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ORIGINAL PAPER

Molecular Characterization and Mitigative Role of Silicon Dioxide Nanoparticles in *Ocimum Basilicum* Under Lead (Pb) Stress

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Abstract
Lead (Pb) accumulation, even in minute quantities, has adverse effects on the morphology, physiology, and biochemistry of almost all plants, resulting in various abnormalities. Silicon dioxide nanoparticles (SiO_2-NPs) are used excessively to reduce abiotic stresses in a large variety of plant species. The present research work was designed to explore the role of SiO_2-NPs in the mitigation of Pb toxicity in *Ocimum basilicum*. SiO_2-NPs were green-synthesized from *Arando donax* plant extract. Characterization of green synthesized SiO_2-NPs was assessed with UV-vs, XRD, FTIR, and SEM–EDS. To analyze the morphology and antioxidant enzyme activities in *O. basilicum*, 8 days old plants were subjected to 3 different concentrations of Pb and SiO_2-NPs (50, 500, and 1000 ppm). Results of UV-vs, XRD, FTIR, and SEM–EDS showed the capping of SiO_2-NPs by different functional groups (Si $(CH_3)_3$, and Si–O-Si) together with its crystalline structure. The average size of the nanoparticles was 26 nm which was confirmed by XRD analysis. Morphological analysis revealed that treatment with 500 ppm concentration of Pb resulted in a significant decrease in the length of root, shoot, and weight, in the ratio of 19, 14, and 10%, respectively. But treatment with 500 ppm (SiO_2- NPs) significantly promoted root, shoot length, and weight of the plant, at the rate of 13, 22, and 7%, respectively. After the confirmation of ameliorative effect of SiO_2-NPs, combined application of Pb + SiO_2-NPs was tested. Root damage and Pb concentration in all the plant parts were much reduced. It was revealed that antioxidant activities of POD and APX were markedly decreased while those of the CAT and SOD increased. Results revealed that SiO_2-NPs are an anti-stressor, that removes Pb from *O. basilicum*, by enhancing its antioxidant activity.

Keywords Pb · SiO_2 –NPs · Antioxidants Enzymes · *Ocimum basilicum* · Stress

1 Introduction

Heavy metals (HMs) are responsible for health and environmental problems throughout the world. Lead (Pb) is one of the highly toxic heavy elements, but its biological role in connection with the human body has not been fully explored yet [1]. Even minor concentrations of Pb affects the water balance, morphology, and physiology of plant [2]. According to Kumar [3] and Youssef [4], Pb is not biologically degradable and is the most toxic element after Arsenic. According to Alfaraas [5], *O. basilicum* plants are susceptible to HMs uptake, and as a result cell damage, thickening of cell walls, and rupture in the parenchyma tissues occur. *O. basilicum* plant is greatly susceptible to toxic HMs from the soil and environment, causing abnormal changes in the anatomy and physiology of these plants [6]. Pb adversely affects seed germination of *O. bacilicum* [7]. Even at a very low concentration, Pb reduces the amount of Proline, the relative water content, Chlorophyll a, antioxidant activity of the plant, and the amount of Phenol [8]. Permissible limits for Pb in herbal medicines and plant bodies is 10 ppm [9]. In Pakistan, herbal and medicinal products are contaminated up to 4.6–46.4 ppm and the relative abundance in the soil ranges from 1.95 to 4.74 ppm [10]. Main reasons ascribed

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for Pb contamination of soil in Pakistan, are mining, smelting, electronic, sewage sludge, scrapping, and chipping. Pb is very tightly bound to the soil particles and can easily exchange ions. It accumulates excessively in plant tissues due to the absorption of water from the soil [11]. According to Kohli [12], the uptake of Pb mainly involves H^+/ATPases for its translocation upto endodermis of leaves, following apoplastic as well as symplastic pathways.

According to the study of Stratu and Lobiuc [13], Pb interferes with amylase and protease enzymes of the *O. bacilicum* seed very easily, thereby inhibiting seed germination. HMs such as Pb, generate oxidative stress, damaging plants directly or indirectly [14]. Pb and other toxic HMs produce Reactive Oxygen Species (ROS), found in nature as singlet oxygen, H_2O_2, or Hydroxyl radical. According to the study by Singh [15], Pb is redox-inactive, disrupts the electron chain, and increases ROS activity. HMs greatly affect the biochemical activities of antioxidant enzymes by enhancing H_2O_2, because of reduction in the activities of Superoxide Dismutase (SOD) and Catalase (CAT). Studies by Liu [16], and Aydin [17], conducted on wheat reported that CAT activity of Pb stressed plants were very low due to oxidative stress. SOD protects biomolecules and tissues against free radical toxicity [18]. Ascorbate Peroxidase (APX) is an Ascorbate- dependent enzyme which can break down H_2O_2 into components, thus preventing plants from its damage. It was reported by Azarakhsh [19], that under the Cobalt stress, APX activity was increased in *O. officinalis*, while under the Pb stress, APX activity was markedly decreased [20]. Glutathione Reductase (GR) reduces the oxidized glutathione GSSG into GSH in the presence of NADPH [21]. Kumar [22] has revealed that at 0.25, 5, 0.75, and 1.25 mP Pb concentrations, the ratio of GSH/GSSG was much reduced in plants.

SiO_2-NPs are widely used these days as agricultural products due to their intimate interaction with plants. NPs application is more appropriate and safer for non-edible medicinal, oil-yielding, and aromatic plants. Silicon (Si), a beneficial nutrient minimizes harmful impact of oxidative stress, created under biotic and abiotic stresses on plants [23]. SiO_2-NPs reduce the toxicity in plants under Pb stress [24]. SiO_2-NPs alleviate the stress effects, such as different diseases, nutrient imbalances, lodging, and heavy metal toxicities [25]. Bharwana [26] reported that SiO_2-NPs inhibit the toxic effects of Pb on cotton plants. The study of Araujo [27] revealed that the effect of SiO_2-NPs inhibited the maize growth retardation due to Pb. SiO_2-NPs accelerate the antioxidant activities of plants [28]. Silica (Si) overwhelms the ROS, created due to HMs stress, by stimulating the enzymatic antioxidants (SOD, APX, GR) and non-enzymatic ant-oxidants such as Ascorbate and Glutathione, thus strengthening the defensive mechanisms of the plants [29]. Chandra [30], assessed the effect of green synthesized SiO_2-NPs on *Cicer arientinum* plant under HMs stress. SiO_2-NPs regulate the expression of the antioxidant genes thus positively affecting the antioxidant enzymes. Gheshlaghpour [31] has shown that SiO_2-NPs greatly alleviate the Cd-induced phytotoxicity in *O. bacilicum* by accelerating APX activity. Imtiaz [32] studied the effects SiO_2-NPs on cotton plants under Pb stress and it was revealed that Si markedly increased the antioxidant enzymes such as GR.

O. basilicum is widely used as a food, perfume, medicine, cosmetic and is the universal source of minerals, vitamins, and different other organic compounds utilized in industries. The contaminated vegetable and oil of this plant are hazardous to health and continuously pose health problems, particularly in Pakistan. After the experiment, it can be hypothesized that SiO_2-NPs treatment in an exogenous way can help the plant tolerate HMs stress, particularly Pb stress. This study provides a new approach to the green synthesis of SiO_2-NPs from *Arando dunax* plant. The study aimed to investigate the SiO_2-NPs mediated morphological changes (root length, shoot length, and plant fresh weight) in *O. bacilicum* under Pb stress. Furthermore, antioxidant enzyme activities such as CAT, SOD, POD, and APX were also investigated under Pb stress.

2 Materials and Methods

2.1 Experimental Design

The experiment was conducted in the Plant Proteomic laboratory, Quaid-i- Azam University, Islamabad. A completely randomized design was used during the experimental process. The experiment was performed in three independent biological replicates.

2.2 SiO_2NPs Green Synthesis

The SiO_2-NPs were prepared from *A. donax* leaves, by using the method previously reported [33]. The extract was prepared by using 20 g leaves in 100 mL distilled water and heated at 80°C until its color changed into dark green. Then the mixture was filtered with Whatman No 42-filter paper to remove the plant residue. For SiO_2-NPs synthesis 100 mL of 1 M SiO_2 salt was mixed with 100 mL of plant extract and pH was maintained at 5. The mixture was stirred for 6 h at 120°C on hot plate until the color changed from pale yellow to dark brownish-red. Then the sample was centrifuged at 14,000 rpm for 10 min and the pallet was oven-dried.

2.3 SiO_2-NPs Characterization

The absorption spectra of SiO_2-NPs were monitored with a spectrophotometer from 400–500 nm while keeping 5 intervals of 20 nm for its quantification and identification. To

identify the functional group of SiO_2NPs, infrared spectra in transmission mode were identified with the help of Fourier-transform infrared spectroscopy (FT/IR-610, JASCO) in the range of 400–4000 cm^{-1} wave number. X-ray diffraction of SiO_2 in the powder form was measured by using an X-ray diffractometer in the range of 20-80° at 2 theta which is equal to 0.154 nm wavelength by using an X-ray diffractometer (Schimadzu Model: XRD 6000). The morphology and elemental analysis were assessed with EDS analysis by using scanning electron microscopy (SEM: JEOL JSM-5800 LV/EDS).

2.4 Experimental Conditions

The sand was selected as a growth medium, soaked in bleach for 24 h, and washed 15 times to remove all impurities. Seeds of *O. basilicum* were obtained from Pakistan Agricultural Research Council and were sterilized in 25% Sodium hypochlorite solution for 2 min and thrice rinsed with distilled water. 20 seeds were sown in pots containing 800 mL of sand. Irrigate with 230 mL of distilled water. Plants were grown in Growth Chamber under 16/8 h light and dark period, with temperature 24 °C-25°C, watered daily with distilled water, and the relative humidity of the chamber kept at 51%. After 8 days of germination, pots were treated with 4 different concentrations of (Pb $(NO_3)_2$, and SiO_2-NPs to induce phytotoxicity in the plant. Plants were successively harvested 2, 4, 6, and 8 days after treatment. They were categorized into four different groups [T_0 = control; T_1 = 500 ppm (Pb $(NO_3)_2$; T_2 = 500 ppm (SiO_2); T_3 = 500 + 500 ppm $Pb(NO_3)_2 + SiO_2$]. Three independent replicates were used for morphological and enzymatic experiments.

2.5 Growth Parameters

Growth parameters, i.e., shoot length, root length, and plant fresh weight, were measured by using a common measuring scale, and electronic weight balance respectively.

2.6 Analysis of Pb in *O. bacilicum*

Using diacid–digestion method for Pb detection, all replicates were divided into roots and shoots and oven-dried for 60 to 72 h. 100 mg of each sample was ground into fine powder, transfer into conical flask, containing mixture of Perchloric and nitric acid ($HCLO_4 + HNO_3$) (10 mL), and left for 24 h. It was heated up to 150°C, changing from brown fumes to white fumes. Distilled water was added and then filtered with Whatman No.42 filter paper and the mixture was added to D.H_2O to determine Pb concentration.

2.7 Histochemical Analysis

Evan's Blue Staining Procedure was adopted to quantify the membrane damage to the plants by Pb, SiO_2-NPs, and combined treatment. Both control and treated plants were collected and then transferred to Eppendorf tubes containing 2 mL volume of Evan's Blue Dye and left overnight [34]. The leaves and roots were thoroughly rinsed with distilled water and the samples were observed under a light microscope.

2.8 Antioxidant Enzymes Activity Assay

To determine the antioxidant activity of the plant enzymes (CAT, APX, SOD, and POD), 10 mg of roots and shoots were ground in liquid nitrogen. Samples were added to 4 mL extraction buffer, containing 1.4 mL of 0.07 mM of Potassium phosphate buffer solution, 20 µL of 200 mM Ascorbic acid, 16 µL of 100 mL of EDTA, and 2% Polyvinyl pyridine (0.08 g for each sample). This whole slurry was added to the Eppendorf tube and centrifuged at 4 °C for 20 min at the speed of 15,000 rpm. The pellet was discarded, and the supernatant was used as enzyme extract. Enzymatic activity of CAT was calculated through Aebi method [35], based on reduction in absorbance at 240 nm, as a result of consumption of H_2O_2; and its optical density was measured by a spectrophotometer. The reaction mixture consists of 50 mM Potassium phosphate buffer, 0.1 mM/L EDTA, 10 mM of H_2O_2, and 50 µL of enzyme extract. The activity of APX was measured through the time-tested procedure of Nakano and Asada [36], based on slowly monitoring the decline rate of Ascorbate absorbance at 25°C and 290 nm wavelength. SOD activity was assessed through the method followed by Verma and Dubey [37]. Both the blank and test solution's optical density was measured at 560 nm wavelength. POD activity was assayed through the method of Velikova [38], and absorbance was observed at 485 nm wavelength.

2.9 Statistical Analysis

The statistical significance of comparisons between multiple groups was evaluated with one-way ANOVA test and comparison between two groups was evaluated by Student's t-test. All calculations were performed using SPSS software (Version 22).

3 Results

3.1 Green Synthesis and Characterization of SiO_2-NPs

The most reliable time-tested techniques (UV–Vis FTIR, XRD, and SEM) were used for characterization of SiO_2-NPs. The reduction of unadulterated SiO_2-NPs was assessed by measuring the UV–Vis spectrum of the reaction mixture. Change in the color of reacting medium was recorded with visual observation, which indicates the synthesis of

SiO_2-NPs. The color of the mixture changed from light yellow to brownish red, a sign of SiO_2-NPs synthesis (Fig. 1a). The absorption was measured in ranged from 200 to 440 nm and the maximum absorbance was recorded at 340 nm (Fig. 1b). Analysis through Fourier-transform infrared spectroscopy (FTIR) showed 3 distinct downward bands, ranging from 600–1100 cm-1 wavenumber. The broad and sharp bands at 1048 cm-1 represent the functional group of Si–O-Si ring. Stretching vibration of the ring is due to Si–O, due to its very strong absorption, and the bending at 773.3 cm^{-1} is due to the presence of Si as Si $(CH_3)_3$. In this molecule, CH_3 functional group represents rocking vibration and Si–C revealed stretching vibrations of SiO_2-NPs (Fig. 1c). The X-Ray Diffraction (XRD) method of analysis using the Originpro 9.0 64 Bit Software. The graph revealed a sharp peak at 25–30° from 2Θ in the SiO_2-NPs graph and the narrow long peak in the range of 26.7° is due to the amorphous nature of SiO_2-NPs (Fig. 1d). The SEM analysis showed that the SiO_2-NPs have spherical shapes with particle sizes ranging from 15 to 47 nm (Fig. 2a-e). Moderate level of agglomeration was also seen in the images. Results of EDS spectra confirmed the presence of chlorine (31%), oxygen (25%), carbon (20%), sodium (16%), and silicon (5%) in green synthesized SiO_2-NPs. Figure 4a-d provides SEM–EDS elemental mapping of Silicon (kα), Oxygen (Kα), and Sodium (kα).

3.2 Morphological Characterization of *O. bacilicum* under Pb stress

Four days old *O. bacilicum* plants were treated with 3 different concentrations (50 ppm, 500 ppm, and 1000 ppm) of Pb. Significant reduction occurs in root length on the first day under 50 ppm treatment. Marked decrease in root length was recorded under 500 ppm and 1000 ppm treatment on 2nd and 3rd days. Great damage to the root length (19%) was caused by 500 ppm treatment as compared to 1000 ppm (3%) treatment of Pb. Reduction in shoot length was not significant in the 50 ppm Pb treatment on the 1st day but on the other two days reduction in the shoot was considerable. The shoot length was decreased (14%) at 500 ppm and (0.5%) at 50 ppm Pb concentration (Fig. 3). Plant fresh weight was decreased at all Pb concentrations as compared to control. The maximum decrease in fresh weight was (10%) and (4%) under 500 and 1000 ppm Pb concentrations respectively.

3.3 Morphological Characterization of *O. basilicum* Under SiO_2-NPs Application

Morphological changes in *O. basilicum* following treatment with SiO_2-NPs (50, 500, and 1000 ppm) were evaluated for a four-day bioassay. After statistical analysis, significant variations were shown in all parameters except plant weight. Root length at all concentrations of SiO_2-NPs (50, 500, & 1000 ppm) for all days has shown an enhancing effect as compared to control. The most enhancing effect (13%) was observed for the 500 ppm SiO_2-NPs concentration. Moreover, 50 and 1000 ppm SiO_2-NPs have shown enhancing effect on root length in the first two days and then caused suppression for the remaining days as compared to control (Fig. 4a). This increase in length might be due to hormetic or stimulatory effect that represents an overcompensation response to disruption in homeostasis. After prolonged exposure to stress toxicity increases due to which suppression in plant growth occurs. Shoot length was observed to increase on all days except for 50 ppm SiO_2-NPs as this concentration did not show any remarkable change on 1stday in comparison to the control. A similar effect was observed for 1000 ppm SiO_2-NPs as it did not show any enhancing effect on shoot length on the 2nd day of treatment. The maximum increase in shoot length, recorded was 22% on the 3rd day at 500 ppm (Fig. 4b). Plant fresh weight was measured under different concentrations of SiO_2-NPs (50, 500, and 1000 ppm). At all concentrations, reduction was observed in fresh weight on the 1st day of stress. On the 2nd day, 50 ppm showed an increase in fresh weight of root. Similarly, 50 ppm and 1000 ppm (SiO_2-NPs) showed remarkable reduction on the 4th and 3rd day as compared to control. The maximum plant fresh weight (7%) was observed for 500 ppm SiO_2-NPs and the lowest (-11%) for 1000 ppm on the 4th day (Fig. 4c). *O. baslicum* was subjected to the same concentrations (50, 500, and 1000 ppm) of Pb and SiO_2-NPs. In response to both treatments 500 ppm Pb damaging, and 500 ppm SiO_2-NPs enhancing effect on basil morphology was most pronounced than the remaining concentration and hence was selected for combined application. In this study, three concentrations of SiO_2-NPs i.e., 50, 500, and 1000 ppm were analyzed for their growth promoting effect. The lower concentration i.e., 50 ppm did not enhance the growth of *O. basilicum* as compared to control. Whereas 1000 ppm concentration caused growth retardation and toxicity in *O. basilicum*. At 500 ppm, *O. basilicum* exhibit maximum growth that's why this concentration was further used in this study to check its mitigative role under lead stress.

3.4 Morphological Characterization After Pb, SiO_2-NPs, and Pb + SiO_2-NPs Application

To evaluate the effects of SiO_2-NPs on *O. basilicum* under Pb stress, morphological changes were analyzed by treating 4 days old plants with SiO_2-NPs 500 ppm, Pb 500 ppm, and Pb + SiO_2-NPs (500 ppm). The root/shoot length and plant fresh weight were measured on 1, 2, 3,

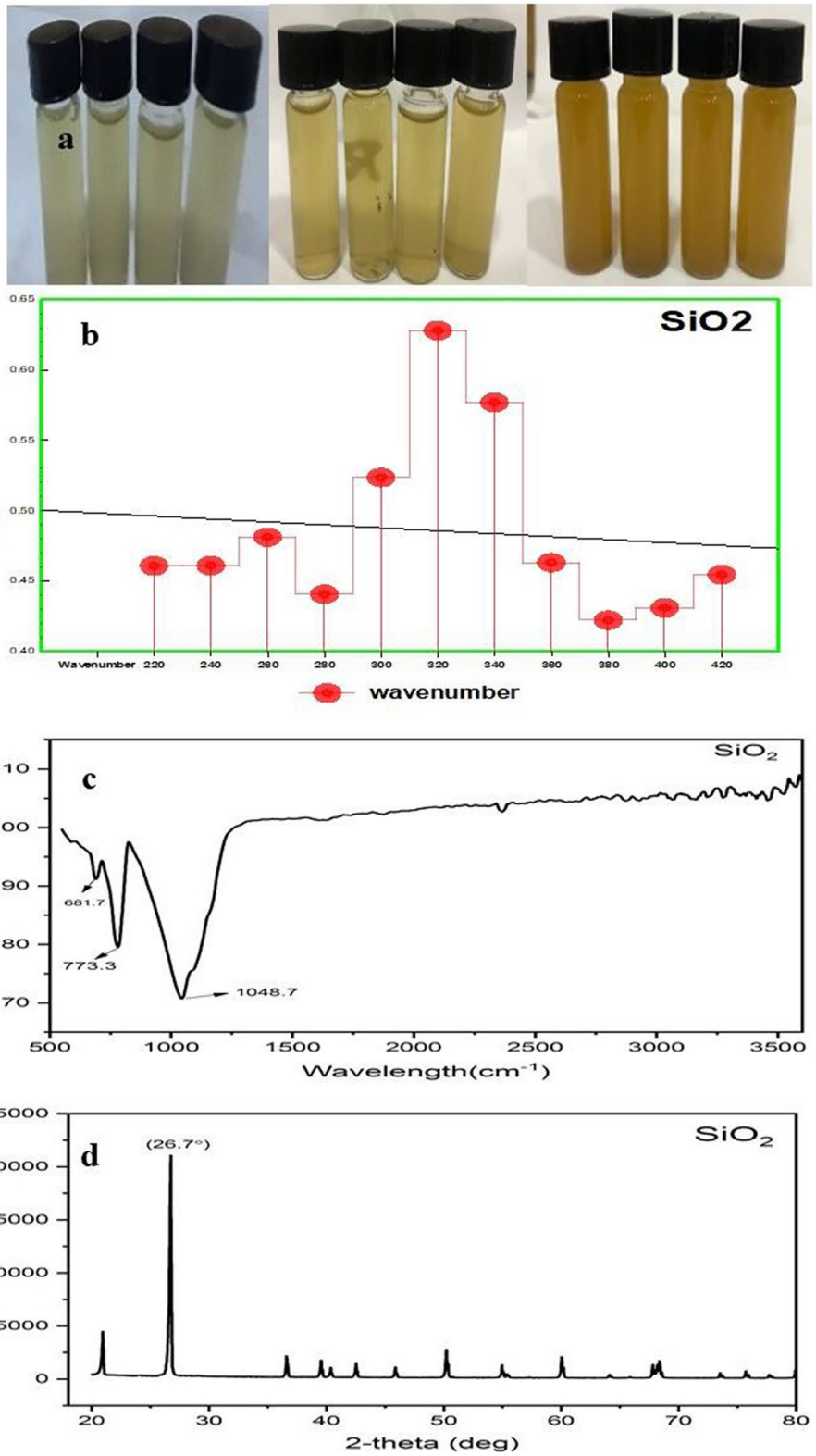

Fig. 1 Gradual color change, typical graphs of Uv–Vis, FTIR, and XRD of green synthesized SiO_2-NPs

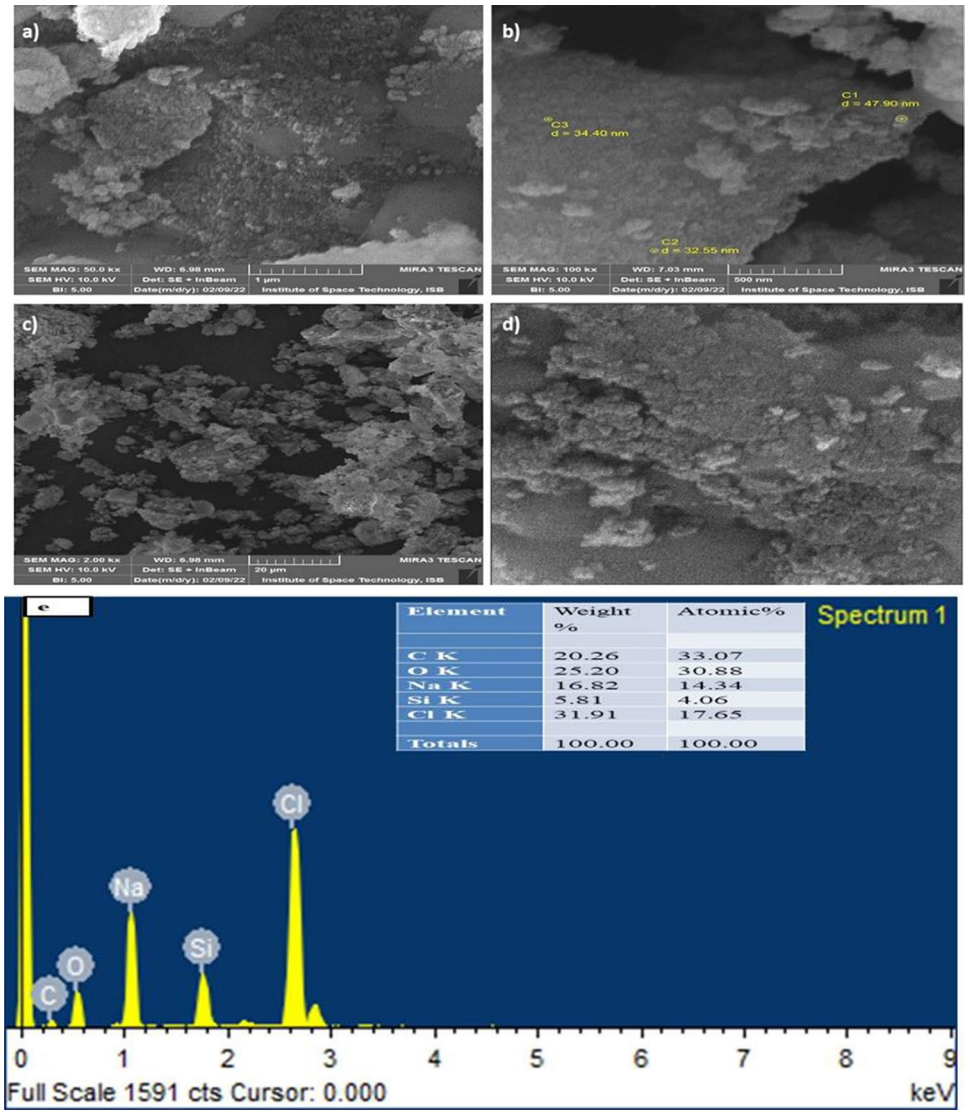

Fig. 2 Characterization of SiO_2-NPs by using SEM and EDS analysis to reveal the size, shape, and elemental composition of NPs. SEM analysis of SiO_2-NPs [scale bar _ 500 nm, {(**a**) 1 µm, (**b**) 5 µm, (**c**) 10 µm and (**d**) 20 µm)}], (**e**) EDS analysis of SiO_2-NPs

and 4 days of treatments. Three mutually independent replicates, treated with Pb, SiO_2-NPs, and Pb + SiO_2-NPs were prepared**.** To evaluate the ameliorative effect of SiO_2-NPs on *O. basilicum* morphology, 4 days old plants were treated with 500 ppm Pb, 500 ppm SiO_2-NPs, and Pb + SiO_2-NPs (500 ppm). The root length was decreased at 500 ppm Pb for all days as compared to the control. SiO_2-NPs (500 ppm) have shown an enhancing effect, while the effect of both Pb + SiO_2-NPs (500 ppm) on root length was similar to control (Fig. 5a).

Shoot length was negatively affected by Pb while positively affected by both SiO_2-NPs alone and SiO_2-NPs in combination with Pb as compared to the control. The minimum shoot length upto 8% was observed for Pb (500 ppm) treated plant on the 4th day of stress application. The maximum shoot length (10%) was seen for SiO_2-NPs (500 ppm) treated plant as compared to the control (Fig. 5b). Results demonstrated that the fresh weight of the plant in all treatments showed significant differences on all days. On the first day of treatment, Pb (500 ppm) decreased the plant biomass, SiO_2-NPs (500 ppm) enhanced the fresh weight while both Pb (500) and SiO_2.NPs (500 ppm) jointly did not cause any significant variation as compared to the control. A decrease in fresh weight was observed in *O. basilicum* on the 1st and 4th day and then no variation was observed for the rest of the days under Pb stress. SiO_2-NPs (500 ppm) showed an overall enhanced effect throughout the stress period, while combined application of Pb and SiO_2-NPs first reduced and then increased plant biomass. The maximum decrease in plant biomass (16%) was observed in Pb (500 ppm) treated plants while SiO_2-NPs (500 ppm) enhanced the fresh weight by 10% (Fig. 5c).

3.5 Pb Uptake in *O. bacilicum*

Pb-uptake in the root and shoot of *O. bacilicum* was evaluated under Pb, SiO_2-NPs, and Pb $_+$ SiO_2NPs (500 ppm) stress. Significant variation was observed in all the 3 samples. The

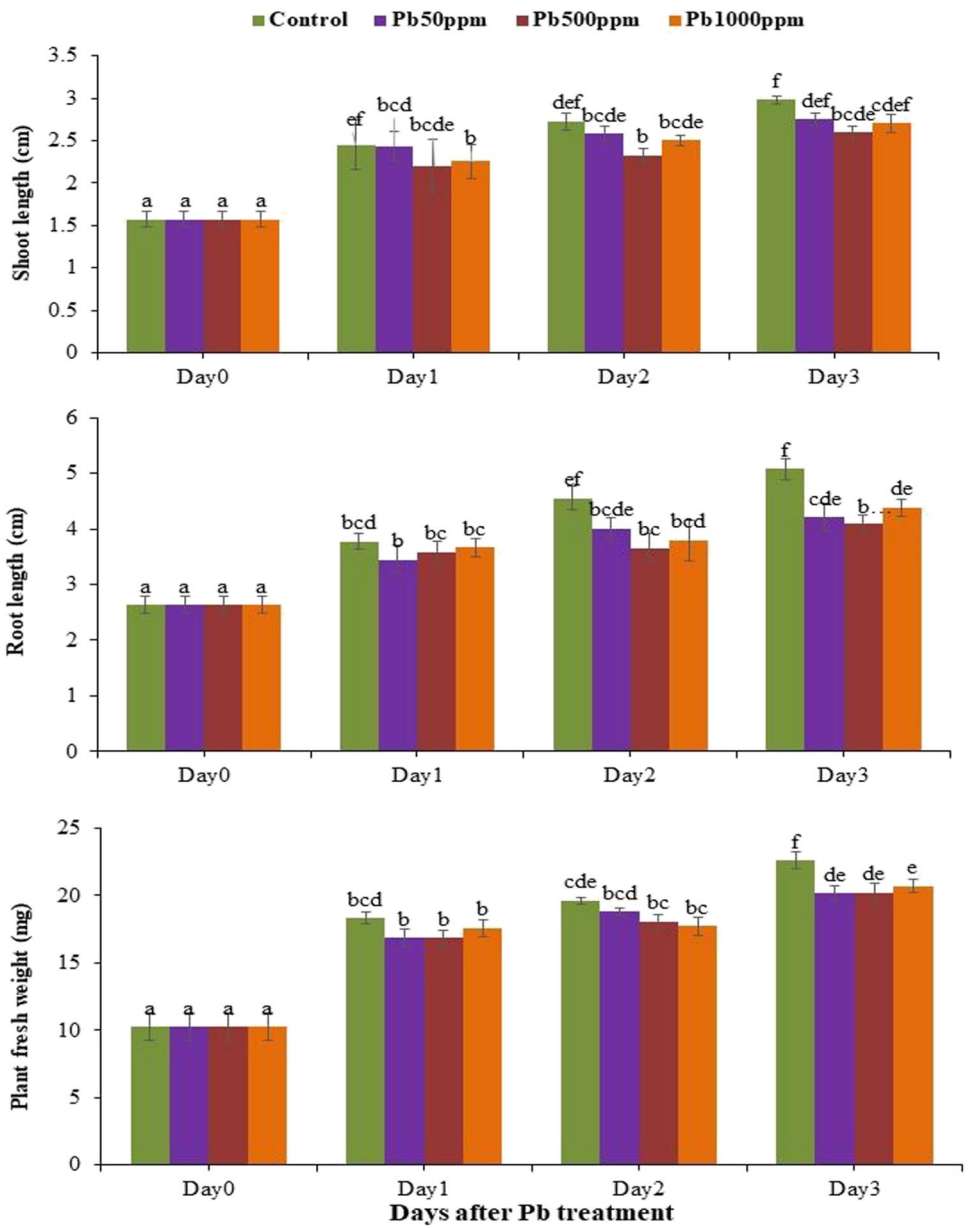

Fig. 3 Effects of various concentrations of Pb on *O. basilicum* growth and biomass. Six days old *O. basilicum* was subjected to the 50, 500, and 1000 ppm concentrations of Pb. Root/shoot (**a** & **b**) length and total plant fresh biomass (**c**) was measured. The presented data exhibited the mean ± S.D. of three biologically independent replicates (each replicate includes five plants). Various astrisks demonstrated a significant variation of mean values according to Tukey's Multiple Range test ($p \leq 0.05$)

maximum contents of Pb were observed in the plant treated with Pb, followed by Pb + SiO_2-NPs (500 ppm) and then SiO_2-NPs (500 ppm) as compared to the control. The content of Pb in *O. basilicum* treated with SiO_2-NPs (500 ppm) was negligible as compared to the control (Fig. 6).

3.6 Histochemical Analysis

The plant was stained with Evan, Blue Dye and the results revealed that the plant tissues had been damaged in Pb (500 ppm), followed by Pb + SiO_2-NPs (500 ppm) as compared to the control. SiO_2NPs (500 ppm) did not affect the anatomy of *O. bacilicum* (Fig. 7).

3.7 Characterization of Antioxidant Enzyme Assay

A significant decrease was noticed in the CAT activity in response to 500 ppm Pb. While CAT activity increased in the plant treated with 500 ppm SiO_2-NPs. In the combined effect of Pb (500 ppm) and SiO_2-NPs (500 ppm), the activity of the CAT markedly increased pointing to the ameliorative effect of SiO_2-NPs (Fig. 8a). APX activity increased in plants treated with Pb. While there was no significant difference between SiO_2-NPs and Pb + SiO_2-NPs (500 ppm) treated plants (Fig. 8b). SOD activity increased in plants when exposed to both Pb and SiO_2-NPs. While the combined treatment of SiO_2-NPs + Pb (500 ppm) markedly decreased the SOD activity as compared to the control (Fig. 8c).

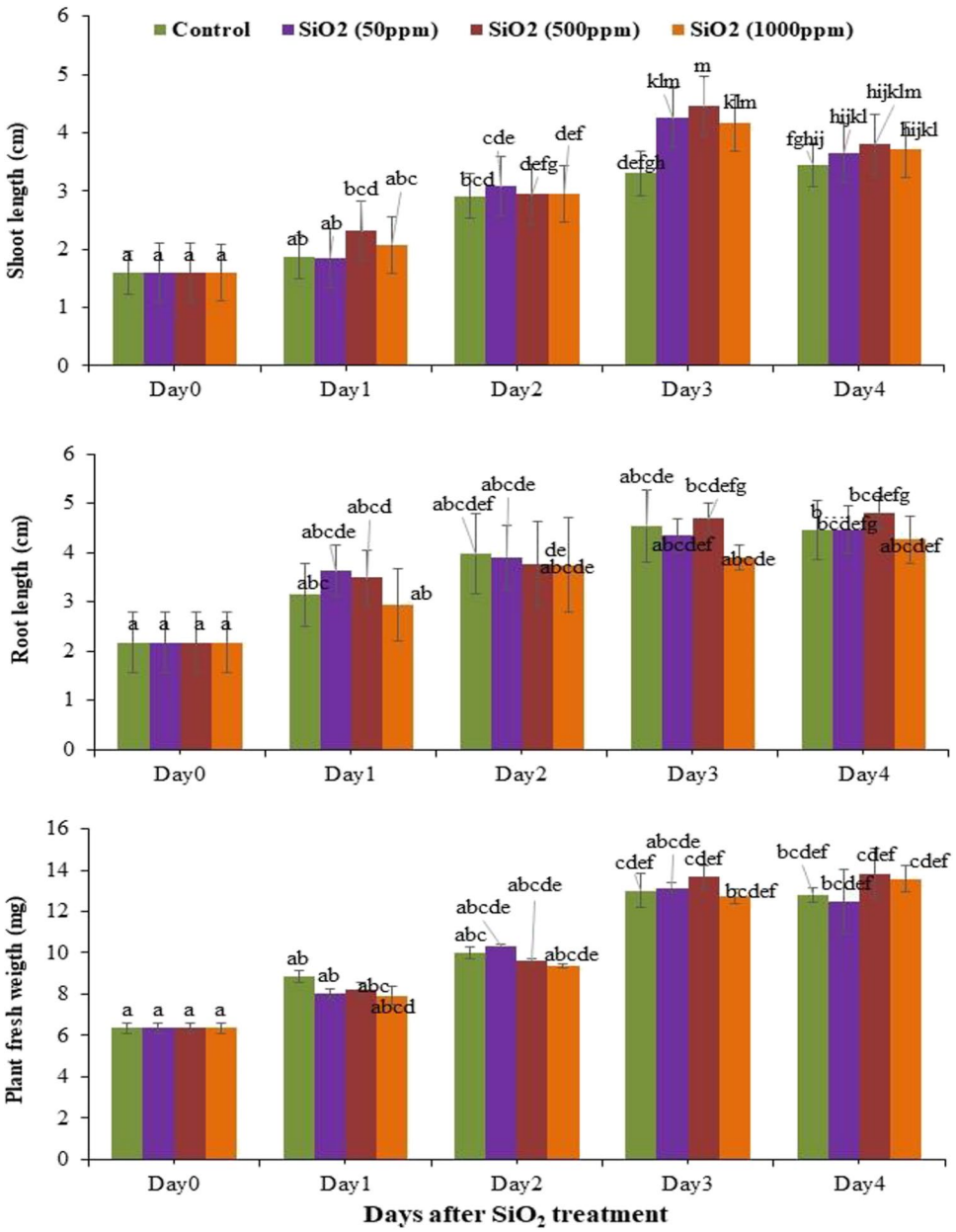

Fig. 4 Effects of various concentrations of SiO_2-NPs on *O. basilicum* growth and biomass. 50, 500, and 100 ppm concentrations of SiO_2-NPs were applied to the Eight days-old *O. basilicum*. Root/shoot length (a & b) and total plant fresh biomass (c) was investigated in response to the applied treatments. The prescribed data is the mean ± S.D. of biologically independent triplicates whereby each replicate includes 5 plants. Asterisks variation at different points exhibits a significant difference in mean values according to Tukey's Multiple Range test ($p \leq 0.05$)

Furthermore, the activity of POD was decreased under Pb (500 ppm), and SiO_2-NPs (500 ppm) treated plants as compared to control. The combined treatment of SiO_2-NPs + Pb (500 ppm) increases the activity of POD (Fig. 8d).

4 Discussion

4.1 SiO_2-NPs Characterization

Gardea-Torresdey [39] illustrated the first approach of using plants for the synthesis of metallic NPs was done by using *Alfalfa sprouts*. SiO_2-NPs were prepared from SiO_2 salt and *Arando dunax* extract because *A. dunax* contains 0.92 to 2.03% of Si and is a rich source of metabolites [40]. At 5 pH, SiO_2-NPs produce with a large amount and high surface area, as reported by Setyawan and Wulanawati [41]. The SiO_2-NPs were characterized by UV–Vis spectroscopy to determine the characteristic peak spectrum of NPs. The absorption peaks were centered around from 200 to 440 nm, and the characteristic peak was at 340 nm indicating the synthesis of SiO_2-NPs. Proteins and secondary metabolites are mainly involved in both the reducing and capping mechanism for NPs formation, as reported by Pansuksan [42], *A. Donax* is a rich source of secondary metabolites.

This study revealed that the FTIR-spectra represented 3 modes of bending and stretching of 3 different bands in the range of 600–1100 cm^{-1} wavenumbers. These bands

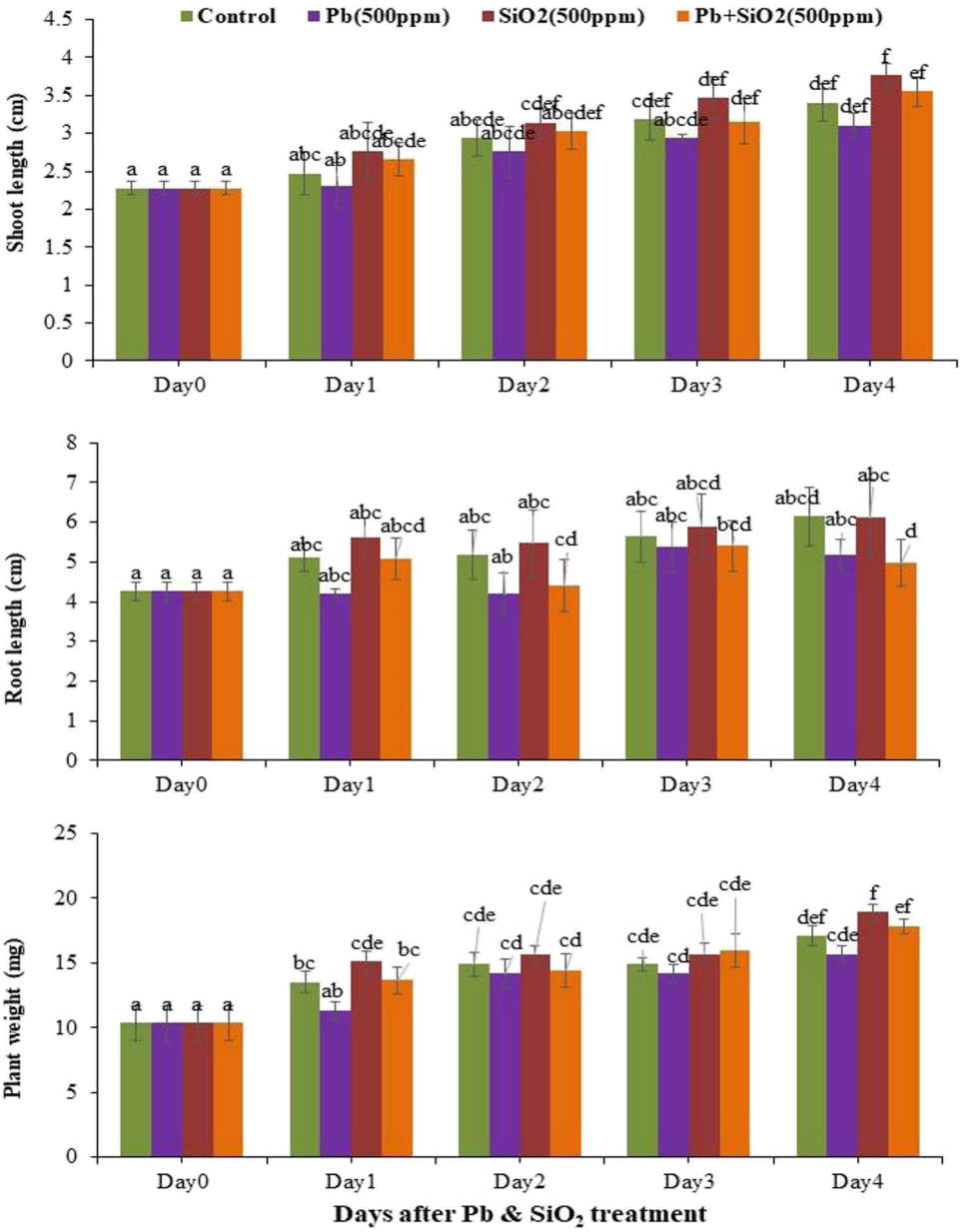

Fig. 5 Effects of Pb and SiO_2-NPs on the growth and biomass of *O. basilicum*. Eight days-old plants were treated with Pb 500 ppm, SiO_2-NPs 500 ppm, and Pb + SiO_2-NPs (500 ppm). Elongation in the root/shoot length and total plant biomass were measured, respectively. Values describe the mean ± S.D (n = 3) of biologically independent triplicate, whereby each includes five plants. Annotation demonstrated the significant difference was analyzed according to Tukey's Multiple Range test ($p \leq 0.05$)

are due to the different vibrations, represented by a specific group; Si–O-Si functional group is represented by the sharp bend at 1048.7 cm^{-1} which is due to stretching vibration of Si–O. Furthermore, as previously reported by Wang [43], the stretching vibrations at the 1048.7 cm^{-1} indicate tetrahedral units of SiO_4 instead of SiO_3 and SiO_2, Si $(CH_3)_3$ functional group is represented in the range of 773.3 cm^{-1} whereby Si–C represented stretching, while CH_3 represented by rocking vibrations and SiO_2-NPs is expressed by 681.7 cm^{-1}. The remaining spectra indicate that the pretreatment showed no impact on the chemical structure of synthesized SiO_2-NPs. These findings are confirmed by the study of Setyawan and Wulanawati [41]. The amorphous nature of SiO_2-NPs is represented in the sharp narrow peak by 2θ and the average size 26.7 nm of SiO_2-NPs, through XRD. Similar results are reported from the work of Kumar [44] nearly the same average size (22 nm) of silica nanoparticles at 2θ. Additionally, the broadness of the peak indicates that the prepared SiO_2-NPs were nanoscale in size [45].

4.2 Morphological Characterization

The underlying study determined the application of Lead Nitrate on the uptake of Pb by roots and shoots of *O. basilicum*. Roots are the first part that is in direct contact with the different components of rhizosphere [46]. The reduction in roots/shoot length and plant weight is also

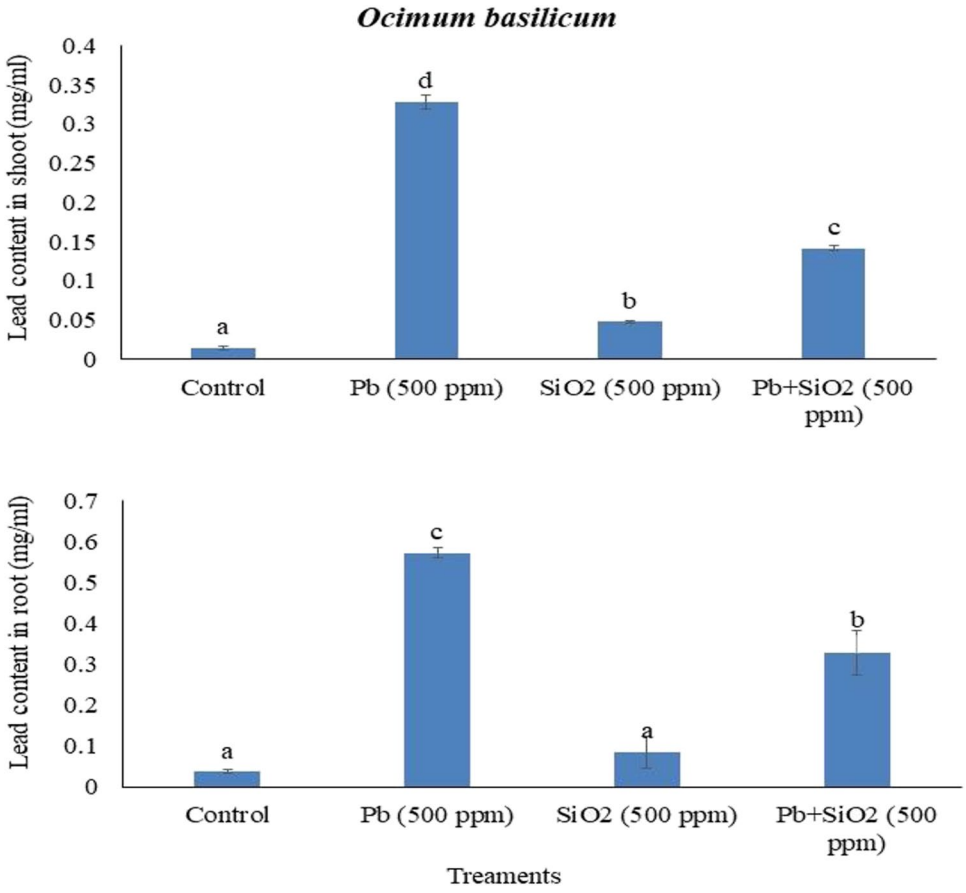

Fig. 6 Effects of SiO_2-NPs were evaluated in the accumulation of Pb by *O. basilicum*. Eight days-old plants were subjected to the Pb 500 ppm, SiO_2-NPs 500 ppm, and Pb + SiO_2-NPs (500 ppm). The content of Pb uptake by both root and shoot was measured after the application of stressors. The data of three biologically independent replicates as the mean ± S.D whereby each replicate includes 5 plants. The different letters at each point are the mean values that exhibit significant difference according to Tukey's Multiple Range test ($p \leq 0.05$)

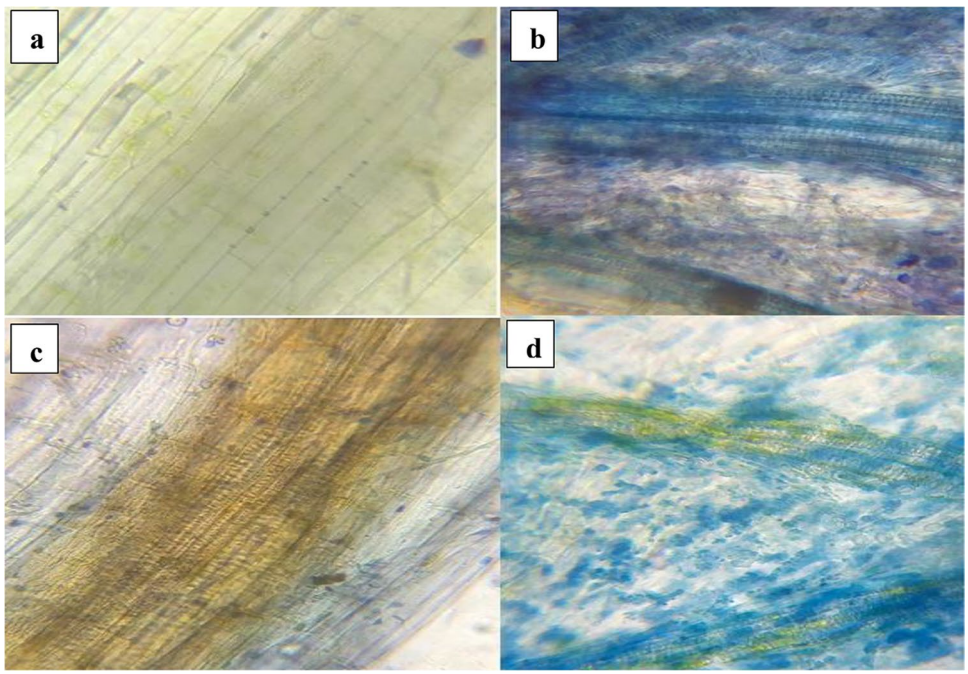

Fig. 7 Effects of Pb and SiO_2-NPs and Pb + SiO_2-NPs on the damaging of *O. basilicum* root. Eight days-old plants were treated with Pb 500 ppm, SiO_2-NPs 500 ppm, and Pb + SiO_2-NPs (500 ppm). After the treatments, the damage was observed under the microscope

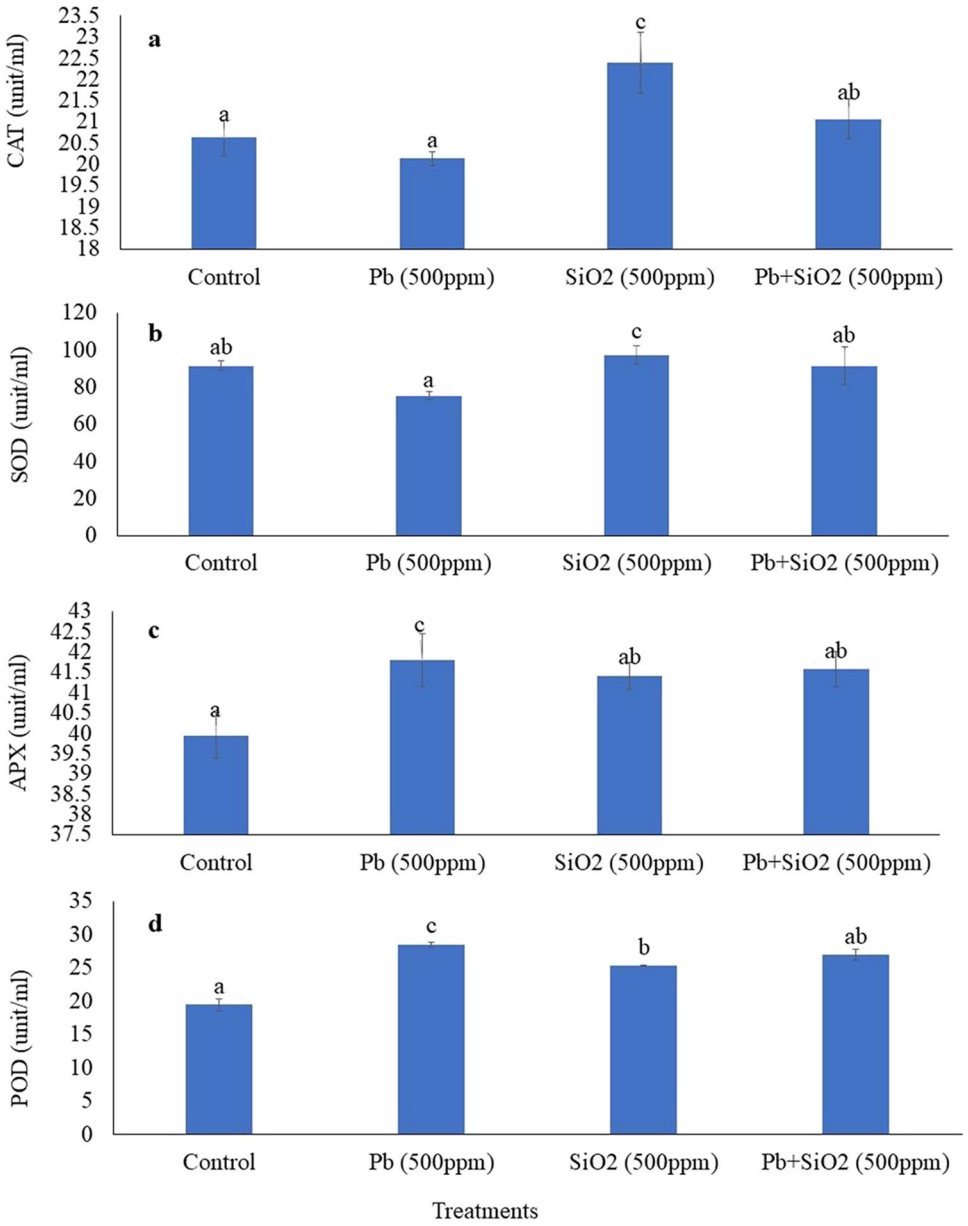

Fig. 8 Effects of SiO_2-NPs on *O. basilicum* antioxidants under Pb on (**a**) CAT, (**b**) APX, (**c**) SOD, and (**d**) POD activities. Eight days-old plants were applied with Pb 500 ppm, SiO_2-NPs 500 ppm, and Pb + SiO_2-NPs (500 ppm). The data as the mean ± S.D per replicate, containing 5 plants. Bars with variables alphabets are significantly different according to Tukey's Multiple Range test ($p < 0.05$)

attributed to excessive agglomeration of Pb in the soil [47]. Growth and productivity of Pb-treated *O. basilicum* are negatively affected [48]. As plant root is in intimate contact with the soil, it is the first part that directly uptakes HMs from the soil. The present study revealed that root length was adversely affected due to a high concentration of Pb, this result is in pair with the work of Youssef [4], who reported a similarly damaging effect in *O. basilicum* under Cd. Thus, higher uptake of Pb by root caused more damage to root than shoot. Decreased plant weight due to Pb has been confirmed by several researchers [7, 49]. The root itself contains metals transporter and releases different chelators which are involved in Pb uptake [50–53]. Decrease in photosynthesis results in the reduction of carbohydrates metabolism, plant fresh weight, root, and shoot length [54]. It has been previously reported that Pb causes inhibition in the uptake of essential nutrients and protein degradation leading to fresh weight reduction [55]. The effect of SiO_2-NPs is mechanical rather than physiological. The present results showed that root, shoot length, and plant weight is increased by SiO_2-NPs stress. The enhancing effect of SiO_2-NPs might be due to improved resistance and better nutrient uptake. SiO_2-NPs increased root length, shoot length, and plant weight. SiO_2-NPs either provide mechanical strength to the cell wall or alter its cationic binding capacity, down-regulating their transporter

genes and blocking the way of HMs [56, 57]. Si enhances soil pH and changes metals speciation through the formation of silicate complexes in soil [58]. Whereas Al-Garni [59] reported that Si enhanced *Coriander sativum* growth through modulation of antioxidant activities. Our findings also depict that SiO_2-NPs enhanced the activities of antioxidants in *O. basilicum* exposed to Pb stress.

Our findings demonstrated that Pb accumulation was higher in roots as compared to shoots in *O. basilicum* under Pb stress. It is because Pb binds with the carboxyl group of galacturonic and glucuronic acid in cell wall, leading to the low transportation rate of Pb via apoplast pathway [60]. These results are justified according to the study by Patel [61]. The increased levels of Pb in the soil cause rhizosphere acidification, which leads to enhancing the bioavailability and mobility of Pb. Rhizosphere contamination with Pb causes its agglomeration in the edible plant, subsequently imparts the food chain and can cause various human and animal health complexities [54]. Concerning the statement, there should be a strong strategy needed to mitigate Pb toxicity and its accumulation in plants. Based on the obtained data, SiO_2-NPs is a simple and feasible approach to minimize the Pb agglomeration in the edible part of *O.basilicum*. Under HMs stress, plants treated with SiO_2-NPs have low level of Pb in their root and shoot as compared to control. According to Chaudhary [61], heavy metal ATPase2 (HMA2) is an HMs hyper-accumulator gene. It has been previously studied that HMs upregulate the HMA2 gene leading to hyper-accumulation of Pb in the treated plant. Whereas Si downregulates the HMA2 gene, leading to reduces Pb concentration in Pb-treated plants [62]. Liang [63] reported that to alleviate HMs, Si increases soil pH, ultimately reducing the HMs concentration in root and shoot. Moreover, Zhang [64] reported that Si plays a critical role in HMs compartmentalization into root cells vacuole, thereby avoiding their translocation into the shoot.

4.3 Antioxidant Enzymes

HMs polluted environment can lead to generating oxidative stress and changing the normal physiology of plants. Metals produce ROS in plants directly or indirectly. Cuypers [65] reported that HMs directly take part in the Fenton and Haber–Weiss reactions, where they create the most toxic OH• radicals from H_2O_2. The generated ROS damage macromolecules such as lipids, carbohydrates, nucleic acids, and proteins, subsequently causing the aging and death of plants [66]. Whereas, plants have a defensive system consisting of antioxidant enzymes, which can overcome the damaging effect of ROS [67]. Our results depict that Pb stress enhances the activity of antioxidant enzymes including SOD, CAT, APX, and POD in *O. basilicum*. An increase in antioxidant activities has been one of the important strategies to combat oxidative stress [68, 69]. According to the study of Sobrino-Plata [70] Cd stress-induced hormetic response in alfalfa through activating antioxidants. Regarding our findings, SiO_2-NPs play a protective role in *O. basilicum* against Pb stress by enhancing antioxidant enzymes. Our results are in accord with the work of Xuebin [71] who reported the same enhancing effect of Si on activities of antioxidants in Cd stressed wheat. CAT is an antioxidant enzyme that converts H_2O_2 into ½ O_2 and (H_2O) and metal stress markedly decreases the enzymatic activity of CAT while the NPs enhance the antioxidant activity of plants [72]. Downregulation of CAT-producing genes may be the reason for the decrease in enzymatic activity under the Pb stress. Whereas when SiO_2-NPs were applied in combination with Pb, the SiO_2-NPs dominated and increase the CAT activity. Gheshlaghpour [31] has shown that SiO_2-NPs accelerate Enzymatic activity, under HMs stress such as Pb. SOD prevents biomolecules from the harmful effects of free radicals, but its activity is greatly reduced due to Pb stress.

5 Conclusion

Preparation of SiO_2-NPs through green synthesis method is a cost-effective approach that reduces the use of toxic chemicals and minimizes the risk to the environment. Hence SiO_2-NPs were prepared biologically by using green resources. The biosynthetic method is environmentally friendly, and the shape, size, and morphology of the particle can be controlled easily. SiO_2-NPs synthesized with biological method from *A. donax* extract suggest a novel and alternative approach to chemically and physically synthesized nanoparticles. In this research, the green synthesized SiO_2-NPs were of spherical structure with 26 nm average size assessed with XRD, while observed size ranging from 15–45 nm demonstrated by SEM–EDS analysis. The functional groups involved in the caping, reduction, and stabilization of SiO_2-NPs were found with FTIR techniques. Pb-induced toxicity caused growth abnormality and physiological properties like altering the antioxidant activity of *O. basilicum* plant. Morphological analysis revealed that treatment with 500 ppm concentration of Pb resulted in a significant decrease in the length of root, shoot, and weight. While treatment with 500 ppm SiO_2-NPs significantly promoted root, shoot length, and weight of the plant. The combined application of Pb + SiO_2-NPs reduced the Pb concentration in all parts of the plant which suggests its

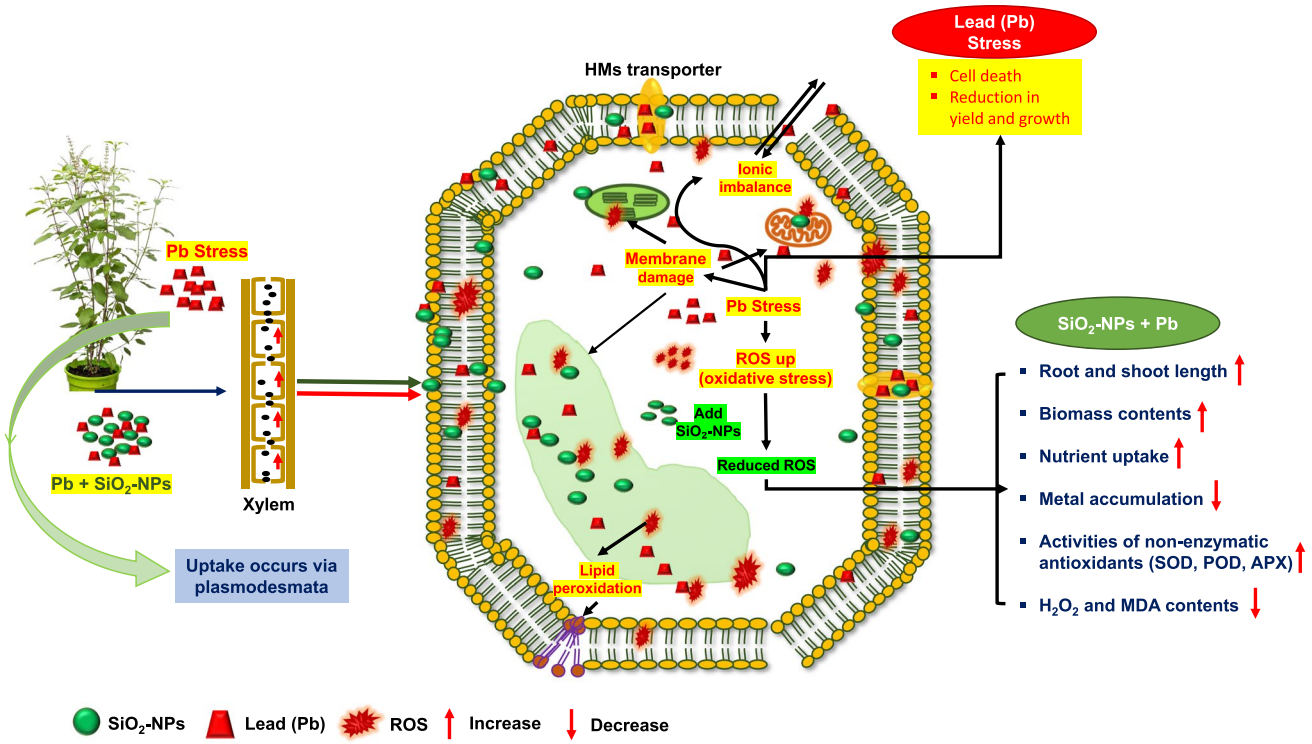

Fig. 9 Mitigative mechanism of SiO_2-NPs under Pb stress in *O. basilicum*

anti-stressor capability (Fig. 9). SiO_2-NPs enhanced the activity of POD and APX and those of CAT and SOD were decreased. SiO_2-NPs have been proved to greatly ameliorate the toxic effect of Pb on *O. basilicum* plant.

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Data Availability All the data generated or analyzed is present in this manuscript in the form of Tables and Figures. So, there is no extra data to present separately.

Declarations

Research Involving Humans/Animals Not applicable.

Informed Consent Not applicable.

Ethics Approval Not applicable.

Consent to Participate Not applicable.

Consent of Publication All the Authors' approved the final version to be published.

Competing Interest The authors have no relevant financial or non-financial interests to disclose.

Conflicts of Interest All the authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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