Greenhouse Plant Production Journal 1(3) (2024) 68–82 **gppj.araku.ac.ir**

Greenhouse Plant Production Journal

Enhancing the absorption of microelements by applying humic acid and zinc sulfate in *Physalis alkekengi*: Improve chlorophyll content and fruit quality

Samira Kazemi [a*], Mohammad Reza Pirmoradi [b], Mahmoud Raghami [b], Mohammad Reza Malekzadeh [b]

[a] *Department of Plant Sciences, University of Idaho, Moscow, Idaho, 83843, USA.*
[b] *Department of Horticultural Sciences, School of Agriculture, Vali-e-Asr University of Rafsanjan, Rafsanjan, Iran.*

Original Article

Use your device to scan and read the article online

Citation: Kazemi, S., Pirmoradi, M. R., Raghami, M. R. and Malekzadeh, M. R. 2024. Enhancing the absorption of microelements by applying humic acid and zinc sulfate in *Physalis alkekengi*: Improve chlorophyll content and fruit quality. Greenhouse Plant Production Journal, 1(3): 68–82.

https://10.61186/gppj.1.3.68

KEYWORDS

Foliar application
Growth promotion
Medicinal plants
Nutrient uptake

ABSTRACT

Physalis alkekengi L. is a valuable medicinal plant from the Solanaceae family that is used in traditional medicine and has numerous medicinal properties. This experiment was conducted to investigate the effects of humic acid and zinc sulfate on the uptake of key microelements (Cu, Zn, Fe, Mn) in *Physalis alkekengi*. The study employed a factorial design based on completely randomizes design with three replications, examining three levels of humic acid (0, 1, 2 g L^{-1}) and three levels of zinc sulfate (0, 0.5, 1 g L^{-1}) applied as foliar sprays. Results demonstrated that the combined application of 2 g L^{-1} humic acid and 0.5-1 g L^{-1} zinc sulfate significantly enhanced microelement uptake, with zinc and iron concentrations showing the greatest improvements. These increases were positively correlated with improved plant physiological traits. This study highlights the potential of integrating humic acid and zinc sulfate applications to optimize nutrient uptake and improve the nutritional value of medicinal plants under controlled greenhouse conditions. Furthermore, these findings have practical implications for sustainable agriculture and traditional medicine, offering strategies to enhance crop productivity, resilience, and the therapeutic quality of *Physalis alkekengi*.

ARTICLE HISTORTY

Received: 02 August 2024
Revised: 07 September 2024
Accepted: 31 September 2024

---

* Corresponding author: S. Kazemi
E-mail address: samira.kazemi20000@gmail.com

Publisher: Arak University

1. Introduction

Physalis alkekengi L. commonly known as the winter cherry or Chinese lantern plant, is indeed a valuable medicinal plant from the Solanaceae family, recognized for its bioactive compounds like withanolides, alkaloids, and flavonoids. These secondary metabolites are recognized for their therapeutic properties, such as antioxidant, anti-inflammatory, and anticancer properties, making the plant an invaluable resource in traditional and modern medicine (Hassanpour, 2024) (Yang *et al*., 2022) (Ge *et al*., 2009).

The quality and medicinal value of *Physalis alkekengi* are closely tied to its nutrient profile, particularly micronutrients such as copper (Cu), zinc (Zn), iron (Fe), and manganese (Mn). These essential micronutrients play critical roles in enzymatic activities, photosynthesis, and the synthesis of secondary metabolites. For instance, Zn acts as a cofactor for numerous enzymes and is crucial for protein synthesis, while Fe is a core component of chlorophyll synthesis and electron transport chains. Similarly, Mn participates in redox reactions, and Cu supports lignification and oxidative stress regulation (Rai *et al*., 2021) (Ahmed *et al*., 2024) (Bhat *et al*., 2020) (Roosta *et al*., 2018). Despite their importance, deficiencies in these micronutrients are a common challenge in greenhouse cultivation due to limited bioavailability in soilless media. Addressing these limitations is essential to optimizing nutrient uptake and enhancing the quality of medicinal plants.

Optimizing nutrient uptake in greenhouse systems has both environmental and economic importance. Efficient nutrient use reduces fertilizer waste and the risk of nutrient leaching, which can lead to environmental pollution (Hong *et al*., 2014; Zhou *et al*., 2022). Economically, better nutrient uptake enhances plant growth and productivity, reducing input costs and increasing profitability for growers (Dobermann *et al*., 2022). These benefits highlight the critical need for sustainable and precise nutrient management practices, particularly for high-value medicinal plants like *Physalis alkekengi*.

Among potential solutions, humic acid has emerged as a potent biostimulant. Humic acid derived from organic matter, enhances nutrient availability, improves soil fertility, and promotes root development through its ability to increase cation exchange capacity and facilitate active nutrient absorption (Ampong *et al*., 2022; Martins *et al*., 2024; Roosta *et al*., 2017). Complementing this, zinc sulfate act as a critical source of Zn, bypassing soil-based limitations and directly addressing Zn deficiencies. Zinc plays a pivotal role in enzymatic activation, chlorophyll synthesis, and other physiological processes critical to plant growth and productivity (Hamzah Saleem *et al*., 2022) (Shanmugavel *et al*., 2023).

While both humic acid and zinc sulfate are well-documented for their individual benefits, their synergistic potential remains underexplored, particularly in medicinal plants like *Physalis alkekengi* (Kazemi *et al*., 2023). Preliminary studies on other crops suggest that combining humic acid and zinc sulfate can significantly enhance nutrient efficiency and plant growth in controlled environments such as greenhouses (Morais *et al*., 2021). Humic acid has been shown to chelate micronutrients, increasing their solubility and mobility (Zanin *et al*., 2019), while zinc sulfate provides essential Zn to support physiological and metabolic functions. This synergy has demonstrated improved plant growth, nutrient uptake, and resistance to environmental stresses, offering a sustainable solution for precision agriculture (Morais *et al*., 2021) (de Moura *et al*., 2023). Research has further highlighted that this combination optimizes fertilizer efficiency, reduces nutrient leaching, and promotes healthier plant development, contributing to sustainable and environmentally friendly cultivation practices (Ampong *et al*., 2022) (Kazemi *et al*., 2023). However, despite these promising findings, the combined effects of humic acid and zinc sulfate on micronutrient uptake (Cu, Zn, Fe, Mn) and key physiological parameters in *Physalis alkekengi* remain largely unexplored.

Moreover, most existing studies focus on food crops or model plants, leaving a critical knowledge gap in how these treatments influence the nutritional and biochemical traits of medicinal species grown in controlled environments. Addressing this gap is essential, as medicinal plants like *Physalis alkekengi* require precise nutrient management to optimize their therapeutic compound production and resilience under greenhouse cultivation.

This study aims to bridge this gap by evaluating the synergistic effects of humic acid and zinc sulfate on *Physalis alkekengi*. By examining key physiological parameters, including micronutrient uptake, chlorophyll content, root development, and antioxidant activity, we provide new insights into sustainable nutrient management strategies tailored for medicinal plants. These findings not only contribute to the broader field of precision agriculture but also advance environmentally friendly practices in controlled cropping systems.

Specifically, this study investigated the effects of humic acid and zinc sulfate application on micronutrient uptake, chlorophyll content, root volume, and antioxidant activity in *Physalis alkekengi* under greenhouse conditions. We hypothesized that the application of humic acid and zinc sulfate would improve the vegetative and reproductive traits and fruit quality of *Physalis alkekengi* by affecting nutrient uptake. We demonstrated that humic acid and zinc sulfate improved growth and increased fruit antioxidant activity by improving nutrient uptake and increasing plant chlorophyll content. These findings not only enhance the nutritional and medicinal potential of *Physalis alkekengi*, but also help to advance environmentally friendly and efficient nutrient management practices in controlled cropping systems.

2. Materials and Methods

2.1 Plant Material and Growth Conditions

This study was conducted during the 2019–2020 growing season in a research greenhouse at the College of Agriculture, Vali-e-Asr University of Rafsanjan, Iran. The objective was to evaluate the effects of humic acid and zinc sulfate on micro-nutrient uptake in *Physalis alkekengi***.** Rooted plants were obtained from Techno Cultivation Co., Shiraz, Iran. On November 1, 2019, one-month-old seedlings at the four-leaf stage were transplanted into 4-kg pots containing a 1:1 mixture of cocopeat and perlite. Cocopeat and perlite were chosen for their excellent water retention, aeration, and drainage properties, creating an ideal balance for root development and nutrient uptake in controlled greenhouse conditions. The greenhouse was maintained at optimal conditions, with daytime temperatures of 23–25 °C, nighttime temperatures of 18–22 °C, a photoperiod of 11/13 h (light/dark), and relative humidity of 50 ± 10%.

To further minimize environmental variability, the controlled conditions ensured uniform temperature, humidity, and light exposure across all treatments. Pots were leached with 1 L of distilled water every 10 days to prevent salt accumulation, and irrigation was performed with standardized volumes of Hoagland nutrient solution, adjusted according to plant growth stage and environmental conditions. Additionally, pest control was managed using sticky traps and netting to eliminate variability caused by pest infestation. These measures ensured that the observed treatment effects on nutrient uptake and physiological traits were attributable solely to the experimental variables.

The experiment was arranged in a factorial design based on a completely randomized design (CRD) with three replications. Each replication consisted of three pots, each containing one plant. The treatments included three levels of humic acid (0, 1, and 2 g L^{-1}) and three levels of zinc sulfate (0, 0.5, and 1 g L^{-1}). These concentrations were selected based on prior studies and preliminary trials, which demonstrated their efficacy in improving nutrient uptake and physiological traits in plants without causing phytotoxicity. The range of concentrations allows for evaluating the individual and combined effects of these treatments under greenhouse conditions. Humic acid, derived from leonardite and containing 55% humic acid and 4% potassium, was sourced from Danesh Sabze Mahan Co. Zinc sulfate, containing 32% zinc, was obtained from Konjale Sabz Tehran Co. A surfactant (Tween 20) was added to all treatment solutions at a rate of five drops per liter. Control plants were sprayed with distilled water. Foliar application of humic acid and zinc sulfate began 30 days after transplantation (December 1, 2019) and continued weekly for 12 weeks until February 10, 2020. A handheld sprayer was used to apply 200 mL of solution per plant, ensuring uniform coverage while avoiding contamination of non-target parts. Pots were leached with 1 L of distilled water every 10 days to prevent salt accumulation. Irrigation was performed with 200–800 mL of Hoagland nutrient solution, adjusted according to plant growth stage and environmental conditions (Table 1).

After 100 days (February 10, 2020), plants were harvested. Shoots, roots, and fruits were collected and processed for micro-nutrient, growth, and physiological analyses. Plant tissues were dried at 70 °C, ground, and digested in a mixture of nitric acid and perchloric acid for microelement analysis. Microelements, including copper (Cu), zinc (Zn), iron (Fe), and manganese (Mn), were measured using Atomic Absorption Spectroscopy (AAS, model Analytikjena, Specord 200 Plus using Atomic Absorption Spectroscopy (AAS, GBC Avanta, Italy).

Table1. Nutrient concentrations in the solution used for this experiment.

Nutrient Type	Nutrient	Concentration ($mg\ L^{-1}$)
Macronutrients	N	210
	P	31
	K	235
	Ca	200
	Mg	48
	S	64
Micronutrients	Fe	2.5
	Mn	0.5
	Zn	0.05
	B	0.5
	Cu	0.02
	Mo	0.01

2.2 Measurement of Physiological Traits

2.2.1. Chlorophyll Content

Total chlorophyll content was determined using the method of Porra (Porra *et al*., 1989). A random sampling of mature leaves was conducted, and chlorophyll was extracted with acetone. Briefly, 0.25 g of fresh leaf tissue was ground in a mortar with 5 mL of 80% acetone until a uniform solution was formed. The extract was centrifuged at 3500 rpm for 10 minutes, and absorbance was measured using a spectrophotometer (Specord 200 Plus, Analytikjena) at 646.6 and 663.6 nm. Total chlorophyll concentration was calculated using the following equation.

$$Total\ chlorophyll\ (\mu g/g\ FW) = \frac{[(17.76 \times OD_{646.6}) + (7.34 \times OD_{663.6})] \times V}{W}$$

Where: $OD_{646.6}$ and $OD_{663.6}$ are the optical densities at respective wavelengths, V is the volume of acetone (mL), and W is the sample fresh weight (g).

2.2.2. Chlorophyll Index (SPAD)

The chlorophyll index (SPAD) was measured using a portable handheld SPAD-502 Chlorophyll Meter (Minolta Camera Co. Ltd., Osaka, Japan). This device provides non-destructive measurements of leaf greenness, which correlates with chlorophyll content, which reflects photosynthetic efficiency and nutrient uptake. SPAD measurements were integral to evaluating the impact of humic acid and zinc sulfate treatments on *Physalis alkekengi*, supporting the study’s goal of optimizing nutrient management.

2.3 Measurement of Antioxidant Activity

Antioxidant activity was assessed using the method of Brand-Williams (Brand-Williams *et al.*, 1995), with modifications. DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity was measured. Four concentrations of prepared plant extracts were used to plot the percentage of inhibition versus concentration to calculate the IC50 value. For this analysis, 300 µL of 1 M DPPH solution was mixed with 100 µL of diluted extract and brought to a final volume of 2 mL using methanol. After incubation in darkness for 30 minutes, absorbance was measured at 517 nm using a spectrophotometer. The percentage inhibition of DPPH was calculated as follows:

$$DPPH\ percentage\ of\ inhibition = [1 - (ADPPH - ASample)/ADPPH)] \times 100$$

Where ADPPH is absorption in the absence of DPPH samples, and ASample is absorption in the presence of DPPH.

2.4 Microelement Analysis

To evaluate the concentrations of Fe, Mn, Zn, and Cu in plant tissues, four mature leaves were randomly collected from each treatment at harvest. Dried and ground leaf samples (0.5 g) were ashed at 550 °C for 3 hours. After cooling, 5 mL of 2N hydrochloric acid (HCl) was added, and the solution was diluted to 50 mL with distilled water. Micro-nutrient concentrations were measured using Atomic Absorption Spectroscopy (AAS, GBC Avanta, Italy). Calibration standards were prepared for each element to ensure accuracy and precision.

2.5 Root Volume

Root volume was measured using the water displacement method based on Archimedes’ principle. The volume of water displaced after immersing the root in a defined volume of water was recorded as the root volume.

2.6 Experimental Design and Data Analysis

The experiment was conducted using a factorial arrangement in a completely randomized design (CRD) with three replications per treatment under greenhouse conditions. Each replication consisted of three pots, with each pot containing one plant. Data were analyzed using Python version 3.12 for analysis of variance (ANOVA) and post-hoc testing. A two-way ANOVA was conducted to assess the independent and interactive effects of humic acid and zinc sulfate treatments on the measured parameters. The analysis was based on the mean of three replicates for each treatment. Post-hoc analysis was performed using Tukey's Honest

Significant Difference (HSD) test to compare treatment means and determine statistically significant differences among groups. All statistical analyses were conducted at a 95% confidence level ($p \leq 0.05$) to ensure robust and reliable results.

Principal component analysis (PCA) and biplots were generated using Python's scikit-learn library for PCA computation and Matplotlib for visualizations. Standardization of variables was performed using the StandardScaler module to ensure all variables had a mean of zero and unit variance prior to conducting PCA.

3. Results

3.1. Chlorophyll Content

The results obtained from this experiment demonstrated that the foliar application of humic acid, zinc sulfate, and their interaction effects had a significant effect on physiological traits and micronutrients uptake characteristic of *Physalis alkekengi* at the level of 5 %.

Chlorophyll content (SPAD values) was significantly affected by the treatments. The highest SPAD value (75) was achieved with the combined application of 2 g L^{-1} humic acid and 1 g L^{-1} zinc sulfate, while the lowest SPAD value (40.45, 46.43) was observed in the 1 g L^{-1} zinc sulfate and control treatment respectively ($p < 0.05$) (Fig. 1A).

3.2. Total chlorophyll

The total chlorophyll content was significantly influenced by the foliar application of humic acid, zinc sulfate, and their interaction. The highest total chlorophyll content (1820 µg g^{-1}) was observed with the simultaneous application of 2 g L^{-1} humic acid and 1 g L^{-1} zinc sulfate, which was significantly higher than all other treatments ($p < 0.05$). In contrast, the lowest chlorophyll content (1128.33 µg g^{-1}) was recorded in the control treatment. The application of 1 g L^{-1} humic acid combined with 0.5 g L^{-1} or 1 g L^{-1} zinc sulfate also resulted in a marked increase in total chlorophyll content compared to the control. These findings indicate that the combined application of humic acid and zinc sulfate enhances chlorophyll biosynthesis, with higher concentrations yielding the best results (Fig. 1B).

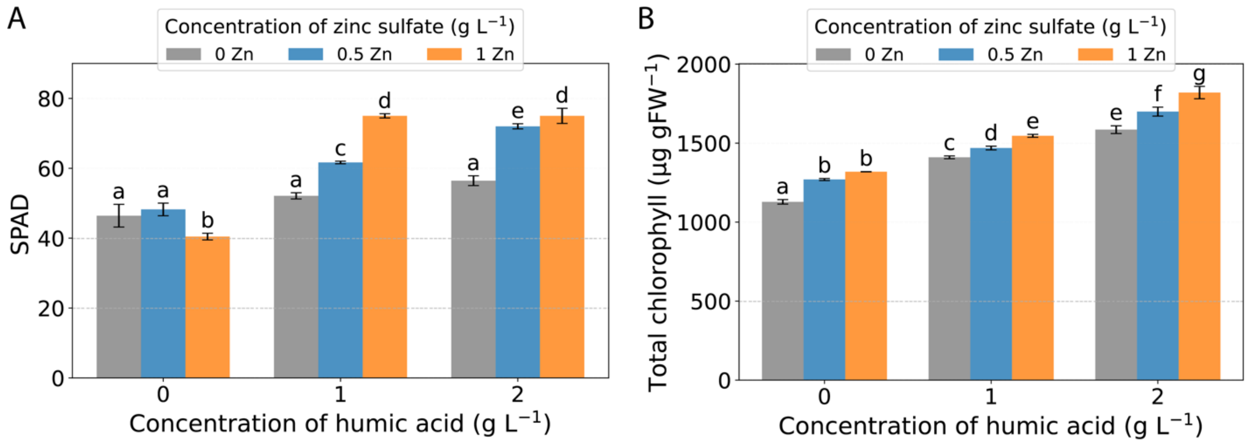

Figure 1. Effect of the interaction of humic acid and zinc sulfate on SPAD (A) **and Total chlorophyll** (B) **in *Physalis alkekengi* L. Values represent the mean of three replicates, and error bars indicate the standard deviation. Significant differences between the means are denoted by different letters, as determined by Tukey's test (p ≤ 0.05**).

3.3. Root Volume and Growth Characteristics

Root volume was significantly influenced by the treatments. The largest root volume (210 cm^3) was obtained with the simultaneous application of 2 g L^{-1} humic acid and 1 g L^{-1} zinc sulfate, whereas the control treatment resulted in the smallest root volume (65 cm^3). Interestingly, no significant differences were observed between treatments combining 2 g L^{-1} humic acid with either 0.5 or 1 g L^{-1} zinc sulfate (Fig. 2A).

3.4. Fruit Antioxidant Activity

The antioxidant activity of *Physalis alkekengi* fruit was significantly influenced by the interaction between humic acid and zinc sulfate. The highest antioxidant activity was recorded with the combination of 2 g L^{-1} humic acid and 1 g L^{-1} zinc sulfate. A statistically significant difference was observed between treatments combining 2 g L^{-1} humic acid with 0.5 g L^{-1} and 1 g L^{-1} zinc sulfate, indicating that the higher zinc concentration (1 g L^{-1}) was more effective. The control treatment exhibited the lowest antioxidant activity, underscoring the essential role of humic acid and zinc sulfate in enhancing the fruit's antioxidant properties (Fig. 2B).

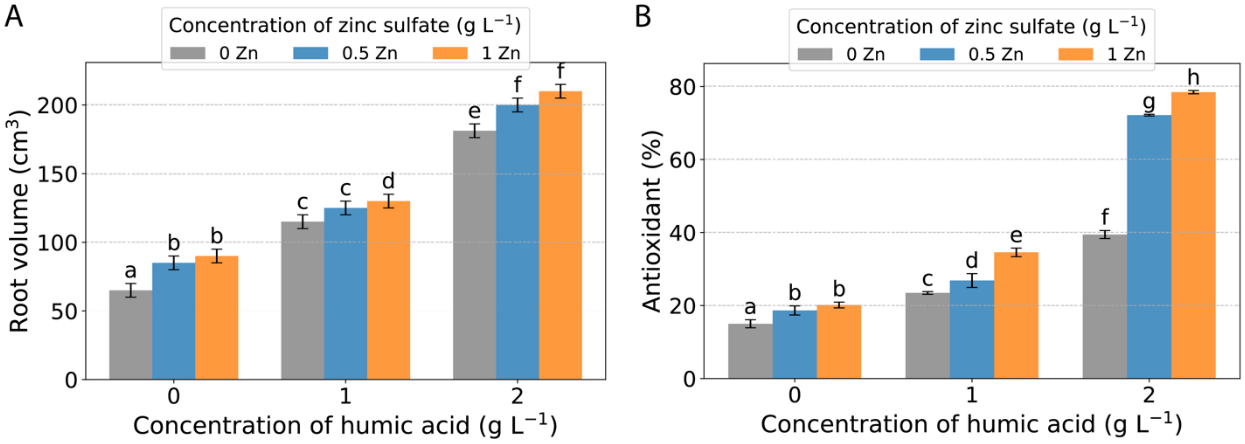

Figure2. Effect of the interaction of humic acid and zinc sulfate on Root volume (A), Antioxidant (B) in *Physalis alkekengi* L. Values represent the mean of three replicates, and error bars indicate the standard deviation. Significant differences between the means are denoted by different letters, as determined by Tukey's test ($p \le 0.05$).

3.5. Microelement Uptake

The uptake of copper (Cu), zinc (Zn), iron (Fe), and manganese (Mn) was significantly enhanced by humic acid and zinc sulfate treatments. The highest Cu uptake (0.11 mg kg^{-1}) was recorded at 2 g L^{-1} humic acid and 1 g L^{-1} zinc sulfate, while the lowest Cu uptake (0.02 mg kg^{-1}) was observed in the control (Fig. 3A). For Zn, the peak concentration (0.91 mg kg^{-1}) occurred with 2 g L^{-1} humic acid and 1 g L^{-1} zinc sulfate (Fig. 3B). Similarly, Fe and Mn uptake were maximized at intermediate and high combinations of humic acid and zinc sulfate, with the highest Fe concentration achieved at 2 g L^{-1} humic acid and 1 g L^{-1} zinc sulfate (Figs. 3C and 3D).

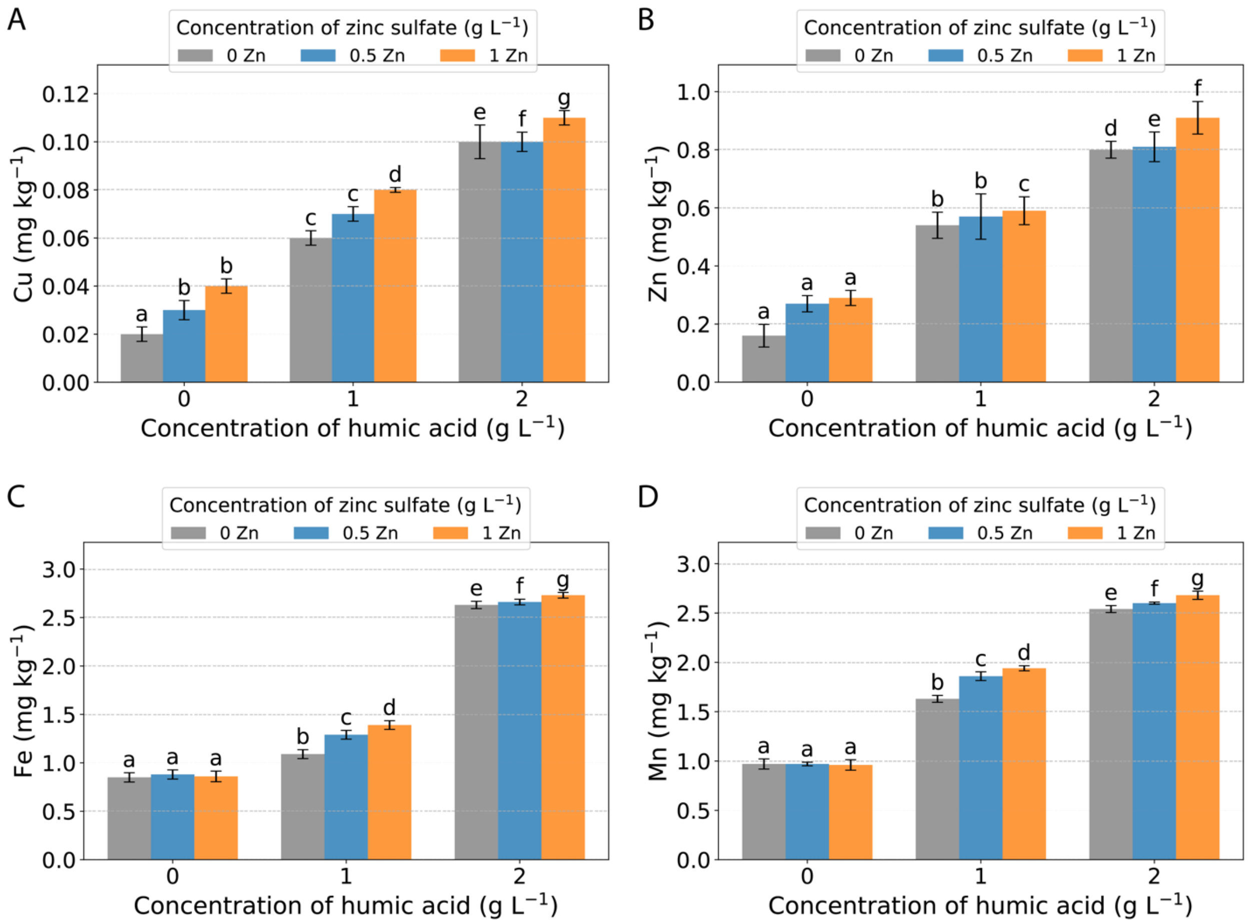

Figure 3. Effect of the interaction of humic acid and zinc sulfate on leaf micronutrients Cu (A), Zn (B), Fe (C), and Mn (D),) in *Physalis alkekengi* L. Values represent the mean of three replicates, and error bars indicate the standard deviation. Significant differences between the means are denoted by different letters, as determined by Tukey's test ($p \leq 0.05$).

3.6. Principal component analysis

The PCA biplots (Fig. 4) illustrate the relative contributions of each variable to the formation of the first two principal components (PC1 and PC2). The size and direction of the vectors indicate the magnitude and orientation of each variable's contribution. These results provide insights into the relationships among variables and their sensitivity to treatment effects. Data were first standardized to a mean of zero and a unit variance to ensure comparability among variables. PCA was then performed to reduce dimensionality and summarize the variation in physiological and biochemical parameters across the treatments. The analysis was conducted separately for each treatment group: Control, zinc sulfate (Zn), humic acid (Ha), and the interaction of humic acid and zinc sulfate (Ha + Zn). In the control group, PCA explained the total variance, with PC1 and PC2 accounting for 57.94% and 42.06% of the variance, respectively (Fig. 4A). For the zinc sulfate treatment group, PCA explained 71.14% of the variance, with PC1 and PC2 contributing 45.76% and 25.38%, respectively (Fig. 4B). Similarly, for the humic acid and the interaction of humic acid and zinc sulfate Ha + Zn treatment groups, PCA captured 69.00% and 89.98% of the total variance, respectively, across the first two components (Figs. 4C and 4D).

Regardless of direction, SPAD and Total Chlorophyll had the strongest contributions to PC1 across all treatment groups, indicating their sensitivity to treatments and their role in explaining the majority of the variance. In contrast, Cu and Zn exhibited lower contributions to PC1 in the control group but showed moderate effects in the zinc sulfate treatment. For the interaction of humic acid and zinc sulfate (Ha + Zn) treatment group, Antioxidant and Root

Volume had the largest contributions, reflecting the impact of combined treatments on these parameters. Interestingly, SPAD consistently influenced PC2, reflecting its variability under all treatments. The clear clustering of samples in the biplots also suggests distinct group-specific patterns, particularly in the interaction of humic acid and zinc sulfate (Ha + Zn) treatment, where the variability was largely explained by the measured parameters contributing to PC1.

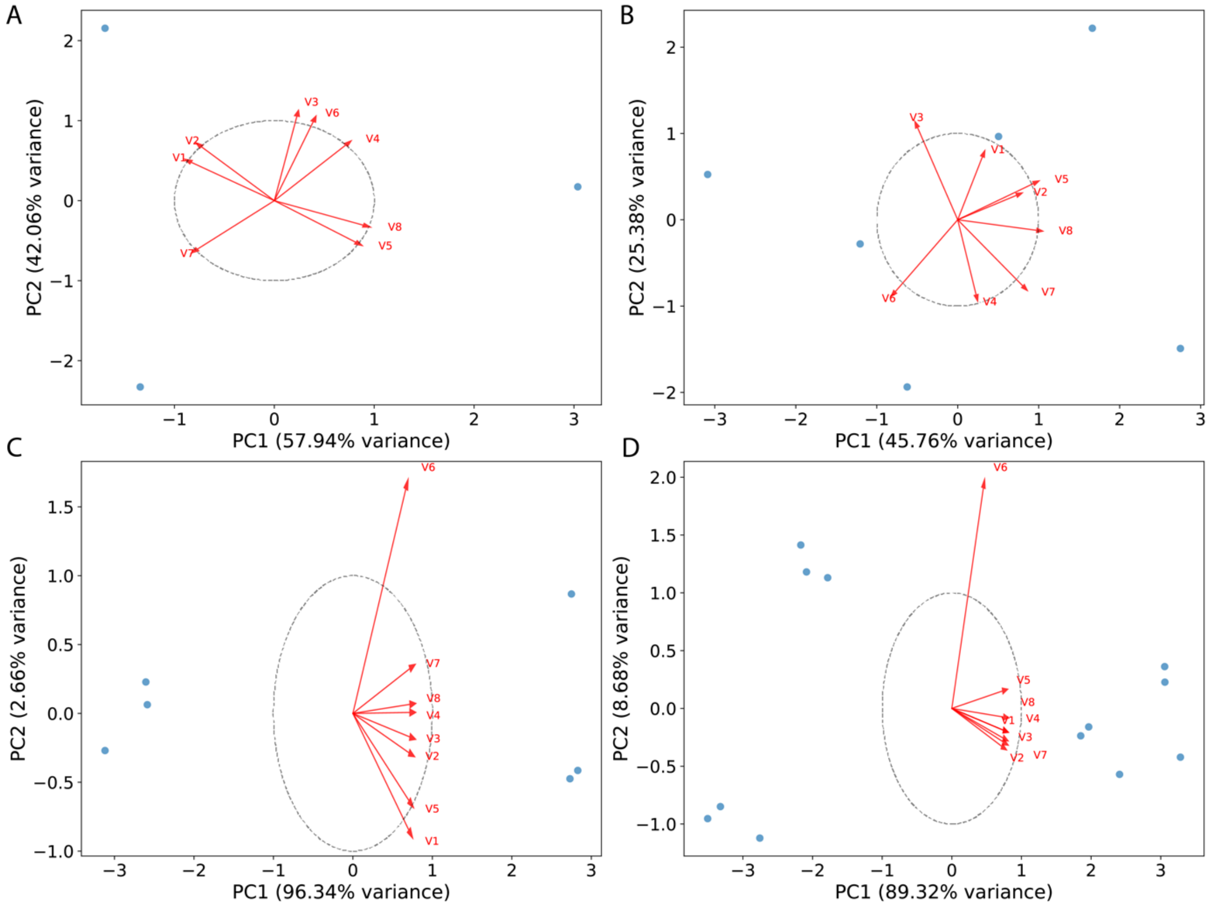

Figure 4. PCA biplots illustrating the relative contribution of each variable to the formation of the principal components (PC1 and PC2). Principal component analysis and biplots were generated using Python version 3.12. Data were analyzed across four treatments: (A) Control, (B) zinc sulfate (Zn), (C) humic acid (Ha), and (D) interaction of humic acid and zinc sulfate (Ha + Zn). Variables included: V1: Cu; V2: Zn; V3: Fe; V4: Mn; V5: Total chlorophyll; V6: SPAD; V7: Root volume; V8: Antioxidant.

4. Discussion

The findings of this study demonstrate that foliar application of humic acid and zinc sulfate significantly improved the growth, nutrient content, and biochemical characteristics of *Physalis alkekengi*. This highlights the critical role of these treatments in enhancing plant physiological and biochemical processes, which aligns with previous research on other plant species. The study demonstrated a substantial increase in chlorophyll content, as indicated by SPAD values, following foliar applications of humic acid and zinc sulfate. The variability in SPAD values across treatments can be attributed to the differential effects of humic acid and zinc sulfate concentrations on nutrient availability and uptake. Treatments with higher humic acid concentrations (2 g L^{-1}) and zinc sulfate (1 g L^{-1}) exhibited superior SPAD values due to enhanced chlorophyll biosynthesis and photosynthetic activity, whereas lower concentrations resulted in moderate improvements. This variability underscores the dose-dependent response of plants to nutrient treatments, reflecting the critical balance required for optimal photosynthetic pigment production. These observations align with previous research that documented the beneficial influence of humic acid on chlorophyll content and plant metabolism (Abd Al Ameer and Bushra, 2018) (Manas *et al*., 2014; Samadimatin and Hani, 2017) thus reinforcing the significance of these treatments. Humic acid's promotion of chlorophyll biosynthesis could be attributed to its role in enhancing nitrogen uptake and the synthesis of porphyrins, the precursors to chlorophyll (Canellas and Olivares, 2014). Likewise, zinc is integral to chlorophyll biosynthesis and maintaining chloroplast structure, playing a role in the activation of critical enzymes involved in the production of chlorophyll (Alloway, 2008) (Vanitha and Mohandass, 2014) (Rabeh *et al*., 2021) (Avinash *et al*., 2017) (Lakshmipathi *et al*., 2018). The synergistic effects of humic acid and zinc sulfate on chlorophyll content observed in this research corroborate findings by (Mohsenzadeh and Moosavian, 2017) on *Rosmarinus officinalis* and (Yılmaz *et al*., 2013) on broccolis, who noted that combined treatments enhance nitrogen availability and enzymatic activity needed for chlorophyll synthesis. Moreover, the correlation between increased chlorophyll content and SPAD values confirms the reliability of the SPAD meter as a non-destructive measure of chlorophyll concentration. Moreover, zinc supports the stabilization of chloroplast membranes and protects them from oxidative damage. This explains the significant increase in SPAD values observed in treatments with zinc sulfate, as also reported by (Manas *et al*., 2014). These studies emphasize the role of zinc in increasing photosynthetic pigment concentration and enhancing photosynthetic efficiency. These findings suggest that foliar applications of humic acid and zinc sulfate can be a practical strategy for improving photosynthetic pigments in crops, particularly under nutrient-deficient or stress-prone conditions. The observed improvements in SPAD values highlight the potential of these treatments to enhance photosynthetic performance, leading to better biomass accumulation and yield.

The significant increase in root volume observed in this study highlights the critical role of humic acid and zinc sulfate in promoting root development. This improvement can be attributed to the biochemical and hormonal activities of humic acid and the nutrient availability provided by zinc sulfate. Humic acid has been widely documented to exhibit auxin-like activity, which stimulates root elongation and branching (Amerian *et al*., 2024) (Trevisan *et al*., 2010). By promoting cell elongation and division, humic acid effectively increases root surface area, enhancing nutrient and water uptake, as demonstrated in studies by (Aman and Rab, 2013) (Olivares *et al*., 2017) and (Elmongy *et al*., 2018).

Increased root volume not only improves nutrient uptake but also enhances plant resilience to environmental stresses such as drought and salinity. A larger root system enables plants to access water and nutrients from a greater soil volume, buffering them against fluctuations in environmental conditions. Zinc sulfate also plays a pivotal role in supporting root growth by enhancing protein synthesis and cellular division, critical processes for root development (Alam *et al*., 2020) (Manas *et al*., 2014). The increase in zinc availability in the rhizosphere likely enhances root cell metabolism and overall growth, aligning with the findings of (Yılmaz *et al*., 2013) on broccoli and similar results observed in phlox (Memon *et al*., 2014). These insights underscore the synergistic benefits of incorporating both humic acid and zinc sulfate as a strategy for improving root development and nutrient uptake efficiency, and plant resilienceas, as highlighted by (Kutlu and Gulmezoglu, 2020) (Elshamly and Nassar, 2023).

The pronounced increase in antioxidant activity in *Physalis alkekengi* due to the applications of humic acid and zinc sulfate illustrates the potential of these treatments to elevate plant resilience against oxidative stress. The highest antioxidant activity was recorded in plants treated with 2 g L^{-1} humic acid and 1 g L^{-1} zinc sulfate, indicating a synergistic effect on biochemical pathways responsible for antioxidant production. Zinc's critical function in the antioxidant defense system as a cofactor for vital enzymes such as superoxide dismutase (SOD) and catalase, which mitigate reactive oxygen species (ROS), supports the observed enhancement in antioxidant activity. The observed increase in antioxidant activity aligns with studies by (López-Morales *et al*., 2020) on cowpea bean and (Mohsenzadeh and Moosavian, 2017) on *Rosmarinus officinalis*, which demonstrated the role of zinc in enhancing enzymatic antioxidant activities against abiotic stress.

Moreover, the involvement of humic acid in stimulating the production of phenolic compounds offers an additional explanation for the increase in antioxidant activity. Phenolic compounds are crucial non-enzymatic antioxidants that neutralize ROS, protecting plants from oxidative stress. This aligns with findings by (Chamani *et al*., 2015) (Vafa *et al*., 2015) (Allahvirdizadeh and Deljou, 2014) (Rahbari *et al*., 2019) (Naguib *et al*., 2012) (Alizade Ahmad Abadi *et al*., 2017), which similarly reported increased antioxidant activity and phenolic content following humic acid application. These findings not only emphasize the role of humic acid and zinc sulfate in improving plant resilience and stress tolerance but also highlight the potential health and pharmaceutical applications of *Physalis alkekengi*. Antioxidants are widely recognized for their ability to reduce oxidative stress in human cells, which is implicated in the prevention of chronic diseases such as cancer, cardiovascular disorders, and neurodegenerative conditions (Houldsworth, 2024) (Sharifi-Rad *et al*., 2020). By enhancing the antioxidant capacity of *Physalis alkekengi*, this study suggests that optimized fertilization practices can improve the therapeutic value of this medicinal plant, promoting its use in pharmaceutical and nutraceutical applications.

The application of humic acid and zinc sulfate significantly enhanced the uptake and accumulation of micronutrients (Cu, Zn, Fe, and Mn) in the leaves of *Physalis alkekengi*. This is consistent with the well-documented ability of humic acid to chelate micronutrients, making them more bioavailable to plants (Gautam *et al*., 2021) (Abay and Pirlak, 2017). The observed increase in micronutrient levels, particularly at higher concentrations of humic acid (2 g L^{-1}), aligns with the findings of (Khaled and Fawy, 2011) (Maruf and Rasul, 2019) (Vafa *et al*., 2015) (Çimrin *et al*., 2010), who reported similar results in wheat and maize plants.

Zinc sulfate plays a critical role in increasing Zn accumulation, which is essential for numerous enzymatic and physiological processes. Zinc is a cofactor for enzymes involved in protein synthesis and metabolic pathways, and its foliar application ensures direct availability to plant tissues. This highlights its pivotal role in nutrient efficiency and root development, as corroborated by studies such as (Tadayyon *et al*., 2017) (Yılmaz *et al*., 2013) (Memon *et al*., 2014), which emphasized zinc's pivotal role in nutrient efficiency and root development. Similarly, iron concentrations showed substantial improvements due to humic acid’s ability to chelate iron, improving its solubility and mobility within plant tissues. Iron plays a central role in chlorophyll synthesis and electron transport in photosynthesis, processes that demand consistent availability of this micronutrient (Kobayashi *et al*., 2019). The synergistic effects of humic acid and zinc sulfate likely facilitated the higher uptake of Zn and Fe by enhancing nutrient solubility, root permeability, and tissue absorption. Moreover, the significant increase in Fe and Mn levels observed in this study suggests that humic acid improves root permeability, allowing for better nutrient absorption. These findings corroborate the conclusions of (de Moura *et al*., 2023) (Elmongy *et al*., 2018) (Olivares *et al*., 2017). These results not only corroborate existing literature on various plant species, including evergreen azalea (Elmongy *et al*., 2018) and tomato (Kumar *et al*., 2017) but also highlight the potential of integrating humic acid and zinc sulfate into fertilization strategies for improving nutrient content in crops, particularly in nutrient-deficient soils.

This research contributes significantly to the field of precision agriculture by demonstrating how the targeted application of humic acid and zinc sulfate enhances plant growth, nutrient efficiency, and resilience to environmental stress. The observed improvements in photosynthetic pigments, root development, and antioxidant activity provide practical strategies for optimizing resource use in crops like *Physalis alkekengi*. These findings align with the principles of precision agriculture, which emphasize sustainable nutrient management, improved productivity, and resilience under stress- prone or nutrient-deficient conditions (Canellas and Olivares, 2014) (Opoku-Ware *et al*., 2024) (Alloway, 2008).

5. Conclusions

The results of this study demonstrate that the simultaneous foliar application of humic acid and zinc sulfate significantly enhanced the growth, nutrient uptake, and biochemical characteristics of *Physalis alkekengi* cultivated under soilless culture conditions. The synergistic interaction between these treatments resulted in improved micronutrient accumulation (Cu, Zn, Fe, Mn), higher photosynthetic pigment levels (chlorophyll and SPAD), enhanced root architecture, and increased antioxidant activity. The enhanced root development observed in this study underscores the role of humic acid and zinc sulfate in improving water and nutrient uptake efficiency, which is critical for plant growth in soilless systems. The findings further revealed that humic acid and zinc sulfate treatments significantly increased the antioxidant content of the fruits. Accordingly, the increased antioxidant activity and phenolic compound content highlight the potential of these treatments to boost plant resilience to abiotic stress and enhance the production of valuable secondary metabolites. These promising results support the adoption of humic acid and zinc sulfate foliar treatments as a strategy to achieve sustainable crop production in soilless agricultural systems. This approach holds significant potential for optimizing plant nutrition and enhancing the production of secondary metabolites in other medicinal plants. Future studies are encouraged to explore the application of this treatment across various soilless cultivation systems, including hydroponics, and assess its long-term effects on plant productivity and quality.

Conflict of interest

The corresponding author must inform the editor of any potential conflicts of interest that could influence the author's interpretation of the data.

References

Abay, S., Pirlak, L., 2017. Effects of iron sulfate, zinc sulfate, iron chelate, powder sulphur and humic acid applications on vegetative growth of sweet cherry (Prunus avium L.). Erwerbs-Obstbau 59, 71–75.

Abd Al Ameer, W.A., Bushra, M., 2018. Effect of mineral and organic fertilization and spraying of humic acid on growth and yield of maize (Zea mays L.). Int. J. Agric. Stat. Sci. 14, 261–265.

Ahmed, N., Zhang, B., Chachar, Z., Li, J., Xiao, G., Wang, Q., Hayat, F., Deng, L., Bozdar, B., Tu, P., 2024. Micronutrients and their effects on horticultural crop quality, productivity and sustainability. Sci. Hortic. 323, 112512.

Alam, N., Anis, M., Javed, S.B., Alatar, A.A., 2020. Stimulatory effect of copper and zinc sulphate on plant regeneration, glutathione-S-transferase analysis and assessment of antioxidant activities in Mucuna pruriens L. (DC). Plant Cell Tissue Organ Cult. PCTOC 141, 155–166. https://doi.org/10.1007/s11240-020-01776-8

Alizade Ahmad Abadi, A., Khorasaninejad, S., Hemmati, K., 2017. The effect of limited irrigation stress and humic acid on the some morphological and root phytochemical characteristics of purple coneflower. J. Crops Improv. 19, 1–14.

Allahvirdizadeh, N., Deljou, M.N., 2014. Effect of humic acid on morph-physiological traits, nutrients uptake and postharvest vase life of pot marigold cut flower (Calendula officinalis cv. Crysantha) in hydroponic system.

Alloway, B.J., 2008. Zinc in Soils and Crop Nutrition. Int. Zinc Assoc. Int. Fertil. Assoc. 16.

Aman, S., Rab, A., 2013. Response of tomato to nitrogen levels with or without humic acid. Sarhad J. Agric. 29, 181–186.

Amerian, M., Palangi, A., Gohari, G., Ntatsi, G., 2024. Humic acid and grafting as sustainable agronomic practices for increased growth and secondary metabolism in cucumber subjected to salt stress. Sci. Rep. 14, 15883.

Ampong, K., Thilakaranthna, M.S., Gorim, L.Y., 2022. Understanding the role of humic acids on crop performance and soil health. Front. Agron. 4, 848621.

Avinash, S.N., Srinivasamurthy, C.A., Bhaskar, S., 2017. Effect of foliar application of humic acid fortified with zinc and boron on growth and yield of capsicum.

Bhat, B.A., Islam, S.T., Ali, A., Sheikh, B.A., Tariq, L., Islam, S.U., Hassan Dar, T.U., 2020. Role of Micronutrients in Secondary Metabolism of Plants, in: Aftab, T., Hakeem, K.R. (Eds.), Plant Micronutrients. Springer International Publishing, Cham, pp. 311–329. https://doi.org/10.1007/978-3-030-49856-6_13

Brand-Williams, W., Cuvelier, M.-E., Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci. Technol. 28, 25–30.

Canellas, L.P., Olivares, F.L., 2014. Physiological responses to humic substances as plant growth promoter. Chem. Biol. Technol. Agric. 1, 3. https://doi.org/10.1186/2196-5641-1-3

Chamani, E., Karimi Ghalehtaki, S., Mohebodini, M., Ghanbari, A., 2015. The effect of Zinc oxide nano particles and Humic acid on morphological characters and secondary metabolite production in Lilium ledebourii Bioss. Iran J Genet Plant Breed 4, 11–19.

Çimrin, K.M., Türkmen, Ö., Turan, M., Tuncer, B., 2010. Phosphorus and humic acid application alleviate salinity stress of pepper seedling. Afr. J. Biotechnol. 9.

de Moura, O.V.T., Berbara, R.L.L., de Oliveira Torchia, D.F., Da Silva, H.F.O., de Castro, T.A. van T., Tavares, O.C.H., Rodrigues, N.F., Zonta, E., Santos, L.A., García, A.C., 2023. Humic foliar application as sustainable technology for improving the growth, yield, and abiotic stress protection of agricultural crops. A review. J. Saudi Soc. Agric. Sci. 22, 493–513.

Dobermann, A., Bruulsema, T., Cakmak, I., Gerard, B., Majumdar, K., McLaughlin, M., Reidsma, P., Vanlauwe, B., Wollenberg, L., Zhang, F., 2022. Responsible plant nutrition: A new paradigm to support food system transformation. Glob. Food Secur. 33, 100636.

Elmongy, M.S., Zhou, H., Cao, Y., Liu, B., Xia, Y., 2018. The effect of humic acid on endogenous hormone levels and antioxidant enzyme activity during in vitro rooting of evergreen azalea. Sci. Hortic. 227, 234–243.

Elshamly, A.M., Nassar, S.M., 2023. Stimulating growth, root quality, and yield of carrots cultivated under full and limited irrigation levels by humic and potassium applications. Sci. Rep. 13, 14260.

Gautam, K., Rajvanshi, M., Chugh, N., Dixit, R.B., Kumar, G.R.K., Kumar, C., Sagaram, U.S., Dasgupta, S., 2021. Microalgal applications toward agricultural sustainability: Recent trends and future prospects. Microalgae 339–379.

Ge, Y., Duan, Y., Fang, G., Zhang, Y., Wang, S., 2009. Polysaccharides from fruit calyx of Physalis alkekengi var. francheti: Isolation, purification, structural features and antioxidant activities. Carbohydr. Polym. 77, 188–193.

Hamzah Saleem, M., Usman, K., Rizwan, M., Al Jabri, H., Alsafran, M., 2022. Functions and strategies for enhancing zinc availability in plants for sustainable agriculture. Front. Plant Sci. 13, 1033092.

Hassanpour, H., 2024. Optimized medium composition in Physalis alkekengi callus culture altered nitric oxide level for inducing antioxidant enzyme activities and secondary metabolites. Sci. Rep. 14, 16425.

Hong, E.-M., Choi, J.-Y., Nam, W.-H., Kang, M.-S., Jang, J.-R., 2014. Monitoring nutrient accumulation and leaching in plastic greenhouse cultivation. Agric. Water Manag. 146, 11–23.

Houldsworth, A., 2024. Role of oxidative stress in neurodegenerative disorders: a review of reactive oxygen species and prevention by antioxidants. Brain Commun. 6, fcad356.

Kazemi, S., Pirmoradi, M.R., Karimi, H., Raghami, M., Rahimi, A., Kheiry, A., Malekzadeh, M.R., 2023. Effect of Foliar Application of Humic Acid and Zinc Sulfate on Vegetative, Physiological, and Biochemical Characteristics of Physalis alkekengi L. Under Soilless Culture. J. Soil Sci. Plant Nutr. 23, 3845–3856. https://doi.org/10.1007/s42729-023-01305-4

Khaled, H., Fawy, H.A., 2011. Effect of different levels of humic acids on the nutrient content, plant growth, and soil properties under conditions of salinity. Soil Water Res. 6, 21.

Kobayashi, T., Nozoye, T., Nishizawa, N.K., 2019. Iron transport and its regulation in plants. Free Radic. Biol. Med. 133, 11–20.

Kumar, H., Kaushik, R.A., Ameta, K.D., Regar, A.L., Singh, K., Rajawat, K.S., Kumari, P., 2017. Effect of humic acid and nutrients mixture on quality parameter of Tomato (Lycopersicon esculentum Mill.) under polyhouse condition. J. Appl. Nat. Sci. 9, 1369–1372.

Kutlu, I., Gulmezoglu, N., 2020. Morpho-agronomic characters of oat growing with humic acid and zinc application in different sowing times. Plant Sci. Today 7, 594–600.

Lakshmipathi, J.D., Adiga, D., Kalaivanan, B., Muralidhara, M., Preethi, P., 2018. Effect of zinc and boron application on leaf area, photosynthetic pigments, stomatal number and yield of cashew. Intl J Curr Microbiol Appl Sci 7, 1795.

López-Morales, D., De La Cruz-Lazaro, E., Sánchez-Chávez, E., Preciado-Rangel, P., Márquez-Quiroz, C., Osorio-Osorio, R., 2020. Impact of agronomic biofortification with zinc on the nutrient content, bioactive compounds, and antioxidant capacity of cowpea bean (Vigna unguiculata L. Walpers). Agronomy 10, 1460.

Manas, D., Bandopadhyay, P.K., Chakravarty, A., Pal, S., Bhattacharya, A., 2014. Effect of foliar application of humic acid, zinc and boron on biochemical changes related to productivity of pungent pepper (Capsicum annuum L.). Afr J Plant Sci 8, 320–335.

Martins, E.M., Pillajo, J.Q., Jones, M.L., 2024. Humic and Fulvic Acids Promote Growth and Flowering in Petunias at Low and Optimal Fertility. HortScience 59, 235–244.

Maruf, M.T., Rasul, G.A.M., 2019. Influence of humic acid and sulfur on the bioavailability of some micronutrients in calcareous soils. Plant Arch 19, 1785–1794.

Memon, S.A., Baloch, R.A., Baloch, M.H., 2014. Influence of humic acid and micronutrients (zinc+ manganese) application on growth and yield of phlox (Phlox paniculata).

Mishra, P.P., n.d. Impact of Nutrients and Biostimulant on Growth, Flowering and Postharvest Life of Different Cultivars of African Marigold (Tagetes erecta L.).

Mohsenzadeh, S., Moosavian, S.S., 2017. Zinc sulphate and nano-zinc oxide effects on some physiological parameters of Rosmarinus officinalis. Am. J. Plant Sci. 8, 2635.

Morais, E.G. de, Silva, C.A., Jindo, K., 2021. Humic acid improves zn fertilization in oxisols successively cultivated with maize–brachiaria. Molecules 26, 4588.

Naguib, A.E.-M.M., El-Baz, F.K., Salama, Z.A., Hanaa, H.A.E.B., Ali, H.F., Gaafar, A.A., 2012. Enhancement of phenolics, flavonoids and glucosinolates of Broccoli (Brassica olaracea, var. Italica) as antioxidants in response to organic and bio-organic fertilizers. J. Saudi Soc. Agric. Sci. 11, 135–142.

Olivares, F.L., Busato, J.G., De Paula, A.M., Da Silva Lima, L., Aguiar, N.O., Canellas, L.P., 2017. Plant growth promoting bacteria and humic substances: crop promotion and mechanisms of action. Chem. Biol. Technol. Agric. 4, 30. https://doi.org/10.1186/s40538-017-0112-x

Opoku-Ware, K., Kazemi, S., Liang, X., Li, L., 2024. Drone Remote Sensing and Evapotranspiration Modeling for Intercropping and Irrigation strategies Study, in: 2024 ASABE Annual International Meeting. American Society of Agricultural and Biological Engineers, p. 1.

Porra, R.J., Thompson, W.A., Kriedemann, P.E., 1989. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochim. Biophys. Acta BBA-Bioenerg. 975, 384–394.

Rabeh, H., El-Salam, A., Badawy, S.H., 2021. Effect of Zinc Foliar Application Splits and Rates Integrated with Humic Acid on Growth, Yield, and Grain Quality of Broadcast-Seeded Rice (Oryza sativa L.) in Northern Nile Delta Region, Egypt. J. Plant Prod. 12, 505–515.

Rahbari, A., Masoud Sinaki, J., Damavandi, A., Rezvan, S., 2019. Responses of castor (Ricinus communis L.) To foliar application of zinc nano-chelate and humic acid under limited irrigation. J. Agric. Sci. Sustain. Prod. 29, 153–171.

Rai, S., Singh, P.K., Mankotia, S., Swain, J., Satbhai, S.B., 2021. Iron homeostasis in plants and its crosstalk with copper, zinc, and manganese. Plant Stress 1, 100008.

Roosta, H.R., Estaji, A., Niknam, F., 2018. Effect of iron, zinc and manganese shortage-induced change on photosynthetic pigments, some osmoregulators and chlorophyll fluorescence parameters in lettuce. Photosynthetica 56, 606–615. https://doi.org/10.1007/s11099-017-0696-1

Roosta, H.R., Safarizadeh, M., Hamidpour, M., 2017. Effect of humic acid contained nano-fertile fertilizer spray on concentration of some nutrient elements in two lettuce cultivars in hydroponic system. J. Soil Plant Interact.-Isfahan Univ. Technol. 7, 51–59.

Samadimatin, A., Hani, A., 2017. Effect of ethanol and humic acid foliar spraying on morphological traits, photosynthetic pigments and quality and quantity of essential oil content of Dracocephalum moldavica L. Iran. J. Plant Physiol. 8, 2299–2306.

Shanmugavel, D., Rusyn, I., Solorza-Feria, O., Kamaraj, S.-K., 2023. Sustainable SMART fertilizers in agriculture systems: A review on fundamentals to in-field applications. Sci. Total Environ. 904, 166729.

Sharifi-Rad, M., Anil Kumar, N.V., Zucca, P., Varoni, E.M., Dini, L., Panzarini, E., Rajkovic, J., Tsouh Fokou, P.V., Azzini, E., Peluso, I., 2020. Lifestyle, oxidative stress, and antioxidants: back and forth in the pathophysiology of chronic diseases. Front. Physiol. 11, 694.

Tadayyon, A., Beheshti, S., Pessarakli, M., 2017. Effects of sprayed humic acid, iron, and zinc on quantitative and qualitative characteristics of niger plant (*Guizotia abyssinica* L.). J. Plant Nutr. 40, 1644–1650. https://doi.org/10.1080/01904167.2016.1270321

Trevisan, S., Francioso, O., Quaggiotti, S., Nardi, S., 2010. Humic substances biological activity at the plant-soil interface: From environmental aspects to molecular factors. Plant Signal. Behav. 5, 635–643. https://doi.org/10.4161/psb.5.6.11211

Vafa, Z.N., Sirousmehr, A.R., Ghanbari, A., Khammari, I., Falahi, N., 2015. Effects of nano zinc and humic acid on quantitative and qualitative characteristics of savory (Satureja hortensis L.).

Vanitha, K., Mohandass, S., 2014. Effect of humic acid on plant growth characters and grain yield of drip fertigated aerobic rice (Oryza sativa L.). The bioscan 9, 45–50.

Yang, J., Sun, Y., Cao, F., Yang, B., Kuang, H., 2022. Natural products from Physalis alkekengi L. var. franchetii (Mast.) Makino: A review on their structural analysis, quality control, pharmacology, and pharmacokinetics. Molecules 27, 695.

Yılmaz, E., Naif, G., Sezer, Ş., Ayşegül, D., Necdettin, S., Mine, A., 2013. Interactive effects of humic acid and zinc on yield and quality in broccoli. Soil Water J. 1, 287–293.

Zanin, L., Tomasi, N., Cesco, S., Varanini, Z., Pinton, R., 2019. Humic substances contribute to plant iron nutrition acting as chelators and biostimulants. Front. Plant Sci. 10, 675.

Zhou, W., Lv, H., Chen, F., Wang, Q., Li, J., Chen, Q., Liang, B., 2022. Optimizing nitrogen management reduces mineral nitrogen leaching loss mainly by decreasing water leakage in vegetable fields under plastic-shed greenhouse. Environ. Pollut. 308, 119616.