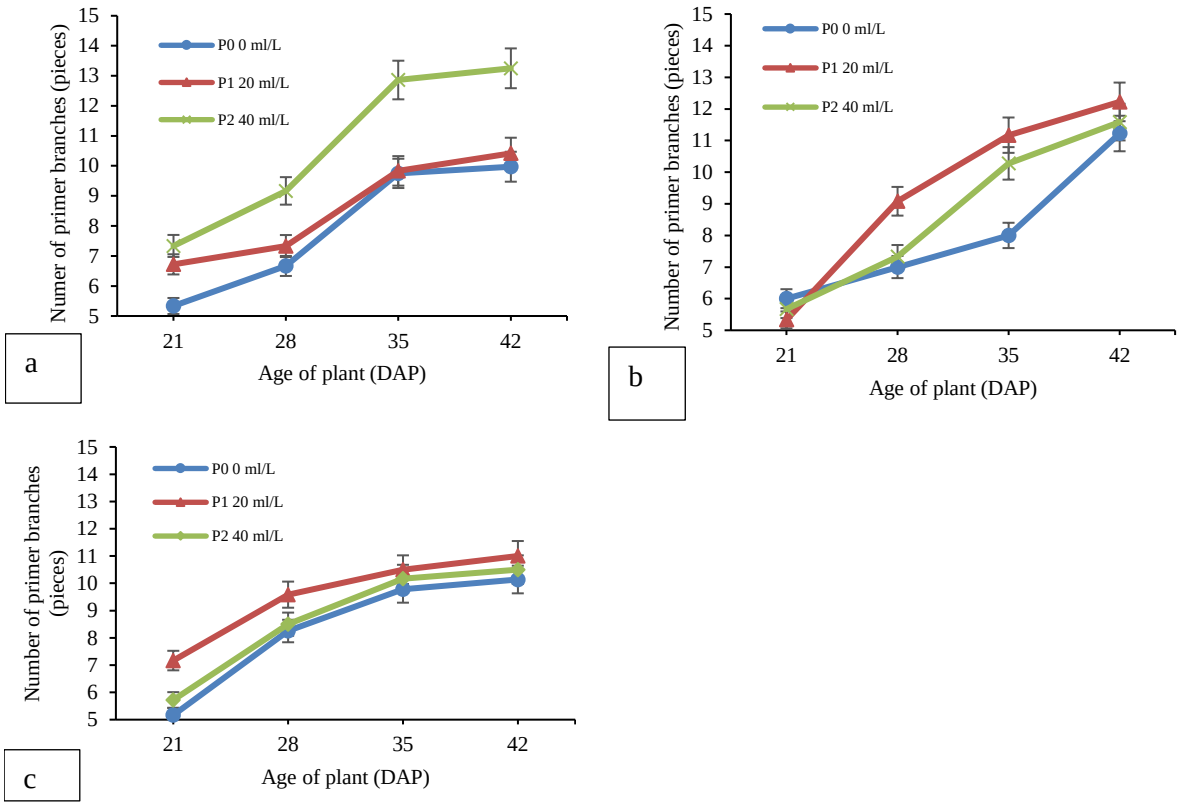

Figure 4. The growth pattern of *O. sanctum* number of primer branches at doses of N fertilizer (a) 92 kg/ha; (b) 138 kg/ha; (c) 184 kg/ha with the addition of various concentrations of PGPR.

The results showed that the application of N fertilizer dose of 92 kg/ha with PGPR concentration of 40 mL/L produced a higher number of primary branches and was significantly different from the application of PGPR concentration of 20 mL/L and even without PGPR. In addition, the application of N fertilizer doses of 138 kg/ha and 184 kg/ha both with PGPR and without PGPR did not affect the number of primary branches (Table 5). This is presumably because at higher doses of N fertilizer, *O. sanctum* plants are no longer responsive to the application of PGPR because their N needs have been met.

Table 5. Number of primer branches (units)

Treatment	Number of primer branches (units) at 42 DAP		
	PGPR concentration (mL/L)		
	0	20	40
N fertilizer dosage (kg/ha)			
92	9.97 a	10.42 a	12.86 b
138	11.22 ab	12.22 ab	11.58 ab
184	10.14 a	11.00 ab	10.17 a
HSD 5%		2.34	
CV (%)		7.29%	

Numbers sharing the same letter within the identical column and treatment do not display significant differences according to the 5% HSD test; DAP = days after planting; HSD = honestly significant difference; CV = coefficient of variation

3.2. Growth Variables

PGPR application did not produce significant differences in fresh weight and flavonoid levels, but N fertilization produced significant differences. *O. sanctum* fertilized with N at a dose of 92 kg/ha produced a higher fresh weight of *O. sanctum* than the N fertilizer dose of 138 kg/ha but not significantly different from the N fertilizer dose of 184 kg/ha. There was an increase of 44.99% by using 184 kg/ha N fertilizer dose compared to 138 kg/ha fertilizer dose at 49 DAP (Table 6).

Table 6. Fresh weight (g)

Treatment	Fresh weight basil plants (g) at observation age (DAP)	
	49	56
N fertilizer dosage (kg/ha)		
200	19.36 b	22.32
300	13.66 a	20.54
400	19.67 b	21.42
HSD 5%	5.30	ns
PGPR concentration (mL/L)		
0	14.36	19.35
20	20.08	22.43
40	18.26	22.50
HSD 5%	ns	ns
CV (%)	28.01	17.19

Numbers sharing the same letter within the identical column do not display significant differences according to the 5% HSD test; DAP = days after planting; HSD = honestly significant difference; CV = coefficient of variation

The flavonoid content analyzed via the standard curve (Figure 5) shows that *O. sanctum* plants fertilized with a dose of 92 kg/ha produced higher flavonoid levels of 16.33% compared to the N fertilizer dose of 184 kg/ha but not significantly different compared to the N fertilizer dose of 138 kg/ha at 56 DAP (Table 7).

Table 7. Flavonoid levels (mgQE/g)

Treatment	Flavonoid levels of basil plants (mgQE/g) at observation age (DAP)	
	49	56
N fertilizer dosage (kg/ha)		
200	0.41	0.57 b
300	0.39	0.49 a
400	0.40	0.49 a
HSD 5%	ns	0.074
PGPR concentration (mL/L)		
0	0.41	0.54
20	0.40	0.52
40	0.39	0.49
HSD5%	ns	ns
CV (%)	10.33	12.03

Numbers sharing the same letter within the identical column do not display significant differences according to the 5% HSD test; DAP = days after planting; HSD = honestly significant difference; CV = coefficient of variation

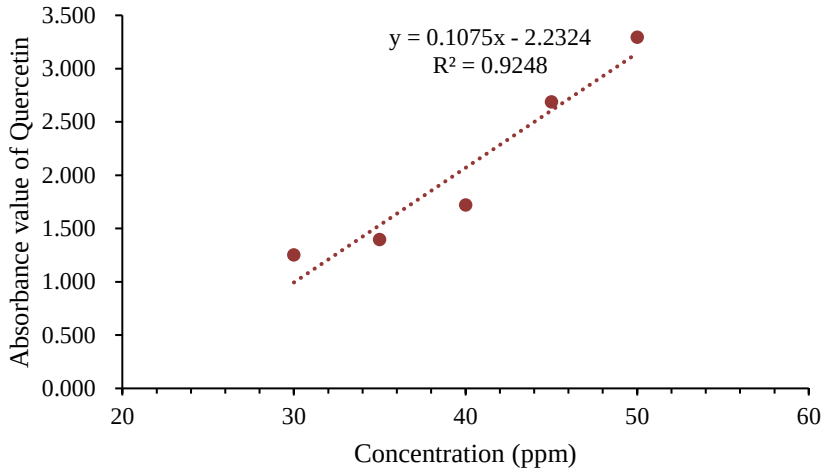

Figure 5. Standard curve of Quercetin

4. DISCUSSION and CONCLUSION

The results showed that the interaction between the dose of N fertilizer and PGPR concentration significantly affected the number of leaves, leaf area and number of primary branches of *O. sanctum*. The dose of N fertilizer 92 kg/ha and PGPR with a concentration of 40 mL/L produced higher growth of *O. sanctum* plants compared to the fertilizer doses of 138 kg/ha and 184 kg/ha. This shows that the application of PGPR with higher concentrations can help the absorption of plant nutrients and increase the absorption efficiency of N fertilization. Paungfoo-lonhenne *et al*. (2019) explain that the addition of PGPR can increase nutrient absorption while reducing the leaching of N fertilizer. The increased efficiency of N fertilizer absorption has an impact on *O. sanctum* growth variables such as the number of leaves, leaf area and number of primary branches. Leaf area is directly proportional to the number of leaves, where the more the number of leaves, the wider the leaf area will be. On the other hand, the number of leaves is also related to the number of primary branches because *O. sanctum* leaves grow from the primary branches. The treatment of 92 kg/ha N fertilizer dose coupled with 40 mL/L PGPR concentration gave good results on the number of leaves, leaf area and the number of primary branches. PGPR with the right concentration can produce and facilitate the absorption of nutrients needed in plant growth (Kurniahu *et al*., 2018). Therefore, giving a dose of N fertilizer 92 kg/ha accompanied by PGPR with a concentration of 40 mL/L can provide better results.

N fertilization at a dose of 92 kg/ha accompanied by PGPR 40 mL/L produced a higher number of leaves and was significantly different from the dose of N fertilizer 138 kg/ha without PGPR, the dose of N fertilizer 138 kg/ha + PGPR 40 mL/L and the dose of N fertilizer 184 kg/ha + PGPR 40 mL/L but not significantly different from the other treatments. Fertilizer dose with the lowest level, accompanied by PGPR with the highest PGPR concentration, resulted in interaction and increased higher *O. sanctum* growth. *O. sanctum* that has dense leaves will produce more photosynthate. The large number of leaves on *O. sanctum* is due to adequate nutritional needs as well as favorable environmental conditions. Poorter *et al*. (2019) explained that environmental factors such as temperature, humidity, light intensity and availability of nutrients can affect leaf growth and development. In addition, the nutrient N can also affect the number of leaves because the Nitrogen plays a part in the development of leaf organs and chlorophyll pigments that play a role in the photosynthesis process, and then the results of photosynthetic assimilation will be directed to plant growth and development (Marschner, 2012). Nutrient availability is related to feeding, where PGPR bacteria are able to enhance the accessibility of nutrients in the media while producing phytohormones such as auxins, cytokinins and gibberellins that play a role in regulating the process of leaf formation and development (Rosyida & Nugroho, 2017). PGPR can increase soil nutrients through N fixation

and phosphate solubilization mechanisms (Choliq *et al.*, 2020). Therefore, the interaction between N fertilizer and PGPR can affect the number of *O. sanctum* leaves.

The number of leaves is related to the leaf area, where the observation results show that the wider leaf area is produced from the treatment of 92 kg/ha N fertilizer dose plus 40 mL/L PGPR concentration. The large number of leaves will increase the accumulation of photosynthate to be used for plant growth and development, one of which increases leaf area. When plants have more leaves, they capture more light, leading to increased photosynthesis and subsequently higher photosynthate production. This surplus energy and carbon source fuels cellular activities and biomass accumulation, contributing to overall growth and greater leaf area (Weraduwage *et al.*, 2015). This is also due to the fulfilment of nutritional needs, one of which is the element N, as the formation of chlorophyll, in this case, the element N is added through the provision of N fertilizer from urea. In addition to N fertilizer, the provision of nutrients is also assisted by PGPR, where PGPR is able to provide nutrients to the planting media while helping the absorption of nutrients through the mechanism of stimulating root growth. Kurniahu *et al*. (2018) explained that the application of PGPR with the right concentration can produce and facilitate the absorption of nutrients needed in plant growth, including an increase in the number of leaves, which then affects the leaf area.

The number of primary branches is a growth variable that affects the number of leaves because the leaves on the *O. sanctum* emerge from the primary branches where the dose of N fertilizer 92 kg/ha accompanied by PGPR with a concentration of 40 mL/L produces a higher number of branches and is significantly different compared to the provision of PGPR at a concentration of 20 mL/L and without PGPR, the dose of N fertilizer 184 kg/ha accompanied by PGPR with a concentration of 40 mL/ and without PGPR, but not significantly different from the other treatments. The number of primary branches is influenced by the availability of nutrients, suitable environmental conditions and genetic factors. According to Gigir *et al*. (2014) nitrogen is an important element in supporting plant vegetative growth, including primary branches. Adequate nitrogen fertilization will increase photosynthate production, which will then affect the number of primary branches of *O. sanctum*. In addition, PGPR also contributes to determining the number of primary branches of *O. sanctum*, where groups of bacteria contained in PGPR can increase plant growth through the mechanism of growth hormone production and increased nutrient availability (Tahami *et al.*, 2017). The interaction between N fertilizer and PGPR can affect the number of primary branches where N fertilizer supports vegetative growth while PGPR increases the efficiency of N fertilizer use.

N fertilization of 92 kg/ha gave higher results in increasing flavonoid levels compared to the N fertilizer dose of 184 kg/ha. There is an opposite relationship between the application of N fertilizer and flavonoid levels, the observation results indicate that the application of less N fertilizer can increase flavonoid levels. In accordance with the research of Yang *et al*. (2018), which states that nutrient deficiencies, including nitrogen, increase the accumulation of flavonoid compounds, the more nitrogen given to plants, the less flavonoid compounds produced. Flavonoid biosynthesis in the plant body is significantly affected by environmental factors, one of which is nutrients, namely nitrogen. Plant growth with low nitrogen concentration shows increased expression of flavonoid biosynthesis genes, the enzymes chalcone synthase and isoflavone reductase (Pratiwi, 2017). However, in flavonoid biosynthesis, N is still needed for the formation of amino acids as precursors in flavonoid biosynthesis (Saifudin, 2014).

The research results reveal that there was an interaction between the dose of N fertilizer and PGPR concentration on the number of leaves, leaf area and number of primary branches. The form of interaction between the dose of N fertilizer and PGPR concentration was in the form of antagonistic interaction, where the addition of PGPR concentration could reduce the dose of N fertilizer, which was indicated by the lowest dose of N fertilizer with a higher concentration of PGPR that could increase the growth of *O. sanctum*. On the other hand, there was no interaction

between the dose of N fertilizer and PGPR concentration on the variable yield of *O. sanctum*, but N fertilization produced significant differences in fresh weight and flavonoid content where the dose of N fertilizer 92 kg/ha gave 16.33% higher flavonoid content than the dose of N fertilizer 184 kg/ha.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Sofika Rahmadani: Conception, Data Collection, Resources, Visualization, Interpretation, and Writing. **Anna Satyana Karyawati**: Methodology, Supervision, and Validation.

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