Effect of fulvic acid on the growth of hydroponic pea (*Pisum sativum* L.) microgreens

Efecto del ácido fúlvico en el crecimiento de microvegetales hidropónicos de guisante (*Pisum sativum* L.)

Efeito do ácido fúlvico no crescimento de microgreens de ervilha hidropônica (*Pisum sativum* L.)

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Abstract

Fulvic acid is a widely recognized biostimulant due to its benefits in traditional crops; however, its application in hydroponic systems, particularly in microgreen production, is not well documented. This study evaluated the effect of fulvic acid on the growth of hydroponic pea microgreens (*Pisum sativum* L.). The experimental design was completely randomized and consisted of four treatments (n=5): nutrient solution (NS), fulvic acid solution 0.01 % (FA), NS + FA, and water (control). After 12 days, growth and biochemical parameters were measured. The results showed that NS and NS+FA treatments significantly increased stem length (7.73 cm and 7.28 cm), fresh weight (0.613 g and 0.618 g), and yield (6.15 $kg.m^{-2}$) compared to the FA treatment or control. The FA treatment increased stem diameter (2.38 mm) but did not significantly increase biomass. Biochemical analysis showed that FA and control had higher nitrate content, while NS and NS+FA reduced nitrate accumulation. Antioxidant capacity, chlorophyll content, and color index were similar among treatments. However, the pH increased with the application of fulvic acid. Fulvic acid alone moderately improved growth but was less effective than the nutrient solution. The combination of fulvic acid with a complete nutrient solution did not produce additive effects, highlighting the importance of balanced nutrition in hydroponic microgreen production.

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Resumen

El ácido fúlvico es un bioestimulante reconocido por sus beneficios en cultivos tradicionales; sin embargo, su aplicación en sistemas hidropónicos, particularmente en la producción de microvegetales, no está bien documentada. Este estudio evaluó el efecto del ácido fúlvico sobre el crecimiento de microvegetales de chícharo (*Pisum sativum* L.) cultivados en hidroponía. El diseño experimental fue completamente al azar y consistió en cuatro tratamientos (n=5): solución nutritiva (SN), solución de ácido fúlvico al 0,01 % (AF), SN+AF y agua (control). Después de 12 días, se midieron parámetros de crecimiento y bioquímicos. Los resultados mostraron que los tratamientos SN y SN+AF incrementaron significativamente la longitud del tallo (7,73 cm y 7,28 cm), el peso fresco (0,613 g y 0,618 g) y el rendimiento (6,15 $kg.m^{-2}$) en comparación con AF o el control. El tratamiento AF incrementó el díametro de tallo (2,38 mm), pero no aumentó significativamente la biomasa. El análisis bioquímico mostró que el AF y el control presentaron un mayor contenido de nitratos, mientras que los tratamientos SN y SN+AF redujeron la acumulación de estos. La capacidad antioxidante, el contenido de clorofila y el índice de color fueron similares entre tratamientos. Sin embargo, el pH aumentó con la aplicación de ácido fúlvico. El ácido fúlvico mejoró moderadamente el crecimiento, pero fue menos efectivo que la solución nutritiva. La combinación de ácido fúlvico con una solución nutritiva completa no produjo efectos aditivos, lo que resalta la importancia de una nutrición equilibrada en la producción hidropónica de microvegetales.

Palabras clave: sustancia húmica, leguminosa, cultivo sin suelo, bioestimulantes.

Resumo

O ácido fúlvico é um bioestimulante reconhecido por seus benefícios em culturas tradicionais; Entretanto, sua aplicação em sistemas hidropônicos, particularmente na produção de microgreens, não está bem documentada. Este estudo avaliou o efeito do ácido fúlvico no crescimento de microgreens de ervilha (*Pisum sativum* L.) cultivados hidroponicamente. O delineamento experimental foi inteiramente casualizado e consistiu em quatro tratamentos (n=5): solução nutritiva (SN), solução de ácido fúlvico 0,01 % (AF), SN+AF e água (controle). Após 12 dias, foram medidos os parâmetros de crescimento e bioquímicos. Os resultados mostraram que os tratamentos SN e SN+AF aumentaram significativamente o comprimento do caule (7,73 cm e 7,28 cm), o peso fresco (0,613 g e 0,618 g) e o rendimento (6,15 $kg.m^{-2}$) em comparação ao AF ou ao controle. O tratamento AF aumentou o diâmetro do caule (2,38 mm), mas não aumentou significativamente a biomassa. A análise bioquímica mostrou que AF e controle apresentaram maior teor de nitrato, enquanto os tratamentos SN e SN+AF reduziram o acúmulo de nitrato. A capacidade antioxidante, o teor de clorofila e o índice de cor foram semelhantes entre os tratamentos. Entretanto, o pH aumentou com a aplicação de ácido fúlvico. O ácido fúlvico melhorou moderadamente o crescimento, mas foi menos eficaz que a solução nutritiva. A combinação de ácido fúlvico com uma solução nutritiva completa não produziu efeitos aditivos, destacando a importância da nutrição balanceada na produção de microgreens hidropônicos.

Palavras-chave: substância húmica, leguminosa, cultivo sem solo, bioestimulantes

Introduction

The production of microgreens has become increasingly important in recent years due to their high nutritional content, short cultivation cycle, and growing demand in gourmet and functional food markets (Choe *et al*., 2018; Rouphael *et al*., 2021). These small vegetables harvested at early developmental stages are rich in vitamins, minerals, antioxidants, and bioactive compounds, making them attractive to consumers interested in healthy and sustainable foods (Sharma *et al*., 2022; Xiao *et al*., 2012). Among the species grown as microgreens, pea (*Pisum Sativum* L.) stands out for its nutritional profile, high protein content, and culinary versatility, justifying the exploration of strategies to optimize its growth and quality in hydroponic systems (Xiao *et al*., 2019; Ebert, 2022).

Biostimulants, such as fulvic acid, have emerged as an innovative practice to enhance agricultural production (Canellas *et al*., 2015; Bell *et al*., 2022). Fulvic acids, soluble fractions of organic matter, possess unique properties that positively affect plant metabolism by enhancing nutrient uptake, improving photosynthetic efficiency, and increasing tolerance to abiotic stress (Canellas *et al*., 2015; Hasanuzzaman *et al*., 2021; Mosaad *et al*., 2024). Although their efficacy has been extensively studied in traditional crops, their application in microgreens, especially in hydroponic systems, requires further research to fully understand their effects on growth and quality parameters (Drobek *et al*., 2019; Sharma *et al*., 2022). In the case of pea microgreens, harvesting is typically recommended between 10 and 14 days after germination, when the shoots reach a height of 7 to 10 cm, and exhibit an intense green color, which are considered key quality indices for market acceptance (Tallei *et al*., 2024). These morphological characteristics are critical in determining harvest timing and consumer preference, as they are directly related to visual appeal, texture, and nutritional content. Therefore, optimization of growth conditions and inputs such as biostimulants is essential to meet quality standards and improve yield consistency in commercial production.

Previous studies have shown that biostimulants can induce significant changes in plant development through physiological and biochemical mechanisms, such as increased chlorophyll production, enzymatic activity, the accumulation of bioactive compounds (Graziani *et al.*, 2022; Sharma *et al*., 2022; Anastacio-Angel *et al*., 2024).

Howeverplant response to fulvic acid can vary depending on the cultivated species and production system conditions (Zhang *et al*., 2021). In this context, it is important to evaluate how fulvic acid affects the growth and yield of pea microgreens in hydroponic systems, considering the growing need for sustainable and efficient agricultural practices.

This aim of this study was to evaluate the effect of applied fulvic acid, on the growth, biochemical composition, and yield of pea tendril microgreens grown hydroponically, in order to determine its potential as a biostimulant for sustainable microgreen production. This work will contribute to the understanding of the potential benefits of fulvic acid in sustainable production systems and provide a scientific basis for its application in urban agriculture and functional food production.

Materials and methods

Localization experiment

The experiment was conducted at the Applied Microbiology, Plant Pathology, and Post-harvest Physiology Laboratory of the Autonomous University of Chihuahua, Chihuahua, MX (28°39'24'' N, 106°05'12'' W) during November and December 2024.

Plant and fulvic acid material

Organic tendril pea microgreens (*Pisum sativum* L.) seeds (Johnny's Selected Seeds, USA) were used for the test. An aqueous solution of fulvic acids derived from leonardite (K-Tionic®, Arysta LifeSience México, MX) containing 25 % organic fulvic acid.

Experimental setup

Pea seeds were washed twice with tap water, soaked in a 0.12 % H_2O_2 solution for 6 h, drained and placed directly in polystyrene trays (13x13x8 cm) (S-22911, Uline México, MX) containing a plastic mesh (1.8 mm) (B0BXKV98MF, Spkaodngo, USA) 2 cm above the bottom, without substrate, at 1 seed per cm^2 (Verlinden, 2020), and placed in the dark at 24 °C. After germination, two-day-old tendril pea plants were transferred to a growth chamber with a photoperiod of 16 h light/8 h dark at 28 °C/ 18 °C, 3,500 lux LED light (Goodwill az-energy®, 20460, MX), and 70 ± 2 % relative humidity. The microgreens were watered every two days with different solutions: A) Steiner nutrient solution (NS) composed of (ppm): 126 NO_3^-, 42 NH_4^+, 31 PO_4^{3-}, 274 K^+, 181 Ca^{2+}, 48.6 Mg^{2+}, 112 SO_4^{2-}, 1.3 Fe-EDTA, 0.8 Mn-EDTA, 0.3 Zn-EDTA, 0.06 Cu-EDTA, 0.4 B, and 0.06 Mo (pH 6.0, EC 2.3 $mS.cm^{-1}$); B) Fulvic acid solution (0.01 %, pH 6.0, EC 0.45 $mS.cm^{-1}$), based on the dosage recommended in the technical data sheet of the product; C) NS + FA (pH 6.0, EC 2.5 $mS.cm^{-1}$); and D) destilled water.

Parameters evaluated

The growth and biochemical parameters of the microgreens were evaluated on day 12 after sowing.

Growth parameters

Ten seedlings from each replicate were cut at the collar region. Stem length and diameter were measured using a digital caliper (Starret®, EC799A-6/150, USA). Stipular leaf area was determined using ImageJ 1.46r software. The number of tendrils was recorded, and fresh weight (FW) and dry weight (DW) were measured using an analytical balance (XT-220A, Precisa Instruments®, Switzerland) after drying at 60 °C for 48 h, in a forced-air convection oven (SMO3, Shel Lab®, USA). The water content (WC) of the microgreen seedlings was determined using the following equation (Eq. 1):

$$WC\ (\%) = \left(\frac{FW\ (g) - DW(g)}{FW(g)}\right) * 100 \quad \text{(Eq. 1)}$$

Yield: The yield of pea microgreens was calculated based on a seeding density of 1 seed per cm^2, using the following equation (Eq. 2):

$$\text{Yield } (kg.m^2) = \text{Fresh weight of seedlings (kg) x seedlings per } m^2 \quad \text{(Eq. 2)}$$

Biochemical parameters

pH: 5 g of seedlings were macerated, and the pH was measured using a pH meter (Checher® pH Tester HI98103, Hanna Instruments, USA). Total soluble solids (TSS) were expressed in °Brix: A drop of microgreen juice was placed on a digital refractometer (Automatic Refractometer Smart-1, Japan).

Color index (CI): Color was measured using the CIE L*a*b* system with a digital colorimeter (Minolta Chroma Meter CR-310; Konica Minolta Optics, Japan). The CI was calculated with the following equation (Eq. 3):

$$CI = (1000 * a) / (L * b) \quad \text{(Eq. 3)}$$

Photosynthetic pigment content

A 0.1 g sample of fresh leaves was macerated with 4 mL of 80 % acetone (v/v) and centrifuged at 3,000 rpm for 5 min. The supernatant was measured at 663, 470, and 645 nm using a UV spectrophotometer (Model 60S Evolution, Thermo Scientific, USA) (Lichtenthaler & Wellburn, 1983). Pigment concentrations were calculated as follows (Eq 4, 5 and 6):

$$\text{Chlorophyll a } (mg.g^{-1}FW) = (12.21 \times A_{663} - 2.81 \times A_{645}) \times V/(1000 \times W) \quad \text{(Eq.4)}$$

$$\text{Chlorophyll b } (mg.g^{-1}FW) = (20.13 \times A_{645} - 5.03 \times A_{663}) \times V/(1000 \times W) \quad \text{(Eq.5)}$$

$$\text{Carotenoids } (mg.g^{-1}FW) = \left[\frac{(1000 \times A_{470} - 3.27 \times Chl_a - 104 \times Chl_b)}{229}\right] \times V/(1000 \times W) \quad \text{(Eq.6)}$$

Where: V= volume (mL) of 80 % acetone, W is the fresh weight (FW) of the sample (g).

Antioxidant activity

Fresh samples (10 g) were homogenized with 20 mL of 80 % ethanol and diluted to 100 mL with destilled water. The mixture was stirred for 10 min, filtered (Whatman No. 1), and 0.1 mL of the extract was mixed with 3.9 mL of 2,2-diphenyl^{-1}-picrylhydrazyl (DPPH; 0.025 $g.L^{-1}$) ethanolic solution. After 60 min in the dark at 25 °C, the absorbance was measured at 515 nm using a UV-vis spectrophotometer. The results were expressed as the percentage of DPPH radical inhibition, calculated using the following equation (Eq. 7) (Rodríguez-Roque *et al*., 2013):

$$\text{DPPH inhibition } (\%) = \left(\frac{C_{abs} - SM_{abs}}{C_{abs}}\right) * 100 \quad \text{(Eq.7)}$$

Where: C_{abs} =absorbance of the control, SM_{abs} = absorbance of the sample extract.

Nitrate content: A 1 g sample of fresh leaves was homogenized in 3 mL of distilled water, centrifuged at 4,000 rpm for 15 min, and 20 µL of the supernatant was mixed with 80 µL of 5 % sulfuric acid-salicylic acid and 3 mL of 1.5 N NaOH. After 10 min, the absorbance was measured at 410 nm using a UV-visible spectrophotometer, and the nitrate concentration was calculated using a KNO_3 standard curve (0, 1, 2.5, 5, 7.5, 10 mM; $R^2 = 0.995$) (Toscano *et al*., 2021).

Statistical analysis

The experiment was set up as a completely randomized design with four treatments: FA, NS + FA, NS, and purified water (control), each replicated five times. Growth and biochemical data were subjected to Shapiro-Wilk and Levene tests for normality and homoscedasticity. Depending on these results, data were analyzed by analysis of variance (ANOVA) with the Tukey test or non-parametric Kruskal-Wallis with Dunn test ($p<0.05$). A Principal Component Analysis (PCA) was performed on key variables to evaluate the influence of fulvic acid on pea microgreens, validated by Bartlett′s test ($p<0.01$) and Kaiser-Meyer-Olkin (KMO) measure (>0.60). Data analysis was performed with Jamovi software 2.5.2.0.

Results and discussion

Growth parameters

The application of different treatments significantly affected the growth of tendril pea microgreens (table 1).

Table 1. Growth parameters of tendril pea microgreens treated with fulvic acid cultivated in a hydroponic system for 12 days.

Parameters	Treatments				CV
	Control	FA	NS	NS + FA	
Stem length (cm)[2]	3.70[c]	4.65[b]	7.73[a]	7.28[a]	18.04
Stem diameter (mm)[1]	2.34[ab]	2.38[a]	2.17[c]	2.26[b]	7.53
Tendrils Number[2]	4.32[a]	3.76[b]	4.22[a]	4.36[a]	12.59
Leaf area stipulate (cm^2)[2]	3.08[c]	3.63[b]	5.40[a]	5.35[a]	18.11
Fresh shoot weight (g)[2]	0.225[c]	0.333[b]	0.613[a]	0.618[a]	17.84
Dry shoot weight(g)[2]	0.028[c]	0.040[b]	0.059[a]	0.061[a]	17.14
Water content (%)[2]	86.88[b]	87.48[b]	90.08[a]	89.84[a]	3.43

Different superscript letters in the same row indicate significant differences according to the Games-Howell test[1] or Dunn test[2] at the 0.05 level. Control= destillated water, FA= fulvic acid, NS= Nutrient solution, CV= coefficient of variation.

For stem length, the NS and NS+FA treatments showed statistically higher values (7.73 cm and 7.28 cm, respectively) compared to FA (4.65 cm) and control (3.70 cm). These results indicate that nutrient supplementation, with or without fulvic acid, enhances elongation, probably due to the increased availability of essential ions such as K^+ and NO_3^-, which are known to promote cell expansion and division (Sano *et al*., 2009). However, the addition of fulvic acid to the nutrient solution (NS+FA) did not further increase stem length compared to NS alone, suggesting no synergistic effect, which may be due to altered solubility or nutrient uptake interactions, as noted by Wang *et al*. (2022).

In terms of stem diameter, the FA treatment (2.38 mm) was statistically superior to NS (2.17 mm), while NS+FA (2.26 mm) showed intermediate values. This may reflect the gibberellic acid-like effects of fulvic acid that promote secondary growth (Pizzeghello *et al*., 2001), although the NS alone appeared to be less effective, possibly due to the prioritization of elongation over thickening in nitrogen-rich environments.

The number of tendrils was significantly reduced in FA compared to the other treatments. NS, NS+FA, and control showed no significant differences, with an average of 4.3 tendrils per plant. This suggests that fulvic acid alone may differentially affect morphogenetic patterns, possibly tangentially by modulating the auxin-cytokinin ratio (Muscolo *et al*., 2007). Stipular leaf area increased significantly in all treatments compared to the control, with the highest values in NS and NS+FA (5.40 and 5.35 cm^2 , respectively) and a moderate increase in FA (3.36 cm^2). These improvements could be related to enhanced nitrogen assimilation and carbon metabolism, as suggested by He *et al*. (2021), especially in nutrient-enriched systems.

For fresh and dry shoot weight, NS and NS+FA achieved the highest biomass values (0.613 g and 0.618 g fresh; 0.059 g and 0.061 g dry, respectively), significantly higher than FA and control. While FA alone improved biomass compared to control, its effect was inferior to NS-based treatments. These results suggest that the combination of macro-and micronutrients is more critical for biomass accumulation than fulvic acid alone, although FA may still contribute to early-stage development.

Water content was not significantly different among treatments, although NS and NS+FA presented slightly higher averages (~90 %) than FA and control (~87 %). This indicates that fulvic acid did not adversely affect water retention and that the high water content in nutrient-treated seedlings reflects better turgor and hydration under optimal mineral nutrition.

Overall, these results show that while fulvic acid alone improves some growth parameters compared to the control, the nutrient solution treatments (NS+FA) provide the most substantial benefits. The lack of additive effects in NS+FA may be related to chemical interactions that limit FA availability or action.

Yield

The yield of tendril pea microgreens was significantly affected by the treatments (figure 1a).

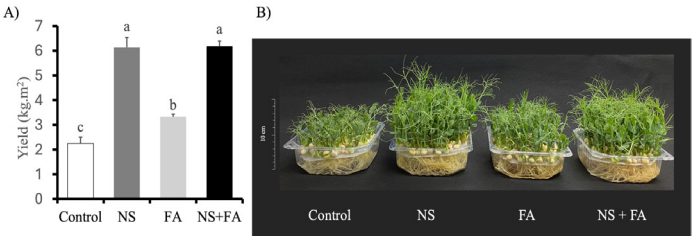

Figure 1. Yield of tendril pea microgreens treated with fulvic acid (FA) cultivated in a hydroponic system for 12 days. Control= destillate water, NS= nutrient solution. Bars with same letters show no significant differences ($p<0.05$, Tukey test).

The NS and NS+FA treatments achieved the highest yields, with statistically similar values averaging 6.15 $kg.m^{-2}$, indicating that the application of Steiner nutrient solution either alone or in combination with fulvic acid, enhanced the increased biomass production. In contrast, the application of FA alone resulted in a moderate yield of 3.3 $kg.m^{-2}$, an increase of 43.47 % over the control (2.3 $kg.m^{-2}$), but was significantly lower than the NS-based treatments. This suggests that while fulvic acid has a stimulatory effect on plant growth, probably due to its role in improving nutrient uptake and metabolic activation (Muscolo *et al*., 2007), it cannot match the contribution of a complete nutrient formulation in supporting maximum biomass accumulation.

The NS+FA treatment did not significantly exceed the performance of NS alone, consistent with observations from the morphological parameters. This lack of additive effect may be due to chemical interactions that reduce the bioavailability or functional efficiency of fulvic acid in nutrient-rich environments (Wang *et al*., 2022), or possibly to saturation effects where the nutrient solution already meets or exceeds the nutritional needs of the plant.

These results support the idea that while fulvic acid can be beneficial, its most effective use may be in nutrient-limited systems or in the early stages of growth, rather than as an additive to already balanced nutrient solutions. Additionally, the yield levels observed for the NS and NS+FA treatments are within the upper range of microgreen productivity under hydroponic conditions reported in the literature (Xiao *et al*., 2012), further validating the effectiveness of the selected nutrient regime.

Visual quality differences among treatments were evident (figure 1b). NS and NS+FA produced denser, more upright, and visually stronger microgreens compared to the control and FA treatments, which appeared sparser and shorter. These visual differences are consistent with the yield and morphological data and reflect more robust development under nutrient-enriched conditions. Minor mechanical damage during harvest was observed in all treatments due to differences in plant height; however, this did not compromise the overall appearance for market purposes.

Biochemical parameters

The biochemical profile of tendril pea microgreens was generally not significantly affected by the application of FA, except for pH, which showed significant variation among treatments (table 2).

Table 2. Biochemical parameters of tendril pea microgreens treated with fulvic acid and grow in a hydroponic system for 12 days.

Parameters	Treatments				
	Control	FA	NS	NS + FA	CV
pH	5.85[b]	6.26[a]	6.24[a]	6.28 [a]	1.64
TSS	11.81[a]	11.11[a]	9.81[b]	9.32[b]	5.55
Color Index	-21.51[a]	-24.65[a]	-24.95[a]	-24.82[a]	25.50
Chlorophyll a	1.27[a]	1.26[a]	1.26[a]	1.026[a]	0.93
Chlorophyll b	0.76[a]	1.04[a]	0.97[a]	1.02[a]	17.19
Carotenoids	0.61[a]	0.64[a]	0.63[a]	0.64[a]	3.25
Antioxidant Capacity	48.90[a]	50.70[a]	51.00[a]	50.90[a]	3.28
Nitrates	2,572.42[a]	2,616.25[a]	1,400.56[b]	1,434.17[b]	19.91

Different superscript letters in the same row indicate significant differences according to the Tukey test at the 0.05 level. Control= destillate water, FA= fulvic acid, NS= Nutrient solution. CV= coefficient of variation.

The pH of the microgreens increased with the FA, NS, and NS+FA treatments compared to the control, with values ranging from 6.24 to 6.28, significantly higher than the control (5.85). This increase may be attributed to the carboxylic and phenolic groups present in fulvic acids, which can alter the rhizosphere pH and internal tissue chemistry (Muscolo *et al*., 2007). However, no significant differences were observed among FA, NS and NS+FA, suggesting that fulvic acids and mineral nutrients may have overlapping effects on pH modulation. Higher pH in plant tissues has been associated with improved sensory quality and shelf life in microgreens, contributing to reduced acidity, improved flavor perception, and microbial stability (Tallei *et al*., 2024). These factors are important for increasing market value and consumer acceptance in commercial production (Seth *et al*., 2025).

Total soluble solids (TSS) were significantly higher in the control and FA treatments (11.81 and 11.11 ºBrix, respectively), while NS and NS+FA treatments had lower values (9.81 and 9.31 ºBrix, respectively). This suggests that nutrient-rich environments may dilute sugar concentrations due to increased vegetative growth. The higher TSS in the control may also reflect stress-related sugar accumulation due to nutrient deficiency, as noted by Lin *et al*. (2016).

No significant differences in color index, chlorophyll a, chlorophyll b, or carotenoids were observed among treatments, indicating that neither FA nor nutrient solutions significantly affected pigment synthesis. Although NS and NS+FA treatments showed a trend toward higher chlorophyll b and carotenoid content, the variation was not statistically significant. These results are consistent with studies suggesting that chlorophyll biosynthesis requires not only adequate nitrogen but also light quality cues, which may have remained stable among treatments (Gao *et al*., 2023).

Antioxidant capacity remained statistically similar across treatments, averaging around 50 %, suggesting that FA and nutrients did not stimulate secondary metabolite production under the given conditions. This is consistent with the findings of Márquez-García *et al*. (2011), where limited abiotic stress did not activate antioxidant pathways in hydroponically grown legumes.

A significant difference was observed in nitrate accumulation, with the control and FA treatments showing significantly higher concentrations (2,572 and 2,616 mg.kg^{-1}, respectively) compared to NS and NS+FA (1,400 and 1,434 mg.kg^{-1}, respectively). This indicates a limited capacity for nitrate assimilation in the absence of a complete nutrient profile. As noted by Nardi *et al*. (2002), the conversion of nitrate to amino acids requires cofactors and energy sources provided by a balanced nutrition, which were absent in the control and FA-only treatments.

Principal Component Analysis (PCA) provided further insight into treatment effects (figure 2).

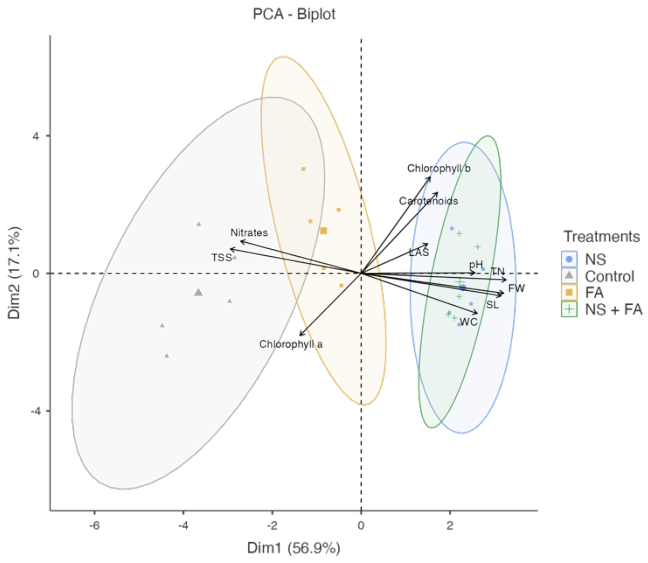

Figure 2. Principal components analysis of tendril pea microgreens treated with fulvic acid (FA) and grown in a hydroponic system for 12 days. TSS= Total soluble solids, LAS= leaf area stipular, WC= water content, SL=Stem length, TN= tendril number, FW= fresh weight.

The first two components (PC1 = 56.9 %, PC2 = 17.1 %) explained 74.0 % of the total variance. The control was associated with higher nitrate and TSS levels, suggesting a biochemical profile typical of nutrient-deficient but metabolically stressed plants. The FA treatments showed a weak association with chlorophyll a, indicating a limited photosynthetic enhancement. In contrast, NS+FA was associated with improvements in morphological traits such as stem length (SL), tendril number (TN), fresh weight (FW), and water content (WC). The NS treatment, although more dispersed in its responses, was associated with chlorophyll b and carotenoids, suggesting a slight advantage in pigment biosynthesis and light-harvesting efficiency.

These results suggest that while fulvic acids alone may slightly alter internal pH and nitrate metabolism, their combination with nutrient solution does not improve biochemical characteristics beyond what is achieved by nutrients alone. This highlights the importance of nutrient completeness over biostimulant supplementation in optimizing biochemical composition, especially under non-stressful hydroponic conditions.

Conclusions

The application of fulvic acid at 0.01 % in pea tendril microgreens grown in a hydroponic system showed a positive, although limited,

effect, especially under nutrient-limited conditions. The FA treatment improved morphological variables such as stem diameter by 25.6 % and fresh weight by 48 % and dry weight by 42.8 % compared to the control, and increased yield by 43 %, demonstrating its potential as a biostimulant in systems without mineral fertilization.

However, when considering all variables were considered simultaneously through multivariate analysis, FA did not outperform the complete nutrient solution and showed no synergistic effect when combined with it. Biochemically, its application was associated with higher nitrate and soluble solids accumulation, without improvements in pigments, antioxidant capacity, or visual quality.

These results suggest that fulvic acid can partially modulate growth and metabolism during early development, but the nutrient context strongly conditioned its efficacy. Therefore, fulvic acid represents a viable alternative to stimulate microgreen development under limited nutrient conditions or as a complementary strategy. However, it does not replace the need for balanced mineral fertilization when the goal is to maximize productivity, quality, and commercial consistency of the crop.

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