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Research Article

The Physiological Effect of Foliar Spraying of Zinc Nanoparticles in Reducing the Harmful Effects of Cadmium on Faba Bean Plants

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Abstract:

Heavy metal pollution is the one of the most important factors that limit the growth and yield of faba bean plants in the world. In recent years, nanotechnology has showen promising result in reducing the harmful effects of heavy metal stress on various crops such as peas (*Pisum sativum*), faba beans (*Vicia faba leguminosae*). This experiment aimed to study the effect of foliar spray of zinc nanoparticles (ZnO NPs) at two concentrations (100 and 200 ppm) on the growth parameters shoot length, root length, fresh and dry weight of shoots and roots, and leaf area of faba bean plants. In addition, the content of chlorophyll and carotenoids were measured under Cd stress (20 and 100 ppm). The samples were collected twice, 15 and 30 days after treatments. The results showed that Cd had adverse effects on shoot and root lengths, shoot fresh and dry weights, root fresh and dry weights, and leaf area. The treatment with ZnO NPs significantly reduced the side effects of Cd on the level of growth parameters at two stages. ZnO NPs 200 was more effective than ZnO NPs 100. From our results, ZnO NPs can be used to mitigate the Cd stress in agricultural soils.

1. Introduction

Heavy metal contamination poses a significant threat to all kinds of life, and it has hazardous effects on the global environment and plant growth (Sharaf-Eldin et al., 2023). These metals can be held in food webs and are exceedingly poisonous at low concentrations; there is a significant risk to the public's health. Most of the heavy metals and organic contaminants are non-degradable and persist in their surroundings for extended periods. According to (Li et al., 2023; Zhao et al., 2023) Cadmium (Cd) is a highly toxic heavy metal that poses a risk to food safety due to its extended biological half-life. Cd is a non-essential heavy metal that poses a significant environmental risk and lowers plant productivity (El-Beltagi and Mohamed, 2013; Charkiewicz et al., 2023).

Nanotechnology is becoming more and more significant as a possible tool for environmental cleanup. Frequently exhibiting a substantial surface area, nanoparticles possess distinct characteristics and hold promise in mitigating the deleterious impact of heavy metals on natural resources (Konate et al., 2017).

Nanotechnology is thought to play a crucial role in the process of cleaning up contaminated soil and improving the fertility and quality of the soil. Because nonmaterial’s have smaller particle sizes and are therefore easier to introduce into the soil, they can achieve high specific area and high reactivity (Salem et al., 2019).Researchers' interest in nanotechnology has grown in recent years due to its widespread application in a variety of industries, including agriculture. The use of nanoparticles (NPs) as a source of nutrients, particularly micronutrients, for plants, is known as nano fertilizers, and this could be a promising strategy since plants only use a small amount of the chemical fertilizers that are applied to the soil, and any remaining fertilizers could seriously harm the environment (Hussain et al., 2018; Rizwan et al., 2017)

Nanoparticles offer great promise as an environmentally friendly method of managing diseases and diagnosing plant diseases. Plant disease detection and management may benefit significantly from the use of nanosensors and mini-detection instruments for pathogen detection. In order to prevent crop loss from pests and diseases, nanoparticles used in disease management and the potential for large-scale adaptability of nanoparticles by integrating them into current practices. ((Siddiqui et al., 2015) (Nazir et al., 2022)

Particles that are at least one dimension in size and range from 1 to 100 nm are referred to as nanoparticles (NPs). They can alter physio-chemical properties more favorably than bulk materials because of their higher surface area, which increases their solubility and surface reactivity. (Nasrallah et al., 2022; Attia and Elsheery, 2020)

Nanoparticles produce a specific kind of material that is smaller than 100 nm. Plant growth and development are ultimately impacted by the interactions between nanoparticles (NPs) and plant cells, which lead to modifications in biological pathways and gene expression pat-

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terns, NPs impacted the growth of V. faba under salinity stress(Omar et al., 2023; Zayed et al., 2017)

Due to the unique qualities of nonmaterial's (NMs), nanotechnology presents a viable substitute for traditional products in the field of plant protection. These benefits include increased efficacy, decreased input, and decreased ecotoxicity. The creation of appropriate growth-stimulating substances, like nano fertilizers, can significantly increase agricultural output. Increasing crop yields and maintaining global food security primarily depend on applying fertilizers containing sources of calcium, phosphorus, potassium, or nitrogen. The function of calcium and other necessary beneficial elements in plants support plant vigor. (Ayyaz et al., 2022)

Zinc oxide nanoparticles (ZnO NPs) are highly suitable for use as fertilizers, fungicides, and pesticides in the agricultural and food industries (Hussain et al., 2018; Ghosh et al., 2016). Plant science considers ZnO NPs to be an essential particle due to their exceptional property. Because of the promising role in plant, the use of ZnO NPs in agriculture is increasing. However, the effect of ZnO NPs is altered depending on certain factors such as NPs properties, plant types and dose and duration of exposure (Thounaojam et al., 2021). Low concentrations of ZnO NPs activate the photosynthetic machinery in plants, speeding up the critical physiological process of photosynthesis and increasing photosynthetic yield. Additionally, ZnO NPs stimulate the plant's defense mechanism against stress, acting as an effective abiotic stress regulator in plants. They also show promise as an antimicrobial for managing a variety of plant diseases in agriculture (Thounaojam et al., 2021; Elsheery et al., 2020a; Pedruzzi et al., 2020). ZnO-NPs prevent HMs from absorbing and accumulating metals, which reduces the expression of genes linked to stress. Moreover, NPs build up in the cell walls of plants and, through the assembly of complexes, render HM ions unavailable, limiting HM transportation and diminishing biological activity(Alhammad et al., 2023) Zinc enhanced the amount of chlorophyll by controlling the transport of magnesium, a crucial element in the structure of chlorophyll. (Alenezi et al., 2022) (Venkatachalam et al., 2017) NPs speed up the electron transport chain and water's photolysis to increase the rate of photosynthetic processes.(Faizan et al., 2021)

ZnONPs may be responsible for improving the efficiency of nutrient utilization. Lessening the poisonous effects of excessive fertilizer application. Enhancing the activity of antioxidant enzymes to protect plants from the damaging effects of reactive oxygen species (Ragab et al., 2022; Shareef et al., 2023). According to previous studies, foliar application of Zno NPs reduces ROS by raising antioxidant enzyme levels. Controlling the electron flow between photosystems, which is crucial to preventing photoinhibition and additional photodamage (Elsheery et al., 2020b). This study aims to evaluate the effect of zinc nanoparticles on the growth parameters of Vicia faba under Cd stress at two growth stages

2. Materials and Methods

The experiments were carried out at the farm of the Agriculture Faculty at Tanta Univ. Bean seeds were obtained from the Food Legumes Research Section, Tanta Agricultural Research Station, Gharbiya, Egypt. Cadmium chloride was used at two concentrations (20 and 100 ppm). Zinc nanoparticles (Zno NPS) M.W.81.408) were used at two concentrations (100 and 200 ppm). The present investigation was conducted during the winter season of 2021.

Experimental design:

Bean seeds were tested with Cd at two concentrations (20 and 100 ppm). In addition to negative control (without ZnO NPs), the plants were treated with ZnO NPs at two concentrations (100 and 200 ppm). Seeds were planted in black plastic bags filled with soil; each treatment was three replicates (ten seeds per replicate). The bean seeds were planted in bags filled with soil and rinsed with water until germination began. Then, some of the bags were treated with cadmium by adding it to the soil in specific concentrations. Foliar spraying was then done (after two weeks of planting) using zinc nanoparticles at concentrations of 100 and 200 ppm. The results were taken after 15 days as a first growth stage and then after 30 days as a second growth stage.

Growth measurements:

Five seedlings of each replicate were taken for the following traits: plant heights (cm), fresh root weight (g), dry root weight (g), shoot length (cm), shoot fresh weight (g), and shoot dry weight (g) and leaf area (cm2).

Chlorophyll content:

Chlorophyll levels were estimated by the spectrophotometric (UV1901PC) method according to Lichtentaler and Wellburn (1985); chlorophyll was expressed as μg/ml methanol by this equation;

Chlorophyll a = 15.65 A666 - 7.340 A653

Chlorophyll b = 27.05 A653 - 11.21 A666

Carotenoids = (1000*A470) - (2.86 – Ch. a) – (129.2*Ch. b /245)

Statistical analysis

The data were presented as means ±SD. The statistical significance was calculated by two-way ANOVA (analysis of variance) using SPSS 20 software, and the individual comparisons between treatments were measured by the Turkey multiple range test at $P<0.05$.

3. Results

3.1. Effects of zinc nanoparticles on shoot and root lengths on faba bean plants under Cd stress at two growth stages.

Table (1) shows the effect of zinc nanoparticles (Zno NPs) with Cd stress on the shoot and root length at two growth stages. There was a significant effect of using zinc nanoparticles on shoot and root length at two growth stages. Zn 100 was more effective than Zn 200 under Cd 200 stress in the shoot length. It gave an increase of 29.37 and 53.35 at the first and second times, respectively in comparison with the control. In the root length, Zn 200 gave a non-significant increase in the first time (7.69 compared with control), whereas in the second time, Zn

200 was more effective than Zn 100 and gave a significantly increase (62.95% in comparison with the control).

3.2. Effects of zinc nanoparticles on shoot and root fresh weight on faba bean plants under Cd stress at two growth stages.

There were no significant effects of ZNO NPs on the shoot and root fresh weight at the two growth stages except root at the second stage. ZNO NPs (200) had a high effect in comparison with the other treatments (56.65%) (Table 2).

3.3. Effects of zinc nanoparticles on shoot and root dry weights and leaf area on faba bean plants under Cd stress at two growth stages.

Table (3) showed a significant effect of ZNO NPs - foliar applications under Cd stress. Mean comparisons showed that Nano-fertilizers treatments increased shoot and root dry weight at two stages except root dry at first stage. Zn 200 had the high impact in reduced the toxicity of Cd.

Nano zinc (both two concentrations) was more effective on leaf area at the second stage. At the first stage, Zn 200 was more effective in reducing the toxicity of Cd 20 with the percentage of 15.52 and 36.87 at the first and second time respectively in comparison with the control (Table 4)

3.4. Effect of Cd stress with or without ZnO nanoparticles on the total chlorophyll and carotenoid of *Vicia faba* at two stage of growth.

Nano-particles increase the plant's chlorophyll under Cd stress at two growth stages. Cd stress gradually decreases the plant's chlorophyll content by increasing the Cd stress level. The high concentration of ZNO NPs was more effective than the low concentration (Figure1).

Carotenoids content showed high significant in Cd treatments, these increase are directly proportional with Cd increase. At the same time, nanoparticles led to reducing in carotenoids content. Carotenoids

Table 1. Effects of ZnO NPs on shoot and root lengths of faba bean plants under Cd stress.

Shoot length (cm)						
	At first stage (15DAS)			**At second stage (30DAS)**		
	Cd 20	**Cd 100**	**mean**	**Cd 20**	**Cd 100**	**Mean**
Control	26± 1.73[ab]	21.67±2.89[b]	23.83±3.19[B]	30.67±1.15[c]	28.67±.58[c]	29.67±1.37[C]
ZnO 100	28.33±1.15[ab]	33.33±1.15[a]	30.83±2.93[A]	43.00±1.00[b]	48.00±1.73[a]	45.50±3.02[A]
ZnO 200	29.00±2.65[ab]	24.67±5.86[b]	26.83±4.71[AB]	43.33±1.15[b]	40.33±1.15[b]	41.83±1.94[B]
mean	27.78±2.17[A]	26.56±6.21[A]		39.00±6.32	39.00±8.50	
Root length (cm)						
	At first stage (15 DAS)			**At second stage (30 DAS)**		
	Cd 20	**Cd 100**	**mean**	**Cd 20**	**Cd 100**	**Mean**
Control	7.33±0.58	5.67±.58	6.50±1.05	5.33±1.53[c]	6.33±0.58[c]	5.83±1.17[C]
ZnO 100	8.00±1.73	6.00±1.00	7.00±1.67	8.67±0.58[b]	7.33±0.58[bc]	8.00±0.89[B]
ZnO200	7.67±0.58	7.67±0.58	7.67±.52	7.33±0.58[bc]	11.67±0.58[a]	9.50±2.43[A]
Mean	7.67±1.00 [A]	6.44±1.13[B]		7.11±1.69[B]	8.44±2.51[A]	

Small letters indicate the interaction between treatments while capital letters indicate the interaction in treatment and the same letters are not significantly at P<5%

Table 2. Effects of ZnO NPs on shoot and root fresh weights of faba bean plants under Cd stress.

Small letters indicate the interaction between treatments while capital letters indicate the interaction in treatment and the same letters are not significantly at P<5%

Shoot fresh weight (g plant^{-1})						
	At first stage (15DAS)			**At second stage (30DAS)**		
	Cd 20	**Cd 100**	**mean**	**Cd 20**	**Cd 100**	**Mean**
Control	14.20±1.04^{a}	9.87±.51^{a}	12.03±2.48	22.67±1.15	18.07±5.85	20.37±4.54
ZNO100	15.77±5.01^{a}	16.57±1.02^{a}	16.17±3.27	18.40±.69	24.17±4.05	21.28±4.09
ZNO200	8.23±.78^{a}	10.97±2.05^{a}	9.60±2.04	26.10±1.15	22.90±2.55	24.50±2.49
Mean	12.73±4.31^{A}	12.47±3.33^{B}		22.39±3.46	21.71±4.70	
Root fresh weight (g plant^{-1})						
	At first stage (15 DAS)			**At second stage (30DAS)**		
	Cd 20	**Cd 100**	**mean**	**Cd 20**	**Cd 100**	**Mean**
Control	3.10±0.90	3.30±.87	3.20±.80	2.87±.78^{ab}	1.80±.10^{b}	2.33±.77^{B}
ZNO 100	3.10±2.00	1.60±.30	2.35±1.52	3.50±.26^{a}	2.97±.85^{ab}	3.23±.63^{A}
ZNO200	2.40±1.44	2.27±1.00	2.33±1.11	3.70±.20^{a}	3.60±.26^{a}	3.65±.22^{A}
Mean	2.87±1.36	2.39±1.01		3.36±.57^{A}	2.79±.91^{B}	

Table 3. Effects of ZNO NPs on shoot and root dry weights of faba bean plants under Cd stress

Shoot dry weight (g plant^{-1})						
	At first stage (15DAS)			**At second stage (30 DAS)**		
	Cd 20	**Cd 100**	**mean**	**Cd 20**	**Cd 100**	**Mean**
Control	1.50±0.30^{ab}	0.83±.12^{b}	1.17±.42^{B}	4.37±.32^{ab}	2.77±.93^{b}	3.57±1.07^{B}
ZNO 100	2.13±.57^{a}	1.87±.58^{ab}	2.00±.53^{A}	2.73±.32^{b}	4.57±1.22^{ab}	3.65±1.28^{B}
ZNO 200	1.40±.36 ^{ab}	0.97±.35^{b}	1.18±.40^{B}	5.53±.49^{a}	4.80±.20^{a}	5.17±.52^{A}
Mean	1.68±.50^{A}	1.22±.60^{B}		4.21±1.26	4.04±1.24	
Root dry weight (g plant^{-1})						
	At first stage (15DAS)			**At second stage (30DAS)**		
	Cd 20	**Cd 100**	**mean**	**Cd 20**	**Cd 100**	**Mean**
Control	0.68±0.19	0.43±0.15	0.56±0.21	0.87±0.06^{ab}	0.50±0.10^{cd}	0.68±0.21
ZNO 100	0.53±0.49	0.27±0.12	0.40±0.35	0.63±0.12^{bcd}	0.80±0.20^{abc}	0.72±0.17
ZNO 200	0.43±0.23	0.33±0.15	0.38±0.18	0.40±0.10^{d}	1.03±0.06^{a}	0.72±0.35
Mean	0.55±0.31	0.34±0.14		0.63±0.22^{B}	0.78±0.26^{A}	

Small letters indicate the interaction between treatments while capital letters indicate the interaction in treatment and the same letters are not significantly at P<5%

Table 4. Effects of ZNO NPs on leaf area of faba bean plants under Cd stress.

leaf area (cm^2 $plant^{-1}$)						
	At first stage (15 DAS)			**At second stage (30 DAS)**		
	Cd 20	**Cd 100**	**mean**	**Cd 20**	**Cd 100**	**Mean**
Control	24.67±1.15^{b}	32.17±4.01^{ab}	28.42±4.88	28.33±5.86^{bc}	22.33±6.66	25.33±6.50^{B}
ZNO 100	26.67±1.44^{b}	28.50±2.60^{b}	27.58±2.13	37.67±1.15^{ab}	26.67±1.15^{c}	32.17±6.11^{A}
ZNO200	39.67±7.18^{a}	26.00±1.73^{b}	32.83±8.82	29.67±2.08^{abc}	39.67±3.79^{bc}	34.67±6.12^{A}
Mean	30.33±7.97	28.89±3.70		31.89±5.40	29.56±8.72	

Small letters indicate the interaction between treatments while capital letters indicate the interaction in treatment and the same letters are not significantly at P<5%

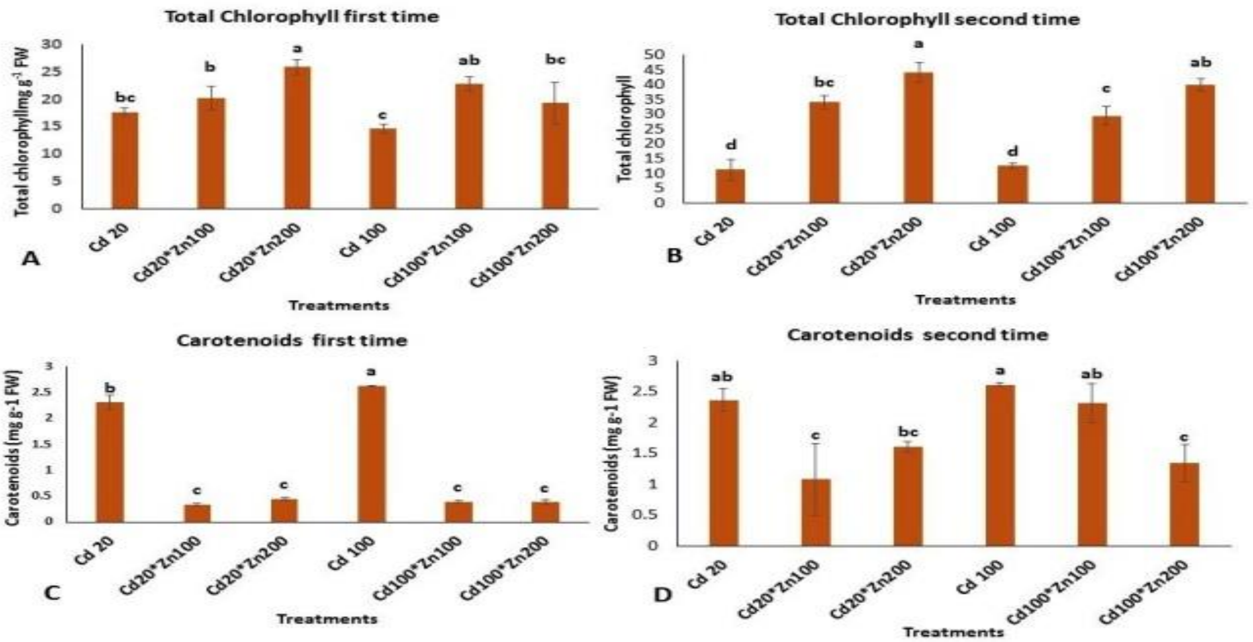

Fig.1. Effect of Cd stress with or without ZNO nanoparticles on the total chlorophyll at the first (A) and second time (B) and carotenoid at the first (C) and second time (D) of Vicia faba. The same letters are not significantly at P<5%

4. Discussion

Cd is a non-essential metal that poses a significant environmental risk and lowers plant productivity, (El-Beltagi and Mohamed, 2013; Charkiewicz et al., 2023) cadmium (Cd) is a highly toxic heavy metal that poses a risk to food safety due to its extended biological half-life (Wang and Zhao, 2023) In the current study, faba bean plants treated with Cd showed a significant reduction in growth parameter when compared to control plants. These findings concur with those of (El-Mahdy et al., 2021), who noted a noteworthy decline in the morphological traits of bean plants following Cd treatment. Additionally, our research supports other studies that found Vigna mungo exposed to Cd had shorter roots and shoots (Dutta et al., 2018). Reduced water potential, lower nutrient content, and blockage in the proton pumps can all contribute to a decrease in plant growth under the stress of Cd and Pb. This further hinders cell division and elongation, which lowers the dry weight of plants (Sarathambal et al., 2017). A significant decrease in common bean's root and shoot dry weight and leaf area was observed with an increase in Cd concentration. These results agree with (Hammami et al., 2022; Aslam et al., 2014; Nouairi et al., 2019). In addition to its high-water solubility and mobility, Cd can be absorbed by plants even at low soil concentrations, much like some other essential elements.

The pot experiment was carried out to test the effect of ZnO NPs at two concentrations (100 and 200 ppm) on the growth traits (length, fresh and dry weight of shoot and root and leaf area) of faba bean plants (Vicia faba) at two growth stages 15 and 30 days after under Cd stress (20 and 100 mg CdCl2/ kg soil). There were noticeable impacts of ZNO NPs on the growth parameters of Vicia faba under Cd stress.

Zinc, or Zn nanoparticles, is a micronutrient that is vital to both plants and animals because it is involved in many enzyme activities and metabolic processes. Plants that are zinc deficient produce lower yields. The result agree with (Srivastav et al., 2021; Irfan and Bhatti, 2023), they reported that ZnO NPs, increased plant biomass and length. Zinc is a necessary element for plants because it affects gene expression, membrane integrity, metabolic processes, protein synthesis, and the activity of multiple enzymes. Therefore, a higher Zn level was advantageous to the plants, improving their growth and these agree with the obtained results with the increasing of ZnO NPs concentration.

Plant growth and development were found to be improved by ZnO NPs, which affect important physiological parameters and act as a natural activator for plants in both stressed and non-stressed environments. These findings showed that by promoting plant growth and biomass production, ZnO NPs decreased Cd accumulation in a dose-dependent pattern for Triticum aestivum L. and consequently created metal stress resistance in plants (Hussain et al., 2021). ZnO NPs applied topically effects on the uptake of Cd from the soil into the shoots. Exogenous application of ZnO NPs has been recommended as a potential means of enhancing photosynthetic efficiency and lowering Cd accumulation in rice plants under Cd stress. (Faizan et al., 2020) mention that Zn improved plant survival and growth by lowering the amount of Cd in the roots and leaves of Cajanus cajan. Zinc supplementation can mitigate oxidative stress caused by Cd in plants by adjusting their redox status.

Zinc is useful for cell elongation, membrane function, and protein synthesis so it appears that ZnO NPs has increased leaf area. Zinc is essential for controlling osmotic activities, preserving the structural stability of the cell's membrane under stressful conditions, and safeguarding and preserving the cell water balance (Elsheery et al., 2020a; Mahawar et al., 2023)

Mahawar et al. (2023) found that the growth in leaf area from one period to the next; this could be the result of ZnO NPs building up in plant tissue following the NPs' subsequent spray.

Zinc enhanced the amount of chlorophyll by controlling the transport of magnesium, a crucial element in the structure of chlorophyll. (Alenezi et al., 2022) (Venkatachalam et al., 2017) NPs speed up the electron transport chain and water's photolysis to increase the rate of photosynthetic processes.(Faizan et al., 2021). THE RUSLT AGREE WITH(Ragab et al., 2022) ZnO NPs may be responsible for improving the efficiency of nutrient utilization. lessening the poisonous effects of excessive fertilizer application. enhancing the activity of antioxidant enzymes to protect plants from the damaging effects of reactive oxygen species

Chlorophyll a, b and total chlorophyll were found to be improved by the use of ZnO NPs in both pathogen-inoculated and uninoculated plants. may have increased because zinc is essential for protein synthesis, membrane function, cell elongation, and protection and maintenance of the structural stability of cell membranes.(Siddiqui et al., 2019)

ZnO NPS enhanced the chlorophyll that agree with Prior research has demonstrated that commercially available ZnO NPs can reduce the uptake of Cd in tomato, wheat, rice, and lettuce.(Timilsina et al., 2023) Under Cd stress, ZnO NPs had a significant impact on antioxidant enzyme activities and photosynthetic pigments and levels. Antioxidant enzymes were upregulated by ZnO NPs in order to prevent oxidative stress and preserve growth performance.(Rashid et al., 2022)

ZnO nanoparticles decreased carotenoid contents in maize leaves Chlorophylls, proteins, DNA, and lipids are among the cellular constituents that carotenoids are crucial in preventing oxidative damage to and capturing radical oxygen species.(Hashemi et al., 2019) One of the most frequent stress responses to heavy metal exposure in plants is the production of reactive oxygen species (ROS), which can alter metabolism through oxidative cell damage (Venkatachalam et al., 2017).

Hussain et al. (2021) and Srivastav et al. (2021) mentioned that ZnO NPS improves plant growth and development by increasing the activity of antioxidant enzymes that work to inhibit ROS and reduce their production in plants during cadmium stress

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