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Comparative effects of micron-sized silicon sources and Si nanoparticles on growth, defense system and cadmium accumulation in wheat (*Triticum aestivum* L.) cultivated in Cd contaminated soil

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A B S T R A C T

Elevated levels of cadmium (Cd) in soils posesignificant challenges to global agricultural crop production. The potential of silicon (Si) nanoparticles (NPs) and biogenic Si sources of different sizes to mitigate Cd stress in wheat remains largely unexplored. A pot experiment was conducted to evaluate the effectiveness of various sizes of biogenic Si sources: rice husk biochar (RHB), sugarcane bagasse (SB), and rice straw (RS); categorized by particle size (<150 μm, 150–250 μm, and 250–1680 μm, denoted as S1, S2, and S3, respectively), and Si NPs on wheat growth and Cd accumulation. The application of RHB (<150 μm) significantly increased plant height (105 %), spike length (94 %), shoot dry weight (93 %), root dry weight (83 %), and grain weight (89 %) compared to the control. The RHBS1 treatment delivered the greatest reduction in the bioavailable fraction of soil Cd (-74 %), as well as Cd levels in roots (-85 %), shoots (-93 %), and grains (-97 %) compared to the polluted control. Additionally, RHB (<150 μm) showed the highest increase in antioxidant enzyme activities compared to the Cd-spiked treatment. Interestingly, the various biogenic Si sources have the potential to mitigate Cd stress in wheat plants. Further research is required to understand the underlying mechanisms and to investigate the effectiveness of different biogenic Si sources and particle sizes in alleviating Cd stress in crops.

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1. Introduction

Wheat (*Triticum aestivum* L.) is one of the oldest and third most important staple crop globally, with an average annual production of approximately 752 million tons (Erenstein et al., 2022). Due to its content of starch, protein, vitamins (particularly B vitamins), dietary fiber, and phytochemicals, wheat holds a crucial position as a dietary staple (Singh, 2023; Yang et al., 2024). Moreover, wheat is a vital source of vegetable proteins in human nutrition, with a protein content of around 13 % (Flambeau et al., 2024; Shiferaw et al., 2013). However, wheat production faces numerous challenges, including unpredictable climate changes, the use of untreated wastewater for irrigation, and various biotic and abiotic stresses (Chowdhury et al., 2024; Senapati et al., 2019; Suzuki et al., 2014; Verma et al., 2023). Among these stressors, cadmium (Cd) contamination poses a substantial environmental and health risk due to its toxicity and potential for bioaccumulation in plants and animals (Shaghaleh et al., 2024; Rahim et al., 2024; Pan et al., 2023; Ullah et al., 2024). The primary sources of Cd contamination include industrial applications, such as its use in polyvinyl chloride (PVC) products, color pigments, Ni-Cd batteries, Cu and Ni smelting, fossil fuel combustion, and the application of phosphate fertilizers (Ullah et al., 2024; Zhang et al., 2023, 2024; Yang et al., 2023). Cd is also present in non-ferrous metal smelters and in the recycling of electronic waste (Aftab et al., 2023). Cd toxicity causes extensive damage to plants by impairing physiological functions, lowering carbon fixation, reducing chlorophyll content, and inducing oxidative stress (Li et al., 2024; Song et al., 2019; Yan et al., 2023; Zhang et al., 2024). As elevated levels, Cd also reduces nutrient and water uptake, disrupts plant metabolism, and inhibits both plant morphology and physiology, ultimately leading to plant tissue death (Li et al., 2023; Khan et al., 2024). As a phloem-mobile element, Cd is distributed throughout plant tissues, significantly reducing biomass and yield (Genchi et al., 2020; Song et al., 2019). As a toxic heavy metal, Cd can have harmful effects on human health when consumed through contaminated food crops (Liu et al., 2012; Vannini et al., 2024). Consumption of food contaminated with high levels of Cd can lead to stomach irritation, abdominal cramps, nausea, vomiting, and diarrhea (Anwar et al., 2024). Therefore, urgent measures are needed to remediate Cd pollution in agricultural fields and ensuring the production of safe food for human consumption.

Silicon (Si) has the potential to alleviate Cd toxicity in plants and reduce its accumulation in plant tissues (Biswas et al., 2023). Research has shown that foliar application of Si on rice plants under Cd stress increases the activities of certain enzymes, thereby mitigating the adverse effects of Cd (Chang et al., 2023; Jalil et al., 2023; Jin et al., 2023; Li et al., 2022). The application of Si nanoparticles (NPs) has also been reported to reduce Cd toxicity in rice cells, highlighting the potential mechanisms and benefits of this approach (Jalil et al., 2023; Bano et al., 2024). Si NPs can additionally modulate the nutritional status of plants, helping to alleviate the toxic effects of Cd (Zhou et al., 2022). Additionally, Si NPs can enhance the antioxidant properties of plants, helping to counteract the oxidative stress caused by Cd toxicity (Jalil et al., 2023). These findings provided ample evidence that Si application may offer a promising approach to reduce the toxic effects of Cd in agricultural fields. In addition, the application of rice husk biochar (RHB) can reduce the availability of Cd in soils, thereby decreasing its uptake by plants (Shaghaleh et al., 2024). This reduction in Cd uptake can lead to lower Cd accumulation in plant tissues, including edible parts, ultimately reducing the risk of Cd toxicity in humans (Ahmed et al., 2023a).

The presence of RHB in soils will prevent the leaching of Cd into groundwater and lowering the possibility of environmental pollution (Abbas et al., 2018a, 2017). The ability of RHB to adsorb Cd is attributed to its high surface area, porosity, and functional groups (Abbas et al., 2018b). Furthermore, RHB can improve soil fertility and plant growth, which can help mitigate the toxic effects of Cd on plant growth and development (Ali et al., 2018). The presence of C═O stretching in aromatic rings, lignin, C-OH groups, along with a significant Si content of 31.0 %, plays a crucial role in the effectiveness of RHB in reducing Cd toxicity in plants. These combined mechanisms make RHB a promising strategy for Cd remediation in agricultural fields (ur Rehman et al., 2018a). Sugarcane bagasse (SB) primarily consists of cellulose (40–50 %), hemicellulose (25–35 %), lignin (20–25 %), and 30.3 % Si, accounting for over 90 % of its dry weight (Zhou et al., 2016; Nasution et al., 2022). Similarly, rice straw (RS) contains cellulose, hemicellulose, and lignin, along with other components such as ash, silica, and lipophilic compounds. Its typical chemical composition includes cellulose (24.0 %), hemicellulose (27.8 %), lignin (13.5 %), ash (14 %), silica (1.48–67.78 %), and lipophilic compounds (3.4 %) (Qibtiyah et al., 2023). While numerous studies have focused on remediating Cd in agricultural fields to ensure safe wheat production using Si NPs and biogenic Si sources, limited research has explored the use of different sizes of biogenic Si sources for Cd remediation. Moreover, no published work has examined the effects of Si NPs and micron-sized organic Si sources on plant growth under Cd stress. This experiment aims to address the existing research gap. The hypothesis is that different sizes of biogenic Si sources and Si NPs can mitigate the toxic effects of Cd by enhancing antioxidant enzyme activity and improving photosynthesis. The primary objectives of this study are: to evaluate the comparative effectiveness of various Si sources in remediating Cd; to assess the effects of particle size reduction in biogenic Si sources on the growth, physiology, and yield of wheat; and to compare the effectiveness of micron-sized organic Si sources with Si NPs in reducing Cd toxicity.

2. Materials and methods

2.1. Experimental material preparation and characterization

The RHB was prepared anaerobically in a muffle furnace. In brief, the rice husk feed stock was collected from a local farm, dried at a temperature of 55±5 °C for three days, and ground in a mechanical grinder. The ground feed stock underwent pyrolysis at a temperature of 400±50 °C for 6 hours, without oxygen, in the muffle furnace. The prepared biochar was then milled, sieved through a 2 mm mesh size sieve, and thoroughly characterized prior to experimentation. Biochar properties, such as pH, EC, organic carbon, total carbon, total nitrogen, plant-available phosphorus, and plant-available silicon, were analyzed following methods outlined by (Sohail et al., 2020a) and (Al-Huqail et al., 2023), as presented in Table 1. The pH and EC of the biochar were determined by suspending the biochar in distilled water at a ratio of 1:20 (w/v) and utilizing pH and EC meters. The plant-available phosphorus was determined through a sodium bicarbonate extraction method, and the resulting supernatant solution was analyzed using a spectrophotometer. The plant-available Si was determined using a 5-day extraction method, as described by (Sohail et al., 2020a). The organic carbon content within the biochar was determined using the loss ignitions method. The SB was obtained from Crescent Sugar Mills & Distillery Ltd. Faisalabad. It was dried at a temperature of 70 °C for 24 hours, crushed using a Willy mill with stainless steel blades, and passed through a 2 mm sieve. The characteristics of SB were determined using the methods mentioned above and summarized in Table 1. The RS was collected from the Farm area of the University of Agriculture Faisalabad, dried at a temperature of 70 °C for 24 hours in an electrical oven, chopped using a Willy mill to create a powder of RS, sieved through a 2 mm mesh size sieve, and characterized as previously mentioned. The Si NPs were purchased from Alfa Acer, a Chinese company, and were of analytical grade with a purity of 98 %. The size of the NPs ranged from 1 to 100 nm.

The soil used in the experiment was obtained from the research farm of the University of Agriculture Faisalabad (Latitude: 31.4306° N, Longitude: 73.0699° E). It was then brought to the wirehouse, where it was air dried under shade. After drying, the soil was crushed using a wooden pestle and mortar; and sieved to remove pebbles and plant parts. A representative soil sample of 1 kg was collected for pre-contamination analysis. The saturation percentage of the soil was calculated by preparing a saturated paste. The soil texture was assessed using the hydrometric method, based on Stocks' law (Bouyoucos, 1962).

Soil pH and EC were measured using respective meters. Soluble cations (Ca+Mg) and anions (CO_3^{2-}, Cl^-, HCO_3^{1-}) were assessed via titration. Sodium (Na) and potassium (K) levels were determined using a flame photometer. The availability of Si and phosphorus for plants was determined using a spectrophotometer (Table 1). The wheat (*Triticum aestivum* L.) seeds used in the experiment were of the variety Akber 2019, and obtained from the University's seed bank. The chemicals used in the experiment were all of analytical grade with maximum purity. The pots used in the experiment had dimensions of 39 cm in diameter and 43 cm in height. They were made of ceramics and were specifically chosen for this experiment.

2.2. Experimentation

The soil was prepared and spiked with Cd at a rate of 20 mg/kg using $CdSO_4.8/3H_2O$. The Cd solution was prepared in distilled water based on the soil saturation percentage, and then applied to the soil and thoroughly mixed. The Cd-spiked soil was incubated in polythene bags for 30 days, adding distilled water to aid in Cd equilibration. After the incubation period, the spiked soil was air-dried, ground, and sieved through a 2 mm mesh sieve. The spiked soil was filled into polythene-lining pots at a rate of 10 kg/pot. The biogenic sources of Si, namely RHB, SB, and RS, were graded into three different sizes (150, 150–250, and 250–1680 µm represented as S1, S2, and S3) using a stack of sieves. The SiNPs and different sizes of biogenic Si sources were applied at a rate of 150 kg/ha of Si, according to (Al-Huqail et al., 2023) and (Rizwan et al., 2023). After applying the amendments to their respective pots, the treated soil was incubated for 30 days before sowing the wheat seeds. Two control treatments were established for comparison: one representing uncontaminated control and the other representing contaminated control. These controls were maintained to assess and contrast the effects of contamination. All treatments were randomly applied using a lottery method, and each treatment was replicated three times. The experiment followed a completely randomized design. The pots were placed in the wirehouse with controlled conditions including temperature (15–20 °C during sowing, and 30–35 °C during crop harvesting) and average relative humidity (70–75 % at sowing, and

Table 1
Properties of experimental soil and biogenic sources of silicon.

Properties of soil				
Parameters	**Units**	**Value**		
Saturation percentage	%	34.65		
Soil texture		Sandy clay loam		
pH of soil paste		7.85		
Electrical conductivity of extract	dS/m	3.45		
Catio exchange capacity	cmolc/kg	12.75		
Organic mater	%	0.78		
Cadmium	mg/kg	Not detected		
Soil available Si	mmol/L	0.50		
Properties of biogenic sources of silicon				
		RHB	**SB**	**RS**
Carbon	%	43.56	18.56	23.55
Nitrogen	mg/g	0.08	0.03	0.04
Phosphorus	mg/g	0.07	0.02	0.03
Potassium	mg/g	4.45	2.12	2.55
Silicon	mg/g	0.87	0.42	0.53
pH		7.87	7.21	7.11
Cation exchange capacity	cmolc/kg	18.75	15.66	16.56
Specific surface area	m^2/g	2.57	1.89	1.65

55–60 % during harvesting). The roof of the wirehouse was covered with glass. Wheat seeds were sown at a rate of 7 seeds/pot, and 5 plants were retained per pot after germination. Recommended doses of fertilizers including N, P, and K were applied at rates of 120, 90, and 60 kg/ha respectively, using urea, DAP, and SOP. The plants were irrigated with canal water, applied at regular intervals based on soil moisture levels and crop water requirements. Water quality parameters, such as pH (7.45), EC (0.55 dS/m), and concentrations of dissolved salts (5.50 meq/L), were regularly monitored to ensure suitability for agricultural use.

2.3. Determination of foliar photosynthetic pigments and gaseous exchange attributes

The chlorophyll a, b, and carotenoid concentrations were determined using a Hitachi U-1800 spectrophotometer, following the methodology of Lichtenthaler (1987). This method involves extracting chlorophyll and carotenoids from plant samples using a solvent, such as 80 % acetone, and then measuring their absorbance at specific wavelengths using a spectrophotometer. The non-destructive gas exchange parameters for mature leaves of the wheat crop were evaluated using a portable and open system gas exchange system with an infra-red gas analyzer (IRGA) (Analytical Development Company, Hoddesdon, England), between 10:00 a.m. and 12:00 p.m., and 50 days after sowing (Yong et al., 2010, 2014). Specifically, the gas exchange parameters for the portable IRGA instrument were calculated in accordance to Farquhar et al. (1980): Photosynthetic or CO_2 assimilation rate, μmol_{CO2} m^{-2} s^{-1}; transpiration rate, $mmol_{H2O}$ m^{-2} s^{-1}; stomatal conductance, mol_{H2O} m^{-2} s^{-1}; and the sub stomatal CO_2, μmol mol^{-1}.

2.4. Assessment of H_2O_2, MDA, and antioxidant enzymes

The H_2O_2 levels in fresh leaf samples were measured following (Lipkin, 1968)., while MDA content was assessed spectrophotometrically at OD 532 and 600 nm from fresh leaf tissue (Heath and Packer, 1968). Fresh leaves of wheat plant were homogenized in pH 7.0 phosphate buffer with 1 % polyvinyl pyrrolidine and 1 mM EDTA, followed by centrifugation to obtain frozen supernatant. SOD, CAT, APX, and POD activities were determined using specific assay mixtures and spectrophotometric measurements at different wavelengths.

2.5. Plant morphological parameters determination

At physiological maturity, agronomic parameters such as plant height, spike length, fresh weights of shoots and roots, were measured. Spikes were manually separated into grain and husk, air-dried, then oven-dried at 65°C for 72 hours, and weighed.

2.6. Assessment of Cd in soil and plants

Dried plant samples were digested with di-acid mixtures (HNO_3: $HClO_4$) and filtered before analysis on an atomic absorption spectrometer (AAS) to determine Cd concentration (Soltanpour, 1985). Soil samples were extracted with AB-DTPA, filtered, and then analyzed on the AAS for post-harvest Cd concentration (Soltanpour, 1985).

2.7. Uptake, translocation and bioaccumulation factors calculation

To calculate the Cd uptake by plant tissues, dry tissue weight was multiplied by Cd concentration in tissues. The Cd translocation factors (TF) from root to shoot and shoot to grain were computed by dividing respective Cd concentrations tissues. The bioaccumulation factor (BAF) was determined by dividing Cd concentration in plant tissue by Cd concentration in soil.

2.8. Translocation, harvest, immobilization, and health risk indices calculation

The translocation index (TI) was calculated as Cd concentration in grains divided by the sum of Cd concentration in tissues, multiplied by 100.

$$TI = \frac{\text{Cd concentration in grains}}{\text{Cd concentration in (shoots + grains)}} \times 100$$

The harvest index (HI) was computed as the sum of Cd concentration in grains and shoots divided by Cd concentration in roots, shoots, and grains, multiplied by 100.

$$HI = \frac{\text{Cd concentration in (shoots + grains)}}{\text{Cd concentration in (roots + shoots + grains)}} \times 100$$

The immobilization index (II) of Cd was determined by subtracting Cd concentration in the control treatment from Cd concentration in the amended treatment, divided by Cd concentration in the control treatment, and multiplied by 100.

$$II = \frac{\text{Cd concentration in (amended soil − contaminated control soil)}}{\text{Cd concentration in contaminated control soil}} \times 100$$

The health risk index (HRI) of Cd was calculated by dividing the daily intake of Cd by the reference dose (0.01) of Cd.

$$\text{Daily intake of Cd} = \frac{\text{Cd concentration in grains} \times 0.085 \times \text{average daily feeding (0.5 kg/person)}}{\text{Average body weight of an adult human (70 kg)}}$$

$$\text{HRI} = \frac{\text{Daily intake of Cd through grains}}{\text{Reference dose of Cd (0.01)}}$$

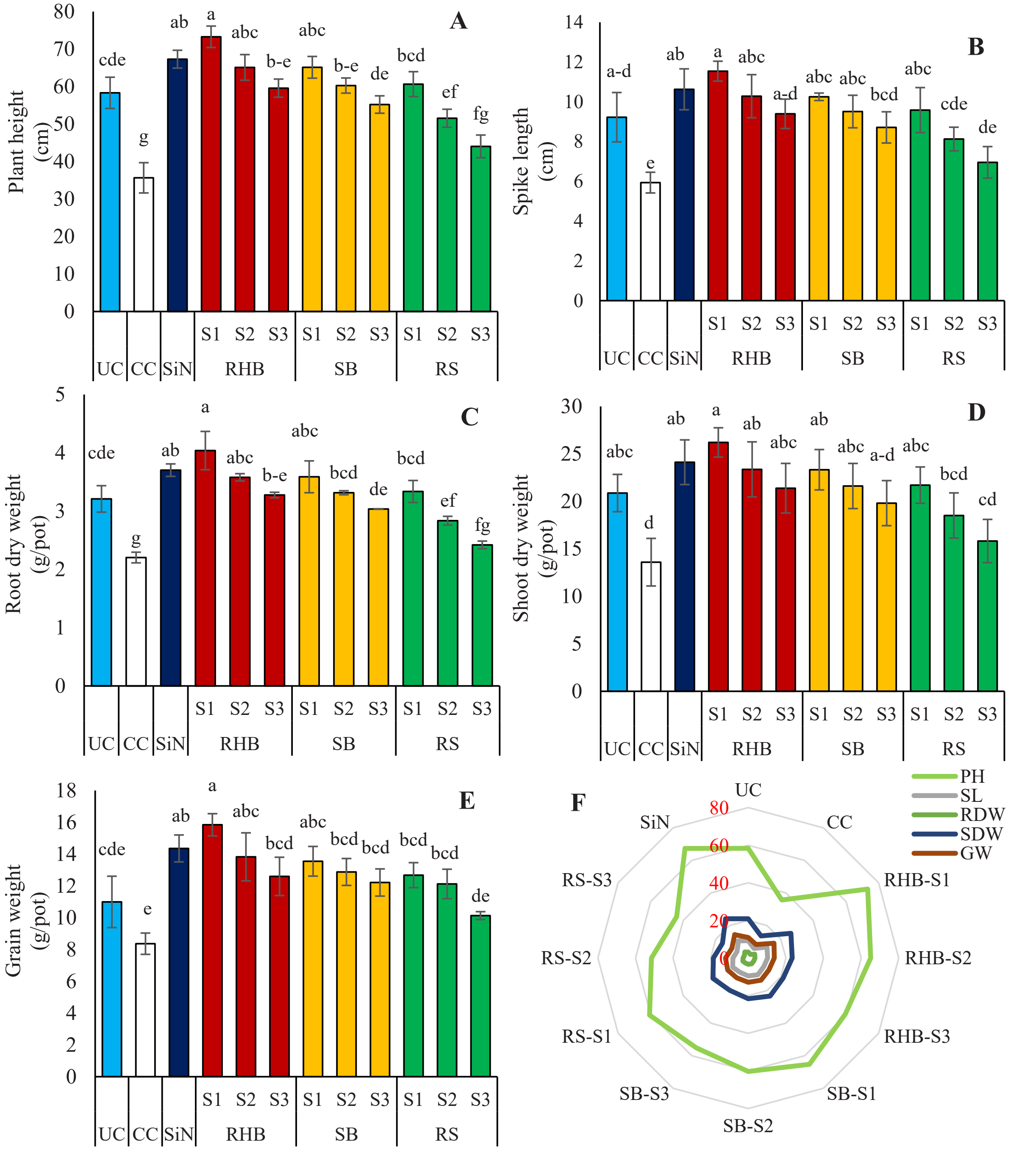

Fig. 1. The comparative effects of Si NPs and different sizes of biogenic sources of Si on plant height (A), spike length (B), root dry weight (C), shoot dry weight (D), grain weight (E), and morphological traits (F) of wheat plants under Cd stress. Bars indicating mean values, error bars showing standard deviation, and different lettering showing the significant different ($P < 0.05$) among the applied treatments. On X-axis, UC; uncontaminated control, CC; contaminated control, SiN; silicon nanoparticles, RHB; rice husk biochar, SB; sugarcane bagasse, RS; rice straw, S1, S2, and S3 representing 0–150, 150–250, and 1680 µm sizes of biogenic sources of Si.

2.9. Statistical analysis

The data were analyzed using SPSS 21.0 with analysis of variance, and treatment significance was determined via Tukey test at a 95 % confidence interval. Pearson correlation and PCA analysis were performed using Origin Pro 2019 and RStudio, respectively.

3. Results

3.1. Effect of different sources of Si and their sizes on morphology of wheat plant

The contamination of Cd significantly reduced the morphological characteristics of wheat plants, as illustrated in Fig. 1. The figure demonstrates that the contaminated control treatment has a negative impact on the growth and yield of the crop. The results indicate that the height of the plants and spike length was reduced by Cd stress to 35.66±4.04 and 5.94±0.52 cm, respectively. Similarly, the dry weight of the shoot, root, and grain was observed to be at its lowest in the Cd-contaminated treatment, measuring 13.60±2.51, 2.20±0.09, and 8.37±0.67 g/pot, respectively. The application of RHB in the smallest size (<150 μm) significantly increased plant height (73.28±2.86 cm), spike length (11.54±0.50 cm), shoot dry weight (26.21±1.54 g/pot), root dry weight (4.04±0.33 g/pot), and grain weight (15.85±0.70 g/pot) in Cd-contaminated soil. The SiNPs shown a minimal response compared to RHBS1 but were greater than the Cd control treatment. Results show that SiNPs increased plant height, spike length, shoot dry weight, root dry weight, and grain weight by 89 %, 79 %, 77 %, 68 %, and 72 %, respectively, compared to the Cd control treatment. Biochar application at 150–250 μm (RHBS2) significantly increased plant height (83 %), spike length (73 %), shoot dry weight (72 %), root dry weight (62 %), and grain weight (65 %) compared to the contaminated control treatment. Larger biochar size (1680 μm) showed intermediate results compared to RHBS1 and RHBS2 but outperformed the contaminated control treatment in wheat plant growth and yield parameters. Different sizes of SB (<150, 150–250, and 1680 μm) significantly enhanced wheat plant growth and yield, with notable variations among sizes. SBS1 resulted in the highest increase in plant height (83 %), spike length (73 %), shoot dry weight (72 %), root

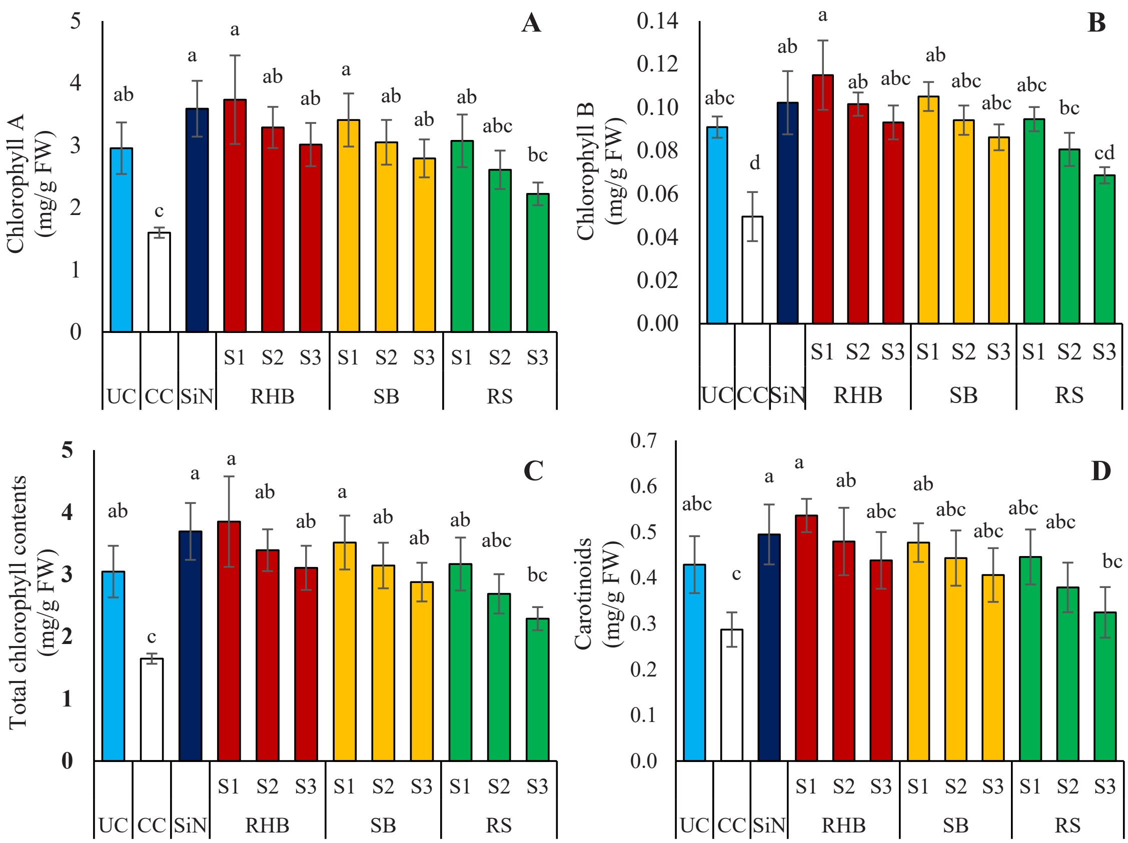

Fig. 2. The comparative effects of Si NPs and different sizes of biogenic sources of Si on chlorophyll A (A), chlorophyll B (B), total chlorophyll contents (C), and carotenoids (D) in wheat plants under Cd stress. Bars indicating mean values, error bars showing standard deviation, and different lettering showing the significant different (P < 0.05) among the applied treatments. On X-axis, UC; uncontaminated control, CC; contaminated control, SiN; silicon nanoparticles, RHB; rice husk biochar, SB; sugarcane bagasse, RS; rice straw, S1, S2, and S3 representing 0–150, 150–250, and 1680 μm sizes of biogenic sources of Si.

dry weight (63 %), and grain weight (62 %) compared to the control and other sizes. Similarly, RS in different sizes (RSS1, RSS2, and RSS3) significantly affected plant growth and yield, with RSS1 exhibiting the greatest response, increasing plant height, spike length, shoot dry weight, root dry weight, and grain weight by 70 %, 61 %, 60 %, 51 %, and 52 %, respectively, compared to the Cd-contaminated control treatment.

3.2. Effect of different sources of Si and their sizes on photosynthetic pigments in leaves of wheat plant

The findings unveiled the detrimental impact of Cd on the chlorophyll and carotenoids in wheat plants, as demonstrated in Fig. 2. This indicates that the Cd-control treatment resulted in the lowest measurements of chlorophyll a, b, total chlorophyll, and carotenoid levels, which was 1.60±0.08, 0.05±0.01, 1.65±0.08, and 0.29±0.04 mg/g FW, respectively. Conversely, the application of RHB, SB, and RS in different sizes (<150, 150–250, and 250–1680 μm) and SiNPs significantly improved the photosynthetic pigments in wheat plants under Cd-induced stress. Among these, the smallest size (<150 μm; S1) of RHB, SB, and RS exhibited the most substantial increase in chlorophyll a, b, total chlorophyll, and carotenoid contents in wheat leaves, greater the effects observed in the S2 and S3 sizes (Fig. 2). Specifically, RHBS1 increased the chlorophyll contents (a, b, and total) by 134 %, 132 %, and 134 % respectively, 87 % increase in carotenoids compared to the polluted control. Similarly, SBS1 demonstrated enhancements of 113 %, 112 %, 113 %, and 66 % in photosynthetic pigments relative to the control. By using the smallest RS size (RSS1), chlorophyll a, b, total chlorophyll, and carotenoids increased by 92 %, 91 %, 92 %, and 55 % respectively, in comparison to the Cd-control treatment. Moreover, SiNPs demonstrated superior results compared to the contamined control, RHBS2, RHBS3, SBS1, SBS2, SBS3, RSS1, RSS2, and RSS3, but lower than RHBS1, as exhibited in Fig. 2. The SiNPs increased the chlorophyll contents (a, b, and total) by 125 %, 106 %, and 124 % respectively, and 72 % increase in carotenoids relative to the polluted control.

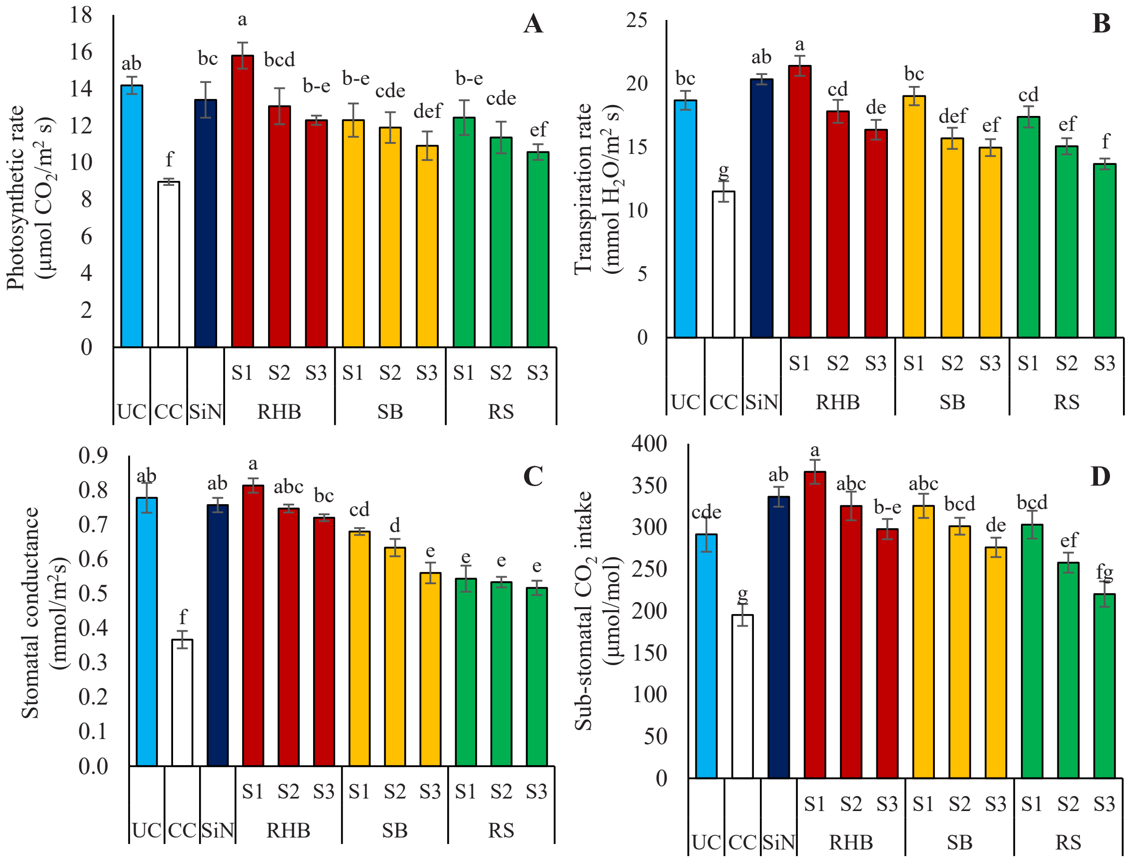

Fig. 3. The comparative effects of Si NPs and different sizes of biogenic sources of Si on photosynthetic rate, $\mu mol_{CO2}\ m^{-2}\ s^{-1}$ (A), transpiration rate, $mmol_{H2O}\ m^{-2}\ s^{-1}$ (B), stomatal conductance, $mol_{H2O}\ m^{-2}\ s^{-1}$ (C), and sub-stomatal CO_2 intake, $\mu mol \cdot mol^{-1}$ (D) in wheat plants under Cd stress. Bars indicating mean values, error bars showing standard deviation, and different lettering showing the significant different ($P < 0.05$) among the applied treatments. On X-axis, UC; uncontaminated control, CC; contaminated control, SiN; silicon nanoparticles, RHB; rice husk biochar, SB; sugarcane bagasse, RS; rice straw, S1, S2, and S3 representing 0–150, 150–250, and 1680 μm sizes of biogenic sources of Si.

3.3. Physiological response of wheat plant by the application Si sources and their sizes under Cd stress

The Cd-linked phytotoxicity significantly decreased the physiological attributes in wheat plants compared to the uncontaminated control treatment ($p < 0.05$). In Cd-spiked soils, the photosynthetic rates, transpiration rates, stomatal conductance, and sub-stomatal CO_2 intake in crop leaves decreased by 37 %, 38 %, 53 %, and 33 %, respectively, compared to the uncontaminated control treatment. However, application of SiNPs and various sizes of biogenic Si sources enhanced gaseous exchange attributes in wheat plants. The

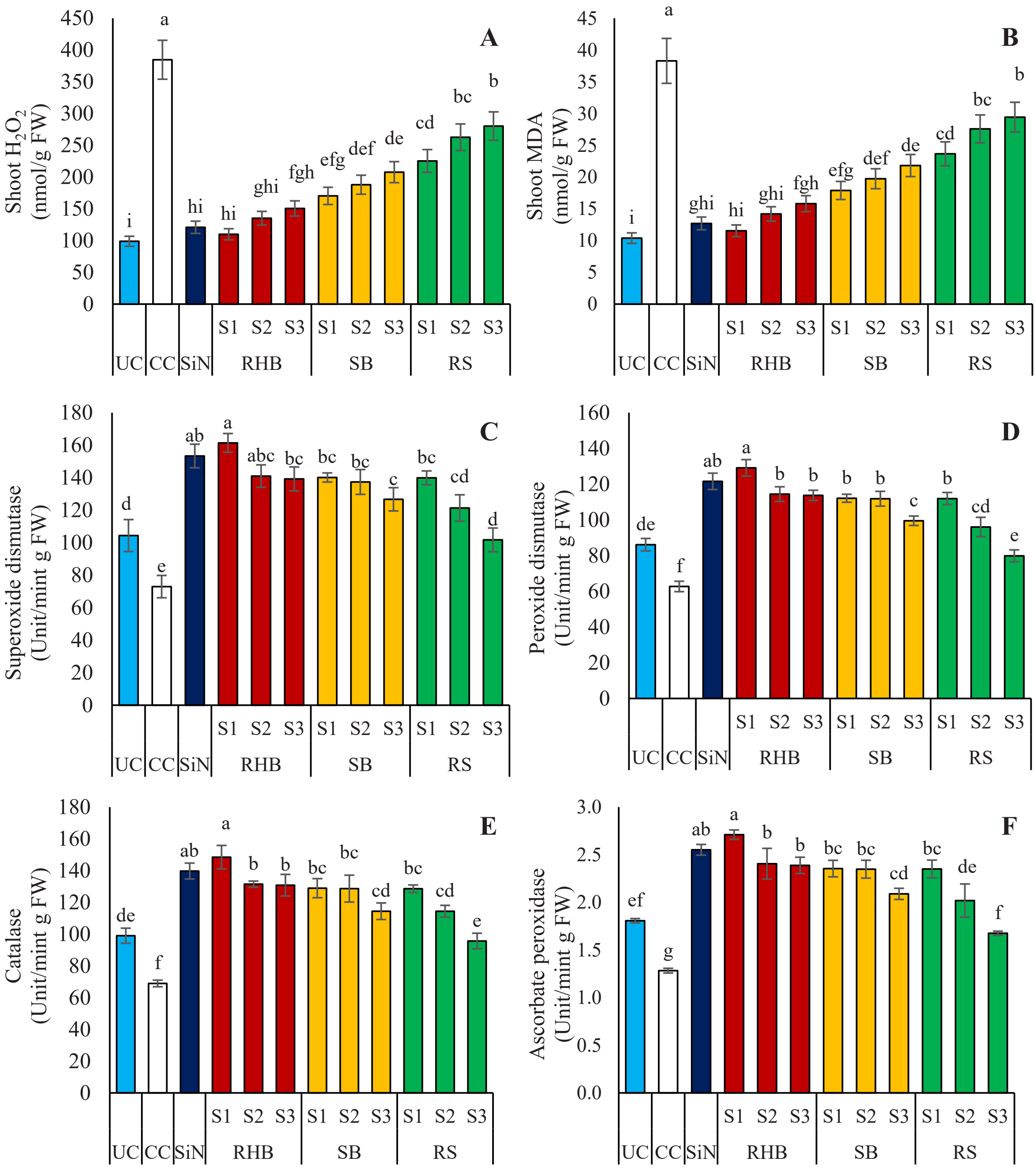

Fig. 4. The comparative effects of Si NPs and different sizes of biogenic sources of Si on shoot H_2O_2 (A), shoot MDA (B), superoxide dismutase (C), peroxidase dismutase (D), catalase (E), and ascorbate peroxidase (F) in wheat plants under Cd stress. Bars indicating mean values, error bars showing standard deviation, and different lettering showing the significant different ($P < 0.05$) among the applied treatments. On X-axis, UC; uncontaminated control, CC; contaminated control, SiN; silicon nanoparticles, RHB; rice husk biochar, SB; sugarcane bagasse, RS; rice straw, S1, S2, and S3 representing 0–150, 150–250, and 1680 μm sizes of biogenic sources of Si.

smallest biogenic Si sources, including RHBS1, SBS1, and RSS1, delivered significant improvements in photosynthetic rates, with increases of 76 %, 37 %, and 39 %, respectively, compared to the Cd-contaminated control. Similarly, these sources enhanced transpiration rates, stomatal conductance, and sub-stomatal CO_2 intake. Specifically, RHBS1 led to increases of 86 % in transpiration rates, 122 % in stomatal conductance, and 88 % in sub-stomatal CO_2 intake. SBS1 showed increases of 65 % in transpiration rates, 85 % in stomatal conductance, and 67 % in sub-stomatal CO_2 intake, while RSS1 resulted in increases of 51 % in transpiration rates, 48 % in stomatal conductance, and 55 % in sub-stomatal CO_2 intake compared to the Cd-spiked control treatment.

Although SiNPs exhibited higher performance compared to the control treatments and other sizes of biogenic Si sources, they were still less effective than RHBS1. SiNPs application resulted in increases of 49 % in photosynthetic rates, 77 % in transpiration rates, 106 % in stomatal conductance, and 72 % in sub-stomatal CO_2 intake compared to the Cd-control treatment. For other sizes of biogenic sources of Si, the ranking was as follows: RHBS2 > RHBS3 > SBS2 > RSS2 > SBS3 > RSS3 for photosynthetic rate; RHBS2 > RHBS3 > SBS2 > RSS2 > SBS3 > RSS3 for transpiration rate; RHBS2 > RHBS3 > SBS2 > SBS3 > RSS2 > RSS3 for stomatal conductance; and RHBS2 > SBS2 > RHBS3 > SBS3 > RSS2 > RSS3 for sub-stomatal CO_2 intake, compared to the Cd-spiked control treatment. Fig. 3

3.4. Production of H_2O_2, MDA, and antioxidant enzymes in wheat plant by Si sources and their sizes under Cd stress

The contamination of soil with Cd enhances the production of ROS such as H_2O_2 and MDA, as illustrated in Fig. 4. This indicates higher levels of H_2O_2 and MDA in the leaves of wheat plants. The maximum levels of H_2O_2 and MDA were observed in wheat plants cultivated in soil spiked with Cd, measuring 384.7±30.6 and 38.3 ±3.5 nmol $gFW+^{-1}$, respectively. The application of various sources and sizes of biogenic Si significantly reduced the production of H_2O_2. The trend of treatments applied for H_2O_2 levels remained as follows: RHBS1 (-71 %), SiNPs (-69 %), RHBS2 (-65 %), RHBS3 (-61 %), SBS1 (-56 %), SBS2 (-51 %), SBS3 (-46 %), RSS1 (-41 %), RSS2 (-32 %), and RSS3 (-27 %), compared to the Cd-control treatment. Similarly, in the case of MDA, RHBS1 showed the highest reduction (-70 %), followed by SiNPs (-67 %), RHBS2 (-63 %), RHBS3 (-59 %), SBS1 (-53 %), SBS2 (-48 %), SBS3 (-43 %), RSS1 (-38 %), RSS2 (-28 %), and RSS3 (-23 %), compared to the Cd-control treatment.

In terms of antioxidant enzymes such as SOD (-30 %), POD (-27 %), CAT (-30 %), and APX (-29 %), a decrease was observed in the

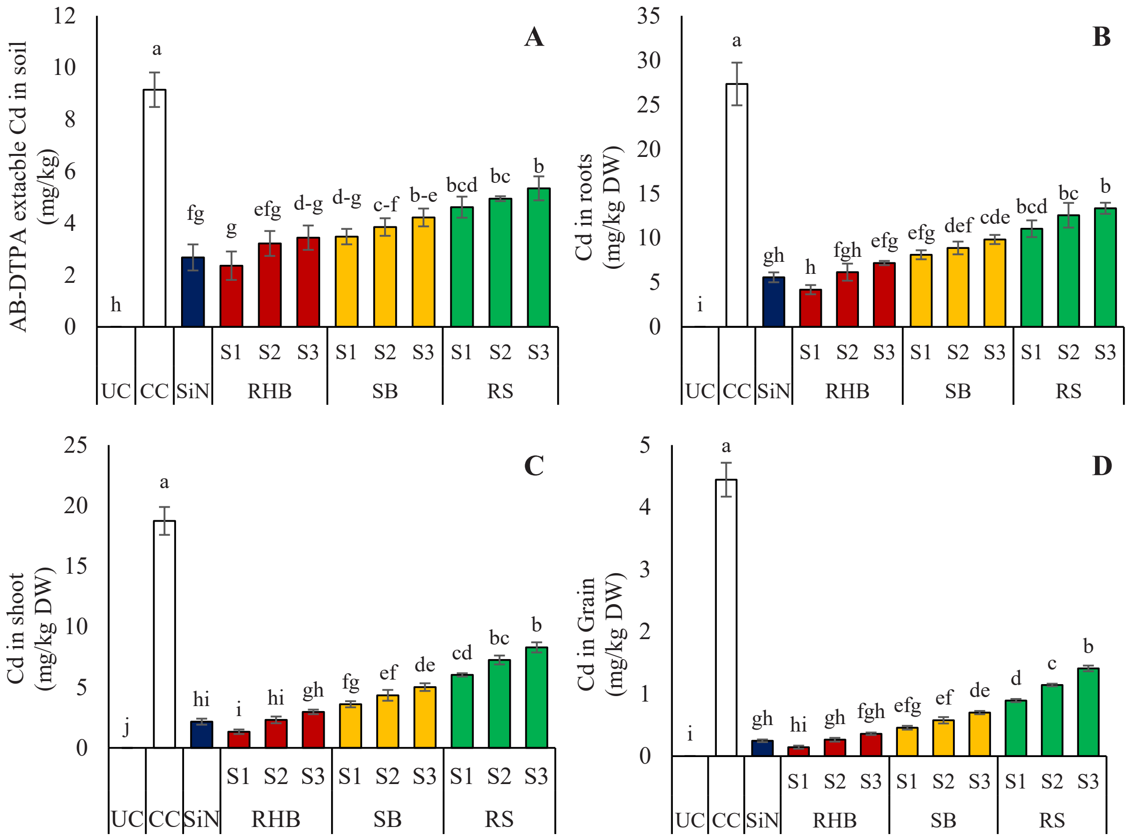

Fig. 5. The comparative effect of Si NPs and different sizes of biogenic sources of Si on AB-DTPA extractable soil Cd (A), Cd in root (B), Cd in shoot (C), and Cd in grain (D) of wheat plants under Cd stress. Bars indicating mean values, error bars showing standard deviation, and different lettering showing the significant different ($P < 0.05$) among the applied treatments. On X-axis, UC; uncontaminated control, CC; contaminated control, SiN; silicon nanoparticles, RHB; rice husk biochar, SB; sugarcane bagasse, RS; rice straw, S1, S2, and S3 representing 0–150, 150–250, and 1680 µm sizes of biogenic sources of Si.

Cd-control treatment compared to the uncontaminated control treatment (Fig. 4). Among the smallest biogenic Si sources, RHBS1 exhibited the highest increases in antioxidant enzyme activities, showing improvements of 121 % for SOD, 106 % for POD, 115 % for CAT, and 111 % for APX compared to the Cd-spiked treatment. SiNPs also demonstrated superior performance compared to the uncontaminated control, Cd-control, and all other biogenic Si treatments, including SBS1, RSS1, RHBS2, SBS2, RSS2, RHBS3, SBS3, and RSS3. Specifically, SiNPs led to increases of 110 % for SOD, 94 % for POD, 103 % for CAT, and 99 % for APX compared to the Cd-control.

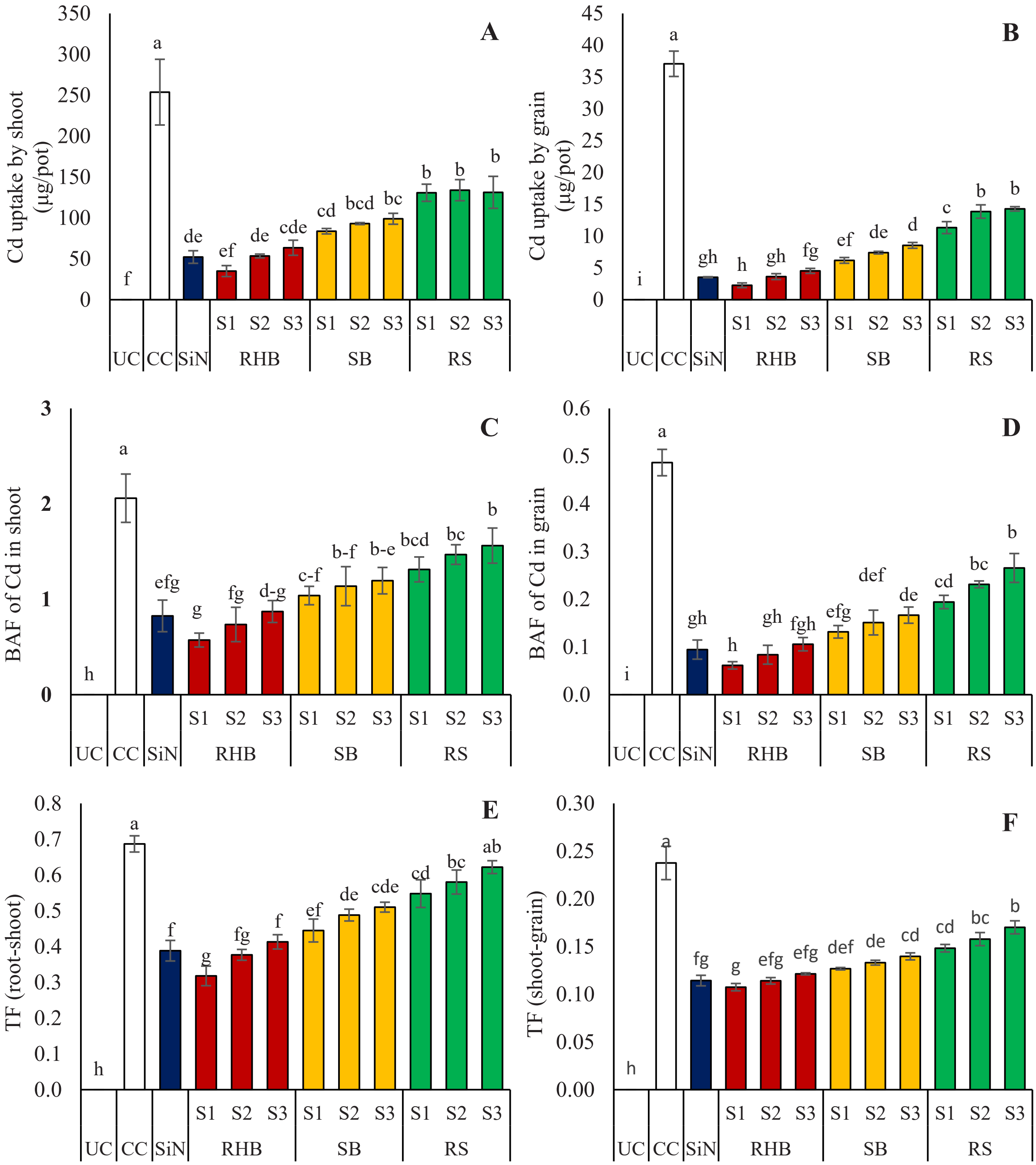

Fig. 6. The comparative effects of Si NPs and different sizes of biogenic sources of Si on Cd uptake by shoot (A), root (B), BAF of Cd in shoot (C), root (D), TF of Cd from root to shoot (E), and shoot to grain (F) of wheat plants under Cd stress. Bars indicating mean values, error bars showing standard deviation, and different lettering showing the significant different (P < 0.05) among the applied treatments. On X-axis, UC; uncontaminated control, CC; contaminated control, SiN; silicon nanoparticles, RHB; rice husk biochar, SB; sugarcane bagasse, RS; rice straw, S1, S2, and S3 representing 0–150, 150–250, and 1680 µm sizes of biogenic sources of Si.

When comparing other biogenic Si sources and sizes, RHBS2 showed the highest improvements in SOD activity (93 %), followed closely by SBS1 and RSS1 (92 %). RHBS3 and SBS2 also performed well, with increases of 91 % and 88 %, respectively. In contrast, SBS3, RSS2, and RSS3 showed more modest increases, ranging from 74 % to 39 %. For POD activity, RHBS2 achieved the greatest improvement (82 %), followed by RHBS3 (81 %) and SBS1 (79 %). RSS1 and SBS2 both showed increases of 78 %, while SBS3, RSS2, and RSS3 had lower increases ranging from 59 % to 27 %. For CAT activity, RHBS2 again led with a 91 % increase, closely followed by RHBS3 (90 %) and both SBS1 and RSS1 (87 %). SBS2 also showed a 87 % increase, while SBS3, RSS2, and RSS3 had more limited

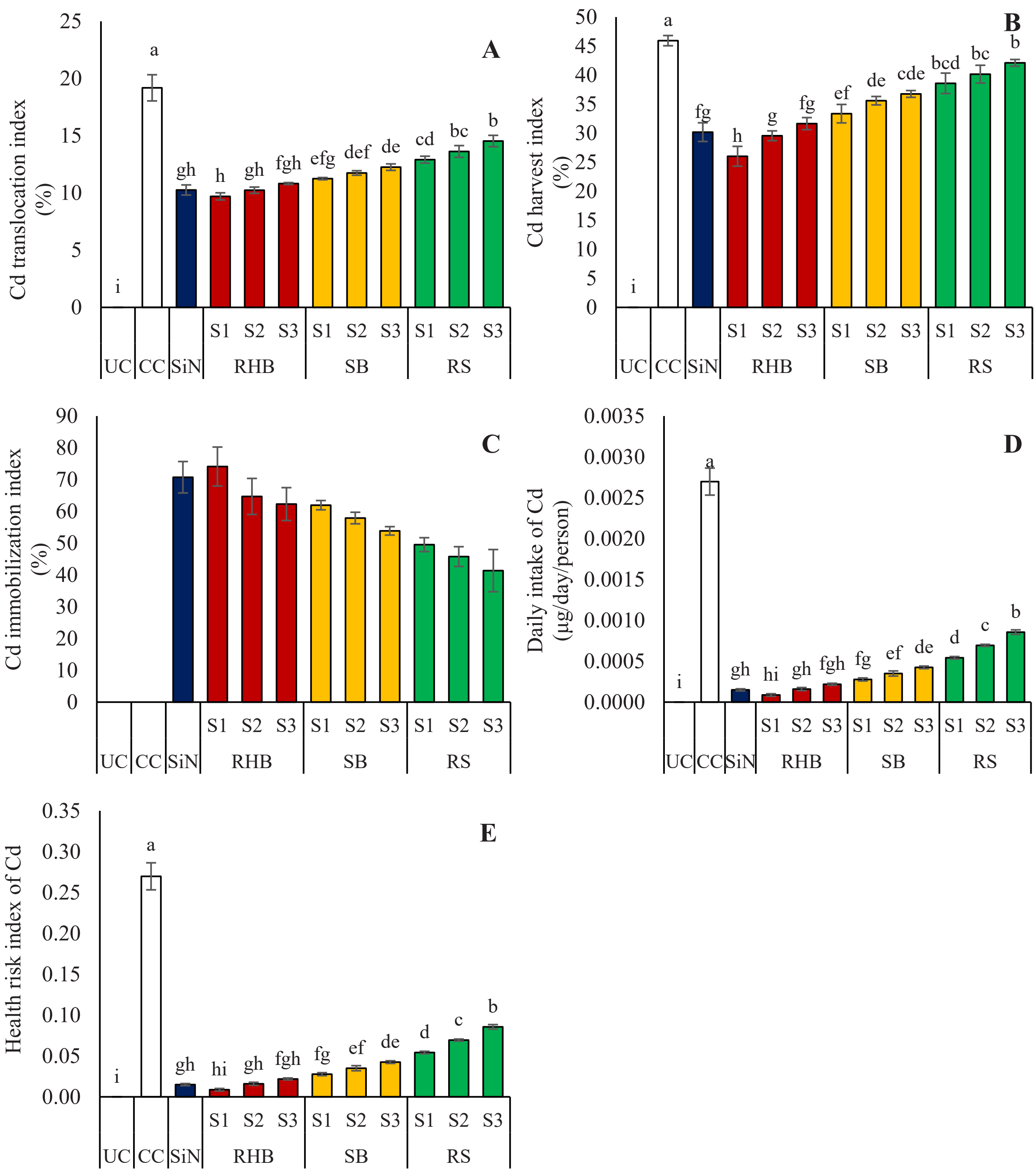

Fig. 7. The comparative effects of Si NPs and different sizes of biogenic sources of Si on translocation index of Cd (A), harvest index of Cd (B), immobilization index of Cd (C), daily intake of Cd (D), and health risk index of Cd (E) in wheat plants under Cd stress. Bars indicating mean values, error bars showing standard deviation, and different lettering showing the significant different ($P < 0.05$) among the applied treatments. On X-axis, UC; uncontaminated control, CC; contaminated control, SiN; silicon nanoparticles, RHB; rice husk biochar, SB; sugarcane bagasse, RS; rice straw, S1, S2, and S3 representing 0–150, 150–250, and 1680 µm sizes of biogenic sources of Si.

improvements, from 66 % to 39 %. Lastly, for APX activity, RHBS2 demonstrated the highest increase (87 %), followed by RHBS3 (86 %) and SBS1 (84 %). RSS1 and SBS2 both showed increases of 83 %, while SBS3, RSS2, and RSS3 had lower improvements ranging from 63 % to 31 %, all compared to the Cd-spiked control treatment.

3.5. Concentration of Cd in soil and plants affected by Si sources and their sizes

Significant variations in Cd distribution in soil and wheat plants were observed between the Cd-control and different Si application sources and sizes (Fig. 5). The Cd-control treatment exhibited the highest Cd concentrations in soil, roots, shoots, and grains. Among Si sources and sizes, RHBS1 demonstrated the highest reduction in Cd concentration in soil (-74 %), roots (-85 %), shoots (-93 %), and grains (-97 %) compared to the Cd-spiked control treatment. SiNPs showed a more favorable response in reducing Cd compared to other Si sources and sizes. Smaller sizes of SB and RS (<150 μm) resulted in lower Cd concentrations in soil and plants compared to larger sizes (150–250 and 1680 μm).

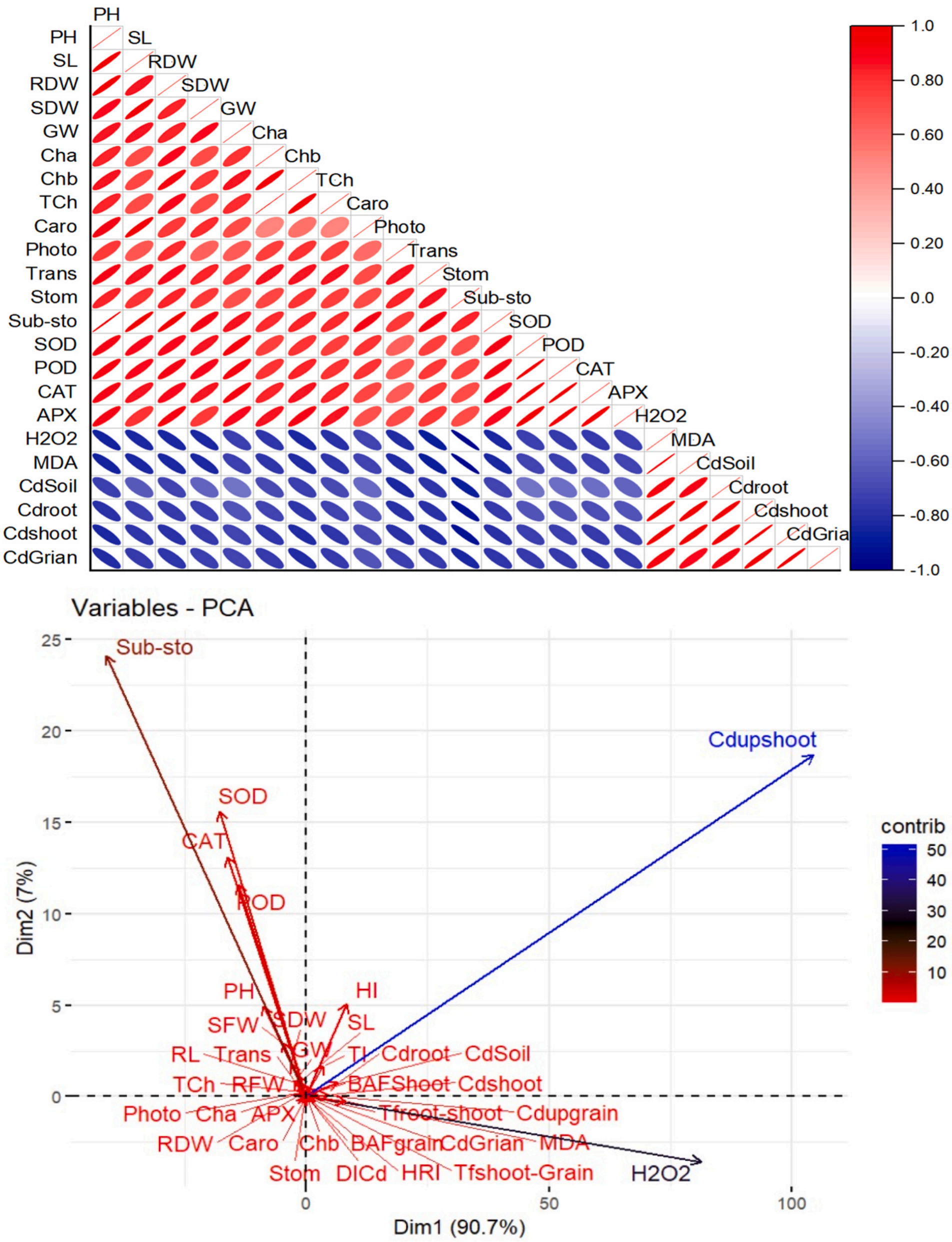

Fig. 8. Correlation and PCA analysis of studied parameters.

3.6. Uptake, translocation factor and accumulation of Cd in plants affected by Si sources and their sizes

The Cd contamination led to a significant increase in Cd uptake, translocation, and bioaccumulation in wheat plants. The highest Cd uptake was observed in the shoots (253.76±40.16 µg/pot) and grains (37.08±1.99 µg/pot) of the Cd-control treatment. This treatment also showed the maximum Cd translocation from roots to shoots (0.69±0.02) and from shoots to grains (0.24±0.02). The Cd bioaccumulation factor was highest in the contaminated control, with values of 2.06±0.25 for shoots and 0.49±0.03 for grains, compared to the uncontaminated control.

The application of Si from various sources and sizes significantly reduced these parameters in wheat plants grown in Cd-spiked soil. Among these, RHBS1 was the most effective in decreasing Cd uptake, translocation, and bioaccumulation, outperforming other Si sources and sizes, including SiNPs, RHBS2, RHBS3, SBS1, SBS2, SBS3, RSS1, RSS2, and RSS3 (Fig. 6).

3.7. Translocation, harvest, health risk, immobilization indices and daily intake of Cd in plants affected by Si sources and their sizes

The TI of Cd increased in wheat crops grown in contaminated soil, with the highest values observed in the contaminated control treatment (Fig. 7). This indicates a higher movement of Cd from the soil into the plant tissues. In contrast, the application of Si from various sources and sizes effectively reduced the Cd TI in wheat plants, highlighting the potential of Si in mitigating Cd translocation. Among the different Si amendments tested, RHBS1 (rice husk biochar with the smallest particle size) demonstrated the most substantial reduction in the Cd TI compared to SiNPs and other organic Si sources of varying sizes. This significant decrease suggests that RHBS1 was the most effective at limiting the movement of Cd from the soil into the wheat plants. Additionally, the HI of Cd, which reflects the proportion of Cd accumulated in the harvestable parts of the plant, decreased with the application of RHBS1. This indicates that RHBS1 was superior to SiNPs and other Si sources in reducing both Cd intake by the plants and the associated health risk. Furthermore, RHBS1 was also found to enhance the Cd immobilization index, which measures the extent to which Cd is immobilized in the soil rather than being taken up by the plants. RHBS1 outperformed SiNPs and other Si sources in this regard, demonstrating its effectiveness in immobilizing Cd and preventing its uptake by wheat plants. These findings underscore the efficacy of RHBS1 in reducing Cd translocation, lowering health risks, and improving Cd immobilization in contaminated soil (Fig. 7).

4. Discussion

The study demonstrated that Cd stress inhibits plant growth by disrupting photosynthetic pigments, reducing growth, and increasing Cd ion uptake. This toxicity significantly reduced wheat crop growth and yield (Fig. 1) due to elevated levels of H_2O_2 and MDA (Fig. 4), alongside nutrient uptake imbalances (Genchi et al., 2020). The Cd toxicity induces oxidative stress in plants, leading to ROS overproduction, lipid peroxidation, and hindered crop yield (Li et al., 2023; Liu et al., 2023; Al-Khayri et al., 2023; Goncharuk and Zagoskina, 2023). It is proposed that Cd enters plant cells through non-selective cation channels and essential cationic nutrient transporters, such as Fe-regulated transporter 1, Zn-regulated transporter-like proteins, and resistance-associated proteins (Chmielowska-Bąk et al., 2014; Sasaki et al., 2012). Once inside the plant cells, Cd accumulates in the cytoplasm, leading to increased production of ROS like superoxide and hydrogen peroxide (Chmielowska-Bąk et al., 2014). In plants exposed to the contaminated control treatment, excessive Cd accumulation resulted in elevated ROS production. These ROS induce oxidative damage within the plant cells, including the destruction of proteins and phospholipids, which ultimately leads to membrane damage and enzyme inactivation (Sasaki et al., 2012; Suzuki and Mittler, 2006). Fig. 8

The ROS production under Cd stress directly damages plant cells, reducing biomass production (Dhaliwal et al., 2021; Afonne and Ifediba, 2020). Additionally, Cd stress enhances active oxygen accumulation, leading to chloroplast damage and inhibition of vital photosynthetic enzymes (ur Rehman et al., 2018b, 2019a). The Cd absorption disrupts plant metabolism, chlorophyll synthesis, and antioxidant defense systems by increasing ROS production (ur Rehman et al., 2015, 2019; Usman et al., 2023; b, 2020). Cuypers et al. (2023) reported that Cd toxicity modulated the physiology, cause damages in the cellular structure of plants by producing ROS. Similarly, (Han et al., 2016) examined that oxidative stress caused by Cd contamination in soil, adversely affects plant development, resulting in the reduction of plant height and biomass. This experiment revealed that the different sizes of biogenic sources of Si and SiNPs have detrimental effects on mitigating Cd uptake and toxicity, enhancing plant growth, and antioxidant defenses. This approach could be harnessed to alleviate Cd soil contamination and foster sustainable agriculture. Numerous studies have reported the potential application of SiNPs for the remediation of Cd and improvement of plant growth under Cd stress (Jalil et al., 2023; Bano et al., 2024; Ahmed et al., 2023a). At present, there is little information available on the efficacy of the amendments (origin of materials) and their associated effects with Si NPs. This pioneer study further demonstrated the effectiveness of different particle sizes of Si and SiNPs.

The smallest size (<150 µm) of biogenic sources (RHB, SB, and RS) showed a better response than other sizes. The smallest size of RHB substantially increased plant growth relative to SiNPs under Cd stress (Fig. 1). The literature indicates that RHB contains a significant amount of Si (Ahmed et al., 2023b; Sohail et al., 2020a, 2020b). Reducing the size of RHB increases its surface area, which enhances its ability to release nutrients and provides more exposed surfaces for the adsorption of Cd (Shen et al., 2021). The size reduction of RHB can lead to an increase in the specific surface area of the amendment, which in turn can enhance its adsorption capacity for Cd ions (Liu et al., 2017). Studies have demonstrated that modifying the RHB by reducing its particle size can substantially increase its specific surface area, thereby enhancing its adsorption capacity for heavy metals such as Cd (Shen et al., 2021; Liu et al., 2017). Additionally, the presence of functional groups on the surface of RHB makes it favorable for the adsorption of pollutants, including heavy metals (Shen et al., 2021). The increased surface area and the presence of functional groups resulting from size reduction and modification offer more exposed sites for Cd ion adsorption. This enhanced surface area and functionality make RHB more effective in mitigating Cd stress in both soil and water environments (Shen et al., 2021; Liu et al., 2017; Li et al., 2019). In the current experiment, similar findings were observed. The smallest size of RHB demonstrated superior performance and exhibited the highest concentration of Si, as shown in Table 1. Moreover, the application of RHBS1, the smallest particle size of RHB, effectively reduced the production of H_2O_2 and MDA, both of which are indicators of oxidative damage to plant cell structures. This reduction in H_2O_2 and MDA suggests that RHBS1 helps mitigate cellular damage in plant leaves caused by Cd stress, reinforcing its effectiveness in reducing oxidative stress and improving plant health.

Research has demonstrated that the application of RHB can enhance plant defense mechanisms when plants are subjected to Cd stress (Ahmed et al., 2023a). This enhancement occurs through the improvement of chlorophyll content, photosynthesis rates, and other key physiological markers (Ahmed et al., 2023a; Gu et al., 2023; Li et al., 2024). Such enhancements are pivotal in counteracting the detrimental impacts of Cd toxicity on plant life. Studies indicate that the incorporation of RHB results in elevated chlorophyll contents, which are essential for facilitating photosynthesis and maintaining overall plant vigor (Al-Huqail et al., 2023; Shaghaleh et al., 2024). Furthermore, the application of biochar has been associated with heightened photosynthetic efficiency, a critical factor in supporting plant growth and fortifying resilience against stressors (Ahmed et al., 2023a). By positively impacting these physiological aspects, RHB aids plants in managing Cd-induced stress more effectively, thus promoting superior growth and development even amidst challenging environmental conditions.

The SiNPs have been shown to enhance plant growth when plants are subjected to Cd stress, employing diverse mechanisms (Yan et al., 2023; Bano et al., 2024). Primarily, SiNPs contribute to increased plant biomass in Cd-stressed environments, resulting in improved growth outcomes. This enhancement is associated with the altered functionality of SiNPs to positively influence photosynthesis in plants, thereby fostering enhanced growth (Li et al., 2022; Rizwan et al., 2023). Furthermore, SiNPs contribute to the reduction of Cd accumulation within plants, thus mitigating the toxic effects of Cd on growth. Through the combined actions of enhancing photosynthesis, diminishing Cd uptake, and fostering overall plant health, SiNPs effectively restore growth under elevated Cd conditions that are unfavourable for growth (Khan et al., 2024). Additionally, SiNPs influence the antioxidant defense system of plants when encountering elevated Cd by enhancing the activities of antioxidant enzymes and diminishing oxidative damage. There is further evidence to demonstrate that SiNPs can enhance the antioxidant defense mechanisms in plants by augmenting the activities of enzymes such as SOD, CAT, and POD (Zia-ur-Rehman et al., 2023). These enzymes are pivotal in neutralizing the ROS and protecting the plant cells from oxidative stress triggered by elevated Cd levels. Similar results were observed in the current experiment. Moreover, SiNPs have been observed to elevate the levels of antioxidants such as ascorbate and glutathione, further fortifying the plant's resistance against oxidative damage (Li et al., 2018). Through the regulation of the antioxidant defense system, the SiNPs protected tge wheat plants by counteracting the adverse effects of Cd stress, thereby preserving their physiological equilibrium and restoring growth.

5. Conclusions

The study highlighted the significant potential of RHB, a biogenic Si source, in alleviating Cd stress in wheat plants. The RHB, particularly in the smallest particle size category (<150 μm), showed notable effectiveness in restoring wheat growth under unfavourable Cd stress conditions. Application of RHB (<150 μm) led to substantial improvements in wheat growth parameters, including height, spike length, biomass, and grain weight, compared to Cd-contaminated treatments. The smallest sized particles of the biogenic Si sources, particularly those from RHBS1, improved wheat plant growth parameters, including photosynthetic rates, transpiration rates, stomatal conductance, and sub-stomatal CO_2 intake. These observations indicated that RHBS1 not only mitigated Cd stress but also restored the overall physiological performance. The application of RHBS1 resulted in a substantial decrease in Cd toxicity-related indicators. It significantly lowered the TI, HI, and Cd bioaccumulation in wheat plants. Additionally, RHBS1 was most effective in increasing antioxidant enzyme activities; with concomitant reduction in oxidative stress markers, such as H_2O_2 and MDA. RHBS1 delivered the highest reduction in bioavailable Cd in soil and plant tissues, highlighting its efficacy in reducing Cd uptake by wheat plants. Furthermore, RHB (<150 μm) significantly enhanced the activities of antioxidant enzymes, essential for mitigating oxidative stress induced by elevated Cd. The SiNPs, while effective, did not perform as well as RHBS1 in several aspects, including the reduction of Cd toxicity and the enhancement of plant growth parameters. These observations demonstrated the advantages of using RHBS1 over SiNPs and other Si sources, in managing elevated Cd levels on crops. These findings highlighted the potential of RHB as an effective amendment for mitigating Cd stress in wheat plants. Further research is needed to understand the underlying mechanisms (pathways of Cd transport) and to examine the efficacy of Si applications in various crop species. Moving forward, assessing the long-term effects of RHB, SB, RS, and SiNPs application on crop yield, soil health and microbial communities is essential to develop effective and sustainable agricultural practices in soils with containing Cd.

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CRediT authorship contribution statement

Ghulam Mohiuddin: Writing – review & editing, Software, Resources. **Hiba Shaghaleh:** Writing – review & editing, Validation, Software. **Hesham F Alharby:** Writing – review & editing, Visualization, Validation. **Yahya M. Alzahrani:** Writing – review & editing, Software, Funding acquisition. **Awatif M. Abdulmajeed:** Writing – review & editing, Visualization, Software, Investigation. **Hameed Alsamadany:** Writing – review & editing, Software, Resources. **Muhammad Zia-ur-Rehman:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Jean Wan Hong Yong:** Writing – review & editing, Funding acquisition, Conceptualization. **Yousef alhaj Hamoud:** Writing – original draft, Formal analysis, Data curation. **Muhammad Usman:** Writing – review & editing, Visualization, Methodology. **Ishaq A. M. Kakakhel:** Writing – review & editing, Visualization, Validation. **Muhammad Rizwan:** Writing – review & editing, Visualization, Validation, Conceptualization.

Declaration of Competing Interest

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.

Data Availability

Data will be made available on request.

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