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RESEARCH PAPER

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EFFECTS OF FOLIAR-APPLIED FULVIC ACIDS ON YIELD, BIOMASS DISTRIBUTION, AND NITRATE ACCUMULATION IN HYDROPONICALLY GROWN RADISH (*Raphanus sativus* L.)

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ABSTRACT

Fulvic acids are humic-derived biostimulants that can modulate nutrient uptake, carbon metabolism, and biomass allocation in horticultural crops; however, their effect on assimilate partitioning and nitrate accumulation in hydroponically grown root vegetables remains poorly understood. This study evaluated the effect of weekly foliar application of a fulvic acid-based biostimulant (K-TIONIC®, 4 mL L^{-1}) on yield, biomass distribution, nitrate content, and quality of radish (*Raphanus sativus* L. Crimson Giant) cultivated in an ebb-and-flow hydroponic system under greenhouse conditions in Chihuahua, Mexico. A completely randomized design with two treatments (control and fulvic acids) and 36 experimental units per treatment was used. Fulvic acid application increased equatorial diameter by 8.50%, fresh storage root weight by 28%, and total yield by 41.66%. In contrast, vegetative growth parameters and photosynthetic pigments were unaffected. Regarding quality attributes, nitrate concentration in edible tissues decreased by 4.96% and water content by 0.57%, pH, total soluble solids, color index were not significantly affected, and visual quality index improved by 98%. These results indicate that foliar-applied fulvic acids improve radish productivity and commercial quality in soilless systems primarily by promoting biomass distribution to the storage organ.

Keywords: biostimulants, soilless culture, root vegetables, storage organ, vegetative growth parameters

INTRODUCTION

Radish (*Raphanus sativus* L. var. *sativus*) is an economically important horticultural crop due to its rapid production cycle, high adaptability to protected environments, and relevance in fresh vegetable markets worldwide [Gamba et al. 2021, Dos Santos et al. 2023]. Its short growth cycle and high responsiveness to nutrient availability make it a suitable model crop for evaluating agronomic strategies under controlled conditions. However, intensive production systems often face challenges related to nitrate accumulation in edible tissues, negatively affecting nutritional quality and market acceptance [Colla et al. 2018]. This issue is particularly relevant in root vegetables

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such as radish, where nitrate accumulation is directly associated with nitrogen uptake and assimilation processes [Di Gioia et al. 2017].

Hydroponic systems have emerged as efficient alternatives to conventional agriculture due to their efficient use of water and nutrients supply, reduced environmental impact, and ability to optimize crop management [Kechasov et al. 2021, Saldinger et al. 2023]. Among these, ebb-and-flow systems allow periodic root-zone aeration and stable nutrient availability, which are critical for short-cycle species such as radish. Nevertheless, even under optimal nutrient conditions, crop productivity and quality largely depend on internal physiological processes, including nitrogen assimilation and the allocation of assimilates toward storage organs [Tegeder et al. 2018].

In this context, plant biostimulants have gained increasing attention as tools to enhance crop performance without increasing fertilizer inputs [Colla et al. 2017, Conversa et al. 2021]. Fulvic acids, a fraction of humic substances, are characterized by their low molecular weight and high solubility, allowing them to interact with plant physiological processes such as nutrient uptake, enzyme activation, and carbon metabolism [Cataldo et al. 2022, Asif et al. 2023]. Previous studies have reported that fulvic acid-based biostimulants can improve growth and yield in horticultural crops, mainly through enhanced nutrient availability and metabolic activity [Zhang et al. 2021, Barzegar et al. 2022, Keskin et al. 2025]. Nevertheless, their effectiveness may vary depending on plant species, application rate, and method of application [Sun and Shahrajabian 2023]. However, most of these studies have focused on leafy vegetables or aboveground biomass, while limited attention has been given to root crops cultivated under hydroponic conditions.

In root crops such as radish, yield is closely associated with plant's capacity to allocate assimilates toward the storage organ, whereas quality is influenced by factors such as nitrate concentration and water content. A reduction in nitrate levels may reflect a more efficient assimilation of nitrogen into organic compounds, while changes in biomass distribution may indicate modifications in source-sink relationships [Xu et al. 2012]. Despite the increasing use of biostimulants in horticulture, there is limited information regarding their effect on nitrate accumulation and assimilate partitioning in hydroponically grown root vegetables, particularly under ebb-and-flow systems.

Therefore, the objective of this study was to evaluate the effect of foliar application of fulvic acids on yield, nitrate content, and biomass distribution in radish cultivated in an ebb-and-flow hydroponic system. The hypothesis was that foliar application of fulvic acids reduces nitrate accumulation, suggesting improved nitrogen assimilation, and assimilate partitioning toward the storage organ, resulting in increased yield and improved commercial quality.

MATERIAL AND METHODS

The experiment was conducted during the 2024 growing season in a greenhouse at the Faculty of Agrotechnological Sciences, Autonomous University of Chihuahua (28.6434°N, 106.0765°W), Chihuahua, Mexico. *Raphanus sativus* L. var. *sativus* Crimson Giant was grown in four-ounce expanded polystyrene containers filled with perlite as substrate in an ebb-and-flow hydroponic system.

The system operated with flooding cycles of 10 min every 2 h during daylight and every 4 h at night, ensuring adequate nutrient supply and root-zone aeration.

The Steiner nutrient solution (Soluponic®, Inverfarms México, San Pablo, Querétaro, Mexico) was used as the nutrient source. This solution is widely used in commercial hydroponic production systems due to its balanced composition and adaptability to a wide range of horticultural crops. The nutrient concentrations were as follows: N-NO_3 15.7 mmol L^{-1}, N-NH_4 1.3 mmol L^{-1}, P 1.3 mmol L^{-1}, K 1.3 mmol L^{-1}, Ca 4.7 mmol L^{-1}, Mg 3.0 mmol L^{-1}, S 3.3 mmol L^{-1}, B 13.9 µmol L^{-1}, Fe-EDTA 28.0 µmol L^{-1}, Zn-EDTA 3.3 µmol L^{-1}, CU-EDTA 1.7 µmol L^{-1}, Mn-EDTA 15.2 µmol L^{-1}, and Mo 0.7 µmol L^{-1}. The nutrient solution pH was maintained between 6.0-6.5, adjusted with HNO_3 (10% *v/v*), and electrical conductivity was kept at 2.3 mS cm^{-1} [Morgan 2000].

The experiment followed a completely randomized design with two treatments: (1) control (distilled water spray) and (2) fulvic acid-based biostimulant application. Each treatment consisted of 36 experimental units, with one plant per experimental unit. Each container thus constituted an independent experimental unit, providing physical independence of the root environment. Plants were arranged in groups of nine per tray, with each tray connected to an independent nutrient solution reservoir. For destructive measurements, 20 plants were randomly selected per treatment.

The fulvic acid-based biostimulant (K-TIONIC®) contained 100 g L^{-1} of total humic carbon extract (10% humic acid and 90% fulvic acids), 6 g L^{-1} nitrogen, and 38 g L^{-1} K_2O. It was applied weekly at a concentration of 4 mL L^{-1}

by foliar spraying using a hand-held atomizer (Specialized Plastic and Packaging, Chihuahua, Mexico), to the point of incipient runoff. This concentration was selected based on the manufacturer's recommended dose and represents the standard application rate established by the manufacturer for horticultural crops, serving as a baseline for evaluating its agronomic efficacy under the conditions of this study. Application started at the appearance of the second true leaf stage and continued until one week before harvesting. All applications were performed at 07:30 a.m.

Vegetative parameters were determined 45 days after sowing. From each treatment, 20 plants were randomly selected for destructive measurement and determine number of leaves, leaf area (cm^2), root length (mm), neck diameter (mm), and polar and equatorial diameters (mm). Fresh and dry biomass were recorded and expressed in grams (g). Dry biomass was obtained by drying the samples at 65°C until constant weight (approximately 72 h) and weighing them using a digital balance (Ohaus, Adventure Pro AV264C, USA). Diameters were measured using a Vernier caliper (Starrett®, EC799A-6/150, USA). Measurements were performed using a digital balance, a digital caliper, and leaf area was determined using ImageJ software. Shape index was calculated as longitudinal diameter / equatorial diameter [Wei et al. 2022].

Quality parameters were determined from fresh tissue extracts. pH was measured using 5 g of macerated tissue, and the extracted juice was used for pH determination using a digital potentiometer (HI98103 pH Tester, Hanna Instruments, Woonsocket, RI, USA). Total soluble solids (TSS) content was determined using a digital refractometer, and expressed as °Brix. Color was evaluated using the CIE Lab* system (Minolta Chromameter CR-310, Konica Minolta, Osaka, Japan), and the color index (CI) was calculated as $CI = (1000 \times a) / (L \times b)$ [Cordoba-Novoa et al. 2018]. Moisture content was calculated as [(fresh weight – dry weight) / fresh weight] × 100 [Jamil et al. 2007].

Photosynthetic pigments were determined according to Lichtenthaler and Wellburn [1983]. Leaf samples (0.1 g) were extracted with 4 mL of 80% (v/v) acetone, centrifuged at 3000 rpm for 5 min, and absorbance was measured at 663, 645, and 470 nm.

Nitrate concentration was determined following Toscano et al. [2021]. Fresh tissue (1 g) was homogenized in 3 mL of distilled water, centrifuged at 4,000 rpm for 15 min. An aliquot of 20 µL of supernatant was reacted with 80 µL of sulphury acid-salicylic solution, followed by neutralization with 3 mL of 1.5 N NaOH. Absorbance was measured at 410 nm, and nitrate concentration was calculated using a KNO_3 standard curve (0–10 mM; $R^2 = 0.9918$). Result was expressed as mg NO_3^- kg^{-1} fresh weight.

Visual quality was evaluated using a five-point scale based on color, shape, texture, and size. Each attribute was scored from 1 (non-commercial quality) to 5 (optimal market quality). The visual quality index was calculated as the mean score. Yield was calculated as fresh storage root weight per plant multiplied by the planting density, determined from the footprint area of each individual four-ounce expanded polystyrene container (21.5 cm^2), and expressed as kg m^{-2}.

The differences between the treatment and control groups were determined using Student's t-test ($p \leq 0.05$) after verifying normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test). Statistical analyses were performed using Jamovi software v. 2.4.8 [The Jamovi Project 2023].

RESULTS

No significant differences were observed in vegetative parameters such as number of leaves, leaf area, root length, or neck diameter between treatments (Table 1). Likewise, chlorophyll *a*, chlorophyll *b*, and carotenoid contents did not differ significantly between treatments (Table 2).

Table 1. Vegetative parameters of radish under fulvic acid application

Parameter	Control	Fulvic acids
Number of leaves	7.80 ±1.50 a	7.50 ±1.40 a
Leaf area (cm2)	462.88 ±128.70 a	483.33 ±291.90 a
Root length (mm)	21.88 ±5.20 a	18.38 ±6.36 a
Neck diameter (mm)	7.64 ±1.42 a	8.29 ±1.70 a
Polar diameter (mm)	30.22 ±4.96 a	32.80 ±4.39 a
Equatorial diameter (mm)	28.00 ±4.23 a	30.98 ±3.14 b
Shape index	0.94 ±0.13 a	0.96 ±0.13 a

Values represent mean ±SE (n = 20). Different letters indicate significant differences according to Student's t-test ($p \leq 0.05$).

Table 2. Physiological and photosynthetic parameters of radish under fulvic acid application

Parameter		Control	Fulvic acids
pH		6.32 ±0.10 a	6.25 ±0.14 a
Total soluble solids (°Brix)		3.74 ±0.35 a	3.75 ±0.63 a
Color index		163.26 ±11.20 a	180.69 ±14.54 a
Water content (%)		97.46 ±0.87 b	96.89 ±0.70 a
Photosynthetic pigments (mg g^{-1} FW)	chlorophyll a	0.51 ±0.17 a	0.52 ±0.23 a
	chlorophyll b	0.21 ±0.12 a	0.20 ±0.19 a
	carotenoids	0.01 ±0.00 a	0.01 ±0.00 a

Values represent mean ±SE (n = 20). Different letters indicate significant differences according to Student's t-test ($p \leq 0.05$).

However, the equatorial diameter of radish roots increased by 8.50% in plants treated with fulvic acids compared to the control (30.98 mm to 28.00 mm respectively; Table 1). In addition, the weight of fresh storage roots increased by 28% with the application of fulvic acid compared to the control, indicating greater biomass accumulation in the storage organ (14.34 g to 11.20 g respectively; Table 3).

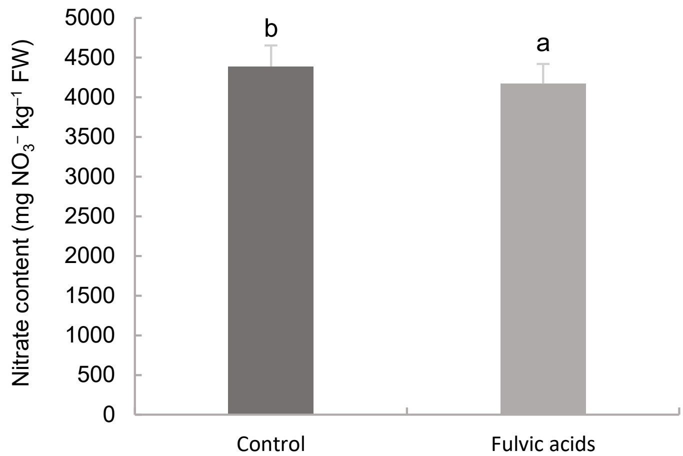
Physiological quality parameters including pH, TSS, and color index were not significantly affected by fulvic acid application (Table 3). In contrast, water content of the storage root decreased by 0.57% in treated radishes (Table 3). Treatment with fulvic acids resulted in a 4.96% reduction in nitrate concentration in radish roots compared to the control (Figure 1).

Table 3. Biomass parameters of radish under fulvic acid application

Parameter	Control	Fulvic acids
Fresh weight of leaves (g)	29.50 ±11.54 a	32.88 ±7.62 a
Fresh weight of storage root (g)	11.20 ±4.25 b	14.34 ±5.28 a
Fresh weight of fibrous root (g)	3.60 ±2.42 a	3.37 ±1.70 a
Dry weight of leaves (g)	1.47 ±0.60 a	1.75 ±0.55 a
Dry weight of storage root (g)	0.31 ±0.23 a	0.44 ±0.17 a
Dry weight of fibrous root (g)	0.21 ±0.10 a	0.20 ±0.11 a

Values represent mean ±SE (n= 20). Different letters indicate significant differences according to Student's t-test ($p \leq 0.05$).

Figure 1. Nitrate content of radish roots under fulvic acid application. Bars represent mean ±SE (n = 20). Different letters indicate significant differences according to Student's t-test ($p \leq 0.05$)



Visual quality also improved markedly, with a 98% increase in the visual quality index compared to the control (Figure 2A, Table 4). Treated radishes exhibited uniform red coloration, smooth surface texture, and optimal commercial size (Figure 2B, Table 4).

Finally, fulvic acid application resulted in a 41.66% increase in total yield compared to untreated plants (figure 3).

Figure 2. Visual quality of radish under fulvic acid application. (A) Visual quality index based on five-point hedonic scale. (B) Representative images of radish roots at harvest. Bars represent mean ±SE (n = 20). Different letters indicate significant differences according to Student's t-test ($p \leq 0.05$)

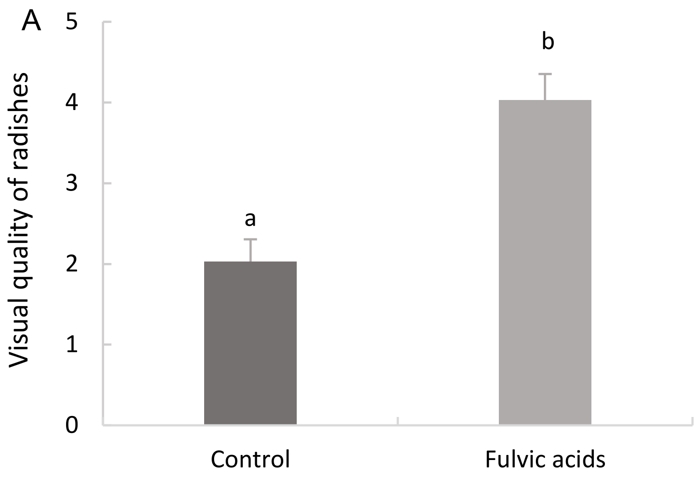


Table 4. Visual assessment scale for quality of hydroponically grown radishes treated with fulvic acid

Score	Description	Visual quality
5	The product is essentially free of defects. • Intense and uniform red color without discoloration. • Symmetrical, cylindrical, or conical root representative of the variety. • Smooth and clean surface. • Optimal size (diameter 3.0–4.0 cm; length 5–6.5 cm).	excellent
4	Minor defects, not objectionable. • Uniform red color with slight variations (<10%). • Slightly irregular but acceptable shape. • Mostly smooth surface. • Commercially acceptable size (2.5–3.0 cm diameter).	good
3	Moderate defects, acceptable in the local markets. • Pink to red color with variability. • Slight curvature or asymmetry. • Slightly rough surface. • Acceptable size (2.0–2.5 cm diameter or >4.5 cm length).	fair
2	Severe defects, low marketability. • Pale or uneven color. • Irregular or curved shape. • Rough surface with visible damage. • Size outside optimal range (<0.8 in. or >2 in.).	poor
1	Non-commercial quality. • Whitish or greenish/brown discoloration. • Severely deformed or bifurcated roots. • Rough surface with cracks. • Unacceptable size.	very poor

Figure 3. Yield of radish under fulvic acid application. Bars represent mean ±SE (n = 20). Different letters indicate significant differences according to Student's t-test (p ≤ 0.05)

DISCUSSION

The results indicate that foliar application of fulvic acids did not modify early vegetative growth of photosynthetic pigment content under the evaluated hydroponic conditions. The absence of significant changes in leaf traits and pigment concentrations suggests that plants in both treatments operate under optimal nutritional and environmental conditions, which tend to stabilize these parameters in soilless systems.

Despite the lack of response in vegetative traits, fulvic acid application promoted significant increase in root diameter, suggesting preferential biomass distribution toward the storage organ. This response is directly associated with commercial quality in root crops, and suggests a modification in source-sink relationships. Similar increases in fruit or root diameter following fulvic or humic acid application have been reported in watermelon [Barzegar et al. 2022], cucumber [Yassin and Al-Zubaidi 2025], and guava [Mosa et al. 2021], and have been attributed to enhanced physiological activity, nutrient uptake, and carbohydrate accumulation.

The observed increase in fresh root weight agrees with previous reports in radish, and other crops treated with humic-based biostimulants. Šlosár et al. [2025] reported increased root weight in radish following humic biostimulant application, while Kaya [2025] observed similar responses under saline stress conditions. Although cultivation systems and stress factors differ, these studies consistently indicate that fulvic-acid-based biostimulants promote biomass accumulation by enhancing activity and resource allocation efficiency. The significant decrease in water content (Table 2) indicates a higher proportion of dry matter in treated roots, aligning with the observed pattern of enhanced biomass allocation toward the storage organ.

The preferential accumulation of biomass in the storage root observed in this study may be explained, at least in part, by the auxin-like activity attributed to fulvic acids. Humic substances, including fulvic acids, contain bioactive fragments that mimic auxin action by interacting with hormonal receptors and activating plasma membrane H^{+-}ATPase proton pumps [Canellas et al. 2015, Nardi et al. 2018]. This proton pump activation generates an electrochemical gradient that energizes secondary transporters involved in nutrient and sugar uptake at the sink level [Canellas et al. 2015]. Furthermore, auxin-mediate signaling has been shown to directly regulate the expression of sucrose transporters, thereby promoting phloem unloading and increasing carbohydrate import into sink organs [Zhang et al. 2022]. In addition, fulvic acid application has been reported to increase endogenous auxin and zeatin concentration in leaves, which may further stimulate assimilate mobilization from source tissues toward developing storage organs [Ampong et al. 2022]. Together, these mechanisms provide a plausible physiological basis for the enhanced assimilate partitioning toward the storage root observed in the present study, although direct evidence from enzyme activity or hormone quantification was not obtained and further studies are warranted.

Fulvic acid application did not significantly affect pH, TSS, or color index, indicating that internal quality attributes associated with basic biochemical composition were preserved. However, the slight reduction in water content suggests an increase in dry matter proportion, which may contribute to improved postharvest quality. Previous studies have shown that higher dry matter content is associated with improved tissue integrity and reduced susceptibility to mechanical damage in root crops [Lockley et al. 2021, Lupu et al. 2024].

The observed reduction in nitrate concentration in treated radishes aligns with the hypothesized role of fulvic acids in enhancing nitrogen assimilation efficiency and regulating nitrate accumulation in edible tissues. This suggests a metabolic redirection of inorganic nitrogen toward organic compounds, a phenomenon consistent with similar reductions reported in radish microgreens treated with plant-derived biostimulants [Toscano et al. 2023]. Internationally, the Joint FAO/WHO Expert Committee on Food Additives has established an Acceptable Daily Intake (ADI) of 3.7 mg nitrate kg^{-1} body weight day^{-1}, which serves as the reference value underlying most national risk assessments; however, no global or food-specific maximum level has been established for nitrate in radish. In its risk-benefit assessment of nitrate from vegetables, the EFSA Panel on Contaminants in the Food Chain [CONTAM 2008] concluded that high-level consumers of vegetables grown under unfavorable production conditions may exceed the ADI by approximately twofold – a concern particularly relevant to crops prone to elevated nitrate accumulation, such as radish. Uddin et al. [2021] reported that, among the root and tuber vegetables analyzed, radish was the only one whose Health Risk Index exceeded the acceptable threshold for both adults and children. Taken together, these findings underscore the practical importance of agronomic strategies, such as foliar fulvic acid application, for mitigating nitrate accumulation in hydroponically grown radish.

Improvements in visual quality further reinforce the positive effect of fulvic acids on commercial performance. Enhanced coloration, surface uniformity, and market size have also been reported in hydroponically grown tomato and cucumber following fulvic acid application [Zhang et al. 2021, Liu et al. 2022, Song et al. 2025], and are commonly linked to optimized nutrient uptake and biomass partitioning.

In terms of yield, the 41.66% increase observed with the application of fulvic acid in this study is consistent with the improvements reported for radishes in other biostimulant-based strategies. Šlosár et al. [2025] observed a 34.8% increase in radish yield following the application of a humic biostimulant, while De Guzman et al. [2026] reported that increasing the application frequency of the hydroponic nutrient solution improved radish yield, reaching a maximum of 6.60 kg m^{-2}.

Overall, the findings demonstrate that foliar application of fulvic acids in an ebb-and-flow hydroponic system enhances radish productivity and commercial quality without altering key physiological quality parameters. The increases in root diameter, fresh biomass, and yield, together with reduced nitrate concentration and water content, suggest a more efficient biomass distribution toward the storage organ. These results support the use of fulvic-acid-based biostimulants as a sustainable agronomic strategy for radish production in soilless systems, while highlighting the need for future studies to elucidate underlying physiological mechanisms and validate these responses under different environmental conditions. Given that the concentration used in this study corresponded to the manufacturer's recommended dose for horticultural crops, future research could evaluate additional concentrations to expand our understanding of crop response, and optimize the agronomic use of the biostimulant in hydroponic systems. Additionally, although the potential contribution of the nitrogen and potassium (1.71 mmol L^{-1} N, 1.61 mmol L^{-1} K, 10%) supplied by biostimulant per foliar application cannot be fully excluded, the observed responses are consistent with the bioactive mechanisms of humic substances documented independently of their mineral content [Canellas et al. 2015, Nardi et al. 2016]. Future studies using purified fulvic acid formulations are recommended to allow more precise attribution of the effect to the humic fraction specifically.

CONCLUSIONS

Foliar application of fulvic acids in an ebb-and-flow hydroponic system improved radish productivity and commercial quality without altering vegetative growth parameters, photosynthetic pigments, or internal quality attributes such as pH, total soluble solids, and color index. The increase in equatorial diameter, fresh root weight, and total yield suggests enhanced biomass distribution toward the storage organ, suggesting a modification in source-sink relationships. The reduction in nitrate concentration within edible tissues indicates a lower accumulation of inorganic nitrogen under the evaluated conditions. Furthermore, visual quality improved markedly, with treated radishes showing enhanced uniformity in coloration, surface texture, and commercial size. The slight decrease in water content may indicate a higher dry matter proportion, which could be advantageous for postharvest quality. Overall, fulvic acids act primarily by promoting biomass distribution toward the storage organ rather than modifying vegetative growth, supporting their use as a sustainable agronomic strategy for hydroponic radish production. Future studies should incorporate direct measurements of nitrogen assimilation activity and evaluate dose-response effects across multiple growing seasons to validate and extend these findings.

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This research did not receive any funding.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DECLARATION OF USING GENERATIVE AI TOOLS

The following generative artificial intelligence tools were used in the process of preparing this manuscript: Consensus and Perplexity AI: used to support the systematic search of scientific literature on the topics covered in the article. DeepL: used for translation support between English and Spanish in the preparation of the manuscript text. Claude: used for language editing, grammar checking, and structural revision of the English manuscript text. Following their use, the authors assumed full responsibility for the publication content, reviewed and edited it as necessary.

ATHORS CONTRIBUTIONS

R.P. – methodologhy; M.R. – project administration and supervision; J.H. – formal analysis, data curation, writing-original draft; A.G. – validation and visualization; K.Q. – investigation; A.A. – conceptualization, writing-review and editing.

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