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Calcium oxide nanoparticles mitigate lead stress in *Abelmoschus esculentus* though improving the key antioxidative enzymes, nutritional content and modulation of stress markers

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ABSTRACT

Lead (Pb) is thought to be one of most injurious metals on the earth. Lead stress in plants enhances synthesis of highly toxic reactive oxygen species (ROS). During present research, impact of calcium-oxide nanoparticles (CaO-NPs) was observed on antioxidative defense mechanism in *Abelmoschus esculentus* plants prone to Pb stress. A CRD experiment was employed with 5 replicates having four treatments (T_0 = Control, T_1 = Pb stress (200 ppm), T_2 = CaO-NPs and T_3 = Pb + CaO-NPs). Pb-stressed seedlings exhibited decreased root growth, shoot growth, chlorophyll concentration and biomass accumulation. Moreover, higher synthesis of hydrogen-peroxide (H_2O_2), malondialdehyde (MDA) and electrolyte leakage (EL) resulting in cellular injuries were noted in plants growing in Pb spiked conditions. Similarly, stressed plants showed higher accumulation of total soluble sugar and proline content besides elevated activity of antioxidant enzymes counting catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR) and ascorbate peroxidase (APX). On the contrary side, CaO-NPs alleviated the Pb induced phytotoxicity through improving activity of antioxidative enzymes. The elevated activity of antioxidant enzymes reduced biosynthesis of H_2O_2 and MDA which was revealed through the increased growth parameters. In addition, CaO-NPs persuaded enhancement in plant defence machinery by decreased chlorophyll deprivation and augmented the uptake of plant nutrients including K and Ca content. Hence, CaO-NPs can be potent regulators of the antioxidative enzymes and stress markers to ameliorate abiotic stresses.

1. Introduction

Abiotic stresses pose major challenge to crop productivity and agricultural sustainability. In the recent, with development and industrialization heavy metal contamination has become more prominent. Rapid industrialization has increased metal pollution in various ecosystems (Ahmad et al., 2022). Various heavy metals, when exceed permissible limits, damage crop production and cause oxidative stress. These includes iron, cobalt, arsenic, mercury and lead; occurring naturally in the earth crust and/or introduced by anthropogenic activities. Lead (Pb) is produced from Earth's crust, naturally. However, some of the anthropogenic activities had speeded up the Pb contamination of the soil. Pb

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contamination sources includes battery smelters, household pipe works (Ayuso and Foley, 2020), automobile engine and exhaust from the vehicles (Borah et al., 2020). Pb toxicity in soils of district Sargodha, Sahiwal and Shahpur; ranged from 2.93 to 3.687 mg/kg (Akhtar et al., 2022). Shooting ranges are another major source of Pb contamination. In soil of Nowshera military shooting range of Pakistan, Pb concentration ranged from 1 to 193 mg/kg (Khan et al., 2021). This challenge persists worldwide, as of an estimation 10–60,000 tons of Pb is added into the environment by different shooting ranges of many countries (Bhai and Zhao, 2020).

Pb is a very common metal pollutant causing injurious effects on all living organisms (Mousavi et al., 2022). This toxic metal doesn't play any positive role in physiochemical activities of plant (Sanaei et al., 2022). Plants don't grow normal in media containing more than 30 mg/kg Pb. Synthetic fertilizers, pesticides, and fuel combustion are the major sources causing Pb contamination of soil (Saha et al., 2022). Plants growing in high Pb concentration exhibit higher levels of ROS such as hydroxyl radical, singlet oxygen, superoxide and peroxide (Mansoor et al., 2022). Higher synthesis of ROS decreases plant growth by deteriorating lipid, nucleic acids, and proteins. Higher concentration of ROS may cause cellular death (Shafi et al., 2022). Several molecular and physiochemical progressions facilitate plants to tolerate metal stress. Plants employ their antioxidant system comprising enzymatic (SOD, APX & CAT) and non-enzymatic (carotenoids, glutathione, tocopherols, ascorbic acid) antioxidants to detoxify ROS and alleviate oxidative stress (Faiz et al., 2022; Usman et al., 2023). Moreover, metal sequestering proteins for example metallothioneins (MTs) and phytochelatins (PCs) along with metal transporters exert a significant role in reducing metal imposed phytotoxic effects in plants (Kumar et al., 2022a,b; Uraguchi et al., 2022). The presence of metal ions in plants raises the activity of phytochelatin synthase enzyme which enhances the biosynthesis of PCs (Hossain et al., 2022).

Particles having a dimension of 1–100 nm are nanoparticles (NPs) (Kashyap and Shekhawat, 2022). Higher reactivity of these particles has attracted the researchers to apply NPs in field of environmental sciences and agriculture (Idrees et al., 2020; Hazarika et al., 2022). The inappropriate use of NPs may cause phytotoxicity in plants. Still, NPs are extensively used in the modern world for the improvement of crop production (Basit et al., 2022). NPs are potentially stress relivers for plants (Groppa et al., 2022).

NPs have also been potentially used in the mitigation of heavy metals toxicity (Ahmad et al., 2022). Nickle oxide nanoparticles intimates oxidative damage encountered by plants (Adeel et al., 2021). NPs in combination with other growth simulators also worked efficiently. Root supplementation of CaO-NPs along with farmyard manure (FM) effectively reduced Cd uptake, compared stressed only plants (Mazhar et al., 2023). Meanwhile, CaO-NPs application significantly enhanced plant biomass; relieved oxidative damage via improved antioxidant defense in plants given Cd stress. CaO-NPs also deprived synthesis of stress markers (MDA and H_2O_2) in these plants (Nazir et al., 2022). NPs triggered and mobilized essential elements, thus improved nutrient availability and uptake (Shakoor et al., 2022). CaO-NPs enhanced nutrient uptake, especially boosted Ca^{2+} uptake and conversely, improved growth, physiological and various biochemical attributes (Abdel-Kader et al., 2023; Farooq et al., 2023).

Okra (*Abelmoschus esculentus* L.) is a horticultural crop of Malvaceae family. Stem, foliage, flowers, pods and seeds of this nutritious plant are used as vegetables and in the pharmaceutical industry (Bencharsi, 2012). Metabolites of this commercial crop have anti-inflammatory, anti-diabetic, antidepressant and antioxidative properties (Agregán et al., 2022). Nevertheless, metal toxicity severely affects yield and quality of this crop. There is urgent need of addressing this prevailing issue. Use of nanoparticles in mitigation of oxidative stress resulted by heavy metal toxicity is a novel approach. In recent, CaO-NPs have gained attention of researchers, as growth simulators of plants. Still, no researcher has expressed the effect of CaO-NPs in mitigation of Pb induced phytotoxicity in a food crop. Similarly, the role of CaO-NPs has never been reported in *A. esculentus* plants' growth.

During the current study, it was assumed that CaO-NPs can improve plant nutrition, maintain growth related activities and alleviate Pb-induced phytotoxicity through reducing its uptake and translocation besides improving the antioxidative system of plants. The main objectives of this examination were to assess impact of Pb toxicity on growth parameters of *A. esculentus*; to examine the role of CaO-NPs in mitigation of Pb toxicity in *A. esculentus* and to scrutinize potential role of CaO-NPs application on nutritional ion uptake and accumulation in *A. esculentus*.

2. Materials and methods

2.1. Preparation of CaO-NPs

Calcium chloride hexahydrate (0.1 M) solution was prepared; stirred vigorously and continuously, using magnetic stirrer. Meanwhile, sodium hydroxide (0.1 M) solution was made and then added in flask having calcium chloride hexahydrate solution, dropwise till that of 1:1 ratio was attained. In condition of continuous stirring, alkali solution was added at 3 mL/min flow rate. Awaited 30 min more to ensure reaction is completed. Filtered (under vacuum) precipitates obtained after reaction and washed filtrate using dis. Water. Filtrate was kept in oven (105 °C) for 7 h, for drying. Dried material was crushed into fine powder and heated over 600 °C for 5 h in furnace. After heating, this material was crushed again.

2.2. FTIR spectral studies

Samples were examined by FTIR spectrometry. For this purpose, reaction was mounted and performed in bottom flask having 5 mM of Ca $(NO_3)_2$ solution (250 mL) and plant extract (27.7 mL). Heated this mixture over 80 °C to proceed further for FTIR analysis of nanoparticles. After the corresponding period, the heating was turned off and mixture was allowed to stand for 48 h. The resultant colloidal suspensions were centrifuged for 15 min at 7830 rpm in an Eppendorf Centrifuge 5430 (Eppendorf, Hamburg, Germany), and the precipitate was rinsed twice with sterile distilled water. At 45 °C, the purified pellets were dried. The KBr pellet approach was utilised to acquire FTIR spectra using the PerkinElmer Spectrum-Two instrument (PerkinElmer SCIEX, Waltham, MA, USA) by scanning in the 400 to 4000 cm^{-1} range.

2.3. XRD analysis

Phase density and size of crystal were analysed using Pan analytical, P pert pro-XRD model Py 2887 operated at 40 mA and 45 KeV with CuKα radiation (Nirmala et al., 2011). Debye Scherrer's formula ($D = k\lambda/B\cos\theta$), was used to mount crystal size (Mascolo et al., 2013). Where, D is average crystal size, k is shape parameter, λ is the X-ray wavelength (0.15406 nm), B is full width at half maximum indicated by FWHM and θ is corresponding Bragg angle (Mascolo et al., 2013).

2.4. Greenhouse experiment

The loamy soil obtained from the Agricultural Farm, University of the Punjab (N 31° 30′ 4.3236″, E 74° 18′ 5.4684″) used for the current study was analysed according to the Rohilla and Salar (2012). This soil showed 7.7 pH and 1.5 dsm^{-1} EC. Greenhouse conditions for this experiment were as follows: 30–35 °C maximum temperature during daytime and 17–20 °C lowest temperature overnight with 14/10 h light/dark period and 60–70 percent humidity level. A proper ventilation was manged for air passage during this whole experimental period. A CRD (completely randomized design) pot experiment with 5 replicates was performed for the evaluation of the capability of (CaO-NPs) to deprive Pb induced stress in *A. esculentus*. Healthy seeds (uniform size) were sterilized for 4 min in the solution of 10% sodium hypochlorite, followed by washing with dis. Water. Ten air dried seeds were sown at equidistance in pots filled with 3.5 Kg loamy soil. There were 4 treatments including Control (normal conditions), Pb stress (Soil amended with 200 ppm $PbCl_3$ solution), CaO-NPs foliar spray (50 mgL^{-1}) and Pb stress + CaO-NPs foliar spray. Each pot was weekly supplied with Hoagland's solution (Hoagland and Arnon, 1950). The experiment was conducted in collaboration with king Saud University, Saudi Arabia.

2.5. Assessment of growth attributes

The 30-d old treated seedlings were carefully uprooted from the allocated pots. Roots of seedlings were rinsed thrice with distilled water to get rid of the soil debris. Root length, shoot length, number of leaves besides fresh and dry biomass of seedlings was evaluated according to Koller (1972).

For estimation of photosynthetic content, a prewashed 100 g fresh foliage sample was extracted with 80% acetone to determine chlorophyll and total carotenoid content (Sarker and Oba, 2018a; Sarker and Oba, 2020a). OD of leaf extract was observed at 646, 663 and 470 nm to evaluate the amount of chlorophyll *b*, and chlorophyll *a*, carotenoids, respectively.

2.6. Estimation of enzymatic activities

Ball mill (MillMix20, Domel) was used to extract protein. For this purpose, a 200 mg foliage sample was extracted with 3.6 mL buffer solution including100 mM potassium phosphate (pH 7.5) and Triton X in presence of polyvinyl pyrrolidone. Followed by centrifugation (14,000 rpm, 10 min, 4 °C), the supernatant was quantified for protein content.

The phosphate buffer (50 mM) at pH 6.8 was used for the extraction of catalase (CAT). Solution was centrifuged at 2360 rpm for 45 min (4 °C). CAT activity from supernatant was measured by employing the hydrogen peroxide-dependent reduction of titanium chloride.

For the extraction of superoxide dismutase (SOD), a 50 mM phosphate buffer at pH = 7.8 was used besides 1.33 mM diethyl-diamino-penta-acetic acid. The SOD activity was assessed by estimating the O_2 induced reduction of nitroblue tetrazolium by xanthine–xanthine oxidase system. The spectrophotometric value was observed at 560 nm.

2.7. Determination of leaf proline

Leaf proline content was determined by a slightly modified method of Grieve and Grattan (1983). Fresh leaves (0.5 g) were grinded and homogenized in 3% aqueous sulfosalicylic acid. Mixture was filtered and filtrate (2 mL) was mixed with equal volume of ninhydrin reagent. 2 mL of glacial acid was added to this solution and let it stay for 1 h at 100 °C. Then this mixture was cooled and toluene (4 mL) was added to it and proline content was found out by finding the absorbance of above said reaction mixture at 520 nm by spectrophotometer (VEB Carl Zeiss). Toulene was used as a blank for this experiment.

2.8. Determination of protein content

To determine protein content of the leaves, Bradford method was employed (Brahmakshatriya and Solanki, 2022). For this purpose, fresh leaves (0.5 g) were rinsed with 10 mL of phosphate buffer of pH 7.8 and molarity 50 mM. Extract was then poured into Eppendorfs and centrifuged for 20 min at 6000 rpm at 4 °C. After centrifugation, supernatant was separated and treated as protein sample. Meanwhile, Bradford reagent prepared by dissolving Coomassie Brilliant Blue dye (100 mg) in 95% of ethanol (50 mL). Afterwards, 100 mL of 8% phosphoric acid was added to Bradford mixture and volume was raised to 1000 mL. Then 0.1 mL of protein extract sample was mixed with 5 mL of prepared Bradford solution by vortexing and absorbance was checked at 595 nm.

2.9. Determination of ion accumulation

0.1 g of ground plant sample was taken each time and was digested with a mixture of sulfuric acid and hydrogen peroxide (2 mL). Digested mixture was filtered and diluted with distilled water and was used for estimating the ionic content of leaves for calcium, sodium and potassium. A flame photometer (Jenway PFP 7) for determining ionic content of leaves by following the method of Wolf (1982). The acid mixture (without plant sample) added with dis. Water was used as a blank.

2.10. Assessment of electrolyte leakage

For the estimation of electrolyte leakage, the uppermost fully stretched foliage sample was washed thrice with distilled water. Leaf sections (1 cm) were submerged in 10 mL distilled water present in stopper vials. These vials were placed on a shaker (100 rpm) at 25 °C for 1d. Afterwards, the initial electrical conductivity (EC1) of the solution was measured. Then vials were placed in an autoclave at 120 °C for 20 min to measure EC (EC2). For evaluation of EL, EC1 was divided by EC2 and expressed as the percentage (Lutts et al., 1995). Formula of EC estimation is as follows.

2.11. Estimation of hydrogen peroxide

Tissue H_2O_2 level was analysed as suggested by Brennan and Frenkel (1977). For estimation of H_2O_2 level from frozen foliage sample, 1000 mg sample was ground along with 20 mL cold acetone. The solution was passed through filter paper and 4 mL filtrate was mixed with 2 mL of 20% titanium reagent ($TiCl_4$ in concentrated HCl, v/v) along with 2 mL of concentrated ammonia solution to precipitate the titanium-hydroperoxide complex. Solution was centrifuged at 5000 rpm for 30 min and the precipitate was mixed in 2N H_2SO_4 and the optical density was observed at 415 nm. The H_2O_2 was quantified by comparing it with the standard curve of known H_2O_2 solutions.

2.12. Estimation of lipid peroxidation

For the estimation of lipid peroxidation, malondialdehyde (MDA) content was evaluated according to Hodges et al. (1999). For this purpose, 0.4 g of frozen foliage sample was vortexed with4 mL of 80% cold ethanol with the help of a tissue homogenizer. The homogenate was centrifuged at 4500 rpm for 20 min at 4 °C. The supernatant was mixed with 20% trichloroacetic acid and incubated in a water bath at 90 °C for 60 min. Then solution was centrifuged and absorbance of the supernatant was observed 600 nm to estimate MDA content.

3. Results

3.1. XRD pattern of CaO-NPs

3.1.1. FTIR spectra

The presence of particular vibrations in metal-oxygen bonds is related with specific patterns or peaks in FTIR spectra, which are graphs that demonstrate how different substances interact with infrared light. These vibrations are detected at wave numbers ranging from 400 to 700 cm^{-1}. The occurrence of a peak at 706 cm^{-1} in the current investigation (Fig. 1, A) is a strong indicator of a specific form of vibration related to calcium oxide (CaO) that validates its production.

3.1.2. X-ray diffractogram

The crystallinity of CaO-NPs was determined using XRD patterns. Fig. 1(B) shows a diffractogram. These results support the synthesis of CaO-NPs. The obtained data was compared with standard database. Peaks at 37.8, 44.20 and 63.93 corresponding to CaO-NPs (111), (200) and (220) diffraction planes, respectively. This revealed CaO-NP production. The average crystal size of CaO-NPs was 35.75 nm.

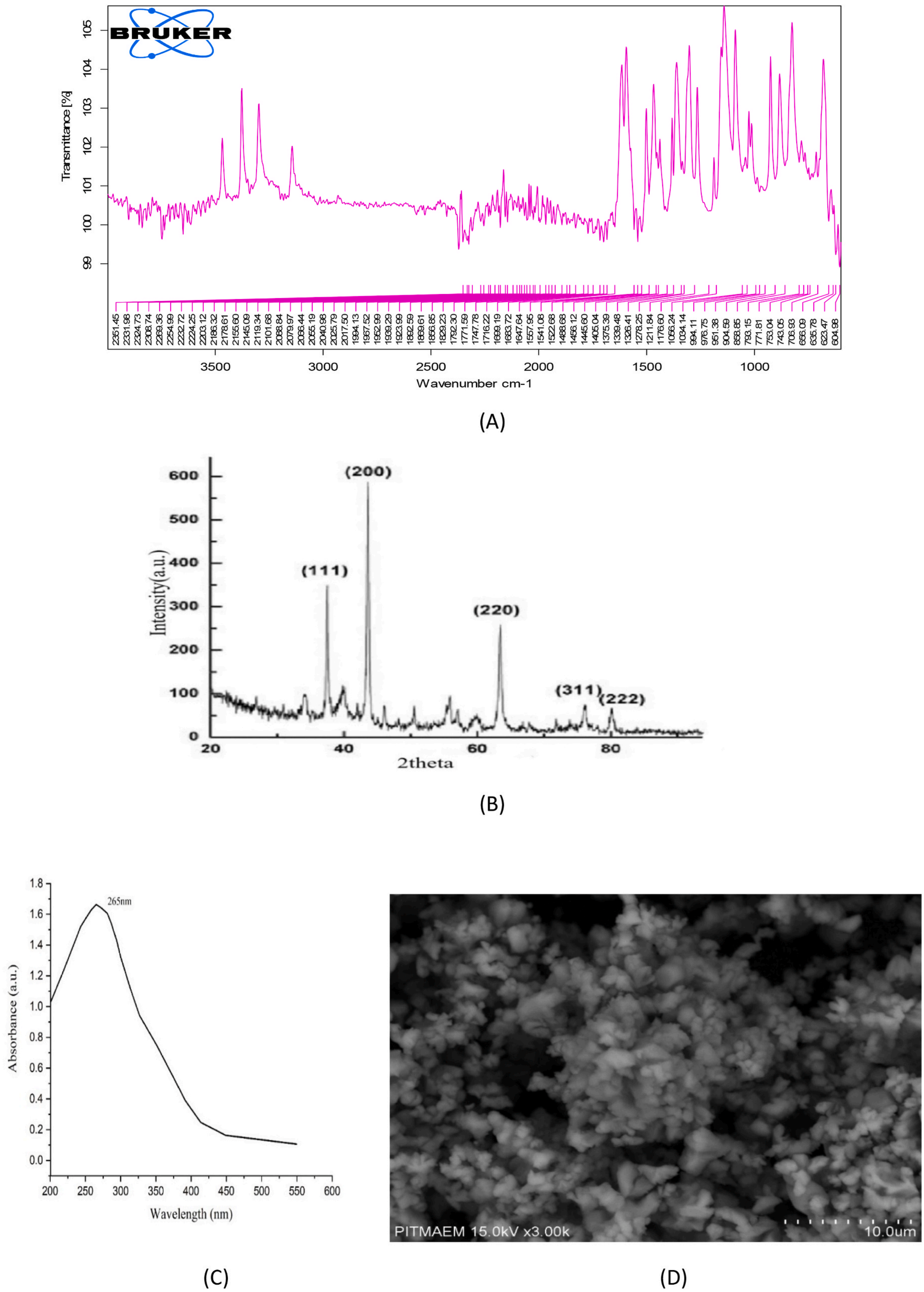

Fig. 1. (A) FTIR spectra of calcium oxide nanoparticle. **(B)** Shows a diffractogram. **(C)** shows the optical absorption spectra of calcium oxide nanoparticles measured with a UV-VIS spectrophotometer. **(D)** SEM micrograph of calcium oxide nanoparticle.

Fig. 1(C) showed measurement of optical spectra of CaO-NPs with a UV-VIS spectrophotometer in the region of 200 nm–550 nm. Calcium oxide was detected by an absorption peak at 265 nm.

3.2. Effect of calcium oxide nanoparticles on growth parameters

Pb stress posed a detrimental impact on growth of *A. esculentus*. The Pb toxicity decreased the Ln, RL, SL, RFW, RFW, RDW and SDW by 26, 23, 41, 5, 6, 17 and 9%, respectively, compared to *A. esculentus* grown in control soil. CaO-NPs foliar spray significantly improved aforementioned growth attributes in *A. esculentus* seedlings, under both control and Pb toxic conditions. *A. esculentus* seedlings supplemented by CaO-NPs and grown in Pb-toxic soil showed increased Ln, RL, SL, RFW, RFW, RDW and SDW by 80, 28, 61, 72, 64, 76 and 91%, respectively, as compared to Pb-only plants (Table-1).

3.3. Effect of CaO-NPs on chlorophyll and carotenoid content

This study also demonstrated that Pb poisoning significantly decreased the quantification of Chl content. Pb stress decreased Chl *a, b* and carotenoid content by 38, 36 and 29%, respectively, as compared to *A. esculentus* grown in non-polluted soil. In case of non-stressed conditions, supplementation of CaO-NPs raised the level of Chl *a, b* and carotenoid content by 60, 66 and 29%, respectively, in *A. esculentus* seedlings. CaO-NPs augmented Chl *a, b* and carotenoid content by 42,95 and 92, respectively, in *A. esculentus* seedlings grown in Pb-spiked soil, in contrast with Pb-only treatment (Fig. 2).

3.4. Effect of CaO-NPs on antioxidative enzyme activities

Pb toxicity decrased activity of POD, CAT and SOD enzyme by 55, 85 and 17%, respectively, in comparison with control treated *A. esculentus* seedlings. Foliar supply of CaO-NPs incremented the activity of POD, CAT and SOD by 57, 45 and 73%, respectively, in *A. esculentus* seedlings, as compared with non-stressed conditions. In case of Pb-contaminated soil, foliar spray of CaO-NPs increased activity of POD, CAT and SOD by 65, 60 and 41%, respectively, in comparison with Pb-only treated *A. esculentus* seedlings (Fig. 3).

3.5. Effect of CaO-NPs on proline and protein content

Pb stress reduced proline and protein content in *A. esculentus* seedlings grown in potted soil. Application of CaO-NPs increased proline and protein levels in *A. esculentus* in control and Pb amended soil. CaO-NPs increased levels of proline and protein by 33 and 9%, respectively, compared to control. CaO-NPs increased proline and protein value by 41 and 6%, respectively, in *A. esculentus* correspondingly to Pb-only treatment (Fig. 4).

3.6. Effect of CaO-NPs on nutritional content

Pb toxicity hindered the mineral absorption capabilities of roots and above ground parts of *A. esculentus*. Pb-stressed seedlings reduced absorption of Na, K and Ca by 53, 33 and 6%, respectively, equated to control. CaO-NPs enhanced the absorption of Na, K and Ca in *A. esculentus* by 27, 5 and 27%, respectively, in comparison with non-polluted conditions. In case of Pb-contaminated soil, application of CaO-NPs escalated Na, K, Cain the roots of *A. esculentus* plants by 82, 64 and 16%, respectively, as equated to Pb-only treatment (Fig. 5).

3.7. Effect of CaO-NPs on electrolyte leakage (EL), hydrogen peroxide (H_2O_2), and malondialdehyde (MDA)

Pb enhanced the level of EL, H_2O_2, MDA by 33, 38 and 62% respectively, in comparison with control treated seedling. CaO-NPs decrease the value of EL, H_2O_2, MDA in *A. esculentus* by 98, 73 and 77%, respectively, as equated to control. Application of CaO-NPs diminished the effect EL, H_2O_2, MDA by 53, 66 and 85% respectively, in *A. esculentus* correspondingly to Pb-only treatment (Fig. 6) (see Fig. 7).

4. Discussion

Lead (Pb) contamination of the soil is widespread issue persisting all over the world. Major sources of Pb includes batteries smelters, household pipe works, ammunitions, automobile engines and exhaust of the vehicles. Pb contaminated soils had adverse effects on plant growth and development. Various studies had revealed negative impact of Pb toxicity on crop production. Various phytoremediation approaches have been launched to cope this issue but these strategies were limited to hyperaccumulators. In this study we analysed CaO-NPs as Pb stress ameliorant in *A. esculentus.* The significant decrease in root and shoot growth confirmed the injurious effects of Pb stress on *A. esculentus* plants (Table 1). *R. pseudoacacia* (Leguminous tree) had gradually lowered growth, photosynthesis rate and exchange with increasing Pb toxicity from 0 to 2000 mg/Kg^{-1} (Yang et al., 2015). Researchers have revealed that NPs treatment may reduce uptake and translocation of Pb in plants (Ghosh and Sarkar, 2022). SiO_2-NPs (100 & 500 μM) maintained cell integrity, improved growth and physiological attributes of bamboo (*P. pygmaeus*) under varying levels (0–1500 μM) of Pb stress (Emamverdian et al., 2020). During the present research, CaO-NPs application decreased Pb accumulation and enhanced the root and shoots growth of Pb-stressed *A. esculentus* plants.

Plants subjected to stress exhibit reduction in their photosynthetic activity (Dobrikova and Apostolova, 2019). Our data revealed that Pb toxicity decreased biosynthesis of photosynthetic pigments and rate of photosynthesis in plants (Fig. 2). The decreased photosynthetic activity exhibited by Pb-stressed plants (Fig. 2) was correlated with the increased uptake and accumulation of Pb in these plants causing oxidative injuries (Bamagoos et al., 2022; Ferati et al., 2022). Pb toxicity decreased chlorophyll content in plants (Hou et al., 2018; Souahi, 2021). Raghib et al. (2020) revealed that wheat plant had lowered chlorophyll content under 100 mg/Kg^{-1} Pb stress. Cenkci et al. (2010) found that higher activity of chlorophyllase, along with lower activity and decreased biosynthesis of photosynthetic pigments through enzymes, also resulted in reduced chlorophyll accumulation in Pb stressed plants. Ultimately, there is a smaller amount of sugar level causing biomass diminution due to the poor commotion of the photosynthetic machinery in plants having higher Pb content. Accordingly, it is assumed that the decreased accumulation of Pb in CaO-NPs treated plants perhaps

Table 1
Effect of Calcium Oxide nanoparticles on growth parameters of *A. esculentus*.

Treatments	Number of leaves	Root length (cm)	Shoot length (cm)	Root fresh weight (g)	Shoot fresh weight (g)	Root dry weight (g)	Shoot dry weight (g)
C	7.6 ± 1.76b	17.76 ± 1.45b	34.6 ± 2.67bc	2.332 ± 0.08b	16.4 ± 1.76c	0.67 ± 0.03b	4.04 ± 0.04bc
CaO-NPs	12 ± 1.34a	18.88 ± 1.87 ab	43 ± 3.67b	3.19 ± 0.04 ab	20.964 ± 1.45b	1.14 ± 0.02 ab	6.872 ± 0.14b
Pb	6 ± 0.76c	15.32 ± 1.13c	30.4 ± 3.89c	2.226 ± 0.07bc	15.422 ± 1.67bc	0.57 ± 0.016bc	3.706 ± 0.07c
Pb + CaO NPs	10.8 ± 1.27 ab	19.7 ± 1.65a	49.2 ± 4.17a	3.594 ± 1.65a	25.4 ± 2.54a	1.24 ± 0.07a	7.082 ± 1.23 ab

C: control; Pb: lead; CaO-NPs: calcium oxide nanoparticles. Different letters indicate significant difference among the treatments ($P \leq 0.05$).

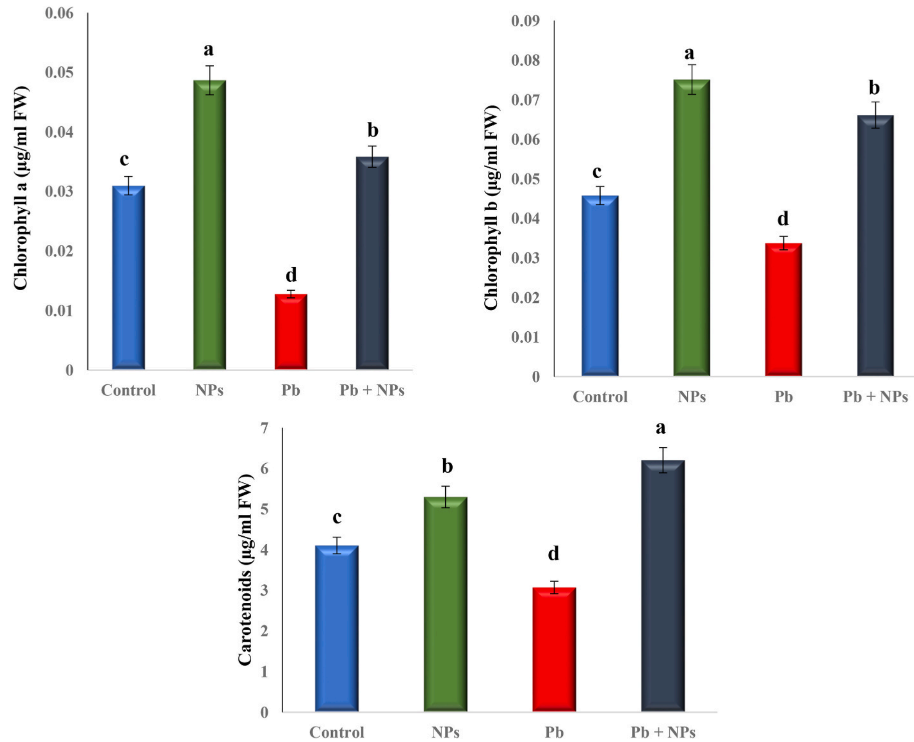

Fig. 2. Influence of calcium oxide nanoparticles on Chlorophyll *a*, chlorophyll *b* and carotenoid content under Pb-stressed soil. C: Control; Pb: Lead; CaO-NPs calcium oxide nanoparticles. Values are means ± SD of five replicates.

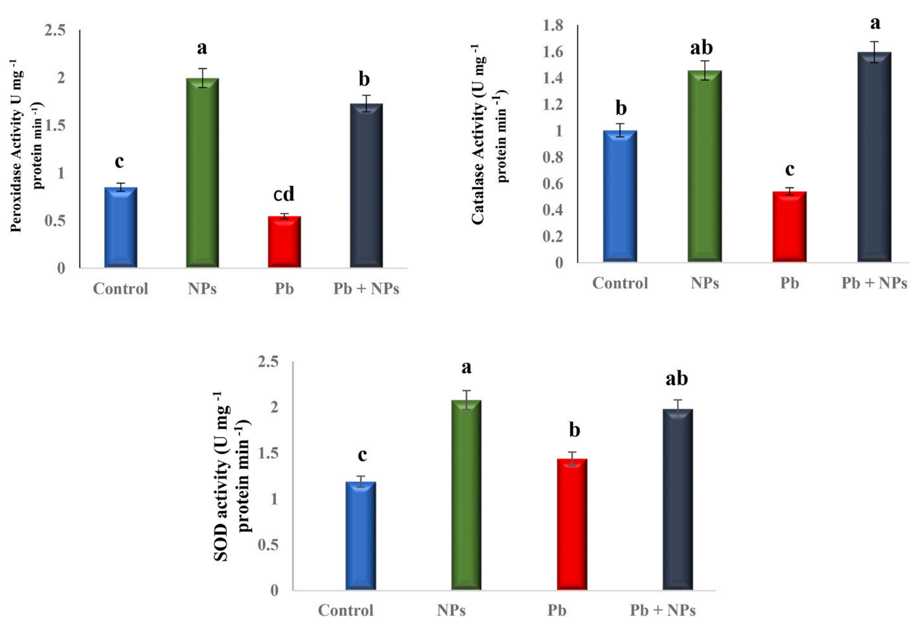

Fig. 3. Influence of calcium oxide nanoparticles on catalase and peroxidase activity under Pb-stressed soil. C: Control; Pb: Lead; CaO-NPs calcium oxide nanoparticles. Values are means ± SD of five replicates.

improved Pb stress tolerance. Likewise, application of CaO-NPs (25 mg L^{-1}) enhanced the photosynthetic activity of barley through sustaining cell homeostasis and by decreasing the degradation of chloroplast thylakoids in As-stressed (50 μM) plants (Nazir et al., 2022).

Plants exposed to Pb toxicity will exhibit higher levels of lipid peroxidation as represented by increased MDA synthesis besides augmented levels of H_2O_2, and O^{2-} content (Malik et al., 2021). Whereas, the better antioxidant system of CaO-NPs treated plants alleviates oxidative stress through scavenging the higher level of ROS in Pb contaminated conditions (Yang et al., 2021). Chen et al. (2022) studied that Pb toxicity (50 μM) significantly impaired growth, photosynthetic capacity and elevated MDA and H_2O_2 in *V. radiata* genotypes. While Ag-NPs (25 mg L^{-1}) treatment improved growth and supressed oxidative damage caused by MDA and H_2O_2 elevated levels, in *V. radiata* genotypes. Here, *A. esculentus* plants having elevated antioxidative activities showed less signs of oxidative injuries and lipid peroxidation level as replicated by decreased level of MDA content (Fig. 6). When plants feel stress, they up-regulate their antioxidant defence machinery to alleviate oxidative

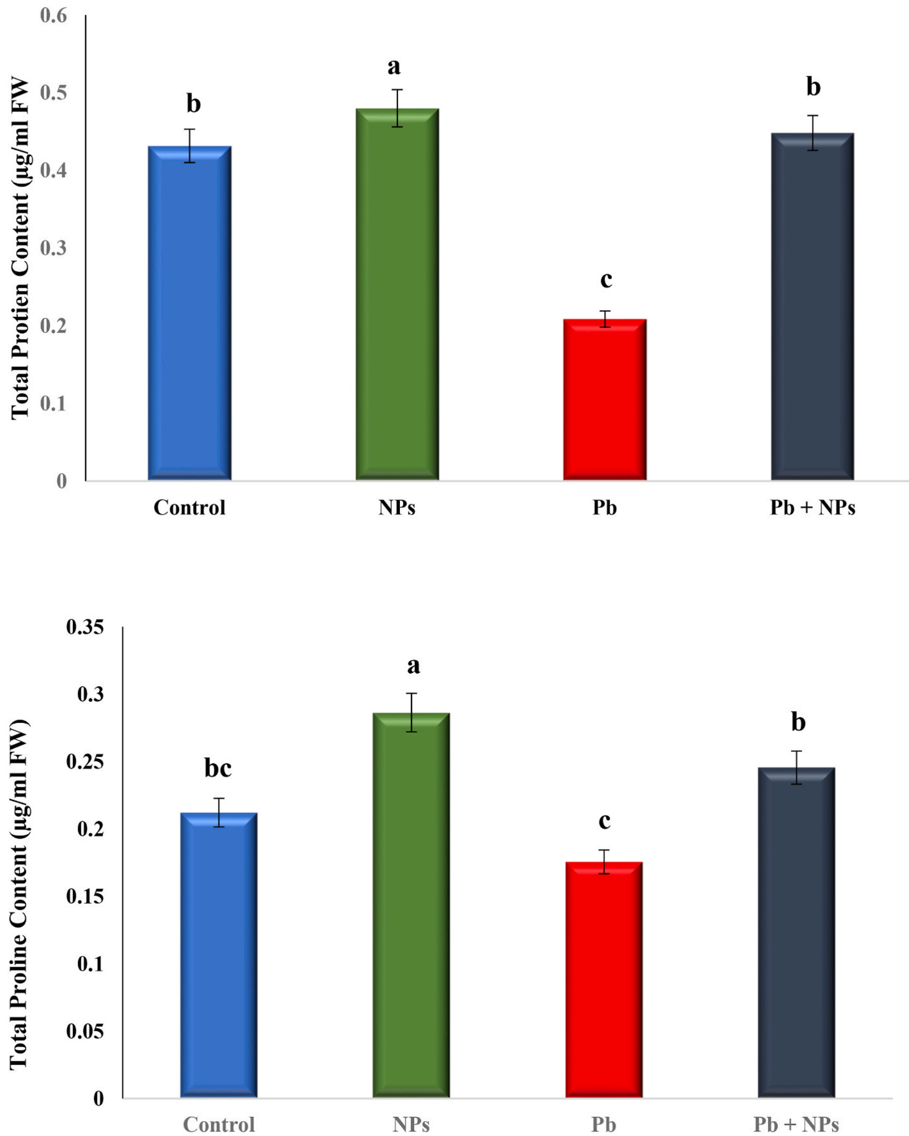

Fig. 4. Influence of calcium oxide nanoparticles on proline under Pb-stressed soil. C: Control; Pb: Lead; CaO-Nps calcium oxide nanoparticles. Values are means ± SD of five replicates.

stress (Hasnain et al., 2023). This antioxidant system comprises enzymatic and non-enzymatic antioxidants. It was the reason that we observed higher activity of SOD, POD, and CAT enzymes, together with the augmented level of $O^{•-2}$, H_2O_2, and MDA content in plants growing under Pb stressed conditions. Detoxification of the elevated synthesis of ROS through amplified activity of antioxidant enzymes during the present study is a defensive approach of plants to decrease the Pb induced oxidative injuries. Yet, this self-defence is not enough to scavenge the increased level of ROS in plants facing severe abiotic stress. To facilitate ROS detoxification in these plants, CaO-NPs further augmented the activities of SOD, POD, and CAT. Therefore, CaO-NPs treated Pb stressed plants exhibit reduced levels of MDA, H_2O_2, and $O^{•-2}$ content during the present study (Fig. 3). Faiz et al. (2022) also found higher activity of antioxidant enzymes decreased MDA, H_2O_2, and $O^{•-2}$ content in MgO-NPs (500 mg L^{-1}) treated soybean plants subjected to As stress (150 µM). These findings propose that exogenous NP supplements may deprived the uptake of toxic metal contaminants and improve antioxidant activities of enzymes by an indefinable mechanism.

Usually, root tissues of plants are more tolerant to metal contaminants as compared to shoot tissues. That's why; plants which are capable of restricting the upward translocation of metal pollutants from xylem tissues of roots to shoots are less susceptible to metal stress (Cui et al., 2019; Tian et al., 2019; Basit et al., 2022). The results of current research showed that CaO-NPs decreased the translocation of Pb as demonstrated by the TF of treated plants. For this reason, it is assumed that CaO-NPs mitigated Pb toxicity in by declined uptake and root to shoot translocation. There exists a probability that CaO-NPs sequestered Pb ions in the root vacuoles, hence limiting root to shoot translocation. Additionally, Ca^{2+} ions present in CaO-NPs possibly mitigated stress by hampering the uptake and translocation of Pb ions in applied plants. The higher accumulation of Ca^{2+} content in CaO-NPs treated plants during the current study supports our postulation. Brahmakshatriya and Solanki (2022) illustrated the antagonistic relationship among Pb and Ca^{2+} ions, which restricted Pb uptake in treated plants. Though, the stress ameliorative effect of Ca^{2+} ions present in CaO-NPs through reducing Pb uptake still need to be elucidated. To sum up, our findings demonstrated that CaO-NPs may mitigate Pb toxicity through declining Pb uptake and translocation besides recuperating the antioxidative system.

Pb stress restricted the uptake and accumulation of plant nutrients. However, the Pb-stressed *A. esculentus* plants treated with CaO-NPs showed improved mineral nutrition (Fig. 5). In fact, Ca^{2+} regulates plant nutrition by interacting with phytohormones/kinase receptors

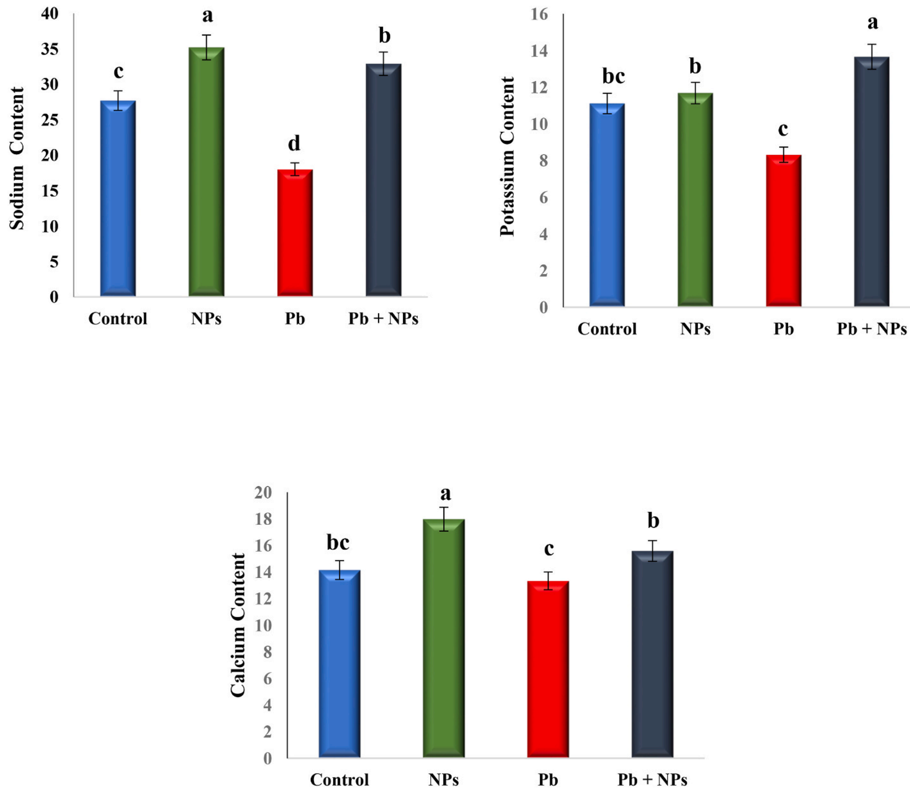

Fig. 5. Influence of calcium oxide nanoparticles on sodium, potassium and calcium content under Pb-stressed soil. C: Control; Pb: Lead; CaO-NPs calcium oxide nanoparticles. Values are means ± SD of five replicates.

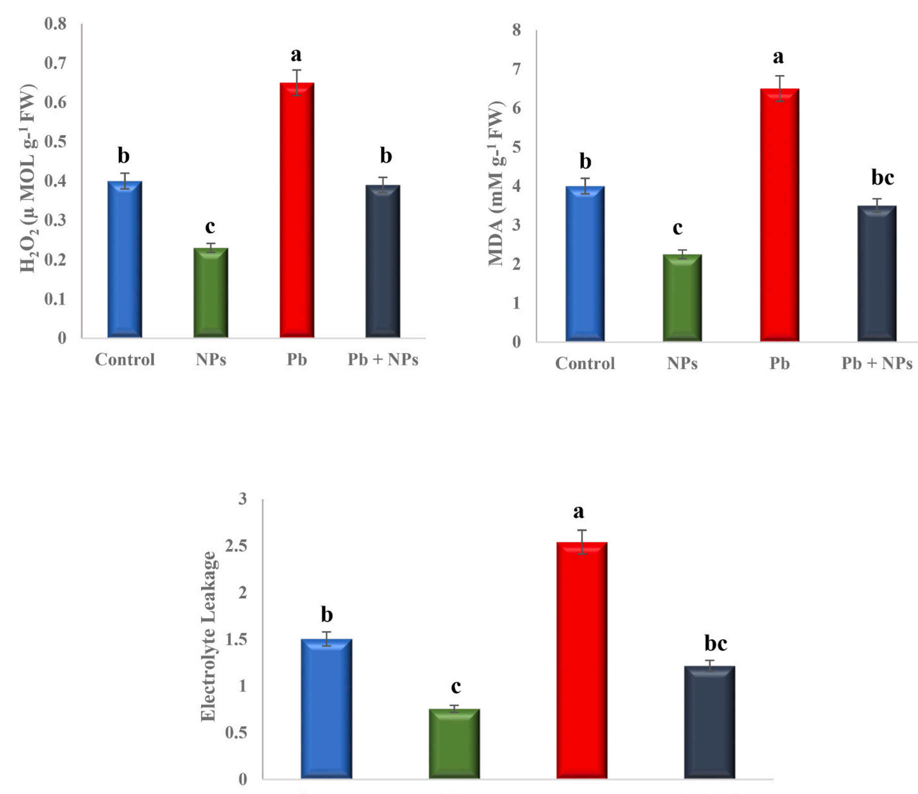

Fig. 6. Influence of calcium oxide nanoparticles on MDA under Pb-stressed soil. C: Control; Pb: Lead; CaO-Nps calcium oxide nanoparticles. Values are means ± SD of five replicates.

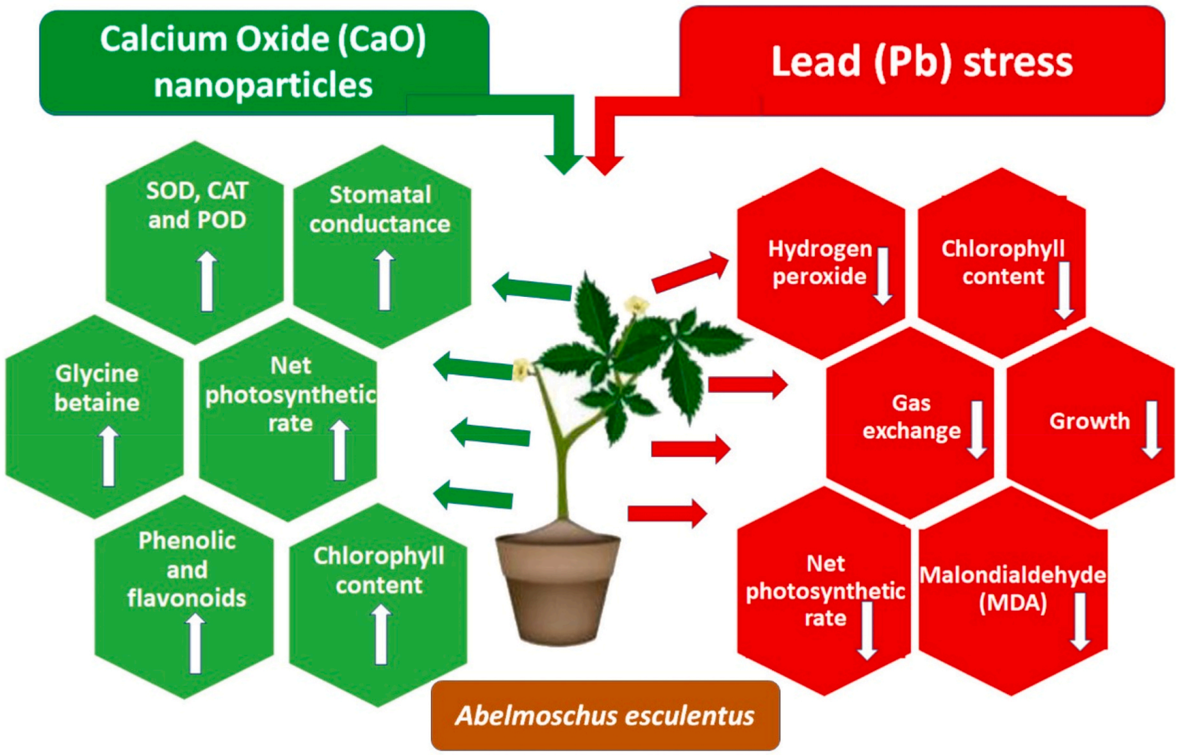

Fig. 7. Schematic diagram showing the effect of calcium oxide nanoparticles on growth and physiological attributes of *Abelmoschus esculentus*.

through adjusting the process of phosphorylation and dephosphorylation (Manishankar et al., 2018; Kudla et al., 2018). Additionally, Ca^{2+} restricts the dispersal of clay and disruption of aggregates by exchanging Mg^{2+} and Na^{+} ions in clay aggregates and microsites (Zhang and Norton, 2002). The exogenously applied Ca^{2+} improves soil structure through formation of cation channels among organic matter and clay particles (Huang et al., 2019; David and Dimitrios, 2002). Similarly, Naveed et al. (2020) found that calcium-fortified manure improved growth of salt stressed canola plants by enhancing the uptake of mineral nutrients including N, P and K.

Overall, results of the present research illustrated that exogenously applied CaO-NPs mitigate Pb stress in *A. esculentus* plants by adjusting various physiochemical activities. Particularly, the elevated activity of antioxidant enzymes consequently detoxified ROS in CaO-NPs treated plants and salvaged them from Pb induced oxidative damage. In addition, regulation in the homeostasis of Ca^{2+} ions increased the photosynthetic activity in CaO-NPs treated plants. Moreover, CaO-NPs decrease Pb uptake, its translocation and accumulation in plant tissues and defend *A. esculentus* plants from Pb-induced toxic effects.

Data availability

The data will be avilable on request.

5. Conclusions

The current study was intended to examine the potential of CaO-NPs in mitigation of Pb-induced toxicity in *A. esculentus* plants. Our results indicated that Pb stress ameliorative efficacy of CaO-NPs may be assigned to the decreased Pb uptake due to increased uptake of Ca ions. Moreover, the improved antioxidant system of CaO-NPs treated plants helped in the detoxification of Pb-stress induced ROS. Conceivably, the exogenous supplementation of CaO-NPs defends A. esculentus plants from Pb induced phytotoxicity through mitigation of Pb-triggered detrimental physiochemical modulations. This study suggests that the exogenously applied CaO-NPs may provide an economical and eco-friendly strategy to cultivate *A. esculentus* in Pb polluted regimes.

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Authors contribution

HZR, SU; Experimentation and Analysis, AAS, ZN; Supervision and Validation, SZ, NAY; Resource acquisition and Writing, FH, MA, H O E; Review, Drafting and Formal analysis, S R.M S, F A. Al-M, AA; Statistical analysis, writing, drafting, validation.

Declaration of competing interest

All the authors declare no conflict of interest.

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