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GROWTH AND YIELD OF BRAZILIAN SPINACH (*ALTERNANTHERA SISSOO*) IN A FLOATING RAFT HYDROPONIC SYSTEM UNDER OPTIMIZED NUTRIENT SUBSTITUTION

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ABSTRACT

Brazilian spinach is gaining attention as a resilient leafy vegetable for controlled cultivation systems. However, information on optimal nutrient management in hydroponics remains limited, particularly regarding the integration of organic and inorganic nutrients. This study aimed to determine the optimal AB Mix and evaluate the potential of liquid organic fertilizer (LOF) to reduce inorganic nutrient use while maintaining growth and yield. The experiment was conducted from March to April 2025 for 42 days using nine treatments and three replications (27 experimental units and 108 plants), arranged in a randomized complete block design. Data were analyzed using ANOVA, Principal Component Analysis (PCA), and Pearson correlation ($P<0.05$). The results showed that 3 mL LOF with 1000 ppm AB Mix (T2) produced the highest total fresh weight (130.33 g plant^{-1}), with a 0.36–10.46-fold increase in fresh consumption weight (FCW) compared with other treatments. PCA explained 89.45% of the total variability and identified T2 as strongly associated with biomass traits (plant height, leaf number, and fresh weight). Pearson correlation indicated significant positive relationships among growth and yield parameters, especially between plant height, leaf number, and fresh weight, indicating coordinated contributions of vegetative traits to biomass accumulation. Optimized nutrient substitution using LOF can enhance plant growth and yield while reducing dependence on inorganic fertilizers. In practice, this approach improves nutrient use efficiency, reduces input costs, and supports more environmentally friendly hydroponic production. The T2 treatment consistently achieved the highest yield (130.33 g plant^{-1}), confirming it as a nutrient management strategy.

Keywords: *Alternanthera sissoo*, Hydroponic, Nutrient management, Principal component analysis.

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1. INTRODUCTION

Brazilian spinach (*Alternanthera sissoo* L.) is a leafy vegetable with high nutritional value, containing essential vitamins (vitamins B9, K, and C), minerals (Ca and Fe), and bioactive compounds such as myricetin, kaempferol glycosides, ferulic acid, p-coumaric acid, and gallic acid, which function as antioxidants. These components make Brazilian spinach a promising functional food with potential health benefits. The species is well adapted to tropical environments and is tolerant to drought and moderate shading (up to 50%). In addition, its rapid vegetative growth and high biomass production make it suitable for intensive cultivation systems (Ferreira et al., 2021; Saengha et al., 2022).

Beyond its stress tolerance, Brazilian spinach is characterized by rapid regrowth and the ability to be harvested repeatedly in short cycles (2–4 weeks). Its ease of propagation through stem cuttings further enhances its suitability for scalable production systems. Despite these advantages, its use in commercial hydroponic production remains limited compared with conventional leafy vegetables. This indicates a need for optimization of cultivation strategies to fully exploit its agronomic and economic potential (Muda et al., 2022; Muda et al., 2024).

The global demand for fresh vegetables continues to increase owing to population growth and urbanization, whereas the availability of fertile agricultural land is declining due to soil degradation and land-use conversion (Muda et al., 2023; Muda et al., 2024; Najibah et al., 2024; Warid & Koniko, 2025). These challenges have accelerated the adoption of soilless cultivation systems such as hydroponics. A hydroponics system can reduce

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water use by 80–90% and enable high productivity in limited spaces through vertical farming and controlled environments. In addition, they allow precise nutrient management, thereby improving crop quality and resource efficiency (Velazquez-Gonzalez et al., 2022; Pomoni et al., 2023; Rajendran et al., 2024).

Globally, hydroponics plays a critical role in supporting sustainable urban agriculture and stabilizing production under climate variability. The performance of these systems is strongly influenced by nutrient management (Fathidarehnijeh et al, 2024; Găgeanu et al., 2024). The productivity of hydroponic system is strongly dependent on precise management. Recirculating systems such as the Nutrient Film Technique (NFT) and Deep Flow Technique (DFT), combined with real-time ion monitoring, improve nutrient use efficiency and reduce losses. Recent developments have emphasized alternative nutrient sources, including organic inputs and beneficial microorganisms, to reduce reliance on synthetic fertilizers (Palmitessa et al., 2024; Renganathan et al., 2024).

In hydroponic systems, mineral nutrient solutions such as AB Mix are widely used because they provide complete macro and micronutrients and support optimal plant growth. The typical concentrations for leafy vegetables range from 600 to 2000 ppm, with an optimal level often between 1200 and 1500 ppm. However, excessive reliance on inorganic fertilizers raises concerns regarding production costs and environmental sustainability. Consequently, liquid organic fertilizers (LOF) have been explored as partial substitutes to reduce chemical inputs while maintaining productivity (Dasgan et al., 2023; Siahaan et al., 2024; Pujiwati et al., 2024).

Nutrient interactions in hydroponic systems play a critical role in determining plant performance. Nutrient uptake is governed by ion balance, pH, and elemental ratios in the solution. Nutrient imbalances can lead to antagonistic and competitive interactions among ions in the root zone. Elevated nutrient concentrations may increase salinity, causing osmotic stress and impairing physiological processes. These conditions reduce water uptake, photosynthesis, and biomass accumulation (Vought et al., 2024).

Root-zone conditions are equally important in hydroponic systems because they directly influence plant growth and productivity. Root morphology, oxygen availability, and nutrient dynamics regulate nutrient uptake and metabolic activity. In floating raft systems, adequate aeration is essential because root respiration depends on dissolved oxygen. Hypoxic conditions can inhibit root function, reduce biomass accumulation, and increase oxidative stress, ultimately affecting photosynthesis. Nutrient imbalances further disrupt root development and reduce nutrient use efficiency (Jiménez et al., 2019; Baiyin et al., 2021; Liu et al., 2024a).

The combined application of mineral nutrients and organic sources has been reported to sustain plant growth and maintain yield quality. However, the exclusive use of organic fertilizers often results in reduced growth due to nutrient imbalance and slower nutrient availability. (Shaik et al., 2022; Cardarelli et al., 2023; Tian et al., 2023). Ahmed et al. (2021) reported that lettuce plants grown using an organic nutrient solution alone exhibited lower growth, biomass, and stomatal density compared with those grown with an inorganic nutrient. However, higher levels of total chlorophyll, carotenoid, phenolic compounds, and flavonoids were observed under organic treatments, indicating improved nutritional quality. Similarly, Kano et al. (2021) found that organic nutrient solutions resulted in lower nitrate content but higher vitamin C levels compared to inorganic treatments.

LOF serves not only as a source of nutrients but also as a biofertilizer that enhances microbial activity to support nutrient mineralization, root development and plant growth. In LOF, groups of plant growth-promoting rhizobacteria (PGPR) such as *Pseudomonas*, *Azospirillum*, and *Azotobacter* play a role in solubilizing phosphate and potassium, oxidizing sulfur, fixing nitrogen, and producing siderophores and phytohormones that increase root surface area and nutrient availability in the rhizosphere (Mohanty et al., 2021; De Andrade et al., 2023). Additionally, *Lactobacillus* contributes to the decomposition of organic matter by producing organic acids and bioactive metabolites, including IAA and siderophores and functions as a biocontrol agent and biostimulant, thereby improving nutrient utilization efficiency (Raman et al., 2022). Information on the response of Brazilian spinach to combined applications of AB Mix and LOF in floating raft hydroponic systems remains limited, particularly regarding the optimal substitution of inorganic nutrients with organic inputs. Moreover, the complex interactions among growth and yield under varying nutrient regimes have not been sufficiently explored using multivariate approaches. This gap limits the development of efficient nutrient management strategies. Given that plant responses to nutrient variations involve interdependent growth traits, multivariate analyses are required to better capture and interpret these relationships (Arteaga et al., 2020; Cui et al., 2025).

The quantitative evaluation of the partial substitution of inorganic nutrients with LOF in Brazilian spinach using integrated multivariate frameworks remains scarce. This study therefore applies multivariate analyses (principal component analysis and correlation analysis) to systematically assess nutrient substitution strategies in a floating raft hydroponic system, enabling a more comprehensive evaluation of growth–yield relationships and nutrient optimization. This study aimed to examine the effects of different concentrations of LOF and AB Mix on the growth and yield of Brazilian spinach and to identify the most efficient nutrient regime.

2. MATERIALS AND METHODS

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2.1. Experimental Site and Materials

This study was conducted in the greenhouse of the Faculty of Agriculture, Syiah Kuala University, Banda Aceh, Indonesia (Fig. 1) at an elevation of 4.85 m above sea level, as determined using Google Earth Pro. The effective fieldwork period was from March 15 to April 27. Environmental data collected during the study, including air temperature, relative humidity, and duration of sunlight, were obtained from the nearest meteorological station operated by the Indonesian Agency for Meteorology, Climatology, and Geophysics (BMKG), namely the Sultan Iskandar Muda Station in Banda Aceh (5°52′ N; 95°41′ E) (Fig. 2).

The materials used included Brazilian spinach cuttings, rockwool growing medium, commercial AB mix nutrients (*Goodplant*), commercial LOF (*Herbafarm*), and water as a solvent. Plant cultivation was carried out using a floating raft hydroponic system. The instruments used included a total dissolved solids meter (TDS meter) and a soil plant analysis development meter (SPAD meter).

2.2 Seedling Preparation and Hydroponic System Setup

Brazilian spinach seedlings were obtained through vegetative propagation using stem cuttings from healthy parent plants approximately one month old. Cuttings approximately 10 cm long were planted in rockwool blocks measuring 5×5×5 cm. The seedlings were grown for seven days and had already developed roots. A closed floating raft hydroponic system with a 90×30×30 cm nutrient container and a solution volume of 10 L was used (Fig. 3).

2.3. Preparation of Nutrient Solutions

The hydroponic nutrient solution used in this study was a commercial AB mix (Goodplant), consisting of stock solutions A and B. The stock solutions were prepared according to the manufacturer's instructions by dissolving each component separately in 5 L of water until completely homogeneous. The working nutrient solutions were then prepared by mixing stock solutions A and B at the desired concentrations. A concentration of 1000 ppm was achieved in a 10-L nutrient container by adding 50 mL of stock A and 50 mL of stock B, while 1800 ppm was achieved by adding 100 mL of stock A and 100 mL of stock B. The nutrient solution concentration was monitored using a TDS meter.

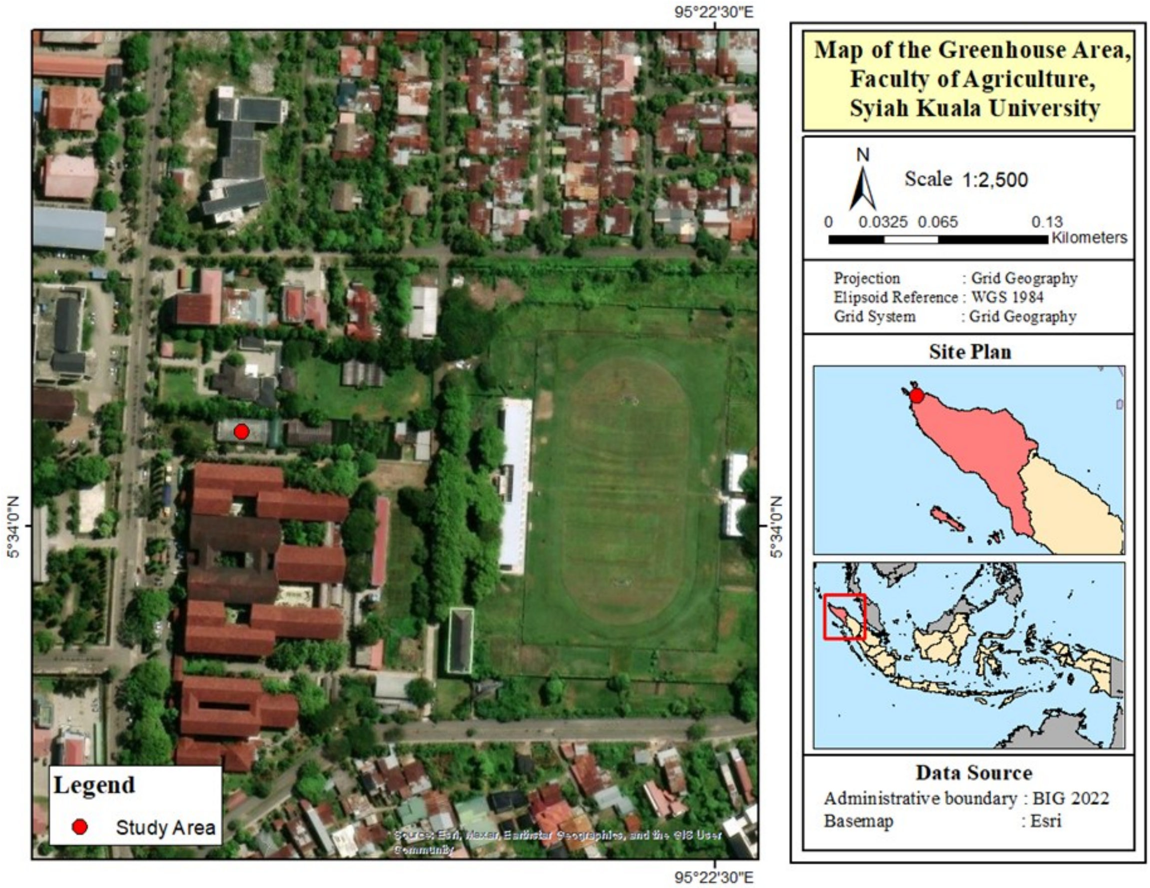

Fig. 1: Location of the experimental greenhouse at the Faculty of Agriculture, Syiah Kuala University, Indonesia (5°34′ N, 95°22′ E).

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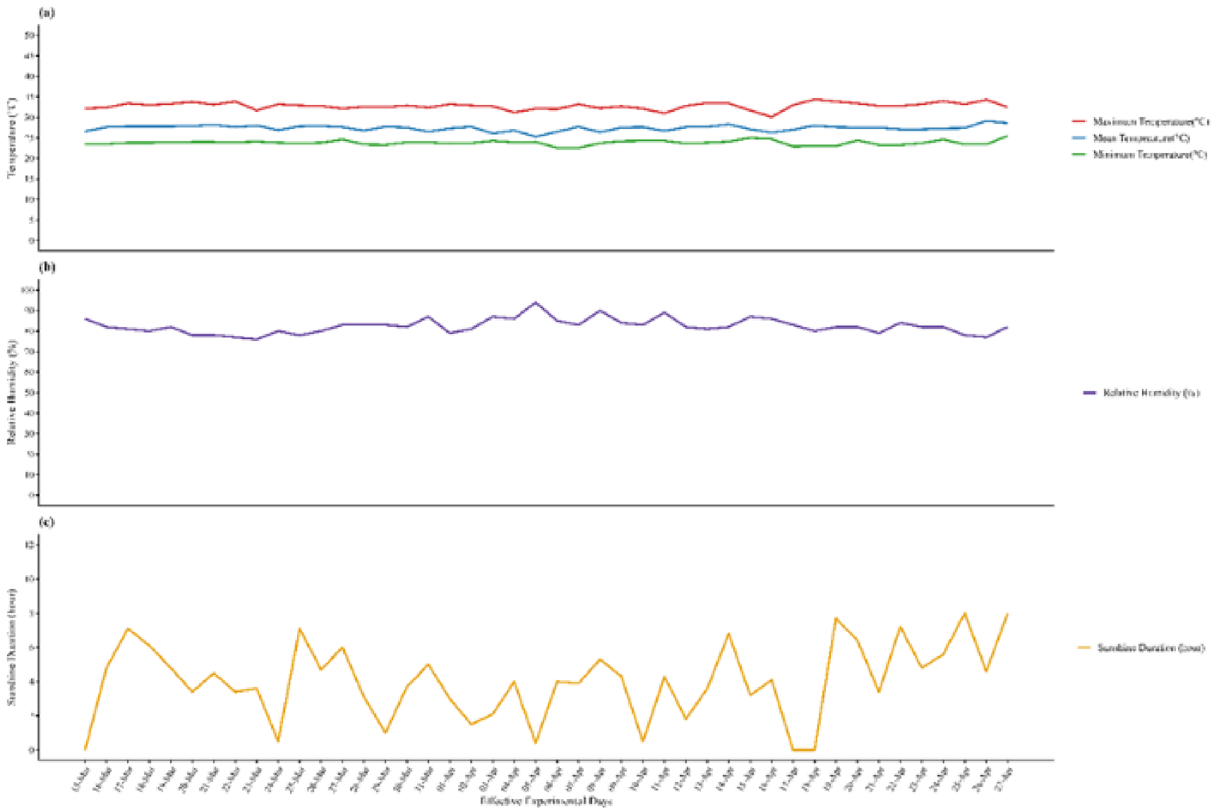

Fig. 2: During the study period, air temperature ranged from 23.0-34.3°C, relative humidity from 76-94%, and sunshine duration from 0 - 8 hours.

(a) (b)

Fig. 3: Experimental setup showing (a) Brazilian spinach seedlings propagated by stem cuttings and rooted in rockwool blocks, and (b) the closed floating raft hydroponic system used for plant cultivation.

2.4. Preparation and Application of Liquid Organic Fertilizer

The LOF used in this study was a commercial product (Herbafarm) and was applied at three concentrations: 3, 6, and 9 mL L^{-1}. In a 10-L nutrient container, these concentrations corresponded to the addition of 30, 60, and 90 mL of LOF. The treatment solutions were prepared by adding AB Mix according to the designated concentration, followed by the addition of LOF. Distilled water was then added to reach a final volume of 10 L, and the solution was mixed thoroughly before application to the hydroponic system.

The nutrient solution in each hydroponic unit was maintained throughout the experimental period without complete replacement. Each container was initially filled with 10 L of nutrient solution and monitored daily. When the solution volume decreased below 5 L due to evapotranspiration, distilled water and nutrient solution were added to restore the initial volume and maintain the designated treatment concentration. Nutrient concentration was monitored using a TDS meter to ensure consistency with the target AB Mix concentration. No pH adjustment was performed during the experimental period. In addition, no supplemental aeration system was applied in the floating raft hydroponic system. A schematic illustration of the experimental workflow for Brazilian spinach grown using a floating hydroponic system is shown in Fig. 4. The chemical compositions of AB Mix and LOF used in this study are presented in Table 1.

LOF (Herbafarm®, Indonesia) is a commercial bio-organic liquid fertilizer derived from by-products of *Sido Muncul* herbal medicine production using medicinal plants and spices as raw materials. The product contains macro-micronutrients, with percentage compositions presented in Table 1, as well as beneficial microorganisms, including

Azotobacter sp., *Azospirillum* sp., *Lactobacillus* sp., and *Pseudomonas* sp., according to the manufacturer's specifications. These microorganisms may enhance nutrient availability through biological processes.

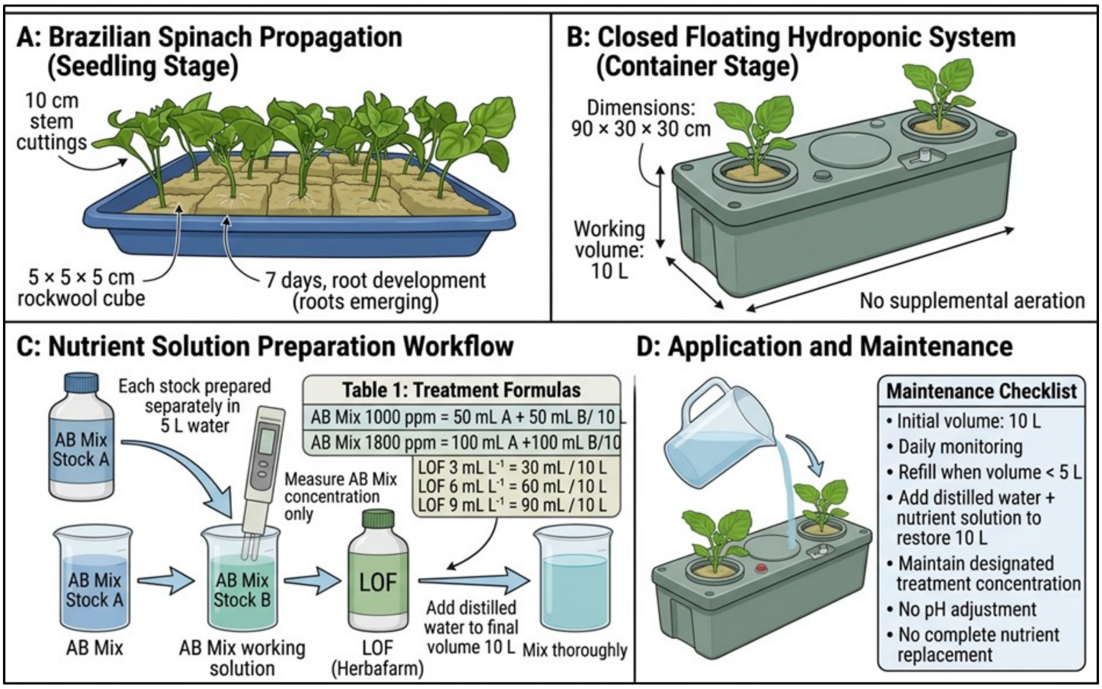

Fig 4: Schematic representation of the experimental workflow for Brazilian spinach grown under a floating hydroponic system, illustrating seedling propagation, closed system setup, nutrient solution preparation, and daily maintenance practices.

Table 1: Comparison of the chemical composition of AB Mix and LOF

Parameter	AB Mix (Goodplant)	LOF (Herbafarm)
C- Organic (%)	-	15.44
Total N (%)	1.51	1.66
P (%)	0.45	1.76
K (%)	2.49	1.87
Ca (%)	0.21	-
Mg (%)	0.31	-
S (%)	0.03	-
Fe (ppm)	129.7	260.9
Mn (ppm)	6.2	30.2
Cu (ppm)	3.4	42.4
Zn (ppm)	6.1	28.1
B (ppm)	9.9	59.4
Mo (ppm)	<0.05	-

Table 2: Treatment combinations of (LOF and AB Mix nutrient concentration

Treatment	LOF Concentration (m L^{-1})	AB Mix Concentration (ppm)
T_1	3	0
T_2	3	1000
T_3	3	1800
T_4	6	0
T_5	6	1000
T_6	6	1800
T_7	9	0
T_8	9	1000
T_9	9	1800

2.5. Experimental Design

The experiment was arranged in a randomized complete block design (RCBD) with nine treatment combinations, as presented in Table 2. Each treatment was replicated thrice, resulting in 27 experimental units. Each experimental unit consisted of four plants, for a total of 108 plants. Data from the two plants within each experimental unit were averaged prior to statistical analysis.

2.6. Growth and Yield Measurements

Plant growth and yield parameters were measured during the experiment. Growth parameters included plant height and the number of leaves, which were recorded at 1, 2, 3, 4, 5, and 6 weeks after transplanting (WAT). Additional growth variables included leaf length (LL), leaf width (LW), chlorophyll content (CC), and root length (RL). Chlorophyll content was measured using a SPAD meter. Yield parameters included total fresh weight (TFW) and fresh consumption weight (FCW). Harvesting was conducted at 6 WAT by removing the entire plant, including the roots, from the net pots. All measurements were performed for each experimental unit.

2.7. Statistical Analysis

The data were analyzed using ANOVA based on RCBD using Microsoft Excel 2021 (Version 2404).

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Prior to ANOVA, assumptions of normality and homogeneity of variance were evaluated using the Shapiro-Wilk test and Levene's test, respectively, in R version 4.4.2. Data variability was assessed using the coefficient of variation (CV), with values below 25% considered acceptable. When significant differences were detected by the F-test ($P<0.05$), treatment means were compared using the Honest Significant Difference (HSD) test at the 5% significance level.

Principal Component Analysis (PCA) was conducted on standardized data (z-score transformation) to identify the main variables contributing to variation in plant growth and yield. Components were retained based on eigenvalues greater than 1 per the Kaiser criterion, and the results were visualized with a biplot to illustrate relationships among variables. PCA was selected to reduce the dimensionality of multivariate data and identify patterns among treatments and parameters. Pearson's correlation analysis was performed to evaluate the strength and direction of relationships among growth and yield variables. Pearson correlation was selected to quantify pairwise associations among variables and complement PCA in interpreting relations among plant growth, physiological, and yield traits. PCA and correlation analysis were performed using R version 4.4.2. PCA visualization and correlation analyses were performed using the *factoextra* and *metan* packages, respectively.

3. RESULTS AND DISCUSSION

3.1. Plant Height

Plant height of Brazilian spinach was significantly influenced by the combination of LOF and AB Mix concentrations across all observation periods (Table 3), highlighting the importance of nutrient management in hydroponic systems. At 6 WAT, the highest plant height was recorded for T2 (3 m L^{-1} LOF + 1000 ppm AB Mix), at 37.58 cm, which differed significantly from all other treatments. This result indicates that a balanced combination of organic and inorganic nutrients at moderate concentrations provides optimal conditions for vegetative growth. Such a nutrient balance likely enhances key physiological processes, including cell division, cell elongation, and photosynthetic activity, which are essential for plant height development (Chrysargyris & Tzortzakis, 2023).

Table 3: Average plant height of Brazilian spinach under varying concentrations of liquid organic fertilizer combined with AB Mix

Treatment	Plant Height (cm)					
	1 WAT	2 WAT	3 WAT	4 WAT	5 WAT	6 WAT
T_1	10.67 cd	14.50 bc	16.42 bc	22.42 cd	31.83 d	34.50 d
T_2	11.25 d	14.83 bc	18.33 d	23.67 d	34.92 e	37.58 e
T_3	8.83 a	11.75 ab	14.08 a	16.92 a	21.17 a	23.83 a
T_4	10.25 bc	15.67 c	17.33 cd	22.67 cd	27.50 bc	32.08 c
T_5	8.83 a	12.08 ab	14.33 a	16.58 a	20.92 a	23.50 a
T_6	10.17 bc	10.08 a	15.33 ab	19.08 b	22.92 a	26.50 b
T_7	10.42 bcd	14.75 bc	17.00 c	21.75 c	28.58 c	32.75 cd
T_8	10.50 bcd	14.00 bc	16.25 bc	21.42 c	26.17 b	31.00 c
T_9	9.67 ab	12.58 abc	15.17 ab	17.58 ab	21.58 a	23.42 a
$HSD_{0.05}$	0.84	3.09	1.29	1.62	2.09	1.96

Note: Values followed by the same letter indicate no significant difference based on the HSD test at 5%.

In contrast, treatments with higher AB Mix concentration (1800 ppm), particularly T3 and T9, consistently resulted in significantly reduced plant height. This reduction can be explained by osmotic stress induced by elevated nutrient concentrations in the hydroponic solution. High nutrient levels increase electrical conductivity (EC), lowering the water potential of the solution and making water uptake by roots more difficult. As a result, plants experience reduced cell turgor pressure, which directly limits cell expansion and elongation. Additionally, osmotic stress can be disrupt nutrient uptake balance, leading to ion toxicity or antagonism, impairing root function and reducing metabolic activity, including photosynthesis (Langenfeld et al., 2022).

Similar trends have been reported in hydroponic leafy vegetables, where excessive nutrient concentrations negatively affect vegetative growth. Hosseini et al. (2021) reported that lettuce and basil grown under EC levels of 0.5, 0.7, 0.9, 1.2, and 2.0 dS m^{-1} achieved the highest vegetative growth at 0.9 dS m^{-1}, indicating this level as the optimum concentration. Increasing EC beyond this point resulted in reduced growth, likely due to increased osmotic stress. Ren et al. (2022) found that basil grown under EC levels of 0.5, 1.0, 3.0, and 5.0 dS m^{-1} exhibited the lowest vegetative growth at the highest EC level (5.0 dS m^{-1}), while optimal growth was observed at a moderate EC level (3.0 dS m^{-1}). Importance of determining an optimal nutrient concentration range in hydroponic systems to avoid osmotic stress and ensure optimal plant growth.

Interestingly, treatments without AB Mix (T1, T4 and T7) produced relatively moderate plant height compared to some high-concentration treatments, indicating that Brazilian spinach is tolerant to low nutrient concentrations.

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However, plant height under these conditions remained lower than that in T2, suggesting that although the plant can tolerate limited nutrient availability, adequate nutrient supplementation is still required to achieve optimal growth.

Although plant height is an important indicator of vegetative growth, it should not be used as the sole parameter for determining the best treatment for a plant. Plant productivity is a complex trait influenced by multiple growth components, including leaf development, root growth, and biomass accumulation. Therefore, a more comprehensive evaluation integrating both growth and yield parameters is necessary to identify the most efficient nutrient management strategy (Najibah et al., 2024).

3.2. Number of Leaves

The number of leaves was significantly influenced by the combination of LOF and AB Mix concentrations (Table 4). At 6 WAT, T2 (3 mL L^{-1} LOF+1000 ppm AB Mix) produced the highest leaf number (140.33 leaves), which differed significantly from all other treatments. This indicates that a balanced nutrient supply at moderate concentrations is optimal for leaf production in Brazilian spinach. The number of leaves is a critical parameter in Brazilian spinach, as leaves constitute the primary edible portion and directly contribute to yield.

Table 4: Average number of leaves on Brazilian spinach at various concentrations of liquid organic fertilizer combined with AB Mix

Treatment	Number of Leaf					
	1 WAT	2 WAT	3 WAT	4 WAT	5 WAT	6 WAT
T_1	10.17 b	14.83 d	38.50 d	62.00 c	90.33 d	120.50 d
T_2	13.50 c	20.50 e	46.33 e	78.83 d	107.17 e	140.33 e
T_3	6.33 a	8.00 a	14.00 a	20.83 a	28.50 a	34.67 a
T_4	10.33 b	15.67 d	30.50 c	44.33 b	74.17 c	89.67 c
T_5	7.00 a	11.67 bc	19.67 b	27.17 a	38.50 ab	50.50 b
T_6	7.83 ab	11.83 c	19.83 b	27.17 a	42.33 b	59.00 b
T_7	10.17 b	16.50 d	35.50 d	63.67 c	89.00 d	106.83 d
T_8	6.67 a	9.67 a	18.50 b	47.17 b	76.00 c	87.00 c
T_9	6.83 a	9.83 ab	16.17 ab	27.83 a	42.00 b	58.33 b
$HSD_{0.05}$	2.98	1.93	3.84	7.28	12.54	14.16

Note: Values followed by the same letter indicate no significant difference based on the HSD test at 5%.

The superior performance of T2 suggests that adequate nutrient availability enhances leaf initiation and expansion through improved physiological processes. Leaf formation is greatly influenced by the sink-source relationship, in which growing young leaves act as sinks that require assimilates produced by mature leaves as sources. A balance of nutrients supports photosynthetic activity and carbohydrate distribution, thereby promoting continuous leaf formation and expansion (Wingler & Henriques, 2022; Fañanás-Pueyo et al., 2025). Treatments with higher combined nutrient concentrations, particularly those involving 1800 ppm AB Mix, resulted in a significant reduction in leaf number. This suggests that excessive nutrient levels in the solution negatively affect plant growth. In hydroponic systems, elevated nutrient concentrations increase the electrical conductivity (EC), which can induce osmotic stress, limit water and nutrient uptake, and ultimately inhibit leaf development. Under such conditions, reduced turgor pressure and impaired cell division in leaf primordia can directly suppress the rate of new formation, further limiting canopy development (Voutsinos-Frantzis et al., 2022).

Furthermore, increasing LOF concentrations within these treatments (6 and 9 mL L^{-1}) further reduced leaf production. This indicates that excessive organic inputs may exacerbate nutrient imbalance and negatively affect plant growth. Although LOF contains beneficial microorganisms that enhance nutrient availability through mechanisms such as nitrogen fixation and nutrient solubilization (De Andrade et al., 2023). Excessive microbial populations increase competition for available nutrients, particularly nitrogen, between microbes and plant roots. This process is known as nutrient immobilization. It converts plant-available nutrients into microbial biomass and reduces nutrient availability for plant uptake, thereby limiting leaf development (Bartelme et al., 2018; Mourouzidou et al., 2023; Patloková & Pokluda, 2024).

Dissolved oxygen (DO) in the root zone plays a crucial role in determining root respiration, nutrient uptake, and canopy growth in hydroponic systems. Roots require oxygen for aerobic respiration and ATP production; thus, low DO conditions (hypoxia) reduce respiratory activity and water uptake and trigger physiological stress in plants. A decrease in DO also inhibits the uptake of essential nutrients such as K, Mg, Ca, P, S, and N in the form of NO_3^- and NH_4^+, resulting in reduced nutrient content in plant tissues and stunted growth (Nițu et al., 2024; Roosta, 2024).

3.3. Yield Components

Leaf morphological traits, including LL and LW, did not differ significantly among treatments (Table 5). This indicates that these parameters remained relatively stable across nutrient regimes and were likely maintained within a non-limiting range of nutrient availability. Leaf size is generally more conservative and is more strongly regulated by genetic factors than by external inputs, particularly under conditions in which nutrients are sufficiently supplied. Moreover, leaf morphological traits tend to exhibit lower plasticity than other growth parameters such as leaf number or biomass accumulation, which are more responsive to environmental changes (Sapkota et al., 2019; Pereira and Marais, 2020). Thus, the absence of significant differences in LL and LW suggests that the applied nutrient treatments did not reach a threshold required to induce morphological variation.

Table 5: Average chlorophyll content and yield components of Brazilian spinach at various concentrations of liquid organic fertilizer combined with AB Mix

Treatment	LL (cm)	LW (cm)	CC (unit mm^{-2})	RL (cm)	TFW (g)	FCW (g)
T_1	5.00	4.67	32.63 cd	32.75 bc	96.09 d	90.31 d
T_2	5.00	5.08	28.33 abc	41.17 c	130.33 e	122.88 e
T_3	4.58	4.25	33.83 d	13.42 a	10.72 a	10.70 a
T_4	4.92	4.50	32.20 bc	33.42 bc	62.48 c	55.43 c
T_5	4.92	3.92	27.42 ab	29.58 b	31.76 ab	27.94 ab
T_6	4.33	4.08	28.87 abc	24.40 ab	31.74 ab	27.76 ab
T_7	5.00	4.75	29.40 abcd	31.50 bc	62.38 c	57.63 c
T_8	4.83	4.58	33.98 d	27.08 b	46.87 bc	45.10 bc
T_9	4.75	3.92	25.70 a	23.67ab	22.31 ab	21.02 ab
$HSD_{0.05}$	-	-	4.92	11.01	29.97	24.54

Note: Values followed by the same letter indicate no significant difference based on the HSD test at 5%, LL (leaf length), LW (leaf width), CC (chlorophyll content), RL (root length), TFW (total fresh weight), FCW (fresh consumption weight).

CC varied among treatments, with the highest value observed in T8, however, this increase was not accompanied by proportional increases in TFW or FCW. This indicates that chlorophyll accumulation alone is not a reliable predictor of plant productivity. Although chlorophyll is essential for light capture, biomass accumulation depends on multiple downstream processes, including photosynthetic efficiency, Rubisco activity, canopy architecture, and assimilate partitioning (Chatzigianni et al., 2019; Lu et al., 2022). Therefore, the lack of a linear relationship between CC and biomass suggests that factors beyond pigment concentration were more influential in regulating growth under these conditions. Higher chlorophyll content may increase light-absorbing capacity, but this does not necessarily translate into higher biomass production when nutrient uptake, root activity, or assimilate translocation is suboptimal. Yang et al. (2021) further reported that under suboptimal nutrient or root conditions, assimilates may not be efficiently transported to growing tissues, thereby weakening the relationship between chlorophyll content and biomass accumulation.

RL showed a clearer and more consistent association with both TFW and FCW, with treatment T2 producing the highest values for both. This finding highlights the central role of root development in supporting plant growth. In hydroponic systems, nutrient uptake depends entirely on root contact with the nutrient solution. Root morphology directly determines nutrient uptake efficiency because longer and well-developed roots provide a larger absorptive surface area for dissolved nutrients. Optimal root development also enhances the formation of active root hairs, which play an important role in absorbing water and nutrients from the hydroponic solution. In addition, a well-developed root system improves oxygen acquisition and ion transport efficiency, both of which are essential for biomass accumulation in a hydroponic system (Liu et al., 2024). Longer roots increase the absorptive surface area and enhance nutrient uptake efficiency by improving contact with dissolved ions. In addition, well-developed roots support better oxygen acquisition, which is critical for root respiration and active nutrient transport. (Nguyen et al., 2022; Liu et al., 2024b). The strong relationship between RL and yield components suggests that nutrient uptake capacity, rather than chlorophyll content or leaf morphology, was the primary driver of biomass production in this study.

The markedly low performance observed in T3, particularly in RL, TFW, and FCW, further emphasizes the importance of a balanced nutrient supply. This treatment likely resulted in suboptimal nutrient availability or an imbalance between LOF and AB Mix concentrations, thereby constraining root development and limiting overall plant growth. Conversely, the superior performance of T2 indicates that this treatment provided a more favorable nutrient composition, supporting both root elongation and biomass accumulation.

The superior performance of T2 may be attributed to a balanced interaction between inorganic and organic nutrient sources. The moderate AB Mix concentration (1000 ppm) likely provided sufficient nutrients without inducing osmotic stress, thereby optimizing nutrient uptake and plant growth. In addition, the presence of beneficial microorganisms in LOF, such as *Azotobacter* sp., *Azospirillum* sp., *Lactobacillus* sp., and *Pseudomonas* sp., may have enhanced nutrient availability through biological processes, including nitrogen fixation and phytohormone production. However, at higher concentrations (1800 ppm), excessive nutrient levels may have increased solution salinity, leading to osmotic stress and reduced plant performance. FCW, representing the edible portion of the plant, followed a trend similar to that of TFW and served as a key indicator of marketable yield. The significant increase in FCW under T2 highlights the practical importance of optimizing nutrient combinations in hydroponic cultivation systems, as this parameter directly reflects economic value and production efficiency. A higher FCW indicates greater harvestable biomass, which can increase marketable output and improve profitability in commercial hydroponic production. In leafy vegetables, fresh biomass is closely associated with consumer acceptance and market price, making FCW an important determinant of economic return (Wang et al., 2023).

These results suggest that plant growth under combined LOF and AB Mix treatments was more strongly governed by belowground traits than by aboveground morphological or physiological characteristics. Although chlorophyll content and leaf size are important components of plant function, they were not reliable predictors of yield under the conditions of this study. Root development emerged as the primary determinant of productivity, underscoring the need to optimize nutrient solutions to enhance root performance in hydroponic systems. The performance of the Brazilian spinach plants following treatment is shown in Fig. 5.

Fig. 5: The performance of Brazilian spinach at various concentrations of liquid organic fertilizer combined with AB Mix.

3.4. Principal Component Analysis

PCA was applied to reduce the dimensionality of growth and physiological traits and to identify the main patterns of variation among treatments (De Carvalho et al., 2022). The results showed that the first two principal components accounted for 89.45% of the total variance, with PC1 explaining 81.24% and PC2 8.21%, indicating that the dataset's structure is well represented in a two-dimensional multivariate space (Fig. 6 and Table 6). Based on the Kaiser criterion (eigenvalue>1) (Kaiser, 1960), only PC1 and PC2 were retained for interpretation, confirming their statistical robustness in explaining treatment-induced variability.

Table 6: Loadings, eigenvalues, and percentage of variance, cumulative percentages of PC1 and PC2 in PCA

Parameters	PC1	PC2
PH1WAT	-0.231	0.057
PH2WAT	-0.211	0.186
PH3WAT	-0.248	0.006
PH4WAT	-0.246	0.187
PH5WAT	-0.257	0.060
PH6WAT	-0.256	0.146
NL1WAT	-0.243	-0.178
NL2WAT	-0.238	-0.262
NL3WAT	-0.250	-0.128
NL4WAT	-0.256	0.006
NL5WAT	-0.257	0.076
NL6WAT	-0.258	-0.006
LL	-0.182	-0.047
LW	-0.240	0.240
CC	-0.023	0.795
RL	-0.232	-0.278
TWB	-0.252	-0.076
FCW	-0.252	-0.072
Eigenvalues	14.62	1.48
Percentage of Variance	81.24	8.21
Percentage Cumulatif	81.24	89.45

Note: PH (plant height), NL (number of leaf), LL (leaf length), LW (leaf width), CC (chlorophyll content), RL (root length), TWB (total fresh weight), FCW (fresh consumption weight).

PC1 exhibited high absolute loadings across all morphological and biomass-related traits, including plant height, number of leaves, leaf length, leaf width, total fresh weight, and fresh consumption weight. The consistently high contribution of these variables indicates that PC1 represents a general plant growth and biomass accumulation axis, reflecting overall vegetative vigor and productivity. The strong clustering of growth and biomass variables within PC1 suggests that vegetative responses in Brazilian spinach were highly coordinated under different nutrient treatments. This pattern indicates that biomass accumulation was primarily regulated by integrated morphological development rather than isolated physiological responses.

PC2 was strongly affected by CC, which showed the highest positive loading (0.795), whereas root length and number of leaves contributed moderately in the opposite direction. This pattern suggests that PC2 reflects a physiological efficiency gradient, particularly associated with photosynthetic capacity and nutrient utilization efficiency. The strong contribution of CC to PC2 indicates that physiological responses to nutrient treatments were partially independent of structural growth responses. This finding suggests that increased chlorophyll accumulation did not necessarily improve biomass production under all nutrient conditions. The separation of chlorophyll content from biomass-related traits indicates that physiological performance may respond independently of morphological growth under different fertilizer concentrations.

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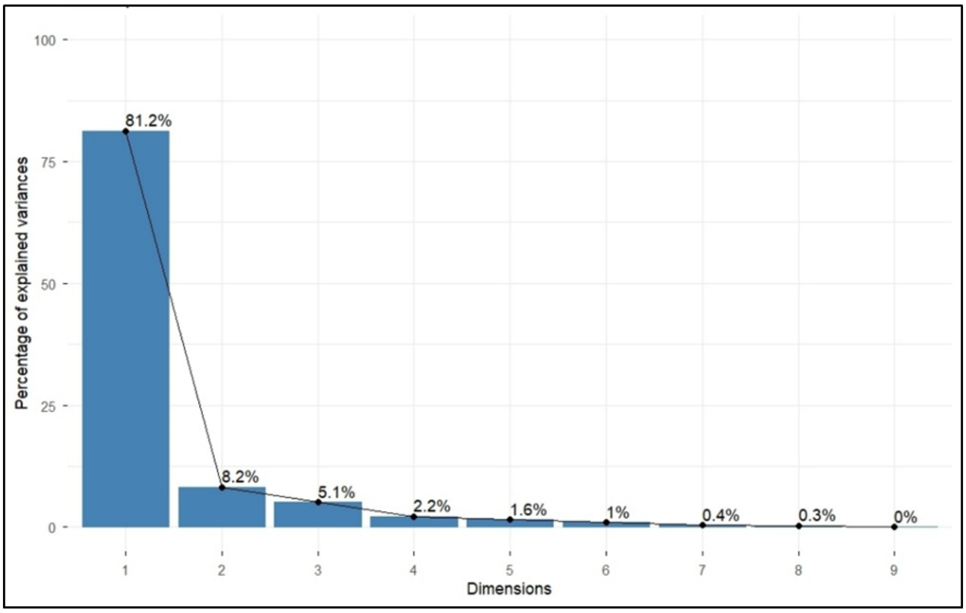

Fig. 6: The scree plot of the PCA results shows that the principal components with eigenvalues > 1 were PC1 (81.2%) and PC2 (8.2%).

The PCA score plot further demonstrated clear differentiation among treatments (Fig. 7 and Table 7). Treatments located in the same direction as growth-related variables on PC1 showed stronger associations with vegetative growth and biomass accumulation. Among these, T2 exhibited the most negative PC1 score, indicating the strongest association with growth-related traits such as plant height, leaf number, and fresh weight, and therefore representing the most favorable treatment in terms of biomass production. Other treatments located in the same direction (T1 and T7) showed similar but less pronounced associations. In contrast, treatments such as T3, T5, and T9, which were positioned toward the positive side of PC1, indicated weaker associations with these growth parameters.

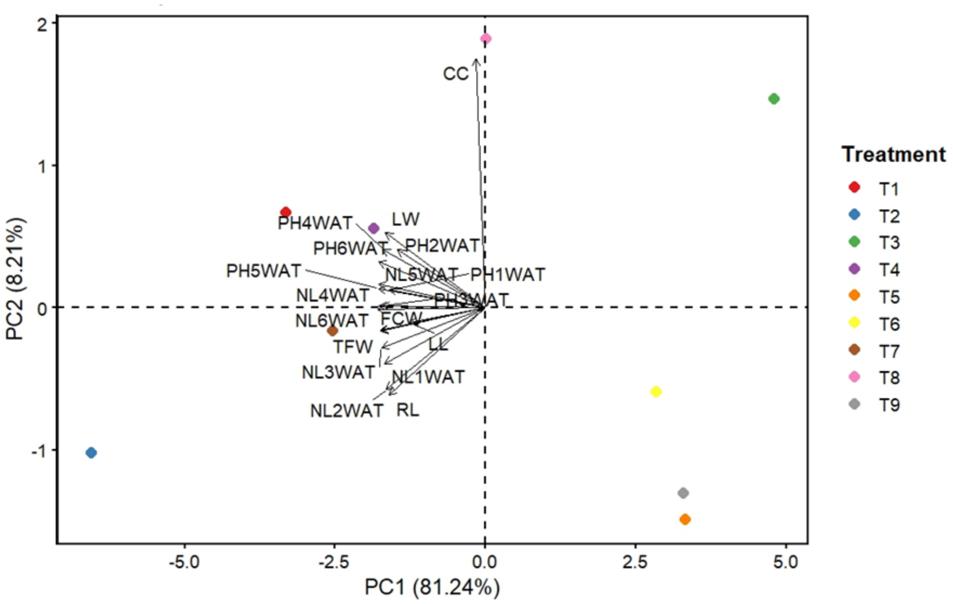

Fig. 7: The PCA biplot visualization shows that the PC1 and PC2 components explains 89.45% of the data variation.

On PC2, treatment T8 exhibited the highest positive score, indicating a strong association with CC as the primary contributing variable. However, this extreme position suggests a tendency toward physiological specialization rather than balanced plant performance. In contrast, treatments with more moderate PC2 scores, such as T1 and T4, reflected a more balanced relationship between chlorophyll content and biomass-related traits. Meanwhile, treatments such as T5 and T9, which showed negative PC2 scores, were more closely associated with biomass accumulation but less with chlorophyll content. This pattern indicates a trade-off between physiological efficiency and structural growth. This suggests that optimal plant performance may depend on balanced trait expression rather than extreme specialization of a single physiological parameter.

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Table 7: The main components of PC1 and PC2 based on various concentrations of liquid organic fertilizer combined with AB Mix

Treatments	PC1	PC2
T_1	-3.319	0.670
T_2	-6.555	-1.026
T_3	4.803	1.466
T_4	-1.858	0.554
T_5	3.322	-1.492
T_6	2.844	-0.593
T_7	-2.545	-0.164
T_8	0.009	1.889
T_9	3.299	-1.304

The PCA results indicate that LOF combined with AB Mix influences plant performance through two distinct response strategies: (i) biomass-driven growth responses captured by PC1, and (ii) physiological efficiency responses related to chlorophyll activity captured by PC2. Nutrient management affects not only affects plant productivity but also regulates the balance between structural development and physiological performance in floating hydroponic system.

3.5 Pearson's Correlation

Pearson's correlation analysis was conducted to evaluate the strength and direction of linear relationships among growth and physiological parameters (Schober et al., 2018). The results showed that most morphological and biomass-related traits were positively and significantly correlated with each other, indicating a strong interdependence among plant growth variables (Fig. 8). In contrast, CC exhibited weak and mostly non-significant correlations with these traits, suggesting a different response pattern compared with structural growth parameters. Strong positive correlations were observed among the key growth variables, particularly between PH6WAT, NL6WAT, FCW, and TFW (r>0.90; P<0.001). These relationships indicate that increases in vegetative growth traits are closely associated with biomass accumulation, reflecting coordinated plant development under the applied treatment.

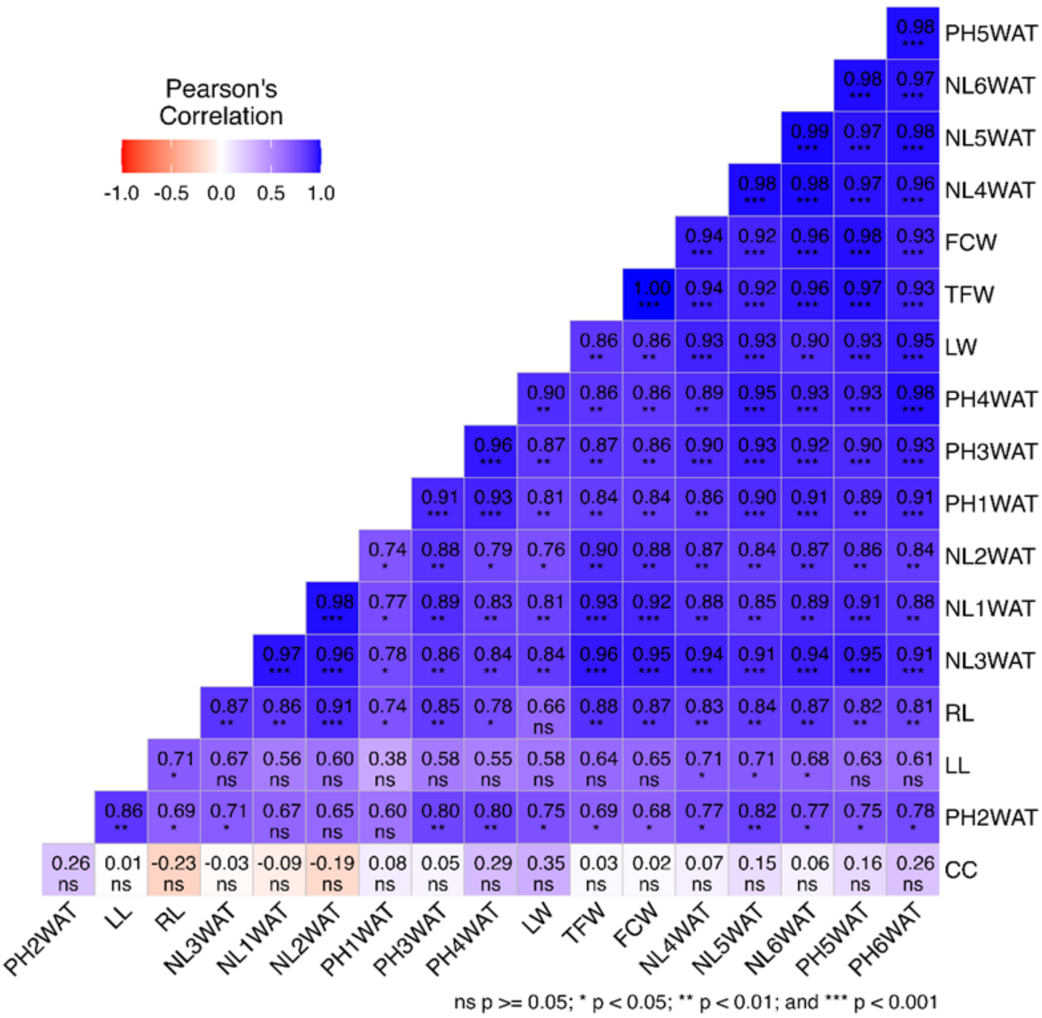

Fig. 8: Pearson correlation between growth and yield variables of Brazilian spinach at different concentrations of liquid organic fertilizer combined with AB Mix.

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RL also showed significant positive correlations with several growth traits, including plant height, leaf number, and biomass variables, suggesting its important role in supporting nutrient uptake and productivity. In a floating hydroponics system, efficient nutrient absorption largely depends on root development and root-solution contact. Therefore, the positive relationship between RL and biomass variables highlights the importance of root morphology in supporting nutrient uptake efficiency and plant growth. However, its correlation with chlorophyll content was weak and negative, further indicating a divergence between physiological and structural responses. CC was weakly correlated and showed non-significant correlation with most growth and biomass parameters. This suggests that chlorophyll accumulation may not be directly linked to increases in plant size or biomass under the tested conditions. The lack of a strong association between CC and growth traits indicates a potential physiological trade-off, in which improvements in photosynthetic pigment concentration do not necessarily translate into enhanced structural growth. The correlation analysis indicates that plant growth is primarily driven by tightly linked morphological and biomass traits, whereas chlorophyll content behaves more independently, reinforcing the distinction between structural growth and physiological efficiency observed in the PCA results.

This study has several limitations. The EC and pH of the nutrient solution were not directly measured, limiting the interpretation of nutrient availability and ionic balance in the hydroponic system. Environmental data were obtained from a nearby meteorological station rather than on-site measurements, which may not fully represent local microclimatic conditions. In addition, the microbial composition of the LOF was not quantified, restricting interpretation of plant–microbe interactions. Future studies should include more comprehensive physiological assessments, such as photosynthetic rate, stomatal conductance, nutrient uptake efficiency, and antioxidant activity, to better elucidate plant responses to combined organic and inorganic fertilization

4. CONCLUSION

Treatment T2 (3 mL POC+1000 ppm AB Mix) demonstrated the best growth and yield performance in Brazilian spinach, as indicated by a 0.36–10.46-fold increase in fresh consumption weight (FCW) compared with other treatments. PCA revealed that PC1 and PC2 explained 89.45% of the total variability in the data, confirming that plant performance was primarily driven by growth- and physiology-related traits. Pearson's correlation analysis further revealed strong positive and significant relationships among most growth and yield variables, whereas chlorophyll content (CC) showed weak, non-significant associations. Optimal nutrient management through the combination of liquid organic fertilizer and AB Mix enhances plant productivity primarily by promoting coordinated vegetative growth and biomass accumulation, while physiological traits such as chlorophyll content may respond independently. The results highlight the importance of a balanced nutrient supply in optimizing both growth performance and physiological responses in Brazilian spinach cultivation.

Declarations

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Author's Contributions: M. Izhar: Conceptualization, Experimental Design, Data collection, Multivariate analysis, and Manuscript drafting. M. Aulia Rahman: Conceptualization, Data collection, and Plant maintenance throughout the experimental period. Dela Arinda: Statistical analysis and Manuscript drafting. Safrizal Safrizal: Statistical analysis and Manuscript drafting.

Generative AI Statements: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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