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Foliar application of Zinc oxide nanoparticles alleviates cadmium toxicity in purslane by maintaining nutrients homeostasis and improving the activity of antioxidant enzymes and glyoxalase system

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Abstract

Cadmium (Cd) reduces plant growth by interfering with important plant metabolic processes at the physiological, biochemical, and molecular levels. Here, the effects of foliar application of zinc oxide nanoparticles (ZnO-NPs) on growth, antioxidant enzymes, glyoxalase system, and macro- and micro-elements levels of purslane (*portulaca oleracea* L.) under Cd toxicity were investigated. The results revealed that Cd toxicity increased the levels of hydrogen peroxide (H_2O_2), methylglyoxal (MG) and malondialdehyde (MDA), resulting in oxidative stress and the induction of electrolyte leakage (EL). Cd stress enhanced the leaf concentration of Cd and declined the leaf concentrations of macro- and micro-elements, resulting in a decrease in the content of photosynthetic pigments and plant growth. However, the foliar application of ZnO-NPs improved the activity of antioxidant enzymes and the glyoxalase system and, consequently, reduced the levels of H_2O_2, MG, MDA, and EL in Cd-stressed plants. ZnO-NPs decreased the leaf concentration of Cd and restored the leaf concentrations of macro- and micro-elements, thereby improving photosynthetic pigments and the growth of Cd-stressed purslane plants. In general, the results revealed that the foliar application of ZnO-NPs improved the growth of purslane plants under Cd phytotoxicity by maintaining nutrient homeostasis, improving the defense mechanisms (antioxidant enzymes and glyoxalase cycle), and increasing the accumulation of proline and glutathione. Therefore, the results of the present study strongly recommend that ZnO-NPs could be used effectively in the cultivation of plants in areas contaminated with toxic Cd metal.

Keywords Zinc-oxide nanoparticles · Purslane · Cadmium toxicity · Foliar application · Essential elements · Oxidative stress

Introduction

In recent years, serious concerns have been raised about the negative effects of environmental pollutants, including heavy metals, on plant quality and yield (Ghorbani et al. 2022). Cadmium (Cd) is known as one of the most important environmental pollutants that is released into ecosystems through metal working industries, power stations, heating systems, urban traffic, waste incinerators, cement factories, and phosphorus fertilizers (di Toppi and Gabbrielli 1999; Nolan et al. 2003). Cd is easily absorbed by the plant and accumulates in different parts of the plant, which not only has adverse impacts on plant growth and development but can also have toxic effects on the organisms that consume them (Hasan et al. 2009; Liu et al. 2007). Cd, as an anti-metabolite, inhibits the activity of enzymes and disrupts the function of biochemical and physiological processes in plants (Stroin´ski 1999). It has been shown that cadmium stress induces oxidative stress by increasing the generation of active oxygen species and thus diminishes plant growth and yield (Rizwan et al. 2016; Ali et al. 2015).

Nanotechnology as an emerging discipline is known as an ideal solution to overcome the adverse effects of heavy

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metals in agriculture. Recently, the application of zinc oxide nanoparticles (ZnO-NPs) has been shown to improve plant nutrition, which has been considered to increase plant growth and yield (Rizwan et al. 2019; Faizan et al. 2021). Zinc is one of the most important essential micronutrients that is involved in the activity of important enzymes such as superoxide dismutase, transphosphorylases, isomerases, aldolases, tryptophan synthetase, dehydrogenases, and RNA and DNA polymerases (Sturikova et al. 2018; Broadley et al. 2007). ZnO-NPs are widely used in biomedical and catalysis applications as well as in agriculture as part of sunscreens, solar cells, ceramics, and wall paints (Santhoshkumar et al. 2017; Suliman et al. 2007). Rizwan et al. (2019) and Singh et al. (2019) revealed that the application of ZnO-NPs has positive impacts on the physiological, nutritional, and quantitative parameters of maize and wheat plants. The application of ZnO-NPs has also been shown to effectively improve plant tolerance to abiotic stresses, especially heavy metal toxicity. Faizan et al. (2021) indicated that the foliar application of ZnO-NPs improved the tolerance of tomato plants to Cd toxicity. Ahmad et al. (2020) revealed that ZnO-NPs modulated the activity of antioxidant enzymes, the glyoxalase system, and the ascorbate-glutathione cycle and reduced arsenic (As) accumulation in the roots and leaves of soybeans, thereby improving plant growth under As stress. The decreased Cd accumulation induced by the application of ZnO-NPs has also been reported in *Triticum aestivum* (Hussain et al. 2018) and *Gossypium hirsutum* (Venkatachalam et al. 2017).

Although several studies have shown that the application of ZnO-NPs can reduce the accumulation of heavy metals, especially Cd, in plants, more research is needed to better understand the role of ZnO-NPs in improving plant tolerance under Cd toxicity. Therefore, in the present study, the effect of foliar application of ZnO-NPs on Cd uptake as well as photosynthetic apparatus, antioxidant defense system, and ionic homeostasis in purslane (*portulaca oleracea* L.) as a model medicinal plant under Cd phytotoxicity was investigated.

Material and methods

Materials and treatments

Purslane seeds germinated after sterilization with mercuric chloride (0.01%) and washing with double distilled water (DDW) (Ghorbani et al. 2019b). Then, 10-day-old purslane seedlings were transferred to pots containing half-strength Hoagland solution (pH 6.0). Every 5 days, the pots were refilled with fresh Hoagland solution. The pots were retained in a growth chamber at 25 °C, 16 h light (150 µmol m^{-2} s^{-1}) and 65–70% relative humidity. Cd treatments and foliar sprays of ZnO-NPs were applied to 20-day-old purslane seedlings (after 10 days of adaptation). Cd treatments were prepared at concentrations of 50 and 100 µM by adding $CdCl_2$ to 1/2-strength Hoagland solution. ZnO-NPs were purchased from USA-Nano with properties of 20–30 nm size, 99% purity, and 5.61 g/cm^3 density and were prepared in concentrations of 50 and 100 mg/L. Plant foliage was sprayed twice (before treatments and 7 days after the start of treatments) with ZnO-NPs. The control plants were sprayed with DDW. 34-day-old seedlings (2 weeks after the start of treatments) were sampled and stored at −80 °C after recording the plant height. To determine the total dry weight, the samples were incubated for 24 h at 70 °C and then weighed (Ghorbani et al. 2009).

Photosynthetic pigments

The contents of chlorophyll *a*, *b*, and carotenoids were determined following the procedure of Arnon (1949). After homogenizing the fresh leaves in 80% ice-cold acetone and centrifuging at 5000 rpm for 10 min, the supernatants were read spectrophotometrically at 470, 645, and 663 wavelengths and calculated as mg/g FW.

Proline, glutathione (GSH) and electrolyte leakage (EL)

To determine the leaf content of free proline, leaf tissues were extracted with sulphosalicylic acid (3%, v/v). After centrifugation, the supernatants were mixed with a ninhydrin solution. The mixtures were incubated in a hot water bath (90 °C) for 30 min and then transferred to an ice bath. Toluene was added to the samples and vortexed for 15 s. After incubating the mixtures in the dark (room temperature) for 30 min, the absorbance of the upper phase was read at 520 nm and the proline content was calculated following the procedure of Bates et al. (1973). Extraction of total GSH was performed using meta-phosphoric acid (6%, pH 2.8) containing EDTA (1 M), and after centrifugation, polyvinylpolypyrrolidone was added to the supernatants and centrifuged at 10,000 rpm for 15 min. The supernatants were mixed with potassium phosphate buffer (0.1 M, pH 7.0), EDTA (5 mM), GR (20 IU mL^{-1}), 5,5′-dithiobis(2-nitrobenzoic acid) (2.4 mg mL^{-1}) and NADPH (1.9 mg mL^{-1}). By recording the absorbance change at 412 nm, the leaf content of GSH was achieved according to Yu et al. (2003). To determine EL, the leaf pieces were immersed in DDW for 24 h. After recording the electrolyte conductivity (C1), they were boiled for 30 min. After recording the electrolyte conductivity (C2) of the cooled solution, EL was obtained according to Huo et al. (2016) and the following equation: $EL = C1/C2 \times 100$.

Hydrogen peroxide (H_2O_2), lipid peroxidation and methylglyoxal (MG)

After extracting the leaves in 0.1% trichloroacetic acid and centrifuging at 10,000 rpm for 20 min, the supernatants were mixed with 1 M potassium iodide and 10 mM potassium phosphate buffer (pH 6.8). After reading the reaction mixture at 390 nm, H_2O_2 content was calculated according to Sinha et al. (2005). The method of Hodges et al. (1999) was utilised to quantify MDA content. Fresh leaf tissue was extracted with trichloroacetic acid (10%) and centrifuged at 10,000 rpm for 15 min. The supernatants were mixed with thiobarbituric acid (0.6%) in trichloroacetic acid (10%) and incubated in a hot water bath for 30 min. After placing the samples in an ice path, the mixtures were centrifuged and the absorbance of the supernatants was read at 532 and 600 wavelengths. To measure MG, fresh leaves were extracted with perchloric acid (5%) and centrifuged at 10,000 rpm for 20 min at 4 °C. Then, the supernatants were decolorized and neutralized using charcoal and saturated potassium carbonate, respectively. By reading the neutralized solution at 288 nm, MG content was determined according to the method previously described by Wild et al. (2012).

Extraction and assay of enzymes

To prepare the enzymatic extract, fresh purslane leaves were homogenized with sodium phosphate buffer (50 mM, pH 7.0), polyvinylpyrrolidone (1%) and EDTA (0.2 mM) and centrifuged at 10,000 rpm for 15 min at 4 °C. Supernatants were stored at 4 °C to measure enzyme activity.

By monitoring the increase in the absorbance of the reaction mixture, including enzyme extract, potassium phosphate buffer (pH 6.8, 10 mM), riboflavin (20 µM), $NaCa_3$ (1.5 M), distilled water, nitro blue tetrazolium (750 µM), EDTA (3 mM) and methionine (0.2 M) at 240 nm, the activity of the superoxide dismutase (SOD) enzyme was calculated as per Beauchamp and Fridovich (1971). Catalase (CAT) activity was obtained by measuring the reduction in absorbance of the reaction solution, which consisted of potassium phosphate buffer (100 mM, pH 7.0), enzyme extract, and H_2O_2 (75 mM) at 240 nm as per the method proposed by Aebi (1984). By recording the change in absorbance of the mixture including potassium phosphate buffer (pH 6.8, 10 mM), enzyme extract, H_2O_2 (10 mM), EDTA (0.4 mM), and ascorbate (1 mM) at 290 nm, the activity of ascorbate peroxidase (APX) was calculated according to the method previously described by Nakano and Asada (1981). The activity of glutathione reductase (GR) was determined based on the Hasanuzzaman et al. (2011) method and recording the change in the absorbance of the reaction solution (enzyme solution, potassium phosphate buffer (pH 7.0, 0.1 mM), NADPH (0.2 mM), GSSG (1 mM) and EDTA (1 mM) at 340 nm.

By monitoring the decline in the absorbance of the assay mixture containing potassium phosphate buffer (pH 7.0, 100 mM), enzyme extract, MG (3.5 mM), GSH (1.7 mM) and magnesium sulfate (15 mM) at 240 nm, glyoxalase (Gly) 1 activity was measured by the method of Hasanuzzaman et al. (2011). The activity of Gly II enzyme was measured by reading the absorbance of the assay solution (Tris-HCl buffer (pH 7.2, 100 mM), enzyme solution, S-D-lactoylglutathione (1 mM) and DTNB (0.2 mM)) at 412 nm as previously published by Principato et al. (1987).

Mineral nutrients

The leaf concentrations of calcium (Ca), potassium (K), magnesium (Mg), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn) and cadmium (Cd) were achieved by ICP-MS (Agilent 7500 cx) and after acidic digestion of dried leaves in a mixture of HNO_3–H_2O (5:1). The leaf contents of nitrogen (N) and phosphorus (P) were determined according to the micro-Kjeldahl (Jackson 1967) and the molybdovanado phosphate (Kitson and Mellon 1944) methods, respectively.

Statistical analysis

The SAS software (V. 9.1.3) was employed for data analysis and the mean comparison was performed based on the least significant difference (LSD) test ($p < 0.05$) (Ghorbani et al. 2011). The values are mean ± SD and $n = 5$.

Results

Effects of Cd and ZnO-NPs on growth and photosynthetic pigments

Cd treatments statistically reduced the morphological traits of purslane plants compared to controls. At 50 and 100 µM Cd the height (9.8 and 26.1%) and the total dry weight (8.7 and 23.3%) declined compared to non-treated plants. However, at both Cd levels, foliar application of ZnO-NPs improved the height and total dry weight, and the maximum improvement was observed in plants treated with 100 mg/L ZnO-NPs (Fig. 1A, B). Chlorophyll *a* and *b* content decreased under Cd toxicity. The highest decrease in chlorophyll *a* (45.8%) and *b* (60.1%) content was observed in plants treated with 100 µM Cd. However, the application of ZnO-NPs restored the contents of chlorophyll *a* and *b* in Cd-subjected plants. The application of 50 and 100 mg/L ZnO-NPs enhanced chlorophyll *a* by 12 and 14.9% in 50 µM Cd-stressed plants and 35.2 and 43.8% in 100 µM Cd-stressed plants, respectively compared to Cd treatment alone (Fig. 1C, D). The carotenoids content was found to be

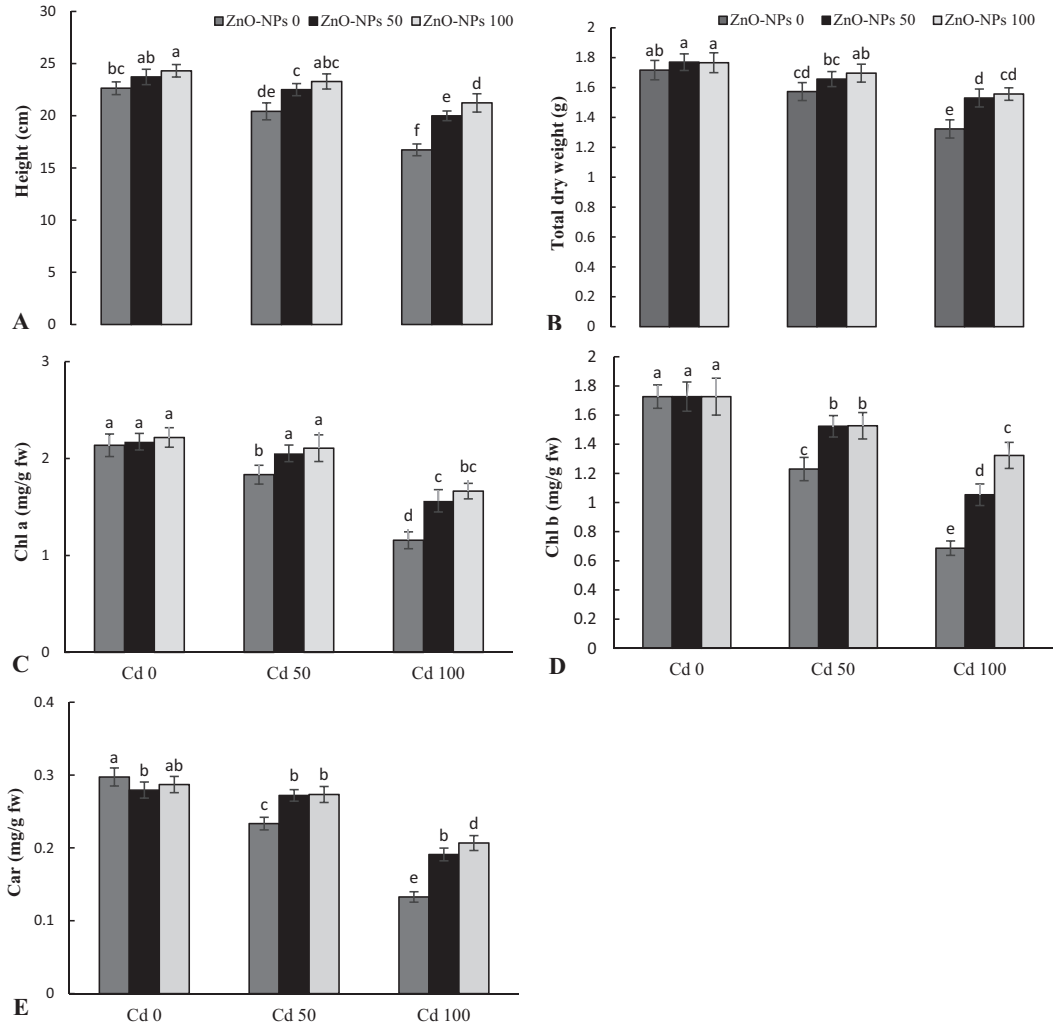

Fig. 1 Effects of zinc oxide nanoparticles (ZnO-NPs, 0, 50 and 100 mg/L) on height (**A**), total dry weight (**B**), chlorophyll (Chl) *a* (**C**), Chl *b* (**D**) and carotenoids (Car, **E**) in purslane plants subjected to cadmium (Cd, 0, 50 and 100 µM). Values (means ± SD, $n = 5$) followed by the same letter are not significantly different ($P < 0.05$; LSD test)

decreased to 21.6 and 55.2% for 50 and 100 µM Cd treatments, respectively, that the controls. The exogenous application of 50 and 100 mg/L ZnO-NPs increased carotenoids by 16.7 and 17.2% in 50 µM Cd-stressed plants and 43.6 and 55.8% in 100 µM Cd-stressed plants, respectively, compared to Cd treatment alone (Fig. 1E).

Effects of Cd and ZnO-NPs on proline and GSH contents

Cd treatment at concentrations of 50 and 100 µM enhanced leaf proline content (2.4- and 3.9-fold) compared with controls. However, the application of ZnO-NPs significantly increased the proline content in the leaves of Cd-stressed plants, and the highest increase was recorded under the treatment of 100 mg/L ZnO-NPs (Fig. 2A). The GSH content of the leaves increased by 19% under 50 µM Cd treatment, while it decreased by 20.3% under 100 µM Cd treatment. However, foliar application of 50 and 100 mg/L ZnO-NPs increased GSH content by 29.5 and 35.6% in 50 µM Cd-stressed plants and 61.7 and 66.5% in 100 µM Cd-stressed plants compared to Cd treatments alone (Fig. 2B).

Effects of Cd and ZnO-NPs on levels of H_2O_2, MG, MDA and EL

The data revealed that H_2O_2 content in purslane leaves showed a significant increase of 28 and 102.4% under 50 and 100 µM Cd, respectively, over the controls. In Cd-stressed plants, the foliar application of ZnO-NPs decreased the leaf H_2O_2 content (Fig. 2C). Treatments of 50 and 100 µM Cd raised leaf MG content by 2.2- and 29-fold, respectively, when compared with controls. Leaf spray of 50 and 100 mgL ZnO-NPs diminished leaf MG content in 50 µM Cd-stressed plants by 24.8 and 31.2%, and in 100 µM Cd-stressed plants by 33.9 and 44.8%, respectively,

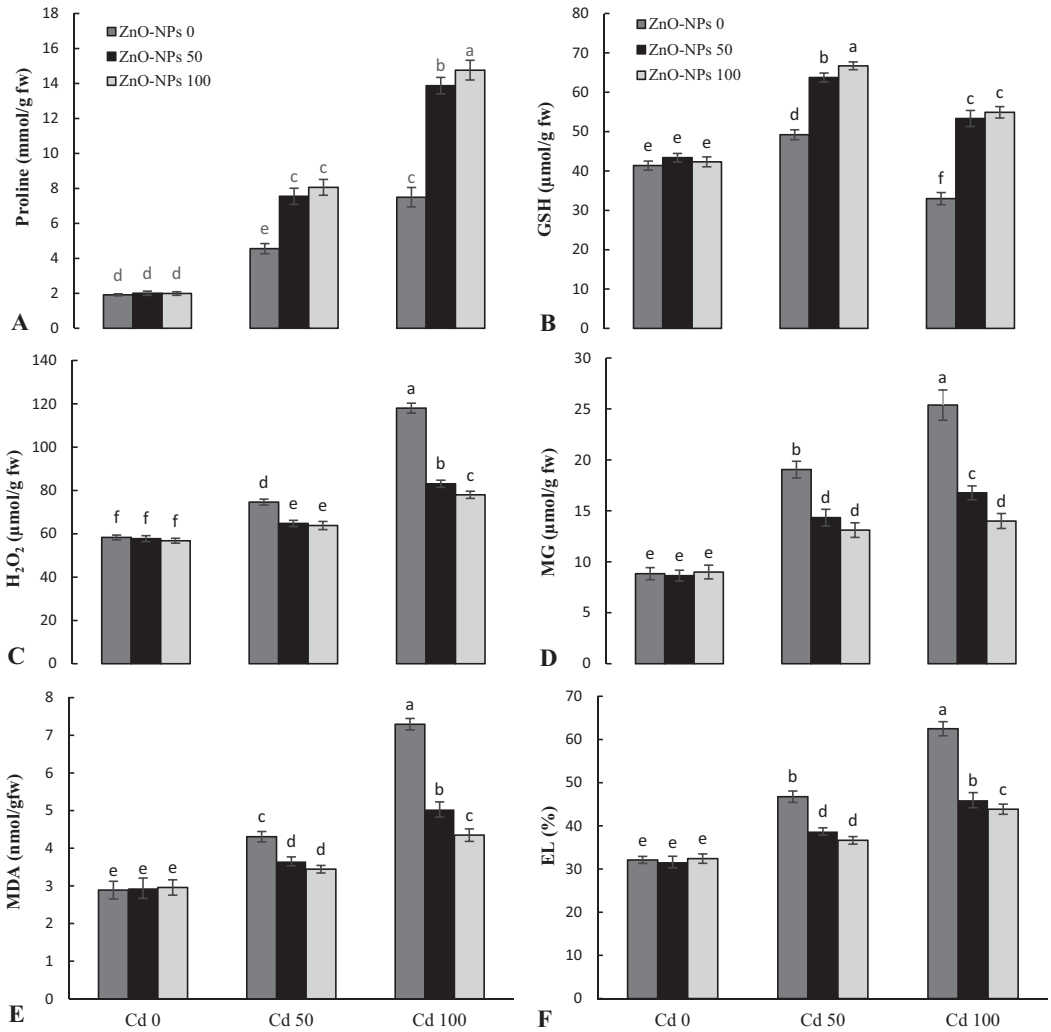

Fig. 2 Effects of zinc oxide nanoparticles (ZnO-NPs, 0, 50 and 100 mg/L) on proline (**A**), glutathione (**B**), hydrogen peroxide (H_2O_2, **C**), methylglyoxal (MG, **D**) and malondialdehyde (MDA, **E**) contents and electrolyte leakage (EL, **F**) in purslane plants subjected to cadmium (Cd, 0, 50 and 100 µM). Values (means ± SD, $n = 5$) followed by the same letter are not significantly different ($P < 0.05$; LSD test)

compared to Cd treatment alone (Fig. 2D). Treatment with 50 and 100 µM Cd led to a meaningful increase in leaf MDA content compared to the controls (49.1 and 152.3%). However, at both Cd levels, foliar application of ZnO-NPs significantly lessened leaf MDA content (Fig. 2E). Compared to untreated plants, Cd stress significantly enhanced leaf EL and the maximum EL was observed in plants treated with 100 µM Cd (94.7%). However, foliar spray with ZnO-NPs decreased leaf EL in Cd-treated plants (Fig. 2F).

Effects of Cd and ZnO-NPs on the activity of antioxidant enzymes and glyoxalase system

The results revealed that Cd treatments upregulated the activity of SOD and CAT enzymes in purslane leaves over the controls, and the highest activity was recorded in plants treated with 100 µM Cd. However, ZnO-NPs treatments caused a greater increase in SOD and CAT activity in Cd-exposed plants than Cd treatments alone (Fig. 3A, B). APX activity was found to be increased by 2.2- and 2.3-fold under 50 and 100 µM Cd toxicity, respectively, when compared to controls. In 50 and 100 µM Cd-stressed plants, foliar application of ZnO-NPs further raised APX activity, although no significant difference was found between the two concentrations of ZnO-NPs (Fig. 3C). GR activity was enhanced by 77.1% under 50 µM Cd and by 105.9% under 100 µM Cd when compared to controls. GR activity was further increased in Cd-treated plants when they were sprayed with ZnO-NPs (Fig. 3D).

The activity of Gly I and Gly II enzymes was found to be raised by 26.2 and 64.3% in 50 µM Cd-exposed plants and by 41.8 and 51.5% in 100 µM Cd-exposed plants, respectively when compared to the controls. At both levels of Cd, foliar application of ZnO-NPs further upregulated the activity of Gly I and Gly II, although no significant

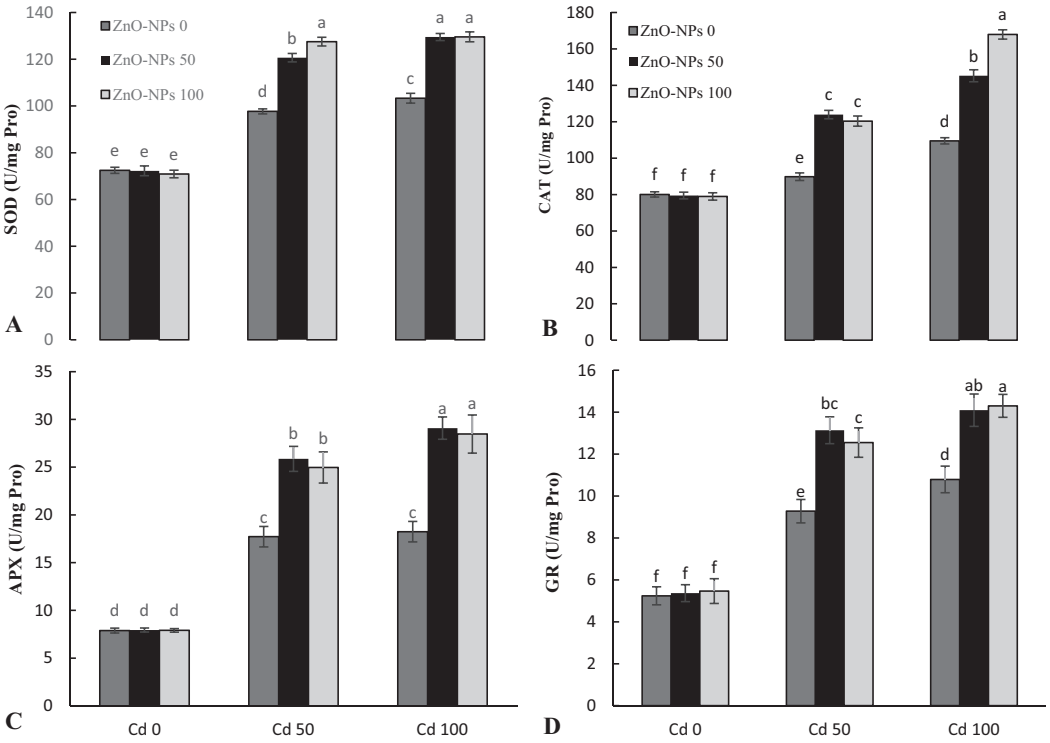

Fig. 3 Effects of zinc oxide nanoparticles (ZnO-NPs, 0, 50 and 100 mg/L) on the activity of superoxide dismutase (SOD, **A**), catalase (CAT, **B**), ascorbate peroxidase (APX, **C**) and glutathione reductase (GR, **C**) in purslane plants subjected to cadmium (Cd, 0, 50 and 100 µM). Values (means ± SD, $n = 5$) followed by the same letter are not significantly different ($P < 0.05$; LSD test)

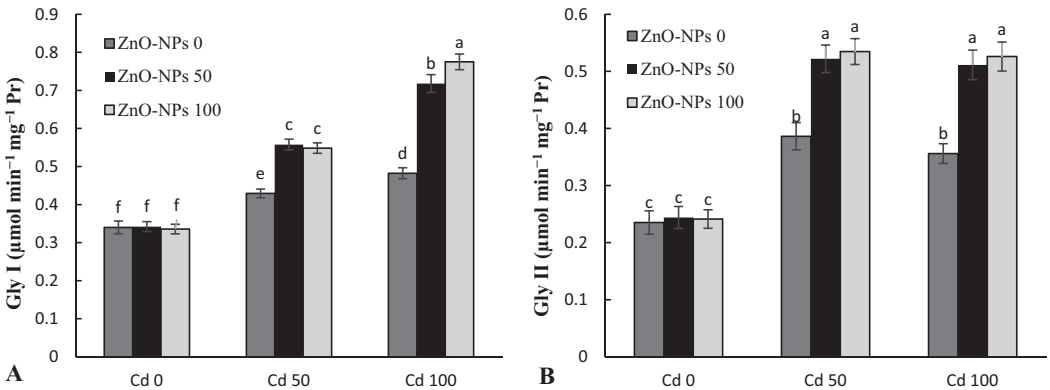

Fig. 4 Effects of zinc oxide nanoparticles (ZnO-NPs, 0, 50 and 100 mg/L) on the activity of glyoxalase 1 (Gly I, **A**) and glyoxalase II (Gly II, **B**) in purslane plants subjected to cadmium (Cd, 0, 50 and 100 µM). Values (means ± SD, $n = 5$) followed by the same letter are not significantly different ($P < 0.05$; LSD test)

difference was found between the two nanoparticle ZnO-NPs (Except for Gly I activity in 100 µM Cd-stressed plants, which had the highest activity under the application of 100 mg/L ZnO-NPs) (Fig. 4A, B).

Effects of Cd and ZnO-NPs leaf macro- and micro-nutrient concentrations

Treatments with 50 and 100 µM Cd lessened the leaf N concentration by 13 and 30.3% and the leaf Ca concentration by 5.2 and 31.9%, respectively, over the control samples. In plants stressed with 50 µM Cd, the foliar application of ZnO-NPs was found to have no significant effect on the leaf concentrations of N and Ca. However, ZnO-NPs significantly enhanced the leaf concentrations of N and Ca in plants exposed to 100 µM Cd, and the highest concentrations of N and Ca were obtained under 100 mg/L ZnO-NPs (Fig. 5A, B). Leaf P concentration diminished by 42.8% under 100 µM Cd treatment compared to the controls. However, the application of 100 mg/L ZnO-NPs

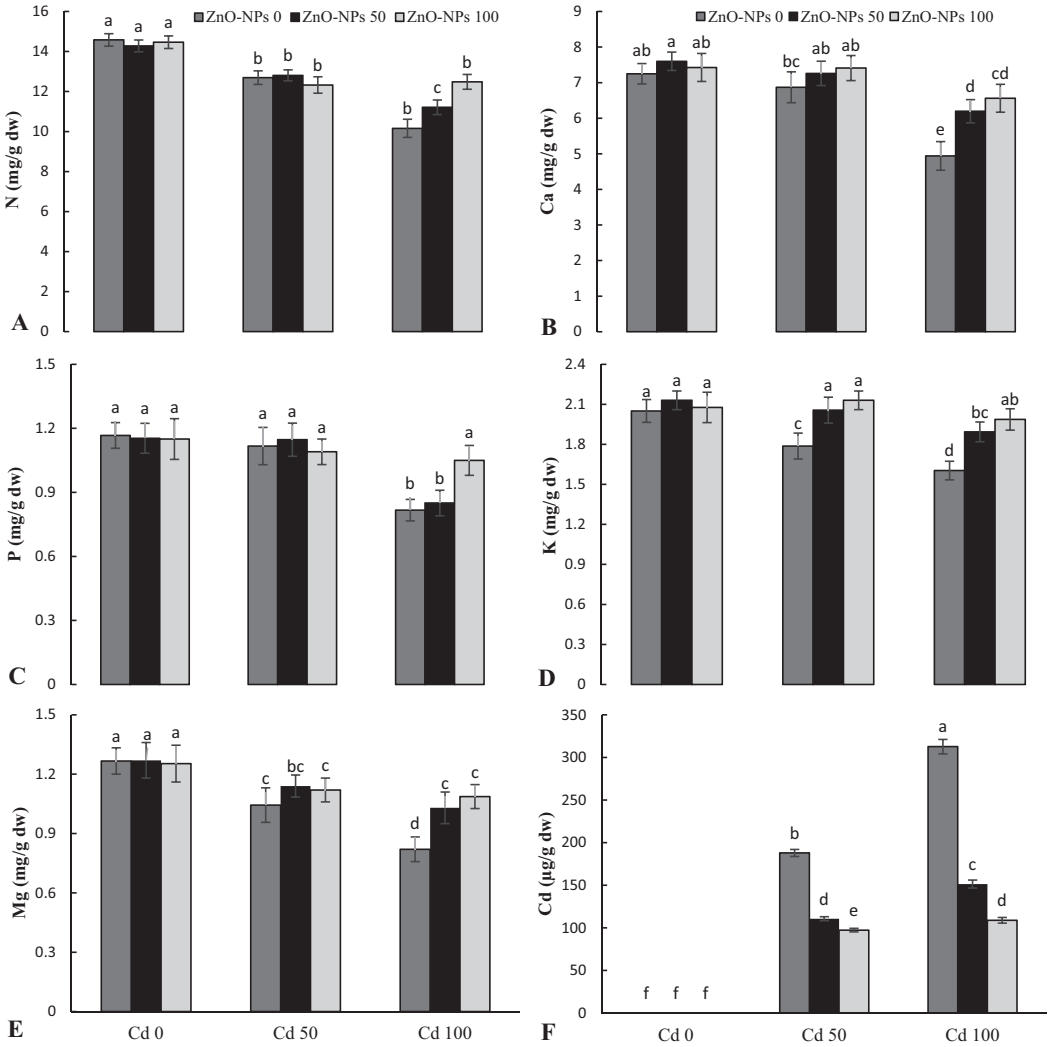

Fig. 5 Effects of zinc oxide nanoparticles (ZnO-NPs, 0, 50 and 100 mg/L) on the leaf concentrations of nitrogen (N, **A**), calcium (Ca, **B**), phosphorus (P, **C**), potassium (K, **D**), magnesium (Mg, **E**) and cadmium (Cd, **F**) in purslane plants subjected to cadmium (Cd, 0, 50 and 100 µM). Values (means ± SD, $n = 5$) followed by the same letter are not significantly different ($P < 0.05$; LSD test)

caused a significant rise in the leaf P concentration of 100 µM Cd-stressed plants compared to 100 µM Cd treatment alone (Fig. 5C). Leaf K concentration lowered with increasing Cd concentration by 12.8 and 21.8% under 50 and 100 µM Cd treatments, respectively, compared to the controls. However, treatments with ZnO-NPs restored the leaf K concentration in Cd-stressed plants, although no significant difference was found between the two levels of ZnO-NPs (Fig. 5D). Treatments with 50 and 100 µM Cd were found to diminish the leaf concentration of Mg by 17.7 and 35.3%, respectively, over the controls. In plants treated with 50 µM Cd, 50 mg/L ZnO-NPs application did not show a significant effect on the leaf concentration of Mg. However, in 100 µM Cd-plants, treatments with 50 and 100 mg/L ZnO-NPs increased the leaf concentration of Mg by 25.6 and 32.6%, respectively, compared to plants treated with 100 µM Cd alone (Fig. 5E).

Cd treatments enhanced the leaf concentration of Cd over the controls and the highest Cd concentration was obtained in plants treated with 100 µM Cd. However, in plants treated with 50 and 100 µM Cd, foliar application of ZnO-NPs diminished Cd accumulation and the lowest Cd concentration was observed in plants treated with 100 mg/L ZnO-NPs (Fig. 5F).

Treatments with 50 and 100 µM Cd significantly decreased the leaf concentrations of Fe and Mn when compared to the control samples, and the lowest concentrations of Fe and Mn were recorded in plants treated with 100 µM Cd. However, foliar application of ZnO-NPs was found to restore leaf concentrations of Fe and Mn in 50 and 100 µM Cd-stressed plants, although no significant difference was found between the two ZnO-NPs levels (Fig. 6A, B). The results also revealed that 50 µM Cd did not have a significant effect on the leaf concentration of Cu compared to the controls. However, 100 µM Cd stress

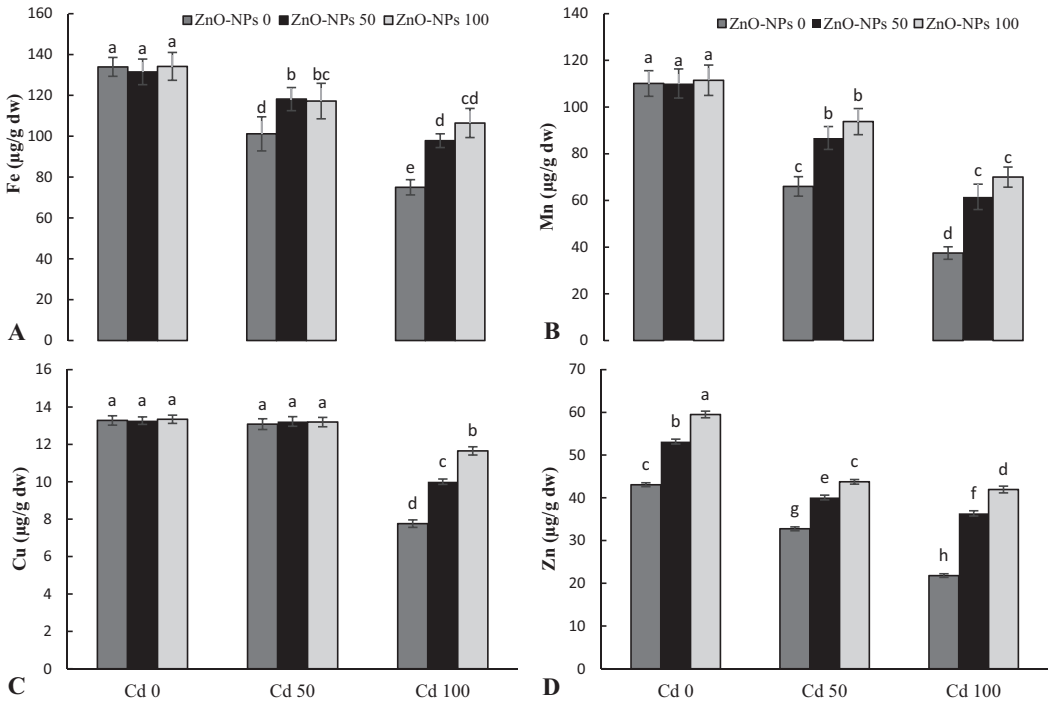

Fig. 6 Effects of zinc oxide nanoparticles (ZnO-NPs, 0, 50 and 100 mg/L) on the leaf concentrations of iron (Fe, **A**), manganese (Mn, **B**), copper (Cu, **C**) and zinc (Zn, **D**) in purslane plants subjected to cadmium (Cd, 0, 50 and 100 µM). Values (means ± SD, $n = 5$) followed by the same letter are not significantly different ($P < 0.05$; LSD test)

significantly decreased the leaf concentration of Cu by 41.6% over the controls. In control plants and 50 µM Cd-treated plants, foliar application of ZnO-NPs did not induce a significant effect on the leaf concentration of Cu. However, in 100 µM Cd-treated plants, 50 and 100 mg/L ZnO-NPs applications enhanced Cu concentration by 28.9 and 50.1%, respectively, compared to plants treated with 100 µM Cd alone (Fig. 6C). Cd toxicity significantly lessened leaf Zn concentration over the controls, and the lowest Zn concentration was observed in plants treated with 100 µM Cd. However, the application of ZnO-NPs enhanced Zn concentration in non-Cd-treated plants and plants treated with 50 and 100 µM Cd, and the maximum zinc concentration was obtained under 100 mg/L ZnO-NPs treatment (Fig. 6D).

Discussion

Although the effective role of NPs in cleaning up environmental pollution is well established, the exact role of NPs in improving the growth and yield of plants under heavy metal phytotoxicity is not fully understood (Tripathi et al. 2015). The main purpose of this study was to investigate the hypothesis that foliar application of ZnO-NPs could be employed as an eco-friendly and effective amendment to reduce the toxic effects of Cd on purslane plants with respect to mineral nutrient homeostasis and antioxidant defense systems. Improving the growth of *Triticum aestivum* (Rizwan et al. 2019) and *Leucaena leucocephala* (Venkatachalam et al. 2017) by the application of ZnO-NPs under Cd toxicity has already been reported. Sharifan et al. (2020) indicated that the application of 100 mg/L ZnO-NPs for two weeks improved the growth of *Spinaciae oleracea* under Cd toxicity by increasing the leaf concentration of Fe, Cu and Zn and diminishing the uptake of Cd. Hussain et al. (2018) showed that the leaf foliar of ZnO-NPs (50, 75, and 100 mg/L) by upregulating the activity of antioxidant enzymes and reducing oxidative stress, increased photosynthetic pigments and, as a result, improved the growth of wheat under Cd toxicity. Improving the tolerance of *Oryza sativa* (Wu et al. 2020) and *Gossypium hirsutum* (Priyanka et al. 2021) plants by ZnO-NPs under the toxicity of As and lead has also been reported, which indicates the positive role of ZnO-NPs in enhancing the tolerance of plants under the toxicity of heavy metals. Therefore, the improvement in the growth and biomass of purslane by foliar application of ZnO-NPs under Cd toxicity may be due to increased concentrations of nutrients such as Zn and reduced leaf Cd accumulation as well as an improved antioxidant defense system (Venkatachalam et al. 2017; Rizwan et al. 2019). However, most studies on the exogenous application of ZnO-NPs on plant resistance to Cd toxicity have been conducted under greenhouse or controlled conditions, and field studies are required for more realistic and practical results.

Estimation of photosynthetic pigment content can indicate the level of oxidative stress induced by heavy metal toxicity (Ghorbani et al. 2018b). The results showed that Cd stress reduced the contents of chlorophyll *a*, *b*, and carotenoids, which is consistent with the results reported by Gerami et al. (2018) in *Salvia officinalis* and Wang et al. (2014) in *Oryza sativa*. Cd stress has been reported to induce oxidative stress and damage the structure of chloroplasts and thylakoid membranes, thereby reducing photosynthetic pigments (Fagioni et al. 2009; Wang et al. 2014). However, the foliar application of ZnO-NPs restored photosynthetic pigments in Cd-exposed plants, which according to the results previously published by Hussain et al. (2018) and Venkatachalam et al. (2017) in wheat and *L. leucocephala*, respectively. Sharifan et al. (2020) reported that ZnO-NPs improve chlorophyll pigments and protect the photosynthetic apparatus from Cd toxicity by reducing the translocation of Cd to photosynthetic organs. It has been reported that increasing the content of photosynthetic pigments induced by metal NPs may be due to their ability to induce photosynthetic pigment biosynthesis, improve chemical energy production and quantum yield of the photosynthetic system (Juhel et al. 2011; Rico et al. 2015).

Proline and GSH have been shown to play an important role in reducing the damage induced by stressful conditions (Ghorbani et al. 2018a; Ghasemi-Omran et al. 2021). Therefore, increasing the accumulation of these compounds can play an important role in improving plant tolerance under Cd toxicity (Ramezani et al. 2021). However, the leaf concentration of GSH decreased under 100 µM Cd compared to non-Cd and 50 µM Cd-treated plants, which could be due to the toxic effects of Cd on GSH-synthesizing enzymes or reduced sulfur availability for GSH synthesis (Lu et al. 2019). The results revealed that the foliar application of ZnO-NPs enhanced the levels of proline and GSH in Cd-stressed plants, which is consistent with the results previously reported by Faizan et al. (2021) and Ahmad et al. (2020) under the toxicity of As and Cd, respectively. Ghorbani et al. (2021) indicated that GHS reduces the toxicity of heavy metals by chelating the toxic metal. Kaya et al. (2020) indicated that increasing proline and GSH accumulation can maintain enzyme function and redox homeostasis, improve the scavenging of toxic radicals, and protect biochemical processes. Ahmad et al. (2020) reported that the exogenous application of 50 and 100 mg/L ZnO-NPs increases the accumulation of proline under the toxicity of heavy metals by inducing the pathway of proline synthesis. Therefore, the application of ZnO-NPs increased the leaf accumulation of proline and GSH, which can play an important role in improving the tolerance of purslane under Cd toxicity.

It has been well established that the phytotoxicity of Cd boosts the accumulation of reactive oxygen species (ROS) and induces oxidative stress, resulting in damage to biomembranes and increased EL (Mittler 2002; Ghorbani et al. 2020). The results showed that Cd stress increased H_2O_2, MG, MDA, and EL in the leaves, which indicates the induction of oxidative stress and damage to membrane lipids. Increases in H_2O_2, MG, and MDA levels as well as enhanced EL have also been reported in wheat (Hussain et al. 2018), cotton (Priyanka et al. 2021) and tomato (Faizan et al. 2021) under Cd toxicity. However, ZnO-NPs upregulated the leaf activity of SOD, CAT, APX, GR, Gly I and Gly II under Cd toxicity, which was associated with decreased levels of H_2O_2, MG and MDA as well as EL, indicating the ability of ZnO-NPs to protect bio-membranes against Cd-induced oxidative stress. These results are consistent with the results previously revealed in other plants (Rizwan et al. 2019; Venkatachalam et al. 2017; Priyanka et al. 2021). Ahmad et al. (2020) reported that the foliar application of 50 and 100 mg/L ZnO-NPs for two weeks reduced the levels of H_2O_2 and MDA by upregulating the activity of CAT, SOD, APX, GR, Gly I and Gly II, thereby improving the tolerance of soybean under As toxicity. These results demonstrated that the application of ZnO-NPs increased the activity of antioxidant enzymes and the glyoxalase cycle, which could effectively help the plant to scavenge toxic radicals under Cd toxicity.

The results confirmed that Cd stress lessened the leaf concentration of macro (N, Ca, P, K, and Mg) and micro (Fe, Mn, Cu, and Zn) nutrients and increased the leaf concentration of Cd in purslane, suggesting the interference of Cd with the translocation of nutrients to the aerial part of the plant. It has been shown that Cd ions may compete with elements such as Ca, Fe, Mn, and Zn to cross the membrane (Llamas et al. 2000). Ca transporters and channels, as well as NRAMP and ZIP members that are involved in nutrient uptake, have been reported to be involved in Cd uptake (Perfus-Barbeoch et al. 2002). Cd-induced nutrient imbalances can be caused by competition between nutrients and Cd ions for binding sites in various compartments, including the cell wall and plasma membrane. Sun and Shen (2007) indicated that Cd toxicity reduced photosynthesis and, as a result, plant growth by reducing the leaf concentrations of P, Mg, S, Fe, and Mn. However, foliar application of ZnO-NPs increased the leaf concentrations of macro- and micro-nutrients and reduced the leaf accumulation of Cd in Cd-stressed plants, which could play an important role in increasing plant tolerance under Cd toxicity. The positive effects of ZnO-NPs on the nutritional parameters have been reported in several plants (Zhao et al. 2014; Kolenčík et al. 2019). However, the interaction between ZnO-NPs and Cd stress on the uptake and transport of nutrients in plants has not been well studied. Palusińska et al. (2020) indicated that the high supply of Zn in the plant could reduce the translocation of Cd from the roots to the shoots by changing the expression pattern of *ZIP* genes. Zn

has been shown to induce the synthesis of S-containing compounds including GSH and phytochelatins, therefore, increasing the endogenous concentration of Zn induced by the application of ZnO-NPs can increase the levels of phytochelatins and GSH, sequestering Cd in the vacuole and reducing its translocation to the shoots. Sharifan et al. (2020) showed that the application of ZnO-NPs by increasing the leaf concentration of Fe, Zn, and Cu improved the growth of leafy greens under Cd stress. Ahmad et al. (2020) indicated that ZnO-NPs decreased the As uptake and increased the Zn uptake in soybean plants under As toxicity. Rizwan et al. (2019) found that the application of ZnO-NPs reduced Cd uptake and enhanced Zn uptake, thereby improving the growth and yield of wheat under Cd toxicity. Although the results of the present study confirmed that the foliar application of ZnO-NPs improved the leaf concentrations of macro- and micro-elements under Cd stress, more studies at the molecular and biochemical levels are needed to better understand the role of ZnO-NPs in the uptake and translocation of nutrients to leaves (Ghorbani et al. 2019a).

Conclusions

The results revealed that the foliar application of ZnO-NPs increased the leaf concentrations of macro- and micro-nutrients and thus improved photosynthetic pigments and the growth of purslane under Cd stress. By improving the activity of antioxidant enzymes and the glyoxalase cycle, ZnO-NPs reduced the levels of toxic compounds H_2O_2 and MG, protected bio-membranes and improved plant tolerance under Cd toxicity. The results indicated that the foliar application of ZnO-NPs at appropriate concentrations (100 mg/L) could reduce Cd translocation to the leaves and improve plant height and biomass under Cd toxicity. Although the results showed that the foliar application of ZnO-NPs significantly increased purslane resistance to Cd toxicity, additional field studies and comparisons with these results are needed for more practical outcomes.

Data availability

All data generated or analyzed during this study are included in this published article.

Author contributions SY and LP conceived the idea and wrote the manuscript. LP and AI corrected the language of the manuscript. SY and LP conducted the literature survey.

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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