Pertanika J. Trop. Agric. Sci. 48 (5): 1529 - 1548 (2025)

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

# Organic Fertilizer Formulation to Improve the Quality and Yield of *Eleutherine palmifolia* L. (Merr) as an Alley Cropping in Coffee Plantation

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## ABSTRACT

*Eleutherine palmifolia* L. (Merr), or what is known as the Bawang Dayak in Indonesia, is a horticultural group of plants that can be used as a medicinal plant. This plant has many benefits because it contains secondary metabolite compounds, such as flavonoids, phenols, naphthoquinones, and antibacterial and anticancer derivatives. This research aims to explain the response of *E. palmifolia* plants to a combination of organic fertilizers on growth, yield, and total flavonoid content as an alley cropping under coffee trees. This research was implemented with randomized complete block design. Providing organic fertilizer uses a minus-one test, which consists of five treatments, namely: Without organic fertilizer; Complete fertilization (cow manure + guano + rice husk ash); cow manure + guano (without rice husk ash); guano + rice husk ash (without cow manure); and cow manure + rice husk ash (without guano). Each treatment was repeated five times. The application of complete organic fertilizer (cow manure + guano + rice husk ash) produces a higher number of leaves, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, tuber weight per plant (21.5 g), and tuber weight per plot (89.8 g) compared to without fertilization. The application of organic fertilizer produces the flavonoid content of *E. palmifolia* bulbs 1.2 – 1.3 times higher than no fertilization. The average percentage of shade obtained was 90.41% with an average air temperature of around 29.7°C. *E. palmifolia* can be planted under the coffee tree as an alley cropping. Based on the characteristics of the growth and yield of *E. palmifolia*, further research on the farming feasibility analysis needs to be considered.

*Keywords:* Bioactive content, *E. Palmifolia*, macronutrients, organic, physiology

ARTICLE INFO

*Article history:*
Received: 07 December 2024
Accepted: 27 February 2025
Published: 29 August 2025

DOI: https://doi.org/10.47836/pjtas.48.5.11

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e-ISSN: 2231-8542

## INTRODUCTION

The body needs medicinal plants to maintain or improve its immune system, so it is not easily attacked by disease (Adelayanti, 2020). The body's immune system fights viruses and diseases and produces antibodies (Chowdury et al., 2020). One type of medicinal plant widely cultivated or cultivated, especially by the people of Central Kalimantan, is *Eleutherine palmifolia*. This plant is native to tropical America and has many health benefits (Mutiah et al., 2019). *E. palmifolia* also has other regional names, including "bawang sabrang" (Sunda), "bawang Tiwai" (Kutai Kartanegara), "bawang hutan" (East Kalimantan), "brambang sabrang" (Java), and "si marbawang-bawang" (North Sumatra) (Poerwosusanta et al., 2019). *Eleutherine palmifolia* is a medicinal plant because it contains several secondary metabolite compounds, including flavonoids as antioxidants that can be used to improve the immune system (Chabib et al., 2018); flavonoids and phenols as antibacterials (Fransira et al., 2019, 2020); and antiacne (Syamsul et al*.,* 2015), antimitotic (Efendi et al*.,* 2015), antifungal (Christoper et al., 2017), and also antioxidants (Andryani et al., 2022; Kuntorini et al*.,* 2016). Several phytochemicals in medicinal plants include flavonoids, naphthoquinones, anthraquinones, alkaloids, saponins, tannins, triterpenoids, and steroids (Kamarudin et al., 2021; Gomes et al., 2021; Yanti et al., 2022). The central part of this plant is the tuber, which is often used as an herbal medicine and consumed either fresh or dried (simplistic).

Fertilization is one way to increase the availability of nutrients in the soil. Nutrients are needed for plant growth and development. The use of organic fertilizer in this research uses common fertilizers, namely cow manure, guano fertilizer, and rice husk ash, each of which is a source of the macro and micronutrients, such as: Nitrogen (N), Phosphorus (P), and Potassium (K), Fe, Cu, Zn, Mn, Mg, and Ca. The macro and micronutrients can promotes of mineralization (Emendu et al., 2021). The N element stimulates the growth of roots, stems, and leaves. Nutrient N is central in cell division, tiller formation, and stem elongation (Saleem et al., 2012). Plants need N because it plays a role in forming chlorophyll and the growth of stems, branches, and leaves, so it has a significant role in photosynthesis (Leghari et al., 2016). The nutrient P is a component of enzymes and proteins, ATP, RNA, and DNA, and has an essential function in photosynthesis and energy transfer. Chotimah et al. (2021) stated that the application of organic fertilizers can improve the performance and yield of *Eleutherine americana* Merr when grown on acidic tropical soils. Potassium plays a role in root development, increases the formation of sugar and starch in photosynthesis, and is essential in plant translocation. Providing potassium affects the growth and yield of garlic plants. The application of 200 kg K $ha^{-1}$ produces the highest weight of fresh and dry garlic bulbs (Jiku et al., 2020).

Coffee plantation land has the potential for intercrop cultivation. Intercrops are cultivated between rows of annual plants, in this case, coffee plants. Coffee plants usually have a spacing of around 2 m × 2 m (Arabica type) or 2.5 m × 2.5 m (Robusta type). This planting distance allows it to be used for other farming businesses, namely cultivating *E. palmifolia* onion. Optimizing land use in coffee plantation areas can increase farmers' income and soil fertility and create a suitable microclimate for the growth of annual crops. Intercropping has improved coffee production by providing an alternative income source to producers, especially in the crop formation phase (Carvalho et al., 2024).

Cultivation of *E. palmifolia* plants in Indonesia still needs to be developed (Ariska et al., 2020). This research has differences, especially in the methods and land conditions used. The combination of organic fertilization using minus one test treatment aims to explain the response of *E. palmifolia* plants as intercrops in coffee plantations to N, P, and K fertilization because these three nutrients are one of the limiting factors in the growth, yield, and physiology of *E. palmifolia* plants. The minus one test also determines the role of the three nutrients by eliminating one of the nutrients used. Apart from that, the results of this research will also be used to prepare Standard Operational Procedures (SOP) for cultivating *E. palmifolia*. The development of SOPs will require extensive additional or advanced research to generate valid and reliable data

The theoretical benefit of this research is that it can apply technical activities for cultivating seasonal crops on limited land to support an integrated agricultural system. The practical benefit that will be obtained later is using land under the shade of coffee by cultivating annual crops using minimum tillage. This research's managerial benefit is obtaining an organic fertilizer formulation to produce growth, yield, and secondary metabolite levels of *E. palmifolia* plants based on field and laboratory tests, which can later be used as additional information to guide good plant cultivation.

## MATERIALS AND METHODS

### Study Area and Materials

The research was conducted from December 2022 to July 2023 (8 months) on a 13-year-old coffee plantation. The coffee plantation belongs to the Tunggak Semi Farmers Group in the Pentingsari hamlet, Umbulharjo Village, Cangkringan District, Sleman Regency, Yogyakarta. This location is 662 m above sea level with rainfall of 660 mm/year (Statistics Sleman Regency, 2021).

The materials used in the research included Kalimantan accession *E. palmifolia* bulbs, cow manure, guano phosphate fertilizer, and rice husk ash. The equipment used for cultivation and harvesting are soil processing equipment, oven, and analytical balances. The equipment used to analyse leaf chlorophyll and total flavonoids includes a visible Spectrophotometer (Genesis 30), 100 mL Erlenmeyer flask, measuring flask, filter paper, test tube, and Ohauss analytical balance. Measurement of light intensity under coffee plants uses a lux meter, while air temperature and humidity measurements use a thermohygrometer.

### Experimental Design

This research was implemented with randomized complete block design. The treatment given is organic fertilizer. Providing organic fertilizer used a minus-one test which consisted of five (5) treatments, namely: Without organic fertilizer; Complete fertilization (cow manure + guano + rice husk ash); cow manure + guano (without rice husk ash); guano + rice husk ash (without cow manure); and cow manure + rice husk ash (without guano). Each treatment was repeated five times so that there were 25 experimental units.

### Procedures

Dayak onion bulbs will first be selected by selecting healthy seeds (shiny colour, compact/not porous, the skin is not injured, and uniform in size). *E. palmifolia* tubers will be planted between or between the coffee planting distances. The coffee trees on the land have a row spacing of ± 1.9 m, while the row spacing is ± 2.4 m, so the plant population per hectare is ± 2157 plants. The distance between coffee plants is ± 25.5 m. Before planting, the soil under the coffee shade is loosened and mulched first to improve soil aeration and make it easier to plant the tubers. For planted seeds, cut the tip of the tuber by approximately 0.5 cm to help break the dormancy period and accelerate plant growth. Then, the tubers are planted by immersing ¾ of the tubers between or on the sidelines of the coffee plantings.

Applications of cow manure, guano fertilizer, and rice husk ash are given according to the treatment. Guano fertilization will be done 3 days before planting (*pre-plant*) to stimulate the growth of *E. palmifolia*'s roots. Maulidan and Putra (2024) stated that phosphorus can stimulate root growth, especially of lateral and hair roots. Meanwhile, cow manure and rice husk ash will be given two weeks after planting/WAP. The dose of cow manure and rice husk ash used in this study refers to the dose used in research (Yustina et al., 2019) on *E. americana* (Merr.), namely 360 kg $ha^{-1}$. The dose of guano fertilizer refers to the dose of biophosphate fertilizer used in research (Sukmasari et al., 2020) on shallot plants, namely 200 kg $ha^{-1}$.

Watering is done at least once daily in the morning/evening or depending on weather conditions. Weeding is done manually once a month. Harvesting will be done five months after planting (MAP). Harvesting *E. palmifolia* at the age of five MAP according to (Rosmawaty et al., 2019), has a single effect on the total flavonoid content of *E. palmifolia* which is the best.

### Observation

Observations include components of plant growth and yield, including (1) Plant height observed every week from 4 - 12 WAP with intervals of once every two weeks; (2) The number of leaves observed every week from 4 - 12 WAP with intervals of once every two weeks; (3) Analysis of total flavonoid content (Spectrophotometry method) at 12 WAP; (4) Number of bulbs per plant and fresh weight of bulbs per plant measured at harvest; (5) Bulb weight per plot calculated at harvest; (6) Fresh weight of shoots and roots measured at harvest (five plants); (7) Dry weight of shoots and roots measured after plant biomass was dried using an oven at 60°C for three days; and (8) Microclimates observation.

Measurements of air temperature and sunlight intensity were measured during observations, both under the coffee tree stands and outside the coffee stands. The intensity of sunlight is measured using a lux meter and the air temperature is also read directly on the tool. The formula for calculating the shade percentage is as follows:

Percentage (%) shade = 100% × (1 - I/D)

Note:
I = intensity in the shade
D = intensity outside the shade

The following is an example of calculating the percentage of shade in coffee stands at the experimental location during observations at 4 WAP:

% shading = 100% × (1 - I/D)
= 100% × (1 - 1652.2/3743.2)
= 100% × (1 – 0.4414)
= 100% × 0.5586
= 55.86%

### Data Analysis

The data analysis was conducted using the analysis of variance (ANOVA) method and statistically significant differences were determined with the DMRT (*Duncan Multiple Range Test*) at the 5% level with the SAS system for Windows 9.0 (English) tools.

## RESULTS AND DISCUSSION

### Quality Characteristics of Organic Fertilizer

The analysis of cow manure shows that the C-organic content meets the minimum technical requirements for solid organic fertilizer based on Minister of Agriculture Regulation Number 261 of 2019 concerning minimum technical requirements for organic fertilizers, biological fertilizers, and soil amendments (Table 1). High C-organic content (more than quality standards) is related to the nitrogen mineralization rate, availability of organic material, and microbial decomposers. Providing cow manure can increase the availability of soil organic matter and microbial activity, which helps break down organic matter

(Nuro et al., 2016). The N-total content also meets the minimum technical requirements for organic fertilizer based on Minister of Agriculture Regulation Number 28/Permentan/SR.130/5/2009. Nitrogen determines the C/N ratio in organic fertilizer. The C/N ratio is a good indicator of the N mineralization process in the soil (Rapisarda et al., 2022).

Table 1
*Results of analysis of several types of organic fertilizer used in research*

| **Fertilizer type** | **Test Variable** | **Result** | **Minimum Technical Requirements for Fertilizer** | **Note** |
|---|---|---|---|---|
| Cow Manure | C-organic (%) | 29.304 | Min. 15 | Appropriate- |
| | N-total (%) | 1.090 | < 6 | Appropriate–<br>Appropriate |
| Guano | $P_2O_5$ (%) | 2.263 | < 6 | Appropriate |
| Rice Husk Ash | $K_2O$ (%) | 0.912 | < 6 | Appropriate |

*Source*: Instiper Laboratory, Yogyakarta (2023)

The Phosphorus (P) nutrient content in guano organic fertilizer meets the minimum technical requirements for organic fertilizer according to Ministry of Agriculture Regulation Number 28/Permentan/SR.130/5/2009 concerning organic fertilizers, biological fertilizers, and soil amendments. Guano fertilizer is fertilizer that comes from bat droppings mixed with soil, which is then composted with the help of microbial activity. Guano fertilizer plays a role in increasing plant growth and stimulating root growth (Muryanto & Lidar, 2020). Phosphorus is needed to development of nucleic acid, energy, and sugar.

Rice husk ash contains the nutrient potassium, which, in this study, the test results met the minimum technical requirements for organic fertilizer according to Minister of Agriculture Regulation Number 28/Permentan/SR.130/5/2009 concerning organic fertilizers, biological fertilizers, and soil amendments. Potassium plays a role in photosynthesis, sugar transportation, protein formation, and enzyme activation.

### Soil Analysis Before Planting

The results of the first soil analysis at the experimental location in Table 2 show that several criteria for soil chemical properties, such as C-organic, N-total, C/N ratio, total $P_2O_5$, total $K_2O$, and pH provide different criteria. The C-organic content is relatively high, indicating that soil organic matter from plant tissue residues decomposed by decomposing microbes is also high. The experimental land used in this research implemented an organic farming system (without applying inorganic fertilizer) to increase the organic C content, total N, and C/N ratio. The organic C and total N content will also determine the C/N ratio in the soil, which is related to the N mineralization process. A lower C/N ratio indicates that the organic material decomposition process will also run better.

Table 2
*The before-planting soil analysis results*

| Test variable | Result | Standard | Criteria |
|---|---|---|---|
| C-organic (%) | 4.173 | 3 – 5 | High |
| N-total (%) | 0.238 | 0.21 – 0.5 | Medium |
| C/N | 17.522 | 16 – 25 | High |
| $P_2O_5$ (%) | 0.289 | > 60 ($P_2O_5$ HCl 25% (mg/100 g) | Very high |
| $K_2O$ (%) | 0.015 | | |
| pH | 7,1 | 6.6 – 7.5 | Neutral |

*Source*: Instiper Laboratory, Yogyakarta (2023)

Phosphorus and potassium nutrient levels in this research field are classified as very high. This is thought to be related to the pH value of the soil. The pH value shows neutral. Neutral pH is also related to the availability of nutrients in the soil. The nutrient P has immobile properties in the soil and can be bound by Al and Fe in land conditions with a low pH value (acid). Neutral pH conditions cause P nutrients to be available in the soil so that plants can absorb these nutrients. Land conditions where organic materials have previously been applied are also thought to reduce the ability of metals, such as Al and Fe, to bind P. This can cause Al, Fe, and Mn in the soil solution to decrease as the soil pH value increases to become neutral. This is in line with the research results that adding organic material can improve soil structure, add nutrients, and enhance availability (Tustiyani et al., 2024).

### After-Planting Soil Analysis Results

The results of the final soil analysis at the experimental location in Table 3 show that applying organic fertilizer with different treatments provides different soil yield assessments. The criteria for assessing soil chemical properties are based on (Eviati & Sulaeman, 2009). C-organic levels show Medium – Very High values. Application of complete organic fertilization (cow manure + guano + rice husk ash) and cow manure + guano fertilization increased the C-organic value from the initial value (before treatment), namely 4.173% to 6.274% and 4.736%. The increase in the value of C-organic levels is in line with the results of research (Adviany & Maulana, 2019). This is because carbon is the main constituent of organic materials. The more organic material given to the soil, the more organic C released into the soil will increase.

The final N-total content value increased from the initial N-total value (0.238%), except for the complete fertilization treatment (0.223%). The increase in N levels is due to the application of organic fertilizers (cow manure, guano, and husk ash), which have complete macronutrients that are thought to increase the uptake of N nutrients. Apart from that, organic fertilizers also play a role in improving the physical properties of the

Table 3
*The final soil analysis results*

| Test Variable | Treatments | | | |
|---|---|---|---|---|
| | **Cow Manure + Guano + Rice Husk Ash** | **Cow Manure + Guano** | **Guano + Rice Husk Ash** | **Cow Manure + Rice Husk Ash** |
| C-organic (%) | 6,274 (Very high) | 4,736 (High) | 2,943 (Medium) | 4,017 (High) |
| N-total (%) | 0,223 (Medium) | 0,492 (Medium) | 0,386 (Medium) | 0,340 (Medium) |
| C/N | 28,1 (Very high) | 9,64 (Low) | 7,62 (Low) | 11,82 (Medium) |
| $P_2O_5$ (%) | 0,349 (Very high) | 0,348 (Very high) | 0,392 (Very high) | 0,395 (Very high) |
| $K_2O$ (%) | 0,282 (Very high) | 0,497 (Very high) | 0,225 (Very high) | 0,056 (Very high) |
| pH | 6,30 (Slightly acid) | 6,21 (Slightly acid) | 6,46 (Slightly acid) | 6,69 (Neutral) |

*Source*: Instiper Laboratory, Yogyakarta (2023)

soil so that they can increase the root development of plants (Bachtiar et al., 2020). The highest increase in the C/N ratio value was shown from the complete organic fertilizer treatment (cow manure + guano + rice husk ash), which was 28.1 from the initial C/N ratio value (17.5). In contrast, the C/N ratio value in the fertilizer treatment of other organics experienced a decline. The increase in the C/N ratio value is likely due to decomposing organic material from organic fertilizer applied to the soil. The process of decomposing or composting organic materials requires carbon as an energy source for decomposing bacteria, while nitrogen was one of the macronutrients that played an essential role in the formation of amino acid, nucleic acid, chlorophyll, proteins, and various secondary and primary metabolites. This indicated that the inadequacy of these nutrients could lead to suboptimal growth in plants (Kishorekumar et al., 2020).

After applying organic fertilizer, the phosphorus and potassium levels increased from the initial phosphorus and potassium levels, namely 0.289% and 0.015%. An increase in phosphorus and potassium levels occurred in all organic fertilizer treatments given. Apart from the application of organic fertilizer itself, the increase in phosphorus and potassium levels is also thought to be due to the effect of phosphorus accumulation in the soil from organic fertilization that has been carried out previously. The accumulation of phosphorus nutrients can be caused by the influence of soil pH, which decreases from the initial pH value (7.1). A decrease in the pH value can cause the soil to become slightly acidic, so Al and Fe bind the immobile phosphorus nutrient. The concentration of $H^+$ ions also influences the decrease in soil pH values. If the concentration of $H^+$ ions in the soil solution increases, the soil pH value will decrease. In addition, the application of organic fertilizer in the decomposition process is thought to produce organic acids, carboxylic acids, and phenolic acid groups as weak acids that release $H^+$ ions, so it is thought to decrease soil pH (Havlin et al., 2017).

### Plant Growth of *E. palmifolia*

The ANOVA results in Table 4 show that applying organic fertilizer resulted in a Dayak onion plant height that was no different from treatment without organic fertilizer at plant ages of 4 to 12 WAP ($P > 0.05$). The increase in plant height from 10 to 12 WAP was 3 - 9 cm with a plant height range of 51 - 54 cm at the end of the observation. The control treatment experienced a decrease at the age of 10 WAP compared to the age of 8 WAP because it had a different number of plants. The number of plants at the age of 10 WAP increased compared to the age of 8 WAP because in general, the growth of *E. palmifolia* was not uniform and experienced slow growth. The same thing also happened to the leaf number variable (Table 5). The application of different types of organic fertilizer did not significantly affect the number of leaves of *E. palmifolia* plants at the age of 4 to 12 WAP ($P > 0.05$). The number of leaves at 6 WAP in the complete fertilization treatment (cow manure, guano, and rice husk ash) was more significant than without organic fertilization. Still, it was similar to other organic fertilizer treatments.

Table 4
*Response of plant height of* E. palmifolia *with the application of different organic fertilizers*

| Treatment | Plant Height (cm)* | | | | |
|---|---|---|---|---|---|
| | 4 | 6 | 8 | 10 | 12 |
| | WAP (Week After Planting) | | | | |
| Without fertilization | 14.3a | 34.5a | 45.8a | 44.6a | 52.6a |
| Cow Manure + Guano + Rice Husk Ash | 15.7a | 30.4a | 41.4a | 48.2a | 50.5a |
| Cow Manure + Guano | 17.1a | 28.6a | 42.5a | 48.3a | 52.6a |
| Guano + Rice Husk Ash | 14.2a | 28.0a | 41.1a | 47.8a | 51.0a |
| Cow Manure + Rice Husk Ash | 16.1a | 29.7a | 44.0a | 45.2a | 53.6a |

*Note*: The numbers in the same column followed by the same letter show that they are not significantly different from the DMRT (*Duncan Multiple Range Test*) test at the significance level $\alpha = 5\%$ ($P > 0.05$).
*: Transformation result = sqrt(x)

Plant height and number of leaves are not different between organic fertilizer treatment and control (without organic fertilizer), which can be expected because the nutrient levels in the soil or experimental field are classified as medium (N-total), high (C-organic), and very high (phosphorus and potassium). This allows plants to be able to take up or absorb nutrients that are already available in the soil. The application of organic fertilizer (cow manure, guano, and rice husk ash), which is slow release, still requires a longer time for

Table 5
*Response of the number of leaves of* E. palmifolia *with the application of different organic fertilizers*

| **Treatment** | **Number of Leaves*** | | | | |
|---|---|---|---|---|---|
| | **4** | **6** | **8** | **10** | **12** |
| | **WAP (Week After Planting)** | | | | |
| Without fertilization | 2.7a | 7.3a | 13.5a | 15.8a | 21.1a |
| Cow Manure + Guano + Rice Husk Ash | 3.9a | 11.1a | 18.4a | 27.1a | 32.6a |
| Cow Manure + Guano | 2.6a | 8.4a | 13.8a | 19.0a | 24.2a |
| Guano + Rice Husk Ash | 2.8a | 8.9a | 14.9a | 19.2a | 26.7a |
| Cow Manure + Rice Husk Ash | 2.6a | 5.6a | 10.9a | 16.9a | 21.3a |

*Note*: The numbers in the same column followed by the same letter show that they are not significantly different from the DMRT (*Duncan Multiple Range Test*) test at the significance level α = 5% (P > 0.05).
*: Transformation result = sqrt(x)

the release of nutrients so that the effect of the application of organic fertilizer is also not visible until the plants are 12 WAP on the growth of plant height and number of leaves. According to Sari et al. (2020), the slow-release application of PUKAP JESTRO SR organic fertilizer is better than conventional fertilizers, single and compound fertilizers.

The increased number of leaves on *E. palmifolia* from 4 to 12 WAP results from the provision of organic fertilizer. Organic fertilizer contains complete macronutrients, one of which is nitrogen. Nitrogen is needed by plants in large quantities in the form of ammonium and nitrate. Nitrogen functions to form or synthesize protein and chlorophyll and stimulates plant vegetative growth. It is thought that the nutrients (N, P, and K) contained in organic fertilizer can be absorbed by plants (Minardi et al., 2020) and increase organic matter and soil structure (Yunilasari et al., 2020). This is supported by the results of the analysis of the N nutrient content in cow manure, which is by the Ministry of Agriculture Regulation Number 28/Permentan/SR.130/5/2009 so that it can increase the nutrients in the soil.

## Plant Biomass Yield of *E. palmifolia*

Table 6 shows that organic fertilizer treatment didn't significantly affect the biomass components of *E. palmifolia* plants (P > 0.05). Application of complete organic fertilizer (cow manure + guano + rice husk ash) resulted in higher shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight than without organic fertilization. Complete organic fertilization provided shoot and root dry weights, respectively, 2.6 and 5.8 times higher than without organic fertilization.

The number of leaves was also accompanied by increased plant biomass weight, especially the wet and dry weight of the *E. palmifolia* plant canopy. The more leaves there are, the greater the plant's ability to carry out photosynthesis. The increased rate of assimilation or photosynthesis processes increases plant biomass weight. Photosynthate

Table 6
*Response of the plant biomass component per plot of* E. palmifolia *with the application of different organic fertilizers*

| Treatment | Fresh Shoot Weight* (g) | Fresh Root Weight* (g) | Dry Shoot Weight* (g) | Dry Root Weight* (g) |
|---|---|---|---|---|
| Without fertilization | 52.6a | 3.6a | 14.6a | 0.6a |
| Cow Manure + Guano + Rice Husk Ash | 151.2a | 21.6a | 37.9a | 3.5a |
| Cow Manure + Guano | 63.6a | 10.4a | 15.9a | 1.9a |
| Guano + Rice Husk Ash | 48.0a | 11.4a | 13.8a | 2.1a |
| Cow Manure + Rice Husk Ash | 51.2a | 4.4a | 14.5a | 0.8a |

*Note*: The numbers in the same column followed by the same letter show that they are not significantly different from the DMRT (*Duncan Multiple Range Test*) test at the significance level α = 5% (P > 0.05).
*: Transformation result = sqrt(x)

will be used for the formation of cell organelles and enzymes and as a substrate in the respiration process for plant growth and development

Providing complete organic fertilizer (cow manure + guano + rice husk ash) can increase the availability of nutrients in the soil and improve the fertility of the soil's physical, chemical, and biological properties. Jenira et al. (2018) stated that fertilizer derived from cow dung can improve soil fertility and lead to a sustainable agricultural system. Organic fertilizer can improve soil chemical properties (pH, available P, and P nutrient uptake) and black rice yields (Yuniarti et al., 2020). The application of additional fertilizer is needed to provide the required nutrients (Tustiyani et al., 2023). Nitrogen is an integral part of chlorophyll which acts as a light-capturing pigment in photosynthesis. The nutrient phosphorus plays a role in the source and transfer of energy, especially in the form of adenosine triphosphate (ATP). Phosphorus is also an important component in the synthesis of nucleic acids, coenzymes, nucleotides, phosphoproteins, phospholipids, and phosphate sugars. The nutrient potassium plays a role in osmotic pressure and ion balance and is involved in the synthesis and transport of photosynthesis products for production and storage in plants (seeds, fruit, and tubers) (Havlin et al., 2017).

## Yield of *E. palmifolia*

The ANOVA results in Table 7 show that the application of organic fertilizer did not significantly affect the yield of *E. palmifolia* (P > 0.05). Overall, the application of complete organic fertilizer (cow manure, guano, and rice husk ash) resulted in higher bulb weight per plant and bulb weight per plant plot compared to without organic fertilization and the other organic fertilizer treatments. Providing complete organic fertilizer (cow manure, guano, and husk ash) resulted in bulb weight per plant plot 2.6 times higher than without organic fertilization.

Table 7
*Response of the weight of the tuber per plant and per plot of* E. palmifolia *with the application of different organic fertilizers*

| **Treatment** | **Tuber Weight per Plant* (g)** | **Tuber Weight per Plot* (g)** |
|---|---|---|
| Without fertilization | 9.9a | 35.2a |
| Cow Manure + Guano + Rice Husk Ash | 21.5a | 89.8a |
| Cow Manure + Guano | 10.1a | 39.2a |
| Guano + Rice Husk Ash | 11.1a | 39.0a |
| Cow Manure + Rice Husk Ash | 10.3a | 42.2a |

*Note*: The numbers in the same column followed by the same letter show that they are not significantly different from the DMRT (*Duncan Multiple Range Test*) at the significance level $\alpha = 5\%$ ($P > 0.05$). *: Transformation result = sqrt(x)

The bulb part of the *E. palmifolia* plant is an organ for storing food reserves. The bulb organ is also part of the sink, which requires photosynthesis to be transported by the leaves in the photosynthesis process. Increasing the rate of photosynthesis is also thought to increase plant weight or yield in the form of bulb weight per plant and plot. Complete fertilization (cow manure + guano + rice husk ash) can increase soil fertility and the availability of nutrients in the soil. This is in line with the research results (Ali et al., 2018) that the increase in the weight of onion bulbs is thought to be due to the role of nutrients, which support the enzyme activation process, protein synthesis, chlorophyll formation, root growth, and cell division from the application of organic fertilizer. Similar results were previously reported by Marlin et al. (2022) that the combination of nitrogen (100 kg N $ha^{-1}$) and kalium (25 kg $K_2O$ $ha^{-1}$) can produce the highest number of bulbs and fresh bulb weight of *E. palmifolia*. Wiendi et al. (2021) also state that the application of NPK fertilizer increases the yield of *E. bulbosa* in various doses.

The bulb weight per *E. palmifolia* plant produced in this study was also lower than the results of the research from Atikah et al. (2021) with organic fertilizer and NPK treatment, namely 62.9 g $plant^{-1}$. This is likely because the shading conditions of the coffee plants cause the low light received by *E. palmifolia*. *E. palmifolia* requires sufficient sunlight in open conditions with lighting of ± 70% to assist in the formation of bulbs as a food reserve from photosynthesis.

## Flavonoid Content of *E. palmifolia*

Anova's results showed that different organic fertilizer treatments had no significant effect on the flavonoid content of *E. palmifolia* bulbs ($P > 0.05$) (Table 8). Application of organic fertilizer with different combinations produces higher flavonoid levels compared to those without organic fertilizer. The increase in flavonoid content is because organic fertilizer treatment can increase the number of soil microbes, making it possible to increase interactions between plant roots and soil microbes. The interaction between microbes and plant roots is thought to increase the formation of chemical components, including flavonoids, which have high antioxidant activity (Shabira et al., 2022). Based on the research of Hamad et al. (2023) and Naguib et al. (2012), the giving of organic fertilizer can increase flavonoid content in the *Moringa oleifera* leaves and broccoli, respectively. The combination of organic and inorganic fertilizer treatments affects flavonoids and catechins contents in tea quality by regulating the phenylpropanoid and flavonoid biosynthesis pathways involved in the modulation of structural genes, such as *PAL* (*phenylalanine ammonia-lyase*) (Raza et al., 2024).

Table 8
*Response of the flavonoid content of* E. palmifolia *with the application of different organic fertilizers*

| **Treatment** | **Flavonoid Content (%)** |
|---|---|
| Without fertilization | 0.159a |
| Cow Manure + Guano + Rice Husk Ash | 0.185a |
| Cow Manure + Guano | 0.209a |
| Guano + Rice Husk Ash | 0.199a |
| Cow Manure + Rice Husk Ash | 0.209a |

*Note*: The numbers in the same column followed by the same letter show that they are not significantly different from the DMRT (*Duncan Multiple Range Test*) at the significant level $\alpha = 5\%$ ($P > 0.05$)

Apart from that, it is thought that the intercropping planting pattern between coffee plants and *E. palmifolia* can also influence flavonoid levels through interactions between *Rhizobium* sp. bacteria and arbuscular mycorrhizal fungi, and plant growth can stimulate rhizobacteria and nematodes. This intercropping planting pattern can increase the supply of organic material, which can increase the number of soil microbes, such as bacteria, fungi, and nematodes, which are useful for the growth of the roots of *E. palmifolia* plants (Rostaei et al., 2018; Salehi et al., 2019; Sutrisno & Yusnawan, 2018). Besides that, light stress from the canopy of coffee trees increases flavonoid content because the enzymes of *PAL* also increase in the phenylpropanoid pathway (Firdaus et al., 2022).

## Microclimates Observation

Apart from observing the growth of *E. palmifolia* plants, air temperature, light intensity, and shade percentage were also measured (Table 9). The average air temperature under the coffee trees at five (5) observations, namely from 4 to 12 WAP, was around 29.7°C. The results of air temperature measurements are the results of air temperature observations from the Climatology station in Figure 1a which show that the temperature range is still suitable for the growth of *E. palmifolia*. The decrease in temperature is followed by increasing in air humidity in Figure 1b which shows the range of 82 – 85% which is still good for the growth of *E. palmifolia*. Purwanti and Taryono (2018) state that *E. palmifolia* can still grow with an air humidity range of 30 – 70%.

Table 9
*Results of measurements of air temperature, sunlight intensity, and percentage of shading*

| Time Measurement (WAP) | The Average Air Temperature Under the Canopy of Coffee Tree (°C) | The Average of Light Intensity (Lux) | | Percentage of Shade (%) |
|---|---|---|---|---|
| | | Under the Coffee Tree | Outside of the Coffee Tree | |
| 4 | 30.5 | 1652.2 | 3743.2 | 55.86 |
| 6 | 30.5 | 661.2 | 157954.6 | 99.58 |
| 8 | 28.6 | 527.4 | 46641.4 | 98.87 |
| 10 | 30.5 | 662.0 | 61402.8 | 98.92 |
| 12 | 28.2 | 539.0 | 46092.0 | 98.83 |
| Average | 29.7 | 808.36 | 63166.8 | 90.41 |

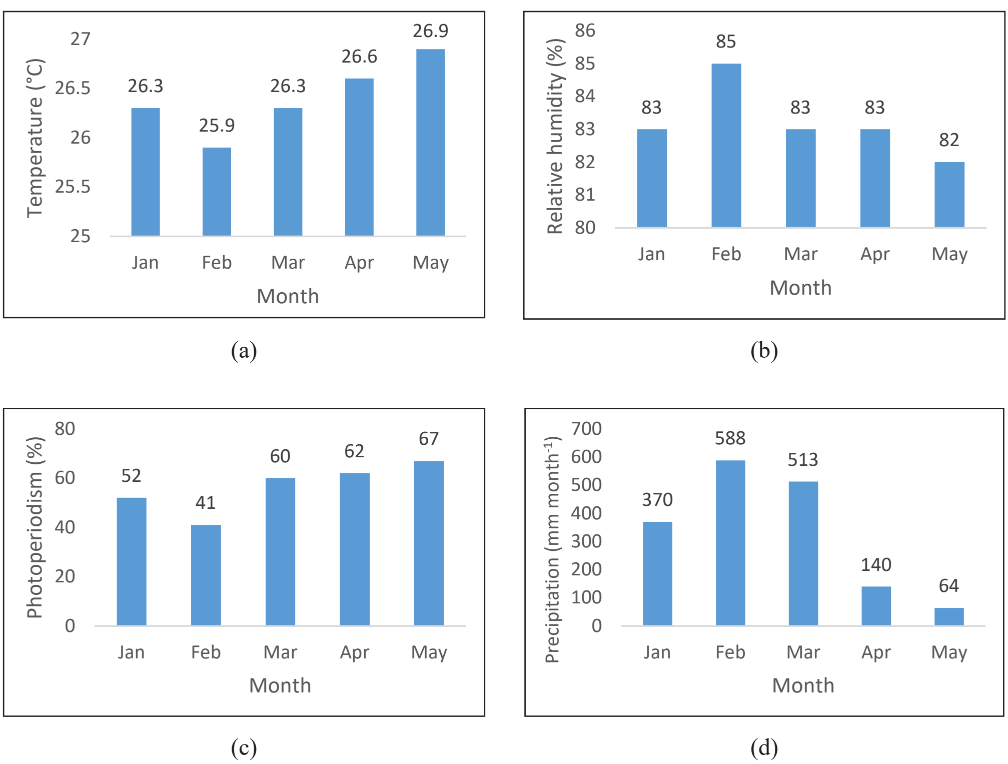


*Figure 1*. The observation of climate elements during research from the Sleman Regency Climatology station. Temperature (a), relative humidity (b), photoperiodism (c), and precipitation (d)

The average light intensity, both under the coffee tree and outside the coffee tree area, shows varying results. The average intensity of sunlight under coffee trees is lower than outside the coffee tree area. The average percentage of shade in observations from 4 to 12 WAP shows around 90.41%. These shade conditions cause low light intensity to be received by *E. palmifolia*. *E. palmifolia* requires sufficient photoperiodism in open conditions with the lighting of ± 70%, meanwhile the results of observing the highest photoperiodism in Figure 1c show a result of 67% which is still by the photoperiodism requirement for the growth of *E. palmifolia*.

The research location, which is located in Umbulharjo Village, Cangkringan District, has an area of approximately ± 103 ha with topography in the form of hills and lowlands at an altitude of ± 662 m above sea level and is ± 12,5 km from the peak of Mount Merapi. The type of coffee cultivated is Robusta which has growing requirements at an altitude of 100 – 600 m above sea level, rainfall of 1250 – 2500 mm/year or < 60 mm/month, and air temperature of 21 – 24°C ( Kementerian Pertanian Republik Indonesia, 2014). Based on data from Climatology Station shows the highest precipitation was 588 mm occurred in February 2023, while the lowest precipitation occurred in May 2023 at 64 mm (Figure 1d) and the average maximum precipitation is 64.8 mm/month (Statistics Sleman Regency, 2021). This shows that *E. palmifolia* plants can still grow and produce in high precipitation with highland topography, although the best results are obtained from lowland topography with air temperatures between 25 - 32°C and dry climates.

## CONCLUSION

The application of complete organic fertilizer (cow manure + guano + rice husk ash) produces a higher number of leaves, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, tuber weight per plant (21.5 g), and tuber weight per plot (89.8 g) compared to without fertilization. The application of organic fertilizer produces the flavonoid content of *E. palmifolia* bulbs 1.2 – 1.3 times higher than no fertilization. The average percentage of shade obtained was 90.41% with an average air temperature of around 29.7°C. *E. palmifolia* can be planted under the coffee tree as an alley cropping. Based on the characteristics of the growth and yield of *E. palmifolia*, further research on the farming feasibility analysis needs to be considered.

## ACKNOWLEDGEMENT

Acknowledgments were given to the Polytechnic of LPP for funding this research through the Competitive Grants Research Program Fiscal Year 2022 on the Lecturer and Student Collaboration Scheme with number 09/Peng/UPPM/XI/2022 on November 13, 2022.

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