

## ARTICLE

# Fulvic acid improves morphophysiological traits and vase life in *Alstroemeria* ‘Orange Queen’ in soilless conditions

Ácido fúlvico melhora características morfofisiológicas e vida de vaso em *Alstroemeria* ‘Orange Queen’ cultivada sem solo

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## Abstract

This study evaluated the potential of fulvic acid as a biostimulant to enhance flowering indices, biochemical traits, and vase life in *Alstroemeria* ‘Orange Queen’. Conducted under controlled greenhouse conditions, the experiment involved foliar applications of fulvic acid at concentrations of 50, 100, and 200 mg L<sup>-1</sup>. The results revealed significant enhancements in key parameters, particularly with the 100 mg L<sup>-1</sup> treatment. This optimal concentration notably increased bud diameter, bud length, and floret fresh and dry weights compared to the control. In addition, fulvic acid application significantly boosted chlorophyll *a*, chlorophyll *b*, total chlorophyll, carotenoid, and anthocyanin contents, thereby improving photosynthetic efficiency and flower pigmentation. Enhanced carbohydrate accumulation was evidenced by increased soluble sugar levels in leaves and petals, particularly at 100 mg L<sup>-1</sup>. Moreover, fulvic acid significantly extended the vase life of cut flowers, with the 100 mg L<sup>-1</sup> concentration providing the greatest benefit. These findings underscore the effectiveness of fulvic acid as a biostimulant for optimizing both aesthetic and physiological traits in *Alstroemeria*. By promoting growth, improving pigment composition, and extending flower longevity, fulvic acid emerges as a valuable tool in ornamental horticulture.

**Keywords:** Biochemical traits, Biostimulant, Flowering indices, Photosynthetic pigments, Vase life.

## Resumo

Este estudo avaliou o potencial do ácido fúlvico como um bioestimulante para aumentar os índices de floração, características bioquímicas e vida de vaso em *Alstroemeria* ‘Orange Queen’. Conduzido sob condições controladas de estufa, o experimento envolveu aplicações foliares de ácido fúlvico em concentrações de 50, 100 e 200 mg L<sup>-1</sup>. Os resultados revelaram melhorias significativas em parâmetros-chave, particularmente com o tratamento de 100 mg L<sup>-1</sup>. Esta concentração ótima aumentou notavelmente o diâmetro do broto, o comprimento do broto e os pesos fresco e seco do florete em comparação com o controle. Além disso, a aplicação de ácido fúlvico aumentou significativamente os teores de clorofila *a*, clorofila *b*, clorofila total, carotenoides e antocianinas, melhorando assim a eficiência fotossintética e a pigmentação das flores. O maior acúmulo de carboidratos foi evidenciado pelo aumento dos níveis de açúcar solúvel nas folhas e pétalas, particularmente em 100 mg L<sup>-1</sup>. Além disso, o ácido fúlvico estendeu significativamente a vida útil do vaso de flores cortadas, com a concentração de 100 mg L<sup>-1</sup> fornecendo o maior benefício. Essas descobertas ressaltam a eficácia do ácido fúlvico como um bioestimulante para otimizar características estéticas e fisiológicas em *Alstroemeria*. Ao promover o crescimento, melhorar a composição do pigmento e estender a longevidade das flores, o ácido fúlvico surge como uma ferramenta valiosa na horticultura ornamental.

Palavras-chave: Características bioquímicas, Bioestimulante, Índices de floração, Pigmentos fotossintéticos, Vida de vaso.

## Introduction

*Alstroemeria* (*Alstroemeria* sp.), commonly known as the Peruvian lily or lily of the Incas, is a prominent ornamental plant celebrated for its vibrant and striking flowers. Native to the Andes region of South America, this species has gained global recognition for its aesthetic appeal, wide range of flower colors, and ease of cultivation, making it a staple in floral arrangements and landscaping (Ranjeetha, 2024). The economic significance of *Alstroemeria* in the floral industry is largely attributed to its long-lasting blooms and intricate floral patterns, which offer an extensive palette of colors and designs, enhancing the visual appeal of bouquets and floral displays. Moreover, its adaptability to diverse environmental conditions and natural resistance to certain pests and diseases make it highly desirable for both growers and consumers (Seyed Hajzadeh et al., 2024). Optimal cultivation of *Alstroemeria* necessitates specific management practices to ensure high-quality growth and flower production. The plant thrives in well-drained, nutrient-rich soils but is sensitive to extreme temperature variations, requiring careful regulation of growing conditions. Recent innovations in soilless cultivation systems, such as cocopeat-perlite substrates, have significantly advanced nutrient management and environmental control, thereby enhancing plant performance and floral quality (Dhiman and Kashyap, 2022).

Fulvic acid, a low molecular weight humic substance characterized by its abundance of acidic functional groups and high oxygen content, plays a pivotal role in promoting plant growth and development. Unlike humic acid, which dissolves only in alkaline conditions, fulvic acid is soluble across a

wide pH range, including both acidic and alkaline environments (Ampong et al., 2022). As a highly effective chelator with a notable ion exchange capacity, fulvic acid enhances the uptake of essential mineral nutrients and strengthens plant resilience against environmental stressors. These attributes contribute to significant improvements in both the quality and quantity of crop yields (Sharaya et al., 2023). The positive effects of humic substances, including fulvic acid, have been extensively documented in ornamental plants. For example, studies on *Gerbera* (*Gerbera jamesonii* "Bolus ex Hook.f.) revealed that the application of humic acid at concentrations of 500, 1000, and 2000 mg L<sup>-1</sup> significantly enhanced leaf length, fresh and dry weight, flower diameter, and stem length, with the most pronounced benefits observed at 2000 mg L<sup>-1</sup> (Mirzaee Esgandian et al., 2020). Similarly, in *Calendula* (*Calendula officinalis* L.), humic acid applications improved various morphological traits, such as increased leaf length, flower number per plant, and flower dimensions (Ahmad et al., 2019).

*Alstroemeria*, celebrated for its vibrant flower colors, intricate patterns, and adaptability to diverse environmental conditions, plays a vital role in ornamental horticulture. Its exceptional aesthetic qualities and significant economic value make it a cornerstone of the floral industry (Seyed Hajzadeh et al., 2024). The continuous refinement of cultivation practices, including optimized nutrient delivery systems and precise postharvest handling techniques, is crucial for unlocking the plant's full commercial potential. Moreover, addressing cultivation challenges such as temperature sensitivity and pest management is imperative to enhance market value and grower satisfaction (Bridgen, 2018).

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In recent years, fulvic acid has gained recognition as a potent biostimulant in ornamental horticulture due to its multifunctional benefits for plant growth and development (Nikoogoftar-Sedghi et al., 2024). Fulvic acid enhances nutrient uptake efficiency, mitigates abiotic stress, and increases carbohydrate reserves in leaves and petals, contributing to superior flower quality and prolonged postharvest vase life (Bayat et al., 2021). This study aims to assess the effects of varying concentrations of fulvic acid on the morphophysiological and biochemical attributes of *Alstroemeria* ‘Orange Queen’. Specific objectives include evaluating its influence on chlorophyll content, carbohydrate reserves, and overall flower quality. Additionally, the research seeks to identify the optimal fulvic acid concentration to maximize these traits and extend vase life, thereby supporting sustainable and high-quality ornamental production.

## Materials and Methods

### Plant material and growth conditions

The rhizomes of *Alstroemeria* ‘Orange Queen,’ distinguished by its vibrant orange flowers adorned with characteristic dark spots on the petals, were utilized in this study (Fig. 1). These rhizomes, procured from Royal Van Zanten in the Netherlands, were supplied by a commercial greenhouse located in Varamin, Iran. The rhizomes were planted in 7-liter pots measuring 29 cm in height and 24 cm in diameter. The experiment was conducted under carefully regulated greenhouse conditions. Daytime temperatures were maintained at 18–21 °C, while nighttime temperatures ranged from 10–13 °C. The light intensity was controlled within a range of 400–500  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , and the relative humidity was stabilized between 60%–70% (Fig. 2).

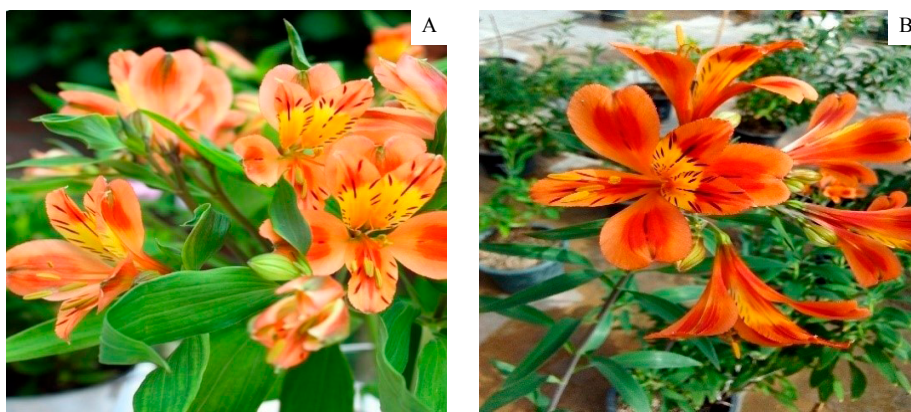


Fig. 1. Flowers of *Alstroemeria* ‘Orange Queen,’ (A, B) characterized by vibrant orange petals with distinctive dark spots.



Fig. 2. *Alstroemeria* plants grown in pots in the greenhouse, as used in the present research.

The experiment was carried out using a completely randomized design with four treatments: three concentrations of fulvic acid (Thomson Fulvico, Humet) at 50, 100, and 200  $\text{mg L}^{-1}$ , and a negative control (0  $\text{mg L}^{-1}$ ), where plants were irrigated with distilled water without any fulvic acid application (Yu et al., 2023). Each treatment was replicated six times, with each replication consisting of one pot containing a single plant, resulting in six pots per treatment, each representing an independent replication. The plants were grown in a soilless culture medium consisting of perlite and cocopeat in a 1:3 (v/v) ratio. Throughout the growth and flowering stages, plants were irrigated with a nutrient solution, as outlined in Table 1. Fulvic acid treatments were applied as a drench every two weeks for two months.

Table 1. Nutrient program used for growing *Alstroemeria* in a 100 L nutrient solution

$\text{Mg}(\text{SO}_4)_2$	$\text{KNO}_3$	$\text{NH}_4\text{NO}_3$	$\text{Na}_2\text{MoO}_4$	$\text{Na}_2[\text{B}_4\text{O}_5](\text{OH})_4$	MAP	$\text{MnSO}_4$	$\text{ZnSO}_4$	$\text{K}_2\text{SO}_4$	Fe chelate 6%	$5\text{Ca}(\text{NO}_3)_2\text{-NH}_4\text{NO}_3 \cdot 10\text{H}_2\text{O}$
10 g	32 g	4 g	0.035 g	0.03 g	5 g	0.2 g	0.15 g	8 g	5 g	10g

### Measurement of morphological traits

Approximately 10 days after the first flower buds became visibly detectable, the buds were measured. The bud diameter was determined at both the base and the middle using a digital caliper, with the average diameter recorded in millimeters (mm) to a precision of 0.01 mm. The flower bud length, measured from the base of petal emergence to the tip, was also recorded with the same caliper, and values were reported to an accuracy of 0.01 mm. For fresh weight determination, floret samples were randomly selected from each pot (two stems per pot, with three flowers per stem) and immediately weighed using a digital scale (METTLER PJ300, Toledo) with a precision of 0.01 g. For dry weight analysis, the samples

were placed in paper bags and dried in an oven at 72 °C for 24 hours. After drying, the samples were reweighed using the same digital scale.

### Measurement of photosynthetic pigments

Chlorophyll content, including chlorophyll *a*, chlorophyll *b*, and total chlorophyll, was measured following the method described by Lichtenthaler (1987). Leaf tissue (0.1 g from fully developed leaves) was ground in a mortar with 5 mL of 100% acetone to create a homogenate. The samples were then centrifuged at 2500 rpm for 10 minutes using a brushless D.C. centrifuge. The spectrophotometer (HALODB-20, Dynamica) was calibrated with acetone. Two mL of the supernatant were

transferred to a spectrophotometer cuvette, and absorbance was measured at wavelengths of 663 nm and 645 nm. Chlorophyll *a*, chlorophyll *b*, and total chlorophyll concentrations were calculated using the following formulas:

- Chlorophyll *a* (mg g<sup>-1</sup> F.W.) =  $11.75 \times (A_{663}) - 2.350 \times (A_{645}) \times (V/(1000 \times wt))$
- Chlorophyll *b* (mg g<sup>-1</sup> F.W.) =  $18.61 \times (A_{645}) - 3.96 \times (A_{663}) \times (V/(1000 \times wt))$
- Total Chlorophyll (mg g<sup>-1</sup> F.W.) =  $20.2 \times (OD_{645}) + 8.02 \times (OD_{663}) \times (V/(1000 \times wt))$

Where wt represents the fresh weight of the leaf sample in milligrams (mg) and V denotes the sample volume in milliliters (mL).

#### Measurement of total anthocyanin

Total anthocyanin content was quantified by grinding 0.1 g of petal sample in a mortar with 10 mL of acidic methanol (methanol containing 1% hydrochloric acid, v/v). The extract was incubated in the dark at 25 °C for 24 hours. After incubation, the extract was centrifuged at 4000 rpm for 10 minutes, and the absorbance of the supernatant was measured at 550 nm using a spectrophotometer. Anthocyanin concentration was calculated using the Beer-Lambert law:  $A = \epsilon bc$  where the extinction coefficient ( $\epsilon$  epsilon) is 33,000 mol cm<sup>-2</sup>. The final concentration was reported in  $\mu\text{mol g}^{-1}$  fresh weight (F.W.) (Wagner, 1979). In this formula, A represents the sample absorbance, b is the path length of the cuvette (in cm), and c is the concentration of the anthocyanin solution.

#### Measurement of sugar content

Soluble sugars were quantified using the anthrone method. To prepare the sample, 0.1 mL of alcoholic extract from leaves and petals was mixed with 3 mL of freshly prepared anthrone reagent (0.675 g anthrone dissolved in 45 mL of 72% sulfuric acid). The mixture was then incubated in a hot water bath at 100 °C for 10 minutes to develop the color. After cooling to room temperature, the absorbance was measured at 625 nm using a spectrophotometer (Irigoyen et al., 1992).

#### Total phenolic contents

Total phenolic content was measured using the Folin-Ciocalteu reagent, as described by Marinova et al. (2005). For the reaction mixture, 0.5 g of petal tissue was ground in a mortar with 5 mL of 85% methanol. The mixture was allowed to incubate in the dark at 25 °C for 24 hours and then centrifuged at 5000 rpm for 15 minutes to separate the supernatant. The supernatant was filtered through fine filter paper or a syringe filter. A 0.5 mL aliquot of the filtered extract was mixed with 2.5 mL of Folin-Ciocalteu reagent (diluted 10-fold with distilled water). After 5 minutes, 10 mL of sodium carbonate solution was added. The mixture was incubated at room

temperature for 90 minutes in the dark. The absorbance was measured at 750 nm using a spectrophotometer. Total phenolic content was determined using a standard curve prepared with gallic acid and expressed as mg gallic acid equivalents (GAE) per g fresh weight (F.W.).

#### Carotenoid contents

Carotenoid content was measured due to its significance in plant tissues. A 0.1 g leaf tissue sample was ground with 5 mL of 100% acetone. The homogenate was then centrifuged at 2500 rpm for 10 minutes, and the total carotenoid content was determined by spectrophotometry at 470 nm, following Lichtenthaler (1987). The results were expressed as mg g<sup>-1</sup> fresh weight using the following formula:

$$\text{Carotenoid} = 1000 A_{470} - 2.270 \text{ Chl } a - 81.4 \text{ Chl } b/227$$

#### Vase life

Cut flowers were harvested early in the morning when one to two florets had partially opened. Immediately upon harvest, the flowers were transferred to the laboratory, where the lower leaves were removed, and the stem ends were cut under running water to a final length of 50 cm. The stems were then placed in vessels containing 250 mL of solution and kept under controlled conditions: a temperature of 22 °C, relative humidity of 75%, and a 12-hour light period with an intensity of 13  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Vase life was determined based on a visual assessment of flower health, which was evaluated daily. Flowers were considered to have reached the end of their vase life when 50% or more of the petals exhibited significant wilting, discoloration, or loss of aesthetic appeal. Additional criteria included 50% leaf yellowing or wilting and the collapse of flower buds (Mutui et al., 2006). Flower health was assessed daily, with visual cues such as wilting of leaves, discoloration of petals, and overall collapse of the flower structure. Flowers were monitored until they met the defined criteria for termination of vase life (Fig. 3).

#### Data analysis

Data analysis was performed using SAS software version 9.2. Mean comparisons were carried out using Tukey's multiple range test at a significance level of 1%.

## Results and Discussion

#### Flower bud diameter and length

As shown in Fig. 4, treatments with fulvic acid at concentrations of 100 and 200 mg L<sup>-1</sup> significantly increased bud diameter compared to the control. Additionally, the 100 mg L<sup>-1</sup> concentration of fulvic acid notably enhanced bud length, exhibiting an approximately 40% increase relative to the control (Fig. 4).

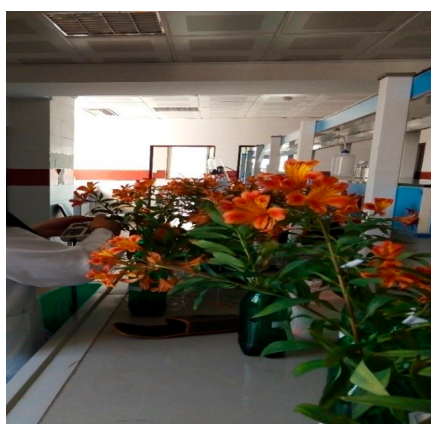
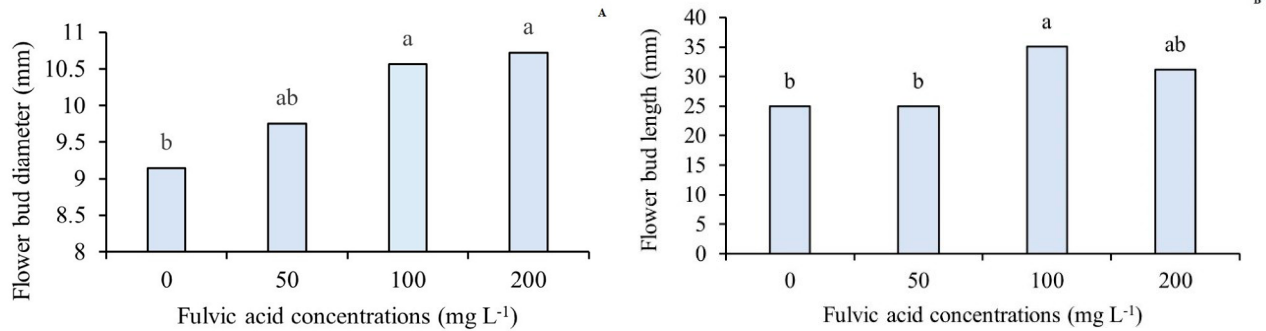


Fig. 3. *Alstroemeria* cut flowers used to evaluate vase life: (A) during vase life and (B) at the end of vase life.



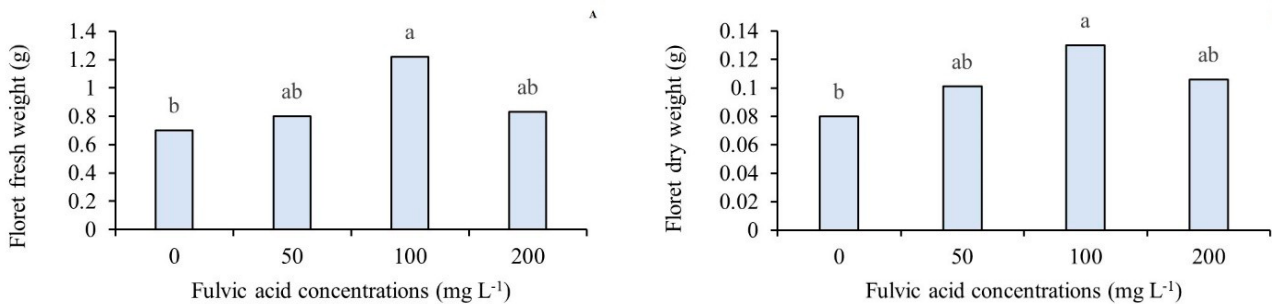
**Fig. 4.** Effect of different fulvic acid concentrations on flower bud diameter (A) and length (B) of *Alstroemeria* 'Orange Queen'. Data represents mean values with different letters indicating significant differences at the 1% probability level, as determined by Tukey's multiple range test.

The application of fulvic acid significantly enhanced flowering indices, including bud diameter and length, by acting as a natural growth enhancer. It modulates the synthesis and activity of key plant growth regulators, such as auxins, gibberellins, and cytokinins, which regulate cell elongation, division, and differentiation - essential processes for bud development. Auxins promote elongation, cytokinins stimulate division, and gibberellins coordinate both, resulting in larger buds (Mazzoni-Putman et al., 2021). Fulvic acid also chelates essential minerals like nitrogen, phosphorus, and potassium, improving nutrient availability and uptake. This supports cellular activities, including division and expansion, crucial for bud growth (Alsudays et al., 2024;

Sahraie et al., 2024). Additionally, it enhances chlorophyll synthesis, boosting photosynthetic efficiency and energy conversion, which further promotes vigorous bud development. Studies confirm that fulvic acid positively influences flower development by optimizing hormonal balance (Kisvarga et al., 2022).

**Floret Fresh and Dry Weight**

Analysis revealed that only the 100 mg L<sup>-1</sup> concentration of fulvic acid significantly increased both floret fresh and dry weight compared to the control, with no significant differences observed for other concentrations (Fig. 5).



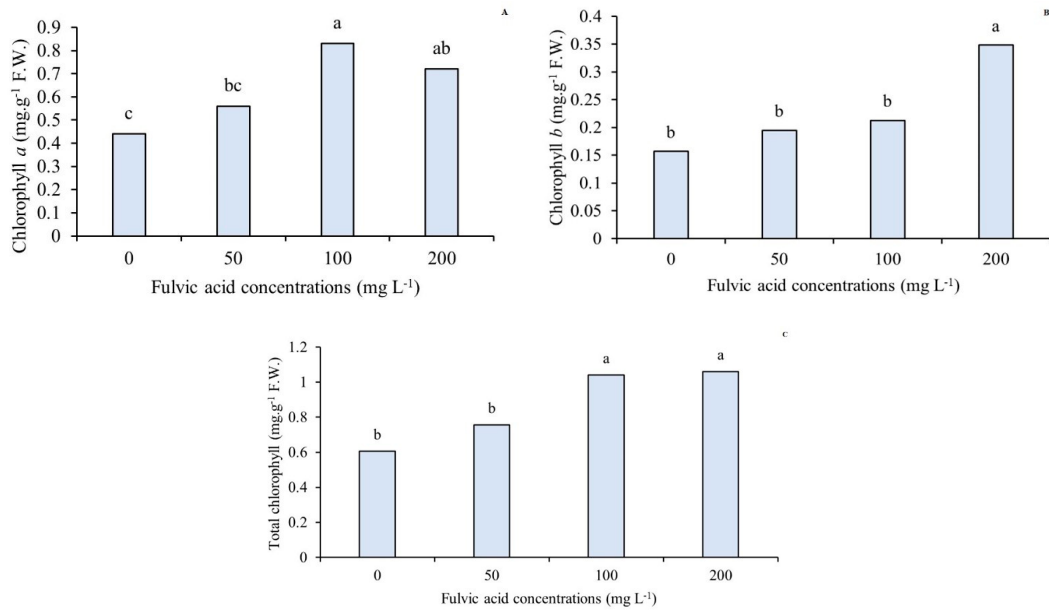
**Fig. 5.** Effect of different fulvic acid concentrations on floret fresh (A) and dry weight (B) of *Alstroemeria* 'Orange Queen'. Data represents mean values with different letters indicating significant differences at the 1% probability level, as determined by Tukey's multiple range test.

Fulvic acid enhances the absorption of essential nutrients, including nitrogen, phosphorus, and potassium, which are critical for plant growth and development (Moura et al., 2023). By chelating these nutrients, it increases their bioavailability, promoting metabolic processes such as photosynthesis and protein synthesis. This, in turn, drives biomass accumulation in flowers through enhanced cell division and expansion (Baltazar et al., 2021). Additionally, fulvic acid facilitates nucleic acid and protein synthesis, essential for cell proliferation and growth. Improved carbohydrate metabolism and energy availability further support the increase in flower biomass. Studies confirm that fulvic acid significantly boosts plant growth and biomass by improving nutrient use efficiency (Liu et al., 2022).

**Photosynthetic pigments**

*Chlorophyll a, b, and total chlorophyll Content*

Figure 6 illustrates that both 100 and 200 mg L<sup>-1</sup> concentrations of fulvic acid significantly enhanced chlorophyll *a* content, with the 100 mg L<sup>-1</sup> concentration showing a 46% increase compared to the control. Additionally, the 200 mg L<sup>-1</sup> concentration significantly boosted chlorophyll *b* content, resulting in a 2.5-fold increase relative to the control. Both fulvic acid concentrations also led to a significant increase in total chlorophyll content, whereas no significant difference was observed between the control and the 50 mg L<sup>-1</sup> concentration.

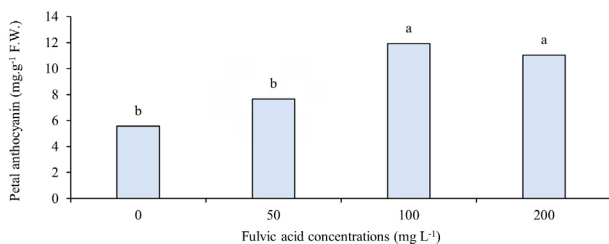


**Fig. 6.** Effect of different fulvic acid concentrations on chlorophyll *a*, *b*, and total chlorophyll (A, B, C) content of *Alstroemeria* 'Orange Queen'. Data represents mean values with different letters indicating significant differences at the 1% probability level, as determined by Tukey's multiple range test.

The application of fulvic acid significantly increased the levels of photosynthetic pigments, including chlorophyll *a*, *b*, and total chlorophyll. The 100 and 200 mg L<sup>-1</sup> concentrations notably affected these indices. Chlorophyll synthesis relies on the availability of essential micronutrients, especially magnesium, a central component of the chlorophyll molecule. Fulvic acid enhances the chelation and transport of magnesium and other micronutrients, improving their uptake and movement within the plant. This promotes chlorophyll biosynthesis, increasing photosynthetic efficiency, deepening green pigmentation, and enhancing growth performance (Turan et al., 2022). Recent studies highlight fulvic acid's role in improving chlorophyll content by boosting micronutrient availability (Nikoogofar-Sedghi et al., 2024).

#### Petal Anthocyanins

Figure 7 illustrates that both 100 and 200 mg L<sup>-1</sup> concentrations of fulvic acid resulted in a two-fold increase in petal anthocyanin levels compared to the control. No significant difference was observed between the control and the 50 mg L<sup>-1</sup> concentration.



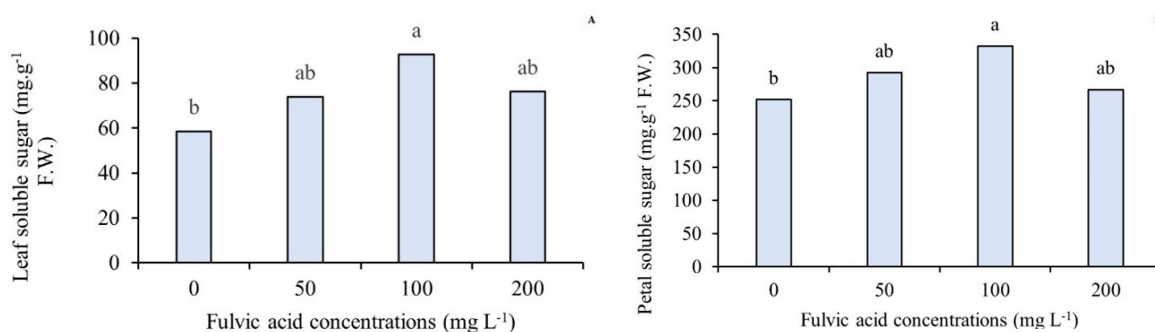
**Fig. 7.** Effect of different fulvic acid concentrations on petal anthocyanin content of *Alstroemeria* 'Orange Queen'. Data represents mean values with different letters indicating significant differences at the 1% probability level, as determined by Tukey's multiple range test.

The application of fulvic acid, especially at a concentration of 100 mg L<sup>-1</sup>, significantly increased petal anthocyanin content compared to the control. Anthocyanins, key flavonoid pigments, are responsible for flower coloration and provide plants with protection against UV radiation and oxidative stress. Fulvic acid stimulates the expression of genes involved in the anthocyanin biosynthetic pathway, including chalcone synthase (CHS) and anthocyanidin synthase (ANS), leading to increased anthocyanin production and more vibrant flower colors, thus enhancing stress tolerance and aesthetic appeal (Mannino et al., 2021). Furthermore, Yao et al. (2023) emphasized the connection between pigment development, anthocyanin production, and carbohydrate levels. Increased sugar availability activates genes associated with anthocyanin synthesis, further promoting its production. Biostimulants like humic substances can enhance the synthesis and secretion of biologically active compounds and hormones, boosting plant organic matter and carbohydrate levels, which in turn elevates anthocyanin content (Garg et al., 2024).

#### Leaf and petal Soluble Sugars

As shown in Fig. 8, the application of fulvic acid resulted in an increase in soluble sugars in both leaves and petals, with significant differences observed only at the 100 mg L<sup>-1</sup> concentration when compared to the control.

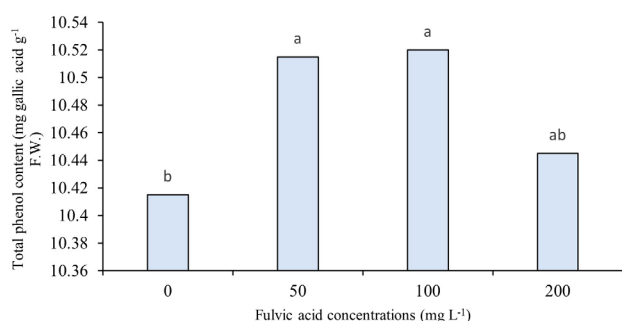
Soluble sugars, including glucose, fructose, and sucrose, are crucial as energy sources for plant cells, as well as signaling molecules and osmoprotectants under stress conditions (Jeandet et al., 2022). Fulvic acid enhances the activity of enzymes involved in carbohydrate metabolism, such as sucrose synthase and invertase, leading to more efficient synthesis and accumulation of soluble sugars. It also improves sugar transport from source tissues (e.g., leaves) to sink tissues (e.g., developing flowers and roots), ensuring a steady energy supply that supports growth (Bayat et al., 2021). Research has shown that fulvic acid significantly increases soluble sugar levels, contributing to overall improved growth and development (Gaber and Kasem, 2022; Yu et al., 2023).



**Fig. 8.** Effect of different fulvic acid concentrations on leaf (A) and petal soluble sugar (B) content of *Alstroemeria* ‘Orange Queen’. Data represents mean values with different letters indicating significant differences at the 1% probability level, as determined by Tukey’s multiple range test.

#### Total phenol content

Figure 9 illustrates that the application of fulvic acid significantly increased the total phenol content in *Alstroemeria* petals. Notably, significant increases were observed at the 50 and 100 mg L<sup>-1</sup> concentrations when compared to the control.

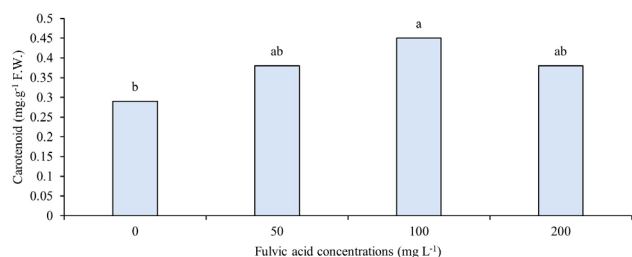


**Fig. 9.** Effect of different fulvic acid concentrations on total phenol content of *Alstroemeria* ‘Orange Queen’. Data represents mean values with different letters indicating significant differences at the 1% probability level, as determined by Tukey’s multiple range test.

Phenolic compounds are crucial for plant defense against both biotic and abiotic stresses (Kumar et al., 2023). Fulvic acid activates the phenylpropanoid pathway, modulating key enzymes like phenylalanine ammonia-lyase (PAL), which are essential for synthesizing phenolic compounds. This pathway produces a variety of phenolic substances that enhance the plant’s antioxidant capacity, structural integrity, and resistance to pathogens. Additionally, fulvic acid increases chlorophyll content and photosynthetic efficiency, providing more energy for synthesizing secondary metabolites, including phenolic compounds (Muhammad et al., 2021). As a result, fulvic acid application leads to higher phenolic content, improving plant resilience and overall health. Recent research confirms the positive impact of fulvic acid on phenolic content and antioxidant capacity (Nikoogoftar-Sedghi et al., 2024).

#### Carotenoid content

Figure 10 shows that the 100 mg L<sup>-1</sup> concentration of fulvic acid significantly increased carotenoid content, resulting in a 1.5-fold increase compared to the control.

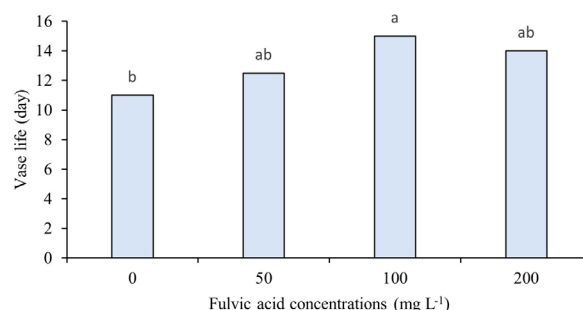


**Fig. 10.** Effect of different fulvic acid concentrations on carotenoid content of *Alstroemeria* ‘Orange Queen’. Data represents mean values with different letters indicating significant differences at the 1% probability level, as determined by Tukey’s multiple range test.

The application of fulvic acid significantly increased carotenoid levels, with the 100 mg L<sup>-1</sup> concentration showing the most pronounced effects. Carotenoids, responsible for yellow, orange, and red pigmentation in flowers, are essential for photosynthesis and photoprotection. Fulvic acid enhances carotenoid biosynthesis by increasing the availability of isoprenoid precursors, which are vital for carotenoid production (Saini et al., 2022). It also modulates the expression of key enzymes in the carotenoid biosynthetic pathway, such as phytoene synthase (PSY) and lycopene beta-cyclase (LCYB) (Kössler et al., 2021). Additionally, fulvic acid’s positive effect on chlorophyll content and photosynthetic efficiency further promotes carotenoid synthesis, essential for light-harvesting and photoprotection (Yan et al., 2023). Recent studies have confirmed that fulvic acid can significantly enhance carotenoid levels in plants (Chen et al., 2022).

#### Vase life

Figure 11 demonstrates that all concentrations of fulvic acid extended the vase life of *Alstroemeria* cut flowers. However, only the 100 mg L<sup>-1</sup> concentration resulted in a significant extension of vase life compared to the control.



**Fig. 11.** Effect of different fulvic acid concentrations on vase life of *Alstroemeria* ‘Orange Queen’. Data represents mean values with different letters indicating significant differences at the 1% probability level, as determined by Tukey’s multiple range test.

The extension of vase life in cut flowers is closely linked to the preservation of cellular integrity and the delay of senescence (Zulfikar et al., 2024; Parvitra et al., 2024). Fulvic acid helps maintain cell membrane stability by reducing lipid peroxidation, a key cause of cell damage during senescence (Hasanuzzaman et al., 2021; Sadeghi and Jabbarzadeh, 2024). It enhances the plant’s antioxidant defense systems and increases the activity of enzymes like superoxide dismutase (SOD) and catalase (CAT), which neutralize reactive oxygen species (ROS) (Feng et al., 2023). By protecting cells from oxidative stress and maintaining turgor pressure, fulvic acid helps prolong flower freshness and vitality (Gaber and Kasem, 2022).

#### Conclusions

This study investigated the effects of fulvic acid on key flowering indices and biochemical traits in *Alstroemeria* ‘Orange Queen’. The application of fulvic acid, particularly at a concentration of 100 mg L<sup>-1</sup>,

significantly enhanced bud diameter, bud length, floret fresh and dry weight, photosynthetic pigments, and soluble sugars in both leaves and petals. These improvements are attributed to the role of fulvic acid in promoting nutrient uptake, enhancing photosynthetic efficiency, and increasing carbohydrate accumulation, all of which contribute to better flower quality and extended vase life. The results suggest that fulvic acid is a promising biostimulant for enhancing growth, ornamental value, and particularly vase life in *Alstroemeria*.

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#### Author Contribution

**FS:** Methodology, Data Curation, Investigation, Formal Analysis. **ZJ:** Methodology, Formal Analysis, Writing – Original Draft. **JA:** Writing – Original Draft, Writing – Review & Editing.

#### Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data Availability Statement

Data will be made available on request.

#### Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT in order to edit the text of the manuscript. After using this tool/service, the authors reviewed and edited the content as needed and took full responsibility for the content of the publication.

#### References

- AHMAD, S.; KHAN, J.A.; JAMAL, A. Response of pot marigold to different applied levels of humic acid. **Journal of Horticulture and Plant Research**, v.5, p.57-60, 2019. <https://doi.org/10.18052/www.scipress.com/jhpr.5.57>.
- ALSUDAYS, I.M.; ALSHAMMARY, F.H.; ALABDALLAH, N.M.; ALATAWI, A.; ALOTAIBI, M.M.; ALWUTAYD K.M.; ALHARBI, M.M.; ALGHANEM S.M.S.; ALZUAIBR, F.M.; GHARIB, H.S.; AWAD-ALLAH, M.M.A. Applications of humic and fulvic acid under saline soil conditions to improve growth and yield in barley. **BMC Plant Biology**, v. 24, n.1, p.191, 2024. <https://doi.org/10.1186/s12870-024-04863-6>.
- AMPONG, K.; HILAKARANTHNA, M.S.; GORIM, L.Y. Understanding the role of humic acids on crop performance and soil health. **Frontiers in Agronomy**, v.4, p.848621, 2022. <https://doi.org/10.3389/fagro.2022.848621>.
- BALTAZAR, M.; CORREIA, S.; GUINAN, K.J.; SUJEETH, N.; BRAGANCA, R.; GONCALVES, B. 2021. Recent advances in the molecular effects of biostimulants in plants: An overview. **Biomolecules**, v.11, n.8, p.1096, 2021. <https://doi.org/10.3390/biom11081096>.
- BAYAT, H.; SHAFIE, F.; AMINIFARD, M.H.; DAGHIGHI, S. Comparative effects of humic and fulvic acids as biostimulants on growth, antioxidant activity and nutrient content of yarrow (*Achillea millefolium* L.). **Scientia Horticulturae**, v.279, p.109912, 2021. <https://doi.org/10.1016/j.scienta.2021.109912>.
- BRIDGEN, M.P. *Alstroemeria*. In: Van Huylbroeck, J. (eds) Ornamental Crops. Handbook of Plant Breeding, vol 11. Springer, Cham, 2018. [https://doi.org/10.1007/978-3-319-90698-0\\_10](https://doi.org/10.1007/978-3-319-90698-0_10).
- CHEN, X.; ZHANG, X.; CHEN, H.; XU, X. Physiology and proteomics reveal fulvic acid mitigates cadmium adverse effects on growth and photosynthetic properties of lettuce. **Plant Science**, v.323, p.111418, 2022. <https://doi.org/10.1016/j.plantsci.2022.111418>.
- DHIMAN, M.R.; KASHYAP, B. *Alstroemeria*: Conservation, characterization, and evaluation in: **Floriculture and Ornamental Plants**. Springer Nature. pp. 117-151, 2022.
- FENG, D.; JIA, X.; YAN, Z.; LI, J.; GAO, J.; XIAO, W.; SHEN, X.; SUN, X. Underlying mechanisms of exogenous substances involved in alleviating plant heat stress. **Plant Stress**, v.10, p.100288, 2023. <https://doi.org/10.1016/j.stress.2023.100288>.
- GABER, M.; KASEM, M. Improving growth characteristics and vase life of *Dendranthema grandiflorum* 'Flyer' using humic and fulvic acids as biostimulants substances. **Scientific Journal of Flowers and Ornamental Plants**, v.9, p.87-102, 2022. <https://doi.org/10.21608/sjfo.2022.248727>.
- GARG, S.; NAIN, P.; KUMAR, A.; JOSHI, S.; PUNETHA, H.; SHARMA, P.K.; SIDDIQUI, S.; ALSHAHRANI, M.O.; ALGOPISHI, U.B.; MITTAL, A. Next generation plant biostimulants & genome sequencing strategies for sustainable agriculture development. **Frontiers in Microbiology**, v.15, p.1439561, 2024. <https://doi.org/10.3389/fmicb.2024.1439561>.
- PARVITRA, S.H.; HELMA, B.N.; CHANDRASHEKAR, S.Y.; KANTHARAJ, Y.; SHIVAPRASAD, M.; HEMANTH, K.P. Effect of vase chemicals at different concentration on longevity of *Alstroemeria* cut flower. **International Journal of Advanced Biochemistry Research**, v.SP-8, n.10, p.1421-1426. <https://doi.org/10.33545/26174693.2024.v8.i10Sq.2736>.
- HASANUZZAMAN, M.; PARVIN, K.; BARDHAN, K.; NAHAR, K.; ANEE, T.I.; MASUD, A.A.C.; FOTOPOULOS, V. Biostimulants for the regulation of reactive oxygen species metabolism in plants under abiotic stress. **Cells**, v.10, n.10, p.2537, 2021. <https://doi.org/10.3390/cells10102537>.
- IRIGOYEN, J.J.; EMERICH, D.W.; SANCHEZ-DIAZ, M. Water stress induced changes in concentration of proline and total soluble sugars in nodulated alfalfa (*Medicago sativa*) plants. **Physiologia Plantarum**, v.84, p.55-60, 1992. <https://doi.org/10.1111/j.1399-3054.1992.tb08764.x>.
- JEANDET, P.; FORMELA-LUBOŃSKA, M.; LABUDDA, M.; MORKUNAS, I. The role of sugars in plant responses to stress and their regulatory function during development. **International Journal of Molecular Science**, v.23, n.9, p.5161, 2022. <https://doi.org/10.3390/ijms23095161>.
- KISVARGA, S.; FARKAS, D.; BORONKAY, G.; NEMÉNYI, A.; ORLÓCI, L. Effects of biostimulants in horticulture, with emphasis on ornamental plant production. **Agronomy**, v. 12, p.1403, 2022. <https://doi.org/10.3390/agronomy12051043>.
- KÖSSLER, S.; ARMAREGO-MARRIOTT, T.; TARKOWSKÁ, D.; TUREČKOVÁ, V.; AGRAWAL, S.; MI, J.; SOUZA, L.P.; SCHÖTTLER, M.A.; SCHADACH, A.; FRÖHLICH, A.; BOCK, R.; AL-BABILI, S.; RUF, S.; SAMPATHKUMAR, A.; MORENO, J.C. Lycopene  $\beta$ -cyclase expression influences plant physiology, development, and metabolism in tobacco plants. **Journal of Experimental Botany**, v.72, n.7, p.2544-2569, 2021. <https://doi.org/10.1093/jxb/erab029>.
- KUMAR, K.; DEBNATH, P.; SINGH, S.; KUMAR, N. An overview of plant phenolic and their involvement in abiotic stress tolerance. **Stresses**, v.3, p.570-585, 2023. <https://doi.org/10.3390/stresses3030040>.
- LI, R.; HE, Y.; CHEN, J.; ZHENG, S.; ZHUANG, C. Research progress in improving photosynthetic efficiency. **International Journal of Molecular Science**, v.24, p.9286, 2023. <https://doi.org/10.3390/ijms24119286>.
- LICHTENTHALER, H.K.; WELLBURN, A.R. Determination of total carotenoids and chlorophylls a and b of leaf in different solvents. **Biochemical Society Transactions**, v.11, n.5, p.591- 592, 1987. <https://doi.org/10.1042/bst0110591>.

- LIU, X.; YANG, J.; TAO, J.; YAO, R. Integrated application of inorganic fertilizer with fulvic acid for improving soil nutrient supply and nutrient use efficiency of winter wheat in a salt-affected soil. **Applied Soil Ecology**, v.170, p.104255, 2022. <https://doi.org/10.1016/j.apsoil.2021.104255>.
- MANNINO, G.; GENTILE, C.; ERTANI, A.; SERIO, G.; BERTEA, C.M. Anthocyanins: biosynthesis, distribution, ecological role, and use of biostimulants to increase their content in plant foods - A Review. **Agriculture**, v.11, p.212, 2021. <https://doi.org/10.3390/agriculture11030212>.
- MARINOVA, D.; RIBAROVA, F.; ATANASSOVA, M. Total phenolic and total flavonoids in Bulgarian fruits and vegetables. **Journal of the University of Chemical Technology and Metallurgy**, v.40, n.3, p.255-260, 2005.
- MAZZONI-PUTMAN, S.M.; BRUMOS, J.; ZHAO, C.; ALONSO, J.M.; STEPANOVA, AN. Auxin interactions with other hormones in plant development. **Cold Spring Harbor Perspective in Biology**, v.13, n.10, p.a039990, 2021. <https://doi.org/10.1101/cshperspect.a039990>.
- MIRZAEI ESGANDIAN, N.; JABBARZADEH, Z.; RASOULI-SADAGHIANI, M.H. Investigation on some morphological and physiological characteristics of *Gerbera jamesonii* as affected by humic acid and nano-calcium chelate in hydroponic culture conditions. **Journal of Ornamental Plants**, v.10, n.1, p.1-13, 2020.
- MOURA O.V.T.; BERBARA, R.L.L.; TORCHIA, D.F.O.; SILVA, H.F.O.; CASTRO, T.A.V.; TAVARES, O.C.H.; RODRIGUES, N.F.; SANTOS, E.Z.L.A.; GARCIA, A.C. Humic foliar application as sustainable technology for improving the growth, yield, and abiotic stress protection of agricultural crops. A review. **Journal of the Saudi Society of Agricultural Sciences**, v.22, n.8, p.493-513, 2023. <https://doi.org/10.1016/j.jssas.2023.05.001>.
- MUHAMMAD, I.; SHALMANI, A.; ALI, M.; YANG, Q.-H.; AHMAD, H.; LI, F.B. Mechanisms regulating the dynamics of photosynthesis under abiotic stresses. **Frontiers in Plant Science**, v.11, p.615942, 2021. <https://doi.org/10.3389/fpls.2020.615942>.
- MUTUI, T.M.; EMONGOR, V.E.; HUTCHINSON, M.J. The effects of Gibberellin<sub>4+7</sub> on the vase life and flower quality of *Alstroemeria* cut flowers. **Plant Growth Regulation**, v.48, p. 207-214, 2006. <https://doi.org/10.1007/s10725-006-0014-6>.
- NIKOOGOFTAR-SEDGHI, M.; RABIEI, V.; RAZAVI, F.; MOLAEI, S.; KHADIVI, A. Fulvic acid foliar application: a novel approach enhancing antioxidant capacity and nutritional quality of pistachio (*Pistacia vera* L.). **BMC Plant Biology**, v.24, n.1, p.241, 2024. <https://doi.org/10.1186/s12870-024-04974-0>.
- RANJEETHA, R. *Alstroemeria*: an exotic cut flower. **Krishi Science**, v.5, n.3, p.80-83, 2024.
- SADEGHI, S.; JABBARZADEH, Z. The effect of pre- and post-harvest sodium nitroprusside treatments on the physiological changes of cut *Alstroemeria aurea* 'Orange Queen' during vase life. **BMC Plant Biology**, v.24, p.678, 2024. <https://doi.org/10.1186/s12870-024-05393-x>.
- SAHRAIE, F.; JABBARZADEH, Z.; AMIRI, J. Effect of fulvic acid on some morphological characteristics and leaf elements of *Alstroemeria* (*Alstroemeria aurea* 'Orange Queen'). **Flower and Ornamental Plants**, v.9, n.1, p.17-30, 2024.
- SAINI, R.K.; PRASAD, P.; LOKESH, V.; SHANG, X.; SHIN, J.; KEUM, Y.-S.; LEE, J.-H. Carotenoids: dietary sources, extraction, encapsulation, bioavailability, and health benefits - A review of recent advancements. **Antioxidants**, v.11, p.795, 2022. <https://doi.org/10.3390/antiox11040795>.
- SEYED HAJIZADEH, H.; BAYRAMI AGHDAM, S.; FAKHRGHAZI, H.; KARAKUS, S.; KAYA, O. Physico-chemical responses of *Alstroemeria* spp. cv. Rebecca to the presence of salicylic acid and sucrose in vase solution during postharvest life. **BMC Plant Biology**, v.24, n.121, 2024. <https://doi.org/10.1186/s12870-024-04814-1>.
- SHARAYA, D.R.; NEHRA, A.; AGRAWALA, N.; KHAN, N.A.; TUTEJA, N.; GILL, R.; GILL, S.S. Biostimulants in the alleviation of metal toxicity: an overview. Editor(s): Sarvajeet Singh Gill, Narendra Tuteja, Nafees A. Khan, Ritu Gill, In: **Biostimulants and Protective Biochemical Agents, Biostimulants in Alleviation of Metal Toxicity in Plants**, Academic Press, p.1-19, 2023. <https://doi.org/10.1016/B978-0-323-99600-6.00017-7>.
- TURAN, M.; EKINCI, M.; KUL, R.; KOCAMAN, A.; ARGIN, S.; ZHIRKOVA, A.M.; PERMINOVA, I.V.; YILDIRIM, E. Foliar applications of humic substances together with Fe/Nano Fe to increase the iron content and growth parameters of spinach (*Spinacia oleracea* L.). **Agronomy**, v.12, p.2044, 2022. <https://doi.org/10.3390/agronomy12092044>.
- WAGNER, G.J. Content and vacuole/ extra vacuole distribution of neutral sugars, free amino acids and anthocyanin in protoplasts. **Plant Physiology**, v.64, p.88-93, 1979. <https://doi.org/10.1104/pp.64.1.88>.
- YAN, D.; WANG, J.; LU, Z.; LIU, R.; HONG, Y.; SU, B.; WANG, Y.; PENG, Z.; YU, C.; GAO, Y.; LIU, Z.; XU, Z.; DUAN, L.; LI, R. Melatonin-mediated enhancement of photosynthetic capacity and photoprotection improves salt tolerance in wheat. **Plants**, v. 12, p.3984, 2023. <https://doi.org/10.3390/plants12233984>.
- YAO, L.; LIANG, D.; XIA, H.; PANG, Y.; XIAO, Q.; HUANG, Y.; ZHANG, W.; PU, C.; WANG, J.; LV, X. Biostimulants promote the accumulation of carbohydrates and biosynthesis of anthocyanins in 'Yinhongli' plum. **Frontiers in Plant Science**, v.13, p.1074965, 2023. <https://doi.org/10.3389/fpls.2022.1074965>.
- YU, B.; WANG, L.; CUI, D.; GAO, W.; XUE, X.; NIE, P. Effects of fulvic acid on growth and nitrogen utilization efficiency in M9T337 seedlings. **Plants**, v.12, p.3937, 2023. <https://doi.org/10.3390/plants12233937>.
- ZULFIQAR, F.; MOOSA, A.; FERRANTE, A.; DARRAS, A.; SHETIWI, M.S.; ALI, B.; ALTAI, M.A.; SOUFAN, W.; EL SABAGH, A. Borage leaf extract improves the vase life of cut gladiolus flowers by delaying the senescence process and reducing water stress. **Postharvest Biology and Technology**, v.210, p.112766, 2024. <https://doi.org/10.1016/j.postharvbio.2024.112766>.