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Full Length Research Paper

Zinc Oxide Nanoparticles Applied Topically Reduce Cadmium Stress and Enhance Periwinkle (Catharanthus roseus L.) Growth Performance

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Worldwide, the growth of attractive plants is restricted by the non-essential and extremely hazardous metal cadmium (Cd). A new method for acting as nano-fertilizers and directly reducing the Cd stress is the use of nanoparticles (NPs). However, little is known about how beautiful and medicinal plants, particularly Catharanthus roseus (periwinkle), defend themselves. Through the enhancement of antioxidant activities, physiological improvements, and elemental status in periwinkle plant parts under Cd stress (0.5 mM), the current experiment aims to examine the effects of zinc oxide nanoparticles (ZnO——NPs; 25 mg l-1) on the reduction of oxidative stress. One week following transplanting, the periwinkle plants were subjected to Cd stress through soil soaking, whereas ZnO——NPs were added topically 14 days later. The periwinkle plants' morphological characteristics were greatly diminished by the Cd toxicity, which also had an adverse effect on the pigments and photosynthetic apparatus, inhibited the activities of antioxidant enzymes, and decreased the accumulation of Cd. However, in Cd-stressed plants, exogenous ZnO——NPs supplementation resulted in increased plant height, flower number, root length, fresh and dry biomass, and levels of carotenoid and chlorophyll. Similarly, in Cd-polluted periwinkles, the ZnO——NPs have similarly controlled the levels of free proline, soluble protein, antioxidant enzymes (SOD, CAT, and APX), and gaseous exchange rates. The treatment of ZnO——NPs also significantly decreased the levels of malondialdehyde (—47%) and hydrogen peroxide (—25%) that were triggered by Cd stress. Additionally, ZnO——NPs supplied Cd-stressed plants with higher Zn and lower Cd levels, respectively. In metal-hoarded soil conditions, the current experiment suggests that exogenous ZnO——NPs supplements are a viable and sustainable method of improving the development characteristics and lowering the Cd levels in periwinkle plants.

Keywords: Antioxidants, Floriculture heavy, Metal hydrogen, Peroxide, Medicinal plants, Phytoremediation

INTRODUCTION

The Contamination of soil by heavy metals (HMs) has become one of the most significant worldwide environmental issues. At all pollution levels, the most harmful and poisonous heavy metals (HMs) to plants include cadmium (Cd), chromium (Cr), arsenic (As), lead (Pb), and mercury (Hg) (Angon et al., 2024). The over-standard rates of Cd, As, Cr, Hg, and Pb are 7.7%, 2.7%, 1.7%, and 1.5%, respectively, whereas the over-standard rate of soil pollution is 16.1% (Zhao et al. 2022). Cd's fluidity, water solubility, and toxicity allow it to be easily absorbed by plant roots. It can also alter the phenotypic characteristics of plants and prevent root development and growth (Zhao et al. 2021; Ahmad et al. 2022). Cd can affect plant metabolism by interfering with photosynthesis and the flow of gas and water in the plants (Naveed et al. 2020; Imran et al. 2021). Additionally, Cd can reduce plant biomass, interfere with photosynthetic pigment synthesis, and impact the antioxidative defense system by increasing the generation of reactive oxygen species (ROS) in impacted plants (Sun et al. 2023b). Through symplastic and apoplastic pathways, Cd is taken up by the plant's roots and subsequently transferred to the shoots by xylem loading, phloem redistribution, and long-distance transport (Luo and Zhang, 2021). Cd is stored in plant shoots, and plant cell walls and vacuoles undergo detoxification (Luo and Zhang, 2021). At varying concentrations, Cd has been shown in several trials to produce deadly effects on plants (Chen et al. 2020; Haider et al. 2022; Sattar et al. 2023). Reducing the buildup of Cd concentrations in plants is therefore crucial. Recently, there has been a lot of interest in using environmentally safe and sustainable additives to restore metal-contaminated soils and water supplies (Ma et al. 2020; Ajmal et al. 2022; Sun et al. 2023b). Among these techniques for purifying contaminated soil and water, nanotechnology is getting more and more popular (Rizwan et al. 2019; Mahamood et al., 2023). Nanotechnology applications are extremely important for agriculture's sustainable development (Lawry et al. 2019). Due to the worldwide zinc deficiency in agricultural soils, zinc oxide nanoparticles (ZnO——NPs) have been used as an efficient nanofertilizer (Li et al., 2021b). They can also be used in solar cells, electronics, photocatalysis, and medicine due to their optical, chemical, and biological properties (Wang et al. 2018). Tiny amounts of zinc can be obtained by crop plants from ZnO——NPs (Faizan et al. 2021b). According to a number of studies, ZnO—NPs increased growth and decreased oxidative damage brought on by abiotic stressors in rice (Faizan et al. 2021b), tomato (Sun et al. 2023b), wheat (Hussain et al. 2018), cotton (Venkatachalam et al. 2017), and chickpeas (Mahajan et al. 2011). By improving membrane integrity, promoting cell division, scavenging ROS generated by stress, and regulating concentrations of carbohydrates, protein metabolism, osmoregulation, amino acids, and photosynthetic pigments, ZnO——NPs can reduce oxidative stress, according to Verma et al. (2023). Compared to conventional ZnO fertilizers, the ZnO—NPs' smaller size, facile dispersion, and dissolution allow plants to absorb the zinc more quickly, meeting their nutritional needs and promoting growth and development (Geremew et al., 2023). According to Nazneen and Sultana (2024), the physical and chemical behaviors of ZnO——NPs vary based on a number of materials or the synthesis processes used. The dissolution of Zn2+ ions caused by ZnO——NPs has also been linked to detrimental effects, including disruption of chloroplast organization, reduction of chlorophyll concentrations, and a decrease in grana and thylakoids (Rao and Shekhawat, 2014; Mousavi et al., 2015). According to Rajput et al. (2021), ZnO——NPs may limit the movement of water and nutrients in the tissues of plant sections that are above ground. According to recent study, ZnO—NPs supplementation has significantly reduced Cd stress in a variety of crops by enhancing their growth and acting as a great alternative to prevent micronutrient deficiencies in plants (Liu et al. 2022; Sun et al. 2023b). ZnO——NPs (less than 50 mg l-1) were suggested by Hussain et al. (2024) as the best option for reducing Cd toxicity in safe farming techniques. In terms of agricultural sustainability, the use of ZnO NPs as a new technique to increase crop yields must be investigated as a potentially successful way to ensure ecological sustainability (Sun et al., 2023a). Negative environmental effects result from the unsafe discharge of ZnO——NPs contents into plant tissues and soil (Sheteiwy et al. 2021). Moreover, the bioavailability of zinc in plants increases its toxicity. The soil's pH, organic content, microbial populations, root exudate, and plant species all influence how bioavailable it is (Balafrej et al. 2020). According to Scheid et al. (2017), zinc poisoning caused a decrease in the surface area of the roots, which affects growth and lowers the capacity to absorb nutrients and water. Therefore, it is necessary to address the safety issues related to Zn supplementation in plants and soil.

Native to the Indian Ocean island of Madagascar, Catharanthus roseus, sometimes known as periwinkle, is a highly medicinal and colorful ornamental bedding and ground cover plant that is a member of the Apocynaceae family (Kaur et al. 2021). Traditional remedies for constipation, malaria, diuretics, and hypertension have used periwinkle plants (Dhyani et al. 2022). Saponins, tannins, and other anti-cancer alkaloids, such as vincristine (VCR), vinblastine (VLB), and leurosin, are found in all portions of C. roseus (Nejat et al. 2015). Because of their ability to produce a variety of colors, including red, pink, purple, and white corolla, C. roseus plants are frequently utilized as ornamental crops (Nejat et al. 2015; Ali et al. 2021). According to Pandey et al. (2007), C. roseus can withstand germination stress from Cd up to 500 mM, although greater Cd concentrations may result in less biomass and germination, promoting sterility and lowering the quantity of total alkaloids. According to Sun et al. (2023b), foliar spraying ZnO——NPs at a dose of up to 50 mg l-1 boosted photosynthetic activity and antioxidant effectiveness, which in turn improved Cd tolerance. At the same time, it reduced Cd accumulation by 41% and increased Zn uptake by 79%. Information about the uptake of ZnO——NPs in various plant parts and foliar supplementation of ZnO——NPs in medicinal and decorative plants, particularly C. roseus, under Cd-stress is not adequately reported. Through increased growth, enhanced photosynthetic apparatus, and positive regulation of antioxidative enzyme activities and Zn contents, while lowering Cd levels in plants, we anticipated that ZnO——NPs could alleviate Cd-mediated stress in periwinkles. Novel insights into the application of nanoparticles in metal-hoarded growing conditions will be provided by evaluating the impact of NPs on significant medicinal and decorative plants of C. roseus with Cd toxicity.

2. Materials and methods

2.1. Experimental setup, nanoparticle preparation, and

planting material

At the Islamia University of Bahawalpur in Pakistan, an experiment was carried out in the natural outdoor environment from the early spring to the early winter. In this study, sandy clay loam (sand 46%, silt 28%, and clay 26%) was used as the potting medium. The pH and electrical conductivity were 7.7 and 3.11 dSm-1, respectively. The soil had the following macronutrients: potassium (167.1 g kg-1), phosphorus (9.87 g kg-1), and nitrogen (87 g kg-1). The average temperature was 40°C during the day and 32°C at night, with a 75% relative humidity.

Greenview nursery provided the three-leaf stage seedlings of Catharanthus roseus cv. Titan, which were then moved to clay pots measuring 14 cm in diameter and 30 cm in depth. Because of its wide range of adaptation to the harsh climatic conditions of the Cholistan desert regions of Bahawalpur, which have a subtropical climate with mild winters (10 °C; December to February) and extremely hot summers (above 46 °C; March to September), C. roseus was chosen for this study. 3.75 kg of dirt (one pot per plant) was placed into the pots. A completely randomized design using a two-factor factorial setup was used to group the pots. The plants were placed in pots for two weeks before being exposed to Cd stress (CdCl2; 0.5 mM) in the soil. Following a week of Cd stress, plants were treated with an exogenous foliar spray of ZnO——NPs (25 mg l-1) (six sprays on alternate days). Based on our earlier research, the concentration of CdCl2 (0.5 mM) and ZnO——NPs (25 mg l-1) was chosen because of the observable differences in the effects of ZnO——NPs on various plant species. The ZnO——NPs, which were purchased from Sigma-Aldrich (St. Louis, Missouri, USA), were 30 § 10 nm in size, 99.9% pure, and had a density of 5.103. ZnO——NPs had a mean hydrodynamic size of 277.13 § 12.71 nm and a zeta potential of 3.11 § 0.72 mV.

By combining the necessary amount of NPs with double-distilled water in a 100 mL flask with 10 mL volume, stirring for two hours, and then ultrasonically agitating for 60 minutes at 40 kHz until the NPs were evenly distributed and adding double-distilled water as suggested by Zou et al. (2022), an adequate volume of NPs was prepared at 25 mg l-1. X-ray powder diffraction analysis of the materials using Cu, K1 radiation on a Rigaku diffractometer produced and characterized the expected NP morphology with proper grinding. Additionally, ZnO——NPs' size was evaluated using scanning electron microscopy (SEM; JSM5910, JEOL). To ensure even coverage and retention of the solution on the leaves, a surfactant, Tween 20 (0.05%), was added to the solution (Hofmann et al., 2020). Seven plants were reproduced four times in each treatment, for a total of 28 plants in each treatment. Four groups of pots were created: control (I) with no spray, ZnO——NPs foliar spray at 25 mg l-1 (II), Cd addition at 0.5 mM (III), and Cd addition at 0.5 mM + ZnO——NPs at 25 mg l-1 (IV).

2.2. Measurement of morphological attributes and plant biomass

In the last several days of the experiment, measurements were made of the aesthetic qualities of the plants. Eight plants were carefully removed, separated into their roots and branches, and then washed with tap water. While the overall height of the plant and the length of its roots were measured using a metric scale, the number of leaves and flowers per plant was noted. Fresh plant biomass was measured using a computerized electric balance, and the dry biomass was estimated by drying the fresh samples for 48 hours at 70 °C in an oven (Ahsan et al., 2023).

2.3. Estimation of leaf chlorophyll and gas exchange attributes

Chl-a and Chl-b contents in leaves were measured using the Takaichi et al. (1995) recommended approach. The Arnon (1949) approach was used to assess carotenoid concentrations. For this aim, a fresh leaf sample weighing approximately 0.15 g was collected and homogenized in 80% acetone. Absorption at wavelengths of 645, 663, and 480 nm was measured using a spectrophotometer (Jenway, Staffordshire, UK). To measure the gaseous exchange parameters, including photosynthetic rate (A), transpiration rate (E), and stomatal conductance (gs), an infrared gas analyzer (Li-COR 6400, Li-COR Inc., USA) was employed. These traits were measured in broad sunshine between 10 and 11 a.m., assuming that the plants were fully functioning at the time (Faizan et al., 2021a).

2.4. Determination of hydrogen peroxide (H2O2) and malondialdehyde (MDA)

To determine the H2O2 levels, 1.0 g of fresh leaf material was obtained and crushed in an ice-cooled mortar with 0.1% of 5 mL (w/v) trichloroacetic acid (TCA). The mixture was centrifuged at 12,000 £ g for 15 minutes. Following vortexing, the blend's optical density (OD) was measured at 390 nm, and the Velikova et al. (2000) method was used to calculate the H2O2 concentration. As advised by Li et al. (2010), a 0.5 g leaf sample was submerged in 1.0% trichloroacetic acid (w/v) to determine the MDA levels.

2.5. Estimation of defense enzymes

A spectrophotometer was used to measure the antioxidant enzymes, including ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD). Dixit et al. (2001) state that a fresh leaf sample (1.0 g) was centrifuged at 4 °C for 20 minutes at 12,000 rpm after being standardized in 50 mM phosphate buffer (pH 7.0) and dithiothreitol (DTT). After the supernatant was discarded, a spectrophotometer (Jenway, Staffordshire, UK) was used to record readings for antioxidant enzyme activity at various wavelengths. While Zhang et al. (2012) approach was utilized to quantify POD and CAT activities, with absorbance measured at 470 nm and 240 nm, respectively, Giannopolitis and Ries (1997) recommendations were followed to measure SOD activities, which were seen at 560 nm. The method suggested by Cakmak (1994) was used to calculate the APX value at 290 nm absorbance.

2.6. Quantification of proline and total soluble proteins

To determine the amount of free proline in periwinkle, 1.0 g of newly harvested leaves were mixed with 10 mL (3%) sulfosalicylic acid. At 90 °C, the incubation period lasted 15 minutes. After that, 4 mL of glacial acetic acid and 4 mL of ninhydrin were combined, and the mixture was heated to 90 °C for an hour. 8 mL of toluene was added once it had cooled. The absorbance was measured at 520 nm (Bates et al. 1973). A spectrophotometer was used to measure the fresh leaf sample's total protein content at 595 nm absorbance using Bradford's (1976) methodology.

2.7. Measurement of Zn and Cd levels in plants

According to Kanee et al. (2001), the dried sample (about 1.0 g per part) was digested with HNO3——HClO4 (3:1, v:v) to oxidize the organic material. This was done with the aid of a hot plate (at 550 °C for 2 hours) in order to measure the levels of Zn and Cd in the lower and upper ground parts (four samples of each part) of periwinkle plants. Following heating, 10 mL of H2O2 was added to the flask's contents, heated gradually, and refluxed for an additional hour. The watch glasses were removed from the flask tops and the heating procedure was resumed when the contents' volume had been cut in half. The digests were diluted to 100 mL with the distilled water and then filtered using filter paper (Whatman No 42). The amounts of Zn and Cd in the processed plant samples were measured using an atomic absorption spectrophotometer (iCE 3500 FAAS, Thermo Scientific, USA).

2.8. Statistical analysis

Using the full investigational data, an analysis of variance (ANOVA) was performed using STATISTIX (computer program, version 8.1). The least significant difference test was used to compare treatment means at a 5% probability level.

3. Results

3.1. Effect of ZnO-NPs on physical traits of periwinkle

When compared to unstressed plants without any spray (control), cadmium stress (0.5 mM) significantly reduced the morphological characteristics of periwinkle, such as height of the plant (HP), number of leaves (NL), number of flowers (NF), and root length (RL), by 54%, 63%, 89%, and 62%, respectively. In Cd-stressed plants, the foliar spray of ZnO——NPs demonstrated enhanced performance of these physical parameters, namely HP, NL, NF, and RL, by 23%, 31%, 36%, and 34%, respectively, while in non-stressed periwinkle plants, the corresponding results were 19%, 6%, 15%, and 25% (Fig. 1a,b,c,d).

3.2. Impact of ZnO-NPs on plant biomass

ZnO——NPs supplemented foliarly increased periwinkle fresh weight by 8% in plants under controlled conditions and by 26% in plants after Cd treatment (Fig. 2a). Similarly, foliar supplementation of ZnO——NPs increased dry plant biomass by 6% in normal-conditioned plants and by 35% in Cd-stressed periwinkle plants, despite the fact that the dry weight of Cd-stressed plants decreased by 48% when compared to the control treatment (Fig. 2b).

3.3. Influence of ZnO-NPs on pigments and gas exchange traits of periwinkle

When compared to periwinkle plants that were not treated (control), the Cd-stress considerably ($p < 0.05$) decreased the concentration of Chl-a (—103%), Chl-b (—70%), and carotenoid (—83%) contents.
Nonetheless, periwinkle plants treated with foliar spray of ZnO——NPs demonstrated improvements of 33%, 36%, and 29% in Chl-a (Fig. 3a), Chl-b

(Fig. 3b) and carotenoid (Fig. 3c) concentrations, respectively, in comparison to plants under Cd stress that were not receiving foliar supplements. Improved A, E, and gs in periwinkle leaves showed a similar pattern. Foliar supplementation of ZnO——NPs increased the levels of A, E, and gs in C. roseus plants by 12%, 21%, and 17% in non-stressed plants and by 38%, 24%, and 19% in Cd-stressed plants, respectively (Fig. 3d,e,F).

3.4. Impact of ZnO-NPs on H2O2 and MDA

MDA and H2O2 concentrations in Cd-stressed periwinkle plants were significantly ($p < 0.01$) increased by the Cd stress. When compared to control plants, the H2O2 and MDA concentrations increased by 49% and 52%, respectively. When compared to Cd-subjective non-treated (without ZnO——NPs application) periwinkle plants, foliar supplementation of ZnO——NPs significantly decreased the levels of H2O2 by 24% and MDA by 47% in Cd-stressed plants (Fig. 4a,b,c).

3.5. Influence of ZnO-NPs on antioxidant enzyme activities of periwinkle

Statistically, ZnO——NPs highly significantly ($p < 0.01$) stimulated the activities of antioxidant enzymes under controlled and Cd- stressed conditions. The Cd toxicity enhanced SOD by 30 %, while,
supplementations of ZnO——NPs further stimulated SOD by 21 % under Cd-polluted environments (Fig. 5a). The POD contents were elevated due to ZnO——NPs spray by 3 % under controlled-conditions and reduced by 31 % in Cd-stressed periwinkle plants, whereas, 34 % increment in POD level was recorded in Cd-stressed untreated plants (Fig. 5b). The activities of CAT and APX were enhanced by 32 % and by 77 % in Cd-stressed plants. The supplementation of ZnO——NPs further stimulated CAT by 17 % and APX by 20 % in Cd-toxicity conditions in comparison with untreated Cd-stressed plants (Fig. 5c,d)

3.6. ZnO——NPs influence free proline and soluble protein levels

The supplementation of ZnO——NPs significantly ($p < 0.05$) ele- vated free proline contents by 34 % in Cd-stressed plants which was already increased (35 %) in Cd-subjected without ZnO——NPs applied periwinkle plants compared with control conditioned plants (Fig. 6a). Total soluble protein level was remarkably reduced by 108 % with Cd-stressed plants. However, ZnO——NPs foliar treatment increased protein levels by 44 %, and 21 %, in Cd-stressed and without Cd-sub- jected periwinkle plants, respectively (Fig. 6b).

3.7. Effect of ZnO-NPs on contents of Zn and Cd in periwinkle plant parts

Experimental data showed that ZnO——NPs application remarkably enhanced Zn levels in periwinkle plant parts. Cd-stressed untreated plants noticeably reduced Zn contents by 53 % (in roots), 47 % (in shoots), 25 % (in leaves), and 70 % (in flowers). Foliar spray of ZnO——NPs increased Zn contents in roots of periwinkle plants by 30 % and by 58 %; in shoots by 62 % and by 77 %; in stems by 46 % and by 52 %; and in flowers by 35 % and by 62 %, in controlled-conditioned plants and Cd-stressed plants, respectively (Table 1). Cadmium drenching in soil noticeably enhanced Cd levels by 100 % (in roots), 95 % (in shoots), 110 % (in leaves), and 91 % (in flowers), compared with controlled plants. The ZnO——NPs foliar spray efficiently allevi- ated Cd stress in all examined plant parts, which showed the phytor- emediation tendency of periwinkle plants for Cd-polluted soils. The foliar application of ZnO——NPs reduced Cd accumulation by 32 % in roots, by 20 % in shoots, by 30 % in stems, and by 35 % in flowers, com- pared with Cd-stressed non-treated (without ZnO——NPs spray) peri- winkle plants (Table 1).

4. Discussion

ZnO——NPs, with their minute size (1 to 100 nm), are commonly used in the nanotechnology field (Lv et al., 2022). Due to their large specific surface area and higher solubility rate, they are effortlessly engrossed by different plat parts (Liu et al., 2022). Beig et al., (2023) found that translocation of Zn from root to above-ground parts is higher with the supplementation of $ZnSO_4$ (Zn^{++} ions) in comparison with ZnO——NPs, due to the high solubility of $ZnSO_4$ than the ZnO——NPs, that presents steady and slow availability of dissolved Zn. Subsequently, the speedy release of Zn^{2+} can positively regulate mor- phological, physio-biochemical, and nutrient uptake that finally impact the plant growth performance (Xie et al., 2022; Wang et al., 2023). The findings of this experiment presented that ZnO——NPs heightened the morphological traits and total biomass of C. roseus under Cd-polluted soil (Fig. 1 and 2). Cd-property for growth retarda- tion is also found in this study for periwinkle plants as previously documented in mung bean (Aqeel et al., 2021) and tomato (He´diji et al., 2015). The ZnO——NPs supplementation positively regulated plant length, leaf number, root length, and plant biomass of wheat in Cd-stressed conditions (Hussain et al., 2018). These results are very simi- lar to the current experiment. These findings illustrated that ZnO——NPs not only improved the growth of C. roseus but also enhanced periwinkle tolerance to Cd toxicity. Hussain et al., (2024) reported that foliar spray of ZnO——NPs on leaf surface plays a key role in preserving and defending structure of cell membrane ultimately helpful in Cd-stress tolerance, synthesis of protein, cell enlargement, functioning of the membrane by increasing antioxidant defenses and reducing oxidative damages by increasing activities of antioxidant enzymes. The effectiveness of foliar supplementation of ZnO——NPs in plants is associated with higher nitrate reductase activities and improved accumulation of proline, sugar, and amino acid contents (Hussain et al., 2024). Increased zinc levels in plants may be the cause of the increased plant biomass following foliar treatment (Table 1). Due to several mechanisms, such as Zn and Cd co-precipitation in metabolically sedentary regions and reducing the Zn deficiency, which promotes healthier plant growth, this increased Zn enrichment in various plant parts may lessen the Cd stress (Wang et al., 2023). Moreover, NP feeding that promotes the redox process and the production of photosynthetic pigments in plants may benefit plant growth by resulting in longer roots and increased plant growth (Hussain et al., 2018).

Chlorophyll levels in plants can be used to assess heavy metal-mediated stress (Wang et al., 2023). Chlorophyll, the primary component of chloroplasts, is fully linked to the pace at which plants photosynthesize. The main indicator of metal stress in plants is cadmium stress, which has a major impact on the amounts of chlorophyll in plant leaves (Rehman et al., 2018). Consequently, a change in the amount of chlorophyll may indicate how healthy the plant is and how it reacts to environmental changes. The current investigation showed that supplementing periwinkle leaves with ZnO——NPs improved photosynthetic pigments and gas exchange properties compared to plants under Cd stress (Fig. 3a-F). This decline in chlorophyll pigments may be related to the drop in methionine and cysteine concentrations, which are important components of chloroplast target proteins, during heavy metal stress (Ledda et al., 2019). Reduced plant Cd levels may be connected to periwinkles' higher chlorophyll levels (Faizan et al., 2021b). By reducing Cd stress in tomatoes, Hussain et al. (2018) also showed improved photosynthetic qualities. Furthermore, there is a correlation between reduced oxidative stress and Cd engagement with the protection of chlorophyll and total photosynthesis when ZnO——NP is treated (Rizwan et al., 2019).

The generation of excessive ROS, which causes oxidative stress to lipids, proteins, and some plant components, is the most significant effect of Cd stress (Faizan et al., 2021b). The photo-activation of photosystem II is prevented by Cd stress-induced inhibition of electron transport (Gutsch et al., 2019). Therefore, through interfering with the mitochondrial electron transport chain and the chloroplasts of leaves, Cd poisoning indirectly leads to the generation of ROS (Farooq et al., 2016). The current investigation found that the tissues

of the plants under Cd stress had higher levels of MDA and H2O2 (Fig. 4a—c). Lipid peroxidation in plants is caused by MDA, which has been considered a measure of oxidative damage (Ahsan et al., 2022). However, foliar ZnO——NPs supplementation significantly decreased MDA and H2O2 in plants exposed to Cd, protecting the integrity of the cell membrane in the process.
Furthermore, ROS-mediated damage is lessened in periwinkle plants treated with ZnO—NPs due to a decrease in Cd accumulation in plant tissues (Adrees et al., 2021). The plants have developed a well-organized defense mechanism to detoxify the ROS (Garcia-Gomez et al., 2018). In plants exposed to heavy metals, the ascorbate-glutathione (AsA, GSH) pathway, which includes four enzymes—glutathione reductase, ascorbate peroxidase, dehydroascorbate reductase, and monodehydroascorbate reductase—plays a crucial role in ROS detoxification (Hasanuzzaman et al., 2019). The AsA-GSH pathway protects plants from Cd-mediated damage and interacts with the plant defense system (Hasanuzzaman et al., 2017). Zn-induced plant enzymes are in charge of protein synthesis, auxin production control, carbohydrate metabolism, and cellular membrane integrity (Jabri et al., 2022). Antioxidant enzymes found in the majority of plant species prevent ROS from building up. In the current experiment, foliar spraying ZnO——NPs resulted in increased SOD and CAT activities but decreased POD concentrations in C. roseus leaves (Fig. 5a-d). These findings support the findings of Mahamood et al. (2023), who reported that SOD converts O_2^- to less lethal H2O2, making it the primary defense mechanism in plants' antioxidant mechanism. POD catalyzes H2O2 (Su et al., 2019), while CAT scavenges H2O2 (Rizwan et al., 2019). Similarly, in several plants under abiotic stress, additional antioxidant enzymes as APX also serve as ROS scavengers (Khoshbakht et al., 2018). However, ZnO——NPs react differentially to antioxidative enzymatic activities depending on the kind of plant, soil type, amount of NPs, and exposure duration (Faizan et al., 2021b).
Solutes like proline are accumulated by plants under a variety of abiotic stressors (Srivastav et al., 2021). According to Li et al. (2021b), proline helps with memory stability, smooth water absorption, and the removal of excess ROS. Following ZnO——NPs supplementation, the current experiment showed increased levels of free proline in plants exposed to Cd (Fig. 6a). Similarly, ZnO——NPs foliar supplementation was found to increase the level of free proline in cucumbers (Li et al., 2021a). Stress tolerance has been linked to pro-line accumulation under abiotic stress in several plant species. Stress-tolerant plants have been showing higher proline concentrations than stress-sensitive plants (Zahedi et al., 2023). ZnO——NPs have a large potential to raise proline level mediated expression of genes for proline biosynthesis, according to Faizan et al. (2021b). The concentrations of soluble proteins in plants vary depending on the species (Kaur et al., 2017). This investigation found that plants under Cd stress had a much lower amount of total soluble protein (Fig. 6b). The results of Faizan et al. (2021b), who demonstrated a decrease in protein synthesis in wheat under Cd toxicity, are supported by these findings.
Increased denaturation of proteins under abiotic stress is the result of increased protease activity, which breaks down the structure and activity of the protein (Chanu and Upadhyaya, 2019). Reduced protein synthesis, impaired photosynthetic protein, and nucleic acid and cell membrane disintegration are all examples of Cd-linked DNA damage (Abbas et al., 2018). In extreme situations, oxidative stress brought on by Cd toxicity can harm lipids, proteins, and nucleic acids, which can stop cell growth or even cause cell death (Loix et al., 2017). Therefore, as this study has shown, the administration of ZnO——NPs improves the Cd-mediated degradation of protein in the periwinkle plants. According to Bala et al. (2019), this increase in soluble protein levels may be one of the main ways whereby NPs exacerbate Cd-stress damage.
Because Cd and some other divalent ions are difficult for plants to absorb, stress caused by Cd toxicity in plants can lead to nutritional deficits (Konate et al., 2017). The absorption of roots and the successful transportation and distribution of nutrients in plants can be impacted by the presence of Cd ions in the soil (Haider et al., 2021). N, Ca, Mn, K, P, B, and Zn levels are significantly decreased by cadmium stress, which also affects how these elements are stored and used as well as how much water plants absorb (Kinay, 2018; Zhang et al., 2019). The results of the current investigation showed that under Cd-stressed conditions, Zn accumulation significantly decreased in all plant sections studied (Table 1). Nevertheless, ZnO——NPs supplementation significantly restored this decrease in Zn levels in Cd-stressed periwinkle plants, and comparable findings were observed in wheat (Rizwan et al., 2019) and tomatoes (Sun et al., 2023b). Although they both negatively impact crop growth and development, toxicity and deficiency rarely occur (Suganya et al., 2020). In comparison to control plants, it has been demonstrated that ZnO——NPs increase the bioavailability of Zn in the soil and its concentrations in various plant sections (Munir et al., 2018). According to Saleem et al. (2022), plants may absorb zinc through two different methods. Mechanism-I includes organic acids, H+ ions, and sol-vent efflux, which improve the absorption of Zn-complexes and the release of Zn2+ ions for the absorption of root epidermal cells. On the other hand, mechanism-II entails the release of phytometallophones, which generate complex compounds with zinc and are then absorbed by the roots' epidermal cells. According to Kareem et al. (2022), plants supplemented with ZnO——NPs had a more heterogeneous distribution of Zn ions than plants sprayed with ZnSO4. Following foliar spray, ZnO——NPs enter the leaf through the stomata, aggregate and release Zn-ions in the apoplast, and then enter the mesophyll cells to be absorbed (Sperdouli et al., 2022). Zn has been found to have a negative influence on plants' ability to absorb Cd and to mitigate the negative effects of Cd on plants (Qaswar et al., 2017). Adding ZnO——NPs to the soil may limit the quantity of Cd present and decrease the amount that plants can absorb from it, claim Hussain et al. (2018). In Cd-stressed soils, Zn2+ supplementation increased the energy fraction used for photochemistry (FPSII), maximized PSII efficacy, decreased lipid peroxidation, and shown decreased ROS production (Sperdouli et al., 2022). Hazardous metals

like Cd are immediately exposed to plant roots, where they are more concentrated than in any other area of the plant (Faizan et al., 2021b). According to Ras-saei et al. (2020), Zn increased the iron and magnesium oxides as well as the water-soluble and exchangeable forms, while decreasing the Cd levels in the fractions of carbonate and organic matter in sandy clay loamy soils. Remaining forms were unaffected. Undoubtedly, under Cd stress conditions, the current experiment likewise discovered that periwinkle roots had the highest Cd concentrations when compared to other above-soil plant components (Table 1). ZnO——NPs supplementation reduces the accumulation of Cd in all plant tissues. Moreover, vascular plants' root apoplast, which is thought of as a natural barrier, inhibits the uptake of Cd (Siemianowski et al., 2014). In tobacco plants, lignification of the roots (by NaCl) may also significantly reduce the absorption of Cd (Yang et al., 2020). The morphological and physiological changes brought on by an increase in several antioxidant enzymes, particularly POD, also affected the effectiveness of apoplastic transport for heavy metal ions and macromolecules, which in turn inhibited the absorption of Cd (Yang et al., 2024). According to the current findings, ZnO——NPs slowed down the transportation of Cd in the root-shoot direction. As previously reported by Bhuyan et al. (2020), ZnO——NPs then had a parallel effect on the movement and absorption of Cd in C. roseus. According to Chai et al. (2024), ZnO——NPs activated the expression of genes linked to stress signal transduction, which in turn supported the expression of downstream target genes associated with cell wall production, secondary metabolite synthesis, and metal transport via transcription factors. The activation of this molecular process helped plants become more resistant to Cd toxicity. According to all of the experiment's data, ZnO——NPs undoubtedly restore Cd-mediated stress tolerance in C. roseus by controlling a variety of phenotypic and biochemical characteristics.

5. Conclusion

In the present investigation, we examined how ZnO——NPs improve Catharanthus roseus growth performance under Cd stress. Our experiment's results showed that ZnO——NPs might be crucial in improving Cd tolerance in C. roseus plants. ZnO——NPs-induced improvements in physiological mechanisms through the enhancement of photosynthetic pigments and rates of gaseous exchange were found to be associated with positive regulatory effects of foliar-supplied ZnO——NPs on morphological traits (plant height, number of leaves and flowers, root length) and plant biomass. By decreasing hydrogen peroxide and malondialdehyde levels, which increased antioxidant enzymatic activities, increasing zinc accumulation, and decreasing Cd uptake and accrual in both above- and below-ground plant parts, foliar supplements of ZnO——NPs decreased oxidative stress. Total soluble protein levels were sharply reduced in plants under Cd stress; however, these negative effects may be mitigated by supplementing with ZnO——NPs. Therefore, ZnO——NPs applied exogenously to the leaves prevents the deadly effects of Cd stress and allows periwinkle plants to survive under stress. Since the effect of ZnO——NPs is primarily studied in restricted or controlled conditions, it is strongly recommended that future study consider the role of ZnO——NPs against Cd toxicity in field settings. To investigate and uncover the processes behind ZnO—NPs-mediated increased tolerance to Cd toxicity in plants, a comprehensive molecular approach is required. Furthermore, because of their possible uses in the food, pharmaceutical, and cosmetics industries, research on plant-based nanoparticles is expected to grow significantly in the future.

Declaration of competing interest

The authors affirm that none of the work described in this publication may have been influenced by any known conflicting financial interests or personal ties.

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