



Ecoenzymes as an alternative nutrient for lettuce (*Lactuca sativa* L) in wick hydroponics: Supporting circular agriculture through organic waste recycling

Jihan Bilqis¹, Hertien Koosbandiah Surtikanti^{1*}, Wahyu Surakusumah¹, Afri Irawan¹

¹ Department Biology, Faculty of Mathematics and Natural Sciences Education, Universitas Pendidikan Indonesia, Bandung, West Java 40154, Indonesia.

*Correspondence: hertien_surtikanti@yahoo.com

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ABSTRACT

Background: This study aims to determine the optimal concentration of ecoenzymes as an alternative nutrient in the cultivation of lettuce (*Lactuca sativa* L.) using a wick system hydroponic medium. This effort aligns with the achievement of the Sustainable Development Goals (SDGs), particularly SDG 2 (zero hunger), SDG 12 (responsible consumption and production), and SDG 13 (climate action). Amid the growing challenges of food security and land conversion, the utilization of ecoenzymes derived from organic waste offers an environmentally friendly solution for sustainable agriculture. **Methods:** This quantitative study employed a completely randomized design (CRD) with six treatments: negative control (water), ecoenzyme at 1% concentration (10 ml/L), 1.5% (15 ml/L), 2% (20 ml/L), 2.5% (25 ml/L), and positive control (AB mix 0.5%). Measured parameters included leaf area, plant height, number of leaves, leaf color, as well as fresh and dry weight. **Findings:** Laboratory analysis results showed that the macronutrient (N, P, K, Ca, Mg) and micronutrient content in the ecoenzyme was below that of commercial nutrient standards such as AB mix. Overall, the AB mix treatment yielded the highest growth results. Nevertheless, the use of ecoenzyme at a concentration of 10 ml (1%) showed the most optimal results among the other ecoenzyme treatments as a supplemental nutrient for increasing leaf surface area, plant height, number of leaves, as well as fresh and dry weights. Furthermore, the 15 ml (1.5%) concentration proved most effective for minimizing leaf yellowing. **Conclusion:** In conclusion, organic ecoenzymes have strong potential for use as biostimulants and supplemental nutrients that support the sustainability of hydroponic agriculture. **Novelty/Originality of this article:** This study established specific concentration thresholds for ecoenzymes derived from organic waste as effective hydroponic biostimulants, while also demonstrating their ability to support lettuce growth and promote waste-free circular agriculture and the achievement of SDG targets.

KEYWORDS: ecoenzymes; lettuce (*Lactuca sativa*); wick-based hydroponic system; SDGs, biostimulants.

1. Introduction

The Sustainable Development Goals (SDGs) are a global development agenda aimed at addressing various issues in the social, economic, environmental, and legal and governance sectors to ensure the sustainability of human quality of life. This study specifically supports the achievement of SDG 2 (Zero Hunger), SDG 12 (Responsible Consumption and Production), and SDG 13 (Climate Action). The primary focus of SDG 2 is the eradication of hunger and the promotion of sustainable agriculture. However, in the Indonesian context,

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efforts to achieve food security face significant obstacles in the form of land scarcity, declining productivity, and governance challenges, particularly in peripheral regions (Tening et al., 2025).

This problem is exacerbated by land conversion due to urbanization, which threatens the availability of productive land, particularly in densely populated areas such as Java. These conditions have the potential to increase food vulnerability even if a region has a regional surplus. To address these challenges, strategic steps are needed in the form of implementing climate-smart agriculture to increase productivity while mitigating climate risks (SDG 13). Additionally, the sustainable optimization of resource use is crucial for promoting more responsible consumption and production patterns (SDG 12) (Faoziyah et al., 2024; Retnaningtyas et al., 2025).

The use of ecoenzymes as liquid organic fertilizers is an environmentally friendly alternative with significant potential to support sustainable agricultural systems. Various studies indicate that applying ecoenzymes at optimal concentrations can provide a balance of macro- and micronutrients essential for plants, thereby effectively accelerating the vegetative phase and optimizing the productivity of horticultural crops. In addition to increasing crop yields, ecoenzymes play a role in mitigating the risk of etiolation—a morphological phenomenon in which plant stems grow elongated yet weak due to light deficiency. The comprehensive nutrients contained in ecoenzymes support the formation of a stronger plant tissue structure, while simultaneously enhancing the plant's resilience against various environmental stresses (Gustaman & Riswan, 2022).

The exploration of ecoenzymes in hydroponic systems is a strategic effort to fill a gap in the literature, particularly regarding the effectiveness of organic fermentation in supporting optimal plant growth. Although previous studies have extensively discussed the general use of organic fertilizers, research on the specific application of ecoenzymes as a nutrient source remains very limited. Therefore, this study aims to determine the optimal concentration of ecoenzymes in the cultivation of lettuce (*Lactuca sativa*) in a wick-system hydroponic medium (without water flow). The integration of these ecoenzymes is not only expected to increase crop yields but also plays a crucial role in promoting sustainable agricultural practices through environmentally friendly alternative nutrient innovations.

2. Methods

2.1 Research method

This study was conducted from September 2025 to December 2025 at the greenhouse of the Universitas Pendidikan Indonesia. This study is a quantitative research using a completely randomized experimental design (CRD). The sampling technique employed was random sampling; the samples used in this study consisted of 5 high-quality lettuce plants (with upright stems and fresh green leaves) in each treatment group. The experimental method involved dividing the treatment groups based on varying concentrations of ecoenzymes, with control groups receiving only water and only AB mix, followed by growth data measurements and statistical analysis to compare differences in results among treatments. The overall treatments are E0 for hydroponics without ecoenzyme (water only); E1 for hydroponics with a 1% ecoenzyme concentration (10 ml in 1 liter); E2 for hydroponics with a 1.5% concentration of ecoenzyme (15 ml per 1 liter); E3 for hydroponics with a 2% concentration of ecoenzyme (20 ml per 1 liter); E4 for hydroponics with a 2.5% concentration (25 ml per 1 liter); E5 for hydroponics with a 0.5% AB mix concentration (5 ml per 1 liter). The number of replicates for each treatment was determined using Freederer's formula. Freederer's formula is as follows on Equation 1.

$$[(t-1)(n-1) \geq 15] \quad (\text{Eq. 1})$$

The explanation are t for number of treatments, n for the number of replicates, and “ ≥ 15 ” for the degrees of freedom for error. The calculation of the number of replicates using the

research formula is as follows on Equation 2. Furthermore, based on these calculations, the minimum number of replicates in this study is 4, and the number of replicates to be used is 5 for each treatment.

$$\begin{aligned}
 &[(t-1)(n-1) \geq 15] \\
 &(6-1)(n-1) \geq 15 \\
 &5(n-1) \geq 15 \\
 &5n - 5 \geq 15 \\
 &5n \geq 20 \\
 &n \geq 4 \text{ Replications}
 \end{aligned}
 \tag{Eq. 2}$$

Periodic observations were conducted once a week in conjunction with hydroponic maintenance (water changes) and the observation of test parameters. The test parameters used included leaf surface area; plant height; number of leaves; leaf color; fresh weight; dry weight. In addition to the test parameters, supporting data were also measured weekly. The supporting data measured included hydroponic pH; greenhouse temperature; greenhouse air humidity; light intensity entering the greenhouse; hydroponic ppm.

Then the equipment and materials as follows. The study utilized a population of 108 healthy and fresh lettuce seeds that had been sown in rockwool medium for 45 days. The equipment used in this study included a wick hydroponic system (hydroponic tray, wick fabric, seedling trays, syringes, AB mix solution, rockwool), 3 liters of eco-enzymes (pineapple peel, watermelon peel, orange peel, mustard greens, molasses, well water), a 1,000 ml measuring cup, a digital thermohygrometer, a water quality tester, a 50 cm ruler, a TDS meter, tweezers, and an analytical balance.

2.2 Research workflow

The research workflow begins with the preparation of ecoenzymes, testing the nutrient content of the ecoenzymes and AB mix, sowing seeds and planting lettuce in a hydroponic system, measuring test parameters, and harvesting the lettuce. First, ecoenzyme preparation, the coenzyme production uses the commonly applied concentration ratio of 1 : 3 : 10. Five liters of ecoenzyme were prepared, requiring 300 ml of molasses, 900 grams of organic waste (225 grams of pineapple peel; 225 grams of watermelon rind; 225 grams of orange peel; 225 grams of mustard greens), and 3 liters of well water.

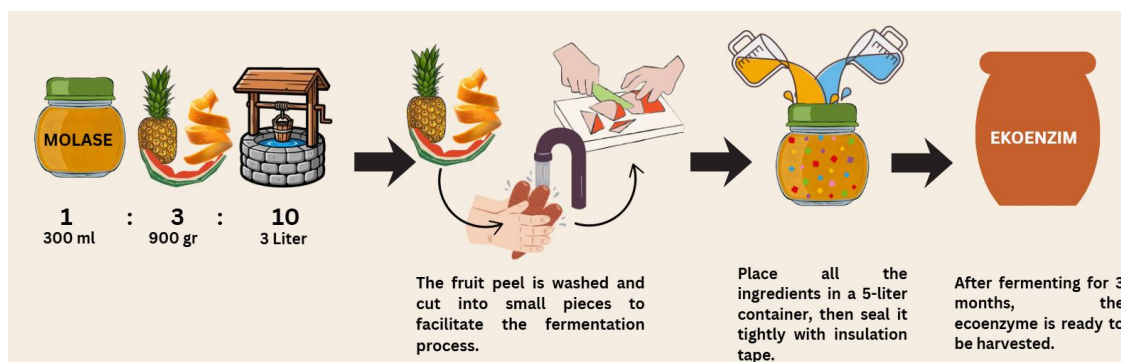


Fig. 1. Process of making ecoenzyme

Second, testing the nutrient content of the ecoenzyme and AB mix. The prepared ecoenzyme is then tested for its nutrient content. Testing of the ecoenzyme's nutrient content will be conducted at the agricultural chemistry laboratory located at Jalan Raya Tangkuban Parahu Km. 22, Cikole, Lembang Subdistrict, West Bandung Regency, West Java. The nutrients to be tested include macronutrients (C; H; O; N; P; K; Ca; Mg) and micronutrients (Mn; Zn; Fe).

Third, sowing seeds and planting lettuce in a hydroponic system. Lettuce seed sowing begins with purchasing seeds from the JM Tani store, produced by PT East West Seed Indonesia. The seeds are then washed and the high-quality ones are selected; to determine seed quality, they are soaked in a 250 ml glass, and the seeds that sink are identified. The selected seeds are sown into 2 cm x 2 cm rockwool cubes. The rockwool cubes containing the seeds are then placed on a germination tray. The tray is then filled with 500 ml of water. Finally, place the tray in a dark, humid location. Let the tray sit for 45 days until the lettuce seedlings begin to emerge. After 45 days of germination, the lettuce seedlings are transplanted into the prepared wick hydroponic medium. There are 12 wick hydroponic units used for 6 treatments (water only; 10 ml; 15 ml; 20 ml; 25 ml; 5 ml AB mix).

Fourth, measurement of test parameters. Test parameter measurements were conducted once a week, and maintenance involved replacing the yellow traps. Test parameters included observing the leaf area, which was calculated using a ruler and plugged into the leaf area formula. The formula is as follows on Equation 3.

$$[w \times l \times h] \quad (\text{Eq. 3})$$

The explanation are w for lettuce leaf length (cm); l for Lettuce leaf width (cm); h for lettuce leaf area constant (0.75). Next, plant height was measured using a ruler, starting from the leaf stalk visible above the rockwool to the tip of the longest leaf stalk. The number of leaves was counted individually. Fifth, lettuce harvesting. Lettuce harvesting was conducted after 49 days in the wick hydroponic system; the harvest was documented and separated by treatment to observe test parameters that had not yet been measured. Test parameters measured after harvest were fresh weight, dry weight, and lettuce leaf color. Fresh weight was determined by weighing the plants on an analytical scale, while dry weight was determined by drying the plants in an oven at 70–80 °C for 48–72 hours. Lettuce leaf observations were conducted after the plants were harvested; if leaf deficiency conditions occurred, they were documented and recorded.

2.3 Data analysis, scope of the study, and hypothesis

Data analysis was performed using quantitative techniques with IBM SPSS software. The analysis began with tests for normality and homogeneity. For data that were normal and homogeneous, parametric tests, specifically one-way Analysis of Variance (ANOVA) and Tukey's test were conducted. However, if the data were non-normal and non-homogeneous, non-parametric tests, specifically the Kruskal-Wallis test followed by Dunn's post hoc test were performed.

To ensure this study remains focused and achieves its intended objectives, the following research limitations have been established: enzyme concentrations are limited to 1%, 1.5%, 2%, and 2.5% as an alternative source of plant nutrients, and application is performed after the lettuce is transplanted into a wick hydroponic system (45 days after sowing). Lettuce plant growth was recorded once a week for 7 weeks (harvest time). The test parameters used were leaf area, plant height, number of leaves, leaf color, fresh weight, and dry weight of the plants. Supporting data measured to assess environmental factors included the pH of the hydroponic medium, greenhouse temperature, greenhouse humidity, light intensity entering the greenhouse, and the concentration of dissolved substances (ppm) in the hydroponic medium.

Based on these research limitations, the following general hypothesis (H1) was formulated: the application of ecoenzymes at different concentrations (1%; 1.5%; 2%; 2.5%) has a significant effect on the growth of lettuce (*Lactuca sativa* L.) in a wick hydroponic system. Null hypothesis (H0): The application of ecoenzymes at different concentrations (1%; 1.5%; 2%; 2.5%) does not significantly affect the growth of lettuce (*Lactuca sativa* L.) in a wick hydroponic system.

3. Results and Discussion

3.1 Results of AB mix and ecoenzyme analysis

The results of the analysis of the AB mix and Ekoenzim content showed that they contain nutrients required by lettuce plants (Table 1). The nutrients contained in the AB mix include macronutrients (N; P; K; Ca; Mg) and micronutrients (Fe; Mn; Zn). Ecoenzymes contain organic carbon, which distinguishes them from AB mix; the organic carbon in ecoenzymes serves to balance the microbial population in liquid organic fertilizer solutions.

Table 1. Comparison table of ecoenzyme and AB mix content

Content	Ecoenzyme	AB mix
Organic C	1.49%	-
Total N (Organic N + NH ₄)	0.03%	9.90%
P ₂ O ₅	0.02%	4.70%
K ₂ O	0.30%	16.50%
Ca	0.08%	8.30%
Mg	0.02%	2.80%
Fe	13.22 ppm	0.04%
Zn	0.10 ppm	0.01%
Mn	2.69 ppm	0.01%

Based on the analysis results, the organic carbon content in the ecoenzyme was recorded at 1.49%. This value is relatively suboptimal when compared to commercial liquid organic fertilizer standards, which require a minimum threshold of 3% to ensure adequate plant nutrition (Fernández-Delgado et al., 2020). From an agronomic perspective, the presence of organic carbon plays a crucial role, particularly in stimulating soil microbial dynamics and regulating the slow release of micronutrients (Dhaliwal et al., 2024).

Analysis results indicate a significant disparity in nitrogen content between the AB mix nutrient solution and the ecoenzyme. The nitrogen content in the AB mix was recorded at 9.90%, a concentration considered optimal for meeting the physiological needs of lettuce plants, particularly for stimulating leaf growth and the formation of a sturdy stem structure. This adequate nitrogen availability positively correlates with increased plant height, leaf proliferation, and biomass weight, given that nitrogen is essential for maximizing photosynthesis rates and tissue formation (Fahmi et al., 2022). Conversely, the total N content (accumulation of organic N and NH₄) in the eco-enzyme is very low, reaching only 0.03% per liter. This value is far below the minimum threshold for commercial liquid organic fertilizers as per SNI 19-7030-2004, which requires a minimum N content of ≥0.40%. However, agronomically, nitrogen plays a crucial role as a precursor for the synthesis of amino acids, proteins, enzymes, and chlorophyll to support vegetative organs, while also functioning as a bioindicator of symbiotic interactions between plants and microbes in the growing medium (Sun et al., 2024).

A comparative analysis of phosphorus content revealed significant disparities between the AB mix solution and the ecoenzyme. The AB mix solution has a phosphorus content of 4.70%, a concentration considered sufficient to support the physiological needs of hydroponic plants, particularly in inducing cell division, as well as stimulating root formation and elongation, which directly impacts increased leaf proliferation and fresh plant weight (Iswiyanto et al., 2022). Conversely, the phosphorus content (in the form of P₂O₅) in the ecoenzyme was recorded as very low, at only 0.02% per liter. Although P₂O₅ accumulated through microbial activity in the ecoenzyme is functionally essential for supporting root development and the efficiency of water and other nutrient uptake, this value is far below the standard range for commercial organic fertilizers (0.5%–4%). This finding confirms that the presence of P₂O₅ in the ecoenzyme primarily reflects its role as a biostimulant, rather than as a primary substitute for liquid organic fertilizer (Leksono et al., 2024).

A comparative analysis of potassium content revealed a significant disparity between the AB mix nutrient solution and ekoenzim. The potassium content in the AB mix was recorded at 16.50%, a concentration deemed adequate and consistent with optimal liquid nutrient standards—ranging from 200–300 mg/L relative to the N, Ca, and Mg ratios—to support hydroponic lettuce cultivation (Gong et al., 2024). Conversely, the potassium content (in the form of K_2O) in the ecoenzyme was recorded as very low, at 0.30% per liter, thus failing to meet the quality standards for liquid organic fertilizer under Ministry of Agriculture Regulation No. 1/2019, which requires a range of 3%–6%. However, physiologically, potassium plays an essential role as the primary cation regulating respiration, osmotic pressure, and cell turgor, which directly impacts transpiration efficiency and water balance (Mostofa et al., 2022; Mulet et al., 2023). Therefore, the availability of potassium in appropriate proportions—as facilitated by AB mix—is crucial for maintaining tissue strength, enhancing plant tolerance to environmental stress, and optimizing crop yield and quality (Fahmi et al., 2022).

The results of the comparative analysis indicate a significant disparity in calcium (Ca) content between the AB mix solution and the ecoenzyme. The AB mix solution has a calcium concentration of 8.30%, a level considered sufficient to meet the essential needs of plants. In contrast, the calcium content in the ecoenzyme was found to be very low, at only 0.08% per liter. Physiologically, calcium acts as an essential cation that is crucial for maintaining membrane stability and cell wall integrity through the formation of calcium pectate (Ca-pectate) compounds, thereby playing a vital role in supporting the structural integrity of tissues including roots, stems, and leaves (Yang et al., 2024). Given its critical role in strengthening cellular structure, an optimal calcium supply—as facilitated by the AB mix—is essential to prevent structural degradation such as tissue fragility and to minimize the risk of physiological abnormalities like tip rot caused by calcium deficiency (Indrani et al., 2024).

Comparative analysis results indicate a significant disparity in magnesium (Mg) availability between the AB mix nutrient solution and the ecoenzyme solution. The AB mix solution contains 2.80% Mg, a concentration deemed sufficient to optimize the activation of enzymes involved in carbohydrate and protein biosynthesis. The adequacy of Mg supply from the AB mix directly correlates with increased leaf area, fresh weight, and photosynthetic rate efficiency in hydroponic lettuce cultivation (Indrani et al., 2024). Conversely, Mg accumulation in the ecoenzyme solution was recorded as very low, at only 0.02% per liter. Despite its minimal concentration, magnesium plays a crucial physiological role in facilitating the translocation of assimilates (s of photosynthesis). More specifically, magnesium functions vitally as a cofactor for the activation of various enzyme complexes and regulates the distribution of sugars from photosynthetic organs (leaves) to the root system (Yang et al., 2024).

A comparative analysis of essential micronutrients—including iron (Fe), manganese (Mn), and zinc (Zn)—reveals significant differences in concentration between the AB mix nutrient solution and the ecoenzyme solution. Although required in minute quantities, micronutrients play a crucial physiological role; Fe acts as an electron carrier in photosynthesis and respiration mechanisms, Mn plays a key role in water photolysis in Photosystem II and the activation of protein-synthesizing enzymes, while Zn is essential for the biosynthesis of auxin hormones that regulate cell elongation (Anwar et al., 2025). The AB mix formulation is considered capable of optimally meeting these physiological needs with Fe content of 0.04%, Mn 0.01%, and Zn 0.01%. In contrast, the accumulation of micronutrients in the ecoenzyme was recorded at significantly lower levels, namely Fe at 13.22 ppm, Mn at 2.69 ppm, and Zn at 0.10 ppm. Although the presence of these micronutrients in the ecoenzyme theoretically supports redox reactions, pathogen resistance, and enzymatic activity, their suboptimal concentrations imply suboptimal plant metabolic performance compared to the use of standard nutrient solutions (Dhaliwal et al., 2024).

3.2 Leaf surface area

Normality and homogeneity tests were conducted using the one-sample Kolmogorov-Smirnov test, and the results showed that the leaf surface data for each treatment were normally distributed and homogeneous; therefore, the analysis proceeded with a one-way ANOVA followed by Tukey's post-hoc test. The results of the interaction between lettuce plants and ecoenzyme application showed a highly significant effect of treatment on leaf surface area. Ecoenzyme treatment exhibiting the lowest leaf surface area (15.3 cm^2) compared to the positive control AB mix (48.85 cm^2) (Table 2).

Table 2. Comparison table of leaf surface area

Treatment	Leaf area (cm^2)
Negative control	$27.65 \pm 14.39\text{a}$
1%	$25.46 \pm 8.56\text{a}$
1.5%	$27.19 \pm 11.09\text{a}$
2%	$27.53 \pm 11.46\text{a}$
2.5%	$25.86 \pm 10.72\text{a}$
Positive control	$41.66 \pm 14.82\text{b}$

Statistical test results indicate that there is a highly significant effect of treatment on leaf surface area, as evidenced by a $p\text{-value} < 0.001$. The 20 ml ecoenzyme treatment produced the highest average leaf surface area ($25.86 \pm 10.72\text{a}$). However, the variation in the ecoenzyme treatments was not statistically significantly different compared to the negative control treatment. This indicates that the application of ecoenzymes within the dose range (10 ml; 15 ml; 20 ml; 25 ml) has not yet been able to provide a better stimulatory effect compared to no treatment (negative control) in promoting the expansion of the leaf surface area of lettuce plants. Based on the statistical analysis described above, there were significant differences in leaf surface area in response to the positive control treatment (AB mix), negative control (water only), and ecoenzyme treatments (10 ml; 15 ml; 20 ml; 25 ml).

Based on observations over seven weeks, the plant height parameter showed a significant increase, while leaf area and number exhibited fluctuating growth. Fluctuations in leaf area as the plant ages essentially reflect cell division and enlargement activity in that organ. Physiologically, this process is induced by the synergy of endogenous phytohormones such as auxin, gibberellin, and cytokinin (Lv et al., 2023). In addition to hormonal factors, environmental microclimate conditions, such as light penetration inside the greenhouse, also play a crucial role. In this study, the recorded light intensity was 5,315 Lux. This value is relatively low compared to the recommendations of Dai et al. (2024), who stated that the optimal light intensity for accelerating leaf area expansion ranges from 10,800 to 16,200 Lux (equivalent to $200\text{--}250 \mu\text{mol m}^{-2} \text{ s}^{-1}$). Nevertheless, this light limitation does not completely inhibit growth. This aligns with the findings of Jin et al. (2025), who explained that lettuce plants possess adaptive mechanisms to low light intensity, whereby leaf blades can still expand with good quality as a form of photon capture optimization.

Another external factor influencing leaf morphology is the availability of nutrients in the growing medium. The data in Table 3 indicate that the ecoenzyme treatment has not yet been able to optimally support the growth of lettuce leaf surface area in the wick hydroponic system. This low effectiveness is strongly suspected to be related to the minimal content of nitrogen (0.03%) and calcium (0.08%) in the ecoenzyme used. Consequently, plants across all ecoenzyme dose variations (10 mL, 15 mL, 20 mL, and 25 mL) experienced a significant reduction in leaf surface area during the final three weeks of observation. This phenomenon confirms the study by Jasmine & Rachmawati (2024), which states that nutrient deficiencies in ecoenzymes can inhibit epidermal cell division, resulting in smaller leaf sizes compared to the positive control. A similar condition was also observed in the negative control, where leaf surface area was lower due to the absence of essential macro- and micronutrients. Conversely, the best performance was demonstrated by the positive control. According to

Hidayat et al. (2025), the application of AB Mix solution at an optimal concentration was proven to produce the highest leaf area growth due to its complete and readily available nutrient supply.

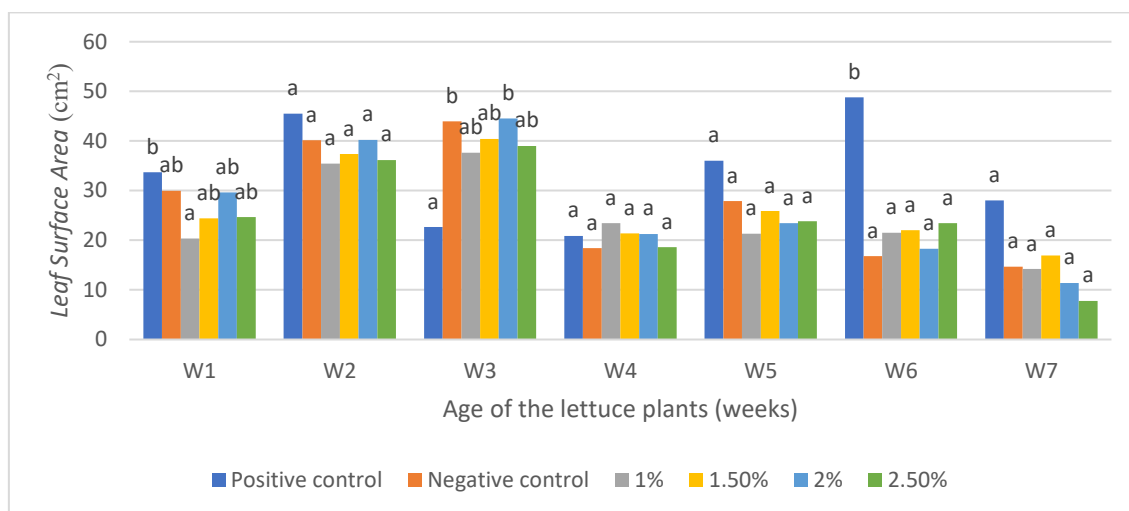


Fig. 2 Average weekly increase in leaf area

In general, the ecoenzyme treatment had no significant effect on the average leaf area of lettuce compared to the AB Mix. However, the potential of the ecoenzyme was evident at a dose of 20 mL/L (2%), which achieved the highest peak leaf area in the third week of observation. Unfortunately, after that phase, leaf growth in all ecoenzyme treatment groups declined steadily until the end of the study. This pattern indicates that the morphological response of lettuce leaves is highly dependent on variations in nutrient concentration and the duration of the observation period (Irsyad et al., 2025).

3.3 Plant height

Normality and homogeneity tests were conducted using the one-sample Kolmogorov-Smirnov test, and the results showed that the lettuce plant height data for each treatment were normally distributed and homogeneous; therefore, the analysis proceeded with a one-way ANOVA followed by Tukey's post-hoc test. Based on the statistical analysis described above, there were no significant differences in plant height in response to the positive control (AB mix), negative control (water only), and ecoenzyme variants (1%; 1.5%; 2%; 2.5%). The results of the interaction between lettuce plants and ecoenzyme application showed that there was no significant effect of treatment on plant height (Table 3). The AB mix positive control produced the tallest plants (47.2 cm) compared to the 15 ml ecoenzyme treatment, which produced the shortest plants (27.4 cm).

Based on the results shown (Table 3), observations of plant height in the negative control treatment showed a tendency toward increased growth, with a consistent, gradual increase in height each week without any decline. At the start of the first week of observation, the negative control values were not significantly different from those of the positive control or the ecoenzyme treatments (10 ml; 15 ml; 20 ml; 25 ml), nor were they different from the negative control during the second, third, fourth, fifth, sixth, and seventh weeks of observation. Lettuce plants in the negative control group exhibited progressive height increases (9.3 cm) from the first week through the seventh week of observation (37 cm). Statistical results using Tukey's post-hoc test indicated that the for the negative control consistently showed the same notation as all treatment groups in every week of observation. This indicates that, statistically, there was no significant difference in lettuce plant height between the negative control and the lettuce plants treated with ecoenzymes (10 ml; 15 ml; 20 ml; 25 ml) or the positive control.

Table 3. Comparison table of plant height

Treatment	Plant Height (cm)
Negative control	24.03 ± 14.28a
1%	26.57 ± 12.99a
1.5%	23.81 ± 10.98a
2%	26.47 ± 11.52a
2.5%	25.16 ± 11.52a
Positive control	29.50 ± 13.44a

In contrast to the leaf area parameter, plant height growth across all treatments showed a positive trend, increasing gradually each week without any downward deviation. Physiologically, this stable vertical growth is triggered by the activity of endogenous cytokinin hormones that stimulate cell division and proliferation in the shoot and stem meristems, thereby inducing continuous stem elongation (Saptiningsih et al., 2025). Although the general trend shows an increase, plant responses to variations in ecoenzyme dosage were highly variable. In the 10 mL/L (1%) ecoenzyme treatment, plant height growth showed no significant effect and tended to be lower than the positive control (AB Mix). However, this 10 mL/L treatment exhibited the most consistent growth trend until the end of the observation period. The low initial response at this dose is suspected to be due to the concentration of bioactive compounds not yet reaching the minimum threshold required to induce a massive biological response in lettuce plants. This finding aligns with the study by Widarawati et al. (2023), which stated that an imbalance in macronutrient content within the ecoenzyme limits its capacity to stimulate optimal vegetative growth.

Although the general trend showed an increase, plant responses to different ecoenzyme doses varied widely. In the 10 mL/L (1%) ecoenzyme treatment, plant height showed no significant effect and tended to be lower than the positive control (AB Mix). However, this 10 mL/L treatment exhibited the most consistent growth trend through the end of the observation period. The low initial response at this dose is likely due to the concentration of bioactive compounds not yet reaching the minimum threshold required to induce a significant biological response in lettuce plants. This finding aligns with the study by Widarawati et al. (2023), which stated that an imbalance in macronutrient content within the ecoenzyme limits its capacity to stimulate optimal vegetative growth.

Conversely, increasing the dose to 15 mL/L (1.5%) actually reduced growth effectiveness compared to the 10 mL/L dose. At a concentration of 15 mL/L, the medium's pH was recorded at 6.95. This phenomenon of reduced performance at higher doses reinforces the findings of Siregar et al. (2025), who confirmed a negative correlation between ecoenzyme dose increases exceeding the threshold and the growth rate of lettuce. This increase in dosage triggers a pH imbalance in the hydroponic growing medium, which in turn disrupts the solubility and availability of essential nutrients to the plant roots.

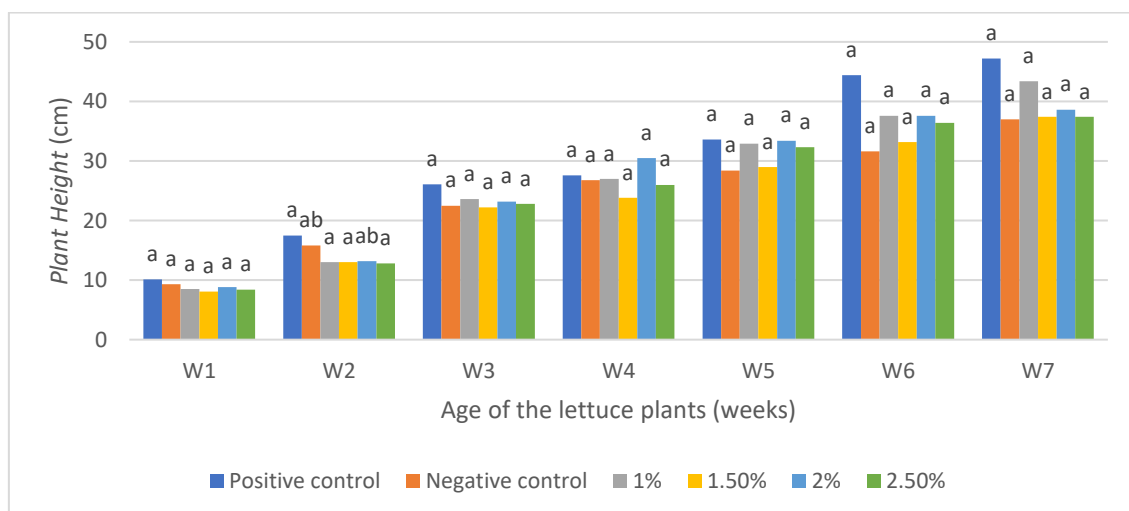


Fig. 3 average weekly increase in plant height

An interesting dynamic was observed in the 20 mL/L (2%) ecoenzyme treatment, where plant height increased steadily from the first to the fourth week and briefly outperformed the 10 mL/L treatment. This acceleration early in the vegetative phase was due to the greater availability of nutrients at that concentration. Nevertheless, the effectiveness of the 20 mL/L dose declined in the late growth phase, where its growth rate was overtaken by the 10 mL/L treatment. This indicates that a high-volume supply of ecoenzyme can stimulate growth initially, but its long-term accumulation may destabilize the pH of the nutrient solution. This phenomenon is supported by Sofian et al. (2024), who reported that the application of ecoenzymes at high doses tends to result in a decline in the growth rate of lettuce plants after a certain period of time.

The most pronounced decline in performance occurred in the highest-dose treatment, namely 25 mL/L (2.5%). The growth response at this dose was lower compared to the 10 mL/L dose, which is suspected to be due to the growing medium becoming overly saturated, thereby disrupting root aeration and respiration. According to De M N Góis et al. (2024), excessively concentrated solutions increase the osmotic pressure of the medium, making it difficult for roots to absorb water. Consequently, plants must allocate more energy to overcome this osmotic stress in order to absorb nutrients, which ultimately results in reduced productivity and plant height.

3.4 Number of leaves

Normality and homogeneity tests were conducted using the one-sample Kolmogorov-Smirnov test, and the results showed that the lettuce plant height data for each treatment were normally distributed and homogeneous; therefore, the analysis proceeded with a one-way ANOVA followed by Tukey's post-hoc test. Based on the statistical analysis described above, there were significant differences in the number of leaves in response to the positive control treatment (AB mix), the negative control (water only), and the various ecoenzyme treatments (1%; 1.5%; 2%; 2.5%). The number of leaves produced showed significant differences, but the negative control yielded a moderate number of leaves (4.4 leaves) between the ecoenzyme treatments and the AB mix positive control treatment (Table 4). The 2% ecoenzyme produced the fewest leaves (3.8 leaves) compared to the other ecoenzymes. Meanwhile, the AB mix produced the highest number of leaves (7.2 leaves).

The leaf number parameter indicated a significant treatment effect (p -value < 0.001). The negative control treatment was classified as moderate, denoted by "ab." The leaf number in the negative control group was not statistically significantly different from that of the ecoenzyme group, but at the same time, it was also not significantly different from that of the positive control group. The most significant ecoenzyme treatment among the other ecoenzyme groups was the 10 ml dose; this group produced the highest number of leaves (4.03 ± 0.95^a) compared to the other ecoenzyme groups.

Table 4. Comparison number of leaves

Treatment	Number of Leaves
Negative control	$3.77 \pm 1.61ab$
1%	$4.03 \pm 0.95a$
1.5%	$3.86 \pm 0.91a$
2%	$3.86 \pm 0.91a$
2.5%	$3.86 \pm 0.65a$
Positive control	$5.43 \pm 1.67b$

Observations over seven weeks indicated that the application of ecoenzymes was not yet able to optimally support the growth of lettuce leaf count in the wick hydroponic system. This limitation is due to disparities in nutrient profiles and availability between the ecoenzyme—an organic fermentation product—and the positive control, which used standard hydroponic nutrients (AB Mix). In the positive control, macronutrients are available in ionic form, ready for direct absorption by plant roots via diffusion and mass

flow driven by transpiration. A sufficient nitrogen supply in the positive control proved effective in stimulating the synthesis of endogenous cytokinin hormones, which are responsible for cell division in the apical meristem, thereby triggering the constant formation of new leaf blades (Chatterjee et al., 2025).

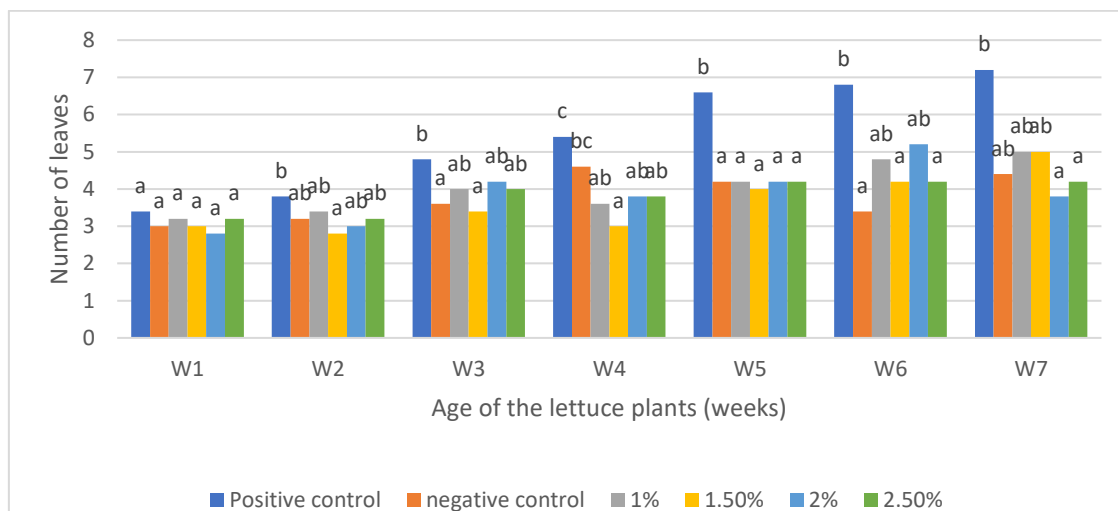


Fig. 4 average weekly increase in number of leaves

Conversely, the nutrients contained in ecoenzymes are mostly still bound in the form of complex organic compounds. These complex compounds require further mineralization and decomposition by microorganisms before they can be converted into inorganic forms ready for absorption by plant roots. Consequently, a physiological response delay (time lag) occurs, where the nutrient release rate in the ecoenzyme-based medium is not as rapid or as intense as that of conventional nutrients in the positive control. This phenomenon caused the accumulation of leaf number in the ecoenzyme treatment to fail to surpass the positive control. This finding aligns with the study by Triana & Enita (2023), which stated that the low concentration of available nutrients in ecoenzymes limits the plant's capacity to optimally pass through the vegetative phase, thus failing to significantly stimulate an increase in leaf number.

3.5 Leaf color

The degree of leaf yellowing differed significantly among the treatments (Fig. 1). The negative control treatment exhibited the highest rate of leaf yellowing (30.3%). As shown in the graph (specify graph), the treatment with a 15 ml concentration of ecoenzyme had the lowest rate (3.3%) of leaf yellowing.

Symptoms of stress, such as leaf yellowing, occurred more frequently in the negative control (water-only) treatment; this symptom was observed in 30.29% of lettuce plants in the negative control group. However, in the ecoenzyme treatments at concentrations of 1%, 1.5%, 2%, and 2.5%, leaf yellowing occurred in less than 50% of the lettuce plants. The 1.5% ecoenzyme treatment resulted in the lowest incidence of chlorosis (3.33%) compared to the other ecoenzyme treatments (1%; 2%; 2.5%). The 1.5% ecoenzyme treatment was the closest to the positive control (0%) because it had the lowest incidence of chlorosis compared to the other treatments.

Based on visual observations of leaf morphology, physiological disturbances were found in lettuce plants in the form of yellowing of the leaf blades (chlorosis). This manifestation of chlorosis is a strong indicator of nutrient deficiency, both macronutrients and micronutrients, which are essential for plant metabolism. Specifically, this yellowing of leaves is triggered by a limited supply of nitrogen (N) in the hydroponic “ ” medium (Dwidjoseputro, 1978). This is reinforced by Wang et al. (2024), who state that nitrogen plays a crucial role as a structural component in protein synthesis, phytohormone

regulation, leaf pigment formation, and the activation of plant defense mechanisms against environmental stress and pathogens.

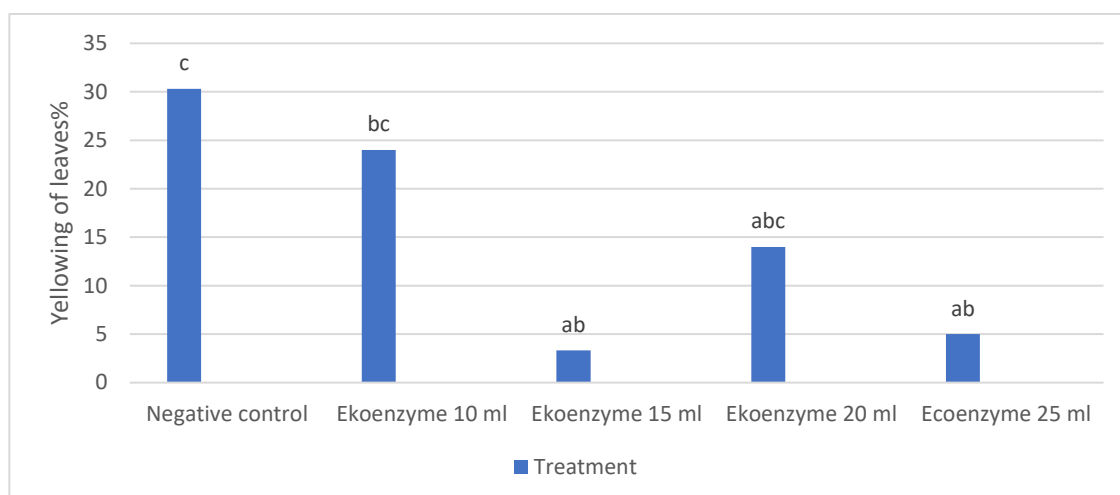


Fig. 5 Yellowing of leaves

Although the analysis in Table 1 indicates that the nitrogen content in the ecoenzyme is relatively low, the 15 mL dose treatment was able to suppress chlorosis more effectively than other ecoenzyme dose treatments. This phenomenon is believed to occur because the bioactive components at that dose are still capable of stimulating chlorophyll biosynthesis in the leaves. Consistent with the findings of Jasmine & Rachmawati (2024), the presence of N, P, and K in the ecoenzyme—even at minimal concentrations—can still trigger enzymatic activity and stimulate the formation of endogenous growth hormones such as auxin, cytokinin, and gibberellin, which support chlorophyll formation, thereby maintaining the green color of the leaves.

Conversely, in treatments experiencing extreme nutrient deficiency, persistent chlorosis symptoms lead to premature aging and the shedding of lettuce leaves. Cytologically, this organ shedding is caused by chlorophyll degradation and damage to chloroplast structure (Zemanová et al., 2024). The limitation of these essential nutrients disrupts the plant's photosynthetic apparatus, thereby reducing the rate of carbon assimilation and forcing the plant to redistribute nutrients from older leaves to meristematic tissues, ultimately leading to leaf abscission (shedding).

3.6 Fresh weight and dry weight

Plant weight is categorized into two types of measurements: fresh weight and dry weight. Fresh weight is the mass of the entire plant weighed directly at harvest before wilting. Fresh weight serves as an indicator of water uptake and metabolic activity. Dry weight is the mass of the plant after all water content has been completely removed. Dry weight can determine how much of the plant's mass is due to photosynthetic accumulation or purely from growth. The results of applying ecoenzyme to the fresh weight and dry weight of lettuce showed that the fresh weight and dry weight of lettuce were still far below those of the AB mix. The 10 ml ecoenzyme treatment yielded the highest results with a fresh weight of 22.36 grams and a dry weight of 1.21 grams compared to the negative control treatment, which produced a fresh weight of 16.97 grams and a dry weight of 0.9 grams.

The data in Table 1 indicate that the application of ecoenzymes has the potential to increase lettuce plant biomass accumulation. Among all ecoenzyme dose variations and the negative control, the treatment with a concentration of 10 mL/L (1%) showed the most optimal results. Conversely, increasing the ecoenzyme dose to higher levels actually reduced growth effectiveness, accompanied by a tendency toward decreased water content in plant tissues. The generally suboptimal performance of ecoenzymes compared to the positive control (AB Mix) stems from their nutrient profile. Jasmine & Rachmawati (2024)

confirmed that the concentration of macronutrients in the ecoenzyme, which is not as high as in commercial nutrients (AB Mix), limits the capacity for structural tissue formation and inhibits the rate of photosynthesis, resulting in lower fresh weight and dry weight.

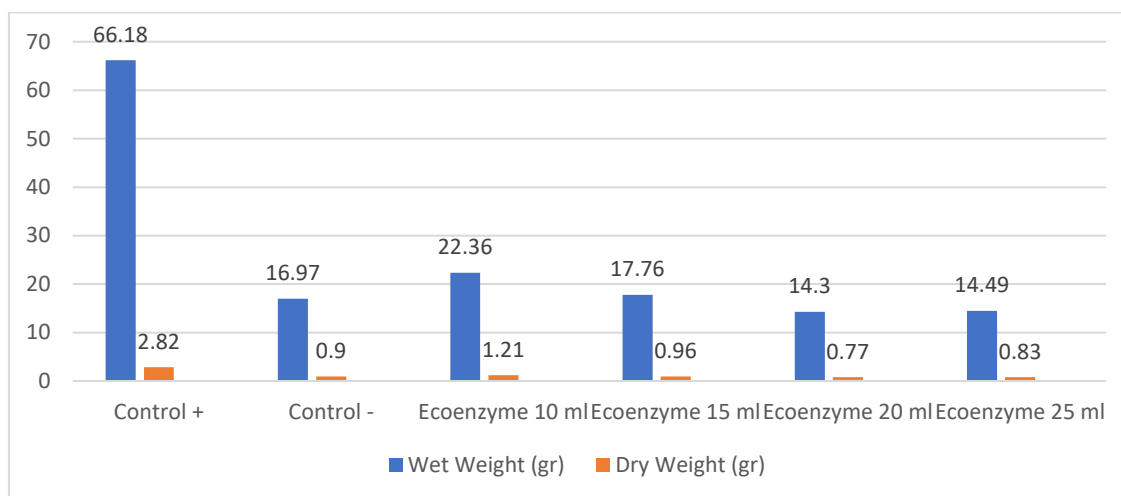


Fig. 6 Comparison of wet wight and dry weight

In addition to nutrient quantity limitations, the stability of the organic nutrient solution also plays a crucial role in lettuce biomass yield. Low nutrient content, exacerbated by suboptimal medium pH, was found to reduce plant fresh weight and dry weight accumulation. In the 10 mL/L (1%) ecoenzyme treatment, the medium pH was recorded at 6.91. The pH range across all ecoenzyme treatment groups in this study was deemed less than ideal for supporting efficient nutrient uptake in the wick hydroponic system. This phenomenon aligns with the findings of Yakuup et al. (2024), who stated that the optimal pH for lettuce cultivation is 6.2. This neutral-to-acidic pH condition is the optimal point that supports nutrient availability, thereby accelerating plant height growth, leaf expansion, and optimizing the fresh and dry weights of lettuce plants.

4. Conclusions

Ecoenzymes derived from organic waste demonstrate strong potential as alternative biostimulants and supplemental nutrients for lettuce (*Lactuca sativa* L.) cultivated in wick-based hydroponic systems. Although the macronutrient and micronutrient content of ecoenzymes remains lower than that of commercial AB mix nutrients, the 1% concentration produced the most optimal outcomes among ecoenzyme treatments for leaf surface area, plant height, leaf number, and fresh and dry weight, while the 1.5% concentration was most effective in minimizing leaf yellowing. These findings confirm that ecoenzymes can serve as a viable, low-input supplement to enhance lettuce growth performance in soilless cultivation.

These results carry meaningful implications for the Sustainable Development Goals. By converting organic waste into a usable plant nutrient source, ecoenzyme-based fertilization supports SDG 2 (Zero Hunger) by offering a low-cost pathway to sustainable food production for urban and land-limited communities. It also advances SDG 12 (Responsible Consumption and Production) through circular valorization of organic waste, and contributes to SDG 13 (Climate Action) by reducing dependence on chemically synthesized fertilizers whose production carries a notable carbon footprint.

Future research should focus on optimizing ecoenzyme formulations to increase macronutrient concentration and stabilize solution pH, both of which were identified as limiting nutrient uptake in this study. Further investigation into fermentation duration, substrate composition, and microbial activity within the ecoenzyme solution is recommended to improve consistency and nutrient bioavailability. Long-term studies

examining coenzyme performance across multiple planting cycles and other leafy vegetable species would further strengthen its applicability. Practically, farmers and hydroponic practitioners are encouraged to adopt coenzyme at the 10 ml/L concentration as a partial nutrient supplement, combined with periodic pH monitoring and adjustment toward the optimal 6.2 range, to maximize lettuce yield while reducing reliance on commercial nutrients and supporting more sustainable, environmentally responsible hydroponic agriculture.

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Author Contribution

J. F. F., S. H. K., K., & I. A. contributed to the literature search, interpretation, writing, and proofreading of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Not available.

Conflicts of Interest

The authors declare no conflicts of interest.

Conflicts of Interest

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Declaration of Generative AI Use

During the preparation of this work, the author used Grammarly to assist in improving the grammar, clarity, and academic tone of the manuscript. After using this tool, the author reviewed and edited the content as needed and took full responsibility for the content of the publication.

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Biographies of Authors

Jihan Bilqis, Department Biology, Faculty of Mathematics and Natural Sciences Education, Universitas Pendidikan Indonesia, Bandung, West Java 40154, Indonesia.

- Email: bilqisjihan048@gmail.com
- ORCID: N/A
- Web of Science ResearcherID: N/A
- Scopus Author ID: N/A
- Homepage: N/A

Hertien Koosbandiah Surtikanti, Department Biology, Faculty of Mathematics and Natural Sciences Education, Universitas Pendidikan Indonesia, Bandung, West Java 40154, Indonesia.

- Email: hertien.surtikanti@yahoo.com
- ORCID: 0000-0003-2743-2578
- Web of Science ResearcherID: N/A
- Scopus Author ID: 57194536681
- Homepage: <https://sinta.kemdiktisaintek.go.id/authors/profile/5995733/>

Wahyu Surakusumah, Department Biology, Faculty of Mathematics and Natural Sciences Education, Universitas Pendidikan Indonesia, Bandung, West Java 40154, Indonesia.

- Email: wahyu.bioupi@upi.edu
- ORCID: 0000-0001-8057-7990
- Web of Science ResearcherID: AAA-4015-2021
- Scopus Author ID: 57201068504
- Homepage: <https://sinta.kemdiktisaintek.go.id/authors/profile/6723267>

Afri Irawan, Department Biology, Faculty of Mathematics and Natural Sciences Education, Universitas Pendidikan Indonesia, Bandung, West Java 40154, Indonesia.

- Email: afri.irawan@upi.edu
- ORCID: 0000-0002-1013-0613
- Web of Science ResearcherID: N/A
- Scopus Author ID: 57224011301
- Homepage: <https://sinta.kemdiktisaintek.go.id/authors/profile/6952614>