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Nanopriming with chitosan-coated SiO_2NPs alleviates cadmium toxicity in wheat (*Triticum aestivum* L.) seedlings: an ecofriendly and sustainable approach

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Abstract

Heavy metals have become a major threat to the yield and grain quality of economically important cereal crops. Cadmium (Cd) is highly toxic and disrupts key physiological functions, biochemical processes, and cellular structures, retarding germination and seedling development. In recent years, SiO_2NPs have shown promising applications in sustainable agriculture. In this study, chitosan (CS)-coated SiO_2NPs were prepared and employed as the nanopriming agents to mitigate toxic effects of Cd in wheat seedlings. Nanopriming with CS-coated SiO_2NPs readjusted the biochemical parameters, which counteracted Cd stress (5 µM) in wheat seedlings compared to the control. They significantly upregulated the hydrolytic enzymes (protease and α-amylase), and enzymatic antioxidants (CAT, POD and SOD). They also induced a significant increase in sugars (reducing sugars, non-reducing sugars, and total sugars) and total soluble proteins, alleviating the Cd toxicity in wheat seedlings. The activated antioxidant potential controlled ROS production, avoiding lipid peroxidation and reducing MDA content under Cd stress. The nanopriming-modulated biochemical attributes as a countermeasure of Cd toxicity, ensuring sustainable seedling development. The highest concentration of Cd (BT3; 1.5 ppm) caused the greatest overall biochemical response, resulting in a 51.7%, 49.5%, 49.8%, and 33.5% increase in TSP, CAT, POD, and SOD, respectively, compared to the non-primed control, but decreasing MDA by 20.1%. The responses were generally more pronounced in BT_3 than the uncoated SiO_2NP treatment (AT_3), indicating the greater efficacy of chitosan coating on nanopriming response under Cd stress.

Keywords: Seed pre-conditioning, Climate-smart, Sustainable agriculture, Heavy metal toxicity, Cadmium stress.

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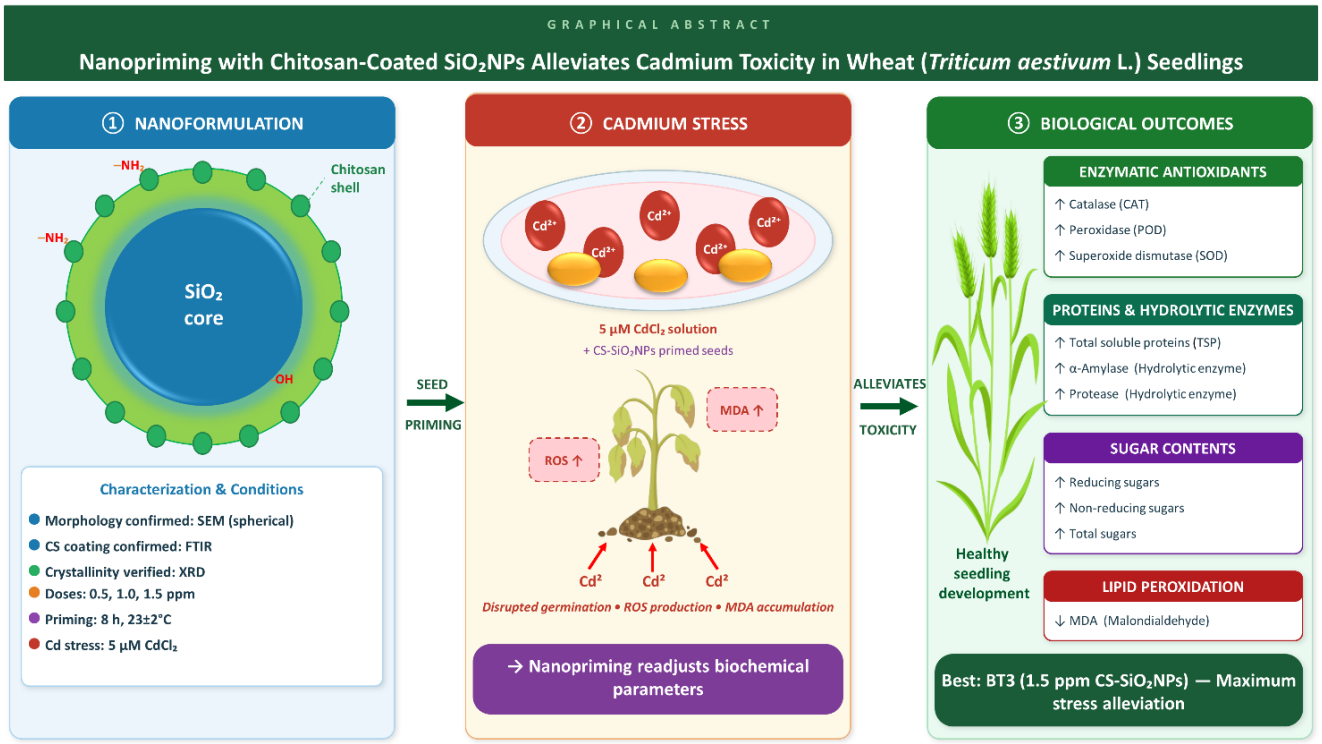
Graphical abstract



1. Introduction

Over the last few decades, heavy metals (HMs) have emerged as main soil pollutants that reduce yield of field crops, posing food security challenge and threatening the recent trends in sustainable agriculture. They are causing huge revenue loss to agro-based economies, especially in developing countries (Hou *et al.*, 2020; Rashid *et al.*, 2023). Cadmium (Cd) metal is frequently used in various industrial processes, such as the production of paints, pesticides, plastics, textile dyes, and rechargeable batteries. Therefore, Cd is causing air, water and soil pollution, resulting in numerous negative impacts on the environment. It shows toxicity to germinating seeds, seedling development and plant growth, leading to low crop yield (Huybrechts *et al.*, 2019). It causes ROS production, damaging the antioxidant machinery and sub-cellular signaling mechanisms (Hassan *et al.*, 2020). Its toxic nature damages metabolism, the respiratory process and energy production (Zhang *et al.*, 2020). It negatively influences the biosynthesis of proteins, sugars and secondary metabolites in germinating seeds and plants. It affects photosynthesis by disturbing the ionic balance and turgor pressure across the cells (Aslam *et al.*, 2023; Liu *et al.*, 2020). Such changes in biochemical attributes due to Cd toxicity retards germination and plant growth, which undermines qualitative and quantitative crop performance (El Rasafi *et al.*, 2022). Wheat (*Triticum aestivum* L.) is a major staple food and economically valuable cereal crop around the globe, especially in Asia. Excessive Cd levels reduce its productivity, causing significant economic losses. Generally, plants upregulate antioxidants and growth hormones to counter Cd toxicity (Gill *et al.*, 2021; Zhu *et al.*, 2021). Recently, nanopriming has become an easy seed pre-conditioning technique that supports them to tolerate abiotic stresses, including the toxic HMs (Bhatia & Gupta, 2022; Kumar *et al.*, 2021).

The primed seeds resist HM's toxicity through several countermeasures, including activation of antioxidants and metabolic activities, readjustment of osmolytes, and cell signaling (Wiszniewska, 2021; Wojtyla *et al.*, 2016). In recent years, nanomaterials have received considerable attention in sustainable agriculture owing to their wide range of applications for managing stress and disease in plants. They have been widely explored as climate-smart priming agents against biotic and abiotic stresses (Sarkar *et al.*, 2022; Singh *et al.*, 2022). Over the last few years, SiO_2NPs have shown promising applications in plant growth regulation owing to their stable and non-toxic nature (Wang *et al.*, 2022; Weisany *et al.*, 2024). Chitosan (CS), a natural biopolymer, has become a promising material with broad applications in plant growth regulation. It positively influences physiological and biochemical attributes in growing plants. It can upregulate antioxidants and increase biosynthesis of phytoalexin and other potent biomolecules (Mukarram *et al.*, 2023). It increases osmolyte levels and stomatal conductance, improving photosynthetic activity. It can act as an antiviral, antibacterial and antifungal agent, thus showing potential applications for disease

management in plants (Nandhini *et al.*, 2025; Suwanchaikasem *et al.*, 2024). The nanoformulation of CS, such as CSNPs, has become an attractive nano-carrier for controlled release of fertilizers, pesticides and other growth-promoting agents (Ma *et al.*, 2023; Saberi Riseh *et al.*, 2024).

The application of SiO_2NPs and CS using foliar spray, soil application and conventional seed treatment has been previously well studied, whereas seed nanopriming represents a different and more targeted mode of delivery (Khan *et al.*, 2023; Nile *et al.*, 2022). It provides active agents directly in the seed matrix in the pre-germination stage, allowing the metabolic reprogramming to occur at an early stage before exposure to Cd (Eevera *et al.*, 2023; Khan *et al.*, 2023). The combined nanoformulation of CS and SiO_2NPs was hypothesized to cumulate the beneficial effects of both the components, which may lead to synergistic antioxidant priming and better imbibition in the seed.

Considering the benefits of nanopriming and promising applications of CS and SiO_2NPs, we envisioned preparing CS-coated SiO_2NPs as an ecofriendly nanopriming agent to evaluate their efficacy against Cd toxicity in wheat seedlings.

2. Materials and Methods

2.1. *Preparation of CS-coated SiO_2NPs as nanopriming agent:*

The 3g of Na_2SiO_3 was dissolved in 15 mL of distilled water and stirred at RT for 6 hr. Then, the solution was neutralized with 1 M HCl, subsequently filtered and dried at 65 °C to obtain the white powder of SiO_2NPs (sample A).

The 50 mg of SiO_2NPs were slowly dissolved in 10 mL of 1% CS solution ((w/v) in acetic acid) followed by sonication and stirred for 24 hr. at RT. Afterwards, the mixture was subjected to centrifugation, rinsed with deionized water, and dried at 65 °C to obtain CS-coated SiO_2NPs (sample B) **(Figure 1)** (Saleh *et al.*, 2022).

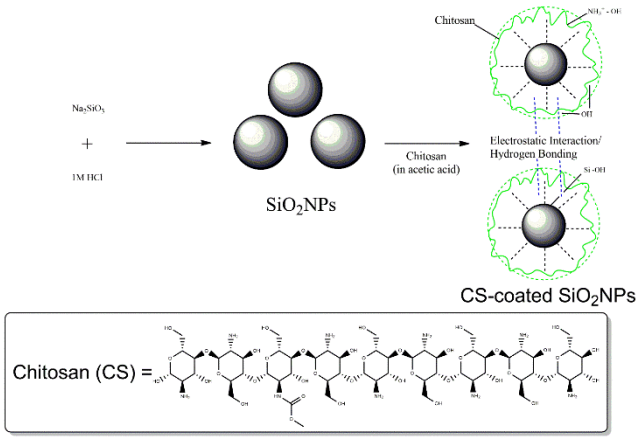


Figure 1. Preparation of CS-coated SiO_2NPs as nanopriming agent

The SEM analysis revealed a layered mesoporous structure of SiO_2NPs and a spherical nature of clustered CS-coated SiO_2NPs **(Figure 2a, b)**. SEM images were obtained from an FEI Nova NanoSEM 450 field emission scanning electron microscope that is fitted with a field-emission gun. Imaging was done at the accelerating voltage of 10 kV, spot size 2.5 and working distance of ~ 5.06 mm under high-vacuum condition, through the Through the Lense Detector (TLD) in secondary electron (SE/CN) signal mode.

The functional groups' interactions were detected using FTIR analysis **(Figure 2c)**. FTIR spectra were collected on the Agilent Technologies MicroLab spectrometer with a spectral resolution of 4 cm^{-1}. A total of 30 sample scans and 10 background scans were recorded in the spectral range 4000-650 cm^{-1}, using the Happ-Genzel apodization function during spectral acquisition.

The XRD validated the crystalline nature of SiO_2NPs and the semi-crystalline nature of CS-coated SiO_2NPs **(Figure 2d)**. The XRD patterns were collected with a Bruker D8 Advance diffractometer with a Theta-Theta goniometer and LynxEye position sensitive detector (PSD). The radiation was Cu-Kα with operating voltage and current of 30 kV and 25 mA, respectively. Continuous PSD fast-scan mode was used to collect data in the range 5° - 80° 2θ and with a step size of about 0.02° and a step time of 0.025 s.

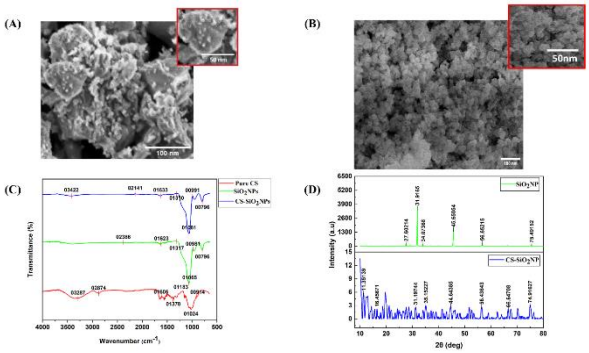


Figure 2. Characterization of CS-coated SiO_2NPs

2.2. *Nanopriming and germination of wheat seeds:*

Spring wheat (*Triticum aestivum* L. cv. Akbar-2019) was obtained from The Ayub Agriculture Research Institute, Faisalabad, Pakistan for this study. The wheat seeds were primed using SiO_2NPs (sample A) and CS-coated SiO_2NPs (sample B). The 0.5 (T_1), 1 (T_2) and 1.5 (T_3) ppm solutions of A and B were prepared for nanopriming of 25 g of seeds for 8 hrs at 23±2°C. Afterwards, they were dried to their original weight.

In parallel, some seeds were hydro-primed for comparison with nanoprimed and non-primed controls. The control, nanoprimed and hydroprimed seeds were allowed to germinate in 12 cm diameter petri plates at 23±2°C (24 seeds in three independent biological replicates (n=3)) (Anonymous, 1990) under Cd stress (5 µM $CdCl_2$) and normal conditions (Ci *et al.*, 2010). The germinating seeds were counted twice a day for 2 weeks. The germination data was recorded and used to determine the percentage of germination, germination index, energy of germination and mean germination time. The entire germination data will be reported in the separate whole-plant study for whole-plant growth responses. The seedling samples were stored at -80 °C before biochemical analyses.

2.3. *Biochemical studies of wheat seedling*

For antioxidant enzyme activity, seedling samples (0.1-0.2 g fresh weight) were homogenized in ice-cold 50 mM potassium phosphate buffer (pH 7.0 for CAT and POD; pH 7.8 for SOD; 1:10 w/v). For protein and sugar content, seedling samples (0.1-0.2 g fresh weight) were homogenized in 0.1% (w/v) TCA (1:10 w/v). Homogenates were centrifuged, at 10,000 x g for 20 mins at 4°C, and the supernatant was used for biochemical assays as described.

2.3.1. Estimation of Total soluble proteins, enzymatic antioxidants and MDA

The bovine serum albumin was employed as a standard for the analysis of total soluble proteins (TSP) (Bradford, 1976). According to a known method, a protein-dye complex was prepared using 5 µL of supernatant, 95 µL of 150 mM NaCl and 1 mL of dye reagent, and then analyzed at 595 nm.

The 40 mM H_2O_2, 50 mM potassium phosphate buffer (pH 7.0) and 20 mM guaiacol were mixed with 0.1 mL enzyme extract. One unit of enzyme activity expressed on a fresh weight basis corresponds to a change in absorbance at 0.01 units/min. The POD activity corresponds to a rise in absorbance at 470 nm, while a decrease in absorbance at 240 nm provides CAT activity (Chance & Maehly, 1955).

The superoxide dismutase (SOD) activity was estimated from photochemical reduction of nitroblue tetrazolium (NBT) as a reference. The amount of enzyme that causes 50% inhibition of photochemical reduction of NBT corresponds to the one unit of SOD activity (Giannopolitis & Ries, 1977). The mixture of 50 µL enzyme extract, 13 mM methionine, 75 nM EDTA, 50 mM potassium phosphate buffer (pH 7.8), and 1.3 µM riboflavin was mixed with 50 µM NBT. Subsequently, the absorbance at 560 nm after irradiating the mixture under a fluorescent lamp.

The lipid peroxidation was estimated from the malondialdehyde (MDA) contents, measured using thiobarbituric acid (TBA). The 0.2 g seedling sample was mixed with 0.1% TCA and subjected to centrifugation for 20 min. In the subsequent step, the supernatant was mixed with 0.05% TBA and 20% TCA and heated at 100 °C before recording the absorbance at 532 nm. The extinction coefficient (155 mM^{-1} cm^{-1}) was used to determine MDA (Heath & Packer, 1968).

2.3.2. Estimation of hydrolytic enzymes:

The TCA was used to measure protease activity following the casein digestion assay. The enzyme activity was defined as the amount of enzyme which released acid soluble fragments corresponding to 0.001 A280 per minute at 37°C and pH 7.8. Enzymes activity was expressed on fresh weight basis (Drapeau, 1977). The mixture of 100 µL of enzyme extract, 2 mL of TCA and 2 mL of 1% casein was incubated, followed by absorbance measurement at 280 nm.

The α-amylase activity was measured according to the well-established methods using DNS reagent. The amount of amylase enzyme that will release 1 mg of maltose per minute under the established assay conditions was defined as 1 unit of enzyme activity. Enzymes activity was expressed on fresh weight basis (Varavinit *et al.*, 2002). The 1 mL of enzyme extract, 1 mL of 1% starch solution and 1 mL DNS reagent were incubated for absorbance measurement at 540 nm.

2.3.3. Estimation of sugars:

The dinitrosalicylic acid was used for measuring of reducing sugars in seedling samples (Miller, 1959). Following the method, with 0.2 mL of extract in 1.8 mL dH_2O was mixed 1 mL DNS reagent and boiled for 10 min. Subsequently, the absorbance was recorded at 540 nm after adding 9 mL of distilled water. The phenol-sulfuric acid method was followed for the estimation of total sugars (Dubois *et al.*, 1951). The 1 ml of extract was mixed with 0.5 ml of 5 % phenol and 2.5 ml of H_2SO_4 before recording the absorbance at 490 nm. Further, a standard curve of glucose was used for the determination of reducing and total sugars. Furthermore, the non-reducing sugars were calculated from the difference between reducing sugars and total sugars.

3. Results

The nanoprimed, hydroprimed and non-primed controls were germinated under normal and Cd stress conditions. The seedlings that originated from these seeds were used for biochemical analyses and compared with the control. The significance of the results at $p \leq 0.05$ and $p \leq 0.01$ was evaluated employing a two-way analysis of variance (ANOVA; factors: priming treatment [8 levels] and cadmium stress condition [2 levels: normal and stress] followed by Tukey's HSD post-hoc test using XLSTAT software. Values of three replicates with S.D and relative percentage have been presented in the **Tables 1-3**. The relative percentage difference (RPD) was calculated by the following formula:

$$\text{Relative Percentage Difference (RPD)}: \frac{(\text{Treatment} - \text{Control})}{\left(\frac{\text{Treatment} + \text{Control}}{2}\right)} \times 100$$

Baseline under normal condition was the corresponding non-stress control; baseline under Cd-stress condition was the corresponding Cd-stress control.

3.1. *Effect of nanopriming on biochemical parameters in wheat seedlings:*

The measured biochemical response was improved by the application of nanopriming under both non-stress and Cd-stress conditions, and the responses were found to be increasing with the increasing concentration of the nanoparticles. Overall, the responses were stronger in CS-SiO_2NP treatments (BT_1-BT_3) compared to the SiO_2NP treatments (AT_1-AT_3) in the presence of Cd stress.

In the presence of nanopriming, the total soluble protein (TSP) was significantly elevated in both conditions with generally a concentration-dependent response. Under control conditions, TSP was observed to be increased by 21-42% and by 23-37% with SiO_2NP (AT_1-AT_3) and CS-SiO_2NP (BT_1-BT_3), respectively. The second increase was 31-33% and 36-51% for the Cd stress. The higher response of the treatments coated with CS (especially BT_3) suggests higher effect of coating under Cd stress **(Table 1)**.

Table 1. Effect of Chitosan- SiO_2NPs priming on enzymatic antioxidants and lipid peroxidation in wheat seedlings under normal and Cadmium stress

Treatments	Enzymatic Antioxidants									
	Normal					Under cadmium stress (5 µM)				
	Total soluble proteins (TSP) (mg/g f. wt.)	Catalase (CAT) (units/ g f.wt)	Peroxidase (POD) (units/g f.wt)	Superoxide dismutase (SOD) (units/g f.wt)	MDA (µM/g f. wt)	Total soluble proteins (TSP) (mg/g f. wt.)	Catalase (CAT) (units/ g f.wt)	Peroxidase (POD) (units/g f.wt)	Superoxide dismutase (SOD) (units/g f.wt)	MDA (µM/g f. wt)
C_0 (Non-primed seeds)	202.167±1.033 h	63± 1.082 b	17046.9± 1.9 c	136.097± 1.903 c	963.14 ± 3.855 ab	208± 1.022 h	38± 1.606 d	15098± 1 d	107.917± 1.083 d	997.790 ±1.581 a
C_H (Hydro-primed)	207.000±1.026 h (2.36 %)	64± 1.513 b (1.57%)	17087.3±1 .7 c (0.23%)	138.076± 1.924 c (1.5%)	903.21 ± 2.774 c (-6.43%)	215.833± 1.167 h (3.77%)	39± 1.429 d (2.60%)	15435.8± 1.2 d (2.21%)	114.697± 1.303d (5.40 %)	984.419 ±1.742 a (-1.349 %)
Sample AT_1 (0.5ppm SiO_2NPs)	251.333±1.667 g (21.68%)	82± 0.882 a (26.21 %)	19630.2±1 .8 bc (14.08%)	144.915± 0.8 b (6.40%)	895.33 ± 1.661 c (-7.32%)	287± 1 e (31.92 %)	58± 1.333 c (41.67 %)	19102.5± 1.5 bc (23.42%)	134.936± 1.064 c (22.23%)	941.145 ±1.855 b (-5.84%)
Sample AT_2 (1ppm SiO_2NPs)	306.000±1.000 c (40.86%)	83± 0.208 a (27.40 %)	24895± 1 a (37.42%)	150.364± 1 a (9.79%)	876.25 ± 1.742 d (-9.46%)	293.167± 0.833 e (33.93 %)	59± 1.155 c (43.30 %)	21165.6± 1.4 b (33.46%)	134.426± 1.574 c (21.48%)	930.048 ±1.952 b (-7.03%)
Sample AT_3 (1.5ppm SiO_2NPs)	311.500±1.500 c (42.57%)	83± 1.028 a (27.40 %)	25796.1± 1 .39 a (40.84%)	152.525± 0.9 a (11.43%)	849.01 ± 1.984 de (-12.58 %)	291.833± 1.167 e (33.6%)	61± 1.764 c (46.47 %)	25866.2± 1.2 a (52.57%)	138.864± 1.136 c (25.10%)	925.452 ±1.855 b (-7.52%)
Sample BT_1 (0.5ppm Chitosan-SiO_2NPs)	254.833±0.767 g (23.05%)	82± 1.528 a (26.21 %)	19744.7± 1.7 bc (14.67%)	146.320± 1.7 b (7.31%)	889.98 ± 1.016 c (-7.88%)	301± 1 d (36.54 %)	54± 0.882 c (34.78 %)	19094.5±1.5 bc (23.37%)	142.740± 1.26 b (28%)	881.597 ±1.548 c (-12.36 %)
Sample BT_2 (1ppm Chitosan-SiO_2NPs)	277.333±0.667 f (31.35%)	81± 1.528 a (25%)	21784.1± 1.9 b (24.40%)	147.311± 1.4 b (7.98%)	868.17 ± 1.823 d (-10.38 %)	326.5± 0.5 b (44.34 %)	56± 0.882 c (38.30 %)	21011.8± 1.2 b (32.75%)	144.926± 1.074 b (29.27%)	870.145 ±1.403 d (-13.67 %)
Sample BT_3 (1.5ppm Chitosan-SiO_2NPs)	296.500±1.5 d (37.83%)	84± 1.155 a (28.57 %)	24830.4± 1.6 a (40.08%)	152.550± 1.6 a (11.42%)	815.09 ± 0.903 f (-16.65 %)	353± 0.985 a (51.70 %)	63± 1.028 b (49.50 %)	25120.6± 1.4 a (49.84%)	151.266± 1.734 a (33.45%)	815.532 ±1.468 f(-20.10 %)

Note: The percentage values were calculated based on the non-stress control and Cd-stress control, respectively.

Table 2. Effect of Chitosan- SiO_2NPs priming on hydrolytic enzymes in wheat seedlings under normal and Cadmium stress

Treatments	Hydrolytic Enzymes			
	Normal		Under cadmium stress (5 µM)	
	α- Amylase (units/g f. wt.)	Protease activity (Units/g f. wt.)	α- Amylase (units/g f. wt.)	Protease activity (Units/g f. wt.)
C_0 (Non-primed seeds)	8.075± 0.187 i	2678.5± 0.45 h	12.104± 0.032 g	2587± 0.671 h
C_H(Hydro-primed)	8.642± 0.106 I (6.78%)	2754.5± 0.5 g (2.79%)	14.179± 0.020 f (15.79%)	2710.5± 0.326 g (4.64%)
Sample AT_1 (0.5ppm SiO_2NPs)	10.708± 0.292 h (28.03%)	2822± 2.5 f (5.24%)	16.547 ± 0.045 d (31.01%)	2837.5± 1.5 f (9.22%)
Sample AT_2 (1ppm SiO_2NPs)	12.132± 0.187 g (40.15%)	2922.5± 1.45 e (8.71%)	19.057± 0.094 c (44.62%)	2995.5± 0.5 e (14.62%)
Sample AT_3 (1.5ppm SiO_2NPs)	16.830± 0.170 d (70.31%)	3467.5± 1.259 c (25.68%)	21± 0.059 b (53.74%)	3612.5± 2.5 b (33.07%)
Sample BT_1 (0.5ppm Chitosan-SiO_2NPs)	11.321± 0.068 h (33.47%)	2854.5± 1.05 f (6.36%)	15.453± 0.055 d (24.30%)	2932.5± 2.5 e (12.50%)
Sample BT_2 (1ppm Chitosan-SiO_2NPs)	16.519± 0.481 d (71.19%)	2992± 1.002 e (11.07%)	16.236± 0.064 d (29.16%)	3212.5± 1.5 d (21.55%)
Sample BT_3 (1.5ppm Chitosan-SiO_2NPs)	22.377 ± 0.062 a (93.93%)	3790.5± 0.5 a (34.38%)	20.519± 0.481 b (51.59%)	3372.5± 0.986 c (26.34%)

Note: The percentage values were calculated based on the non-stress control and Cd-stress control, respectively.

Table 3. Effect of Chitosan- SiO_2 NPs priming on sugar contents in wheat seedlings under normal and Cadmium stress

Treatments	Sugar contents					
	Normal			Under cadmium stress (5 µM)		
	Reducing Sugars (mg/g f. wt.)	Non-reducing Sugars (mg/g f. wt.)	Total Sugars (mg/g f. wt.)	Reducing Sugars (mg/g f. wt.)	Non-reducing Sugars (mg/g f. wt.)	Total Sugars (mg/g f. wt.)
C_0(Non-primed seeds)	10.934± 0.054 f	13.227±0.079 h	24.160± 0.184 h	9± 0.246 g	14.422±0.058 f	23.422± 0.047 i
C_H (Hydro-primed)	11.976± 0.039 e (9.09%)	14.214±0.077 g (7.19%)	26.188± 0.225 g (8.05%)	10.853± 0.147 fg (18.67%)	14.650±0.085 f (1.57%)	25.504± 0.196 h (8.51%)
Sample AT_1(0.5ppm SiO_2NPs)	14.795± 0.205 c (30.01%)	14.904±0.096 f (11.92%)	29.699± 0.3 ef (20.57%)	14.208± 0.092 c (44.88%)	15.973±0.093 d (10.20%)	30.181± 0.219 d (25.22%)
Sample AT_2(1ppm SiO_2NPs)	15.286± 0.014 b (33.20%)	14.902±0.098 f (11.91%)	30.188± 0.312 e (22.18%)	14.491± 0.051 c (46.75%)	17.014±0.986 c (16.49%)	31.506± 0.494 d (29.43%)
Sample AT_3(1.5ppm SiO_2NPs)	16.090± 0.010 ab (38.16%)	15.612±0.388 e (16.54%)	31.702± 0.298 d (27%)	18.024± 0.098 a (66.78%)	18.387±0.613 b (24.17%)	36.411± 0.589 b (43.42%)

Sample BT_1(0.5ppm Chitosan-SiO_2NPs)	13.946± 0.054 cd (24.21%)	15.846±0.065 de (18.02%)	29.792± 0.106 f (20.88%)	13.090± 0.091 cd (37.03%)	15.216±0.284 e (5.36%)	28.306± 0.045 f (18.88%)
Sample BT_2(1ppm Chitosan-SiO_2NPs)	15.110± 0.090 b (32.07%)	16.695±0.305 c (23.18%)	31.805± 0.03 d (27.32%)	15.141± 0.086 b (50.87%)	16.348±0.715 d (12.52%)	31.489± 0.076 d (29.38%)
Sample BT_3(1.5ppm Chitosan-SiO_2NPs)	16.893± 0.107 ab (42.83%)	18.349±0.154 b (32.44%)	35.242± 0.261bc (37.31%)	17.039± 0.096 ab (61.74%)	17.952±0.548 c (21.81%)	34.991± 0.259 bc (39.61%)

Note: The percentage values were calculated based on the non-stress control and Cd-stress control, respectively.

Nanopriming had a general positive effect on the expression of antioxidant enzymes. Under normal conditions, CAT activity was elevated by 25-28% in both SiO_2NPs and CS- SiO_2NPs while under Cd stress there was a higher elevation of CAT activity, 46% for AT_3 and 49% for BT_3. Activity of POD also increased with the concentration of the nanoparticles from 14-40% under normal conditions and 23-52% under Cd stress, with maximum activity at AT_3 and BT_3, respectively. The SOD activity had relatively small increases under the normal condition (6-11%) but the activity was greater under Cd stress (25% for AT_3 and 33% for BT_3). In general, the antioxidant response was stronger under Cd stress, especially at the highest concentration of CS-SiO_2NP **(Table 1).**

However, the MDA content decreased with increasing concentrations of nanoparticles, showing a progressive decrease in lipid peroxidation, whereas the antioxidant enzymes increased with nanoparticle concentration. When no stress was applied the change in MDA was 7-12% for SiO_2NPs and 7-16% for CS- SiO_2NPs. In Cd stress, the reductions were 5-7% and 12-20% respectively, the most considerable decrease being for BT_3. Therefore, CS coating improved (under Cd stress) the reduction of lipid peroxidation **(Table 1)**.

Nanopriming has also increased the activities of hydrolytic enzymes, but the sensitivity of the enzyme to nanopriming varied among the treatments and stress conditions. In normal conditions, the activity of α-amylase increased gradually from 28% for AT_1 to 70% for AT_3, and from 33% for BT_1 to 93% for BT_3 in the CS-coated treatments. The activity of α-amylase under Cd stress was enhanced by 31-53% by SiO_2NPs and 24-51% by CS- SiO_2NPs. In control conditions, protease activity also increased in a concentration-dependent manner, with AT_1-AT_3 concentration, the activity increased by 5-25% and BT_1-BT_3 concentration, 6-34%. The increases were 33% for AT_3 and 26% for BT_3 under Cd stress. The results showed a significant rise in the activity of α-amylase and protease especially at the high concentration of nanoparticles after nanopriming, suggesting an increase in hydrolytic activity after nanopriming (**Table 2**).

Nanopriming also influenced the carbohydrate metabolism, with the concentration of the nanoparticles generally increasing the concentration of the non-reducing sugars and total sugars. In normal conditions, the level of non-reducing sugars increased by 11-16% and 18-32% in SiO2NPs and CS-SiO_2NPs respectively, while in Cd stress it was increased by 10-24% and 5-21% respectively. Results showed that the total sugars concentration was elevated by 20-27% with SiO_2NPs and 43% with AT_3 under non-stress conditions while under Cd stress, the total sugars concentration increased by 39% with CS-SiO_2NPs. The total effect of the nanoparticles on sugar accumulation was greater with higher concentrations, although some parameters were affected more than others by the CS-coating effect **(Table 3)**.

4. Discussion

Cd is a highly phytotoxic element that adversely affects physiological and biochemical parameters in germinating seeds, resulting in yield and quality losses. Therefore, seeds have developed various mechanisms to tolerate Cd and alleviate its toxicity.

Germinating seeds primarily use proteins as a source of C and N, vital for the synthesis of biomolecules during seedling establishment. An array of physiological and biochemical functions, including signal transduction, metabolic activities, and antioxidant defense, is controlled and coordinated by various enzymes, which are essentially proteins. Cd toxicity disrupts the TSP, generally disturbing vital processes, leading to abnormalities during germination and subsequent developmental stages (Bai et al., 2015). The Cd inactivates the enzymes either by replacing essential cofactors or by blocking thiol and carbonyl groups on their surface (Mostofa et al., 2015; Muszynska & Labudda, 2019; Rodriguez-Celma et al., 2010). Generally, seeds and developing plants increase their TSP content to counter the abiotic stresses, including HMs. The rice seedlings increased TSP and counterbalanced the toxic effects of Cd under SiO_2NPs treatments (Faisal et al., 2024). Spinach alleviated the Cd toxicity by increasing TSP following SiO_2NPs foliar application (Ullah et al., 2025). The exogenous application of CS remodeled biochemical parameters in wheat seedlings, including antioxidant enzyme activity, sugar levels, and increased TSP (17-36%) as a measure of Cd stress tolerance (Liu et al., 2021). The CS applications at lower doses upregulated the contents of antioxidants, chlorophyll, and TSP in pea (*Pisum sativum* L.) to alleviate Cd toxicity (Rasheed et al., 2020). The seed priming with CS

activated the antioxidant machinery and increased TSP as a Cd acclamatory response in black seeds (*Nigella sativa* L.) (Arab *et al.*, 2025). In a recent study, the foliar applications of CSNPs in *Solanum lycopersicum* protected against Cd toxicity by regulating enzymatic and non-enzymatic antioxidants, osmoprotectants and TSP contents (Faizan et al., 2021). The CS application controlled the glycolysis and protein expression as a stress-responsive measure in tomato seedlings (Attia *et al*., 2021). The CS increased the aspartate, glutamate and other amino acids, activated enzymes for N reduction, and modulated carbon and nitrogen assimilation in wheat seedlings (Zhang *et al.,* 2017). Accordingly, in our study, TSP content increased significantly following nanopriming with CS-coated SiO_2NPs, counteracting the negative impacts of Cd in wheat seedlings. The overexpression of TSP as key enzymes may have regulated antioxidant defense, metabolic activities, and cellular signaling during germination and seedling development.

Germinating seeds and growing plants activate antioxidant machinery as a main counter-step to resist HM's toxicity. They maintain controlled ROS production for the integrity of biomembranes by upregulating SOD, POD and CAT and neutralize HM toxicity (Moreira *et al.*, 2020; Yang *et al.*, 2012). Rice seedlings counteracted the Cd toxicity by upregulating its antioxidant potential due to SiO_2NPs treatments (Ashraf *et al.*, 2024; Muhammad *et al.*, 2025). The *Momordica charantia* L. seedlings activated the enzymatic antioxidants, showing significant resistance to Cd following nano-SiO_2 treatment (Sun *et al.*, 2023). The *Satureja hortensis* L. also showed tolerance to toxic effects of Cd by upregulating CAT, SOD and POD activities following the foliar application of SiO_2NPs (Farajzadeh Memari-Tabrizi *et al.*, 2021). The exogenous CS applications activated the enzymatic antioxidants and enhanced photosynthetic efficiency, enabling edible rape plants (*Brassica napus* L.) to withstand the detrimental effects of Cd stress (Zong *et al.*, 2017). The CS treatments significantly improved antioxidant status and upregulated photosynthesis, protecting the maize (*Zea mays* L.) seedlings from Cd toxicity (Qu *et al.*, 2019). In our case, the priming treatments activated the SOD, POD and CAT activities as a counterbalancing approach to resist Cd toxicity. The higher antioxidant activities were correlated with the lower level of lipid peroxidation, indicating that the antioxidants either prevent or slow the ROS mediated cellular damage. The significant decrease in MDA levels was correlated with nanopriming, indicating decrease in oxidative stress and Cd toxicity associated with increased antioxidant activity.

The hydrolytic enzymes, including α-amylase and protease, play a vital role in mobilizing stored reserves as a stress-acclamatory response during germination and plant growth. The hydrolytic enzymes exhibit reduced efficiency and adverse impacts due to Cd toxicity, leading to interrupted germination and seedling development (Anwar *et al.*, 2021). The α-amylase loses its efficiency and functioning after the replacement of Ca with Cd (Shiyu *et al.*, 2020). The wheat seedlings exhibited resistance to Cd by modulating the biochemical attributes, including activation of hydrolytic enzymes, following SiO2NPs treatments (Li *et al.*, 2025). The maize seedlings also exhibited sustainable growth after SiO_2NPs treatments (Wang *et al.*, 2024). The SiO2NPs treatments of stevia positively influenced germination by activating the hydrolytic enzymes and other biochemical parameters (Hasanaklou *et al.*, 2023). The upregulated hydrolytic enzymes induced tolerance against Cd toxicity following the foliar application of SiO_2NPs in wheat (Thind *et al.*, 2021). Wheat seed priming with CS increased the activity of protease and α-amylase along with other biochemical features, resulting in improved germination and seedling development (Hameed *et al.*, 2013). In this study, the protease and α-amylase exhibited increased activity, inhibiting Cd toxicity and ensuring sustainable seedling development.

Cd toxicity negatively influences and reduces sugar contents during seed germination and other developmental stages (Mishra *et al.*, 2014). Seeds and growing plants use sugars as vital resources against the negative impacts of HMs. They provide insulation against HM and assist germination and seedling development by modulating biochemical attributes, respiration, and metabolic activities (Jeandet *et al.*, 2022). They insulate the cellular structure by regulating ROS and acting as osmoprotectants (Farooq *et al.*, 2022; O'Hara *et al.*, 2013; Sami *et al.*, 2016). Maize seedlings alleviated the toxic impacts of Cd by ROS scavenging role of sugars (Karalija & Selovic, 2018). Also, the rice seedlings mitigated Cd toxicity by regulating ROS with non-reducing sugars and regulation of metabolism (Wang *et al.*, 2020). The wheat crop performed better with readjusted biochemistry and sugar contents following the silicon NP treatments (Li *et al.*, 2023). In this study, priming treatments significantly increased the sugar contents as a countermeasure to resist Cd toxicity.

The significant improvements of the chitosan coated SiO_2NPs (BT_1, BT_2, BT_3) could be explained by the coating of chitosan, which provides both physicochemical and biological enhancement for the measured biochemical parameters that were consistently better than that of the uncoated SiO_2NPs (AT_1, AT_2, AT_3). The positively charged seed surface carries a negative charge, and chitosan is a cationic biopolymer that contains free amino and hydroxyl groups, which can electrostatically interact with the seed surface, ensuring the retention of nanoparticles on the seed surface and improving the contact between the seed surface and the nanoparticles during imbibition at the initial stage (Hameed *et al.*, 2013). The improved seed–particle interaction might help the Si to be absorbed into the plant and to alleviate Cd toxicity by improving the integrity of cell walls, antioxidant defense and reducing the bioavailability of Cd to the plant via co-precipitation and intracellular compartmentalization (Wang *et al.*, 2022). Furthermore, chitosan has the ability to activate plant defense signaling pathways on its own, and influence expression of antioxidant-related genes, as well as biosynthesis of osmoprotectants (Mukarram *et al.*, 2023).

These synergistic effects would be stronger when coupled with the SiO_2NPs, offering twofold benefits: increased Si-mediated stress protection, decreased Cd bioavailability, in addition to chitosan-mediated defense activation and antioxidant effect. Such a synergistic effect could account for the improved performance of coated nanoparticles relative to the equivalent uncoated SiO_2NPs at the same concentration.

Principal component analysis (PCA) biplots were generated using OriginPro 2025b (OriginLab Corporation, Northampton, MA, USA) to evaluate the collective effects of seed priming treatments on biochemical parameters of wheat seedlings under normal and Cd-stressed conditions (**Figure 3a, b**). The first two principal components explained 94.21% and 96.44% of the total variance under normal and Cd stress conditions, respectively, indicating strong inter-variable correlations and reliability of the PCA model.

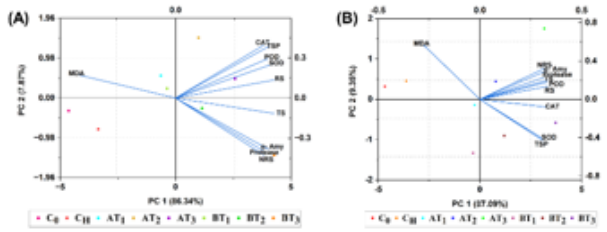


Figure 3. PCA analysis of CS-coated SiO2NPs against Cd

Under normal conditions (**Figure 3a**), primed treatments, particularly CS-coated SiO_2NPs (BT_1-BT_3), were positively associated with total soluble proteins (TSP) and antioxidant enzymes (CAT, POD, SOD), demonstrating that nanopriming induced a metabolically activated state even in the absence of stress. Hydro-primed and control seeds showed weaker associations with these biochemical traits, indicating limited physiological enhancement.

Under cadmium stress (**Figure 3b**), a clear separation was observed between lipid peroxidation (MDA) and antioxidant/metabolic parameters. CS-coated SiO_2NPs -primed seedlings clustered closely with CAT, POD, SOD, and TSP, whereas MDA was oriented in the opposite direction, confirming that nanopriming mitigated Cd-induced oxidative damage. The stronger grouping of antioxidant enzymes suggests a coordinated defense response triggered by CS-coated SiO_2NPs.

Overall, the PCA indicated correlative patterns, suggesting that CS-coated SiO_2NPs seed priming was associated with distinct biochemical profile that included increased antioxidant activities, higher protein and sugar levels and lower MDA concentrations under Cd stress. These patterns are correlations among the measured variables that do not necessarily imply a direct mechanism.

Wheat seed preconditioning with CS-coated SiO_2NPs triggered a biochemically activated seed state that is consistent with a priming response, contributing to Cd tolerance during seedling establishment. It regulated the enzymatic antioxidants, hydrolytic enzymes and other biomolecules to mitigate the Cd toxicity for sustainable growth.

5. Conclusions

Nanopriming has become a facile and innovative approach for managing stress and supporting climate-smart and sustainable plant growth. Seed preconditioning with CS-coated SiO_2NPs significantly increased total soluble proteins and sugars to counteract Cd toxicity. It avoided peroxidation and reduced MDA by controlling ROS production through activation of antioxidant enzymes. Further, it upregulated the protease and α-amylase enzymes which positively influenced the germination and seedling development. The findings showed that nanopriming with CS-coated SiO_2NPs successfully reduced the effects of Cd toxicity by modulating biochemical attributes for sustainable establishment of seedlings.

6. Future Recommendations

This study has a number of limitations that should be considered. First, the direct quantification of Cd uptake in the seedling roots and shoots by ICP-MS or AAS was not conducted, which would give a definite proof of decreased Cd uptake and should be considered as a priority for future research. Second, the uptake of silicon into seedling tissues was not quantified; follow-up studies should confirm delivery of nanoparticles by ICP-OES or ICP-MS analysis. Third, no histochemical ROS localization procedures were performed by DAB or NBT staining nor were fluorescent ROS detection procedures performed in the present study, and the MDA levels and SOD activity data are functional measurements of ROS within the scope of the present study. Fourth, gene expression analysis of antioxidant and stress responsive genes (SOD, POD, CAT, APX) was not feasible within the scope of the present work and it is recommended as an important step to provide molecular level proof of the observed biochemical responses. Fifth, the study was done only in the seedling stage, hence further testing of nanopriming efficacy in pot or soil condition is important for future research. Sixth, there was a lack of a chitosan-only control in the experimental design, which would provide further information regarding the independent contribution of the SiO_2NP core and the CS coating. The study of stability of the nanoparticles under the priming conditions (in aqueous suspension, 23 ± 2°C for 8h) and the release kinetics of the CS-coated SiO_2NPs during seed imbibition were not determined and should be investigated in future studies. Seventh, the tested concentration range (0.5, 1.0 and 1.5 ppm) is only a preliminary proof of concept and needs to be optimized further under greenhouse conditions. Lastly, CS and amorphous SiO_2 are both considered as low-toxicity and biodegradable, but long-term environmental effects after multiple applications of nanoparticles, especially on soil microbiota and aquatic ecosystems, need to be evaluated in the future studies.

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Conflicts of Interest

"The authors declare no conflict of interest".

Credit Author Statement

Arruje Hameed: Conceptualization, Writing, Supervision. **Ayesha Saeed:** Experimentation, data curation and analyses. **Razia Noreen**: Sources, Member of the supervisory committee. **Nosheen Aslam:** Sources, Member of the supervisory committee.

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