University of Tabriz
Journal of Plant Physiology and Breeding
Print ISSN: 2008-5168 Online ISSN: 2821-0174

2026, 16(2): 53-68

Improvement of biochemical, photosynthetic, and morphological traits of snapdragon (*Antirrhinum majus*) using chitosan-coated iron oxide and silver nanoparticles

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Article Info
Article type:
Research article

Article history:
Received: January 28, 2026
Revised: February 23, 2026
Accepted: March 7, 2026
Published online: May 23, 2026

Keywords:
Chlorophyll fluorescence,
Nanoparticles,
Snapdragon,
Stomatal conductance.

Abstract

Objective: Snapdragon (*Antirrhinum majus*), a perennial species in the Scrophulariaceae family, is valued for its colorful flowers and ornamental appeal. This study aimed to evaluate the effects of chitosan-coated iron oxide nanoparticles (chitosan-coated IONPs) and silver nanoparticles (AgNPs) on key morphological and biochemical characteristics of this species.

Methods: The experiment was conducted under greenhouse conditions as a factorial arrangement in a completely randomized design with four replications. Factors included four concentrations of chitosan-coated IONPs (0, 50, 100, and 150 µM) and four concentrations of AgNPs (0, 50, 100, and 150 $mg \cdot L^{-1}$). Measured traits encompassed stem diameter, fresh weight of aerial parts, number of lateral branches, plant height, flower number and diameter, total sugars, total flavonoids, total phenolic content, antioxidant capacity [1,1-diphenyl-2-picrylhydrazyl (DPPH)], stomatal conductance, and chlorophyll fluorescence indices (F_0, F_m, F_v, F_v/F_0, and F_v/F_m).

Results: The combined application of chitosan-coated IONPs and AgNPs significantly enhanced vegetative growth, floral attributes, and most physiological indicators. The greatest improvements in fresh weight, plant height, and number of lateral branches were achieved with 100 µM chitosan-coated IONPs + 50 mg/L AgNPs. The F_v/F_0 and F_v/F_m ratios were enhanced by the combination of 150 µM chitosan-coated IONPs + 150 mg/L AgNPs. Significant interaction effects were observed for total sugars, total flavonoids, and DPPH activity, with the 100 µM chitosan-coated IONPs + 100 mg/L AgNPs treatment showing the most pronounced improvements. Total phenolic content also increased under combined nanoparticle treatments, indicating activation of the antioxidant defense system.

Conclusion: Overall, single or combined applications of chitosan-coated IONPs and AgNPs improved growth performance, flowering, and antioxidant responses in snapdragon. These findings highlight the potential of nanoparticle-based strategies for sustainable, high-quality ornamental plant production. Future research should focus on refining optimal nanoparticle concentrations and assessing their long-term impacts.

Cite this article: Pourbeyrami HirY, Sardari S. 2026. Improvement of biochemical, photosynthetic, and morphological traits of snapdragon (*Antirrhinum majus*) using chitosan-coated iron oxide and silver nanoparticles. J Plant Physiol Breed. 16(2): 53-68. https://doi.org/10.22034/jppb.2026.71310.1405

Introduction

Snapdragon (*Antirrhinum majus*), an ornamental perennial plant from the Plantaginaceae family (previously classified under *Scrophulariaceae*), is native to the Mediterranean region. It is highly valued for its bilabiate, colorful, and diverse flowers-ranging from white, pink, red, and purple to yellow and bicolored- and its attractive elongated spike structure, making it one of the most important cut-flower and bedding plants worldwide. The flowers feature an upper lip forming a hood-like structure and a lower lip acting as a platform, which facilitates pollinator attraction. The plant height typically ranges from 30 to 120 cm depending on the cultivar. In cold climates, it is grown as an annual, while in temperate regions it is cultivated as a biennial plant. The high demand for snapdragons in cut-flower markets and landscaping has highlighted the need for innovative methods to extend vase life, enhance flower color, increase the number of flowering stems, and improve resistance to post-harvest stresses (Tikhomirova *et al.* 2020).

In recent decades, nanotechnology has emerged as a revolutionary scientific advancement, opening new horizons in agriculture and sustainable resource management. Due to their minute size (1-100 nm), extremely high specific surface area (up to several hundred m^2/g), ability to penetrate cell walls, and unique physicochemical properties-such as conductivity, catalytic activity, and selective adsorption- nanoparticles have become promising tools for improving plant growth, pest control, resistance to abiotic stresses (drought, salinity, and heavy metals), and the production of secondary metabolites (Al-Qudah *et al.* 2022). Among these nanoparticles, iron oxide nanoparticles (IONPs, typically Fe_3O_4) and silver nanoparticles (AgNPs) have attracted particular attention due to their targeted nutrient delivery-especially iron, essential for chlorophyll synthesis and enzyme activity-and their strong antimicrobial properties, achieved through the controlled release of Ag^+ions and disruption of microbial membranes), respectively (Krzepiłko *et al.* 2025).

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The application of nanoparticles as bio-pesticides, protective agents, antimicrobials, growth stimulants, and nutritional supplements has transformed agriculture. Behzad *et al.* (2024) demonstrated that AgNPs at low concentrations (e.g. 60 ppm) can increase fresh weight, stem length, and anthocyanin production through the time-dependent stimulation of antioxidant enzyme activity (e.g., SOD, CAT, and APX). According to Mirzaei and Kiarostami (2022), low concentrations of AgNPs also enhance plant growth and boost the production of secondary metabolite, such as flavonoids, thereby improving antioxidant properties, which are particularly beneficial for snapdragon flower color quality, vase life, and post-harvest durability. On the other hand, IONPs (especially Fe3O4) offer better biocompatibility, magnetic properties, and gradual iron ion release, playing a key role in managing environmental stresses and improving plant nutrition. Furthermore, a chitosan coating on these nanoparticles not only enhances colloidal stability and prevents particle aggregation but also reduces potential toxicity and facilitates systemic uptake. In a study on peppermint (*Mentha piperita*) under drought-stress conditions, the chitosan-coated iron oxide nanoparticles (chitosan-coated IONPs) improved chlorophyll fluorescence, chlorophyll content, and photosynthetic indices (Torabi Giglou *et al.* 2022).

This research focused on the synthesis, mode of action, and potential of chitosan-coated IONPs and AgNPs in enhancing snapdragon's performance, with an emphasis on their antimicrobial, nutritional, and growth-stimulating effects.

Materials and Methods

Snapdragon (*Antirrhinum majus*) seeds were prepared and cultivated under greenhouse conditions at the Faculty of Agriculture and Natural Resources, Mohaghegh Ardabili University, Ardabil, Iran. AgNPs and chitosan-coated IONPs were procured from the Azar Tajhiz Company, Tabriz, Iran. The dry nanoparticles were dispersed in the central laboratory of Mohaghegh Ardabili University using an ultrasonic device and applied as foliar sprays. Treatments included factorial combinations of two nanoparticle types: AgNPs and chitosan-coated IONPs, each at four concentrations: 0 (control), 50, 100, and 150 mg/L for AgNPs, and 0 (control), 50, 100, and 150 µM for chitosan-coated IONPs. Treatments were applied via foliar spraying at three key growth stages. Seeds were first sown in trays and then transplanted to plastic pots (4 L volume) containing a 1:1:1 (v/v) mixture of organic matter, sand, and garden soil. The study was carried out as a factorial experiment based on a completely randomized design with four replications.

The measured morphological traits were plant height, number of branches and flowers, and flower diameter. The number of lateral branches and flowers was recorded using direct counting. Flower and stem diameters were determined using a digital caliper with 0.1 mm precision. The fresh weight of the aerial parts was measured with an analytical balance with 0.001 g accuracy.

Total phenol content was measured according to Meda *et al.* (2005), with gallic acid as a standard, and reported as mg gallic acid equivalents per g dry weight. Total flavonoid content was assessed with a spectrophotometer using the method of Krizek *et al.* (1998), by mixing 1.5 mL 80% methanol, 100 µL 10% $AlCl_3$, 100 µL of 1 M sodium acetate, and 2.8 mL of water with 500 µL of extract, with absorbance measured at 415 nm after 40 minutes and reported as mg quercetin equivalents per g dry matter, using quercetin as a standard. For the total sugar measurement, 0.2 g of green plant tissue was combined with 10 mL of 95% ethanol in a sealed test tube and heated in a water bath at 80 °C for 1 hour. After cooling, 1 mL of the sample was mixed with 1 mL of 5% phenol and 5 mL of 98% sulfuric acid, and the absorbance was read at 483 nm using a spectrophotometer. Chlorophyll fluorescence was measured using a Hansatech FMS2 fluorometer (Hansatech Instruments, Norfolk, UK). Leaves were dark-adapted for 30 minutes using leaf clips. The saturating pulse was set at 3000 µmol m^{-2} s^{-1} for 0.8 seconds, and the measuring light was 700 µmol m^{-2} s^{-1}. Then, the parameters F_0 (minimum fluorescence), F_m (maximum fluorescence), F_v (variable fluorescence), F_v/F_0, and F_v/F_m were recorded. Stomatal conductance (mmol m^{-2} s^{-1}) was measured using the SC-1 Leaf Porometer (Decagon Devices, Pullman, WA, USA) on sunny days between 10:00 and 12:00.

All measurements were conducted at specific growth stages (vegetative, budding, and full flowering) under controlled greenhouse conditions (temperature: 22 ± 2 °C during the day and 18 ± 2 °C at night; relative humidity: 60-70%; natural light was supplemented with LED lighting if needed) to minimize environmental variability. Means were compared using Duncan's multiple range test. Data were analyzed using SAS 9.1 software and graphs were prepared using Microsoft Excel.

Results

Analysis of variance showed that both individual and interaction effects of chitosan-coated IONPs and AgNPs on plant height, fresh weight of aerial parts, stem diameter, number of lateral branches and flowers, and flower diameter were significant (Table 1).

Analysis of variance for biochemical and photosynthetic characteristics also revealed that these traits, including Fv/F_0, Fv/Fm, stomatal conductance, total sugars, total flavonoids, and total phenols were significantly affected by the interaction of chitosan-coated IONPs and AgNPs (Table 2).

Plant height

The results indicated that plant height significantly increased in plants treated with the combination of 100 µM chitosan-coated IONPs and 50 mg/L AgNPs compared to the control. However, increasing the concentrations of AgNPs and chitosan-coated IONPs to 150 mg/L and 150 µM, respectively, decreased final plant height. The lowest plant height was observed in the control group (no chitosan-coated IONPs or AgNPs applied) (Figure 1A).

Table 1. Analysis of variance of the effects of chitosan-coated IONPs and AgNPs on morphological traits of snapdragon.

Sources of variation	df	Mean squares					
		Plant height	**Branch number**	**Flower number**	**Flower diameter**	**Stem diameter**	**Fresh weight of aerial parts**
Chitosan-coated IONPs (IO)	3	45.92**	24.87**	518.5**	59.16**	8.76**	1957.51**
AgNPs (Ag)	3	11.49*	56.70**	108.62**	48.41**	1.45**	13.57
IO × Ag	9	26.88**	31.02**	91.23**	107.62**	1.50**	177.33**
Error	48	3.83	2.43	7.80	4.09	0.324	9.94
Coefficient of variation	-	6.68	10.86	13.38	6.99	14.25	5.43

*, **: Significant at the 5% and 1% probability levels, respectively; IONPs: Iron oxide nanoparticles, AgNPs: Silver nanoparticles.

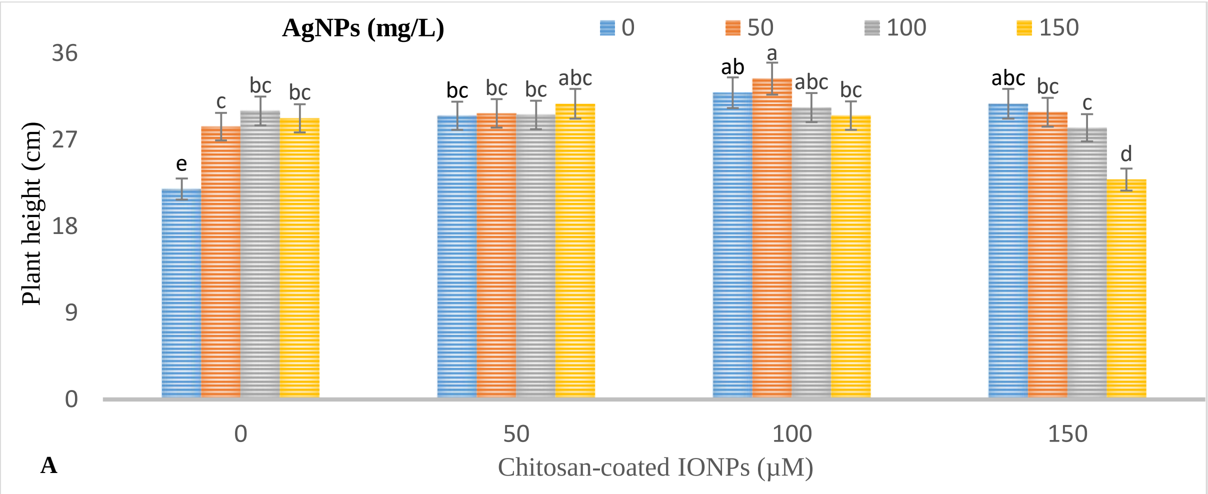
Table 2. Analysis of variance of the effects of chitosan-coated IONPs and AgNPs on biochemical and photosynthetic traits of snapdragon.

Sources of variation	df	Mean squares						
		Total phenols	**Total flavonoids**	**Total sugars**	**DPPH**	**g_s**	**F_v/F_0**	**F_v/F_m**
Chitosan-coated IONPs (IO)	3	470.0**	54.11**	0.068**	1595.6**	2086**	0.81**	0.00061*
AgNPs (Ag)	3	88.0**	4.58**	0.116**	152.7**	407*	5.46**	0.0001*
IO × Ag	9	23.6**	1.05**	0.016**	184.1**	888**	1.39**	0.0013**
Error	48	1.26	0.126	0.003	8.5	223	0.14	0.00021
CV (%)	-	10.17	8.745	8.06	7.26	11.00	8.99	7.83

*, **: Significant at the 5% and 1% probability levels, respectively; IONPs: Iron oxide nanoparticles, AgNPs: Silver nanoparticles, g_s: Stomatal conductance.

Branch number

Branch number showed a response pattern comparable to that of final plant height under the combined application of chitosan-coated IONPs and AgNPs. The highest number of branches was obtained in the treatment combining 100 µM chitosan-coated IONPs with 50 mg/L AgNPs. However, the highest concentrations of these compounds (150 µM chitosan-coated IONPs and 150 mg/L AgNPs) significantly negatively affected this trait (Figure 1B).



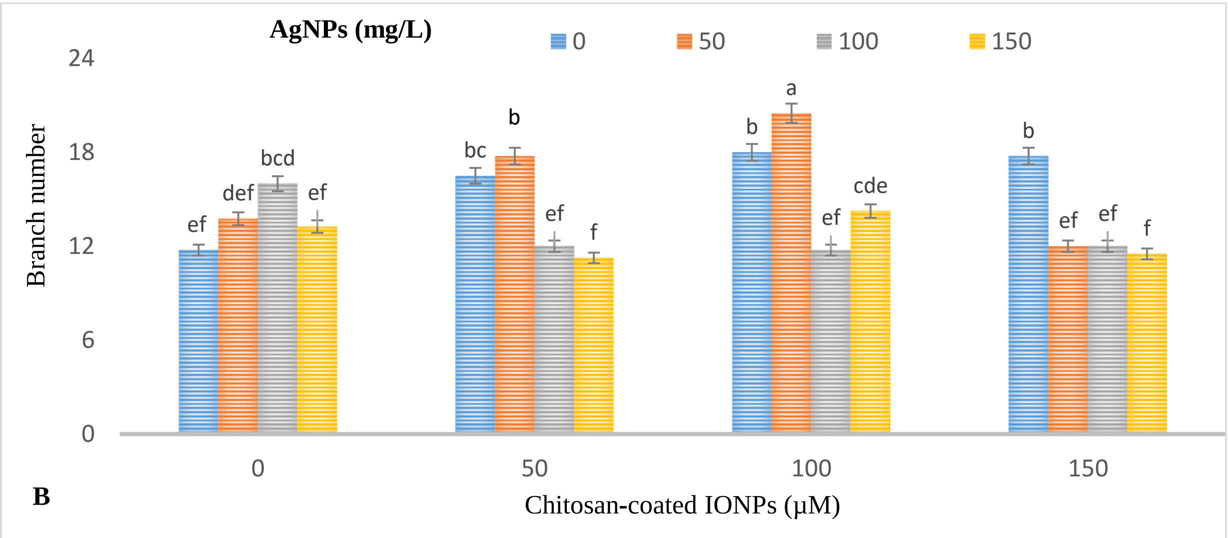


Figure 1. Interaction effects of chitosan-coated iron oxide nanoparticles (chitosan-coated IONPs) and silver nanoparticles (AgNPs) on plant height (A) and branch number (B) of snapdragon.

Fresh weight of aerial parts

The highest fresh weight of aerial parts of snapdragon was observed in plants treated with 100 µM chitosan-coated IONPs and 50 mg/L AgNPs (Figure 2A); however, application of the highest concentrations (150 µM and 150 mg/L) of these two compounds decreased the fresh weight of aerial parts (Figure 2A).

Stem diameter

The results showed a significant effect of chitosan-coated IONPs and AgNPs on stem diameter. The highest stem diameter was observed in plants treated with 50 µM chitosan-coated IONPs and 150 mg/L AgNPs, whereas while the lowest was found in untreated control plants (Figure 2B).

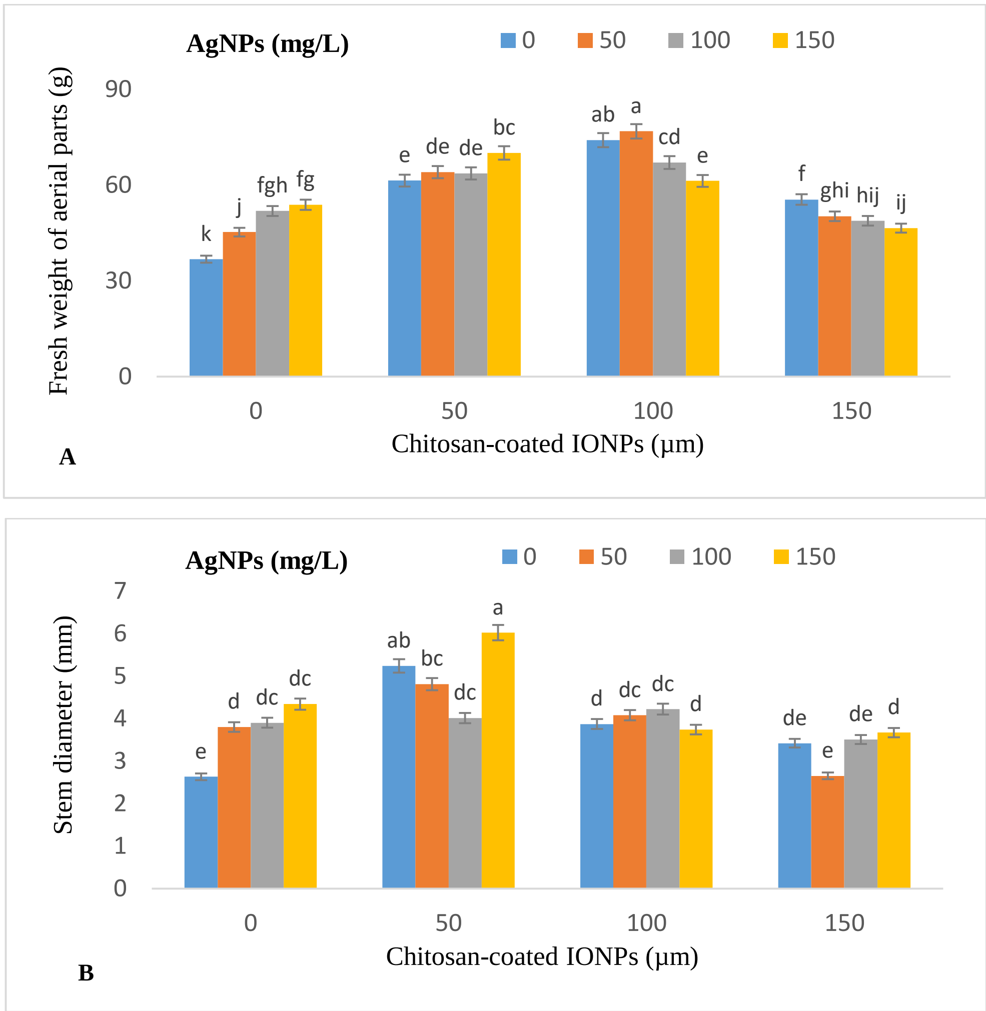


Figure 2. Interaction effects of chitosan-coated iron oxide nanoparticles (chitosan-coated IONPs) and silver nanoparticles (AgNPs) on the fresh weight of the aerial parts (A) and the stem diameter (B) of Snapdragon.

Flower number

According to Figure 3A, the flower number increased with increasing concentrations of chitosan-coated IONPs and AgNPs. The highest number of flowers was observed in plants treated with 100 µM chitosan-coated IONPs combined with 150 mg/L AgNPs, followed by plants treated with 150 µM chitosan-coated IONPs combined with 50 mg/L AgNPs. The lowest flower number was observed in the control (0 µM chitosan-coated IONPs and 0 mg/L AgNPs).

Flower diameter

According to the results obtained from the effects of different concentrations of chitosan-coated IONPs and AgNPs (Figure 3B), the lowest flower diameter was recorded in the control treatment (0 mg/L AgNPs and 0 µM chitosan-coated IONPs), while the highest flower diameter was observed in the treatment with 150 mg/L AgNPs and no chitosan-coated IONPs.

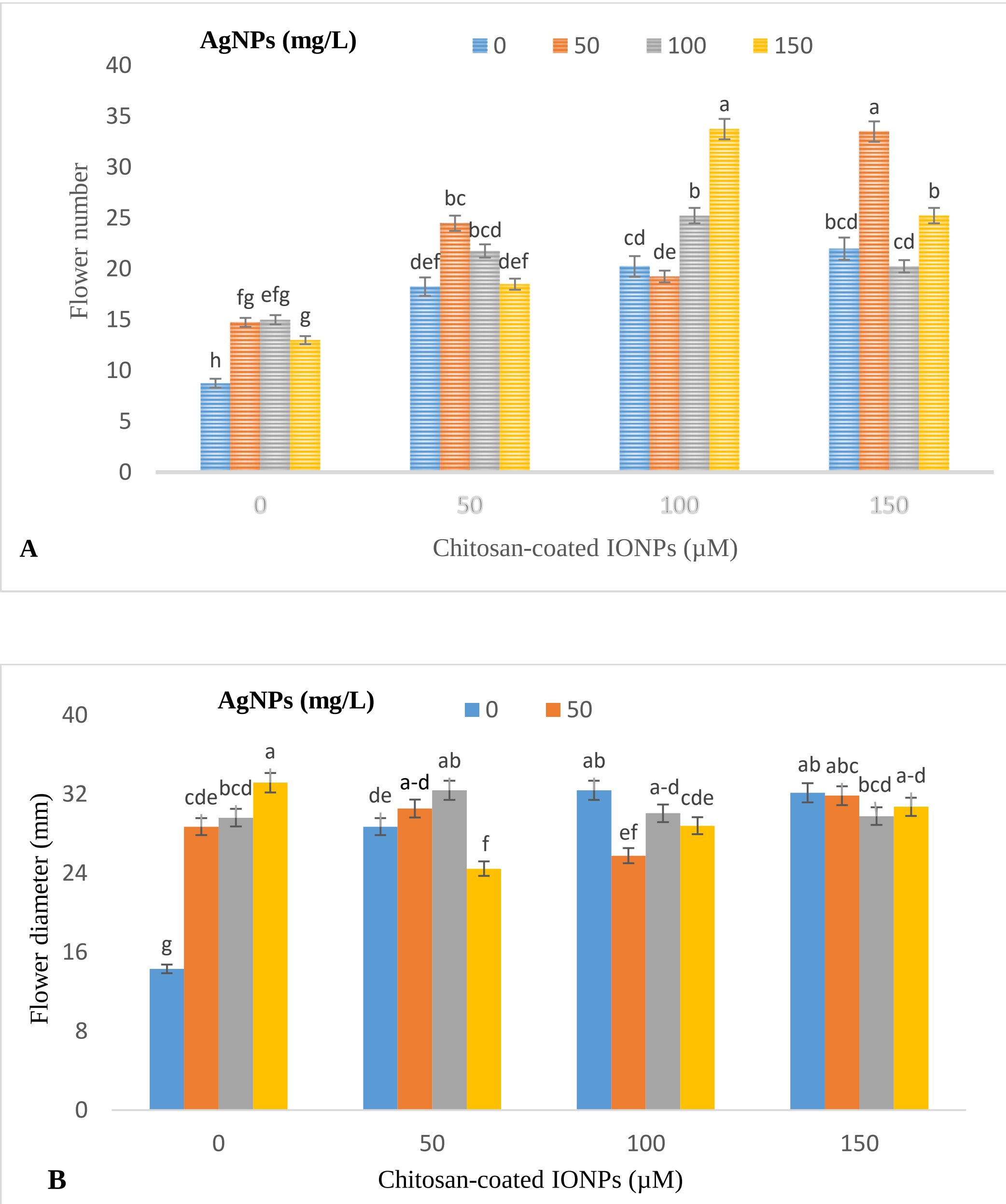


Figure 3. Interaction effects of chitosan-coated iron oxide nanoparticles (chitosan-coated IONPs) and silver nanoparticles (AgNPs) on flower number (A) and flower diameter (B) in snapdragon.

F_v/F_o

The highest F_v/F_0 value was observed in plants treated with 150 mg/L AgNPs combined with 150 µM chitosan-coated IONPs, followed by 150 mg/L AgNPs combined with 50 µM chitosan-coated IONPs. Conversely, a high concentration of chitosan-coated IONPs (150 µM) in the absence of AgNPs resulted in a significant decline in this trait compared with the control (Figure 4A).

F_v/F_m

Similar to the F_v/F_0 ratio, the highest F_v/F_m value was observed in plants treated with 150 mg/L AgNPs combined with 150 µM chitosan-coated IONPs (Figure 4B).

Stomatal conductance

The highest stomatal conductance was observed in the control treatment, and most of the treatments with the combination of chitosan-coated IONPs and AgNPs had significantly lower stomatal conductance in the leaves of snapdragon, except for 100 µM chitosan-coated IONPs alone and 50 µM chitosan-coated IONPs + 150 mg/L AgNPs (Figure 4C).

Total phenols

The results of the experiment revealed that by increasing the concentration of chitosan-coated IONPs from 0 to 100 µM, the phenol content increased significantly. The highest total phenols were observed in 100 µM chitosan-coated IONPs + 150 mg/L AgNPs and 150 µM chitosan-coated IONPs + 50 mg/L AgNPs. In contrast, the control treatment without IONPs and AgNPs showed the lowest phenol content (Figure 5A).

Total flavonoids

The results showed that the flavonoid content in snapdragon was influenced by varying nanoparticle concentrations. The lowest flavonoid content was observed in the control (0 mg/L AgNPs and 0 µM chitosan-coated IONPs), while the highest was achieved with 100 mg/L AgNPs + 150 µM chitosan-coated IONPs or 100 mg/L AgNPs + 100 µM chitosan-coated IONPs (Figure 5B).

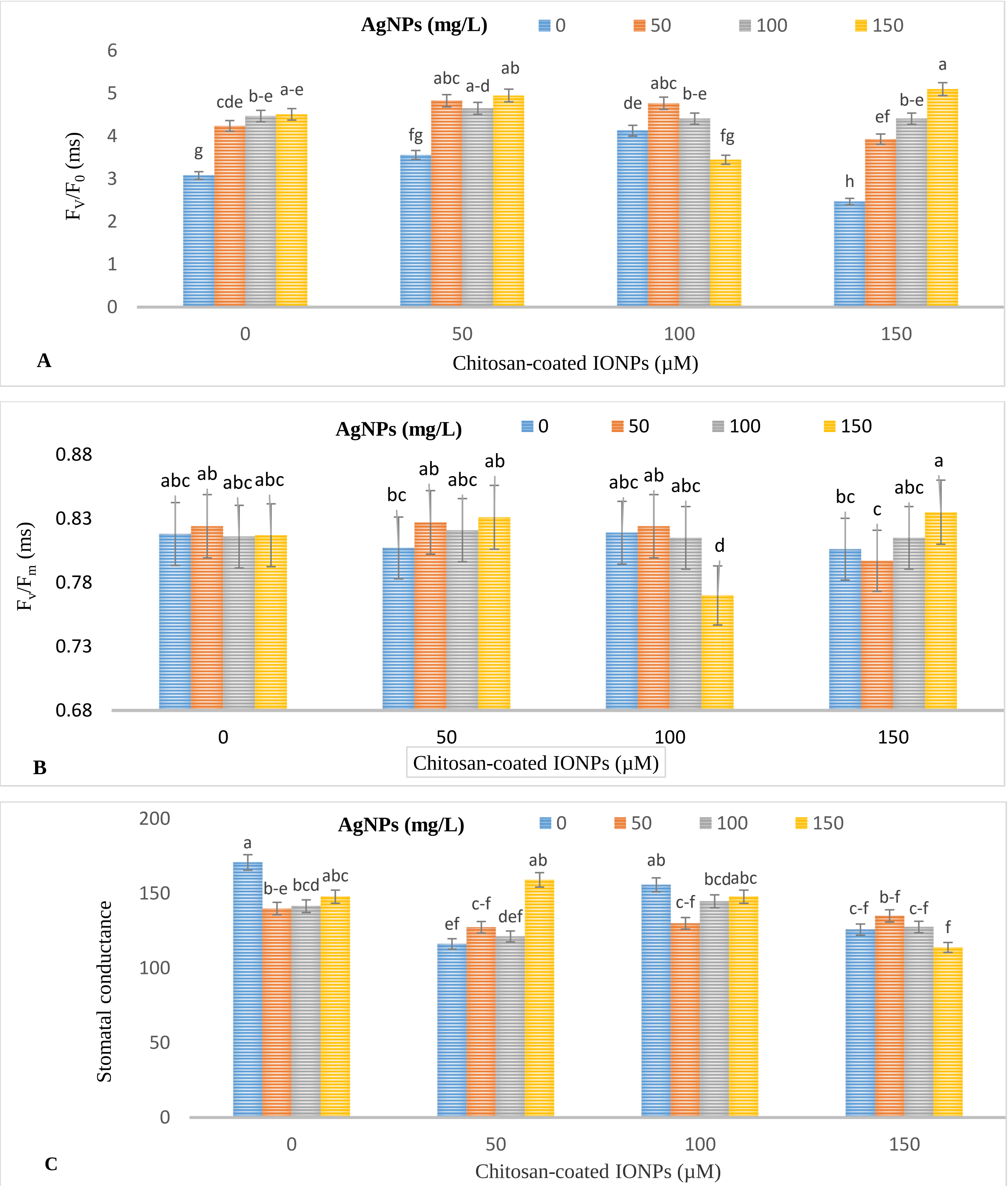


Figure 4. Interaction effects of chitosan-coated iron oxide nanoparticles (chitosan-coated IONPs) and silver nanoparticles (AgNPs) on the F_v/F_0 (A), F_v/F_m (maximum fluorescence) (B), and stomatal conductivity (C) in snapdragon.

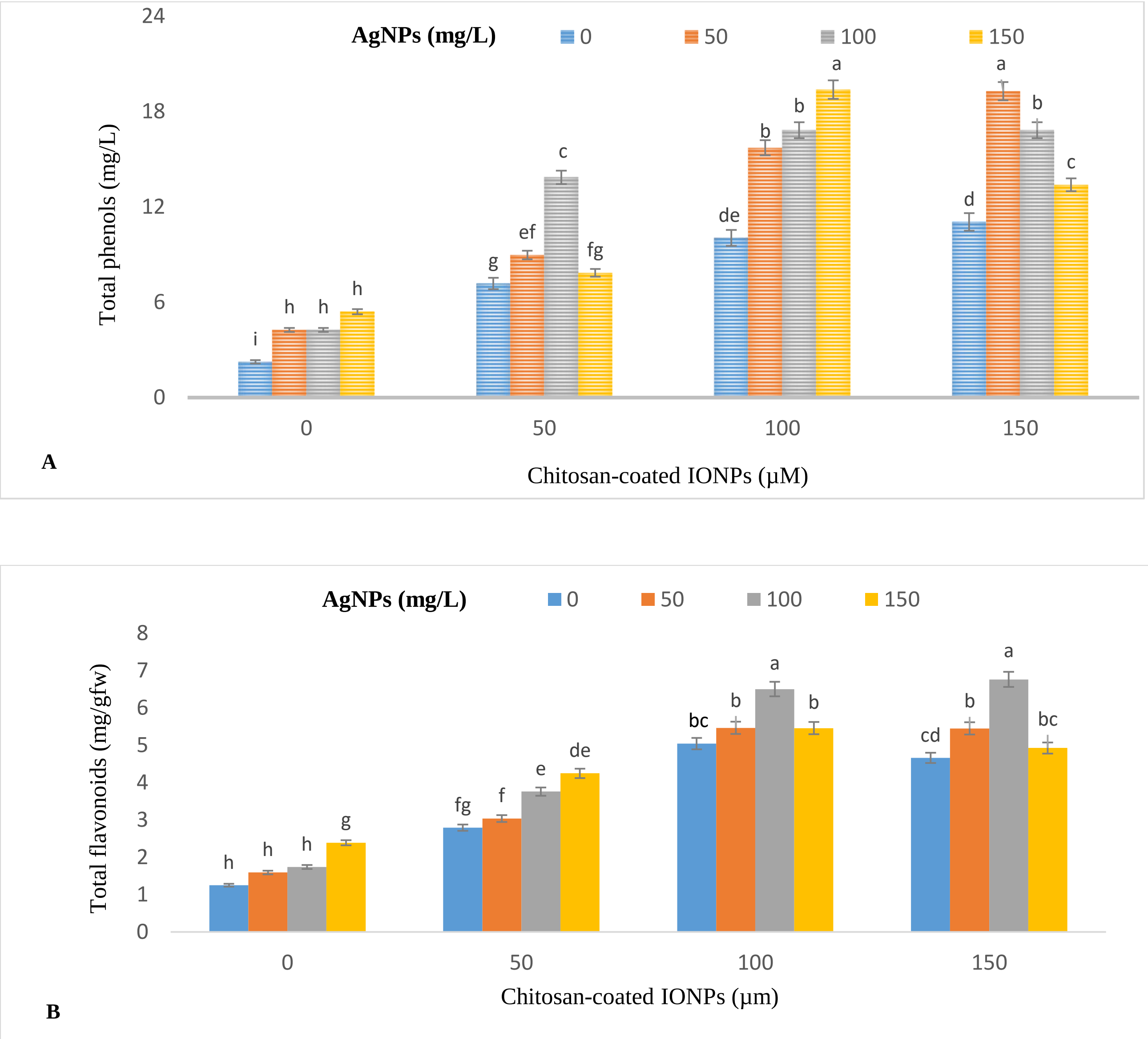


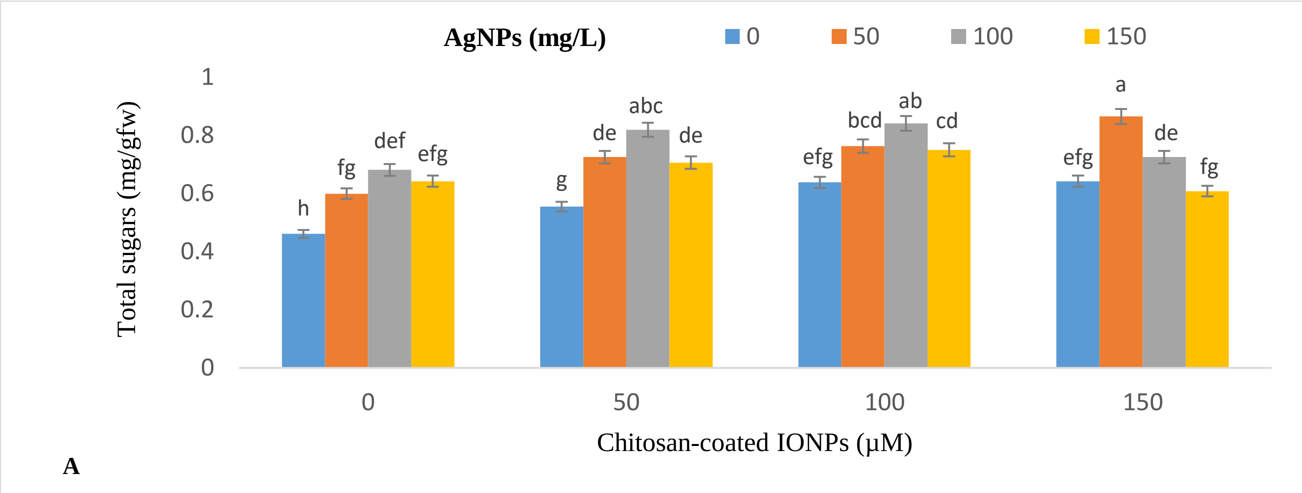
Figure 5. Interaction effects of chitosan-coated iron oxide nanoparticles (chitosan-coated IONPs) and silver nanoparticles (AgNPs) on total phenols (A) and total flavonoids (B) in snapdragon.

Total sugars

The analysis indicated that the total sugar content in snapdragon varied with increasing concentrations of AgNPs and chitosan-coated IONPs. The lowest value was observed in the control treatment (0 mg/L AgNPs and 0 µM chitosan-coated IONPs), while the highest value was recorded in plants treated with 50 mg/L AgNPs + 150 µM chitosan-coated IONPs, followed by 100 mg/L AgNPs + 100 µM chitosan-coated IONPs (Figure 6A).

DPPH

The analysis revealed that increasing concentrations of chitosan-coated IONPs generally enhanced antioxidant capacity. The greatest antioxidant capacity was observed in plants treated with 150 µM chitosan-coated IONPs + 50 mg/L AgNPs and 100 µM chitosan-coated IONPs + 100 mg/L AgNPs (Figure 6B).



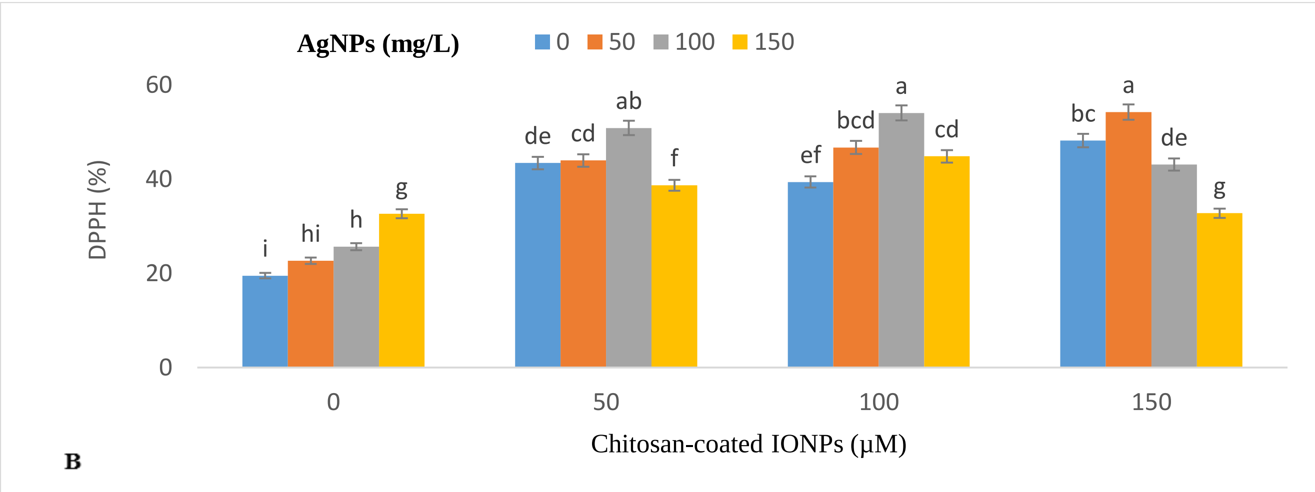


Figure 6. Interaction effects of chitosan-coated iron oxide nanoparticles (chitosan-coated IONPs) and silver nanoparticles (AgNPs) on total sugars (A) and DPPH (B) in snapdragon.

Discussion

The findings of this study demonstrated that foliar application of chitosan-coated IONPs and AgNPs significantly affected the morphological, physiological, and biochemical traits of snapdragon (*Antirrhinum majus*). These results are consistent with previous reports showing that nanoparticles can enhance growth and quality characteristics in ornamental and horticultural plants (Anushi *et al*.

2023; Behzad *et al.* 2024). AgNPs, particularly at 50 mg/L, increased shoot growth, lateral branching, and aerial fresh weight, and also increased other traits at 50-150 mg/L, depending on the trait measured. Chitosan-coated IONPs at 100-150 µM significantly improved vegetative growth, flower production, fresh weight, total sugars, total phenolic content, flavonoids, and antioxidant capacity as compared with the control.

These improvements align with earlier findings in other species. For example, Torabi Giglou *et al.* (2023) reported that chitosan-coated IONPs enhanced photosynthetic capacity in peppermint. AgNPs have been shown to activate antioxidant systems and increase phenolic compounds (Ribeiro *et al.* 2023), while iron-based nanoparticles improved iron availability, chlorophyll synthesis, and biomass in tomato (Rahmatizadeh *et al.* 2019). The chitosan coating likely contributed to nanoparticle stability and reduced potential toxicity, facilitating effective delivery (Shi *et al.* 2019). The results revealed that increasing the concentration of AgNPs significantly increased the total flavonoid content, which is in agreement with the results obtained from *Iris pseudacorus* L. (Pourbeyrami Hir *et al.* 2022). The observed increases in total sugars and flavonoids under combined treatments indicate enhanced carbon allocation and activation of secondary metabolite pathways. Flavonoids and phenolics serve as defensive compounds, and their elevation is consistent with nanoparticle-induced modulation of phenylpropanoid metabolism (Selvakesavan *et al*. 2023). These results are further supported by a previous study on snapdragon by Pourbeyrami Hir and Sardari (2025), which reported significant improvements in vegetative growth, flower diameter, lateral branching, and total phenol content using the same nanoparticle types and concentration ranges.

In conclusion, the combined application of 100-150 µM chitosan-coated IONPs and 50-150 mg/L AgNPs, depending on the traits studied, effectively enhanced most key growth and quality traits of snapdragon. These findings confirm the potential of nanoparticle-based foliar treatments for improving ornamental plant performance under controlled conditions. Future research should explore long-term effects, molecular mechanisms, and environmental safety aspects to support practical applications of nanoparticles in sustainable floriculture.

Conclusion

This study demonstrated that the combined application of chitosan-coated IONPs and AgNPs can effectively improve growth and physiological traits in snapdragon. These nanoparticles enhanced plant height, branching, and flowering while also increasing chlorophyll fluorescence and phenolic compounds. These findings support the strategic use of biocompatible nanoparticles in ornamental plant management as a promising approach for boosting performance and resilience, particularly within sustainable and eco-friendly agricultural systems.

Author Contributions

Pourbeyrami Hir: Conceptualization, methodology, data analysis, data curation, project administration, resources, funding acquisition, writing and editing; Sardari: Data curation. All authors have read and agreed to the published version of the manuscript.

Funding

This research was funded by the University of Mohaghegh Ardabili, Ardabil, Iran.

Data Availability

Data are available on request.

Acknowledgments

The authors would like to thank all the colleagues who assisted us in this study.

Conflict of Interest

The authors declare that they have no conflicts of interest with any individual or organization concerning the subject of this article.

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