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Characterization and Effectiveness of Moringa Leaves (*Moringa oleifera* Lamk.) And *Azotobacter chroococcum* Bio-Organic Fertilizer on the Growth of Green Spinach

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ABSTRACT

The decline in spinach production is a major challenge in efforts to increase sustainable productivity. Bio-organic fertilizer (BOF) formulated from moringa leaves (*Moringa oleifera* Lamk.) and *Azotobacter chroococcum* contains essential nutrients that support plant growth. This study aims to determine the characteristics of BOF in terms of pH, temperature, and fertilizer color, as well as its effectiveness on the growth of green spinach. The study used an experimental design with five treatments, namely P1 (10% moringa leaves), P2 (15%), P3 (20%), K– (without moringa leaves), and K+ (commercial fertilizer). The characteristic data were analyzed descriptively with reference to SNI and relevant scientific literature, while the growth data in the form of plant height and number of leaves were analyzed using ANOVA and ANCOVA at a significance level of 5%. The results showed that BOF had a pH of 6–7, a temperature of 26–27 °C, and a dark brown color. Treatment P3 gave the best results with an average plant height of 26.6 cm and the highest number of leaves, namely 14 leaves. These findings indicate that moringa leaf-based BOF has the potential to be an effective and environmentally friendly organic fertilizer in promoting the sustainable growth of green spinach.
Keyword: Eco-friendly, natural, plant nutrition, sustainable agriculture, quality

ABSTRAK

Penurunan produksi bayam merupakan tantangan besar dalam upaya meningkatkan produktivitas berkelanjutan. Pupuk bio-organik (BOF) yang diformulasikan dari daun moringa (*Moringa oleifera* Lamk.) dan *Azotobacter chroococcum* mengandung nutrisi esensial yang mendukung pertumbuhan tanaman. Penelitian ini bertujuan untuk menentukan karakteristik BOF dalam hal pH, suhu, dan warna pupuk, serta efektivitasnya terhadap pertumbuhan bayam hijau. Penelitian ini menggunakan rancangan eksperimen dengan lima perlakuan, yaitu P1 (10% daun moringa), P2 (15%), P3 (20%), K- (tanpa daun moringa), dan K+ (pupuk komersial). Data karakteristik dianalisis secara deskriptif dengan merujuk pada SNI dan literatur ilmiah terkait, sementara data pertumbuhan berupa tinggi tanaman dan jumlah daun dianalisis menggunakan ANOVA dan ANCOVA pada tingkat signifikansi 5%. Hasil menunjukkan bahwa BOF memiliki pH 6–7, suhu 26–27 °C, dan warna cokelat gelap. Perlakuan P3 memberikan hasil terbaik dengan tinggi tanaman rata-rata 26,6 cm dan jumlah daun tertinggi, yaitu 14 daun. Temuan ini menunjukkan bahwa BOF berbasis daun moringa berpotensi menjadi pupuk organik yang efektif dan ramah lingkungan dalam mendukung pertumbuhan berkelanjutan bayam hijau.
Kata kunci: Ramah lingkungan, alami, nutrisi tanaman, pertanian berkelanjutan, kualitas

1. Introduction

The demand for green amaranth (*Amaranthus tricolor* L.) has increased because it contains vitamins A, E, and C, calcium, fiber, beta-carotene, and iron at 3.9 mg/100 g, which play a role in maintaining health and preventing anemia through the formation of hemoglobin (Kundaryanti et al., 2019; Yudhistira et al., 2018). The 2023 Horticulture Fixed Number Survey reported that spinach productivity in Indonesia tends to be unstable, as seen in the total spinach production which decreased by 0.08%, from 170.82 tons in 2022 to

170.688 tons in 2023, while the spinach harvest area decreased by 0.51% from 47.049 ha in 2022 to 46.81 ha in 2023 (Agriculture, 2024). Research by Khoirurrozzikin et al. (2023) shows that the decline in crop productivity is caused by the continuous use of inorganic fertilizers.

Global use of chemical fertilizers increased from 82 to 112 kilograms per hectare of agricultural land from 2000 to 2021 (Brief, 2023). Chemical fertilizers contain nitric acid, which is often used in the production of mineral fertilizers such as NPK fertilizers and calcium ammonium nitrate fertilizers; as well as ammonium nitrate, which is used in the manufacture of nitrogen-based fertilizers due to its high nitrogen content of around 15.5-34.5% (Alhasan et al., 2024; Ma et al., 2023). Excessive use of these materials can cause acidification, altering the composition and structure of soil microbial communities (Yang et al., 2023; Zhou et al., 2023).

As a solution to this problem, biological organic fertilizer (BOF) made from moringa leaves and azotobacter bacteria is a more sustainable alternative. *Azotobacter chroococcum* converts free nitrogen into ammonium ions (NH_4^+) or nitrate ions (NO_3^-) that can be easily absorbed by plants (Zhu et al., 2021). Meanwhile, moringa leaves are rich in minerals such as sodium (Na), potassium (K), phosphorus (P), calcium (Ca), iron (Fe), copper (Cu), sulfur (S), zinc (Zn), and magnesium (Mg) to provide nutrients (Meireles et al., 2020). The content of 100 grams of fresh moringa leaves includes 1240 mg N, 70 mg P, 259 mg K, 440 mg Ca, 42 mg Mg, and 0.85 mg Fe (Mashamaite et al., 2022).

The combination of organic matter from moringa leaves and Azotobacter bacteria is expected to produce a more effective BOF. Characterization is needed to determine the extent to which this fertilizer can optimally meet plant nutritional requirements. Therefore, this study aims to characterize moringa leaf bio-organic fertilizer through pH, color, and temperature analysis, as well as to test its effectiveness in supporting plant growth.

2. Method

The research was conducted from January to March 2025 in the Green House at coordinates 7°57'41.3" S and 112°37'4.3" E and Microbiology Laboratory of State University of Malang at coordinates 7° 57′ 39.5″ S and 112° 37′ 08.0″ E. Testing the effectiveness of moringa leaf BOF using a Non-Factorial Completely Randomized Design (CRD) with 5 treatments consisting of P1 (10% moringa leaves = 100 g L^{-1}); P2 (15% moringa leaves = 150 g L^{-1}); P3 (20% moringa leaves = 200 g L^{-1}); K- (0% moringa leaves); and K+ (moringa-LOF commercial fertilizer). Each treatment was repeated four times, resulting in 20 experimental units.

2.1 Subculture and inoculant preparation of *Azotobacter chroococcum*

The medium used for the growth of nitrogen-fixing bacteria is Nitrogen Free Bromthymol Blue (NFB) consisting of 1 L of distilled water, with the pH adjusted to 6.5, then sterilized in an autoclave for 30 minutes at a temperature of 121 °C and a pressure of 1 ATM (Baldani et al., 2014). The bacterial subculture process was carried out on Nutrient Agar (NA) media that was tilted aseptically, then incubated at room temperature for 24 hours. The stage of preparing bacterial inoculants for fertilizer production was by inoculating 1% of the parent bacteria *A. chroococcum* into 200 mL of NFB solution in an Erlenmeyer flask. The medium containing the bacteria was then shaken at room temperature for 3 days (Hindersah et al., 2021).

2.2 Preparation of moringa leaf BOF

Fresh moringa leaves are washed thoroughly with running water. Each treatment is fermented in a 2.5 L volume. Formulation P1 consists of 250 grams of crushed moringa leaves, mixed with 100 mL of molasses, 100 mL of EM4, 1.5 L of rice washing water, 150 grams of fermented compost fertilizer, 200 mL of *A. chroococcum* inoculant, and clean water to reach the specified volume. Concentration P2 used 375 grams of moringa leaves, and P3 used 500 grams of moringa leaves, with the same additional ingredients. As a negative control (K-), the ingredients were mixed without adding moringa leaves, but still using the same proportions for the other components. All ingredients were placed in a closed container. According to Sarah et al. (2023), a minimum fermentation time of 14 days is required to produce BOF.

2.3 Sowing of green spinach seeds

Maestro F1 green spinach seeds were sown in trays 1 cm deep, and each hole was filled with 3 seeds and then covered with soil. The tray is covered with clear plastic for 2 days and watered sufficiently to keep it moist. Spinach seedlings that were 7 DAP and had 4 leaves were transferred into polybags measuring 20 cm x 20 cm. Each polybag contained 1 spinach plant.

2.4 Application of moringa leaf BOF and plant maintenance

A total of 10 mL of concentrated liquid BOF is diluted in 1 L of water (1:100) and then sprinkled around the roots (Onyia et al., 2020). Fertilizer is applied once a week with 100 mL in the morning because the temperature is cooler and the air humidity is high, reducing the risk of evaporation. After the plants are fertilized, there is no need to water them with water. Plant maintenance is carried out by watering spinach plants regularly once a day in the morning and cleaning weeds to prevent competition in nutrient absorption between plants.

2.5 Parameter observation

Fertilizer characteristics, including pH, temperature, and color, were observed 3 times, namely on the 0th, 7th, and 14th days of fermentation. Measurements of pH and temperature are carried out using a pH TDS EC Temp Meter tool, while color observations use the eye directly (Tsaniya et al., 2021). Testing the effectiveness of moringa leaf BOF includes the height and number of leaves on green spinach plants. These parameters were measured every 5 days, namely on the 7th to 37th day of DAP.

2.6 Data analysis

BOF characterization data were analyzed descriptively in accordance with Indonesian National Standards (INS) and credible journal articles. Plant height growth data were analyzed using Analysis of Covariance (ANACOVA), while the number of leaves using one way Analysis of Variance (ANOVA) with a significance level of 5%, followed by LSD and Duncan's further test. The data analysis of this study used IBM SPSS Statistics 27.0 software.

3. Result and Discussion

Degree of acidity (pH) of moringa leaf bio-organic fertilizer

Degree of acidity (pH) is an important factor that affects the activity of microorganisms to decompose organic matter. The graph of changes in the pH of moringa leaf BOF during the fermentation process can be seen in Figure 1.

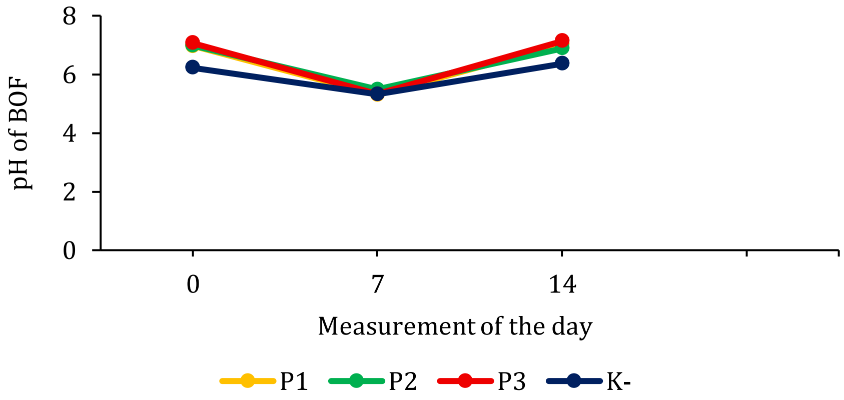

Figure 1. Changes in pH of moringa leaf BOF

Based on Figure 1, the pH of the fertilizer before fermentation was in the neutral range of 6-7. On day 0, it was noted that P3 showed the highest pH of 7.07 while K- showed the lowest pH of 6.23. In the first week, the pH of all treatments decreased until it reached 5. Entering the second week, the pH value increased again to 6-7. The observation results for 14 days showed that P3 had the highest pH of 7.13 and K- showed the lowest pH of 6.36.

Anaerobic fermentation process can take place in the optimum pH range of 6.5 - 7.5 (Sarah et al., 2023). A decrease in pH in fermentation indicates that bacteria are active in decomposing organic matter. This finding is in line with the research of Sarah et al. (2023) who reported that there was a decrease in pH from 6.4 to 5.1 during the fermentation process, where simple organic compounds were converted into various types of acids such as acetic acid, propionic acid, and butyric acid. This is reinforced by the research of Azara et al. (2021) which explains that the decomposition of carbohydrates will produce organic acids so that it will cause a gradual decrease in pH.

The degree of acidity (pH) began to increase on days 8 to 14. An increase in pH has also been reported by (Allaily et al., 2022), namely in the composting process during the incubation period as a result of the release of ammonia. According to Yao et al. (2024), ammonia is the result of microorganism metabolism which also plays a role in increasing pH. Along with the increase in pH, the population of certain microorganisms will increase because pH conditions that are close to normal can support their growth. Fermentation that lasted for 14 days resulted in a final pH of around 6-7. This condition supports the survival of *A. chrooccocum* bacteria contained in the fertilizer. According to Aasfar et al. (2021), the growth of *A. choroococcum* can take place optimally if the pH is in the range of 7-7.5 because it can benefit the physiological functions of bacteria for metabolic processes. Fertilizers that have a pH that is too low can cause microbial death while if the pH is too alkaline it will create a bad environment for microorganism activity to decompose organic matter (Hernández-Fernández et al., 2021; Wang et al., 2022).

The results showed that the pH value of this BOF was within the normal range and safe to apply to plants. The pH value of this fertilizer is around 6-7 in accordance with the Minimum Technical Requirements for Organic Fertilizers, Biofertilizers, and Soil Improvers which states that the pH of fertilizers that are safe to apply is in the range of 4-9 (Ministry of Agriculture, 2019), so it has met the eligibility standards for application in the field.

Temperature of moringa leaf bio-organic fertilizer

Temperature is an important environmental factor for the survival of microorganisms. The changes in the temperature of moringa leaf BOF during 14 days of fermentation can be seen in Figure 2.

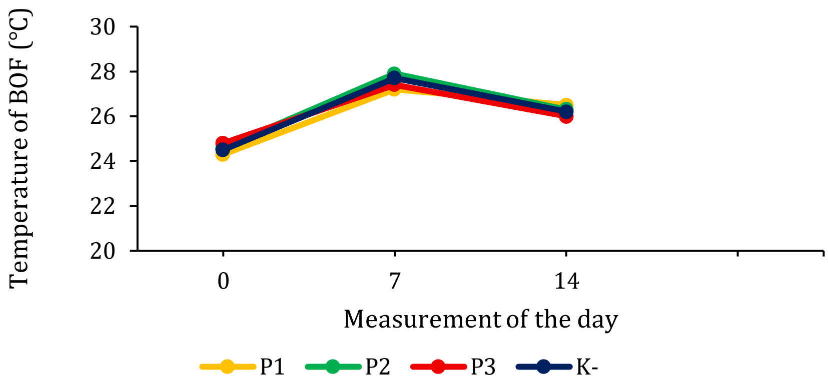

Figure 2. Changes in Temperature of Moringa Leaf BOF

Based on the research results in Figure 2, the temperature changes during fermentation. At the beginning of the observation, it was noted that P3 showed the highest temperature of 24.8 °C while P1's lowest temperature was 24.3 °C. After one week, all treatments experienced an increase in temperature until it reached 27 °C with P2 reaching the highest temperature of 27.9 °C. Entering the second week, the temperature decreased to 26 °C. Observation results for 14 days showed that P1 had the highest temperature of 26.5 °C while P3 showed the lowest temperature of 26 °C.

The observation results on day 0, the initial temperature of the fertilizer was recorded at around 24 °C. This temperature is lower than the optimal range of fermentation which is 25-55 °C (Arifan et al., 2022), which is likely influenced by room temperature and light intensity (Ginting et al., 2022). During the first week of fermentation, the temperature increased due to microbial activity. Research conducted by Napoleon & Sulistiyani (2023) reported that there was an increase in temperature during the fermentation process from 26 °C to 29 °C. Similar findings were also reported by Abidin et al. (2024) that there was an increase in temperature during the fermentation process from 25.9 °C to 29.1 °C as a result of the biochemical reactions that occurred. The study conducted by Afa et al. (2024) explained that there was no increase in temperature during the fermentation process.

The results of observations in the second week, namely on days 8 to 14, the temperature decreased from 27 °C to 26 °C. This decrease indicates that microbial activity has decreased due to the reduction of organic matter as a source of metabolism. A decrease in temperature will occur along with the decomposition of most organic matter (Napoleon & Sulistiyani, 2023). However, the resulting final temperature of 26 °C is within the range that supports the survival of *A. chroococcum*. According to Aasfar et al. (2021), Azotobacter can grow well at a temperature of 25-30 °C which supports its physiological activities.

Color of moringa leaf bio-organic fertilizer

Color acts as one of the important indicators in identifying the maturity of fertilizer. Changes in the color of the fertilizer during the fermentation process for 14 days are presented in Table 1.
Visual observation of fertilizer color as shown in Table 1.

Table 1. Color of moringa leaf biological organic fertilizer for each treatment

Treatments	Colors		
	0	7	14
0% moringa leaves (K-)	Brown	Brown	Dark Brown
10% moringa leaves (P1)	Greenish Brown	Dark Brown	Dark Brown
15% moringa leaves (P2)	Greenish Brown	Dark Brown	Dark Brown
20% moringa leaves (P3)	Greenish Brown	Brown	Blackish Brown

Visual observation of fertilizer color as shown in Table 1 shows a color change from greenish brown to dark brown to blackish brown. On day 0, treatments P1, P2, and P3 were dominated by greenish brown color, while K- showed brown color. Entering the first week of fermentation, the color changed to brown to dark brown, indicating that organic matter decomposition had occurred. At the end of the fermentation process, the color of the fertilizer became more intense to dark brown to blackish brown.

The initial color of fertilizer before fermentation is greenish brown which is influenced by the composition of the basic ingredients (Apriani & Asngad, 2023). The brown color comes from ingredients such as molasses, EM4, and manure, while the greenish color is the natural color of moringa leaves that have not been decomposed. Color changes that occur during the fermentation process are due to the activity of microorganisms in decomposing organic matter in fertilizers. As the process progresses, organic matter undergoes an oxidation reaction which results in a darker color. In accordance with the study of Kartini et al. (2023) which shows that the activity of microorganisms causes changes in the particle size of organic matter to become smaller and then followed by a change in color to darker.

P3 treatment produces a blackish brown final color. This is because Moringa leaves have phenolic compounds that can potentially produce dark pigments. Studies conducted by Gomes et al. (2014) stated that phenol compounds in the substrate can be oxidized by the enzyme polyphenol oxidase (PPO) into dark quinone compounds. This oxidation process is likely to occur when the container is open so that outside air can react directly with the material. The observation results for 14 days of fermentation showed the dominance of dark brown to blackish color. This condition has reached optimal fertilizer maturity, as described by Afa et al. (2024), which states that the dark color indicates that most of the organic matter has been decomposed so that fertilizers containing nutrients can be directly absorbed by plants.

Green spinach plant height

Observation data on the height of green spinach plants during 37 weeks of planting overall showed that the provision of moringa leaf BOF gave significant influence and difference on the growth of green spinach plant height. BOF P3 gave the highest result of 26.57 ± 1.27 but was not significantly different from K+ and P2, while K- gave the lowest result of 19.25 ± 1.27. The results of the analysis of the average height of green spinach plants are in Table 2.

Table 2. Average height of green spinach.

Treatments	Average Height of Spinach (cm)
0% moringa leaves (K-)	19.25±1.27ab
10% moringa leaves (P1)	21.17±1.37b
15% moringa leaves (P2)	23.35±1.29bc
Commercial Fertilizer (K+)	24.84±1.26bc
20% moringa leaves (P3)	26.57±1.27c

Note: In columns with the same letter above the number, the value is not significantly different based on Duncan's test at the 5% significance level (n=5; mean ± SD).

In general, the development of the average height of spinach plants is presented in Figure 3, which illustrates the plant's growth from 7 to 37 WAP (Week After Planting).

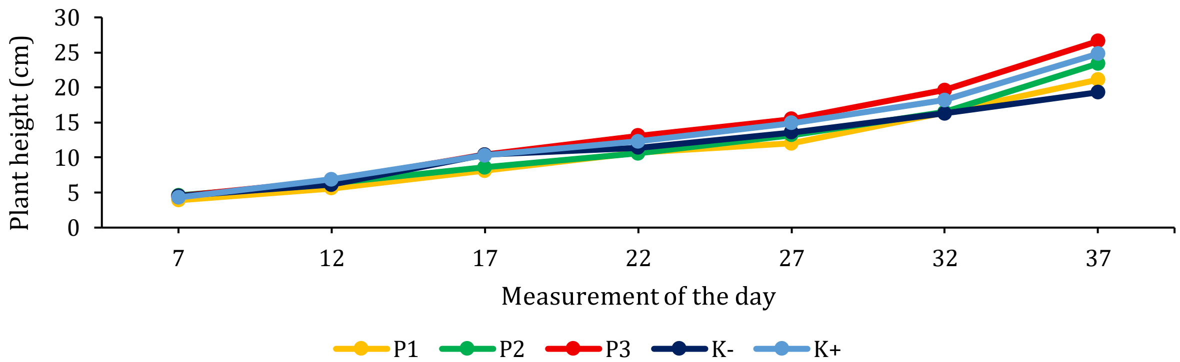

Figure 3. Height of green spinach

Based on the observation in Figure 3, P3 significantly increased the average plant height by 26.6 cm at 37 weeks after planting, while K- showed the lowest average of 19.3 cm. This indicates that P3 is the optimal dose in the growth of green spinach plants. Increasing the concentration of moringa leaves in fertilizer can provide nutrient availability. The P3 treatment showed more optimal results than the K+ treatment, presumably related to the presence of *A. chroococcum* bacteria. Studies conducted by Aasfar et al. (2021), explained that Azotobacter bacteria are able to increase nitrogen availability and synthesize growth hormones such as cytokinins, IAA, and gibberellins.

The P3 variation showed a significant effect on the growth of green spinach plant height, indicating the potential of moringa leaves as fertilizer material. These results are in line with the study of Fiddaroini & Yulianti (2023) which reported that moringa leaf liquid organic fertilizer accelerated the growth rate of red spinach plant height up to 1.4 times faster than plants that were only watered with water with growth rates of 0.36 cm and 0.26 cm per day, respectively. Another study also proved that moringa fertilizer can increase the height of leek plants from 6.20 cm to 20.20 cm (Mare et al., 2023).

Moringa leaves contain phytohormones such as cytokinins zeatin, auxins, and gibberellins that play a role in regulating plant growth (Mashamaite et al., 2022). Zeatin, which is dominant in moringa leaves, has an important role in cell division and enlargement in young tissues, as well as increasing

the absorption and distribution of photosynthetic products (Yuniati et al., 2022). This role is supported by auxins that stimulate cell elongation, stem growth, and lateral roots, as well as gibberellins that contribute to increasing plant height, leaf expansion, and photosynthetic efficiency (Mashamaite et al., 2022).

In addition to phytohormone content, Moringa leaves also contain macro nutrients such as Nitrogen (N), Phosphorus (P), and Potassium (K) which play an important role in supporting plant growth (Mare et al., 2023). Nitrogen functions in stimulating vegetative cell division, including stems so that plant growth can be influenced (Leghari et al., 2016). Other macro elements such as phosphorus contribute to increasing abiotic stress tolerance in plants, supporting growth, and strengthening roots and stems (Khan et al., 2023). Potassium also plays an active role in promoting plant growth by stimulating enzymes involved in photosynthesis, stomatal regulation, protein synthesis, and other metabolic processes (Chrysargyris & Tzortzakis, 2025).

Number of green spinach leaves

Observation data for 37 weeks after planting showed that the provision of moringa leaf BOF had a significant effect on the increase in the number of green spinach leaves. The P3 treatment gave the highest result of 14.25 ± 0.95 but did not show a significant difference with K+ and P2, while the treatment with K- gave the lowest result of 10.50 ± 1.29 but did not differ significantly with P1 and P2. The results of the analysis of the average height of green spinach plants are presented in Table 3.

Table 3. Average number of green spinach leaves.

Treatments	Average number of spinach leaves
0% moringa leaves (K-)	10.50±1.29ab
10% moringa leaves (P1)	11.75±1.89b
15% moringa leaves (P2)	12.25±1.89bc
Commercial Fertilizer (K+)	13.00±1.41bc
20% moringa leaves (P3)	14.25±0.96c

Note: In columns with the same letter above the number, the value is not significantly different based on Duncan's test at the 5% significance level (n=5; mean ± SD).

In general, the development of the average number of green spinach leaves is presented in Figure 4, which illustrates the plant's growth from 7 to 37 WAP (Week After Planting).

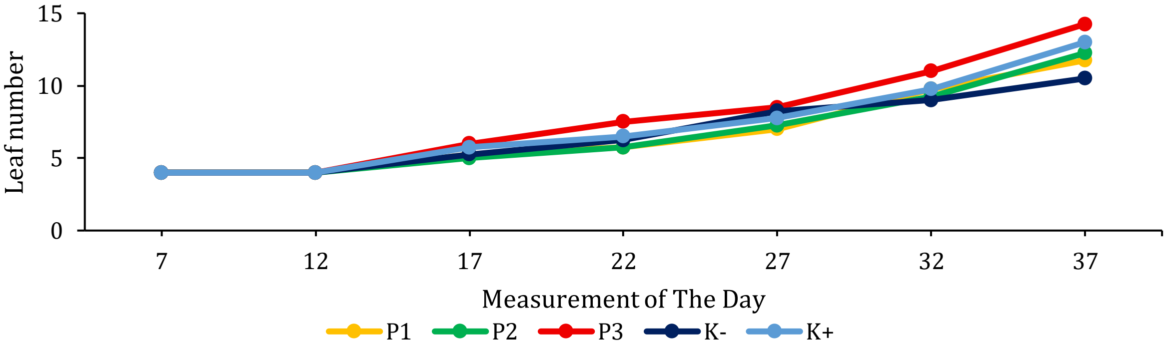

Figure 4. Number of green spinach leaves

The observation results presented in Figure 4 show the number of green spinach leaves has increased significantly. At 7 weeks of planting, all treatments had 4 leaves. After 37 weeks of planting, changes were seen, where P3 had the highest number of leaves, namely 14.25 leaves, while K- had the least with 10.5 leaves. It is suspected that P3 provides optimal results in meeting the nutritional needs of plants. In line with the research of Tomia & Pelia (2021) which reported an increase in the number of purple eggplant leaves with an average of 8.25 strands to 25.25 strands after being given moringa leaf liquid organic fertilizer. Another study also proved similar results in tomato plants with an average of 97.58 leaves at 21 HST (Noer, 2022).

Moringa leaves have a number of natural phytohormones such as auxins, cytokinins, and gibberellins that play an active role in leaf formation and development. Cytokinin contributes to enlarging mesophyll cells so that the leaf surface area increases, strengthening leaf structure by stimulating cell wall thickening in reinforcing tissues, and encouraging the formation of vascular tissue (Sosnowski et al., 2023). Auxin supports the growth of leaf number through cell elongation and enlargement in leaf meristem tissues, and regulates stomatal density (Mashamaite et al., 2022). Gibberellin also plays a role in cell elongation, leaf expansion, new leaf formation, and regulates leaf senescence (Ritonga et al., 2023). The interaction between the three phytohormones contributes significantly to increasing leaf quantity and quality.

In addition to the phytohormone content, Moringa leaves also contain macronutrients such as Nitrogen (N), Phosphorus (P), and Potassium (K) that support leaf formation. Leghari et al. (2016) suggested that nitrogen stimulates the division of plant vegetative cells, one of which is the leaf. Nitrogen plays a role in the synthesis of nucleic acids, proteins, chlorophyll, and several hormones to support the photosynthesis process (Wang et al., 2024). Nitrogen deficiency will cause symptoms such as chlorosis in the leaves, stunted plant growth, thickening of root cell walls, and reduced crop yields (Lu et al., 2021). Phosphorus also plays an important role in the photosynthesis process such as synthesizing ATP, forming Ribulose1,5-Bisphosphate (RuBP), and NADH which supports the Calvin cycle (Khan et al., 2023). The number and quality of leaves will affect the photosynthetic ability of plants because it can increase the efficiency of energy absorption to support overall plant growth (Noer, 2022).

4. Conclusion

Increased concentrations of moringa leaves in Biological Organic Fertilizer (BOF) significant effect on the growth parameters of green spinach plants. BOF with a 20% concentration of moringa leaves showed the highest effectiveness in increasing plant height and number of leaves. Characterization results indicate that this fertilizer has pH, temperature, and color that meet organic fertilizer quality standards. Thus, bio-organic moringa leaf fertilizer has the potential to be an optimal source of nutrients to support sustainable plant growth.

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