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Short-term effect of zinc oxide nanoparticles (ZnO-NPs) on *Carum copticum* growth and physiological effects under hydroponic condition

Maryam Mazaheri-Tirani [1,2*], Soleyman Dayani [3]

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[1] *Department of Biology, Faculty of Science, University of Jiroft, Jiroft 78671-61167, Iran.*

[2] *Department of Biotechnology, College of Agriculture, Isfahan University of Technology, Isfahan, 84156 83111, Iran.*

[3] *Department of Biotechnology, Faculty of Agriculture and Natural Resources, Imam Khomeini International University, Qazvin 34149-16818, Iran*

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***Corresponding author**

mazaheri@ujiroft.ac.ir

ABSTRACT

This study investigated the growth and physiological response in medicinal plant *Carum copticum* exposed to zinc oxide nanoparticles (ZnO-NPs). Treatments of 0.1, 0.2, 1, 5 and 25µM of ZnO-NPs compared with 0.2 µM $ZnSO_4$ (control; Zn form in Johnson solution) were applied in culture media under hydroponic conditions. The plants shoot/root length, and water content, H_2O_2, anthocyanin and activity POD enzyme were measured. The root length was more sensitive to ZnO-NPs than shoot length. The 0.2 and 1 µM NPs increased plant fresh weight by 96.8 and 115.2%, respectively. The shoot and root water content increased at 0.1-25 µM NPs treatments by 40-51% and 26-34% compared to the control group, respectively. The increase in POD enzyme was accompanied by increased length and fresh weight and anthocyanin contents under ZnO-NPs. The 25 µM ZnO NPs (high level) resulted in 36% shoot length, 44% root length, 38% plant fresh weight and 27% anthocyanin decline, while increased 40% shoot water content, 26% root water content, 17% H_2O_2 and 69% POD activity compared with control. Overall, 25 µM ZnO-NPs treatment induced higher damages to the growth parameters due to H_2O_2 increase (oxidative stress) and the failure of the enzymatic and non-enzymatic mechanisms (anthocyanin and POD) to cope with the H_2O_2 enhancement induced by ZnO-NPs.

Keywords: Ajwain, nano metal oxide, nano toxicity

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Introduction

Nanoparticles (NPs; at least one dimension <100 nm) are widely have been widely used in various industries due to their enhanced and multidimensional effects. It is predicted that, the amount of nanotechnology production will reach more than $1 trillion within the next 10 to 15 years. The rapid expansion of nanotechnology in various industries leads to their intentional or unintentional release into soil, air, and ultimately plants, animals and humans. The effects of NPs are being widely studied, with both positive and negative impacts on living organisms

(Ochoa-Chaparro et al., 2026; Islam et al., 2025). Some general mechanisms of interaction of NPs with plant have been identified over the past decade. Some general mechanisms of NPs' interaction with plants have been elucidated over the last decade (Islam et al., 2025; Zeng et al., 2025). Oxide metal NPs are among the most often used forms of NPs in various section of industries. Metallic nanoparticles might release lower concentrations of soluble metal ions in the environment, but they could be more efficiently taken up by plants. Metal oxide NPs have attracted attention due to their potential positive and negative ecological and biological impacts on living organism and ecosystem (Ochoa-Chaparro et al., 2026; Zeng et al., 2025). Zinc element is an important micronutrient that plays important roles in many integrated metabolic processes (Ochoa-Chaparro et al., 2026; Mousavi et al. 2024). Zinc is essential for optimal plant growth regulation; zinc toxicity leads to oxidative stress and disruption of physiology and molecular processes in cells (Ochoa-Chaparro et al., 2026; Mousavi et al., 2024; Singh et al. 2018). The remarkable properties of zinc oxide nanoparticle (ZnO-NPs) may contribute to increase agricultural yield. On the other hand, ZnO-NPs are widely used in many commercial applications and may leach into the surrounding environment (Amin and Erfan, 2025; Lebaka et al., 2025). The ZnO-NPs had cost-effective and less toxic NP types that reportedly benefit from "quantum size effects" as a result of their special physical properties. Plants of response to ZnO-NPs had reported to be dose-dependent, and in various species, it showed positive impacts on seed germination, growth, development and flowering under various concentrations (Lebaka et al., 2020; Mazaheri-Tirani and Dayani, 2020). It has been reported that the life-long effect of ZnO-NPs five months after seed germination at concentrations of 1 and 5 μM on the growth in *Carum copticum* under hydroponic condition (perlite-base) significantly improved growth and flowered earlier than the control, while concentrations of 25 and 125 μM resulted in toxic responses (Mazaheri-Tirani et al., 2024). ZnO toxicity produces significant amounts of H_2O_2, as a strong oxidizing agent and consequently leads to hydroxyl radicals' production. Antioxidant systems scavenged ROS (Such as H_2O_2) and can be classified into main classes including non-enzymatic (carotenoids, flavonoids, ascorbic acid and anthocyanin) and enzymatic (superoxide dismutase (SOD), catalase, ascorbate peroxidase (APX), and peroxidase (POD)) (Mustafa et al., 2024; Mazaheri-Tirani et al., 2024).

Soil supports plant life by providing a physical environment for roots and a source of macro- and micro-nutrients needed for growth and development. However, the nutrient availability it provides varies across soils and depends on conditions at the level of roots and plant tissues. However, soil cultivation environments may have many limitations. The soil limitations such as reduced gas exchange, pesticide residue, high salt concentrations and excessive nitrates, and can impair plant growth and development. Hydroponic conditions can be a suitable alternative approach (Xing et al., 2025; Pandao et al., 2024; Thapa et al., 2024).

Carum copticum L. (known as Ajwain) is an important medicinal plant, native to India and Iran. *C. copticum* is widely cultured because of its beneficial biochemical composition that results in its high antioxidant (Monfared et al. 2020; Firoozi et al. 2019; Nagalakshmi et al. 2000). The production of agro-products under controlled environments, such as hydroponics systems can contribute to food safety, and increase the efficiency of land and fresh water resources (Barbosa et al. 2015). Therefore, this study investigated the impact of ZnO nanoparticles in hydroponics conditions (water base) and reported its influence on plant species growth and physiological traits of ajwain medicinal plant.

Materials and Methods

Plant material and growth conditions

The seeds of *Carum copticum* L. were collected from Pakan-bazr Company, Isfahan, Iran. The seeds were planted in plastic pot containing sterilized perlite. The two-leaf stage of seedlings were transferred to soilless culture with three plants as one replication in 1 L containers. Ten days later, ZnO NPs treatments were applied at 0.1, 0.2, 1, 5, and 25 μM levels (after optimization) in the growth media. The ZnO-NPs [average particle size: 25 nm, specific surface area: 4.8-6.8 m^2 g^{-1}, purity: ≥99.9 wt%, volume density: 0.3 g cm^{-3}, spherical shaped and without covering, Nanotec, Itay] were provided by Nanotec, Italy. The solutions of ZnO-NPs were prepared in deionized water and were sonicated for 20 min, two times at 40˚C, using a sonicator instrument (Ultrasonic cleaner 2200 MH, Soltec, Italy). The base nutrient solution (without $ZnSO_4$) was prepared, then different concentrations of ZnO-NPs were added (solutions pH: 5.7 ± 0.05). The control group was 0.2 µM $ZnSO_4$ (amount in Johnson nutrient solution). The solutions were continuously aerated at a rate of about 0.5 L min^{-1} with the light intensity of ~100 µM m^{-2} s^{-1}. The solutions were replaced once a week. The analyses were conducted under controlled environment at 25-27˚C for 16 h light and 18-21˚C for 8 h darkness. Three weeks later, the seedlings were harvested and dried in an oven for dry weight. Fresh samples were collected and frozen in liquid nitrogen and stored at -80ºC for later analyze.

Growth parameters

The plants root and shoot were cut and separated. The root/shoot (length) and fresh/dry weight (FW/DW) were calculated. Then, the water content of root (WCR) and shoot (WCS) was calculated as fallows (Shah et al. 2002):

$$WC(\%) = \frac{(FW - DW)}{FW} \times 100$$

Measurement of Hydrogen peroxide

The H_2O_2 content was measured according to Tirani and Haghjou (2019). The 50 mg leaves tissues were homogenized in 1 ml of 0.1% trichloroacetic acid (TCA), and were centrifuged at 5000 × g for 15 min. Then, 750 µl supernatant was added to 750 µl of 1 M KI. The absorbance's each sample were read at 390 nm using UV/VIS spectrophotometer (UV-1601, Rayleigh, China). H_2O_2 (30%) was used as the standard.

Measurement of anthocyanin contents

The amount of anthocyanins were evaluated using acidified methanol according to the method reported by Wagner (1979). The 20 mg leaves were homogenized in 5 mL of acidified-methanol [pure methanol: HCl 37% = 99:1 (v/v)]. The sample kept for two days at room temperature in full dark conditions. Then, they were centrifuged at 5000 × g for 15 min at 4 ºC temperature. The absorptions of the supernatants were read by a UV-VIS spectrophotometer (Rayleigh, China) at 550 nm. The anthocyanin contents were measured using an extinction coefficient of 33000 M–1 cm–1.

Determination of POD enzymatic activity

The activity of peroxidases (POD) on plant leaf tissues were determined according to Mazaheri-Tirani et al. (2019). The 100 mg leaves were homogenized in 4 ml solution containing phosphate buffer (50 mM, pH = 7.8). The homogenate was centrifuged at 10,000 rpm for 15 min at 4ºC. The reaction mixture contained 20 mM guaiacol and 40 mM H_2O_2 were measured. The increase in the absorbance at 470 nm were measured for 60 s. Peroxidase (POD) activity was calculated using an extinction coefficient of 26.6 mM^{-1} cm^{-1}.

Statistical analysis

The experiment was planned as completely randomized design (CRD) with three replicates (3 pots). One pot with three individual plants was assigned for each replicate. The analysis of variance (one-way ANOVA) using the Duncan test was applied on mean separations by SPSS software package 22 ($P \leq 0.05$). Error bars are the standard deviation (StD).

Results and discussion

Plant biomass production and water content

The impacts of different levels of ZnO-NPs on shoot/root length (SL/Rl), plant fresh weight (PFW), shoot/root fresh weight (SFW/RFW) ratio, shoot/root water content (SWC/RWC), anthocyanin, H_2O_2 and peroxidase (POD) of *Carum copticum* seedlings compared to the control (0.2 µM $ZnSO_4$) under hydroponic conditions are reported (Table 1). The results showed that the impact of different levels of zinc oxide nanoparticles on the measured parameter was significant

Table 1- The ANOVA results of the effects of different levels of zinc oxide nanoparticles on shoot length (SL), root length (RL), plant fresh weight (PFW), shoot/root fresh weight (SFW/RFW) ratio, shoot water content (SWC), root water content (RWC), anthocyanin (Antho), H2O2 and polyphenol oxidase (POD) of Carum copticum seedlings compared with the control (0.2 μM ZnSO4) grown under hydroponic condition.

		Means square								
Source of variation	**df**	**LS**	**LR**	**PFW**	**FWS/FWR**	**SWC**	**RWC**	**H_2O_2**	**Antho**	**POD**
Treatment	5	37.32**	43.90**	0.129**	5.037**	0.027**	0.019**	0.021**	4599.0**	0.048**
Error	12	4.18	2.33	0.002	0.213	0.001	0.001	0.001	53.1	0.009

** respectively significant at 1 percent probability

Table 2. The effect of ZnO nanoparticles (NPs) treatments on shoot length (SL), root length (RL), plant fresh weight (PFW), shoot/root fresh weight (SFW/RFW) ratio, shoot water content (SWC) and root water content (RWC) of *Carum copticum* seedlings compared with the control (0.2 μM $ZnSO_4$) grown under hydroponic condition.

Treatments (μM)	SL (cm)	RL (cm)	PFW (g)	SFW / RFW	SWC (%)	RWC (%)
Control	16.09 ± 1.57^{a}	20.01 ± 2.03^{ab}	0.336 ± 0.058^{c}	5.22 ± 0.79^{ab}	47.5 ± 2.6^{b}	66.8 ± 3.2^{b}
0.1	19.66 ± 1.81^{a}	18.04 ± 0.56^{b}	0.662 ± 0.034^{ab}	4.70 ± 0.42^{bc}	73.2 ± 2.8^{a}	83.8 ± 3.4^{a}
0.2	17.09 ± 2.16^{a}	21.52 ± 1.71^{a}	0.724 ± 0.069^{a}	5.71 ± 0.29^{a}	70.1 ± 0.8^{a}	83.3 ± 2.0^{a}
1	19.95 ± 1.50^{a}	21.53 ± 1.97^{a}	0.635 ± 0.051^{b}	4.03 ± 0.36^{c}	76.4 ± 1.1^{a}	87.3 ± 1.7^{a}
5	17.83 ± 3.41^{a}	19.08 ± 1.13^{ab}	0.616 ± 0.018^{b}	4.91 ± 0.45^{ab}	74.4 ± 1.5^{a}	83.7 ± 1.8^{a}
25	10.28 ± 0.91^{b}	11.28 ± 1.22^{c}	0.208 ± 0.023^{d}	2.05 ± 0.23^{d}	70.7 ± 1.9^{a}	81.8 ± 3.8^{a}

The values presented are the mean of three independent experiments and different letters indicate significant differences among treatments in each parameter, at $p < 0.05$ according to the Duncan's test.

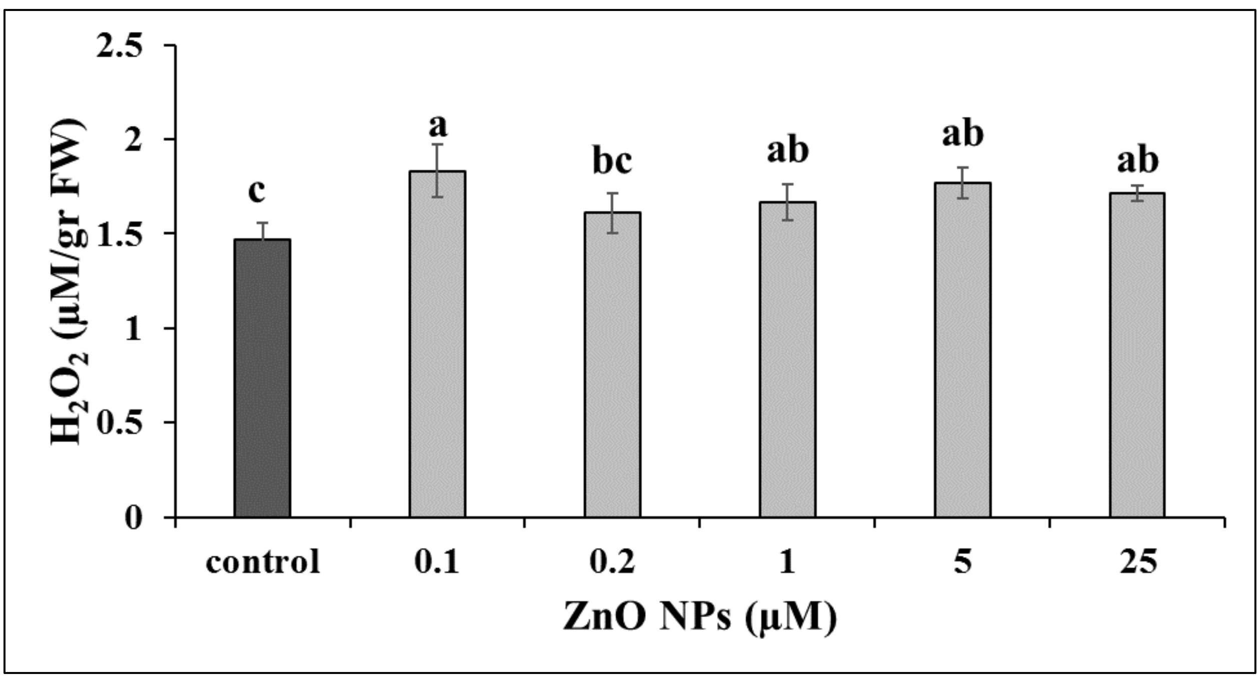

Figure 1. Mean comparison of the effects of different levels of zinc oxide nanoparticles on H_2O_2 content of Carum copticum seedlings compared with the control (0.2 μM ZnSO4) grown under hydroponic condition. The values presented are the average of three

at the 1% level. In this study, the ZnO-NPs treatments at 25 µM negatively affected almost all the growth parameters studied compared to the control. Thus, this level of ZnO-NPs could indicate a toxicity threshold for *Carum copticum* seedlings (Table 2). Exceptionally, the 25µM ZnO-NPs decreased shoot and root length by 36 and 44% compared with the control, respectively. The plant FW increased 97, 115, 89 and 83% in 0.1, 0.2, 1 and 5 µM, respectively but decreased 38% in 25 µM of ZnO-NPs treatments (Table 2). Shoot FW to root FW ratio decreased in 5 and 25 µM of NPs (Table 2). All levels of ZnO-NPs treatments increased the shoot and root water content compared to the control. These parameters were increased by 39-51% in shoot and 26-34% in root compared with control. The lowest and highest water contents reported at control and 1 µM ZnO-NPs groups, respectively (Table 2). There were conflicting results about the effects of NPs on plant growth among various studies. The shoots of ryegrass grown in hydroponic conditions were reduced when treated with 1000 mg/L (about 12.3 mM) of ZnO-NPs. While this concentration caused spot damage in the roots (Lin and Xing 2008). On the other hand, the 50 mg L^{-1} ZnO-NPs improved plant biomass in *Triticum aestivum*. The ZnO-NPs at 20 and 50 mg L^{-1} significantly increased lateral roots by 19 and 32%, respectively (Awasthi et al. 2017). It has been reported that ZnO-NPs lead to higher bioavailability of Zn^{2+} in tobacco callus cultures and show lower metal toxicity compared to non-nano ZnO (ZnO bulk) (Mazaheri-Tirani and Dayani 2020). Zinc regulated auxin bio-synthesis by tryptophan synthetase, and improved in plant cells expansion and growth and development in plant (Begum et al. 2016; Castillo-González et al. 2018). Changes in growth indices may likely due to alteration in the plant growth regulations.

Oxidant and antioxidant system parameters

The oxidative stress under high zinc concertation is due to imbalanced generation and removal of hydrogen peroxide (H_2O_2) in the intracellular cell (Huang et al. 2023; Sachdev et al. 2021; Nadarajah 2020). All levels of ZnO-NPs (except 0.2 µM) significantly increase H_2O_2 content (Figure 1). The H_2O_2 contents were recorded at 0.1, 1, 5 and 25 µM ZnO-NPs by 24, 13, 20 and 17.0% increase compared to the control, respectively. H_2O_2 content showed positive correlation with SWC (r = 0.680) and RWC (r = 0.672) treatments (Table 3). Any stimulant that increased ROS and upset the redox balance; resulted in increased oxidative stress. The oxidative explosion due to increased H_2O_2 has led to cell death (Hasanuzzaman et al. 2020; Nadarajah 2020). Plants enzyme activity (e.g. POX, POD and SOD) non-enzymatic (e.g. phenolic compound and anthocyanin) mechanisms to dampen the NPs toxic impacts by oxidative stress via ROS-scavenging (Ghosh et al. 2016; Hasanuzzaman et al. 2020; Mazaheri-Tirani et al. 2019). The 25 µM ZnO-NPs declined the oxidative stress (H_2O_2) in *Cicer arietinum* L. plant under chromium toxicity (Singh et al. 2024). Under ZnO-NPs treatment, only 25 µM treatment decreased anthocyanin content by 27.4%, but the 0.1, 1 and 5 µM ZnO-NPs treatments were increased by 130.6 58.1 and 56.9% compared to control, respectively (Figure 2). The anthocyanin content showed positive correlations with shoot length (r = 0.762) and plant FW (r = 0.681) (Table 3). The POD enzyme activity showed an increase at 5 and 25 µM nanoparticle treatments by 15.4 and 69.3%, while 1 µM ZnO-NPs decreased it about 30.9% compared with the control, respectively. The others NPs levels didn't meaningfully change the POD enzyme activity compared to the control (Figure 3). The POD enzyme activity showed negative correlations with shoot length (r = -0.790), root length (r = -0.829) and plant FW (r = -0.682) treatments (Table 3). Figure 4 shows the summary of the effects of different levels of zinc oxide nanoparticles on *C. copticum* seedlings under hydroponic condition. Various abiotic and biotic stresses increased H_2O_2 significantly. It was reported that the antioxidant system plays important role in supporting plants against negative impacts induced by ZnO toxicity (Mazaheri-Tirani et al. 2019). Singh et al. (2024) and Pullagurala et al. (2018) reported improved antioxidant defense system

Table 3- Correlation matrices showing relationships between measured parameters of *C. copticum* seedlings grown in hydroponic under ZnO nanoparticles (NPs) treatment. SL: shoot length; RL: root length; PFW: plant fresh weight; SWC: shoot water content; RWC: root water content; Antho: Anthocyanin; H_2O_2: Hydrogen peroxide; POD: Polyphenol oxidase.

	LS	LR	Plant FW	WC shoot	WC root	H_2O_2	Antho
LR	0.794**						
Plant FW	0.769**	0.699**					
WC shoot	0.242	-0.013	0.498*				
WC root	0.216	0.006	0.508*	0.952**			
H_2O_2	0.052	-0.316	0.262	0.680**	0.672**		
Antho	0.762**	0.329	0.681**	0.402	0.353	0.451	
POD	-0.790**	-0.829**	-0.682**	-0.038	-0.08	0.145	-0.564*

*and** respectively significant at 1 and 5 percent probability.

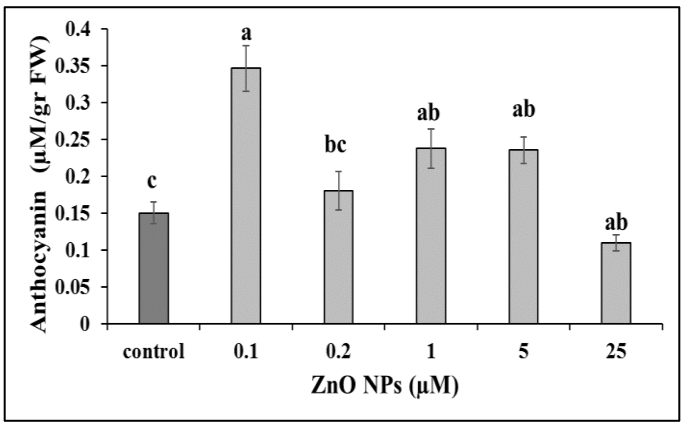

Figure 2. Mean comparison of the effects of different levels of zinc oxide nanoparticles on anthocyanin content of *C. copticum* seedlings compared with the control (0.2 µM $ZnSO_4$) grown under hydroponic condition. The values presented are the average of three independent experiments and different letters indicate significant differences between treatments in each parameter, at $p < 0.05$ according to Duncan's range test.

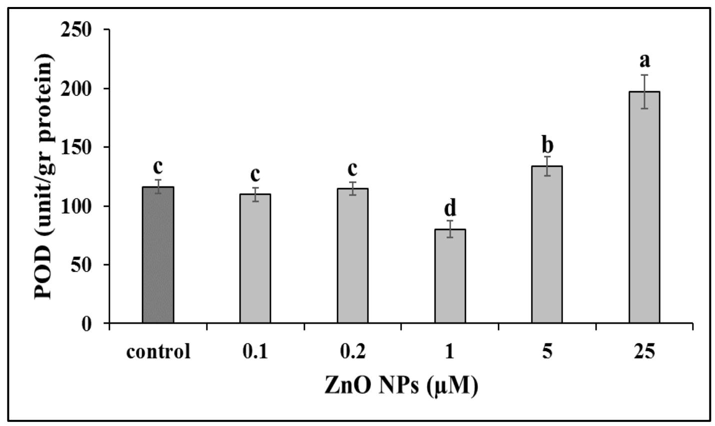

Figure 3. Mean comparison of the effects of different levels of zinc oxide nanoparticles on POD enzyme activity of *C. copticum* seedlings compared with the control (0.2 µM $ZnSO_4$) grown under hydroponic condition. The values presented are the average of three independent experiments and different letters indicate significant differences between treatments in each parameter, at $p < 0.05$ according to Duncan's range test.

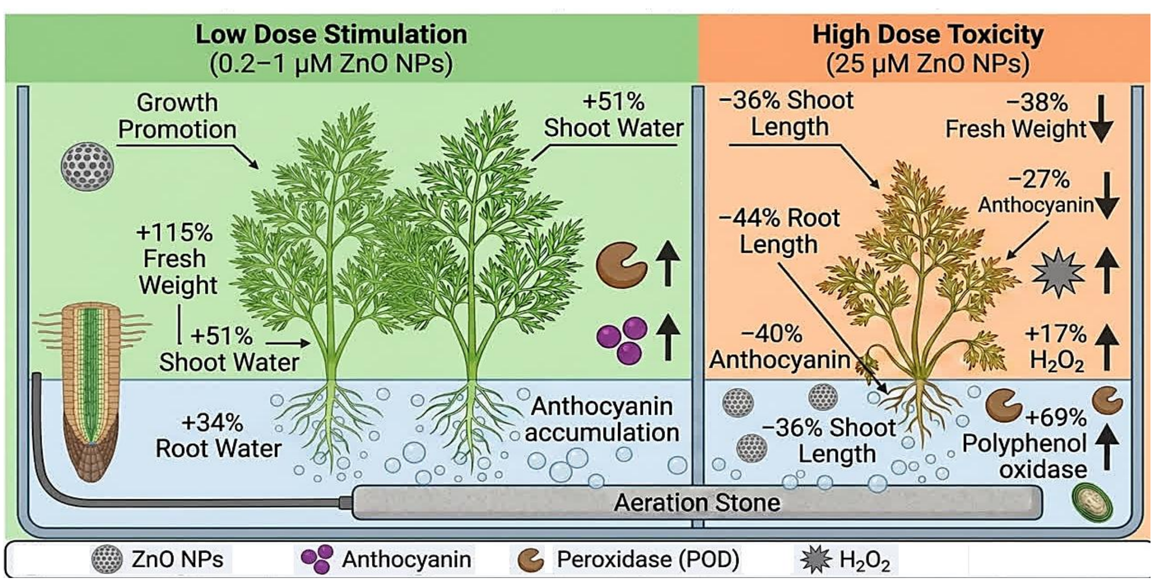

Figure 4. The summary of the effects of different levels of zinc oxide nanoparticles on C. copticum seedlings under hydroponic condition.

with ZnO-NPs treatment on C*icer arietinum* and *Coriandrum sativum*, respectively. It could be the result of the genes expression (Nair and Chung 2017) or the activity of different oxidative processes (Mazaheri-Tirani et al. 2019; Li et al. 2016). The alteration in anthocyanin (major phenolic class) content was compensated with modification in phenolic compounds at ZnO-NPs treatment. Therefore, ZnO-NPs might affect the phenolic compound's biosynthesis pathways. Plants' response to stresses might vary depending on the plant species, forms and intensity of stress, and other environmental parameters (e.g. temperature, media pH and culture substrate).

Conclusion

This study demonstrated that ZnO-NPs increased POD activity but H_2O_2 content in *Carum copticum* seedling under hydroponic (water-based; without solid culture medium) conditions, thus leading to increased H_2O_2 and induction of oxidative stress. Plants of response to ZnO-NPs had reported to be dose-dependent. Important oxidative stress biomarkers, such as H_2O_2 generation, were different under ZnO-NPs concentrations in these plants. It should be noted that due to its thin stem and fragile root systems, *Carum copticum* plant is not suitable for cultivation in a hydroponic (water-base) system.

Conflict of Interest

The authors have not declared any conflict of interests.

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