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The Effect of Three Different Concentrations of a Bio-Stimulant on Lettuce Yield and Quality Variation in NFT System

Edouard Tabet[1], Maria Hosry[1], Suzy Rouphael[1], Dalida Darazy[1], Khaled El Omari[2,3] & Joe Sakr[4]

[1] Faculty of Agriculture, Department of Plant Production, Lebanese University, Dekwaneh, Lebanon

[2] Quality Control Center Laboratories at the Chamber of Commerce, Industry and Agriculture of Tripoli and North Lebanon, Tripoli 1300, Lebanon

[3] Laboratoire Microbiologie Santé et Environnement (LMSE), Doctoral School of Sciences and Technology, Faculty of Public Health, Lebanese University, Lebanon

[4] Van Iperen International B V, WESTMAAS, Smidsweg 24-3260 AH OUD-BEIJERLAND, Netherlands

Correspondence: Edouard Tabet, Faculty of Agriculture, Department of Plant Production, Lebanese University, Dekwaneh, Lebanon. Tel: 961-365-5178. E-mail: Edouard.tabet@ul.edu.lb

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Abstract

This study examines the impact of three different doses (D2: 0.675 mL/week; D3: 3.375 mL/week; D4: 13.5 mL/week) of a bio-stimulant (P4P-Vita), provided by Van Iperen, on lettuce yield and quality variation in a Nutrient Film Technique (NFT) system. The study compared these doses to a control group (D1) and evaluated the most effective dose for maximizing yield and quality. Parameters such as fresh weight, leaf number, leaf size (length and width), and root system volume were measured on day 0, day of harvest to assess productivity. Additionally, weight loss, Brix degree, and pH were measured on post-harvest days: (0,4,7 and 14) during refrigerated storage to assess quality variation. Results indicate that the bio-stimulant positively influenced lettuce growth and storage. The moderate dose (D2) yielded the best results, promoting higher fresh weight, longer and wider leaves, a more developed root system, reduced weight loss, longer shelf life and improved taste compared to other treatments.

Keywords: lettuce locarno, bio-stimulant, NFT, productivity, quality variation

1. Introduction

Lettuce (*Lactuca sativa* L.), a versatile leafy green, cultivated globally across diverse climates. Global production, particularly in countries like China and the United States, underscores its significance in human diets (Shatilov *et al.,* 2019). The European Union also contributes significantly to global supply with Spain as a leading producer (CTIFL, 2022). In Lebanon lettuce is particularly popular in greenhouse cultivation (FAOSTAT, 2017). However, increasing global demand for food coupled with climate change poses challenges to agricultural productivity and food security (Al-Karaki *et al.,* 2023). Soilless cultivation systems like Nutrient Film Technique (NFT) offer promising solutions for sustainable crop production (Veronique, 2021; Kumar *et al.,* 2023). By optimizing nutrient solutions, these systems can enhance both yield and quality (Yaseen and Takacs-Hajos, 2022). Bio-stimulants derived from natural sources have gained increasing attention as environmentally friendly tools for improving plant growth and yield (Colla *et al.,* 2019; Desoky *et al.,* 2021; Zhang *et al.,* 2024). They enhance nutrient uptake, stress tolerance, and root development (Colla *et al.,* 2017; Muhammad *et al.,* 2023). The global bio-stimulants market was valued at $2.19 billion in 2018 and is projected to grow significantly, with a compound annual growth rate of 12.5% from 2019 to 2024. Europe currently holds the largest market share for bio-stimulants (Albrecht, 2019). The objectives of our study are to investigate the impact of three different doses of a bio-stimulant on lettuce yield and quality in an NFT system, to identify the ideal concentration of it, and to evaluate the efficiency and sustainability of using this bio-stimulant in order to enhance both lettuce yield and its quality on post-harvest days in refrigerated storage.

2. Material and Methods

2.1 Work Description

The experiment was conducted from April 1st and 25th, 2024, at a lettuce farm in Okaibeh, Keserwan, Lebanon. The greenhouse covered an area 25 square meters (5m x 5m), with three replications of NFT systems each containing four rows of gutters with a slope of approximately 1%. The "Locarno" variety of Lolo type lettuce, and loose-leaf lettuce was cultivated under uniform conditions across all treatments. All conditions affecting the cultivation of Locarno lettuce were the same. The four tanks were filled from a 1000 mL mother tank where the fertigation recipe was prepared at the beginning of our experiment. The irrigation system pump was activated for 12 hours daily, from 6 a.m. till 6 p.m. Each 30 minutes interval, included a 2 minutes pumping period.

2.2 Experimental Design

A Randomized Complete Block Design (RCBD) was employed for comparing the effects of three different doses of the bio-stimulant (P4P-Vita) against a control treatment. This design involved in three replications (R1, R2, and R3) for each treatment as shown in the figure 1.

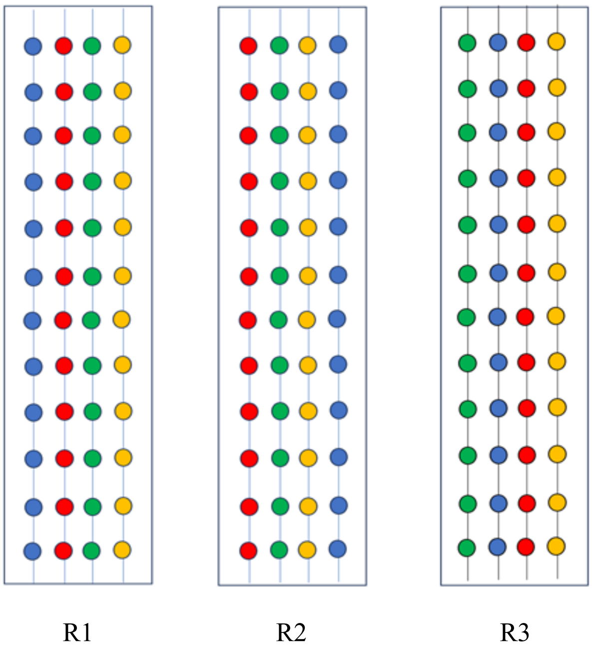

Figure 1. Randomized Complete Block Design of the applied experiment with three Blocks (R1, R2 and R3) and four Treatments of used different consecutive doses (1, 2, and 3) of the P4P-Vita bio-stimulant (D2 in yellow, D3 in green and D4 in red) and the control treatment with no bio-stimulant D1 (in blue).

2.3 Growing lettuce in NFT

Each tank used in the experiment contained the same fertilization recipe but differed in bio-stimulant dosage. A total of 144 lettuce plants were cultivated across three units.

2.4 Fertigation

The four tanks were filled from a 1000 mL mother tank, where the nutrient solution was prepared at the start of the experiment and acid was added to prevent clogging (Wang *et al.*, 2022). The nutrient solution was prepared at the start using a recipe based on farm recommendations. The exact fertigation program and types of applied fertilizers are shown in table 1.

Table 1. Fertigation recipe of lettuce in NFT system.

Fertilizers	Formulation	Total grams needed per 4 weeks
Calcium-Magnesium	13% N, 6% Mg, 13% Ca	1000
Potassium nitrate	13/00/46	300
Iron	6% Chelated EDDHA	50
Potassium sulfate	00/00/51	150
Mono potassium phosphate	00/52/34	150
Trace elements	CU, Fe, Mn, Zn, MgO, Na, Mo, As, Cd, Cr, Pb, Hg, Cl	50

2.5 pH and EC Measurement

The pH and electrical conductivity (EC) of the four tanks were measured every two days using a pH/EC meter combined in one simple device (Bluelab Combo Meter), in order to control them as best as possible to ensure the best pH range for maximum nutrient absorption. The pH was maintained between 5.5 and 6.8 while electrical conductivity ranged from 490 to 620 ppm (Dharti *et al.,* 2021). The device was calibrated weekly using buffer solutions.

2.6 Bio-Stimulant Manipulation and Composition

Doses were administered weekly using a syringe. The doses differ from tank to tank and are shown in table 2.

Table 2. The different bio-stimulant doses according to the tanks.

Tank	BS Recommended Dose in L/Ha	Dose in L/ 36 lettuce	Dose in L/ Lettuce	Dose/week in mL
D1	0	0	0	0
D2	6	0.0027	0.000075	0.675
D3	30	0.0135	0.000375	3.375
D4	120	0.054	0.0015	13.5

The table provides details on the application of recommended bio-stimulant (BS) doses for four different tanks, labeled D1, D2, D3, and D4. It includes information on the recommended dose per hectare (L/Ha) equivalent to dose applied to 8000 lettuces, the corresponding dose for 36 lettuce plants, the dose per individual lettuce plant, and the weekly dose in milliliters (mL). Tank D1: No bio-stimulant is applied in this tank. Tank D2: The recommended dose for this tank is 6 L/Ha. Tank D3: In this case, the recommended bio-stimulant dose is 30 L/Ha. For 36 lettuce plants. Tank D4: The highest recommended dose is 120 L/Ha. This table highlights the increasing bio-stimulant doses from D1 to D4, with the amount applied to both individual lettuce plants and the total for 36 lettuce plants increasing in a stepwise fashion. Additionally, the weekly dose applied to each tank also rises correspondingly, which could impact the growth and development of the lettuce plants. P4P-Vita bio-stimulant, is a 100% natural dark red color liquid that will optimize water supply ensuring better performance throughout the season, allowing for higher yield and crop quality. Having an organic certification approved by ECOCERT INPUTS, the composition of this product is shown in the table 3.

Table 3. P4P-Vita's composition. **(%w/w:** % weight per weight)

Components	(%w/w)
Potassium Oxide	1.2
Flavonoids	1
Organic Acids	20
Organic Matter	43
Dry matter	54

2.7 Harvesting and Sampling

Lettuces were harvested after four weeks at 8 a.m., with samples transported under controlled conditions for analysis. From the 144 samples, 36 plants were selected randomly for productivity analysis (9 from each treatment) on the day of harvest and were stored at 5°C and 85-90% relative humidity (França *et al.,* 2018).

2.8 Measured Parameters

Parameters were divided into productivity measurements at harvest and quality variation during post-harvest storage.

2.8.1 Productivity Measurements of Lettuce

The productivity parameters were measured on day 0 corresponding to the day of harvest for all four treatments.

Fresh weight

On day 0, immediately after harvest, the fresh weight of the aerial part of the lettuce was separated from the root volume of each plant by cross-sectioning of the plant crown and weighing it on a precision balance (AE ADAM, PGW 603e, Max 600g d=0.001g).

Number of leaves

On day 0, right after harvest, the number of leaves of lettuce was manually counted.

Leaf length and width of the 5 outer leaves

On day 0, immediately after the harvest, the length and width of the 5 outer leaves were measured using a measuring tape, from the two largest ends of each leaf, vertically and horizontally.

Root system volume

On the day of harvest, the root system volume was measured using the water displacement method. The root system was rinsed and then immersed in a 16 Oz Philips cylinder (with a known volume of 44.18 cm^3), and the water displacement was measured. The root volume was calculated using the formula:

Root system volume (cm^3) = cylinder air (cm^2) x water displacement (cm)

2.8.2 Quality Variation of Lettuce

During the post-harvest life of lettuce, the quality parameters were measured at the laboratories on day 0; 4; 7 and 14 respectively.

Weight loss

The weight loss (%) was calculated using the formula:

$$\text{Weight loss (\%)} = \frac{Initial\ weight\ (g) - Final\ weight\ (g)}{Initial\ fresh\ weight\ (g)}\ x\ 100$$

Total soluble solids or Brix degree:

Measurements were conducted using a refractometer (BOECO Germany, PC-324)**,** at room temperature (23-25°C) in direct sunlight (AOAC, 2002).

pH:

Measurements were conducted using a pH meter (EUTECH instruments, pH 700). Lettuce leaves were blended with distilled water to create a homogeneous mixture.

2.9 Statistical Analysis

Data analysis used General Linear Model (GLM) and SIGMASTAT software with One-way ANOVA for productivity parameters: fresh weight, number of leaves, leaf length, leaf width, and root system volume. And Two-way ANOVA for quality parameters. Duncan test was operated to identify the significant differences between the means whenever it was shown at 0.05% in a descending way.

3. Results and Discussion

3.1 Productivity Measurements

The influence of bio-stimulant doses on fresh mass is summarized below:

Fresh mass of the aerial part

The dose effect responses of the different bio-stimulant doses on the fresh mass of the aerial part of each treatment in every replication R of the lettuces on the day of harvest appeared in Table 4.

Table 4. The dose effect responses of the different bio-stimulant concentrations on the fresh mass of the aerial part of each treatment in every replication R, of lettuce on the day of harvest

		Fresh aerial part mass (g)				p-value
		D1	D2	D3	D4	
Day 0	R1	187	245.2	197.5	186	
	R2	150.6	231.9	178.5	181.3	
	R3	120	171	182.8	166. 1	
	Mean	129.6 ^{b}	216.9 ^{a}	188 ^{a}	162.2 ^{b}	0.011

*In columns and rows the numbers with similar exponent represent the absence of a significant difference at p>0.05.

The D2 and D3 groups showed a significant increase in fresh aerial mass compared to D1 and D4 (P<0.05). This suggests that the BS positively affected plant growth. This finding aligns with Ertani *et al.,* (2018) and Chaski *et al.,* (2022), who also reported increased fresh weight in lettuce treated with BS. This increase may be attributed to the bio-stimulant's ability to mitigate stress, optimize water use efficiency, and enhance photosynthesis. Furthermore, the fresh aerial mass of the D2 treatment was clearly the highest among all treatments with a value of 216.9g compared to the other groups D3, D4 and D1 with values of 188g, 162.2g and 129.6g respectively. This suggests that a moderate BS dose (D2) optimized plant growth, while a higher dose (D4) had a similar effect to the control (D1). Indeed, in our case, an increase in fresh weight by 67.35% was observed in D2 when compared to the control and in parallel an increase of 65% in fresh weight was reported with another bio-stimulant when tested by Bulgari *et al.,* in 2019. In addition, many studies revealed that lower bio-stimulant doses often yielded better fresh aerial mass results especially that fresh weight is a crucial factor in the commercial value of lettuce. (Kunicki *et al.*, 2012; Bulgari *et al.,* 2019; Chebil *et al.,* 2019; Santos *et al.,* 2009).

Number of leaves

The dose response effects obtained with different dilutions of the bio-stimulant on the number of leaves of lettuce of each treatment in every replication R, on the day of harvest appear in table 5.

Table 5. The dose response effects obtained with the different bio-stimulant dilutions on the number of leaves of lettuce of each treatment in every replication R, on the day of harvest

		Number of leaves				p-value
		D1	D2	D3	D4	
Day 0	R1	21	25.3	23.3	23.3	
	R2	24.7	23.3	22.7	24	
	R3	20.7	21.3	24.3	24.3	
	Mean	22	23	23	24	0.011

*In columns and rows the numbers with similar exponent represent the absence of a significant difference at p>0.05.

D4 exhibited the highest average number but showed no significant difference from other treatments with values of 23, 23 and 22 respectively, with a slight difference (P > 0.05). This suggests that the bio-stimulant had a minimal impact on leaf number, contrary to the findings of Francesca *et al.,* (2020), who reported an increase in fruit and leaf number in tomato plants treated with a bio-stimulant. This suggests that while bio-stimulants can positively impact various plant development traits, their effect on leaf number may be influenced by specific environmental conditions, bio-stimulant formulations or targeted plants. Chaski *et al.,* (2022) demonstrated an increase in lettuce leaf number with a silica-based bio-stimulant. This aligns with Khan *et al.,* (2009), who reported that silica can promote cell division and elongation by altering plant water relations. While there was no statistically significant difference between treatments, the bio-stimulant-treated plants, particularly D4, showed a slight increase (9%) in leaf number compared to the control. This suggests that higher bio-stimulant concentrations may positively influence leaf number, contrary to the findings of Chebil *et al.,* (2019), who reported better results with lower concentrations. According to Araujo *et al.,* (2016), a higher leaf number can lead to increased leaf area, fresh weight, and yield. Even though, there was no significant difference between treatments, the bio-stimulant-treated plants, especially D4, showed a slight increase in leaf number. This suggests that the BS may have a positive impact on leaf number, potentially leading to increased plant growth and yield.

Leaf length and width

The effects of different doses of the bio-stimulant on leaf length of each treatment in every replication R, on the average length and width of 5 outer lettuce leaves are shown in table 6.

Table 6. Effects of the different bio-stimulant concentrations of each treatment in every replication R, on the average length of 5 outer lettuce leaves on the day of harvest

		Average length of the 5 outer lettuce leaves (cm)				p-value
		D1	D2	D3	D4	
Day 0	R1	12.1	16.3	15.93	14.73	
	R2	14.2	15.87	16.73	15.6	
	R3	14.2	16.93	16.3	17.3	
	Mean	13.5 ^{b}	16.37 ^{a}	16.32 ^{a}	15.88 ^{a}	0.018

*In columns and rows the numbers with similar exponent represent the absence of a significant difference at p>0.05.

With values of 16.37, 16.32, 15.88, and 13.5 correspondingly, the average length of the five outer lettuce leaves in groups D2, D3, and D4 was significantly different from that of the D1 group (P<0.05). It is evident that plants that have been given the bio-stimulant are far more developed than those that have not, demonstrating that the application of bio-stimulant has a beneficial impact on plant physiology, including leaf length. This aligns with Gu *et al.,* (2014) and Albrecht, (2019). This could be explained by the bio-stimulant ability to enhance plant tolerance to abiotic stress and water use efficiency, promoting photosynthesis and leaf growth. However, as Sibomana *et al.,* (2020) and *Monireh et al.,* (2022) suggest, the impact on leaf length can vary. And this could also be explained by the bio-stimulant's ability to enhance nutrient uptake, particularly potassium. Potassium deficiency can lead to poor leaf quality (Soltaniband *et al.,* 2022). The 5 outer leaves in D2 and D3 had approximately similar lengths, with D2 slightly longer but not significantly (P>0.05). This suggests that minimal bio-stimulant doses are optimal for leaf length, aligning with Kunicki *et al.,* (2010) and Chebil *et al.,* (2019). D2 treated lettuces showed a 21.25% increase in leaf length compared to the control. D2 treatment yielded the longest 5 outer leaves. Concerning the leaf width, the effects of variations in different doses of the bio-stimulant of each treatment in every replication R, on leaf width on the average width of 5 outer lettuce leaves results in the table below (Table 7).

Table 7. Dose response effects of the different bio-stimulant concentrations of each treatment in every replication R, on the average width of 5 outer lettuce leaves on the day of harvest

		Average width of the 5 outer lettuce leaves (cm)				p-value
		D1	D2	D3	D4	
Day 0	R1	15. 13	20.27	18.93	18.53	
	R2	16.2	19.53	19.93	17.93	
	R3	16. 13	19	18. 13	19.13	
	Mean	15.82 [b]	19.6 [a]	18.99 [a]	18.53 [a]	<0.001

*In columns and rows the numbers with similar exponent represent the absence of a significant difference at p>0.05.

The average width of the 5 outer leaves in D2, D3, and D4 was significantly higher (P<0.05) than D1, with values of 19.6, 18.99, 18.53, and 15.82 respectively. This indicates that the application of bio-stimulant has a positive impact on plant physiology, including leaf width. These findings are consistent with Abdelgalil *et al.,* (2021), who also observed a positive effect of bio-stimulants on growth parameters such as leaf width. This improvement can be attributed to enhanced water use efficiency y (Sibomana *et al.,* 2020) and increased nutrient uptake, particularly potassium (Soltaniband *et al.,* 2022). D2 and D3 treatments showed the highest leaf widths, with D2 being slightly wider but not significantly different (P>0.05). This suggests that lower doses of bio-stimulant may be optimal for increasing leaf width, which is in line with the findings of Kunicki *et al.,* (2010) and Chebil *et al.,* (2019). Leaf length and width are important criteria in vegetable selection (Santos *et al.,* 2009). Lettuces treated with D2 exhibited a 23.9% increase in leaf width compared to the control. The D2 treatment resulted in the widest 5 outer leaves.

Root system volume

The table 8 presents the effects of different doses of the bio-stimulant on the root system volume of each treatment in every replication R.

Table 8. Effects of different bio-stimulant doses of each treatment in every replication R, on the root system volume, on the day of harvest.

		Root system volume (cm^3)				p-value
		D1	D2	D3	D4	
Day 0	R1	1.43	2.17	1.77	1.9	
	R2	1.1	2	1.87	1.83	
	R3	1.37	1.53	1.47	1.4	
	Mean	1.3	1.9	1.7	1.71	0.092

The average root system volume of the treatments did not show a significant difference between all four groups (P>0.05) but a slight superiority was noticed in groups D2, D3 and D4 comparing to D1 with values of 1.9, 1.7, 1.71, and 1.3 respectively. Bio-stimulants likely positively impact root system volume, and this may be due to their natural substances that mimic or supplement plant hormones. These hormones promote root hair development and density, enhancing nutrient absorption (Gu *et al.,* 2014; Colla *et al.,* 2017; Monireh *et al.,* 2022; Li *et al.,* 2023). A robust root system is crucial for lettuce quality, which is a key factor in market selection (Santos *et al.,* 2009). While not statistically significant, D2 exhibited the highest root system volume among the bio-stimulant treatments. This suggests that even minimal concentrations of the Bio-stimulants can yield positive results, aligning with Chebil *et al.,* (2019), who found optimal results with lower bio-stimulant concentrations. D2-treated lettuces exhibited a 46.15% increase in root system volume compared to the control. The D2 treatment yielded the largest root systems.

3.2 Quality Variation of Lettuce

This phase describes the influence of the different bio-stimulant doses on the quality variations of lettuce during its post-harvest lifespan.

Weight Loss

The effects of different doses of the bio-stimulant on the total percentage of weight loss of lettuces during their post-harvest lifespan in refrigerated storage, appear in table 9.

Table 9. The effects of different doses of the bio-stimulant on the total percentage weight loss of lettuces during its post-harvest lifespan, on refrigerated storage.

	Weight loss (%)				
Number of days	D1	D2	D3	D4	P-value
Day 0	0	0	0	0	0
Day 4	4.739	1.235	2.670	2.836	0.582
Day 7	2.316	1.271	2.015	5.147	0.471
Day 14	0.488	1.296	1.528	2.145	0.141
P-value	0.214	0.993	0.855	0.571	

While there were no statistically significant differences in total weight loss between treatments (P >0.05), it's evident that D1 and D4 exhibited more variable weight loss patterns during post-harvest storage compared to the more stable trends observed in D2 and D3, particularly D2. Weight loss, a common post-harvest phenomenon, is associated with water loss and increased respiration rates (Chitarra and Chitarra, 2005; Martinez *et al.,* 2007). This leads to wilting, wrinkling, and accelerated deterioration (Schvambach *et al.,* 2020). Many factors influence water loss in vegetables, but the most significant one is the percentage of moisture lost, which, as noted by Manolopoulou and Varzakas (2011), causes a loss of freshness, look, and texture. Financial problems are directly linked to weight loss and typically lower the market value of vegetables by more than 5%; it is a very significant factor (Brown and Bourne, 2002). To optimize marketability, it's crucial to minimize post-harvest weight loss. D2 and D3 treatments, particularly D2, exhibited more stable weight loss patterns. This may be due to the BS ability to mitigate oxidative stress, even post-harvest, leading to reduced water loss and a more consistent weight. The moderate BS dose appears to be most effective in this regard. The best weight loss results were in favor of the use of a moderate amount of BS, especially the D2 treatment.

Total Soluble Solids or Brix Degree (°Brix)

The dose response effects of different doses of the bio-stimulant within the same day on total soluble solids (TSS) or Brix level during the post-harvest lifespan of lettuce, on refrigerated storage, appear in table 10.

Table 10. The dose response effects of different doses within the same day of the bio-stimulant on total soluble solids (TSS) or Brix degree during the post-harvest lifespan of lettuce, in refrigerated storage.

	Total Soluble Solids (TSS) - ° Brix				
Number of days	D1	D2	D3	D4	P-value
Day 0	1.27	0.97	1.26	1.03 [B]	0.792
Day 4	1.99	1.53	1.77	2.16 [A]	0.064
Day 7	2.12 [a]	1.46 [ab]	1.64 [ab]	1.94 [aA]	0.036*
Day 14	1.52	1.3	1.49	1.69 [A]	0.606
P-value	0.176	0.147	0.07	0.021*	

*In columns and rows the numbers with similar exponent represent the absence of a significant difference at p>0.05. Small letters represent similarities between doses within the days and capital letters are the similarities between the days within doses.

On day 7, the total soluble solids (TSS) of D2 and D3 treatments showed a significant difference (P<0.05) and were lower compared to D1 and D4 treatments, with values of 1.46, 1.64, 2.12, and 1.94 respectively. There was no significant difference between the treatments on days 0, 4, and 14 (P>0.05). This difference can be explained by the moderate amount of bio-stimulant used in the D2 and D3 treatments; during refrigerated storage, resulting in a moderate amount of organic acids present in them. In contrast, the high amount of bio-stimulant in the D4 treatment did not show any significant difference with the control group. According to Amorim *et al.,* (2010) the bitterness of lettuce is influenced by the ratio of TSS to organic acid content, and a balance between sweetness and acidity is required for a more pleasing flavor. Additionally, TSS is influenced by environmental factors such as light and temperature, as demonstrated by Andriolo *et al.,* (2005).

In lettuce the taste is primarily determined by bitterness, which is influenced by organic acids, lipids and phenols, and sweetness, which is determined by glucose, fructose, sucrose and fibers. Although lettuce is naturally low in sugar and rich in phenolic compounds, its TSS content is not considered a significant quality indicator. However, TSS is directly correlated with preserving a sweet taste over time (Martinez, 2010). TSS values decreased over time in all treatments, and which is consistent with Rashidi *et al.,* (2015). However, this contradicts Cândido *et al.,* (2015), who observed increased TSS with BS. This discrepancy may be due to the different lettuce variety used, highlighting the complex and not always positive impact of BS on post-harvest TSS. A significant difference was also noticed in D4 treatment, with the moderate value on day 0, and the highest on day 4, followed by a decline. Factors such as senescence, starch, and organic acid depletion may contribute to this reduction, as suggested by Kaewklin *et al.,* (2018). TSS peaked on day 4 for all treatments indicating mature lettuce. As noted by Balaguera-López *et al.,* (2016), TSS content often increases during fruit and vegetable ripening processes. However, over-maturity can lead to bitterness and reduced marketability (Alemu & Kim, 2024). Nevertheless, D2 treatment maintained the lowest TSS values throughout the refrigerated storage period. This could be attributed to a slower metabolic activity in the post-harvest days, resulting in higher quality after storage compared to the control. According to Shezi *et al.,* (2024), higher metabolic activity in leafy vegetables accelerates the loss of their quality. The best TSS results were observed in the D2 treatment.

pH

The effects of different bio-stimulant doses on the pH of lettuce during and after refrigerated storage are shown in table 11.

Table 11. The effects of different bio-stimulant doses on the pH of lettuce during and after refrigerated storage.

	pH				
Number of days	D1	D2	D3	D4	P-value
Day 0	5.97	5.97 ^{B}	5.99	5.99	0.955
Day 4	5.96	5.91 ^{B}	5.94	5.91	0.845
Day 7	6.04	6.14 ^{A}	6.03	5.99	0.161
Day 14	6.02 ^{a}	6.2 ^{aA}	5.93^{ab}	5.95 ^{ab}	0.043*
P-value	0.623	0.005*	0.464	0.389	

*In columns and rows the numbers with similar exponent represent the absence of a significant difference at p>0.05.

Small letters represent similarities between doses within the days and capital letters are the similarities between the days within doses.

D3 and D4 treatments exhibited significantly lower pH on day 14 ($P < 0.05$) compared to D1 and D2, with values of 5.93, 5.95, 6.02, and 6.20 respectively. Meanwhile there was no significant difference between the treatments on days 0, 4, and 7 ($P > 0.05$). This suggests that higher bio-stimulant doses may influence pH over time, possibly due to the BS's ability to help plants tolerate abiotic stresses, which can impact metabolic processes and pH. Ascorbic acid, the primary form of vitamin C in lettuce, constitutes 55-65% of the total vitamin C content (Patil *et al.,* 2017). Increased storage duration and respiration can contribute to ascorbic acid reduction, as reported by Sharma *et al.,* (2011). The acidity of ascorbic acid significantly impacts lettuce flavor (Aroucha *et al.,* 2015). As the total acid content decreases, pH increases, and vice versa, influencing the overall taste profile. The D2 treatment showed a significant difference in pH between days 0 and 4 compared to days 7 and 14 ($P < 0.05$). The highest pH values were observed on days 7 and 14, indicating a potential increase in sweetness during post-harvest storage. The D2 treatment exhibited lower ascorbic acid content and higher pH levels during post-harvest storage, potentially leading to a sweeter taste. This suggests that a minimal BS dose can improve taste, even post-harvest. However, these findings contradict Slavica *et al.,* (2016), who observed a pH decrease with BS application. The best results that give a sweeter taste after storage were in favor of the D2 treatment, after 7 days of refrigerated storage.

4. Conclusion and Perspectives

Our study demonstrates that regarding the phase of lettuce productivity, moderate doses of bio-stimulants can significantly enhance both yield and post-harvest quality in lettuce cultivated under NFT systems. The D2 treatment, led to a 67.35% increase in fresh mass, 21.25% longer leaves, 23.9% wider leaves, and 46.15% larger root systems compared to the control. While the number of leaves wasn't significantly different, the D4 treatment (highest dose consisting of 13.5mL/week) still yielded 9% more leaves. The best results of fresh aerial mass, leaf length, width, and root volume were all in favor of D2 treatment (lowest dose consisting of 0.675mL/week). This suggests that a moderate application of P4P-Vita has a positive impact on lettuce productivity, outperforming other treatment groups (D1, D3, and D4). In the post-harvest phase of lettuce shelf life in cold storage, P4P-Vita bio-stimulant significantly improved lettuce quality, especially at moderate doses. Lettuces treated with D2 experienced minimal weight loss and maintained relatively low TSS levels, indicating better quality during post-harvest refrigerated storage at 5°C and 85-90% relative humidity. Additionally, D2 treatment resulted in higher pH levels after 7 days of storage, suggesting a potential improvement in taste. These findings suggest that a moderate application of P4P-Vita especially D2 treatment can extend the shelf life of lettuce. In summary, we can conclude that D2 treatment was the best among all treatments, playing a positive role in the productivity and various quality aspects of lettuces grown in a hydroponic system. D2, at a most moderate and economical dose, yielded the best and higher results. These results suggest potential benefits for hydroponic lettuce production in similar agricultural contexts, warranting further field-scale validation. The effectiveness of bio-stimulants in enhancing crop quality and yield varies significantly based on the lettuce variety, crop species, and environmental conditions. Lettuce cultivars exhibit diverse physiological responses to bio-stimulants, influenced by traits like root structure and nutrient absorption capacity. These variations require careful cultivar selection to ensure reliable and applicable findings, as generalizing results across varieties can lead to inaccuracies. Additionally, bio-stimulants interact uniquely with different crops due to distinct physiological and biochemical pathways, highlighting the importance of crop-specific research. (Ertani *et al.,* 2021; Li *et al.,* 2022). Environmental factors, such as temperature, salinity, and stress conditions, further affect bio-stimulant performance, underscoring the complexity of their application (Hasanuzzaman *et al.*, 2021).

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