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ARTICLE

Dose-Dependent Effects of ZnO Nanoparticles on Growth, Gas Exchange, and Inflorescence Quality in Marigold (*Tagetes erecta* L.)

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ABSTRACT: In soils and substrates, the bioavailability of Zn^{2+} is generally low. The use of zinc oxide nanoparticles (ZnO-NPs) has emerged as a promising strategy in agriculture to improve zinc availability and stimulate plant growth. Their physicochemical properties and biocompatibility promote greater micronutrient uptake and the regulation of physiological processes in plants. In this study, the effects of five concentrations of ZnO-NPs (0, 100, 250, 500, and 1000 mg L^{-1}) and two application methods (foliar or drench) on the growth, gas exchange, and flowering quality of marigold were evaluated. ZnO-NPs significantly influenced plant growth; plant height and total dry weight increased by 28.6% and 35.2%, respectively, at a concentration of 100 mg L^{-1}. Similarly, the flower diameter increased by 28.6%. The number of inflorescences increased 1.6-fold at 1000 mg L^{-1}, whereas photosynthesis, stomatal conductance, and transpiration increased at 250 mg L^{-1} (84.4, 242.8, and 168.8%, respectively). Intercellular carbon increased by 79.3% with the application of 100 mg L^{-1}, and SPAD units increased by 27.2% with the application of 1000 mg L^{-1}. The application of ZnO-NPs significantly improved plant growth and physiological processes, demonstrating their potential as an efficient strategy to optimize crop productivity and quality.

KEYWORDS: Biostimulants; zinc nanomaterials; photosynthesis

1 Introduction

The application of biostimulants in agriculture has gained considerable relevance as a sustainable strategy to improve crop productivity, nutrient use efficiency, and plant physiological performance. Biostimulants are capable of stimulating physiological processes that enhance nutrient absorption and assimilation, thereby promoting plant growth and development [1]. In this context, inorganic biostimulants based on nanotechnology have emerged as innovative alternatives with high potential for application in modern agricultural production systems [2]. In particular, NPs (dimensions $\leq$ 100 nm) have attracted increasing attention because of their unique physicochemical properties, including high specific surface area, high reactivity, and nanoscale size, characteristics that facilitate their interaction with plant tissues and various physiological processes [3,4].

NPs can be absorbed through the roots, stomata, and cuticular pathways, facilitating their mobility and translocation within plant tissues [5]. Once internalized, they may act as physiological modulators capable

of influencing plant metabolism, photosynthetic performance, nutrient uptake, and growth responses [6,7]. Two main mechanisms have been proposed to explain NP-induced biostimulation. The first involves the initial interactions between NPs and the cell surface, which can trigger signaling pathways that activate positive physiological responses. The second mechanism is associated with NP internalization and the subsequent availability of their components to participate in different plant metabolic functions [8,9].

Among the nanoparticles most widely studied in agriculture are ZnO-NPs, which are considered among the most versatile metal oxides because of their broad range of applications in biomedicine, biosensors, cosmetics, water treatment, controlled drug delivery, and agriculture [10–12]. Zn is an essential micronutrient involved in several physiological and biochemical processes, including chlorophyll synthesis, enzyme activation, auxin metabolism, membrane stability, and protection against oxidative damage [13–15]. Consequently, compared with conventional Zn sources, ZnO-NPs have attracted considerable attention as potential biostimulants since their nanoscale properties may increase Zn bioavailability, absorption efficiency, and mobility within plant tissues [16,17]. Several studies have demonstrated the biostimulant potential of ZnO-NPs in different crops, promoting increases in yield, biomass accumulation, plant growth, fruit quality, and the accumulation of bioactive and antioxidant compounds [18,19]. Similarly, improvements in physiological traits related to chlorophyll content, root development, productivity, and the accumulation of phenols and flavonols in horticultural species have also been reported [20,21]. Despite these positive responses, the effects of ZnO-NPs are not universal, as they may also induce toxic responses depending on factors such as NP size and shape, applied dose, application method, exposure time, environmental conditions, and plant species [22,23]. Previous studies have reported phytotoxic effects associated with ZnO-NPs, including reductions in biomass, root growth, and shoot development, as well as physiological, transcriptomic, and cellular alterations accompanied by oxidative stress in different plant species [24–26]. These contrasting responses highlight the importance of establishing appropriate concentration ranges and application methods specific to each plant species, particularly under nonstress conditions where biostimulant effects can be more accurately evaluated.

Despite the growing interest in agricultural nanotechnology, studies focused on ornamental species remain relatively limited. In ornamental horticulture, plant quality is primarily determined by traits such as flower size, number of inflorescences, color intensity, and postharvest longevity, which directly influence the commercial value and market acceptance of plants [27,28]. Therefore, identifying sustainable strategies capable of simultaneously improving physiological performance and ornamental quality represents important challenges for floriculture production systems. Although ZnO-NPs have been shown to have growth-promoting effects on several agricultural crops, information regarding their dose-dependent physiological effects on ornamental species, particularly under optimal growth conditions, remains scarce. Understanding these physiological responses is essential for establishing optimal application ranges and evaluating the potential of ZnO-NPs as biostimulants in ornamental horticulture.

Marigold is among the most important ornamental species worldwide because of its high ornamental value and its applications in the pharmaceutical, cosmetic, and food industries, which are associated with its elevated content of carotenoids and bioactive compounds [29]. In addition to its ornamental importance, this species is widely cultivated for landscaping and commercial flower production, where inflorescence quality and plant vigor constitute critical parameters for marketability [30,31]. Therefore, evaluating the physiological and ornamental responses of marigold to ZnO-NP application may contribute to the development of more sustainable and efficient production strategies for ornamental horticulture.

Therefore, the objective of this study was to evaluate the dose-dependent effects of the application of ZnO-NPs through foliar spray and drench methods on the growth, gas exchange, and inflorescence

quality of marigold cultivated under greenhouse conditions. It was hypothesized that ZnO-NPs act as biostimulants capable of enhancing photosynthetic activity, plant growth, and ornamental quality, with responses depending on the NP concentration and application method.

2 Materials and Methods

2.1 Synthesis and Modification of the ZnO-NPs

Spherical ZnO-NPs were obtained using the precipitation method [32]. For this purpose, 26.33 g of zinc acetate [$Zn(O_2CCH_3)_2$] was dissolved in a mixture of 1700 mL of ethanol and 300 mL of deionized water. Subsequently, 5.36 mL of triethanolamine (TEA) and 1.42 mL of n-propylamine (NPA) were added, and the reaction was maintained at 65°C under reflux conditions with constant stirring for 12 h.

After the reaction was complete, the mixture was allowed to cool to room temperature. The resulting material was subsequently washed, centrifuged, and vacuum-dried for 12 h at 80°C.

2.2 Characterization of the ZnO-NPs

The crystalline structure of the ZnO-NPs was determined by X-ray diffraction (XRD) using a diffractometer (Siemens D-5000 diffractometer, Munich, Germany). The morphology and particle size distribution were examined by high-resolution transmission electron microscopy (HRTEM) using an FEI Titan 80–300 kV HRTEM (Hillsboro, OR, USA). The functional groups were identified by infrared spectroscopy using an FT-IR spectrometer (Thermo Scientific Nicolet iS50, Thermo Fisher Scientific, Waltham, MA, USA).

2.3 Plant Material and Growth Conditions

The experiments were conducted in greenhouses at the Department of Horticulture of the Universidad Autónoma Agraria Antonio Narro, located in Saltillo, Coahuila, Mexico. During the study period, the average maximum temperature was 23.2°C, the average minimum temperature was 12.4°C, and the seasonal mean temperature was 18.1°C. The relative humidity averaged 95% (maximum mean), 47% (minimum mean), and 72% (seasonal mean). The seasonal average photosynthetically active radiation was 370 μmol m^{-2} s^{-1}. The hybrid marigold material F1 Big Duck Orange, obtained from the commercial seed company AmeriSeed, was used. Seeds were sown in 200-cell polystyrene trays using a substrate composed of peat moss and perlite (1:1, v/v). Afterward, the seedlings were transplanted into 4-L containers filled with a mixture of soil collected from the study site and coconut fiber at a 1:1 ratio (v/v). Chemical analysis of the soil revealed a pH of 8.07, an electrical conductivity of 1.09 dS m^{-1}, and macronutrient contents of 13.8 mg kg^{-1} N–NO_3^-, 34.9 mg kg^{-1} P, 221 mg kg^{-1} K^+, 2774 mg kg^{-1} Ca^{2+}, and 346 mg kg^{-1} Mg^{2+}. All the plants, including those treated with ZnO-NPs, were irrigated with a complete nutrient solution according to Steiner [33], which contained 12 NO^{3-}, 1 $H_2PO_4^-$, 7 K^+, 9 Ca^{2+}, 4 Mg^{2+}, and 7 SO_4^{2-} in meq L^{-1}. Micronutrients were supplied in mg L^{-1} as follows: 5.3 Fe-EDTA, 0.4 Zn-EDTA, 2.6 Mn-EDTA, 0.5 Cu-EDTA, 0.2 B ($Na_2[B_4O_5(OH)_4]8H_2O$), and 0.2 Mo (Na_2MoO_4). The pH of the nutrient solution was adjusted to 6.0 ± 0.1, and the electrical conductivity (EC) was adjusted to 2.3 dS m^{-1}. The nutrient solution was adjusted according to the crop phenology. During the establishment stage, the solution was applied at 50% strength; four weeks later, it was increased to 100% and maintained at this level during the floral induction and flowering stages. Irrigation was supplied through a drip irrigation system with two emitters per container, each with a flow rate of 1 L h^{-1}.

The crop remained in the greenhouse for 110 days after transplanting, the period in which the plants reached the commercial flowering stage, and the corresponding evaluations were conducted.

2.4 Foliar and Drench Application

The applications of the ZnO-NPs began 20 days after transplanting (DAT) and were repeated at 15-day intervals, for a total of six applications. The concentrations used were 0, 100, 250, 500, and 1000 mg L^{-1} of ZnO-NPs, which were applied either foliage or as a drench depending on the treatment. Considering the total number of applications, the total amount of Zn^{2+} supplied through the ZnO-NPs corresponded to the cumulative sum of the six applications at each concentration. Additionally, all the plants received Zn^{2+} from the nutrient solution in the form of Zn-EDTA at a concentration of 0.4 mg L^{-1}, which remained constant across all the treatments throughout the entire crop cycle. For the foliar application, treatments were applied manually, ensuring complete foliage coverage with 100 mL per plant of the ZnO-NP solutions at different concentrations (0, 100, 250, 500, and 1000 mg L^{-1}), using a manual pressure sprayer. The substrate application (drench) consisted of directly pouring 100 mL of each ZnO-NP solution into the root zone of each experimental plant. Prior to application, the ZnO-NP dispersions in distilled water were subjected to sonication using an ultrasonic bath (BRANSON 2510 ultrasonic cleaner; Connecticut, Waltham, MA, USA) for 15 min at 38% amplitude.

2.5 Evaluated Variables

Plant height was measured using a measuring tape (TRUPER) from the base of the stem to the highest point of the plant. Stem diameter was measured at the base of the main stem using a digital caliper (STAINLESS). These growth parameters were evaluated at 30, 60, and 90 DAT, with 12 plants per treatment assessed. Dry weight was determined by evaluating six plants per treatment at 90 DAT. The plant material was subjected to a drying process in a forced-air oven (NOVATECH HS45-AIA drying oven, Murrieta, CA, USA) at 65°C for 48 h. After this period, the dry weight was recorded using a digital balance (CGOLDENWALL). Floral dome diameter and height were evaluated for 12 plants per treatment at 70, 80, and 90 DAT. Floral diameter was measured as the maximum diameter of the fully open inflorescence using a digital caliper. Floral dome height was measured from the base of the capitulum to the highest point of the flower apex. The number of flowers per plant was determined by directly counting all fully developed inflorescences present on each plant. Only flowers that had reached full anthesis were considered, excluding developing buds or senescent inflorescences. Records were taken manually for each experimental unit at the end of the crop cycle (110 DAT). Color measurements were performed on randomly selected petals. For each treatment, three flowers were selected, and three readings were taken per flower; the values were recorded in the CIE L*, a*, and b* system, where L* corresponds to luminosity, a* to the red–green axis, and b* to the yellow–blue axis. Measurements were obtained using a colorimeter (KONICA MINOLTA CR-300 colorimeter, Osaka, Japan). SPAD units were measured using a chlorophyll meter (KONICA MINOLTA SPAD-502, Osaka, Japan).

Measurements were taken between 10:00 and 12:00 h under conditions of maximum irradiance, with photosynthetically active radiation of approximately 1705 μmol m^{-2} s^{-1} inside the greenhouse. Finally, the photosynthetic rate, transpiration rate, stomatal conductance, and intercellular carbon content were measured using a portable photosynthesis system (LI-COR 6400XT; Lincoln, NE, USA). Gas exchange measurements were performed on fully expanded leaves located in the middle third of the plant, which were directly exposed to light and without visible damage, to ensure comparability among treatments. To avoid fluctuations among measurements, the conditions inside the infrared gas analyzer (IRGA) chamber of the instrument were kept constant during all the readings, with a CO_2 concentration of 390.4 μmol mol^{-1}, an air temperature maintained at 34.4°C, and a relative humidity of 68.4%.

2.6 Experimental Design and Statistical Analysis

The study included five concentrations of ZnO-NPs (0, 100, 250, 500, and 1000 mg L^{-1}) and two application methods (foliar or drench). The treatments were arranged in a 5 × 2 factorial randomized complete block design, resulting in a total of 10 treatments, 12 independent plants were used per treatment and distributed across three experimental blocks. Each plant was individually grown in a separate container, considered an experimental unit, and evaluated independently. Statistical analysis was performed using analysis of variance (ANOVA), and when significant differences were detected, a multiple comparison test was conducted using the Tukey procedure ($p < 0.05$) with SAS 9.0.

3 Results

3.1 Characterization of the ZnO-NPs

HRTEM analysis revealed that the nanoparticles exhibited a predominantly spherical morphology, and the histogram showed a particle size distribution with an average diameter of 75.05 nm (Fig. 1a,b). XRD analysis of the ZnO-NPs revealed characteristic diffraction peaks corresponding to the wurtzite crystalline phase, confirming their high structural purity (JCPDS 36-1451) (Fig. 1c). Furthermore, FT-IR analysis revealed the presence of a typical band at 520.5 cm^{-1}, attributed to the stretching vibration of Zn–O bonds characteristic of metal oxides (Fig. 1d).

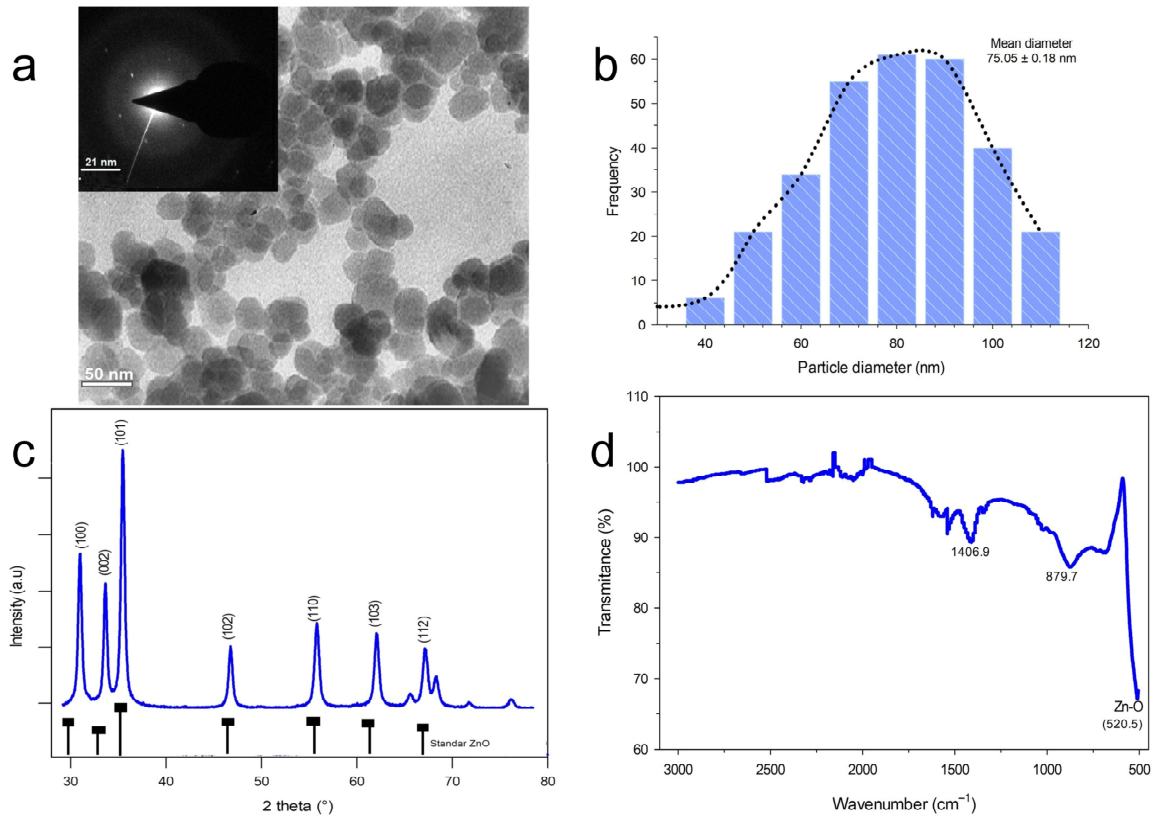

Figure 1: HRTEM micrographs (**a**), particle size distribution (**b**), XRD patterns (**c**), and FT-IR spectrum of the ZnO-NPs (**d**).

3.2 Effect of ZnO-NPs on Growth and Development

In general, compared with the control treatment, the ZnO-NP treatment tended to increase the evaluated growth parameters, although the magnitude and statistical significance of the responses depended on the variable and concentration evaluated; the highest values were observed at a concentration of 100 mg L^{-1} (Table 1). The foliar application method resulted in 8.22% and 3.53% increases in plant height and stem diameter, respectively. However, for root dry weight, shoot dry weight, and total dry weight, the application method did not have a significant effect (Table 1).

Table 1: Effects of different concentrations of ZnO-NPs and two application methods on the growth and development of marigold plants.

	Plant Height (cm)	Stem Diameter (mm)	Root Dry Weight (g)	Shoot Dry Weight (g)	Total Dry Weight (g)
Concentration (mg L^{-1})					
Control	29.6 ± 0.4^{c}	8.7 ± 0.1^{b}	11.9 ± 0.4^{c}	21.7 ± 1.0^{b}	33.5 ± 1.3^{b}
100	35.2 ± 0.5^{a}	10.4 ± 0.2^{a}	16.9 ± 0.8^{a}	28.4 ± 1.9^{a}	45.3 ± 2.3^{a}
250	34.5 ± 0.5^{ab}	9.9 ± 0.3^{a}	14.4 ± 0.6^{b}	25.8 ± 2.3^{ab}	40.2 ± 2.4^{a}
500	33.6 ± 0.5^{b}	9.9 ± 0.3^{a}	16.9 ± 0.6^{a}	25.4 ± 2.3^{ab}	42.3 ± 2.5^{a}
1000	35.1 ± 0.4^{ab}	9.8 ± 0.4^{a}	17.8 ± 0.9^{a}	27.3 ± 1.9^{a}	45.1 ± 2.4^{a}
ANOVA	<0.001	<0.001	<0.001	0.01	<0.001
Application					
Foliar	34.9 ± 0.4^{a}	9.6 ± 0.20^{b}	15.5 ± 0.5^{a}	25.2 ± 1.1^{a}	40.7 ± 1.3^{a}
Drench	32.3 ± 0.3^{b}	9.9 ± 0.19^{a}	15.6 ± 0.7^{a}	26.2 ± 1.4^{a}	41.8 ± 1.7^{a}
ANOVA	<0.001	0.0397	0.7498	0.4084	0.3706
Interaction					
C ∗ AM	0.7354	<0.001	<0.001	0.4228	0.0232

Note: C ∗ AM = concentration ∗ application mode. Different letters within the same column indicate significant differences according to Tukey's multiple comparison test ($p \leq 0.05$).

3.3 Effect of ZnO-NPs on Inflorescence Quality

The diameter or size of the inflorescence significantly increased with the application of 100 mg L^{-1}, representing an increase of 28.66% compared with that of the control. Compared with the control treatment, the 1000 mg L^{-1} treatment increased the height of the inflorescence dome by 65.61%. Compared with the control treatment, the application of 250 and 1000 mg L^{-1} ZnO-NPs significantly increased the number of flowers per plant by approximately 1.6-fold (Table 2). The CIELAB color system is widely used to characterize floral coloration in different plant species [34,35]. In this study, colorimetric evaluation revealed that the parameters of the color space (L*, a*, and b*) were directly associated with the luminosity, chromatic intensity, and hue of the inflorescence. Compared with that of the control, the L* value was greater at all the evaluated concentrations, with the greatest increase occurring at a concentration of 1000 mg L^{-1}. The a* and b* values represent the degree of redness and yellowness, respectively, of the inflorescence color. In our study, the a* and b* values increased at all concentrations compared with those of the control; however, both values were greater at a concentration of 1000 mg L^{-1} (Table 2).

The foliar application method significantly improved the parameters of floral dome height, number of flowers, and a* color value. These results contrast with the L* and b* values, which improved with drench application. Similarly, the inflorescence diameter variable was not affected by either application method (Table 2).

Table 2: Effects of different concentrations of ZnO-NPs and two application methods on the inflorescence quality of marigold plants.

	Inflorescence Diameter (mm)	Floral Dome Height (mm)	Flowers per Plant	L*	a*	b*
Concentration (mg L^{-1})						
Control	53.1 ± 0.8^{c}	28.8 ± 0.7^{b}	5.3 ± 0.1^{c}	63.7 ± 0.3^{b}	21.6 ± 0.4^{b}	95.9 ± 0.2^{d}
100	68.3 ± 0.9^{a}	28.6 ± 0.9^{b}	7.2 ± 0.2^{b}	69.9 ± 0.4^{a}	25.8 ± 0.6^{a}	100.4 ± 0.4^{b}
250	61.5 ± 0.8^{b}	27.3 ± 1.1^{b}	8.6 ± 0.3^{a}	70.8 ± 0.2^{a}	25.4 ± 0.3^{a}	101.8 ± 0.3^{ab}
500	62.4 ± 0.9^{b}	19.6 ± 0.6^{c}	7.8 ± 0.4^{ab}	71.1 ± 0.3^{a}	25.9 ± 0.3^{a}	98.5 ± 0.5^{c}
1000	62.8 ± 0.8^{b}	47.7 ± 0.8^{a}	8.6 ± 0.5^{a}	71.6 ± 0.4^{a}	26.0 ± 0.2^{a}	102.5 ± 0.5^{a}
ANOVA	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Application						
Foliar	61.2 ± 0.7^{a}	29.3 ± 1.4^{b}	7.0 ± 0.3^{b}	70.3 ± 0.6a	24.6 ± 0.4b	100.2 ± 0.5^{a}
Drench	62.1 ± 0.9^{a}	31.5 ± 1.3^{a}	8.0 ± 0.3^{a}	68.8 ± 0.7b	25.3 ± 0.4a	99.4 ± 0.5^{b}
ANOVA	0.1517	0.0009	<0.001	0.0125	0.0396	0.0187
Interaction						
C*AM	<0.001	0.0002	<0.001	0.5925	0.4005	0.3402

Note: Different letters within the same column indicate significant differences according to Tukey's multiple comparison test ($p \leq 0.05$). C ∗ AM = concentration ∗ application mode.

3.4 Effect of ZnO-NPs on Gas Exchange Parameters

Compared with the control, the concentration of 250 mg L^{-1} significantly increased the photosynthetic rate, representing an increase of 84.4% (Table 3). Similar trends were observed for the stomatal conductance and transpiration rate, which increased by approximately 3.4 (242.8%) and 2.7 (168.8%) times, respectively, compared with those of the control (Table 3). The intercellular carbon concentration significantly increased with the application of 100 mg L^{-1} and 500 mg L^{-1} ZnO-NPs, representing increases of 79.3% and 66.3%, respectively, compared with that of the control. SPAD units increased at all the ZnO-NP concentrations compared with those of the control; however, the highest value of this variable was recorded at a concentration of 1000 mg L^{-1}, representing a 27.1% increase compared with that of the control (Table 3). With respect to the application method, the parameters of the photosynthetic rate and intercellular carbon increased with drench application compared with those with foliar application, in contrast to the stomatal conductance, which improved with foliar application. The parameters of the transpiration rate and SPAD units did not differ between the application methods.

Table 3: Effects of different concentrations of ZnO-NPs and two application methods on gas exchange parameters in marigold plants.

	Photosynthetic Rate (μmol CO_2 m^{-2} s^{-1})	Stomatal Conductance (mol H_2O m^{-2} s^{-1})	Internal CO_2 Concentration (μmol mol^{-1})	Transpiration Rate (mmol H_2O m^{-2} s^{-1})	SPAD Units
Concentration (mg L^{-1})					
Control	7.7 ± 0.3^{c}	0.0623 ± 0.0056^{b}	155.4 ± 4.8^{d}	0.5367 ± 0.0344^{d}	46.29 ± 0.56^{b}
100	12.9 ± 0.6^{ab}	0.0883 ± 0.0091^{b}	278.6 ± 7.8^{a}	0.7564 ± 0.436^{cd}	56.19^{a}
250	14.2 ± 0.7^{a}	0.2137 ± 0.0721^{a}	238.5 ± 5.9^{b}	1.4430 ± 0.1259^{a}	57.12 ± 0.75^{a}
500	11.7 ± 1.2^{b}	0.1288 ± 0.0147^{ab}	258.4 ± 19.3^{ab}	1.2245 ± 0.0929^{ab}	58.32 ± 0.89^{a}
1000	11.9 ± 0.8^{b}	0.0923 ± 0.0105^{b}	212.3 ± 27.9^{c}	0.9433 ± 0.1045^{bc}	58.85 ± 0.67^{a}
ANOVA	<0.001	0.0093	<0.001	<0.001	<0.001
Application					
Foliar	10.1 ± 0.5^{b}	0.1475 ± 0.0307^{a}	194.4 ± 10.1^{b}	0.9793 ± 0.0791^{a}	55.87 ± 0.74^{a}
Drench	12.9 ± 0.7^{a}	0.0865 ± 0.0060^{b}	262.8 ± 11.7^{a}	0.9822 ± 0.0823^{a}	54.83 ± 0.75^{a}
ANOVA	<0.001	0.0286	<0.001	0.9675	0.0946
Interaction					
Conce ∗ Application	<0.001	0.0304	<0.001	0.0022	0.0094

Note: Different letters within the same column indicate significant differences according to Tukey's multiple comparison test ($p \leq 0.05$). C ∗ AM = concentration ∗ application mode.

3.5 Factor Interactions on Agronomic Parameters

The interaction between ZnO-NPs C × AM, significantly affected stem diameter, root dry weight, and total dry weight, indicating that the plant response depended not only on the nanoparticle concentration but also on the application pathway. Compared with that of the corresponding control, the stem diameter increased by 40.6% when 1000 mg L^{-1} ZnO-NPs were applied via foliar application; however, at concentrations of 250 mg L^{-1} and 500 mg L^{-1}, this variable also increased, but with foliar application (Fig. 2a). Compared with that of the corresponding control, the dry weight of roots increased by 52.7% with the foliar application of 100 mg L^{-1} ZnO-NPs; likewise, compared with the corresponding control, the concentration of 1000 mg L^{-1} increased this variable by 82.7% with drench application (Fig. 2b). The total dry weight of the plants was greater at concentrations of 100 mg L^{-1}, 500 mg L^{-1}, and 1000 mg L^{-1} when the application was performed by drenching, with increases of 43.2%, 41.3%, and 57.5%, respectively, compared with those of the corresponding control. However, compared with the corresponding control, the application of 100 mg L^{-1} via foliar application increased the total dry weight (Fig. 2c). These results indicate that foliar and drench applications induced distinct growth responses depending on the evaluated variable and ZnO-NP concentration.

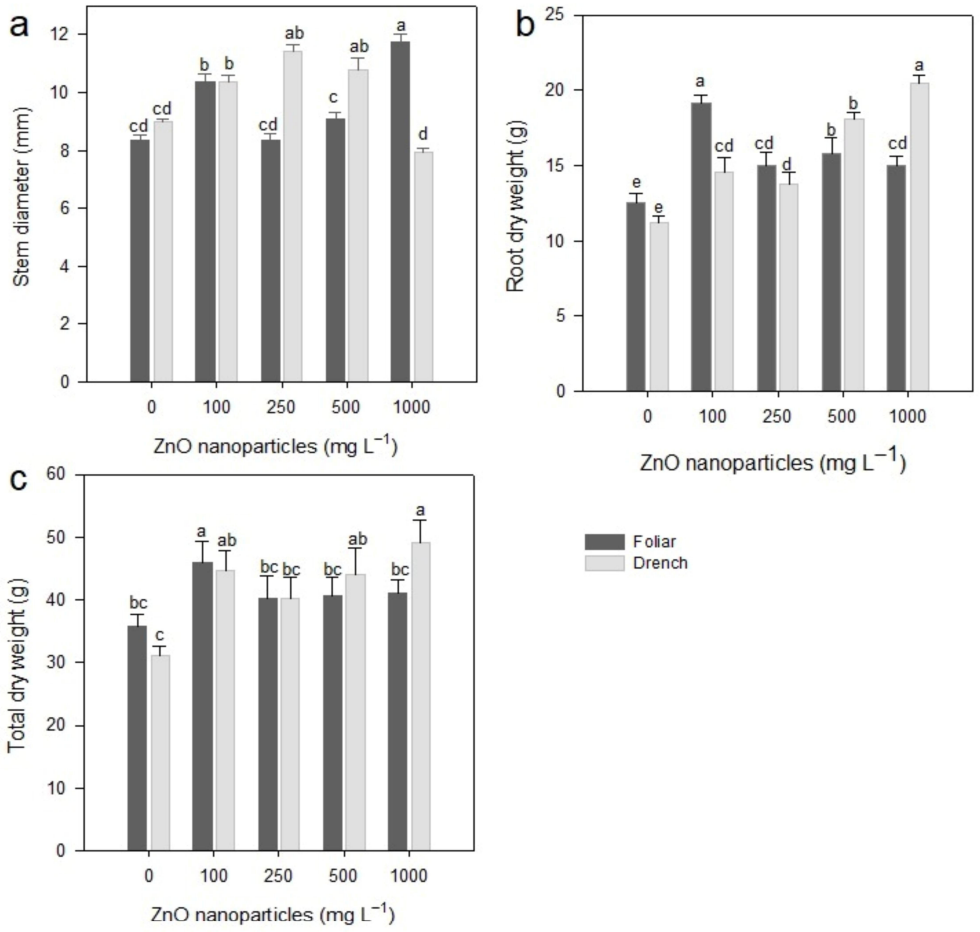

Figure 2: Effects of the C × AM interaction of ZnO-NPs on growth variables in marigold plants: stem diameter (**a**), root dry weight (**b**), and total dry weight (**c**). Bars = standard error. Different letters indicate significant differences according to Tukey's multiple comparison test ($p < 0.05$).

3.6 Interaction Effects of Factors on Inflorescence Quality

Significant interactions were observed between the evaluated factors for the variables inflorescence diameter, floral dome height, and number of flowers per plant. The inflorescence diameter increased by 36.9% compared with that of the corresponding control at a concentration of 100 mg L^{-1} ZnO-NPs when drenching was applied (Fig. 3a). Compared with that of the corresponding control, the height of the inflorescence dome improved at a concentration of 1000 mg L^{-1} without the influence of the application method, since both the foliar and drench applications increased by 61.9% and 69.5%, respectively (Fig. 3b). The number of inflorescences per plant doubled with drench application at a concentration of 1000 mg L^{-1}, increasing from 5.33 flowers in the control to 10.16 flowers per plant (Fig. 3c). However, a significant increase was also observed at the other concentrations: compared with the control treatment, foliar application at 250 mg L^{-1} increased the number of flowers per plant by 75%, whereas drench application at 500 mg L^{-1} increased it by 68.8% (Fig. 3c).

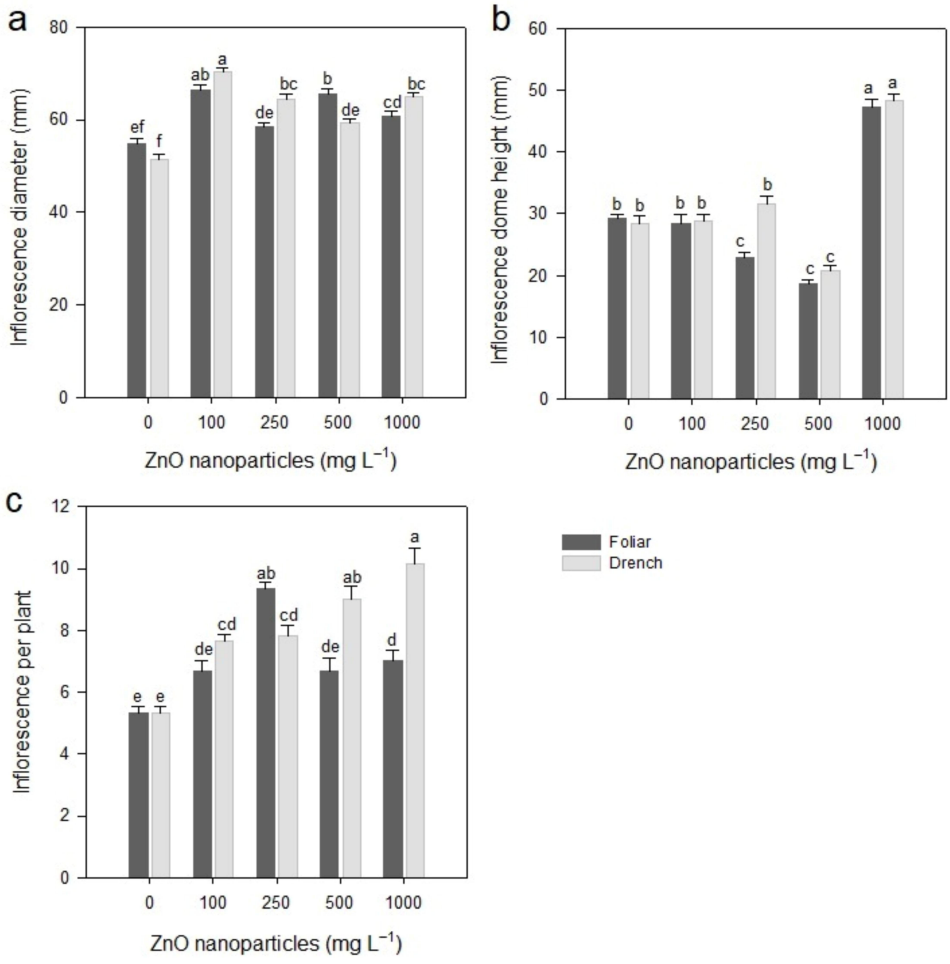

Figure 3: Effects of the C ∗ AM interaction of the ZnO-NPs on the inflorescence quality of marigold plants: inflorescence diameter (**a**), inflorescence dome height (**b**), and number of inflorescences per plant (**c**). Bars = standard error. Different letters indicate significant differences according to Tukey's multiple comparison test ($p < 0.05$).

3.7 Interaction Effects of Factors of ZnO-NPs on Gas Exchange Parameters

A significant interaction between C*AM was detected for all the gas exchange variables under study. The photosynthetic rate increased at all ZnO-NP concentrations when drenching was applied; however, the greatest increase was recorded at a concentration of 250 mg L^{-1}, which was 2.4 times greater than that of the corresponding control (Fig. 4a). Similarly, the concentrations of 100 mg L^{-1} and 250 mg L^{-1} with foliar application also resulted in an increase in this parameter (Fig. 4a). Compared with that of the corresponding control, the stomatal conductance increased by 163% when 250 mg L^{-1} ZnO-NPs were applied via foliar application (Fig. 4b). In terms of the intercellular carbon concentration, the greatest increase (71.8%) was recorded with the drench application of 500 mg L^{-1} ZnO-NPs; however, this parameter also increased at 100 mg L^{-1} and 1000 mg L^{-1} (Fig. 4c). Compared with that in the corresponding control plants, the transpiration rate in the plants treated with drench application of 250 mg L^{-1} ZnO-NPs increased by 3.07 times (Fig. 4d). Finally, SPAD units increased at concentrations of 250 mg L^{-1}, 500 mg L^{-1}, and 1000 mg L^{-1} when the application was performed via foliar spraying, representing increases of 23.7%, 29.1%, and 24%, respectively, compared with those of the corresponding control (Fig. 4e).

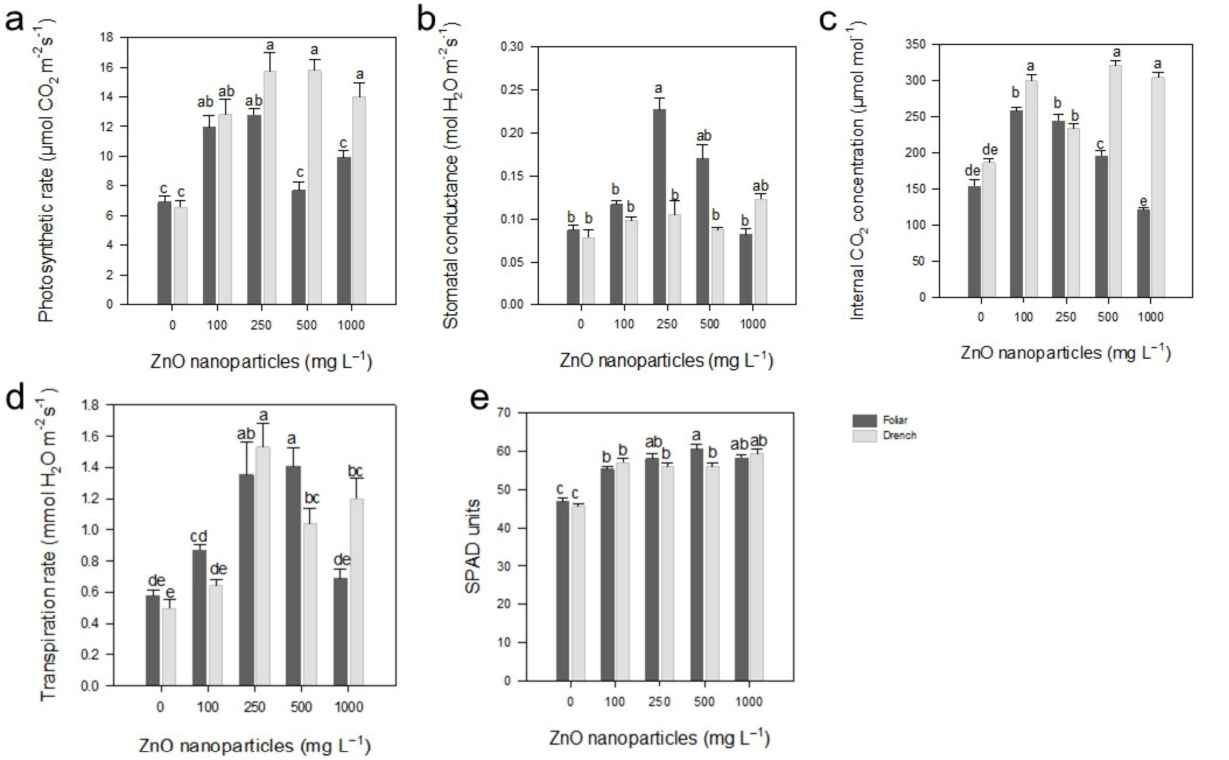

Figure 4: Effects of the C ∗ AM interaction of ZnO-NPs on physiological parameters in marigold plants: photosynthetic rate (**a**), stomatal conductance (**b**), intercellular carbon concentration (**c**), transpiration rate (**d**), and SPAD units (**e**). Bars = standard error. Different letters indicate significant differences according to Tukey's multiple comparison test ($p < 0.05$).

4 Discussion

Following the application of ZnO-NPs, the growth, inflorescence quality, and gas exchange parameters of marigold plants increase; the beneficial effects of ZnO-NPs have been attributed to their chemical composition, nanometric size (which allows rapid penetration and distribution within the cell), surface

area, stability, and biochemical reactivity [36]. The growth parameters showed the greatest response to 100 mg L^{-1} of ZnO-NPs, indicating that this concentration provided an adequate Zn^{2+} availability to stimulate plant metabolism without inducing toxic effects. Although all the treatments received Zn^{2+} supplied as Zn-EDTA (0.4 mg L^{-1}) through the nutrient solution, including the control treatment, the differential responses observed among the different concentrations of ZnO-NPs suggest that additional effects are associated with the application of ZnO-NPs. Furthermore, the significant interaction between concentration and application method indicated that the physiological response of marigold plants depended not only on the ZnO-NP dose but also on the nanoparticle uptake pathways and Zn distribution within plant tissues. In the present study, drench application promoted greater increases in root dry weight and total biomass, whereas foliar application exerted stronger effects on stem diameter. These differences may be associated with the distinct absorption and translocation mechanisms of the ZnO-NPs. Root-applied nanoparticles may partially dissolve in the rhizosphere, releasing Zn^{2+} ions that are subsequently absorbed and transported through the xylem, thereby increasing nutrient availability and biomass accumulation. In contrast, foliage-grown ZnO-NPs may enter leaves through stomatal and cuticular pathways, resulting in faster localized effects on aerial growth and leaf metabolism [37–39]. Haga clic o pulse aquí para escribir texto. Previous studies in crops such as bell pepper plants (*Capsicum annuum* L.), habanero pepper plants (*Capsicum chinense* Jacq) and lettuce have consistently reported positive effects of ZnO-NPs on plant height, stem diameter, and fresh and dry biomass as well as Zn^{2+} content in plant tissue [8,40,41], supporting the stimulatory responses observed in the present study. Similarly, in *Ginkgo biloba*, improvements in plant growth, root dry biomass, and grain yield have been reported following the application of ZnO-NPs [20], whereas in potato (*Solanum tuberosum* L.), increases in yield, tuber dry weight, starch content, total soluble solids (TSS), and vitamin C content have also been observed [42]. This beneficial effect may be attributed to Zn^{2+} being an essential micronutrient involved in multiple metabolic processes, including protein synthesis, cell division, membrane integrity maintenance, and the regulation of phytohormones such as auxins; in addition, Zn^{2+} promotes the activity of enzymes associated with plant growth, stimulating radicle development and increasing biomass accumulation in plants [43,44]. Despite the growing interest in the application of nanomaterials in agriculture, research on the effects of ZnO-NPs on ornamental species remains limited. The response of inflorescence quality variables was dose dependent, since different floral traits exhibited distinct optimal concentrations. For instance, the increase in inflorescence diameter was greatest at 100 mg L^{-1}, whereas the number of inflorescences per plant increased from 250 to 1000 mg L^{-1}, and the inflorescence dome height was greatest at 1000 mg L^{-1}. In addition, the interaction between concentration and application method revealed that drench application promoted greater improvements in flower number and inflorescence diameter, suggesting that root-mediated Zn^{2+} uptake may favor the allocation of photoassimilates and nutrients toward reproductive structures. Zinc plays important roles in cell division, protein biosynthesis, and hormone regulation, particularly in terms of auxin metabolism, which may contribute to enhanced floral development and inflorescence formation [45,46]. Furthermore, improved reproductive performance may also be associated with the enhanced photosynthetic activity induced by ZnO-NPs, since greater carbon assimilation can increase the availability of assimilates required for flower formation and growth [47,48]. However, studies in ornamental species such as Haga clic o pulse aquí para escribir texto. sunflower (*Helianthus annuus* L.), lisianthus (*Eustoma grandiflorum* (Raf.) Shinn) and safflower (*Carthamus tinctorius* L.) have demonstrated improvements in flower size and number, as well as increased chlorophyll content following the application of ZnO-NPs [49–51]. These findings are consistent with our results, in which ZnO-NPs increased flower number and improved the inflorescence quality of marigold. Haga clic o pulse aquí para escribir texto. An adequate supply of Zn^{2+} promotes greater

biomass, improved chlorophyll content, earlier flowering, and larger flowers, whereas Zn^{2+} deficiency causes delayed flowering and small flowers, and Zn^{2+} excess may cause toxicity [46]. Thus, the application of ZnO-NPs could represent a promising alternative for increasing the yield and quality of ornamental plants; however, further systematic research in this area is still needed.

The gas exchange variables were greater at 250 mg L^{-1} of ZnO-NPs. This behavior suggests that different physiological responses exhibit distinct sensitivity thresholds to ZnO-NPs. Previous studies have demonstrated that relatively high concentrations of ZnO-NPs can temporarily stimulate the photosynthetic machinery through increases in chlorophyll content and the activation of key photosynthesis-related enzymes such as Rubisco and carbonic anhydrase [23,52–54]. In the present study, the interaction between concentration and application method also influenced the photosynthetic rate, stomatal conductance, transpiration, and SPAD index, indicating that physiological responses depended on both Zn availability and the route of nanoparticle uptake. Drench application promoted greater increases in the photosynthetic rate, stomatal conductance, and transpiration, suggesting that root-mediated Zn absorption favored systemic physiological activity associated with carbon assimilation and stomatal regulation [55]. In contrast, foliar application resulted in greater SPAD values, possibly because of the direct interaction of ZnO-NPs with leaf tissues and chlorophyll-associated metabolic processes [56,57]. Similar differences between foliar and root nanoparticle applications have been reported previously, where foliar treatments induced faster localized responses, whereas root applications promoted more sustained systemic effects on plant metabolism and biomass accumulation [58,59].

Our results are consistent with those of several studies conducted in agricultural crops, where ZnO-NPs promoted significant improvements in processes associated with gas exchange and photosynthetic activity. In crops such as rice (*Oryza sativa* L.), tea plants (*Camellia sinensis* L.), coriander (*Coriandrum sativum* L.) and potato, increased photosynthetic rates and photosynthetic pigment contents have been reported following the application of ZnO-NPs [42,60–62]. Similarly, improvements in SPAD values and chloroplast structure have also been observed in rice [63], supporting the positive effects on gas exchange observed in the present study in marigold.

Zn^{2+} is an essential micronutrient that directly participates in multiple physiological processes related to gas exchange in plants [64]. Previous studies have suggested that adequate Zn availability may promote stomatal function, which increases stomatal conductance, allowing greater entry of CO_2 into the leaf interior and consequently increasing the net photosynthetic rate [65]. In addition, by promoting the integrity of cellular membranes and the structure of chloroplasts, Zn^{2+} is associated with the maintenance of chlorophyll content, which is reflected by increased SPAD measurements and improved light capture capacity [46,66,67]. When zinc is used in the form of NPs, these effects may be enhanced because of their greater specific surface area, higher chemical reactivity, and improved bioavailability.

In this context, the increase in photosynthesis, stomatal conductance, and transpiration observed at 250 mg L^{-1} may indicate a physiological activation associated with enhanced metabolic activity and a temporary improvement in CO_2 fixation [68]. Singh et al. demonstrated that low and intermediate doses of ZnO-NPs increase photosynthetic efficiency and performance, thereby promoting plant energy metabolism [69]. The responses induced by ZnO-NPs are highly dependent not only on the applied dose but also on their physicochemical properties, particularly NP size and morphology. In general, moderate concentrations tend to stimulate plant growth and photosynthesis, whereas high doses may induce oxidative stress, cellular damage, and physiological disturbances [70,71]. For instance, high concentrations of ZnO-NPs have been reported to increase ROS generation, induce lipid peroxidation, reduce photosynthetic activity, and impair cellular integrity [72–75]. In contrast, other studies have reported positive responses even at

considerably higher concentrations. Although the 500 mg L^{-1} and 1000 mg L^{-1} concentrations did not consistently improve any of the evaluated parameters, they did not drastically reduce growth, biomass, or gas exchange compared with those of the control. These findings suggest that, in marigold, high concentrations of ZnO-NPs may exceed the optimal range for certain physiological processes without inducing severe toxicity. The variability in the response among variables likely reflects differences in physiological sensitivity and metabolic regulatory mechanisms associated with Zn^{2+} absorption and accumulation. Moreover, the distinct responses observed between foliar and drench applications reinforce the importance of NP uptake pathways in determining physiological efficiency and dose–response behavior [76–78]. Although the 500 mg L^{-1} and 1000 mg L^{-1} concentrations did not consistently increase all the evaluated parameters, they did not drastically reduce growth, biomass, or gas exchange compared with those of the control. These findings suggest that, in marigold, high concentrations of ZnO-NPs may exceed the optimal range for certain physiological processes without inducing severe toxicity. The variability in the response among variables likely reflects differences in physiological sensitivity and metabolic regulatory mechanisms associated with Zn^{2+} absorption and accumulation. For example, improvements in growth variables and gas exchange parameters have been observed in tomato (*Solanum Lycopersicon* L.), peanut (*Arachis hypogaea* L.), pea (*Pisum sativum* L.), and bell pepper (*Capsicum annuum* L.) following the application of 500 mg L^{-1} up to 2000 mg L^{-1} ZnO-NPs [64,79–81]. These findings demonstrate that the optimal response range is not universal and largely depends on the tolerance and physiological capacity of each plant species. Therefore, the effects of ZnO-NPs cannot be generalized, as concentrations that are beneficial for certain crops may be toxic to others. Consequently, establishing species-specific doses under particular cultivation conditions is essential for maximizing biostimulant effects while preventing potential phytotoxicity.

From this perspective, interest in the use of nanoparticles in agriculture has also expanded to the ornamental sector, particularly floriculture, and nanotechnology studies are largely focused on the postharvest life of ornamental plants, which represents a critical challenge that reduces their longevity and quality [82]. Thus, owing to their antibacterial and antifungal properties, NPs have demonstrated promising potential as alternatives for postharvest management [83].

The findings of this study highlight the potential of ZnO-NPs in ornamental horticulture and emphasize the need for further research on their mechanisms of action, environmental behavior, and long-term safety under field conditions. This is particularly important because significant challenges still remain, including understanding the mechanisms of action of NPs and their environmental impact associated with the accumulation of NPs in soil, water, and trophic chains as a consequence of the increasing agricultural and commercial applications of nanomaterials [84–86]. There are reports indicating that ZnO-NPs may alter soil microbial communities, enzymatic activity, and nutrient dynamics because of their high reactivity and Zn^{2+} ion release, highlighting the need for careful evaluation of their long-term ecotoxicological effects under agricultural conditions [87–89]. Furthermore, the accumulation of ZnO-NPs in edible plant tissues could represent a potential pathway for entry into the human food chain [90–92]. Although ZnO-NPs exhibit considerable potential as efficient nanofertilizers, their excessive or inappropriate use may pose long-term ecological risks. Therefore, several authors agree that the optimization of dose, particle size, and exposure time is essential to maximize their benefits while minimizing adverse effects.

5 Conclusions

The application of ZnO-NPs significantly influenced the growth, development, and inflorescence quality of the evaluated plants. In terms of vegetative growth, the concentration of 100 mg L^{-1} promoted relevant increases in plant height, stem diameter, and total dry weight. With respect to reproductive

parameters, a concentration of 100 mg L^{-1} increased inflorescence diameter, whereas 1000 mg L^{-1} markedly increased floral dome height and the number of flowers and improved color attributes (L*, a*, and b*), reflecting a positive impact on ornamental quality.

At the physiological level, ZnO-NPs enhanced gas exchange and photosynthetic efficiency, with notable increases in the photosynthetic rate, stomatal conductance, transpiration, and intercellular carbon, particularly at concentrations of 250 mg L^{-1} and 500 mg L^{-1}. Similarly, the chlorophyll content, evaluated in terms of SPAD units, increased at all concentrations, reaching the highest value at 1000 mg L^{-1}. The application method also played an important role: foliar application favored parameters such as stomatal conductance, whereas drench application was more effective for photosynthesis and intercellular carbon.

Overall, the results confirm that ZnO-NPs not only promote vegetative and reproductive growth but also improve both the photosynthetic physiology and the ornamental quality of flowers. However, the effect depends on both the applied concentration and the application method, highlighting the need to establish optimal doses to maximize benefits while avoiding potential adverse effects.

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