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Effect of PGPM and goat manure doses on biomass yield and rosmarinic acid content in cat's whiskers plant (*Orthosiphon aristatus* Bl. Miq)

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ABSTRACT

Cat's whiskers plant (*Orthosiphon aristatus* B. Miq) is a medicinal plant containing various metabolites that act as antioxidants and anti-inflammatory, including rosmarinic acid. The domestic market demand for dry leaves was 20-40 tons and abroad it was 141-180 tons in the 1990-2005 period, while production from 2013 to 2017 decreased from 116 tons to 56 tons. Therefore, it is necessary to increase the cat's whiskers biomass production, one of which can be done through the engineering of growing media using organic fertilizers, including goat manure and microbes, e.g., Plant Growth Promoting Microorganisms (PGPM). This study aims to determine the optimal combination of PGPM – Goat Manure for biomass and the rosmarinic acid content in *O. aristatus*. The study was conducted using a 2-factor randomized block design (RBD) with 12 treatment combinations and 3 repetitions on cultivated land in the Tanjungsari area and analysis of the results was carried out in the SITH laboratory from October 2020 to March 2021. The rosmarinic acid content in the leaves ranged from 144.43 – 206.31 mg/g or 14.44 – 20.63%, with the highest content found in leaves treated with 3 ml/L PGPM and 86.2 g/plant goat manure fertilizer (P_2O_2).

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1. Introduction

Cat's whiskers plant (*Orthosiphon aristatus* M.) is a woody perennial plant from the Lamiaceae family with morphology in the form of roots, stems, leaves, flowers, and seeds (Febjislami et al., 2019). *O. aristatus* leaves can be used as ingredients for medicines (Faramayuda, 2020). In addition, *O. aristatus* contains various kinds of secondary metabolites, one of which is rosmarinic acid. The compound has been reported to exhibit bioactivities, including anti-inflammatory and antioxidant (Kim et al., 2015). This makes *O. aristatus* a potential source of medicine for the treatment of various diseases.

Based on the data from West Java Center Bureau of Statistic (BPS) in 2017, there was a decrease in the production area of *O. aristatus* from 260 ha to 217 ha during 2013 to 2017. Furthermore, and a decrease in biomass production of *O. aristatus* leaves from 116 tons to 56 tons was recorded during 2013 to 2017. Meanwhile, the average market demand for dry leaves was 20 - 40 tonnes/year for domestic industry (Pribadi, 2009) and 141.1 – 180 tonnes/ha in for export during 1990 – 2005 (Aminudin, 2005). Therefore, it is still necessary to meet the demand for the simplicia production of *O. aristatus* leaves through a sustainable cultivation system.

Soil fertility is an important factor for the sustainability of crop production systems. Plant growth and yields are highly dependent on growing media in providing optimal nutrients (Supangat et al., 2013). Plant Growth Promoting Microorganisms (PGPM) are soil microbes consisting of bacteria and fungi that live in habitats in plant root areas (rhizosphere) which plays a role in increasing plant growth both directly and indirectly (Utami et al., 2018) through its role as biofertilizer (Kundan et al., 2015), bioprotectant (Mishra et al., 2017), and biostimulant (Odoh, 2017). In addition to PGPM, goat manure added to plant growth media also supports the supply of nutrients and organic matter needed by plants and PGPM to grow, reproduce, and produce growth-promoting compounds as well as provide nutrients (Shofiyah et al., 2018). Goat manure can improve soil physical characteristics by improving pore space and soil permeability, increasing soil oxygen levels, and increasing available water holding capacity. Decomposition of goat manure can increase the stability of soil aggregates and soil granules so that plant root growth can be optimal (Hartatik, and Widowati, 2008).

In this study, various doses of PGPM and goat manure mixed with soil were used as a medium for growing *O. aristatus*. This study aims to determine the effects of PGPM and goat manure on the plant biomass of *O. aristatus* and rosmarinic acid content in *O. aristatus* leaves.

2. Materials and methods

2.1. Chemicals and reagents

The materials used in this study were purple flowering *O. aristatus* seeds aged 6 weeks, PGPM "Tricoganik" solution, Decis 25 EC pesticide, polybags measuring 30 x 30 cm, goat manure, and soil as a planting medium. The materials used in the determination of rosmarinic acid contecnt in *O. aristatus* included aluminum foil, 0.1% formic acid, acetonitrile (AcN), standard rosmarinic acid solution, 50% methanol (MeOH), 70% MeOH, and *O. aristatus* leaves samples.

O. aristatus cultivation is carried out at ricefields in Kutamandiri Village, Tanjungsari District, Sumedang Regency, West Java with the location coordinates of being 6°54'43.8"S; 107°47'07.7"E; elevation of 846 meters above sea level (masl). Parameters were observed and tested at the cultivation area, Downstream Process Engineering Laboratory at ITB Jatinangor Campus, and SITH Instrumentation Laboratory at ITB Ganesha Campus.

2.2. Procedures

This study used a factorial randomized block design (RBD) consisting of two factors, namely the dose of PGPM (P) and the dose of goat manure (O). Each treatment consisted of three replications and four units of *O. aristatus*. The combination of treatments can be seen in Table 1. The contents of manure have been analyzed at the Vegetable Crops Research Agency (BALITSA), Lembang, Bandung.

Table 1. Treatment combination of PGPM – goat manure

PGPM (P)	Goat manure dose (O)			
Concentration	0 g (O_1)	86.2 g (O_2)	172.4 g (O_3)	344.8 g (O_4)
0 ml/l (P_1)	P_1O_1	P_1O_2	P_1O_3	P_1O_4
3 ml/l (P_2)	P_2O_1	P_2O_2	P_2O_3	P_2O_4
6 ml/l (P_3)	P_3O_1	P_3O_2	P_3O_3	P_3O_4

2.3. *O. aristatus* cultivation

Cultivation of *O. aristatus* was carried out on an area of 60 m^2 using polybags 30 x 30 cm with 4 kg of soil for each bag. The growth medium used was ricefield soil with the addition of a combination of PGPM and goat manure. The cultivation method followed the Standard Operational Procedure (SOP) for the planting of *O. aristatus* (Widaryanto and Azizah, 2018; Dinas Pertanian Jogjakarta, 2018).

Table 2. Types and concentration of microbes in PGPM

Microorganism type	Concentration
Bacillus sp.	7 x 10^2 cfu/ml
Pseudomonas sp.	4 x 10^2 cfu/ml
Rhizobium sp.	6 x 10^2 cfu/ml
Beauveria sp.	4 x 10^2 cfu/ml
Trichoderma sp.	5 x 10^2 cfu/ml

(Source: Tricoganik, 2020)

2.4. Collection of microclimatic and edaphic data

Microclimatic data in the form of daily average air temperature, daily average air humidity, and daily average rainfall were taken indirectly (secondary data) via the NASA POWER climatology website (https://power.larc.nasa.gov/data-access-viewer/). The edaphic data of cultivation in the field in the form of daily average soil temperature and daily average soil pH were taken directly (primary data) using the 4-in-1 Soil Survey. In addition, laboratory tests were carried out on the soil used at the Vegetable Crops Research Agency (BALITSA), Lembang, Bandung.

2.5. Measurement of growth variables

The height of *O. aristatus* plant was measured once a week by measuring the length from the base of the stem to the tip of the top leaf. Leaf area was measured by taking pictures of the top, middle, and bottom leaf samples next to a ruler and then processing them using the ImageJ application to obtain the leaf area. The number of leaves and the number of nodes were recorded manually by counting and recording them in the observation table.

2.6. Analysis of chlorophyll content is *O. aristatus* leaves

Measurement of total chlorophyll content of *O. aristatus* was carried out in the last week of observation or 8 weeks after planting (WAP). *O. aristatus* leaves were weighed 1 g for each treatment and replication. The sample was crushed in a mortar and then was extracted by adding 50 ml of 96% alcohol until all the chlorophyll was dissolved (5 min). The extract was then filtered using filter paper. The filtrate was transferred into a 100 ml volumetric flask and then 96% alcohol was added to a volume of 100 ml. The solution was analyzed for chlorophyll content using a spectrophotometer at wavelength of 649 nm and 665 nm. The absorbance values were then converted into units of mg/l using the following formula:

$$\text{Total chlorophyll (mg/l)} = (20 \times OD_{649} + 6.1 \times OD_{665})$$

2.7. Biomass measurement of *O. aristatus*

Measurement of the biomass of *O. aristatus* was carried out at 2, 4, 6, and 8 WAP by destruction of each treatment unit and replicates. Biomass measurement was divided into the measurement of wet and dry biomass. Wet biomass measurement was carried out by weighing the wet weight of leaves, stems, and roots, while dry biomass measurement was conducting by drying the sample in oven at 105°C for 24 h.

2.8. Measurement of water content and dried sample yield

The water content is the percentage of water content of the leaf organ calculated using the following formula (Efendi, 2016):

$$Water\ content\ (\%) = \frac{wet\ weight - dry\ weight}{wet\ weight} \times 100\%$$

The dried sample yield was measured using the following formula:

$$Dried\ sample\ (\%) = \frac{dry\ weight}{wet\ weight}\ x\ 100\%$$

2.9. Analysis of rosmarinic acid content

Dried leaves were grounded into powder and 100 mg of them was extracted with 2 ml of 70% methanol in an ultrasonic water bath at 50ºC for 15 min. The methanol extracts were then centrifuged at 1500 rpm for 3 min to separate the solid residue. The supernatants were decanted for HPLC analysis. The chromatography analysis was performed using LC-20 system equipped with UV Vis SPD 20A detector, CTO-20A oven column, SIL-20AC autoinjector, and LC-20AT pump (Shimadzu, Japan). The separation was done using reverse-phased column C18 (Shim-pack GIST 4.6x250 mm, 5μm, Shimadzu) with the mobile phase consisting of 0.1% formic acid (A) and acetonitrile (B). The elution was performed in a gradient mode starting from 15% B (0-1 min); 15-65% B (1-12 min); 65-15% B (12-15 min) and hold 15% B (15-25 min) with a flow rate 1 ml/min. The column temperature was at 40ºC, injection volume was 20 μl, and UV wavelength was at 320 nm. The standard curve of rosmarinic acid was measured at 0.5-100 ppm. The chromatogram data obtained were processed using the Shimadzu Lab Solutions software.

2.10. Data Analysis and Visualization

The data are presented in the form of data tabulations and analysis graphs for each measurement parameter of *O. aristatus* biomass and rosmarinic acid content. Statistical testing was carried out using a 2-factor 2-Way ANOVA (Analysis of Variance) test and continued with the DMRT (Duncan Multiple Range Test) follow-up test. From the results of the 2-Way ANOVA and DMRT analysis, conclusions were given for each parameter tested.

3. Results and discussion

3.1. Edaphic analysis on research site

Based on the actual edaphic data collection, the average temperature at the research site is in the range of 25.67 – 27.33°C, the average soil pH is 4.83 – 6.50, and the average soil moisture is 50 – 93%. Furthermore, the initial laboratory edaphic test results revealed that the soil has a dusty clay texture. The actual and potential pH of the soil were 6.2 and 5, respectively. The C-organic and N (nitrogen) content were 2.34% and 0.24%, respectively, while the C/N ratio value was 10.

Differences in the use of goat manure doses in *O. aristatus* media affected temperature dynamics of the soil. The higher the organic matter given, the higher the water holding capacity of the soil particles. Moreover, the organic matters will increase the heat absorption capacity of the soil, rendering the soil temperature to raise. In addition, the activity of soil microbes such as PGPM in *O. aristatus* media is highly dependent on soil temperature. Soil pH affects the availability of nutrients and the toxicity of compounds in the soil, as well as plays a role in various reaction mechanisms in plant root proptoplasm thereby affecting plant growth (Soti et al., 2015). The addition of organic matter and the decomposition of organic matter in the soil will increase the binding capacity between soil particles and water and increase the pore space in the soil, so that the soil water content can be better maintained.

3.2. Microclimate analysis on research site

Based on the results of NASA's microclimate data collection, the daily average air temperature was 23.5°C, the daily average relative humidity was 85%, and the daily average rainfall was 28 mm/day.

3.3. Chemical and elemental analysis of goat manure

Based on the results of chemical and elemental analysis of the goat manure used, it was revealed that the pH of H_2O and KCl of the fertilizer were 8.2 and 7.6, respectively. The fertilizer moisture content and C – organic fertilizer content were 12.22% and 17.87%, respectively. The levels of N – total, P_2O_5, and K_2O were respectively 1.57%, 0.95% and 2.25%. The C/N ratio of fertilizer is 11.

3.4. Analysis of plant height

Based on the results of the 2-Way ANOVA test at 2 WAP observations, a significant effect on the interaction of PGPM and goat manure was observed. At 8 WAP observations, it was shown that the single effect of goat manure has a significant effect. Whereas at 4 WAP and 6 WAP it did not show significant results. Based on plant height measurements at 2, 4, 6, and 8 WAP, it was shown that the highest average plant height was achieved by P_1O_4 treatment while the lowest was by P_2O_2 in Fig. 1.

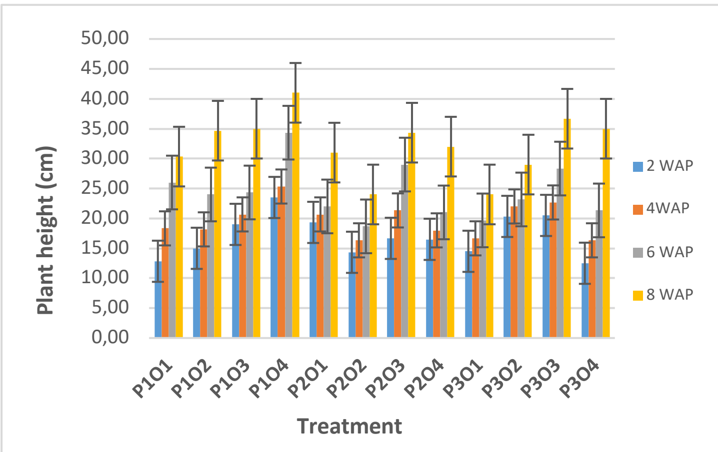

Fig. 1. Effect of treatment on plant height

Effect of goat manure interaction with PGPM, caused by the increasing of metabolic activity of soil microbes by providing organic matter as a source of energy for soil microbes in producing various compounds. The C/N ratio of goat manure will affect the effectiveness and efficiency of soil microbial metabolism. In addition, soil microbes also play a role in the composting process of available organic matter due to the addition of goat manure to the soil (Shofiyah, 2017).

The results of this study are in line with the results of research by Yoviana et al (2019). The results of Yoviana et al (2019) research showed that the bokashi treatment of goat manure showed a significant effect at a rate of 15 tons/ha.

3.5. Analysis of internodes number

Based on the results of measuring the number of internodes at 2, 4, 6, and 8 WAP, it was shown that the highest average number of internodes was achieved by the P_1O_4 treatment while the lowest was by P_3O_1. The number of nodes is directly affected by plant height. Tall plants produce a higher number of internodes and branches, thereby affecting the number of leaves. This is in accordance with the statement of Muliawati (2010) that the number of leaves of the cat's whiskers depends on the number of internodes and branches (Jayanti et al., 2019).

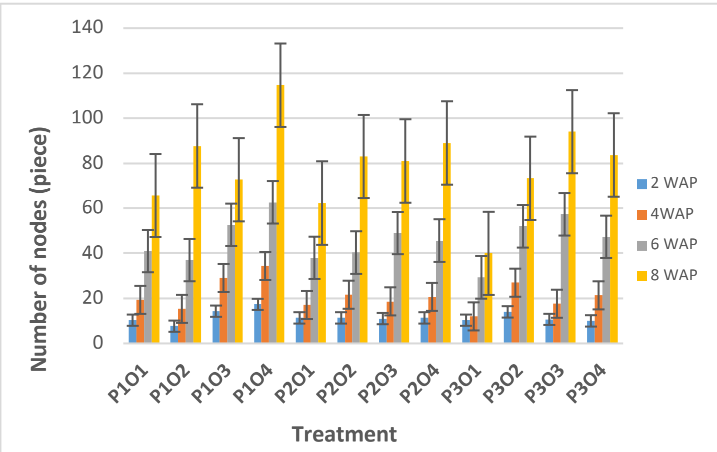

Fig. 2. Effect of treatment on segment count

3.6. Analysis of leaf number

Based on measurements of the number of leaves at 2, 4, 6, and 8 WAP, it was shown that the highest average number of leaves was achieved by the P_1O_4 treatment while the lowest was by P_3O_1 in Fig. 3. The highest treatment obtained from this study did not differ much and even tended to be higher than those reported in another study conducted by Jayanti (2019) with an average number of cat's whiskers leaves of 250 leaves at 8 WAP. According to the results of a study by Yoviana et al (2019), the bokashi treatment of goat manure at a dose of 15 tons ha^{-1} showed the best effect on the number of leaves of the cat's whiskers plant.

3.7. Analysis of leaf area

The interaction effect of PGPM and goat manure on leaf area was significant at the age of 4 and 6 WAP. Based on measurements of the total leaf area at 2, 4, 6, and 8 WAP, it was shown that the highest average total leaf area was achieved by the P_2O_2 treatment while the lowest was by P_2O_4 (Fig. 4). Leaf area is an important indicator of plant growth which determines the rate of photosynthesis per unit time. The greater the leaf area, the higher the expected effectiveness of the leaves in absorbing light as a factor in photosynthesis is, resulting in the production of more photosynthetic products. In addition, greater leaf is useful for the process of plant growth and development (Maulana et al., 2019).

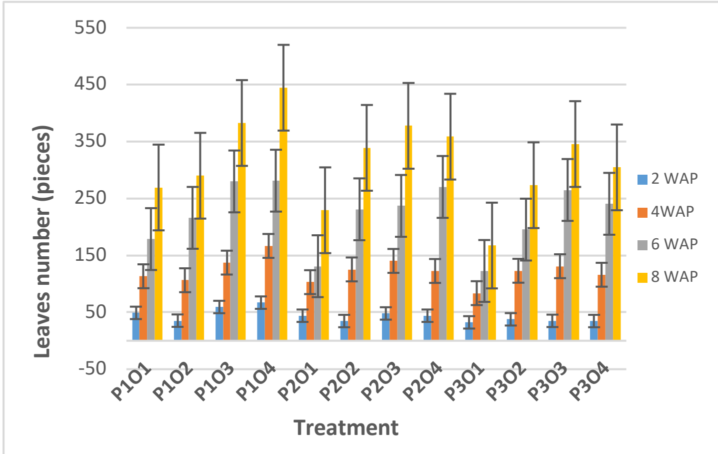

Fig. 3. Effect of treatment on leaf number

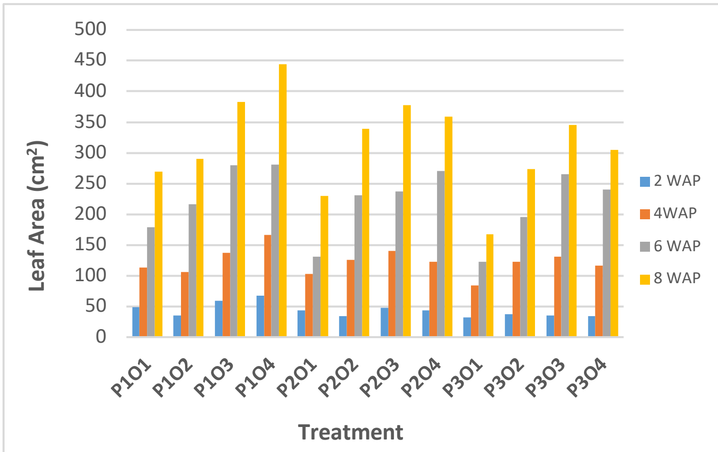

Fig. 4. Effect of treatment on leaf area

3.8. Analysis of shoot – root ratio

Based on the results of measurements of the shoot-root ratio in the field at 2, 4, 6, and 8 WAP, it was found that the value of the shoot-root ratio relatively fluctuated and decreased from 6 to 8 WAP. However, there are several treatments, such as P_2O_2, P_2O_3, P_3O_1, and P_3O_4, which experienced an impairment without prior fluctuations. The interaction effect between PGPM and goat manure was significant at the age of 2 and 6 WAP. The P_3O_3 treatment showed the highest shoot-root value and was significantly different from other treatments. the interaction between PGPM and goat manure occurs as a positive effect of goat manure which can improve the physical and chemical properties of the soil so as to provide optimal conditions for the development of soil microbes.

Shoot – root ratio is influenced by external factors such as the availability of groundwater and available nutrients for roots. The lower the shoot – root ratio, the better the dry matter production. A low shoot-root ratio indicates good root growth so that the area of nutrient absorption by the roots takes place optimally (Rahmawati et al., 2013). The optimal shoot-root ratio for annual plants such as cat's whiskers ranges from 0.41 to 5.6 (Sainju et al., 2017). The increase in the value of shoot – root ratio is caused by limited root growth and plants depend on the energy of sunlight to produce photosynthates which are translocated from the leaves. Excessive nitrogen content in the soil causes stunted roots and makes the shoot-root ratio high (Rahmawati et al., 2013).

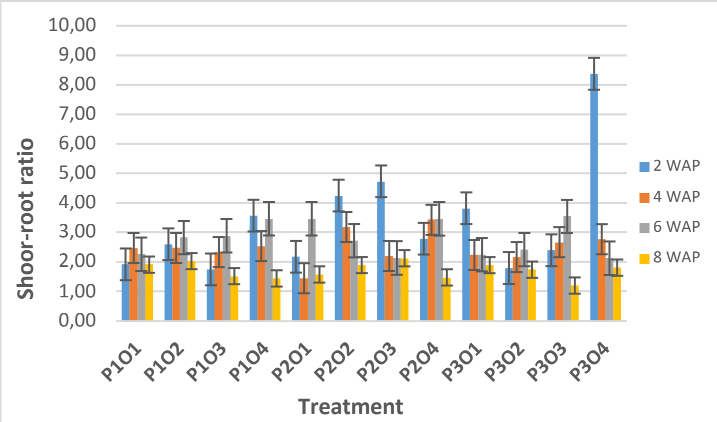

Fig. 5. Effect of treatment on shoot – root ratio

3.9. Analysis of total chlorophyll content in leaves

The total leaf chlorophyll content was measured at 8 WAP. The analysis result revealed the chlorophyll levels in the leaves was in the range of 5.59 – 8.93 mg/g. The P_3O_4 treatment had the highest total chlorophyll content of 8.93 mg/g while the lowest total chlorophyll content was the P_1O_2 treatment of 5.59% mg/g. The results of this study indicated that increasing the dose of PGPM and goat manure had an effect on increasing leaf chlorophyll content. However, the results from this study was lower than that of Ibrahim (2012) which was in the range of 20.13 ± 0.11 mg/g to 34.23 ± 0.98 mg/g (Neina, 2019).

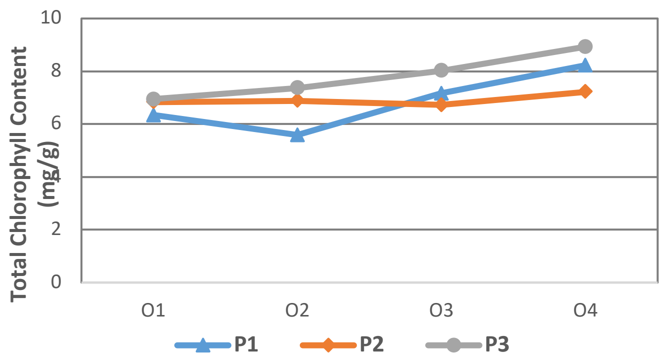

Fig. 6. Effect on treatment on total chlorophyll content in leaves (mg/g)

Table 3. Total chlorophyll content in leaves (mg/g)

Treatment	O_1	O_2	O_3	O_4
P_1	6.34	5.59	7.16	8.23
P_2	6.84	6.89	6.73	7.22
P_3	6.95	7.37	8.03	**8.93**

3.10. Analysis of leaves biomass wet weight

Based on measurements of the wet weight of the leaves at 2, 4, 6, and 8 WAP, the relative wet weight of the leaves underwent an increase in each treatment (Fig. 7). The results of the 2 Way ANOVA test at 2 WAP showed that the single effect of PGPM showed significant results, so that the DMRT follow-up test was carried out. Further test results obtained that the single effect of PGPM treatment P_2 with a weight value of 2.49 g showed a significant difference compared to the single effect of PGPM on other treatments and was the treatment with the smallest value. Meanwhile, the single effect of PGPM treatment P_1 and P_3 demonstrated no significant difference with each other with their wet weight of 4.92 g and 4.08 g, respectively. The results of the 2 Way ANOVA test at 4 WAP found that the single effect of goat manure exhibited significant results, so a further DMRT test was carried out. Further test results revealed that the single effect of O_4 treatment fertilizer displayed a significant difference compared to other treatments and had the largest weight value of 17.93 g, while the single effect of O_1 treatment fertilizer showed the lowest biomass of 7.21 g. The single effect of the O_2 and O_3 treatment fertilizers did not present significant difference from each other. However, both treatments showed a significant difference when compared to the single effect of the O_1 and O_4 treatment fertilizers with biomass weightsof 12.35 g and 11.98 g, respectively.

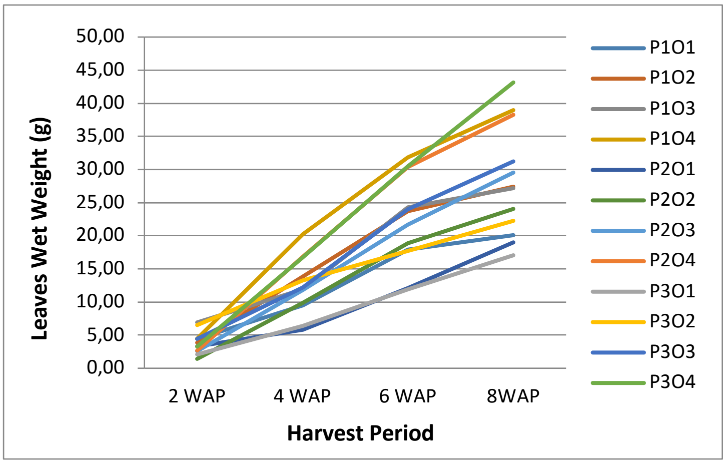

Fig. 7. Effect of treatment on leaves biomass wet weight at different harvest periods

The results of the 2 Way ANOVA test at 6 WAP discovered that the single effect of both PGPM and goat manure showed significant results, so a further DMRT test was carried out. Further test results demonstrated that the single effect of PGPM treatment P_1 showed significant difference and had the largest value of 24.42 g compared to the single effect of PGPM treatment P_2 and P_3. Conversely, the single effect of PGPM treatment P_2 and P_3 did not show significant differences with each other with biomass weights of 20.76 g and 21.04 g, respectively. Meanwhile, the single effect of fertilizer from each treatment O_1, O_2, O_3, and O_4 exhibited significant differences from one another with the highest average weight, namely the single effect of the O_4 treatment of 30.88 g, and the smallest weight, i.e., the single effect O_1 treatment of 13.98 g. The results of the 2 Way

ANOVA test at 8 WAP found that the effect of a single fertilizer showed significant results so that it was continued with the DMRT follow-up test. The results of the DMRT follow-up test, namely the single effect of fertilizer for each treatment both O_1, O_2, O_3, and O_4 showed a significant difference from each other with the highest weight value, namely the influence of O_4 treatment fertilizer, which was 40.13 g and the smallest weight value, namely the effect of O_1 treatment fertilizer that is equal to 18.72 g.

The single effect of PGPM on leaf wet weight at 2 and 6 WAP showed significant results so it was continued with DMRT follow-up tests. After further testing, it was found that there was no additional leaf wet weight which was positively correlated with the addition of PGPM doses. Similarly, there were no significant results in the addition of leaf wet weight due to PGPM when observed at 4 and 8 WAP. This is not in line with the ability of PGPM as a plant growth promoter. This could be caused by the fact that PGPM growth and its bioactivity are influenced by environmental factors, one of which is soil pH (Odoh, 2017). The pH conditions of cultivated soils are in the range of 4.83 – 6.5 and most are recorded at pH 5.5. In the PGPM used, there are 3 types of microbes that have different growth pH requirements compared to the pH of the media used. The fungus *Trichoderma sp.* optimal growth and activity is at pH 4 (Singh et al., 2014), *Beauveria sp.* optimal growth and activity is at pH 6.8 – 7.5 (Padmavathi et al., 2003), *Pseudomonas sp.* optimal growth and activity at pH 5.6 – 7.1 (Dimassi et al., 2020), *Bacillus sp.* optimal growth at pH 5 – 9 (Andriani et al., 2017), and *Rhizobium sp.* optimal growth and activity at pH 4 – 8 (Martani et al., 2011). On the other hand, the fertilizer used showed a significant single effect on the addition of leaf wet weight at 4 to 8 WAP. The significant increase in leaf wet weight due to a single effect between O_1, O_2, O_3, and O_4 treatments was in line with fertilizer decomposition that occurred during period 2 to 8 WAP. Fertilizer decomposition increases the available nitrogen in the soil as a nutrient source (Pramitasari et al., 2016). Accumulation of leaf area and number of leaves positively correlated with the fresh weight of the leaves produced. Nitrogen acts as a raw material for chlorophyll which plays an important role in photosynthesis to produce photosynthates that will be translocated to plant organs and affect the total weight of plants (Utami et al., 2020).

Conducted a study on the effects of PGPM and goat manure fertilizer on the wet weight of leaves over a period of 8 weeks. Found that the relative wet weight of the leaves increased in all treatments. Results of 2 Way ANOVA tests showed significant effects of PGPM and goat manure fertilizer on leaf wet weight at different time points. Further DMRT follow-up tests revealed that the single effect of PGPM treatment P_2 and goat manure fertilizer treatment O_4 showed significant differences compared to other treatments. The addition of PGPM doses did not result in any additional leaf wet weight at 4 and 8 WAP, possibly due to environmental factors such as soil pH. However, the use of goat manure fertilizer showed a significant increase in leaf wet weight from 4 to 8 WAP due to the increased availability of nitrogen in the soil. Fertilizer decomposition increases the available nitrogen in the soil as a nutrient source (Pramitasari et al., 2016). Accumulation of leaf area and number of leaves positively correlated with the fresh weight of the leaves produced, and nitrogen acts as a raw material for chlorophyll which plays an important role in photosynthesis to produce photosynthates that will be translocated to plant organs and affect the total weight of plants (Utami et al., 2020).

3.11. Analysis of roots dry weight

Based on measurements of root dry weight at 2, 4, 6, and 8 WAP, the results obtained were that the relative root dry weight showed an increase in weight which can be seen in Fig. 8. The interaction effect of PGPM and goat manure showed significant results. The interaction effect of PGPM and P_3O_2 fertilizer treatment showed the most significant difference compared to other treatments at the same time with the largest biomass weight of 1.05 g. Then followed by the P_1O_3 treatment which showed a significant difference regarding the effect of fertilizer on PGPM with a weight value of 0.92 g. The P_2O_3 and P_3O_3 treatments showed significant differences regarding the effect of PGPM on fertilizer with weight values of 0.21 g and 0.52 g, respectively. The effect of the interaction between PGPM and goat manure comes from microbes that utilize available organic matter from soil media and from manure as energy for growth and bioactivity (Padmavathi et al., 2003). The higher the dose of PGPM given will increase plant growth through its ability as a biofertilizer, biostimulant, and bioprotectant and the faster the process of decomposition of organic fertilizers in the growing media by the PGPM used (Wahyuningsih et al., 2017).

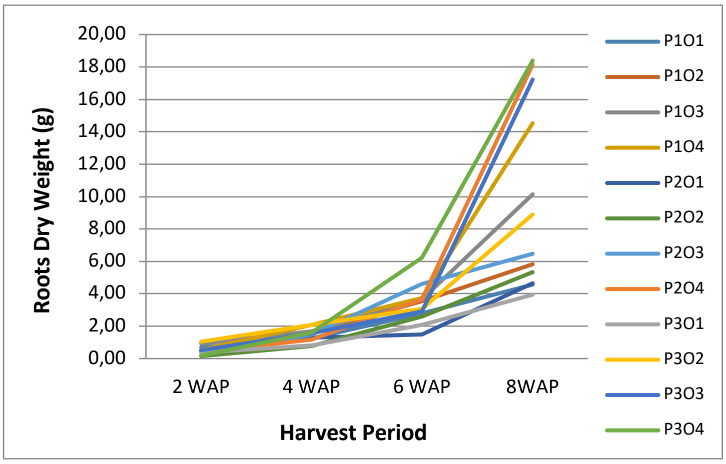

Fig. 8. Effect of treatment on roots biomass dry weight at different harvest people

Decomposition of goat manure can increase the stability of soil aggregates and soil granules so that plant root growth can be optimal (Dinas Pertanian Jogjakarta, 2018). However, the quality of goat manure is determined by its decomposition rate which is influenced by the quality of the basic fertilizer, decomposer, temperature, and humidity (Campbell et al., 2008). As a result, at 4 WAP the effect of fertilizer had not shown significant difference because it was still in the process of decomposition. Root dry weight is the accumulation of photosynthate resulting from photosynthesis translocated from leaves to roots, assimilation of food reserves in the roots, and organic matter resulting from drying of the wet weight of the roots which reflects the nutrient status of the roots (Ahmad et al., 2016).

3.12. Analysis of stems dry weight

Based on measurements of stem dry weight at 2, 4, 6, and 8 WAP, the results obtained were relative stem dry weight indicating an increase in value which can be seen in Fig. 9. The results of the 2 Way Anova test at 2 WAP found that either the single effect of PGPM or goat manure and the interaction effect of the two did not show significant results so that the DMRT follow-up test was not carried out. The P_3O_2 treatment showed the highest weight value of 0.98 g and the P_2O_2 treatment showed the smallest weight value of 0.35 g. The results of the 2 Way Anova test at 4 WAP found that the single effect of goat manure showed significant results, so a further DMRT test was carried out. Further test results obtained that the single effect of O_4 treatment manure showed the most significant difference compared to other treatments and had the largest weight value of 1.96 g. The single effect of O_1 treatment fertilizer showed the smallest weight value of 1.06 g.

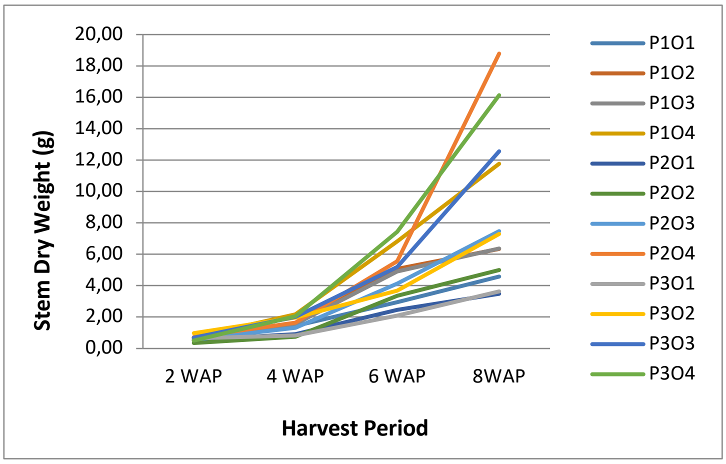

Fig. 9. Effect of treatment on stems biomass dry weight at different harvest people

The dry weight of stems shows the level of efficiency of stem tissue metabolism towards photosynthate resulting from translocation from leaves (Hidayat et al., 2020). The dry weight of the stems shows the levels of organic matter derived from the accumulation and absorption of nutrients sourced from the soil medium and the goat manure used. However, manure requires time to fully decompose. The increase in nitrogen yield from organic fertilizers is in line with the length of the decomposition time (Zhu et al., 2020). Therefore, the effect of a single fertilizer on the increase in stem dry weight did not show significance at 2 WAP.

The study measured stem dry weight at 2, 4, 6, and 8 weeks after planting (WAP) to evaluate the efficiency of stem tissue metabolism and the impact of different fertilizers. The results showed a relative increase in stem dry weight over time, as seen in Fig. 9. At 2 WAP, the 2 Way ANOVA test did not show significant results for the single effect of PGPM or goat manure or their interaction, and the DMRT follow-up test was not carried out. The P_3O_2 treatment resulted in the highest weight value, while the P_2O_2 treatment had the smallest weight value. At 4 WAP, the single effect of goat manure showed significant results, and the O_4 treatment had the most significant difference and the largest weight value, while the O_1 treatment had the smallest weight value. The study suggests that the level of stem dry weight is influenced by the efficiency of stem tissue metabolism, which is affected by organic matter accumulation and nutrient absorption from the soil medium and fertilizers used, with the impact of organic fertilizers increasing over time.

3.13. Analysis of leaves dry weight

Based on measurements of the dry weight of the leaves at 2, 4, 6, and 8 WAP, the results obtained were that the relative dry weight showed an increase in weight which can be seen in the graph of Fig. 10. The interaction effect of PGPM and goat manure showed significant results. The interaction effect of PGPM and P_3O_2 fertilizer treatment showed the most significant difference compared to other treatments at the same time with the largest weight value of 0.91 g. Then followed by the P_1O_3 treatment which showed a significant difference regarding the effect of PGPM on fertilizer and the effect of fertilizer on PGPM with a weight value of 0.89 g. The effect of PGPM on fertilizer in the P_2O_3 treatment showed the smallest weight value of 0.33 g.

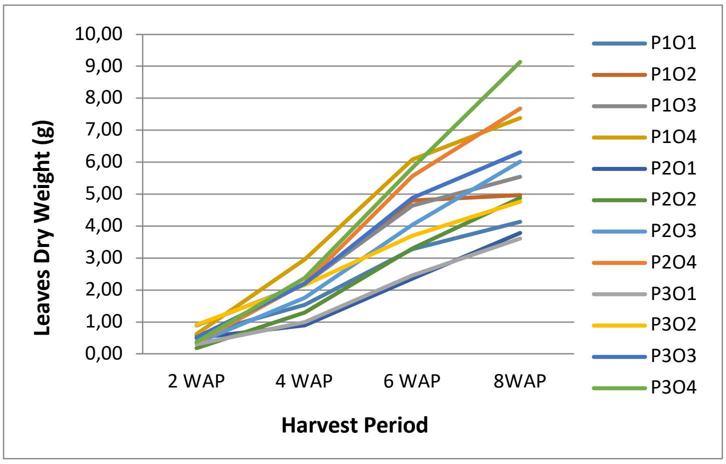

Fig. 10. Effect of treatment on leaves biomass dry weight at different harvest people

The effect of the interaction between PGPM and goat manure comes from microbes that utilize organic matter from soil media and fertilizers as energy for growth and bioactivity (Mulyaningsih and Djumali, 2015). The higher the dose of PGPM given will increase plant growth through its ability as a biofertilizer, biostimulant, and bioprotectant and the faster the process of decomposition of organic fertilizers in the growing media by the PGPM used (Hajoeningtijas et al., 2008). Fertilizer decomposition increases the available nitrogen in the soil as a nutrient source. Nitrogen plays a role in increasing leaf area and number of leaves (Pramitasari et al., 2016). Leaf dry weight is the accumulation of photosynthate from photosynthesis which has been reduced by leaf cellular respiration (Mulyaningsih et al., 2015). Therefore, the decomposition of fertilizers that produce nitrogen available to plant roots affects the leaf dry weight produced.

The study measured the dry weight of leaves at 2, 4, 6, and 8 weeks after planting (WAP) to assess the impact of the interaction between PGPM and goat manure on plant growth. The results showed an increase in relative dry weight over time, as seen in Fig. 10. The interaction effect of PGPM and goat manure was found to be significant, with the PGPM and P_3O_2 fertilizer treatment showing the most significant difference and the highest weight value of 0.91 g, followed by the P_1O_3 treatment with a weight value of 0.89 g. The P_2O_3 treatment showed the smallest weight value of 0.33 g. The study suggests that the interaction between PGPM and goat manure can enhance plant growth through the utilization of organic matter as energy for microbial growth and bioactivity, leading to the faster decomposition of organic fertilizers in the growing media and increasing the available nitrogen as a nutrient source, which plays a role in increasing leaf area and number of leaves, and thus leaf dry weight.

3.14. Analysis of yield simplicial percentage

Based on the results of measuring the yield of leaf simplicia at 2, 4, 6, and 8 WAP, it was found that the yield value of simplicia for each treatment relatively increased and only a few treatments experienced fluctuations at 4 and 6 WAP and then experienced an increase in yield value, namely in the P_1O_2 treatment, P_1O_3, and P_3O_3 which can be seen in the graph of Fig. 11.

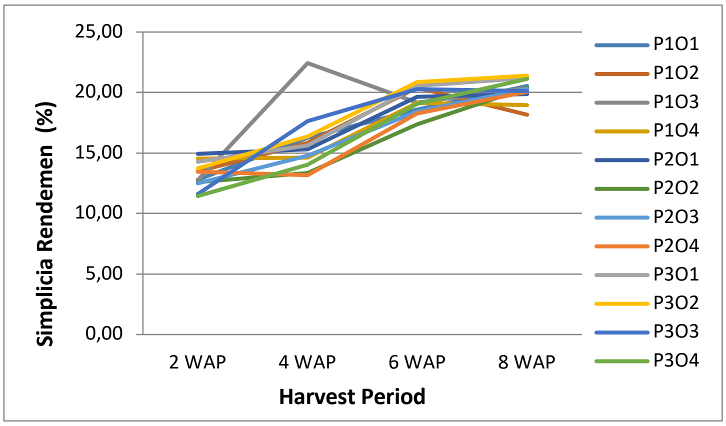

Fig. 11. Effect of treatment on dry leaves yield at different harvest people

The single effect of PGPM treatment P_3 showed a significant difference compared to the single effect of PGPM other treatments and had the highest yield value of 20.19 %. The single effect of PGPM treatment P_2 showed a significant difference compared to other treatments and had the smallest yield value of 18.42%. The simplicia yield value is influenced by various factors, one of which is the fluctuating water content, nutrient absorption, and rainfall. In general, cat's whisker leaves yielded a simplicia yield value of 13.89 – 18.51% in two harvests with a harvest age range of six week (Delyani et al., 2017). Nurhajijah (2014) obtained a wider range of simplicia yield values, namely 11.41 – 29.19% in the second harvest with a harvest age range of 2 – 12 weeks. Because the yield value of simplicia is determined by the dry weight and wet weight of the leaves, the growth of leaf biomass which is influenced by the ability of photosynthesis, respiration, and absorption of nutrients determines the yield value of simplicia (Delyani et al., 2017).

3.15. Analysis of rosmarinic content in leaves

The analysis of rosmarinic acid was performed using HPLC method. As shown in Fig. 12 that the HPLC system was able to separate and identify the presence of rosmarinic acid in plant extract. The analysis of rosmarinic acid content at 8 WAP revealed that the range of rosmarinic acid content in leaves was 144.43 – 206.31 mg/g or 14.44 – 20.63%. The leaves sample treated with P_2O_2 contained the highest rosmarinic acid content of 206.31 mg/g, while the lowest rosmarinic acid content was shown by sample treated with the P_1O_2 fertilizer, which was 144.43 mg/g, as shown in Fig. 13.

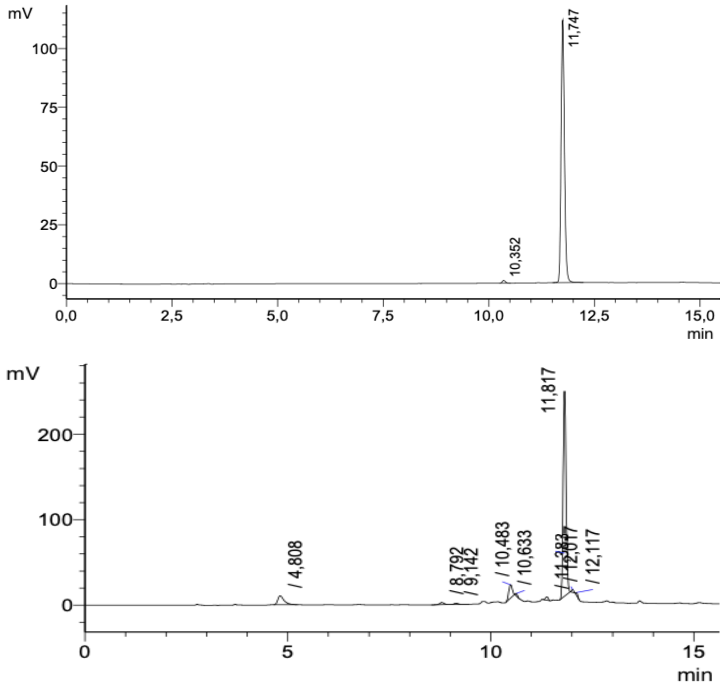

Fig. 12. HPLC chromatogram of rosmarinic acid reference (above) and leave extract of *O. aristatus* (bottom)

The analysis of rosmarinic acid content at 8 WAP revealed that the range of rosmarinic acid content in leaves was 144.43 – 206.31 mg/g or 14.44 – 20.63%. The leaves sample treated with P_2O_2 contained the highest rosmarinic acid content of 206.31 mg/g, while the lowest rosmarinic acid content was shown by sample treated with the P_1O_2 fertilizer, which was 144.43 mg/g.

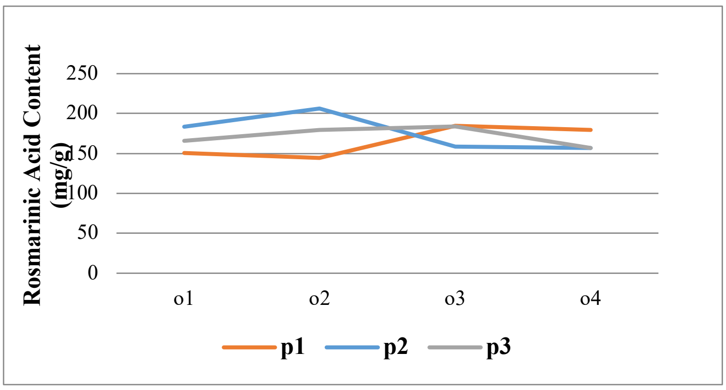

Fig. 13. Effect of treatment on rosmarinic acid content in leaves

The content of rosmarinic acid in *O. aristatus* leaves is affected by the drying temperature used, the higher the drying temperature used, the lower the rosmarinic acid level (Abdullah et al., 2017). In a report by Abdullah et al. (2017), the rosmarinic acid content in samples dried at 30°C (air dried), 40°C, and 50°C had average contents of 171.47 mg/g, 98.69 mg/g, 9.10 mg/g, respectively.

Table 4. Rosmarinic acid content in leaves samples

Treatment	O_1, mg/g	O_2, mg/g	O_3, mg/g	O_4, mg/g
P_1	150.82	144.43	184.68	179.26
P_2	183.85	**206.31**	158.78	156.87
P_3	166.21	179.47	**183.86**	156.92

4. Conclusion

The study found that the combination of PGPM and goat manure had a significant effect on the growth of cat's whiskers plants, but the effect varied depending on the observation time and the variable being measured. The highest plant height, number of segments, number of leaves, and wet weight of leaves were achieved with the P_1O_4 treatment. The highest total leaf area was achieved with the P_2O_2 treatment, while the highest total chlorophyll content was found in the P_3O_4 treatment. The shoot-root ratio fluctuated and decreased from 6 to 8 WAP. The highest percentage of simplicities is at 3 ml/l PGPM and 86.2 g of goat manure (P_2O_2 treatment). The rosmarinic acid content in the leaves ranged from 144.43 – 206.31 mg/g or 14.44 – 20.63%, with the highest content found in leaves treated with P2O2 fertilizer and the lowest in leaves treated with P_1O_2 fertilizer. The results suggest that the use of PGPM and goat manure could be a potential strategy to improve the growth of cat's whiskers plants.

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Conflict of interest

The authors declare there is no conflict of interest in this study.

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