



Amelioration of Chromium-Induced Growth, Physiological, and Biochemical Alterations in Sunflower (*Helianthus annuus* L.) through Foliar Application of Zinc Oxide Nanoparticles

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ABSTRACT

The present study investigated the ameliorative effects of foliar-applied ZnO-NPs on sunflower (*Helianthus annuus* L.) subjected to Cr stress. Sunflower plants were exposed to 50 and 150 ppm Cr, with foliar application of ZnO-NPs at 50 and 100 ppm. The effects of these treatments were evaluated through photosynthetic pigments, root and shoot length, proline, soluble sugars, protein content, membrane stability index (MSI), fresh weight, and dry weight. Chromium stress significantly ($P \leq 0.001$) reduced most growth, physiological, and biochemical attributes. Total chlorophyll decreased by 58.45% and 62.69%, MSI by 77.37% and 84.16%, fresh weight by 42.42% and 54.22%, and dry weight by 68.00% and 74.00% under 50 and 150 ppm Cr, respectively. In contrast, Cr stress increased proline and soluble sugar accumulation, indicating activation of stress-adaptive responses. Foliar ZnO-NP application substantially alleviated Cr-induced damage, with 100 ppm ZnO generally producing greater improvements than 50 ppm. At 100 ppm ZnO, total chlorophyll, chlorophyll b, shoot growth, protein content, and MSI showed pronounced recovery, while MSI amelioration reached 135.85%. Under severe Cr stress (150 ppm), total chlorophyll recovery increased from 22.80% with 50 ppm ZnO to 60.49% with 100 ppm ZnO, whereas chlorophyll b recovery increased from 31.69% to 70.93%. Correlation analysis demonstrated positive associations among growth, biomass, chlorophyll, protein, and MSI, while proline and soluble sugars were associated with stress-related responses. Hierarchical clustering and principal component analysis further separated Cr-stressed plants from ZnO-associated recovery responses, with the first two principal components explaining 91.2% of total variation. Overall, foliar ZnO-NPs, particularly at 100 ppm, substantially improved the physiological and biochemical performance of sunflower under Cr stress.

Introduction

Chromium (Cr) contamination of agricultural soils is an increasing environmental concern because of its persistence, mobility, phytotoxicity, and potential transfer through the soil-plant system. Chromium is released into agricultural and natural ecosystems through several anthropogenic activities, including electroplating, leather tanning, mining, metal processing, pigment production, industrial effluents, and the use of contaminated irrigation water. Among the different oxidation states of Cr, hexavalent chromium [Cr(VI)] is generally considered more mobile and toxic than trivalent chromium [Cr(III)]. Because plants do not possess a specific Cr uptake system, Cr can enter plant tissues through transport pathways normally involved in the uptake of essential nutrients. Once absorbed, Cr may accumulate in roots and shoots and interfere with nutrient acquisition, water relations, photosynthesis, cellular metabolism, and plant growth (Shanker et al., 2005; Shahid et al., 2017a, 2017b; Hayat et al., 2012; Ulhassan et al., 2022). Chromium toxicity is also strongly influenced by Cr speciation, concentration, exposure duration, plant species, and environmental conditions (Shanker et al., 2005; Shahid et al., 2017b). Additional evidence shows that Cr exposure can suppress growth and photosynthesis, disturb mineral nutrition, and generate oxidative injury across crop species (Basit et al., 2022b; Basit et al., 2023b; Paunov et al., 2018; Fattahi et al., 2019).

The phytotoxicity of Cr is closely associated with disruption of cellular redox homeostasis. Chromium exposure can stimulate excessive production of reactive oxygen species (ROS), including superoxide radicals and hydrogen peroxide. When ROS production exceeds the capacity of antioxidant defense mechanisms, oxidative stress develops, resulting in damage to lipids, proteins, nucleic acids, membranes, and photosynthetic structures. Chromium-induced oxidative stress can consequently impair chloroplast function, reduce photosynthetic pigment accumulation, disturb carbon assimilation, and restrict plant growth and biomass production (Dixit et al., 2002; Mittler, 2002; Sharma and Dietz, 2009; Hayat et al., 2012; Shahid et al., 2017b). The magnitude of these responses depends on Cr concentration, exposure duration, plant species and genotype, developmental stage, and environmental conditions (Shanker et al., 2005; Shahid et al., 2017b; Ulhassan et al., 2022). These responses are consistent with the broader concept that stress-generated ROS can function in signaling at controlled levels but become damaging when production exceeds cellular scavenging capacity (Mittler, 2017; Mittler et al., 2022). Amino-acid metabolism and cellular redox imbalance are central components of heavy-metal stress adaptation (Sharma and Dietz, 2006, 2009).

Nanotechnology provides a promising approach for improving plant tolerance to abiotic stresses. Nanoparticles possess distinctive physicochemical properties, including high surface area, enhanced reactivity, and the ability to interact with plant surfaces and cellular systems (Rico et al., 2011; Dimkpa et al., 2012). Among different metal-based nanoparticles, zinc oxide nanoparticles (ZnO-NPs) have received particular attention because zinc (Zn) is an essential micronutrient required for numerous physiological and biochemical processes. Zinc participates in enzyme activation, protein synthesis, membrane stability, gene regulation, photosynthetic processes, and maintenance of cellular structural integrity (Cakmak, 2000; Broadley et al., 2007; Hafeez et al., 2013; Marschner, 2012). Consequently, an appropriate supply of Zn through nanoformulations may improve plant nutritional status and strengthen physiological and antioxidant responses under stressful conditions (Khan et al., 2023). Agricultural nanomaterials can influence nutrient delivery, uptake, translocation, and stress signaling, although their effects depend on particle chemistry, size, dose, and exposure route (Basit et al., 2022a; Basit et al., 2023a; Kah et al., 2018; Rico et al., 2011; Siddiqui et al., 2015). Earlier work also established both beneficial and phytotoxic plant responses to engineered nanoparticles, emphasizing the importance of dose and formulation (Prasad et al., 2012; Sabir et al., 2014; Liu et al., 2022).

Several studies have demonstrated the potential of ZnO-NPs to mitigate heavy-metal toxicity. Metallic nanoparticles, including ZnO-NPs, have been reported to reduce heavy-metal accumulation and toxicity through several mechanisms, including improved photosynthetic activity, regulation of cellular metabolism, enhanced nutrient acquisition, stimulation of antioxidant defense, reduction of oxidative damage, and improvement of cellular integrity (Dimkpa et al., 2012; Ulhassan et al., 2022; Khan et al., 2023). For example, Ramzan et al. (2023) reported that phytostabilized ZnO nanoparticles improved physiological and antioxidative responses of maize exposed to Cr stress and reduced Cr accumulation. Similarly, ZnO-NPs have improved physiological and antioxidant responses in other heavy-metal-stressed crops, including soybean exposed to arsenic (Noman et al., 2020). These findings suggest that ZnO-NPs may act through multiple complementary mechanisms rather than through a single pathway. Beneficial ZnO-NP responses have been reported in chickpea, mung bean, tomato, cilantro, cotton, wheat, maize, and other crops, particularly through improved pigment retention, antioxidant capacity, nutrient relations, and growth (Mahajan et al., 2011; Burman et al., 2013; Raliya et al., 2015; Pullagurala et al., 2018; Hussain et al., 2018; Rizwan et al., 2019b; Venkatachalam et al., 2017b). Conversely, high or poorly matched doses may inhibit development or alter nutritional quality (Wang et al., 2016; Yoon et al., 2014; Peralta-Videa et al., 2014; Stampoulis et al., 2009).

Sunflower (*Helianthus annuus* L.) is an economically important oilseed crop whose productivity depends strongly on efficient photosynthesis, adequate vegetative growth, membrane stability, and effective biomass accumulation. Exposure to Cr can interfere with these processes and consequently reduce plant growth and physiological performance (Shanker et al., 2005;

Hayat et al., 2012). Recent evidence also indicates that zinc-based nanoparticles can improve chlorophyll content, growth, osmotic regulation, and antioxidant defense in sunflower subjected to other abiotic stresses. Despite increasing interest in nanoparticle-mediated mitigation of heavy-metal stress, relatively limited information is available regarding the integrated physiological and biochemical response of sunflower to Cr stress combined with foliar ZnO-NP application. In particular, simultaneous evaluation of photosynthetic pigments, root and shoot growth, osmolytes, protein, membrane stability, and fresh and dry biomass can provide a more comprehensive understanding of ZnO-mediated stress alleviation. Foliar delivery is particularly relevant because particle retention, dissolution, leaf-surface properties, and subsequent transport strongly influence Zn uptake and biological effectiveness (Read et al., 2020; García-López et al., 2019).

The present study was therefore conducted to investigate the ameliorative potential of foliar-applied ZnO nanoparticles against Cr-induced stress in sunflower. Specifically, the study evaluated the effects of 50 and 100 ppm ZnO-NPs under 50 and 150 ppm Cr stress on photosynthetic pigments, root and shoot growth, proline, soluble sugars, protein, membrane stability, fresh weight, and dry weight. In addition, percentage reduction under Cr stress and percentage amelioration and recovery following ZnO-NP application were calculated to quantify the magnitude of stress injury and ZnO-mediated restoration. Multivariate analyses, including correlation analysis, hierarchical clustering, and principal component analysis, were further used to identify relationships among traits and characterize the overall physiological response of sunflower to Cr and ZnO-NP treatments. The experimental objective was to determine whether foliar ZnO-NP application could reduce the physiological and biochemical consequences of Cr toxicity and identify the more effective ZnO-NP concentration for stress mitigation.

Materials and Methods

Experimental site and plant material

The experiment was conducted from February to May at the Plant Physiology Laboratory and Botanical Garden, Department of Botany, Lahore College for Women University (LCWU), Lahore, Pakistan, to evaluate the ameliorative effects of chemically synthesized zinc oxide nanoparticles (ZnO-NPs) on chromium (Cr)-induced stress in sunflower (*Helianthus annuus* L.). Seeds of sunflower cultivar Hi-Sun 33 were obtained from Ayub Agricultural Research Institute (AARI), Faisalabad.

Synthesis and characterization of ZnO nanoparticles

ZnO-NPs were synthesized through a chemical precipitation method using zinc sulfate heptahydrate and sodium hydroxide as precursor materials. A 0.1 M zinc sulfate solution was reacted with 0.2 M NaOH under vigorous magnetic stirring at 100 °C for 2 h, while maintaining the pH at approximately 12 through gradual addition of NaOH. The resulting suspension was allowed to stand overnight, and the precipitate was collected, washed 2–3 times with deionized water, and dried in a hot-air oven at 400 °C. The synthesized ZnO-NPs were characterized using UV-visible absorption and Fourier-transform infrared (FTIR) spectroscopy following established procedures (Al-Aboodi et al., 2020; Ansari et al., 2024).

The use of ZnO-NPs as a potential stress-mitigating treatment is supported by recent studies reporting improvements in plant growth, photosynthetic performance, osmotic regulation, and biochemical responses under abiotic stress (Ramzan et al., 2023; Khan et al., 2023). However, ZnO-NP responses are concentration-dependent, and excessive nanoparticle exposure may cause oxidative stress and physiological disturbances; therefore, their concentration and mode of application should be carefully considered (Kang et al., 2024). Comparable concentration-dependent benefits and limitations have been observed across ZnO-NP studies (Sheteiwy et al., 2021; Singh et al., 2016; Pandey et al., 2010).

Soil analysis and pot experiment

Soil collected from a local nursery in Lahore was analyzed for pH, electrical conductivity (EC), and texture using standard procedures. The soil had a pH of 6.89, EC of 79.7 mS m⁻¹, and sandy clay texture. The pot experiment was conducted under botanical garden conditions using a completely randomized design with three replications. Seeds were soaked overnight in distilled water and sown at a depth of approximately 2.5–5.0 cm, with 10 seeds initially sown per pot. Seedlings were manually thinned at the 4–5-inch stage to maintain one healthy plant per pot. Plants were subjected to the respective Cr and ZnO-NP treatments, while distilled water was applied to the untreated control. ZnO-NPs were applied as foliar treatments, and leaf samples were collected 4–5 days after application. Plants were harvested at physiological maturity, approximately 90–120 days after sowing.

Foliar application was selected because Zn-based nanoparticles can influence plant physiological and biochemical processes through direct leaf exposure and subsequent uptake, while recent studies have demonstrated the potential of foliar-applied ZnO-NPs to alleviate abiotic stress responses in crops (Khan et al., 2023). Foliar ZnO-NP application has also been associated with altered growth, antioxidant responses, and metal accumulation under combined abiotic stresses (Adrees et al., 2021; Rizwan et al., 2019b).

Experimental treatments

The experiment consisted of a 3×3 factorial combination of three Cr concentrations (0, 50, and 150 ppm) and three ZnO-NP concentrations (0, 50, and 100 ppm), resulting in nine treatment combinations. These comprised the untreated control (0 Cr + 0 ZnO-NPs), Cr-stressed treatments (50 and 150 ppm Cr), ZnO-NP treatments (50 and 100 ppm ZnO-NPs), and their respective combinations under Cr stress. Each treatment was replicated three times.

Morphological and physiological measurements

Shoot and root lengths were measured in centimeters using a scale after carefully removing adhering soil. Fresh biomass was recorded immediately after harvest using an electronic balance, whereas dry biomass was determined after oven-drying the plant material at 70 °C for 72 h to constant weight.

Relative water content (RWC) was determined according to Weatherley (1950). Fresh leaf tissue was weighed, hydrated in distilled water for 24 h to obtain turgid weight, and subsequently oven-dried at 70 °C for 72 h to determine dry weight. RWC was calculated as:

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

where FW, TW, and DW represent fresh, turgid, and dry weights, respectively.

Membrane stability index (MSI) was determined following Premachandra et al. (1990). Leaf tissue (0.1 g) was washed with distilled water, subjected to control heating, and electrical conductivity was recorded before and after heat treatment. MSI was calculated from the initial and final conductivity readings.

Photosynthetic pigments

Chlorophyll and carotenoid contents were determined according to Arnon (1949) and Lichtenthaler and Wellburn (1983), respectively. Fresh leaf tissue (0.1 g) was homogenized in 80% acetone, centrifuged, and the absorbance of the extract was recorded at 663, 645, and 470 nm using a spectrophotometer. Chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoid contents were calculated using the respective equations of Arnon (1949) and Lichtenthaler and Wellburn (1983).

The inclusion of photosynthetic pigments as indicators of Cr toxicity and ZnO-NP-mediated recovery is supported by recent studies showing that heavy-metal stress can disrupt photosynthetic processes, whereas appropriate Zn-based nanoparticle application may improve chlorophyll retention and photosynthetic performance (Ramzan et al., 2023). Photosynthetic traits are widely used as sensitive indicators of abiotic and metal stress, while ZnO-NPs can modify pigment content and photosynthetic performance (Faseela et al., 2020; Kataria et al., 2019; Salam et al., 2022).

Biochemical analyses

Total soluble sugars were estimated using the phenol-sulfuric acid method of Dubois et al. (1956). Fresh leaf tissue (0.1 g) was homogenized in distilled water and centrifuged at 3000 rpm for 10 min. The supernatant was reacted with 5% phenol and concentrated sulfuric acid, and absorbance was measured at 420 nm. Sugar concentration was calculated using a glucose standard curve.

Free proline was determined following Bates et al. (1973). Fresh leaf tissue (0.1 g) was extracted with sulfosalicylic acid, reacted with acid ninhydrin, heated at 100 °C for 1 h, and the resulting chromophore was extracted with toluene. Absorbance was measured at 520 nm, and proline concentration was calculated using a standard curve.

Soluble protein content was estimated according to Lowry et al. (1951). Fresh leaf tissue (0.1 g) was extracted in sodium phosphate buffer, centrifuged, and the supernatant was reacted with Lowry reagents. Absorbance was recorded at 650 nm, and protein concentration was determined using a protein standard curve.

The selection of proline, soluble sugars, and protein as biochemical indicators is consistent with recent ZnO-NP studies, in which osmolyte accumulation and biochemical adjustment were associated with plant responses to abiotic stress (Khan et al., 2023; Ramzan et al., 2023). Similar biochemical adjustments involving osmolytes, proteins, and antioxidative processes have been documented under nanoparticle-mediated stress mitigation (Noman et al., 2018; Faizan et al., 2021).

Statistical Analysis

Data were analyzed using R statistical software (R Core Team, 2024). The experiment was analyzed as a 3×3 factorial experiment with Cr concentration (0, 50, and 150 ppm) and ZnO-NP concentration (0, 50, and 100 ppm) as fixed factors and

three replications. Factorial analysis of variance (ANOVA) was performed separately for each morphological, physiological, and biochemical trait to determine the main effects of Cr, ZnO-NP, and their interaction (Cr × ZnO-NP).

The percentage reduction caused by Cr stress relative to the unstressed control was calculated as:

$$Reduction(\%) = \frac{C - S}{C} \times 100$$

where **C** represents the mean value of the control treatment (0 Cr + 0 ZnO-NPs) and **S** represents the mean value of the corresponding Cr-stressed treatment without ZnO-NPs. This calculation was applied to traits for which a decrease represents an adverse response to Cr stress, including growth, chlorophyll, biomass, and membrane stability.

The ameliorative effect of ZnO-NPs under Cr stress was quantified as percentage recovery:

$$Amelioration(\%) = \frac{T - S}{C - S} \times 100$$

where **C** is the mean value of the unstressed control, **S** is the mean value of the corresponding Cr-stressed treatment without ZnO-NPs, and **T** is the mean value of the corresponding Cr + ZnO-NP treatment. A value of 0% indicates no recovery, whereas 100% represents complete restoration to the control level. Values >100% indicate that the ZnO-NP treatment exceeded the control value, whereas negative values indicate a response lower than that of the Cr-stressed treatment. This recovery approach provides a quantitative estimate of the proportion of Cr-induced deviation restored by ZnO-NP application.

For proline, which commonly functions as a stress-associated osmolyte, treatment responses were interpreted according to its biological role rather than assuming that an increase necessarily represents improved plant performance. A decline in stress-induced proline toward the control level was considered evidence of alleviation only when accompanied by improvements in growth, physiological, or other biochemical traits.

Pearson's correlation analysis was performed to evaluate the strength and direction of associations among morphological, physiological, and biochemical traits. Principal component analysis (PCA) was performed using standardized quantitative trait data to identify major sources of multivariate variation and traits contributing most strongly to differential responses to Cr and ZnO-NP treatments. A standardized treatment × trait matrix was also visualized using a heatmap to provide an integrated comparison of treatment responses.

Results

Analysis of variance

Analysis of variance revealed highly significant ($P \leq 0.001$) effects of Cr concentration on all evaluated traits (table 1), with particularly strong effects on MSI, total chlorophyll, fresh weight, dry weight, and chlorophyll *a*. ZnO-NP application also significantly affected all traits except root length, for which the effect was non-significant. The Cr × ZnO-NP interaction was highly significant ($P \leq 0.001$) for all traits except protein, where the interaction was significant at $P \leq 0.05$. The strongest interaction effects were observed for proline, MSI, total chlorophyll, dry weight, chlorophyll *a*, sugar, shoot length, and root length. The significant interaction across most traits indicates that the response to ZnO-NPs varied with Cr concentration and demonstrates a strong ZnO-NP-mediated modification of sunflower responses to Cr stress.

Table 1. Analysis of variance for the effects of chromium (Cr), ZnO nanoparticles, and their interaction on physiological and biochemical traits of sunflower

1. Trait	2. Cr	3. ZnO	4. Cr × ZnO
5. TChl	6. 5295.92***	7. 2261.47***	8. 128.31***
9. Chl _a	10. 979.01***	11. 126.53***	12. 22.53***
13. Chl _b	14. 461.62***	15. 229.07***	16. 8.79***
17. Root length	18. 46.29***	19. 1.77 ns	20. 10.77***
21. Shoot length	22. 467.33***	23. 136.24***	24. 15.23***
25. Proline	26. 26.12***	27. 19.43***	28. 288.36***

29. Protein	30. 127.25***	31. 29.30***	32. 2.93*
33. Sugar	34. 228.37***	35. 76.51***	36. 19.01***
37. MSI	38. 7571.28***	39. 1728.28***	40. 218.61***
41. Fresh weight	42. 1232.37***	43. 42.65***	44. 10.74***
45. Dry weight	46. 590.06***	47. 147.39***	48. 36.89***

ns = non-significant; * = significant at $P \leq 0.05$; ** = significant at $P \leq 0.01$; *** = significant at $P \leq 0.001$.

Effect of Chromium Stress on Physiological, Biochemical, Growth, and Biomass Traits of Sunflower

The results in table 2 indicate that chromium stress markedly reduced most physiological, biochemical, growth, and biomass traits of sunflower, with generally greater reductions at 150 ppm Cr. The highest reductions were observed for MSI, chlorophyll, dry weight, and protein, whereas proline and sugar showed negative reduction values, indicating an increase under Cr stress.

At 50 ppm Cr, total chlorophyll, chlorophyll *a*, and chlorophyll *b* decreased by 58.45%, 78.53%, and 41.09%, respectively. Increasing Cr concentration to 150 ppm resulted in reductions of 62.69%, 73.16%, and 42.60%, respectively. Root and shoot lengths were reduced by 29.03% and 37.89% at 50 ppm, with greater reductions of 32.26% and 47.02% at 150 ppm. Protein content declined by 47.34% and 54.99%, while fresh and dry weights decreased by 42.42% and 68.00% at 50 ppm and by 54.22% and 74.00% at 150 ppm, respectively.

The membrane stability index showed the strongest decline, decreasing by 77.37% at 50 ppm and 84.16% at 150 ppm Cr, demonstrating substantial membrane damage under chromium toxicity. In contrast, proline exhibited negative reduction values (−125.96% and −213.87%), indicating an increase of approximately 126% and 214%, respectively, as Cr concentration increased. Similarly, sugar increased by approximately 26.79% and 34.71%, as indicated by the negative reduction values.

Table 2. Percentage reduction in physiological, biochemical, growth, and biomass traits of sunflower under 50 and 150 ppm chromium (Cr) stress.

49. Trait	50. Reduction at 50 ppm Cr (%)	51. Reduction at 150 ppm Cr (%)
52. Total chlorophyll (TChl)	53. 58.45	54. 62.69
55. Chlorophyll <i>a</i> (Chl _a)	56. 78.53	57. 73.16
58. Chlorophyll <i>b</i> (Chl _b)	59. 41.09	60. 42.60
61. Root length	62. 29.03	63. 32.26
64. Shoot length	65. 37.89	66. 47.02
67. Proline	68. −125.96	69. −213.87
70. Protein	71. 47.34	72. 54.99
73. Sugar	74. −26.79	75. −34.71
76. Membrane stability index (MSI)	77. 77.37	78. 84.16
79. Fresh weight	80. 42.42	81. 54.22
82. Dry weight	83. 68.00	84. 74.00

Ameliorative Effects of ZnO Nanoparticles on Chromium-Induced Stress in Sunflower

The results show that foliar application of ZnO nanoparticles ameliorated several adverse effects of chromium stress in sunflower, with 100 ppm ZnO showing greater recovery for several traits (table 3). Foliar application of 50 ppm ZnO increased total chlorophyll, chlorophyll *a*, and chlorophyll *b* by 38.30%, 14.54%, and 23.51%, respectively. At 100 ppm ZnO, the corresponding amelioration increased to 101.62%, 34.96%, and 52.63%, demonstrating a stronger recovery of the photosynthetic pigments. Similarly, shoot length improved by 22.52% and 43.05%, while protein content increased by 17.47% and 43.23% under 50 and 100 ppm ZnO, respectively.

The membrane stability index showed the greatest positive response, with amelioration of 46.65% at 50 ppm and 135.85% at 100 ppm ZnO, indicating a pronounced improvement in membrane stability under the higher ZnO treatment. Dry weight showed an identical amelioration of 46.15% at both ZnO concentrations. Root length improved by 14.29% with 50 ppm ZnO, whereas a negative response (–52.38%) was observed at 100 ppm. Fresh weight also showed a negative response at 50 ppm (–21.33%) but a slight positive response at 100 ppm (7.64%).

Proline and sugar exhibited negative amelioration values at both ZnO concentrations. Proline showed values of –52.69% and –30.20%, while sugar showed –9.53% and –15.72% at 50 and 100 ppm ZnO, respectively. These negative values indicate that ZnO application did not increase these traits relative to the Cr-stressed plants and may reflect the reduction of stress-induced osmolyte accumulation following stress alleviation.

Table 3. Percentage amelioration of chromium-induced stress in sunflower by foliar application of 50 and 100 ppm ZnO nanoparticles across physiological, biochemical, growth, and biomass traits.

85. Trait	86. ZnO 50 ppm amelioration (%)	87. ZnO 100 ppm amelioration (%)
88. Total chlorophyll (TChl)	89. 38.30	90. 101.62
91. Chlorophyll a (Chl_a)	92. 14.54	93. 34.96
94. Chlorophyll b (Chl_b)	95. 23.51	96. 52.63
97. Root length	98. 14.29	99. –52.38
100. Shoot length	101. 22.52	102. 43.05
103. Proline	104. –52.69	105. –30.20
106. Protein	107. 17.47	108. 43.23
109. Sugar	110. –9.53	111. –15.72
112. Membrane stability index (MSI)	113. 46.65	114. 135.85
115. Fresh weight	116. –21.33	117. 7.64
118. Dry weight	119. 46.15	120. 46.15

ZnO Nanoparticle-Mediated Recovery from Chromium-Induced Stress in Sunflower

The results indicate that ZnO nanoparticles promoted substantial recovery of sunflower plants from the adverse effects of 150 ppm Cr stress, with the magnitude of recovery generally greater at 100 ppm ZnO than at 50 ppm ZnO (table 4). Total chlorophyll recovered by 22.80% with 50 ppm ZnO + 150 ppm Cr and by 60.49% with 100 ppm ZnO + 150 ppm Cr. Similarly, chlorophyll a recovery increased from 5.34% to 12.83%, while chlorophyll b recovery increased markedly from 31.69% to 70.93% with increasing ZnO concentration.

Growth traits also showed considerable recovery. In contrast, shoot length recovered by 25.37% and 48.51% under 50 and 100 ppm ZnO, respectively. Protein content showed recovery of 14.30% at 50 ppm and 35.39% at 100 ppm ZnO. Sugar exhibited substantial recovery, increasing from 36.99% to 61.00% with increasing ZnO concentration.

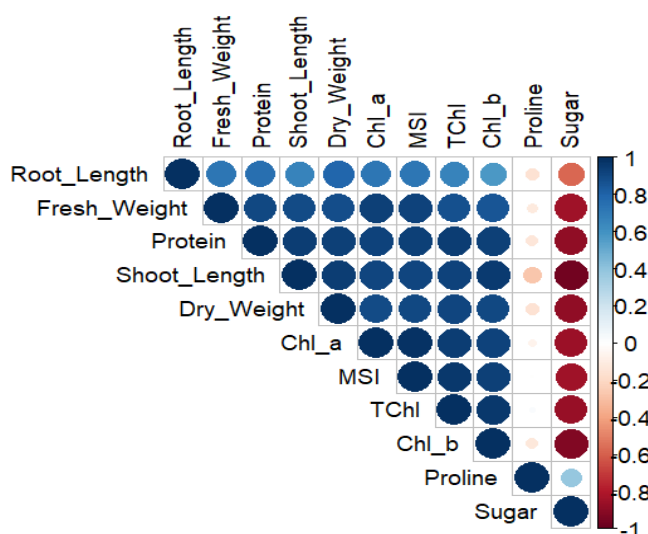
A notable recovery was also observed in stress-related biochemical and membrane parameters. Proline showed 77.33% recovery with 50 ppm ZnO and 44.32% with 100 ppm ZnO, while MSI recovered by 8.78% and 25.58%, respectively. Dry weight showed 16.22% recovery at both ZnO concentrations. Fresh weight showed a negative recovery at 50 ppm ZnO (–18.01%) but a slight positive recovery (6.45%) at 100 ppm ZnO.

Table 4. Percentage recovery of chromium-induced stress in sunflower following ZnO nanoparticle application under 150 ppm Cr

121. Trait	122.ZnO 50 ppm + Cr 150 ppm (%)	123.ZnO 100 ppm + Cr 150 ppm (%)
124.Total chlorophyll (TChl)	125. 22.80	126. 60.49
127. Chlorophyll a (Chl_a)	128. 5.34	129. 12.83
130. Chlorophyll b (Chl_b)	131. 31.69	132. 70.93
133. Root length	134. 30.00	135. -110.00
136. Shoot length	137. 25.37	138. 48.51
139. Proline	140. 77.33	141. 44.32
142. Protein	143. 14.30	144. 35.39
145. Sugar	146. 36.99	147. 61.00
148. Membrane stability index (MSI)	149. 8.78	150. 25.58
151. Fresh weight	152. -18.01	153. 6.45
154. Dry weight	155. 16.22	156. 16.22

Correlation Analysis of Traits Under Cr Stress and ZnO Treatments

The correlation matrix highlights two contrasting physiological clusters in sunflowers under chromium stress and ZnO nanoparticle treatments: The first cluster includes growth parameters (Root_Length, Shoot_Length), biomass traits (Fresh_Weight, Dry_Weight), photosynthetic pigments (Chl_a, Chl_b, TChl), Protein, and membrane stability (MSI) as presented in figure 1. These traits show strong positive correlations with one another (dark blue circles), indicating that these traits were positively associated with plant growth and biomass accumulation. The second cluster centers on stress-responsive osmolytes, specifically Sugar. Soluble sugar content exhibits strong negative correlations (dark red circles) with all growth, biomass, and chlorophyll traits. This inverse relationship indicates that under heavy metal stress, plants divert energy from growth toward sugar accumulation for osmotic defense, whereas effective ZnO nanoparticle treatments reduce stress levels, lowering sugar accumulation while promoting growth.

**Figure 1. Correlation matrix showing the relationships among physiological, biochemical, growth, and biomass traits of sunflower under chromium stress and ZnO nanoparticle treatments.**

Hierarchical Cluster Analysis

The hierarchical clustering heatmap divides the nine treatment combinations (3×3 factorial of Cr stress and foliar ZnO-NPs) into two distinct trait and response profiles (figure 2). Growth parameters (Root_Length, Shoot_Length), biomass traits (Fresh_Weight, Dry_Weight), photosynthetic pigments (Chl_a, Chl_b, TChl), soluble protein, and membrane stability (MSI) cluster tightly together, showing elevated expression (red/orange z-scores) in treatments where foliar ZnO-NPs mitigated chromium toxicity. This demonstrates that ZnO-NP applications protect cellular membranes and chlorophyll synthesis, directly sustaining vegetative growth and biomass accumulation. Conversely, stress osmolytes (Sugar and Proline) form a separate, isolated cluster that peaks primarily under severe, unmitigated chromium stress (T₃). Under high Cr toxicity, sunflower plants divert metabolic resources toward osmoprotectant synthesis for survival, resulting in reduced chlorophyll and biomass. Foliar ZnO-NP treatments effectively reverse this trend, suppressing stress-induced osmolyte reliance while restoring normal photosynthetic and growth processes.

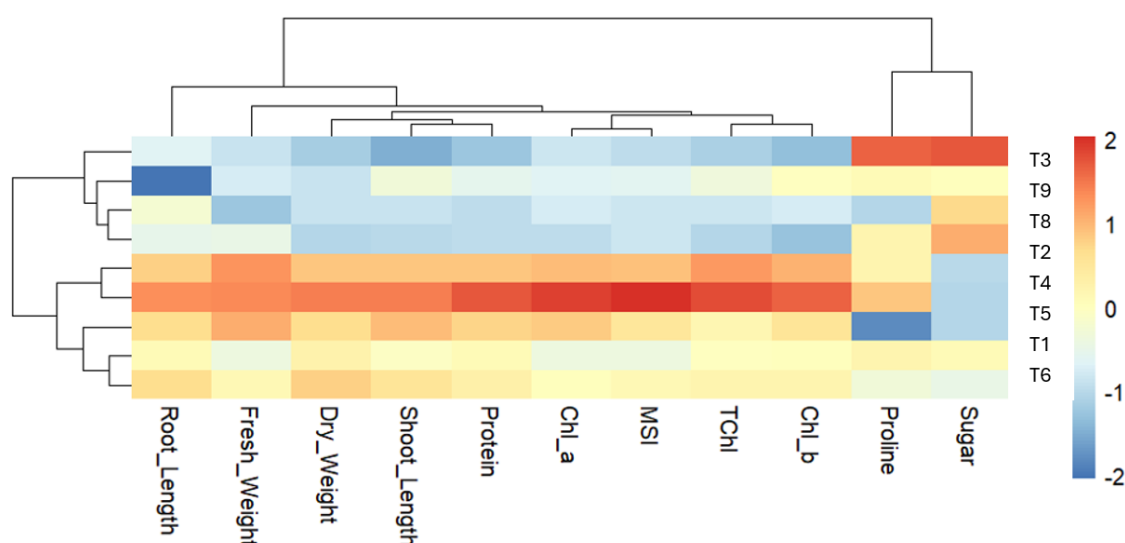


Figure 2. Hierarchical cluster heatmap displaying the differential responses of physiological, biochemical, growth, and biomass traits in sunflower (*Helianthus annuus* L.)

Principal Component Analysis (PCA)

The Principal Component Analysis (PCA) biplot accounts for 91.2% of the total dataset variance (figure 3), effectively partitioning the responses into two main components: Dimension 1 (80.9%) and Dimension 2 (10.3%). Dimension 1 serves as the primary axis differentiating plant vigor from chromium-induced stress, where non-stressed and ZnO-NP-treated plants (T₁, T₄, T₅, T₆) cluster on the positive (right) side alongside vectors for photosynthetic pigments (Chl_a, Chl_b, TChl), biomass (Dry_Weight), growth (Shoot_Length), soluble protein, and membrane stability (MSI). In sharp contrast, chromium-stressed treatments (T₂, T₃, T₈, T₉) plot on the negative (left) side, aligning directly with elevated Sugar content. This spatial divergence confirms that while Cr stress restricts growth and suppresses photosynthetic efficiency, foliar application of ZnO-NPs drives recovery along Dimension 1 toward higher biomass and pigment retention. Meanwhile, Dimension 2 is mainly defined by the orthogonal vector for Proline, capturing specific osmotic adjustments across the treatment groups. The strong opposite orientation between Sugar and growth/pigment vectors further illustrates the metabolic shift from biomass production to stress-induced osmoprotection.

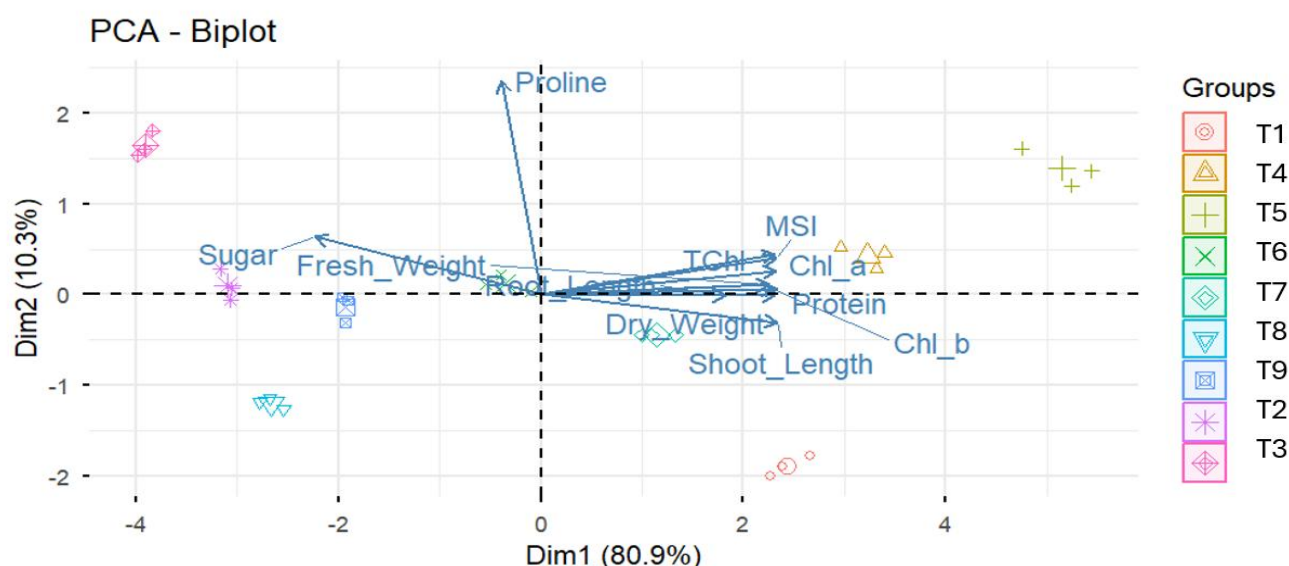


Figure 3. Principal Component Analysis (PCA) biplot illustrating the grouping of sunflower (*Helianthus annuus* L.) traits and treatment combinations (T1–T9) under chromium stress and foliar ZnO nanoparticle applications.

Discussion

Chromium Stress and Physiological Impairment

Chromium (Cr) significantly affected all measured traits ($P \leq 0.001$), particularly membrane stability index (MSI), total chlorophyll, fresh weight, dry weight, and chlorophyll a. These findings agree with reports that Cr toxicity disrupts nutrient uptake, photosynthesis, membrane integrity, and biomass production (Shanker et al., 2005; Shahid et al., 2017; Ulhassan et al., 2022). Comparable Cr-related physiological impairment and nanoparticle-mediated attenuation have been documented in rice and soybean (Basit et al., 2022b; Basit et al., 2023b).

Total chlorophyll declined by 58.45% and 62.69% at 50 and 150 ppm Cr, respectively. Chlorophyll a decreased by 78.53% and 73.16%, while chlorophyll b declined by 41.09% and 42.60%. These reductions may result from nutrient interference, oxidative damage, impaired pigment biosynthesis, and disrupted electron transport. Because chlorophyll a reduction was greater at 50 ppm, the results indicate severe pigment disruption at both concentrations rather than a strictly linear dose response. Such pigment losses are consistent with metal-induced disruption of chloroplast function, electron transport, and chlorophyll fluorescence (Paunov et al., 2018; Faseela et al., 2020).

MSI declined by 77.37% and 84.16% at 50 and 150 ppm Cr, respectively, suggesting substantial oxidative membrane injury, lipid peroxidation, and electrolyte leakage. Root length decreased by 29.03% and 32.26%, shoot length by 37.89% and 47.02%, fresh weight by 42.42% and 54.22%, and dry weight by 68.00% and 74.00%. The greater reduction in dry weight indicates impaired biomass accumulation. The stronger effects on shoots and biomass suggest major disruption of aerial physiological processes; however, Cr accumulation and translocation were not measured. Heavy-metal stress commonly reduces growth and biomass as oxidative damage and nutrient imbalance intensify (Fattahi et al., 2019; Hussain et al., 2018).

Proline increased by approximately 126% and 214%, while soluble sugars increased by 26.79% and 34.71% at 50 and 150 ppm Cr, respectively. These changes likely represent osmotic adjustment, membrane and protein stabilization, and redox regulation rather than improved growth. The simultaneous decline in growth, chlorophyll, protein, MSI, and biomass suggests metabolic investment in stress protection (Shahid et al., 2017; Ulhassan et al., 2022). Protein content decreased by 47.34% and 54.99%, possibly because of inhibited protein synthesis, oxidative damage, altered nitrogen metabolism, and increased proteolysis. Proline and soluble sugars can contribute to osmotic adjustment and cellular protection under stress, although their accumulation often reflects stress intensity rather than productivity (Hayat et al., 2012; Noman et al., 2018).

ZnO nanoparticles (ZnO-NPs) significantly affected nearly all traits, except root length, and the Cr $\times$ ZnO interaction was significant for most traits. Total chlorophyll, chlorophyll a, and chlorophyll b increased by 38.30%, 14.54%, and 23.51% with 50 ppm ZnO, compared with 101.62%, 34.96%, and 52.63% with 100 ppm ZnO. This suggests stronger pigment recovery at 100 ppm, potentially due to Zn-mediated improvement in enzyme activity, protein metabolism, membrane stability, and antioxidant protection (Khan et al., 2023; Ulhassan et al., 2022). Similar improvements under Cr stress have been reported in maize and chickpea. Similar ZnO-NP-induced improvements in growth, pigments, and antioxidant performance have been

reported in chickpea, tomato, cilantro, maize, and wheat (Burman et al., 2013; Pullagurala et al., 2018; Faizan et al., 2021; Salam et al., 2022; Adrees et al., 2021).

MSI amelioration reached 46.65% and 135.85% with 50 and 100 ppm ZnO, respectively. Dry weight improved by 46.15% at both concentrations, while shoot length increased by 22.52% and 43.05% and protein content by 17.47% and 43.23%. These responses indicate coordinated improvements in photosynthesis, growth, protein metabolism, and membrane stability. Root length increased by 14.29% with 50 ppm ZnO but showed a -52.38% response at 100 ppm. This indicates that ZnO effects were concentration- and trait-dependent; excessive ZnO exposure may disturb nutrient balance, increase ROS, or inhibit growth (Kang et al., 2024). Thus, 100 ppm was more effective for most traits but was not universally beneficial. Zinc can support membrane integrity, enzyme function, and stress tolerance, and ZnO-based treatments may also reduce heavy-metal accumulation or improve nutrient balance (Ajouri et al., 2004; Huang et al., 2019; Rizwan et al., 2019a; Rizwan et al., 2019b).

Proline amelioration values were -52.69% and -30.20%, while sugar values were -9.53% and -15.72% at 50 and 100 ppm ZnO. Under 150 ppm Cr, proline recovery reached 77.33% and 44.32%, while sugar recovery reached 36.99% and 61.00%, respectively. Reduced accumulation of these stress-associated metabolites, alongside improved growth, chlorophyll, protein, MSI, and biomass, may indicate reduced stress pressure rather than a negative ZnO effect. Similar shifts in stress metabolites and antioxidant systems following ZnO-NP treatment have been observed in several crop species (García-López et al., 2019; Venkatachalam et al., 2017a; Venkatachalam et al., 2017b).

At 150 ppm Cr, total chlorophyll recovery increased from 22.80% with 50 ppm ZnO to 60.49% with 100 ppm, while chlorophyll b recovery increased from 31.69% to 70.93%. Shoot length recovery increased from 25.37% to 48.51%, and protein recovery from 14.30% to 35.39%. However, root recovery declined from 30.00% to -110.00%, and fresh-weight recovery was -18.01% and 6.45%. These results confirm that 100 ppm generally improved recovery but responses remained trait-specific. The trait-specific nature of recovery is consistent with evidence that ZnO-NP effects depend on dose, plant species, exposure pathway, and the prevailing stress environment (Sheteiwy et al., 2021; Basit et al., 2023a; Ulhassan et al., 2023).

Growth, biomass, chlorophyll, protein, and MSI showed strong positive associations, reflecting their shared roles in photosynthesis, cellular integrity, and biomass production. Soluble sugars were negatively associated with growth, biomass, and chlorophyll, likely reflecting stress-related metabolic allocation. However, correlation indicates association, not causation. Hierarchical clustering grouped growth, biomass, pigments, protein, and MSI separately from proline and sugar, distinguishing performance-related and stress-responsive traits. PCA explained 91.2% of total variation (Dim1 = 80.9%; Dim2 = 10.3%). Dimension 1 separated treatments according to plant vigor and Cr stress, while Dimension 2 was mainly associated with proline. ZnO-treated plants generally shifted toward improved growth and photosynthetic functioning. These coordinated multivariate patterns agree with the close physiological coupling among photosynthesis, membrane integrity, biomass production, and stress metabolism (Kataria et al., 2019; Mittler, 2017).

Cr stress caused substantial reductions in photosynthetic pigments, growth, protein, MSI, and biomass, while increasing proline and soluble sugars. ZnO-NPs modified these responses, with 100 ppm generally producing greater improvement in chlorophyll, shoot length, protein, MSI, and recovery traits. Nevertheless, negative responses in root length and some recovery variables demonstrate that ZnO effects depend on dose, exposure conditions, nanoparticle properties, and plant genotype (Khan et al., 2023; Ulhassan et al., 2022; Kang et al., 2024). Studies across crops likewise show that ZnO-NPs may be beneficial within an effective concentration range while becoming neutral or inhibitory at higher exposure levels (Mahajan et al., 2011; Pandey et al., 2010; Singh et al., 2016; Wang et al., 2016; Yoon et al., 2014).

Conclusion

Overall, the findings indicate that foliar ZnO nanoparticles have considerable potential as a nanotechnology-based strategy for alleviating Cr-induced physiological and biochemical stress in sunflower. The results are particularly encouraging because ZnO treatment improved several interconnected attributes rather than a single trait. However, optimization of nanoparticle concentration and application strategy remains essential to maximize stress alleviation while minimizing possible nanoparticle-associated phytotoxicity.

References:

- Shanker, A.K., Cervantes, C., Loza-Tavera, H., & Avudainayagam, S. (2005). Chromium toxicity in plants. *Environment International*, 31, 739–753. <https://doi.org/10.1016/j.envint.2005.02.003>
- Shahid, M., Shamshad, S., Rafiq, M., Khalid, S., Bibi, I., Niazi, N.K., Dumat, C., & Rashid, M.I. (2017a). Chromium speciation, bioavailability, uptake, toxicity and detoxification in soil-plant system: A review. *Chemosphere*, 178, 513–533. <https://doi.org/10.1016/j.chemosphere.2017.03.074>

- Shahid, M., Dumat, C., Khalid, S., Schreck, E., Xiong, T., & Niazi, N.K. (2017b). Foliar heavy metal uptake, toxicity and detoxification in plants: A comparison of foliar and root metal uptake. *Journal of Hazardous Materials*, 325, 36–58. <https://doi.org/10.1016/j.jhazmat.2016.11.063>
- Hayat, S., Khalique, G., Irfan, M., Wani, A.S., Tripathi, B.N., & Ahmad, A. (2012). Physiological changes induced by chromium stress in plants: An overview. *Protoplasma*, 249, 599–611. <https://doi.org/10.1007/s00709-011-0331-0>
- Dixit, V., Pandey, V., & Shyam, R. (2002). Chromium ions inactivate electron transport and enhance superoxide generation in vivo in pea (*Pisum sativum* L. cv. Azad) root mitochondria. *Plant, Cell & Environment*, 25, 687–693. <https://doi.org/10.1046/j.1365-3040.2002.00843.x>
- Sharma, S.S., & Dietz, K.J. (2006). The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. *Journal of Experimental Botany*, 57, 711–726. <https://doi.org/10.1093/jxb/erj073>
- Sharma, S.S., & Dietz, K.J. (2009). The relationship between metal toxicity and cellular redox imbalance. *Trends in Plant Science*, 14, 43–50. <https://doi.org/10.1016/j.tplants.2008.10.007>
- Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science*, 7, 405–410. [https://doi.org/10.1016/S1360-1385\(02\)02312-9](https://doi.org/10.1016/S1360-1385(02)02312-9)
- Mittler, R., Zandalinas, S.I., Fichman, Y., & Van Breusegem, F. (2022). Reactive oxygen species signalling in plant stress responses. *Nature Reviews Molecular Cell Biology*, 23, 663–679.
- Cakmak, I. (2000). Possible roles of zinc in protecting plant cells from damage by reactive oxygen species. *New Phytologist*, 146, 185–205. <https://doi.org/10.1046/j.1469-8137.2000.00630.x>
- Broadley, M.R., White, P.J., Hammond, J.P., Zelko, I., & Lux, A. (2007). Zinc in plants. *New Phytologist*, 173, 677–702. <https://doi.org/10.1111/j.1469-8137.2007.01996.x>
- Hafeez, B., Khanif, Y.M., & Saleem, M. (2013). Role of zinc in plant nutrition—A review. *Journal of Experimental Agriculture International*, 3, 374–391. <https://doi.org/10.9734/AJEA/2013/2746>
- Marschner, P. (Ed.). (2012). *Marschner's Mineral Nutrition of Higher Plants* (3rd ed.). Academic Press. <https://doi.org/10.1016/C2009-0-63043-9>
- Hayat, S., Hayat, Q., Alyemeni, M.N., Wani, A.S., Pichtel, J., & Ahmad, A. (2012). Role of proline under changing environments: A review. *Plant Signaling & Behavior*, 7, 1456–1466. <https://doi.org/10.4161/psb.21949>
- Premachandra, G.S., Saneoka, H., & Ogata, S. (1990). Cell membrane stability, an indicator of drought tolerance, as affected by applied nitrogen in soybean. *Journal of Agricultural Science*, 115, 63–66. <https://doi.org/10.1017/S0021859600073938>
- Ulhassan, Z., et al. (2022). Efficacy of metallic nanoparticles in attenuating the accumulation and toxicity of chromium in plants: Current knowledge and future perspectives. *Environmental Pollution*, 315, 120390. <https://doi.org/10.1016/j.envpol.2022.120390>
- Khan, A.R., Azhar, W., Fan, X., Ulhassan, Z., Salam, A., Ashraf, M., Liu, Y., & Gan, Y. (2023). Efficacy of zinc-based nanoparticles in alleviating the abiotic stress in plants: Current knowledge and future perspectives. *Environmental Science and Pollution Research*, 30, 110047–110068. <https://doi.org/10.1007/s11356-023-29993-6>
- Ramzan, M., Naz, G., Shah, A.A., Parveen, M., Jamil, M., Gill, S., & Sharif, H.M.A. (2023). Synthesis of phytostabilized zinc oxide nanoparticles and their effects on physiological and antioxidative responses of *Zea mays* L. under chromium stress. *Plant Physiology and Biochemistry*, 196, 130–138. <https://doi.org/10.1016/j.plaphy.2023.01.015>
- Read, T.L., Doolette, C.L., Li, C., Schjoerring, J.K., Kopittke, P.M., Donner, E., & Lombi, E. (2020). Optimising the foliar uptake of zinc oxide nanoparticles: Do leaf surface properties and particle coating affect absorption? *Physiologia Plantarum*, 170, 384–397. <https://doi.org/10.1111/ppl.13167>
- Kah, M., Kookana, R.S., Gogos, A., & Bucheli, T.D. (2018). A critical evaluation of nanopesticides and nanofertilizers against their conventional analogues. *Nature Nanotechnology*, 13, 677–684.

- Wang, X., Yang, X., Chen, S., Li, Q., Wang, W., Hou, C., Gao, X., Wang, L., & Wang, S. (2016). Zinc oxide nanoparticles affect biomass accumulation and photosynthesis in Arabidopsis. *Frontiers in Plant Science*, 6, 1243. <https://doi.org/10.3389/fpls.2015.01243>
- Raliya, R., Nair, R., Chavalmane, S., Wang, W.N., & Biswas, P. (2015). Mechanistic evaluation of translocation and physiological impact of titanium dioxide and zinc oxide nanoparticles on tomato plants. *Metallomics*, 7, 1584–1594. <https://doi.org/10.1039/C5MT00168D>
- Dimkpa, C.O., McLean, J.E., Britt, D.W., & Anderson, A.J. (2012). Bioactivity and biomodification of Ag, ZnO, and CuO nanoparticles with relevance to plant performance in agriculture. *Industrial Biotechnology*, 8, 344–357. <https://doi.org/10.1089/ind.2012.0028>
- Rico, C.M., Majumdar, S., Duarte-Gardea, M., Peralta-Videa, J.R., & Gardea-Torresdey, J.L. (2011). Interaction of nanoparticles with edible plants and their possible implications in the food chain. *Journal of Agricultural and Food Chemistry*, 59, 3485–3498. <https://doi.org/10.1021/jf104517j>
- Prasad, T.N.V.K.V., Sudhakar, P., Sreenivasulu, Y., Latha, P., Munaswamy, V., Raja Reddy, K., Sreeprasad, T.S., Sajanlal, P.R., & Pradeep, T. (2012). Effect of nanoscale zinc oxide particles on the germination, growth and yield of peanut. *Journal of Plant Nutrition*, 35, 905–927. <https://doi.org/10.1080/01904167.2012.663443>
- Yoon, S.J., Kwak, J.I., Lee, W.M., Holden, P.A., & An, Y.J. (2014). Zinc oxide nanoparticles delay soybean development: A standard soil microcosm study. *Ecotoxicology and Environmental Safety*, 100, 131–137. <https://doi.org/10.1016/j.ecoenv.2013.10.014>
- Peralta-Videa, J.R., Hernández-Viezcás, J.A., Zhao, L., Corral-Díaz, B., Ge, Y., Priester, J.H., Holden, P.A., & Gardea-Torresdey, J.L. (2014). Cerium dioxide and zinc oxide nanoparticles alter the nutritional value of soil cultivated soybean plants. *Plant Physiology and Biochemistry*, 80, 128–135. <https://doi.org/10.1016/j.plaphy.2014.03.028>
- Sabir, S., Arshad, M., & Chaudhari, S.K. (2014). Zinc oxide nanoparticles for revolutionizing agriculture: Synthesis and applications. *The Scientific World Journal*, 2014, 925494. <https://doi.org/10.1155/2014/925494>
- Noman, A., Ali, Q., Maqsood, J., Iqbal, N., Javed, M.T., Rasool, N., & Naseem, J. (2020). Zinc oxide nanoparticles application alleviates arsenic toxicity in soybean plants by restricting the uptake of As and modulating key biochemical attributes, antioxidant enzymes, ascorbate-glutathione cycle and glyoxalase system. *Environmental Pollution*, 260, 114046.
- Kang, M., Liu, Y., Weng, Y., Wang, H., & Bai, X. (2024). A critical review on the toxicity regulation and ecological risks of zinc oxide nanoparticles to plants. *Environmental Science: Nano*, 11, 14–35. <https://doi.org/10.1039/D3EN00630A>
- Liu, A., et al. (2022). Plants and rhizospheric environment: Affected by zinc oxide nanoparticles (ZnO NPs). A review. *Plant Physiology and Biochemistry*, 185, 91–100. <https://doi.org/10.1016/j.plaphy.2022.05.032>
- Stampoulis, D., Sinha, S.K., & White, J.C. (2009). Assay-dependent phytotoxicity of nanoparticles to plants. *Environmental Science & Technology*, 43, 9473–9479.
- Siddiqui, M.H., Al-Whaibi, M.H., Firoz, M., & Al-Khaishany, M.Y. (2015). Role of nanoparticles in plants. In *Nanotechnology and Plant Sciences*. Springer, 19–35.
- Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, 28, 350–356. <https://doi.org/10.1021/ac60111a017>
- Bates, L.S., Waldren, R.P., & Teare, I.D. (1973). Rapid determination of free proline for water-stress studies. *Plant and Soil*, 39, 205–207. <https://doi.org/10.1007/BF00018060>
- Lowry, O.H., Rosebrough, N.J., Farr, A.L., & Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, 193, 265–275.
- Lichtenthaler, H.K., & Wellburn, A.R. (1983). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochemical Society Transactions*, 11, 591–592. <https://doi.org/10.1042/bst0110591>
- Weatherley, P.E. (1950). Studies in the water relations of the cotton plant. I. The field measurement of water deficits in leaves. *New Phytologist*, 49, 81–97. <https://doi.org/10.1111/j.1469-8137.1950.tb05146.x>
-

- Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant Physiology, 24, 1–15. <https://doi.org/10.1104/pp.24.1.1>
- Adrees, M., Khan, Z.S., Hafeez, M., Rizwan, M., Hussain, K., Asrar, M., et al. (2021). Foliar exposure of zinc oxide nanoparticles improved the growth of wheat (*Triticum aestivum* L.) and decreased cadmium concentration in grains under simultaneous Cd and water deficient stress. Ecotoxicology and Environmental Safety, 208, 111627. <https://doi.org/10.1016/j.ecoenv.2020.111627>
- Ajouri, A., Asgedom, H., & Becker, M. (2004). Seed priming enhances germination and seedling growth of barley under conditions of P and Zn deficiency. Journal of Plant Nutrition and Soil Science, 167, 630–636. <https://doi.org/10.1002/jpln.200420425>
- Basit, F., Asghar, S., Ahmed, T., Ijaz, U., Noman, M., Hu, J., et al. (2022a). Facile synthesis of nanomaterials as nanofertilizers: A novel way for sustainable crop production. Environmental Science and Pollution Research, 29, 51281–51297. <https://doi.org/10.1007/s11356-022-20950-3>
- Basit, F., He, X., Zhu, X., Sheteiwy, M.S., Minkina, T., Sushkova, S., et al. (2023a). Uptake, accumulation, toxicity, and interaction of metallic-based nanoparticles with plants: Current challenges and future perspectives. Environmental Geochemistry and Health, 45, 1–15. <https://doi.org/10.1007/s10653-023-01561-4>
- Basit, F., Nazir, M.M., Shahid, M., Abbas, S., Javed, M.T., Naqqash, T., et al. (2022b). Application of zinc oxide nanoparticles immobilizes the chromium uptake in rice plants by regulating the physiological, biochemical and cellular attributes. Physiology and Molecular Biology of Plants, 28, 1175–1190. <https://doi.org/10.1007/s12298-022-01207-2>
- Basit, F., Shahid, M., Abbas, S., Naqqash, T., Akram, M.S., Tahir, M., et al. (2023b). Protective role of ZnO nanoparticles in soybean seedlings growth and stress management under Cr-enriched conditions. Plant Growth Regulation, 100, 1–14. <https://doi.org/10.1007/s10725-023-00965-7>
- Burman, U., Saini, M., & Kumar, P. (2013). Effect of zinc oxide nanoparticles on growth and antioxidant system of chickpea seedlings. Toxicological and Environmental Chemistry, 95, 605–612. <https://doi.org/10.1080/02772248.2013.803796>
- Faizan, M., Bhat, J.A., Noureldeen, A., Ahmad, P., & Yu, F. (2021). Zinc oxide nanoparticles and 24-epibrassinolide alleviates Cu toxicity in tomato by regulating ROS scavenging, stomatal movement and photosynthesis. Ecotoxicology and Environmental Safety, 218, 112293. <https://doi.org/10.1016/j.ecoenv.2021.112293>
- Faseela, P., Sinisha, A., Brestič, M., & Puthur, J. (2020). Chlorophyll a fluorescence parameters as indicators of a particular abiotic stress in rice. Photosynthetica, 58, 293–300. <https://doi.org/10.32615/ps.2019.147>
- Fattahi, B., Arzani, K., Souri, M.K., & Barzegar, M. (2019). Effects of cadmium and lead on seed germination, morphological traits, and essential oil composition of sweet basil (*Ocimum basilicum* L.). Industrial Crops and Products, 138, 111584. <https://doi.org/10.1016/j.indcrop.2019.111584>
- García-López, J.I., Niño-Medina, G., Olivares-Sáenz, E., Lira-Saldivar, R.H., Barriga-Castro, E.D., Vázquez-Alvarado, R., et al. (2019). Foliar application of zinc oxide nanoparticles and zinc sulfate boosts the content of bioactive compounds in habanero peppers. Plants, 8, 254. <https://doi.org/10.3390/plants8080254>
- Huang, G., Ding, C., Zhou, Z., Zhang, T., & Wang, X. (2019). A tillering application of zinc fertilizer based on basal stabilization reduces Cd accumulation in rice (*Oryza sativa* L.). Ecotoxicology and Environmental Safety, 167, 338–344. <https://doi.org/10.1016/j.ecoenv.2018.10.044>
- Hussain, A., Ali, S., Rizwan, M., Ur Rehman, M.Z., Javed, M.R., Imran, M., et al. (2018). Zinc oxide nanoparticles alter the wheat physiological response and reduce the cadmium uptake by plants. Environmental Pollution, 242, 1518–1526. <https://doi.org/10.1016/j.envpol.2018.08.036>
- Iqbal, M.N., Rasheed, R., Ashraf, M.Y., Ashraf, M.A., & Hussain, I. (2018). Exogenously applied zinc and copper mitigate salinity effect in maize (*Zea mays* L.) by improving key physiological and biochemical attributes. Environmental Science and Pollution Research, 25, 23883–23896. <https://doi.org/10.1007/s11356-018-2383-6>
- Kataria, S., Jain, M., Rastogi, A., Živčák, M., Brestic, M., Liu, S., et al. (2019). Role of nanoparticles on photosynthesis: Avenues and applications. In Nanomaterials in Plants, Algae and Microorganisms, Vol. 2, pp. 103–127. <https://doi.org/10.1016/B978-0-12-811488-9.00006-8>

- Mahajan, P., Dhoke, S.K., & Khanna, A.S. (2011). Effect of nano-ZnO particle suspension on growth of mung (*Vigna radiata*) and gram (*Cicer arietinum*) seedlings using plant agar method. *Journal of Nanotechnology*, 2011, 696535. <https://doi.org/10.1155/2011/696535>
- Mittler, R. (2017). ROS are good. *Trends in Plant Science*, 22, 11–19. <https://doi.org/10.1016/j.tplants.2016.08.002>
- Noman, A., Ali, Q., Maqsood, J., Iqbal, N., Javed, M.T., Rasool, N., et al. (2018). Deciphering physio-biochemical, yield, and nutritional quality attributes of water-stressed radish (*Raphanus sativus* L.) plants grown from Zn-Lys primed seeds. *Chemosphere*, 195, 175–189. <https://doi.org/10.1016/j.chemosphere.2017.12.059>
- Pandey, A.C., Sanjay, S.S., & Yadav, S.R. (2010). Application of ZnO nanoparticles in influencing the growth rate of *Cicer arietinum*. *Journal of Experimental Nanoscience*, 5, 488–497. <https://doi.org/10.1080/17458081003649648>
- Paunov, M., Koleva, L., Vassilev, A., Vangronsveld, J., & Goltsev, V. (2018). Effects of different metals on photosynthesis: Cadmium and zinc affect chlorophyll fluorescence in durum wheat. *International Journal of Molecular Sciences*, 19, 787. <https://doi.org/10.3390/ijms19030787>
- Pullagurala, V.L.R., Adisa, I.O., Rawat, S., Kalagara, S., Hernandez-Viezcas, J.A., Peralta-Videa, J.R., et al. (2018). ZnO nanoparticles increase photosynthetic pigments and decrease lipid peroxidation in soil grown cilantro (*Coriandrum sativum*). *Plant Physiology and Biochemistry*, 132, 120–127. <https://doi.org/10.1016/j.plaphy.2018.08.037>
- Rizwan, M., Ali, S., Rehman, M.Z.U., & Maqbool, A. (2019a). A critical review on the effects of zinc at toxic levels of cadmium in plants. *Environmental Science and Pollution Research*, 26, 6279–6289. <https://doi.org/10.1007/s11356-019-04174-6>
- Rizwan, M., Ali, S., Ur Rehman, M.Z., Adrees, M., Arshad, M., Qayyum, M.F., et al. (2019b). Alleviation of cadmium accumulation in maize (*Zea mays* L.) by foliar spray of zinc oxide nanoparticles and biochar to contaminated soil. *Environmental Pollution*, 248, 358–367. <https://doi.org/10.1016/j.envpol.2019.02.031>
- Salam, A., Khan, A.R., Liu, L., Yang, S., Azhar, W., Ulhassan, Z., et al. (2022). Seed priming with zinc oxide nanoparticles downplayed ultrastructural damage and improved photosynthetic apparatus in maize under cobalt stress. *Journal of Hazardous Materials*, 423, 127021. <https://doi.org/10.1016/j.jhazmat.2021.127021>
- Sheteiwy, M.S., Shaghaleh, H., Hamoud, Y.A., Holford, P., Shao, H., Qi, W., et al. (2021). Zinc oxide nanoparticles: Potential effects on soil properties, crop production, food processing, and food quality. *Environmental Science and Pollution Research*, 28, 36942–36966. <https://doi.org/10.1007/s11356-021-14542-w>
- Singh, A., Singh, N., Hussain, I., Singh, H., Yadav, V., & Singh, S. (2016). Green synthesis of nano zinc oxide and evaluation of its impact on germination and metabolic activity of *Solanum lycopersicum*. *Journal of Biotechnology*, 233, 84–94. <https://doi.org/10.1016/j.jbiotec.2016.07.010>
- Ulhassan, Z., Yang, S., He, D., Khan, A.R., Salam, A., Azhar, W., et al. (2023). Seed priming with nano-silica effectively ameliorates chromium toxicity in *Brassica napus*. *Journal of Hazardous Materials*, 458, 131906. <https://doi.org/10.1016/j.jhazmat.2023.131906>
- Venkatachalam, P., Jayaraj, M., Manikandan, R., Geetha, N., Rene, E.R., Sharma, N., et al. (2017a). Zinc oxide nanoparticles (ZnONPs) alleviate heavy metal-induced toxicity in *Leucaena leucocephala* seedlings: A physiochemical analysis. *Plant Physiology and Biochemistry*, 110, 59–69. <https://doi.org/10.1016/j.plaphy.2016.08.022>
- Venkatachalam, P., Priyanka, N., Manikandan, K., Ganeshbabu, I., Indiraarulseli, P., Geetha, N., et al. (2017b). Enhanced plant growth promoting role of phycomolecules coated zinc oxide nanoparticles with P supplementation in cotton (*Gossypium hirsutum* L.). *Plant Physiology and Biochemistry*, 110, 118–127. <https://doi.org/10.1016/j.plaphy.2016.09.004>
- Hussain, M., Kaousar, R., Haq, S.I.U., Shan, C., Wang, G., Rafique, N., Shizhou, W., & Lan, Y. (2024). Zinc-oxide nanoparticles ameliorated the phytotoxic hazards of cadmium toxicity in maize plants by regulating primary metabolites and antioxidants activity. *Frontiers in Plant Science*, 15, 1346427. <https://doi.org/10.3389/fpls.2024.1346427>



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