

Effect of Liquid Organic Fertilizer (LOF) Derived from *Azolla pinnata* on the Growth and Productivity of Red Spinach (*Amaranthus tricolor* L.)

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ABSTRACT

This study aims to analyze the effect of applying liquid organic fertilizer (LOF) derived from *Azolla pinnata* on the growth and productivity of red spinach (*Amaranthus tricolor* L.), as well as to determine the most effective application rate. The study was motivated by the high reliance on inorganic fertilizers, which have the potential to degrade soil quality; therefore, an environmentally friendly organic fertilizer alternative capable of providing nutrients particularly nitrogen is needed. The study employed a quantitative experimental method using a Randomized Block Design (RBD) consisting of four treatments: P0 (control), P1 (100 mL/L), P2 (150 mL/L), and P3 (200 mL/L), with three replicates, resulting in 12 experimental units. The observed parameters included plant height, number of leaves, leaf area, root length, and fresh plant weight. The data were analyzed using analysis of variance (ANOVA), followed by the Honest Significant Differences (HSD) test at a 5% significance level. The results showed that the application of *Azolla pinnata* based LOF had a significant effect on all growth and productivity parameters of red spinach plants. Treatment P3 (200 mL/L) yielded the best results, with an average plant height of 34.60 cm, 24.29 leaves, a leaf area of 48.96 cm, a root length of 12.63 cm, and a fresh weight of 30.60 g. Thus, the application of *Azolla pinnata* based LOF at a dose of 200 mL/L was the best treatment among those tested for enhancing the vegetative growth and productivity of red spinach.

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1. INTRODUCTION

The agricultural sector plays a strategic role in providing food and nutritional sources to support national food security. In general, the agricultural sector consists of the food crops, plantation, and horticulture subsectors, with the horticulture subsector particularly vegetables continuing to grow in line with increasing food demand and public awareness of healthy eating patterns (Syahrial *et al.*, 2024). One vegetable with high potential for development is red spinach (*Amaranthus tricolor* L.) because it contains nutrients beneficial to health and has a relatively short harvest period approximately 30 days after planting (Hasyimi *et al.*, 2025). Nevertheless, spinach production in North Sumatra continues to fluctuate. Data from the Statistics of North Sumatra Province show

that amaranth production reached 17,979.9 metric tons in 2021, then decreased to 16,797.5 metric tons in 2022, increased to 16,894.9 metric tons in 2023, and rose again to 17,581.0 metric tons in 2024. This fluctuation indicates that amaranth production has not remained stable and highlights the importance of improving crop growth and productivity through appropriate cultivation practices.

Plant growth and productivity are influenced by several factors, including genetic characteristics, environmental conditions, and nutrient availability. Adequate nutrient availability is particularly important for amaranth because the crop has a relatively short growth cycle and requires sufficient nutrients to support rapid vegetative growth. Nitrogen, phosphorus, and potassium play important roles in leaf formation, photosynthesis, root development, and biomass production. Several studies have reported that fertilizer application and improved nutrient availability can enhance the growth and yield of *Amaranthus tricolor* L.. However, the continuous and inappropriate use of inorganic fertilizers may potentially lead to nutrient imbalance and adversely affect soil quality. This condition highlights the need for alternative and environmentally friendly nutrient sources, one of which is the utilization of organic fertilizers. Organic fertilizers can supply nutrients while adding organic matter to the soil, thereby potentially supporting improvements in soil conditions and plant growth. One organic material with potential as a source of organic fertilizer is *Azolla pinnata*. This plant contains nutrients that can support plant growth and can be processed into liquid organic fertilizer (LOF). The utilization of *Azolla pinnata* as a raw material for LOF is expected to provide an alternative nutrient source for red spinach. Therefore, further research is needed to determine the effect of *Azolla pinnata* based LOF application on the growth and productivity of red spinach (*Amaranthus tricolor* L.).

The growth and productivity of red spinach are influenced by the availability of nutrients, particularly nitrogen, which plays a role in chlorophyll formation, protein synthesis, vegetative tissue development, leaf formation, and photosynthetic activity. Nitrogen deficiency can stunt plant growth and reduce yields. In addition to nutrients, soil pH is also an important factor because it affects the availability and uptake of nutrients by plants. Red spinach generally grows well at a soil pH of approximately 6.0-7.0, where as soil conditions that are too acidic or too alkaline can inhibit the availability of certain nutrients, including nitrogen (Tusi *et al.*, 2025). Thus, providing nutrients through proper fertilization is one approach that can be taken to support the growth and productivity of red spinach. One alternative with potential for development is the use of liquid organic fertilizer (LOF) made from *Azolla pinnata* as a source of organic matter and nutrients.

Azolla pinnata is an aquatic fern that forms a symbiotic relationship with *Anabaena azollae*, a microorganism capable of nitrogen fixation. *Azolla* biomass contains nitrogen, organic matter, and macro- and micronutrients that decompose relatively easily, making it a potential source of organic nutrients for plants (Prayoga *et al.*, 2019). The use of *Azolla* as a fertilizer ingredient has shown positive results for several crops. Monareh *et al.*, (2023), reported that *Azolla pinnata* LOF at a concentration of 150-200 mL/L resulted in better growth and yield in pakcoy (*Brassica rapa* L.) based on the parameters of plant height, number of leaves, leaf area, and fresh weight. Research from Setiawati *et al.*, (2023) also showed that applying *Azolla pinnata* compost to rice plants can increase the number of productive tillers and tends to increase grain yield, while Soelaksini *et al.*, (2022) reported increased growth and yield of mung beans following the application of *Azolla pinnata*. These results indicate that *Azolla* has potential as a source of organic matter to support plant growth and productivity.

Although various studies have demonstrated the potential of *Azolla pinnata* as a source of organic nutrients, the reviewed literature still primarily discusses its use on crops such as rice and legumes in the form of solid organic fertilizer, whereas research on *Azolla pinnata* in the form of fermented liquid organic fertilizer (LOF) for red spinach remains limited. Furthermore, research results on other crops cannot be directly applied to determine effective application rates for red spinach, as each plant has distinct physiological characteristics, growth patterns, nutrient requirements, and responses to fertilization. Differences in environmental and agroecological conditions can also influence plant responses to fertilizer application. Therefore, experimental research is needed to test red spinach's response to *Azolla pinnata* based LOF at various doses, particularly regarding vegetative growth parameters and crop productivity.

This study aims to analyze the effect of *Azolla pinnata* based application on the growth and productivity of red spinach (*Amaranthus tricolor* L.). The approach involved applying *Azolla pinnata* based LOF at various doses and observing growth indicators, including plant height, number of leaves, leaf area, and root length, as well as productivity indicators such as fresh plant weight. The novelty of this study lies in the testing of *Azolla pinnata* based LOF derived from fermentation on red spinach, which has been studied only to a limited extent compared to research on other crops, as well as in the assessment of plant responses to various LOF doses under the agroecological

conditions of Gunungsitoli City. The research results are expected to expand scientific knowledge regarding the use of *Azolla pinnata* as a source of organic fertilizer and to serve as an alternative, more environmentally friendly, and sustainable fertilization technology in red spinach cultivation.

2. METHOD

2.1 Type of Research

This research employed a quantitative experimental approach to evaluate the response of red spinach (*Amaranthus tricolor* L.) to different application rates of liquid organic fertilizer (LOF) produced from *Azolla pinnata*. The experiment was arranged in a Randomized Block Design (RBD) comprising four LOF treatments and three replications, giving a total of 12 experimental units. The treatments consisted of P0 (control, without LOF), P1 (100 mL LOF/L water), P2 (150 mL LOF/L water), and P3 (200 mL LOF/L water). The RBD was applied because the experimental area and growing environment were considered insufficiently uniform; therefore, blocking was used to minimize variation attributable to environmental factors outside the treatments (Syilfi & Hidayat, 2024). The independent variable (X) was the application dose of *A. pinnata*-based LOF, whereas the dependent variables (Y) comprised plant height (cm), number of leaves (leaves), leaf area (cm²), root length (cm), and productivity expressed as fresh weight (g). The LOF was produced using fresh *A. pinnata* biomass that was green, healthy, and free from decomposition. The collected biomass was washed with clean water to eliminate soil particles, mud, and other contaminants before being blended with sufficient clean water to reduce particle size and facilitate the subsequent decomposition process.

The homogenized *Azolla* material was then combined with clean water at a ratio of 1 kg fresh biomass to 1 L water, followed by the addition of 100-200 g of brown sugar or molasses and 10-20 mL of EM4 bioactivator for each fermentation mixture (Taradifa *et al.*, 2022). The mixture was transferred into an airtight plastic container to create anaerobic fermentation conditions and allowed to ferment for 7-14 days. During fermentation, the mixture was stirred once or twice daily to maintain uniformity and facilitate the decomposition process. Following fermentation, the liquid was filtered and stored in tightly sealed bottles or plastic jerry cans in a cool and shaded location away from direct sunlight. The fermented LOF was characterized as a liquid organic fertilizer derived from *A. pinnata* containing organic matter and dissolved plant nutrients and was subsequently used as the treatment material. The total LOF requirement for the three application periods was approximately 1.35 L for all treatments, equivalent to approximately 1.35 kg of fresh *A. pinnata* based on the 1:1 fresh biomass-to-water ratio used in the formulation. Prior to planting, the experimental field was cleared of weeds and residual plant material and then manually tilled using a hoe until the soil was adequately friable. Twelve plots measuring 1.2 m² were established according to the experimental design, with a 50 cm distance between adjacent plots. Each plot contained 25 plants arranged at a spacing of 20 cm × 20 cm. Initial soil characterization was conducted before planting by assessing soil pH, nitrogen availability, and soil texture. The initial soil pH was 6.0, which was considered suitable for red spinach cultivation.

Seedlings were initially raised in seedling trays containing a uniformly mixed soil and compost substrate at a ratio of 2:1. The seed was placed at a depth of 0.5-1.0 cm, with 1-2 seeds per tray cell, and the substrate was maintained under moist but non-saturated conditions. Planting in the experimental plots was performed in the afternoon using the dibbling method, with planting holes prepared at a depth of 1-2 cm and a spacing of 20 cm × 20 cm. Each hole received 2-3 seeds, which were then lightly covered with soil to maintain moisture and protect the seeds. Thinning was performed when the plants reached approximately 7-14 days after planting by retaining one healthy and uniform plant per planting hole. Replacement seedlings of comparable age were used when plants failed to emerge or died during establishment. Routine crop management included watering every morning and afternoon, manual weed removal, and mechanical or natural pest and disease management when necessary. The LOF treatments were administered three times during the growing period using a uniform application technique. Before application, the LOF was diluted with water according to the designated treatment concentration and then distributed evenly onto the soil surrounding each plant while avoiding direct application to the foliage.

The volume applied during each application was 33.33 mL LOF per plot for P1, 50 mL per plot for P2, and 66.67 mL per plot for P3. With 25 plants per plot, these volumes corresponded to approximately 1.33, 2.00, and 2.67 mL LOF per plant per application, respectively. Over three applications, the cumulative LOF volume received by each plant was approximately 4.00 mL for P1, 6.00 mL for P2, and 8.00 mL for P3. Plant observations were conducted at 7, 14, 21, and 28 days after planting (DAP). The measurement at 7 DAP was treated as the baseline observation because

the LOF treatment had not yet been applied. Eight plants from the central portion of each plot were designated as observation units, while border plants were excluded to reduce edge effects, resulting in 96 observation plants from the 12 experimental units. Plant height, number of leaves, leaf area, and root length were evaluated as growth parameters, whereas fresh weight was used to represent crop productivity. At approximately 30-40 DAP, the plants were harvested carefully to minimize physical damage, cleaned of adhering soil and debris, and weighed to determine fresh biomass. All observations were recorded systematically, tabulated, and subsequently analyzed statistically according to the RBD to determine the response of red spinach growth and productivity to the different doses of *A. pinnata*-based LOF.

2.2 Research Location and Schedule

The study was conducted in Ombolata Ulu Village, Gunungsitoli District, Gunungsitoli City. The study lasted approximately 2 months. Research activities included land and growing medium preparation, preparation of *Azolla pinnata* LOF, seed sowing, planting, application of LOF treatments, maintenance and weeding, observation and measurement of growth, harvesting, and measurement of fresh weight. After all data were collected, data processing and analysis were carried out in accordance with the research design. The stages of the study were carried out sequentially from preparation through harvest and data analysis.

2.3 Population and Sample

According to Amin *et al.*, (2023), a population is the entire set of objects or subjects of a study that share specific characteristics defined by the researcher for study, from which conclusions are drawn. Determining the population size of plants in agronomic research is typically based on the planting spacing used (Kustanto, 2022). The population in this study consists of all red spinach plants grown in the experimental plots, which comprise 4 treatments and 3 replicates, resulting in a total of 12 experimental plots. Each plot was planted with 25 red spinach plants, so $12 \times 25 = 300$ red spinach plants constitute the total population. Sampling was conducted from the center of each plot to minimize *the border effect*, as plants located at the plot edges generally experience different environmental conditions-such as having more growing space, higher light intensity, and greater access to nutrients compared to plants in the interior thereby ensuring that the sample plants more accurately represent the response to the *Azolla pinnata* liquid biofertilizer treatment (Reprint *et al.*, 2023). From each plot, 30% of the plants that met Arikunto's (2006) observation criteria a total of 8 plants were selected as research samples. Each experimental plot measured 1.2 m^2 with a planting spacing of $20 \text{ cm} \times 20 \text{ cm}$, resulting in 25 plants per plot.

2.4 Equipment and Materials

The equipment used in this study included measuring tools such as rulers or measuring tapes, digital scales, buckets and measuring cups, a pH meter, writing utensils and observation sheets, a camera or cell phone, plot signs/labels, and cultivation tools consisting of a machete, hoe, shovel, , and watering can. Research materials included *Azolla pinnata* as the raw material for LOF, red spinach seeds of the Mira Cap Panah Merah variety, brown sugar as a carbon source, EM4 as a bioactivator, clean water, a blender, and a jerry can as a fermentation container.

2.5 Data Analysis Techniques

Observation data were tabulated and analyzed using Microsoft Excel. The data were first checked for completeness and consistency, then tested for normality using the Shapiro-Wilk test and for homogeneity using Levene's test. Data meeting these assumptions were analyzed using Analysis of Variance (ANOVA) based on the Randomized Block Design (RBD) model, namely:

$$Y_{ij} = \mu + \tau_i + \beta_j + \varepsilon_{ij} \quad (1)$$

The effect of a treatment was considered significant at the $F_{hitung} > F_{tabel}$ level at the 5% significance level and highly significant at the 1% level. If a significant or highly significant effect was found, the analysis continued with the Honest Significant Differences (HSD) test at the 5% level to identify differences between treatments and determine the dose of *Azolla pinnata* LOF that yielded the best response.

3. RESULTS AND DISCUSSION

3.1 Results

This subsection shows that the application of LOF derived from *Azolla pinnata* had a positive effect on the growth and yield of red spinach. The parameters observed included plant height,

number of leaves, leaf area, root length, and fresh weight. In general, an increase in the LOF dose from P0 to P3 was accompanied by an increase in the mean values of all observed parameters, with treatment P3 (200 mL/L) yielding the highest values.

Table 1. Average Plant Height of Red Spinach at 7-28 Days After Sowing

Treatment	Age of Observation Week			
	7 DAP	14 DAP	21 DAP	28 DAP
P0	7.72d	12.84d	18.81d	24.12d
P1	7.82c	13.71c	20.63c	27.02c
P2	7.92b	14.43b	22.77b	29.94b
P3	8.03a	16.13a	25.69a	34.60a

Note: Numbers followed by the same letter in the same column are not significantly different, while different letters indicate significant differences based on the Tukey HSD ^{a,b}test at the 5% level.

Table 2. Mean Number of Leaves on Red Spinach Plants Aged 7-28 Days After Sowing

Treatment	Age of Observation Week			
	7 DAP	14 DAP	21 DAP	28 DAP
P0	4.37c	8.54d	11.58d	14.08d
P1	5.00b	9.75c	13.83c	17.00c
P2	5.17ab	10.69b	15.62b	20.58b
P3	5.50a	12.21a	18.50a	24.29a

Note: Numbers followed by the same letter in the same column are not significantly different, while different letters indicate significant differences based on the Tukey HSD ^{a,b}test at the 5% level.

Table 3. Mean Leaf Area of Red Spinach Plants Aged 7-28 Days After Sowing

Treatment	Age of Observation Week No.			
	7 DAP	14 DAP	21 DAP	28 DAP
P0	1.62c	9.91c	12.40c	34.62d
P1	2.18b	11.41b	20.94b	40.16c
P2	2.57a	12.21ab	23.58b	42.91b
P3	2.77a	13.44a	27.22a	48.96a

Note: Numbers followed by the same letter in the same column are not significantly different, while different letters indicate significant differences based on the Tukey HSD ^{a,b}test at the 5% level.

Table 4. Mean Root Length of Red Spinach Plants Aged 7-28 Days After Sowing

Treatment	Root Length per Plot
P0	8.01d
P1	9.29c
P2	10.83b
P3	12.63a

Note: Numbers followed by the same letter in the same column are not significantly different, while different letters indicate significant differences based on the Tukey HSD ^{a,b}test at the 5% level.

Table 5. Mean Fresh Weight per Sample of Red Spinach Plants Aged 7-28 Days After Sowing

Treatment	Fresh Weight per Sample (g)
P0	16.65d
P1	21.10c
P2	25.26b
P3	30.60a

Note: Numbers followed by the same letter in the same column are not significantly different, while different letters indicate significant differences based on the Tukey HSD ^{a,b}test at the 5% level.

Growth observations showed a trend of increase in all parameters as the dose of *Azolla pinnata* LOF increased. At 28 days after sowing (DAS), plant height increased from 24.12 cm in the P0 treatment to 34.60 cm in the P3 treatment. The number of leaves also increased from 14.08 to 24.29, while leaf area increased from 34.62 cm² to 48.96 cm². In addition, the P3 treatment produced the highest root length and fresh weight, at 12.63 cm and 30.60 g, respectively. These findings indicate that the application of *Azolla pinnata* LOF at a dose of 200 mL/L resulted in the highest growth response and yield of red spinach compared to the other treatments.

Table 6. Summary of statistical assumption tests

Parameter	Observation time	Shapiro-Wilk Sig.	Sig. Levene	Conclusion
Plant height	7-28 HST	0.157-0.963	0.824-0.999	Normality and Homogeneity
Number of leaves	7-28 HST	0.210-1.000	0.245-0.424	Normality and Homogeneity
Leaf area	7-28 HST	0.114-0.886	0.066-0.201	Normality and Homogeneity
Root length	end of research	0.780-1.000	0.958	Normality and Homogeneity
Fresh weight	end of research	0.524-1.000	0.994	Normality and Homogeneity

Note: Data are considered normal and homogeneous if the Sig. value is > 0.05.

The Shapiro-Wilk test shows that all data have a significance value > 0.05, indicating a normal distribution. The Levene test also shows a significance value > 0.05 for all parameters and

observation times, indicating that the data variances are homogeneous. Thus, all data meet the assumptions for analysis using ANOVA.

Table 7. Results of the analysis of variance (ANOVA) for the growth and productivity of red spinach treated with *Azolla pinnata* LOF

Variables	Observation time	Calculated F-value for Treatment	Sig.	Notes
Plant height	14 HST	631.830	<0.001	**
	21 HST	8,464.408	<0.001	**
	28 HST	48,604.872	<0.001	**
Number of leaves	14 HST	128,981	<0.001	**
	21 HST	520,475	<0.001	**
	28 HST	368,966	<0.001	**
Leaf area	14 HST	23,649	<0.001	**
	21 HST	115,684	<0.001	**
	28 HST	1,385,508	<0.001	**
Root length	-	189,555	<0.001	**
Fresh weight	-	8,579.161	<0.001	**

Note: ** = highly significant at the 1% level ($p < 0.01$).

Table 8. Tukey's Post-hoc Test Table for the Growth and Productivity of Red Spinach with *Azolla pinnata* LOF

Variable	Time	P0 (0 mL/L)	P1 (100 mL/L)	P2 (150 mL/L)	P3 (200 mL/L)
Plant height (cm)	14 HST	12.85 ^a	13.71 ^c	14.43 ^b	16.13 ^a
	21 HST	18.82 ^a	20.63 ^a	22.77 ^b	25.69 ^a
	28 HST	24.12 ^a	27.02 ^a	29.94 ^b	34.60 ^a
Number of leaves (sheets)	14 HST	8.54 ^d	9.75 ^c	10.69 ^b	12.21 ^a
	21 HST	11.59 ^a	13.84 ^a	15.63 ^b	18.50 ^a
	28 HST	14.08 ^a	17.00 ^a	20.58 ^b	24.29 ^a
Leaf area (cm)	14 HST	9.91 ^a	11.42 ^c	12.21 ^b	13.44 ^a
	21 HST	12.40 ^a	20.94 ^a	23.58 ^b	27.22 ^a
	28 HST	34.62 ^a	40.17 ^a	42.91 ^b	48.97 ^a
Root length (cm)	-	8.02 ^d	9.30 ^c	10.83 ^b	12.63 ^a
Fresh weight (g/sample)	-	16.65 ^d	21.10 ^c	25.26 ^b	30.60 ^a

Note: Numbers followed by different letters in the same row indicate significant differences based on the Tukey HSD test at the 5% level.

The results of the analysis of variance showed that the application of *Azolla pinnata* LOF had a highly significant effect ($p < 0.01$) on plant height, number of leaves, leaf area, root length, and fresh weight of red spinach plants. Initial observations at 7 days after sowing (DAS) showed differences in values among treatments, but these data were obtained before the application of *Azolla pinnata* LOF; therefore, they were used as baseline data and not interpreted as a response to the treatment. The effect of the treatment was evident as early as 14 days after sowing (DAS) and became increasingly clear by 28 DAS. Tukey's 5% post-hoc test indicated that treatment P3 (200 mL/L) produced the highest values for all observed variables and differed significantly from the other treatments.

3.2 Discussion

3.2.1 Effect of *Azolla pinnata* LOF Application on Plant Height

The analysis results showed that the application of *Azolla pinnata* LOF had a highly significant effect on the height of red spinach plants at 14, 21, and 28 HST. Increasing the LOF dose resulted in a trend toward increased plant height, with treatment P3 (200 mL/L) yielding the highest values, while P0 yielded the lowest. This response is related to nutrient content, particularly nitrogen, which plays a role in chlorophyll formation, protein synthesis, and cell division and elongation. Phosphorus supports energy supply for growth, while potassium plays a role in enzyme activity and the translocation of photosynthetic products. The mechanism of action of *Azolla pinnata* LOF begins when the fertilizer solution is applied to the plants or growing medium. In the study Emelia *et al.*, (2022), it is stated that nutrients in dissolved form are more easily absorbed by roots compared to organic matter that has not yet undergone decomposition.

Growth enhancement is also supported by the organic matter in the LOF, which promotes microbial activity and nutrient availability in the root zone. These conditions improve water and nutrient uptake, thereby supporting vegetative plant growth. These findings align with the study by Monareh *et al.*, (2023) which reported that *Azolla* LOF at a concentration of 150-200 mL/L can increase the height of pak choi plants. Aisyah *et al.*, (2024), also demonstrated that the

use of *Azolla* LOF can enhance vegetable plant growth. The differences observed at 7 days after sowing (DAS) represent preliminary data since the LOF had not yet been applied; therefore, the discussion of treatment effects focuses on observations made after application, specifically from 14 to 28 DAS.

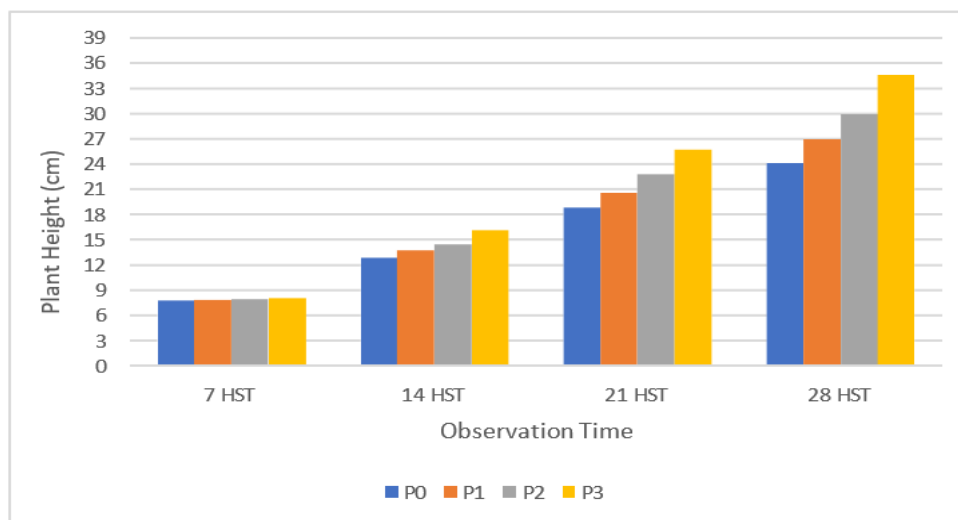


Figure 1. Bar chart of the average height of red spinach plants

3.2.2 Effect of *Azolla pinnata* LOF Application on Leaf Number

The application of *Azolla pinnata* LOF had a highly significant effect on the number of leaves at 14, 21, and 28 days after sowing (DAS). The number of leaves increased with higher LOF doses, with P3 yielding the highest number of leaves and P0 the lowest. This increase is related to nitrogen availability, which supports the formation of chlorophyll, protein, and vegetative tissue. Better nutrient availability allows photosynthesis and the formation of vegetative organs to proceed more optimally.

The differences between treatments became increasingly evident as the plants aged, particularly at 21 and 28 days after sowing. These results are consistent with the findings of Monareh *et al.* (2023), who reported that *Azolla*-based organic liquid fertilizer (LOF) can enhance the growth of leafy vegetables. Aisyah *et al.* (2024), also reported an increase in the number of leaves and plant vigor following the application of *Azolla*-based LOF. Thus, *Azolla pinnata* LOF is capable of supporting the formation of red spinach leaves, particularly at a dose of 200 mL/L.

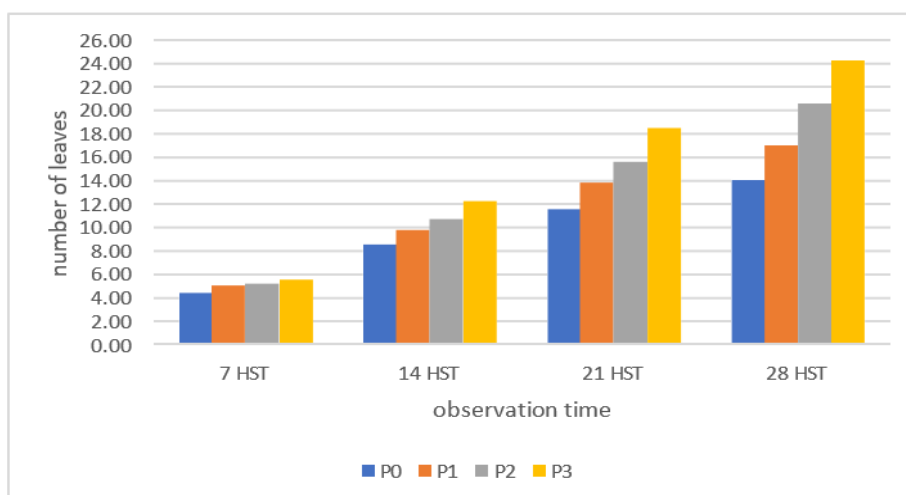


Figure 2 . Bar chart of the average number of red spinach leaves

3.2.3 Effect of *Azolla pinnata* LOF Application on Leaf Area

Analysis results show that the application of *Azolla pinnata* LOF had a significant to very significant effect on leaf area at 14, 21, and 28 days after sowing. Leaf area tended to increase with higher LOF doses, and treatment P3 produced the highest leaf area. The increase in leaf area is related to the availability of nutrients that support cell division and enlargement as well

as chlorophyll formation. These conditions enhance photosynthetic capacity, thereby supporting plant biomass formation.

The treatment response became increasingly evident at 21 and 28 days after sowing, indicating that plant growth occurred cumulatively as the plants aged and LOF was applied. These results are consistent with Monareh *et al.*, (2023), who reported that *Azolla pinnata* LOF can increase the leaf area of vegetable plants. Thus, the P3 dose provided the best response for leaf area development under the conditions of this study.

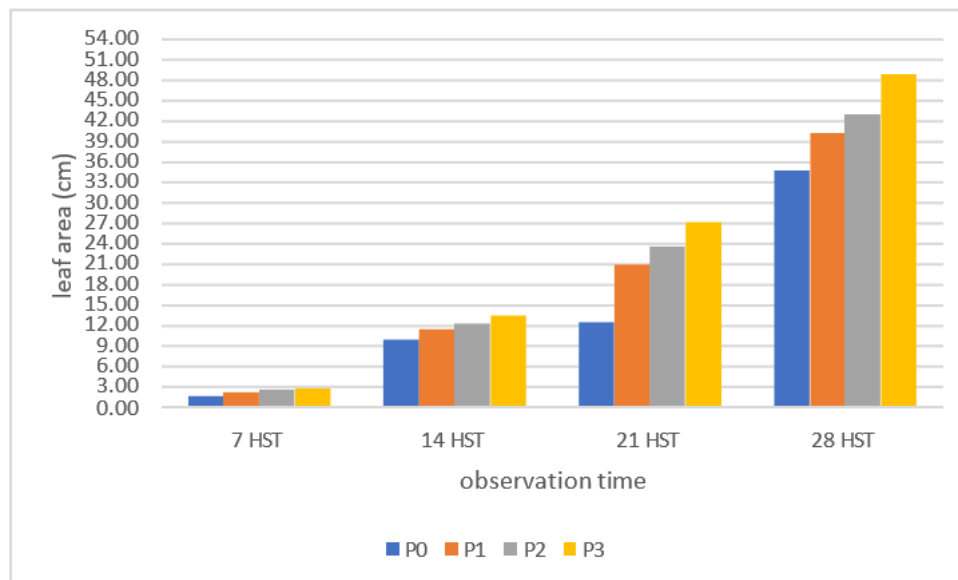


Figure 3. Bar chart of the average leaf area of red spinach

3.2.4 Effect of *Azolla pinnata* LOF Application on Root Length

The application of *Azolla pinnata* LOF had a highly significant effect on the root length of red spinach plants. Treatment P3 produced the longest roots, followed by P2, P1, and P0. The increase in root length is related to the availability of nutrients, particularly phosphorus, which plays a role in the formation and development of the root system. Organic matter content can also support the biological and physical conditions of the growing medium, thereby optimizing root development and the uptake of water and nutrients.

These results are consistent with the findings Setiawati *et al.*, (2023) who stated that liquid organic fertilizer can increase nutrient availability and support root development. Better root growth can subsequently enhance the plant's ability to absorb water and nutrients to support the growth of the aboveground parts.

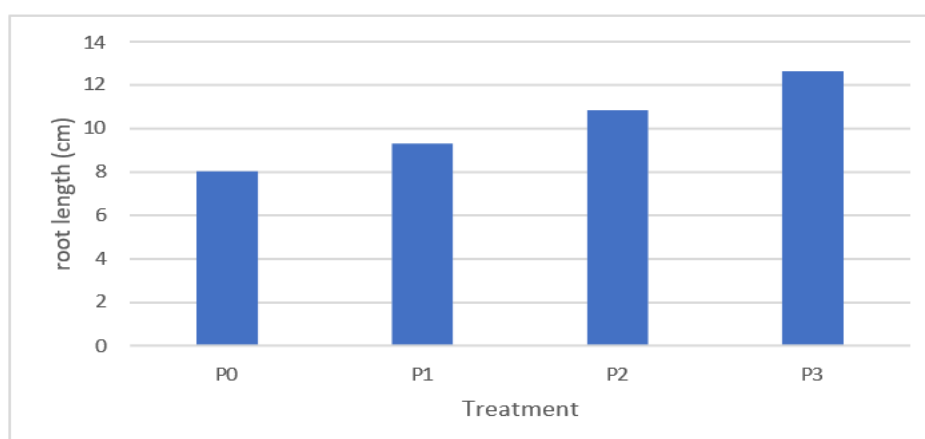


Figure 4. Diagram of red spinach root length

3.2.5 Effect of *Azolla pinnata* Liquid Organic Fertilizer Application on Fresh Weight

The application of *Azolla pinnata* liquid organic fertilizer had a very significant effect on the fresh weight of red spinach plants. Treatment P3 (200 mL/L) produced the highest fresh weight, while P0 produced the lowest. The increase in fresh weight resulted from improved vegetative growth, as indicated by increases in plant height, number of leaves, leaf area, and root length. More

optimal nutrient availability supported photosynthesis and plant tissue formation, thereby increasing fresh biomass accumulation. These findings are consistent with those of Monareh *et al.* (2023) and Aisyah *et al.* (2024) which showed that the use of *Azolla*-based LOF can enhance the growth and fresh weight of vegetable crops. Treatment P3 yielded the best response without showing any signs of reduced growth; therefore, a dose of 200 mL/L is the optimal treatment under the conditions of this study. However, these results are still limited to the specific location and study period; thus, testing in different environments and seasons is necessary to derive broader recommendations.

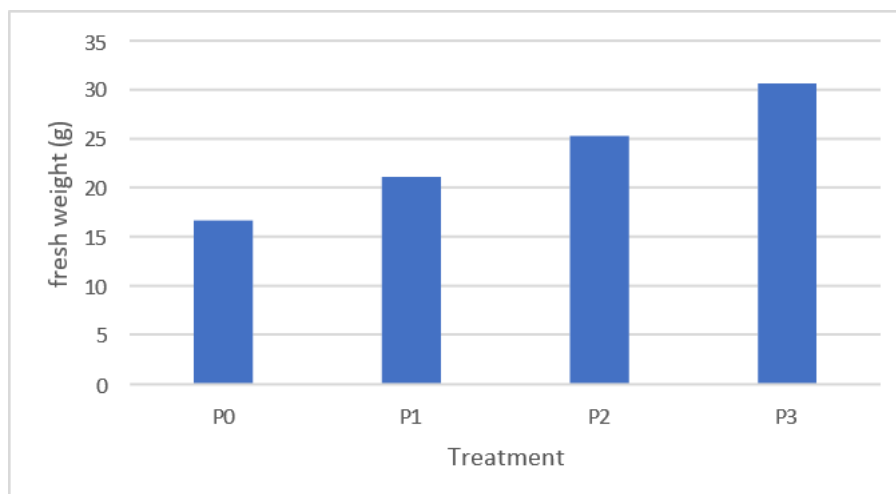


Figure 5. Bar chart of fresh weight of red spinach

4. CONCLUSION

Based on the results and discussion presented, this study demonstrates a consistency between the objectives outlined in the introduction and the research findings. The application of *Azolla pinnata*-based LOF was shown to influence the growth and productivity of red spinach (*Amaranthus tricolor* L.), as evidenced by increases in plant height, number of leaves, leaf area, root length, and fresh weight. Treatment P3, with a dose of 200 mL/L, yielded the best response, resulting in a plant height of 34.60 cm, 24.29 leaves, a leaf area of 48.96 cm², a root length of 12.63 cm, and a fresh weight of 30.60 g. Thus, the 200 mL/L dose was the most effective among the treatment levels tested under the conditions of this study. These results indicate that *Azolla pinnata* based LOF has the potential to be developed as a source of organic nutrients to support the growth and productivity of red spinach. Further application can be conducted through testing at different locations, seasons, and cultivation conditions with a larger number of replicates. Future research should also analyze the nutrient content of the LOF, plant nutrient uptake, chlorophyll content, and dry biomass to clarify the mechanisms of plant response and to provide broader and more precise recommendations for the use of *Azolla pinnata* LOF.

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