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Exploring the synergistic benefits of biochar and gibberellic acid in alleviating cadmium toxicity

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Cadmium (Cd) toxicity significantly threatens agricultural productivity and food safety. Developing effective strategies to enhance plant tolerance to Cd stress is essential. This study investigates the synergistic effects of biochar (BC) and gibberellic acid (GA3) on mitigating Cd toxicity in maize (*Zea mays*), focusing on their impact on oxidative stress markers and antioxidant enzyme activities. Soil samples were collected from the Cholistan Institute of Desert Studies (CIDS) and analyzed for trace metal ions and other properties. Biochar was produced from fruit and vegetable waste, washed, washed, deashed, and mixed with 10 ppm GA3. FH-1036 hybrid maize seeds were sterilized and planted in pots containing soil with varying Cd levels (0, 8, and 16 mg Cd/kg soil). Twelve treatments were established, including control, GA3, BC, and their combinations under different Cd stress levels. Plants were irrigated to maintain 60% field capacity and harvested at the V10 growth stage. Hydrogen peroxide (H_2O_2) contents and activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) were measured in roots, stems, and leaves. Statistical analysis was performed using OriginPro 2021, with ANOVA and Fisher's LSD test used to determine significant differences. GA3 and BC treatments significantly reduced H_2O_2 levels in maize roots, stems and leaves under Cd stress. The combined treatment of GA3 + BC showed the most significant reduction in H_2O_2 levels across all plant parts, reducing root H_2O_2 by 50%, stem H_2O_2 by 55%, and leaf H_2O_2 by 53% under severe Cd stress (16 mg Cd/kg). SOD activity increased under non-stress conditions but decreased under Cd stress, with the highest activity observed in the combined treatment. POD activity followed a similar pattern, with GA3 + BC treatment resulting in the most significant increases under non-stress conditions and the least reductions under Cd stress. CAT activity showed substantial increases with GA3 + BC treatment, particularly under severe Cd stress, with a notable rise over the control. APX activity also exhibited enhancements with GA3 and BC treatments, especially in the combined treatment under various Cd stress levels. This study highlights the potential of combined BC and GA3 treatments in improving Cd stress tolerance in maize. Future research should focus on field trials and the long-term impacts of these treatments on crop productivity and soil health.

Keywords Agricultural resilience, Cadmium stress, Environmental mitigation, Maize cultivation, Plant growth promoters, Soil remediation

Introducing heavy metals (HMs) into the environment through industrial activities, mining operations, and improper waste disposal poses a significant threat to ecosystems and human health. Metals such as lead (Pb), cadmium (Cd), and mercury (Hg) accumulate in soil and water, leading to soil pollution, water contamination, and severe health issues, including neurological disorders and organ impairment[1]. Persistent heavy metals in agricultural soils are taken up by crops, entering the food chain and presenting risks to human health.

Maize (*Zea mays* L.), a staple crop, is notably a significant source of dietary Cd for humans. Despite its importance, maize exhibits resilience to Cd stress and is often utilized in the phyto-management of contaminated soils[2]. However, prolonged Cd exposure profoundly impacts maize growth by compromising its structural

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integrity and disrupting essential morphological, physiological, and biochemical processes. This includes detrimental effects on photosynthesis, chloroplast ultrastructure, and increased oxidative damage through ROS overproduction. Cd uptake through plant roots adversely affects agricultural production by disrupting photosynthesis, modifying chloroplast ultrastructure, enhancing lipid peroxidation, and increasing oxidative damage due to ROS generation. These effects vary based on plant type, exposure duration, absorption amount, sequestration, and geographical location[3–5].

Cadmium (Cd) accumulation in maize is a complex process influenced by various factors including soil Cd concentration and plant physiology[6]. Cd is primarily taken up by the maize roots from contaminated soils, where it initially accumulates due to its affinity for root tissues. The extent of Cd uptake and its subsequent distribution within the plant is governed by soil conditions such as pH and organic matter content, which affect the metal's bioavailability[7]. Once absorbed, Cd is translocated through the xylem to the above-ground parts of the plant, including the stems and leaves. Although the roots generally accumulate higher concentrations of Cd, significant amounts can also be found in the stems and leaves. The maize grains, or kernels, may also contain Cd, which raises concerns about food safety. The accumulation of Cd in maize tissues leads to various physiological and biochemical disruptions, such as impaired root development, reduced nutrient uptake, and oxidative stress[8]. These disruptions manifest as stunted growth, chlorosis, and reduced biomass, reflecting the plant's struggle to manage and detoxify the heavy metal.

Gibberellic acid (GA3) emerges as a crucial plant growth regulator. GA3 initiates various physiological and developmental processes, including root production, flowering, cell division, maturity, and seed germination. It enhances plants' resilience to environmental challenges such as salt, cold, drought, and heavy metal stress by modulating antioxidant enzyme activity[9]. GA3 reduces excessive intracellular ROS generated during stress, protecting plants from environmental stressors. Exogenous GA3 effectively alleviates oxidative stress in wheat induced by salt stress, enhancing soil nutrients, and increasing plant output. Gibberellins regulate various plant growth processes, including seed germination, stem elongation, and flowering[10–12].

Simultaneously, biochar (BC) emerges as a promising solution to mitigate the detrimental effects of heavy metal contamination in soils. Produced by controlled pyrolysis of organic material in an oxygen-limited environment, BC has shown significant positive outcomes in pot and field testing, enhancing growth, biomass, and other factors in Cd-contaminated soils. Incorporating BC into soil is a viable solution to mitigate harmful heavy metal impacts[13]. BC improves plant growth and soil productivity and is effective in absorbing and reducing HM concentrations in soils, particularly in HM-contaminated areas. As an organic soil amendment, BC aids in soil immobilization, showcasing favorable characteristics such as a fundamental nature, porous texture, energetic functional groups, and greater cation exchange capacity (CEC). Soils treated with BC exhibit reduced Cd transport and accumulation[14–16]. Through compound generation and cation exchange techniques, BC rapidly absorbs Cd, Pb, and Cu. The use of BC to remove Cd from soil has gained considerable interest, improving biological, chemical, and physical soil characteristics. Recycling discarded straws for BC production is ecologically friendly and economically practical. BC rapidly improves soil physicochemical characteristics, promoting plant photosynthetic capacity, enhancing growth, and increasing the retention ability for hydration, nutrients, and nutrition levels[17,18].

Gibberellic acid and biochar contribute significantly to the regulation of antioxidant enzymes, which play a critical role in scavenging reactive oxygen species (ROS) and mitigating oxidative stress in plants exposed to cadmium toxicity[19]. GA3 enhances the activity of essential antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD)[20]. SOD catalyzes the dismutation of superoxide radicals into hydrogen peroxide, which is then further broken down by CAT and POD into water and oxygen, thereby reducing ROS levels and protecting cellular components from oxidative damage. GA3 influences the expression and activity of these enzymes, thereby boosting the plant's ability to manage oxidative stress and maintain cellular redox balance. Concurrently, biochar improves soil health and plant growth by increasing cation exchange capacity (CEC), enhancing soil structure, and supporting beneficial microbial communities[21]. These improvements contribute to reduced availability and uptake of Cd, decreasing the overall oxidative stress on plants. Additionally, biochar can adsorb heavy metals, including Cd, effectively reducing their bioavailability in the soil and limiting their entry into plant tissues. The combined application of GA3 and BC is hypothesized to offer a synergistic effect by both directly enhancing the antioxidant defense mechanisms and indirectly reducing the oxidative stress imposed by Cd contamination, leading to improved plant growth and resilience.

This study aims to evaluate the potential positive effects of gibberellic acid GA3 and BC on maize growth under Cd stress. Specifically, it seeks to investigate the individual and combined applications of GA3 and BC to determine their effectiveness in mitigating the adverse impacts of Cd on maize. The objectives of this research are to assess the individual effects of GA3 on maize growth, physiological parameters, and biochemical responses under Cd stress, and to evaluate the individual effects of BC on soil properties, Cd bioavailability, and maize growth under Cd stress. Furthermore, the study aims to investigate the combined effects of GA3 and BC on maize growth, physiological parameters, and biochemical responses under Cd stress in maize. By addressing these objectives, this research endeavors to bridge existing knowledge gaps and provide insights into the efficacy of GA3 and BC as potential interventions for mitigating Cd stress in maize. The findings are expected to contribute to the development of practical and sustainable strategies for managing heavy metal contamination in agricultural systems, ultimately supporting food security and environmental sustainability. The novelty of this research lies in its comprehensive approach, integrating GA3 and BC to address Cd stress in maize. This study aims to elucidate the individual and combined effects of these treatments and provide a sustainable, practical solution for improving crop resilience and productivity in Cd-contaminated soils. The findings will contribute to the broader understanding of phyto-management strategies and their potential applications in agricultural practices, particularly in areas affected by heavy metal contamination.

Materials and methods

Soil sampling and analysis

The soil samples for the experiments were sourced from the Cholistan Institute of Desert Studies (CIDS), The Islamia University of Bahawalpur, Pakistan. They consisted of calcareous sandy loams, known for their fertility and low levels of organic matter. Procedures outlined by[22] were followed to identify trace metal ions in the soil samples. After collection, the samples underwent a series of steps. Ten milliliters of nitric acid (HNO_3) were added to an Erlenmeyer flask containing one gram of dried soil, and the mixture was allowed to stand overnight. Controlled heating up to 200 °C, followed by cooling and treatment with HNO_3 and perchloric acid ($HClO_4$), was performed[23]. Further heating at 280 °C continued until fumes from $HClO_4$ became apparent. After cooling, hydrochloric acid was introduced, and another heating-cooling cycle was conducted. The resulting solution, mixed with 1% hydrochloric acid (HCl), was filtered using Whatman filter paper number 42.

Trace metal ions in the filtered solution were measured using an atomic absorption spectrophotometer (AAS), specifically the Thermo Scientific iCE 3000 Series. Distinct soil attributes were determined using specific methods: pH determination involved analyzing soil-saturated paste with a pH meter (model: Hanna HI2211), soil organic content (SOC) was quantified using the potassium dichromate ($K_2Cr_2O_7$) method; electrical conductivity (EC) was assessed by mixing soil and deionized water at a 1:10 ratio followed by analysis on an EC meter (model: Hach HQ40d); soil phosphorus (P) analysis followed the Olsen method; for soil potassium (K) analysis, extraction with ammonium acetate ($NH_4CH_3CO_2$) and flame photometry were employed (model: Jenway PFP7)[24–26]. The properties of the soil are presented in Table 1.

Biochar's composition and nutritional content

BC was generated using waste materials sourced from local vendors selling fruits and vegetable juices. The gathered waste underwent drying under sunlight and subsequent fragmentation into small pieces. During BC preparation, the maximum temperature reached was 570 ∘C after 20 min of ignition with an average temperature of ~ 325 ± 5 °C throughout the process in a stainless-steel kiln. After the pyrolysis process was completed, the substance was cooled, crushed, and ground into particles smaller than < 2 mm. The properties of BC are presented in (Table 1).

Depletion of Ash from Biochar

It was first washed with tap water to prepare the BC to eliminate any impurities. Once the ash content was eliminated, the BC underwent a thorough rinsing with deionized water to ensure the removal of any remaining ash residues. Subsequently, it was air-dried in a well-ventilated space until it reached complete dryness[18]. Finally, the deashed BC was properly stored for further use.

Procurement and sterilization of seeds

FH-1036 hybrid seeds were acquired from a local market. The collection of plant material complies with relevant institutional, national, and international guidelines and legislation. The seed sterilization involved three rinses with 95% ethanol followed by the application of 5% sodium hypochlorite (NaOCl). The procedure entailed immersing the seeds in a NaOCl solution for 30 min followed by three washes using 95% ethanol[27–29].

Experimental setup

Powdered deashed BC was blended with 90% pure GA3. The process commenced with finely grinding the prepared deashed BC into powder. Subsequently, using an analytical balance, 10 ppm GA3 was precisely weighed and mixed with the BC. This resulting powder was promptly employed as a soil amendment after processing. As per the treatment plan, 0.75% BC was manually added to the soil followed by pot filling. Three different Cd levels were applied detailed below in the treatment protocol[30]. Cadmium nitrate tetrahydrate ($Cd(NO_3)_2{\cdot}4H_2O$) (ALDRICH, CAS number − 10022-68-1, MDL number - MFCD00149626, product number − 642045 and batch number - MKCT3996) was employed to induce Cd toxicity[22]. The spiking technique involved carefully

Soil	Values	Biochar	Values
pH	8.26	pH	8.21
EC*e* (dS/m)	3.11	EC*e* (dS/m)	3.05
SOC (%)	0.65	Volatile Matter (%)	25
TN (%)	0.04	Fixed carbon (%)	45
TCd (µg/g)	30	TN (%)	0.07
EP (mg/kg)	9.87	TP (%)	0.19
AK (mg/kg)	139	TK (%)	0.33
Sand (%)	25	TCd (µg/g)	0.09
Silt (%)	40	Particle Size	< 2 mm
Clay (%)	35	–	–
Texture	Clay Loam	–	–

Table 1. Soil and biochar attributes before the commencement of the experiment. TN = Total Nitrogen; EP = Extractable Phosphorus; AK = Available Potassium; CEC = Cation Exchange Capacity; EC = Electrical Conductivity; TCd = Total Cadmium.

adding cadmium nitrate solution to 35 kg of soil over 21 days to ensure a consistent distribution. The soil, placed in a plastic container for the spiking process, was covered after spiking to prevent contamination and a 21-day incubation period was followed. This incubation duration was selected to facilitate prolonged interaction between Cd and the soil matrix.

Treatment protocol

A completely randomized design served as the foundation for the experimental setup. All treatments were replicated three times for robustness. The investigations included twelve different treatments. These are; T1: Control (No Cd stress), T2: Mild Cd stress (8 mg Cd/kg soil), T3: Severe Cd stress (16 mg Cd/kg soil), T4: 10 ppm GA3 (No Cd stress), T5: 10 ppm GA3 + Mild Cd stress, T6: 10 ppm GA3 + Severe Cd stress, T7: 0.75% Biochar (No Cd stress), T8: 0.75% Biochar + Mild Cd stress, T9: 0.75% Biochar + Severe Cd stress, T10: 10ppm GA3 + 0.75% Biochar (No Cd stress), T11: 10ppm GA3 + 0.75% Biochar + Mild Cd stress and T12: 10ppm GA3 + 0.75% Biochar + Severe Cd stress. The optimization of different doses in the experiment was based on a combination of previous research, pilot studies, and a desire to cover a range of Cd stress levels. The selection of specific concentrations such as 8 mg Cd/kg soil for mild stress, 16 mg Cd/kg soil for severe stress and 10 ppm GA3, as well as the combination of BC and GA3, have been influenced by established literature on Cd toxicity thresholds, known effective concentrations of GA3 and the aim to observe a spectrum of responses in maize plants under varying stress conditions. The goal was to create a comprehensive experimental design that allows for a thorough evaluation of the effects of different treatments on plant growth under diverse Cd stress scenarios.

Fertilizer application

The application of recommended fertilizer rates for macronutrients involved 200 kg N, 150 kg P, and 100 kg K per hectare[31–33]. Urea, single superphosphate and sulfate of potash were used as sources for nitrogen (N), phosphorus (P) and potassium (K) respectively to supply these nutrients. The experimental design considered the application of normal fertilization practices to ensure proper plant nutrition across all treatment groups. This approach aimed to isolate the specific impact of BC and GA3 on Cd stress alleviation without confounding factors related to nutrient deficiencies. By providing a consistent nutritional baseline, the study aimed to assess the unique contributions of BC and GA3 in mitigating Cd stress, ensuring that any observed effects could be attributed to these amendments rather than variations in nutrient availability.

Irrigation practices

Water was provided to the pots three to four days a week until the maize plants reached full physiological development. According to Danish and Zafar-ul-Hye, maize plants were irrigated to maintain 60% field capacity (FC) during the growth season[34,35].

Crop Harvest

Maize plants were harvested at the V10 Zadoks growth stage. Samples of roots, shoots, and leaves were obtained to collect data.

Determination of relative water content (RWC) of leaf

RWC of fresh leaves was determined using Weatherley's method[36]. First, when the leaf discs had been detached from the young leaves, their fresh weight was measured. After being positioned in petri plates filled with distilled water and given time to saturate the identical discs' turgid weights were calculated. The leaf discs' dry weight was computed following oven drying. The formula below was used to determine the RWC:

$$\mathrm{RWC}\,(\%) = (\mathrm{FW} - \mathrm{DW})/(\mathrm{TW} - \mathrm{DW}) \times 100$$

where: FW = fresh weight, DW = dry weight, TW = turgid weight.

Enzyme extraction

Enzyme extraction was carried out from 200 mg of fresh root and shoot samples. These samples were homogenized in 5 ml of 50 mM potassium phosphate buffer (pH 7.8) containing 1% polyvinylpyrrolidone (PVP), 1 mM ascorbic acid and 1 mM phenylmethylsulfonyl fluoride (PMSF). The homogenate was centrifuged at 22,000 g for 10 min at 4 °C. The resulting supernatant was collected and used for enzyme activity assays.

Protein content determination

The protein content of the samples was determined using the Bradford method. Briefly, a standard curve was prepared using bovine serum albumin (BSA). The supernatant obtained from the enzyme extraction was mixed with Bradford reagent, and the absorbance was measured at 595 nm. The protein concentration was then calculated using the standard curve.

Determination of catalase (CAT) activity

Catalase activity was measured by following the decrease in absorbance at 240 nm due to the decomposition of H_2O_2. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 10 mM H_2O_2, and the enzyme extract. The change in absorbance was recorded for 2 min. Catalase activity was calculated using an extinction coefficient (ε) of 0.036 mM^{-1} cm^{-1} and expressed as EU mg^{-1} protein[37].

Determination of peroxidase (POD) activity

Peroxidase activity was measured using guaiacol as the substrate. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 1 mM guaiacol, 1 mM H_2O_2, and the enzyme extract. The increase in absorbance at 470 nm was recorded for 3 min. POD activity was calculated using an extinction coefficient (ε) of 26.6 mM^{-1} cm^{-1} and expressed as EU mg^{-1} protein[38].

Determination of Superoxide dismutase (SOD) activity

Superoxide dismutase activity was determined by measuring the inhibition of photochemical reduction of nitroblue tetrazolium (NBT). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.8), 13 mM methionine, 75 µM NBT, 0.1 mM EDTA, 2 µM riboflavin, and the enzyme extract. The reaction was initiated by exposing the mixture to light, and the reduction of NBT was measured at 560 nm. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of NBT reduction and expressed as EU mg^{-1} protein[39].

Determination of ascorbate peroxidase (APX) activity

Ascorbate peroxidase activity was measured by monitoring the decrease in absorbance at 290 nm due to the oxidation of ascorbate. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.2 mM ascorbic acid, 0.2 mM EDTA, 1 mM H_2O_2, and the enzyme extract. The change in absorbance was recorded for 7 min at 30-second intervals. APX activity was calculated using an extinction coefficient (ε) of 2.8 mM^{-1} cm^{-1} and expressed as EU mg^{-1} protein[40].

Statistical analysis

Statistical analysis was performed using OriginPro 2021 software. Analysis of variance (ANOVA) followed by Fisher's LSD test was used to determine significant differences between treatments.

Results

Effect of gibberellic acid (GA3) and Biochar (BC) on maize hydrogen peroxide (H_2O_2) contents in root, stem, and leaves under Cd stress

The application of GA3 and BC significantly influenced hydrogen peroxide (H_2O_2) contents in maize roots, stems, and leaves under Cd stress (CdS). According to the two-way ANOVA results, treatments (GA3, BC, GA3 + BC, and Control) and Cd stress levels (0, 8, 16 mg Cd/kg soil) had substantial effects on H_2O_2 contents. In the control plants, H_2O_2 content increased with higher Cd levels.

Specifically, root H_2O_2 content increased by 100% under 8 mg Cd/kg and 16 mg Cd/kg, respectively, compared to control. Similar trends were observed in stems and leaves. GA3 and BC treatments individually reduced H_2O_2 levels compared to control plants. Under 8 mg Cd/kg, GA3 reduced root H_2O_2 by 20%, stem H_2O_2 by 22%, and leaf H_2O_2 by 18%, while BC reduced root, stem, and leaf H_2O_2 by 25%, 30%, and 28%, respectively. Under 16 mg Cd/kg, GA3 reduced root, stem and leaf H_2O_2 by 30%, 35%, and 33%, while BC reduced these levels by 35%, 40%, and 38%. The combined GA3 + BC treatment showed the most significant reduction in H_2O_2 levels across all plant parts. Under 8 mg Cd/kg, GA3 + BC reduced root H_2O_2 by 40%, stem H_2O_2 by 45%, and leaf H_2O_2 by 42%. Under 16 mg Cd/kg, this treatment reduced root, stem, and leaf H_2O_2 by 50%, 55% and 53%, respectively. This indicates a synergistic effect of GA3 and BC in mitigating oxidative stress induced by Cd, highlighting their potential to enhance Cd stress tolerance in maize by significantly reducing H_2O_2 accumulation (Fig. 1).

Effect of gibberellic acid (GA3) and Biochar (BC) on maize Superoxide Dismutase (SOD) contents on root, stem and leaves under Cd stress

The application of GA3 and BC significantly influenced SOD activity in the roots, stems, and leaves of maize under Cd stress. Under control conditions (0 Cd), root SOD activity increased by approximately 30% with GA3, 25% with BC and 35% with combined GA3 + BC treatment. However, under moderate Cd stress (8 Cd), root SOD activity decreased by 10% for GA3, 15% for BC, and 20% for the combined treatment (Fig. 2).

At high Cd stress (16 Cd), the reductions were more pronounced, with decreases of 40%, 35%, and 50% for GA3, BC and the combined treatment, respectively. Stem SOD activity under control conditions increased by

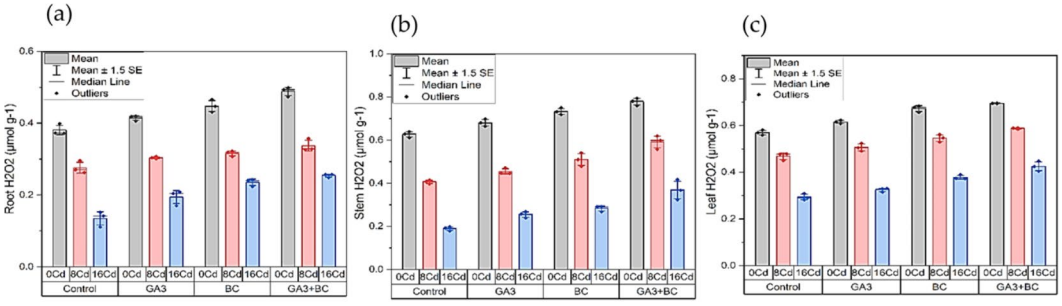

Fig. 1. Effect of the BC-GA3 intervention for enhancing Cd tolerance in maize growth parameters: (**a**): root H_2O_2 (µmol g^{-1}), (**b**): stem H_2O_2 (µmol g^{-1}), (**c**): leaf H_2O_2 (µmol g^{-1}). (GA3 = Gibberellic acid; BC = Biochar).

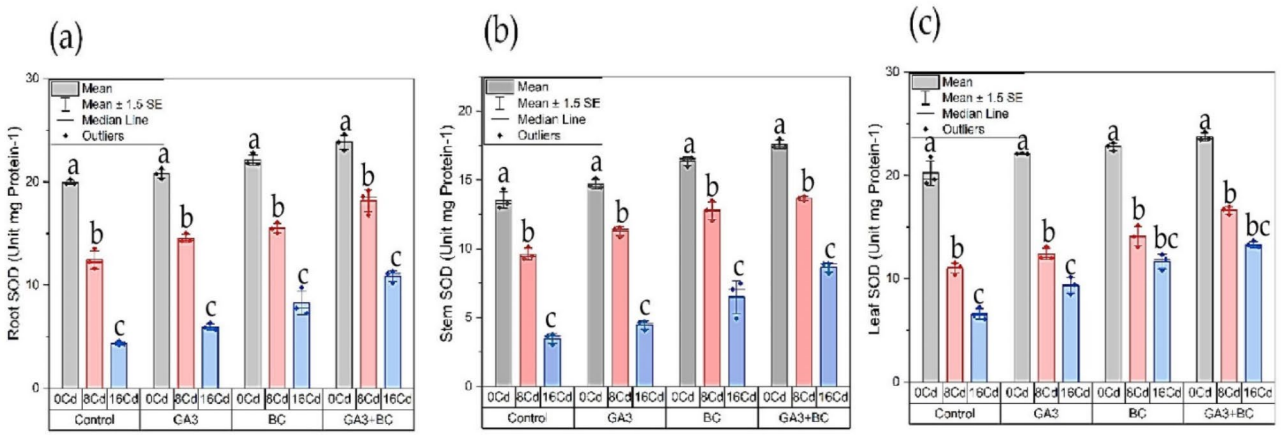

Fig. 2. Effect of the BC-GA3 intervention for enhancing Cadmium tolerance in maize growth parameters: (**a**): root SOD (Unit mg Protein^{-1}), (**b**): stem SOD (Unit mg Protein^{-1}), (**c**): leaf SOD (Unit mg Protein^{-1}). (GA3 = Gibberellic acid; BC = Biochar).

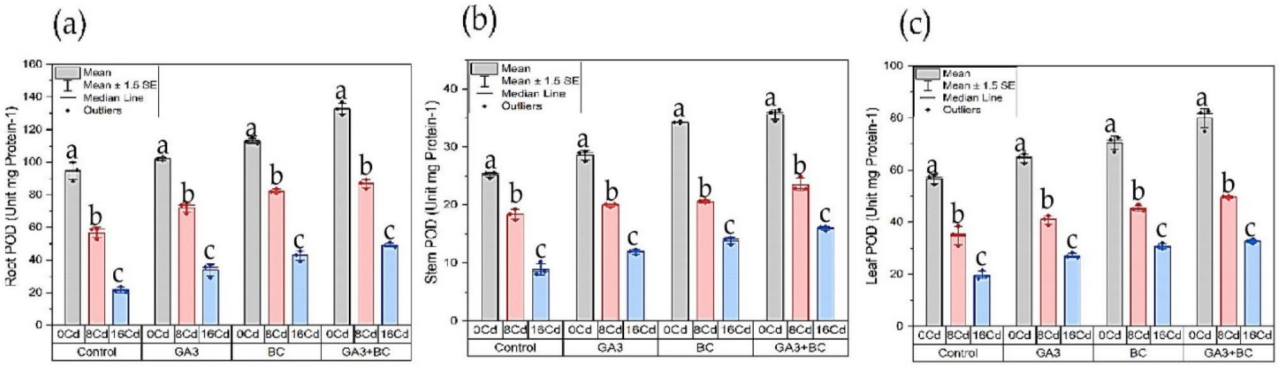

Fig. 3. Effect of the BC-GA3 intervention for enhancing Cadmium tolerance in maize growth parameters: (**a**): root APX (μmol g^{-1}), (**b**): stem APX (μmol g^{-1}), (**c**): leaf APX (μmol g^{-1}). (GA3 = Gibberellic acid; BC = Biochar).

20% with GA3, 15% with BC, and 25% with GA3 + BC, but decreased under moderate Cd stress by 10%, 5%, and 15%, and under high Cd stress by 30%, 25%, and 35% for GA3, BC and the combined treatment, respectively. Similarly, leaf SOD activity under control conditions rose by 25% for GA3, 20% for BC, and 30% for GA3 + BC. Under moderate Cd stress, it decreased by 15% for GA3, 10% for BC, and 20% for the combined treatment, while under high Cd stress, the reductions were 35%, 30%, and 40% for GA3, BC, and combined treatment, respectively. The combined GA3 and BC treatment demonstrated the highest SOD activity under non-stress conditions but also the most significant decline under severe Cd stress, with statistical analysis confirming highly significant differences in treatment effects, Cd stress levels, and their interactions on SOD activity across root, stem and leaf tissues.

Effect of gibberellic acid (GA3) and Biochar (BC) on maize peroxidase (POD) activity of root, stem and leaves under Cd stress

In the control group, a substantial 94.40% increase in root POD was noticed, which decreased to 56.88% under 8Cd stress and further to 21.56% in high Cd stress (16Cd). GA3 treatment without stress increased root POD by 102.1%, while GA3 with 8Cd stress raised it to 71.93%, and with 16Cd stress, it measured by 33.84%. BC treatment without stress significantly enhanced root POD to 113.02% and under 8Cd and 16Cd stress, it reached 82.15% and 42.55%, respectively. Combining GA3 + BC treatment during 0Cd stress led to a remarkable 132.83% increase in root POD, while under 8Cd and 16Cd stress, it measured 86.80% and 49.18%, respectively (Fig. 3).

Similarly, in the stem, 0Cd stress resulted in a 25.20% increase, dropping to 18.36% under 8Cd and further to 8.93% in 16Cd stress. GA3 treatment without stress raised stem POD by 28.57%, and with 8Cd stress, it reached 19.96%, while with 16Cd stress, it measured by 11.91%. BC treatment without stress significantly enhanced stem POD to 34.27%, and under 8Cd and 16Cd stress, it reached 20.61% and 13.84%, respectively. Combining GA3 + BC treatment during 0Cd stress resulted in a substantial 35.61% increase in stem POD, while under 8Cd and 16Cd stress, it measured 23.51% and 15.96%, respectively. In the leaves, 0Cd stress increased POD

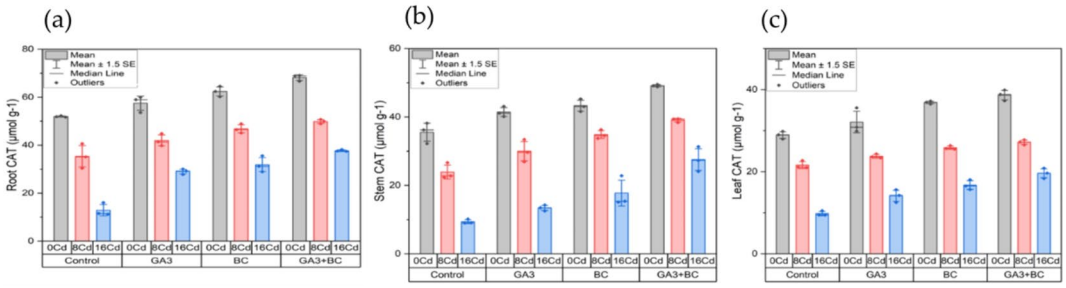

Fig. 4. Effect of the BC-GA3 intervention for enhancing Cadmium tolerance in maize growth parameters: (**a**): root CAT (µmol g^{-1}), (**b**): stem CAT (µmol $^{-1}$), (**c**): leaf CAT (µmol $^{-1}$). (GA3 = Gibberellic acid; BC = Biochar).

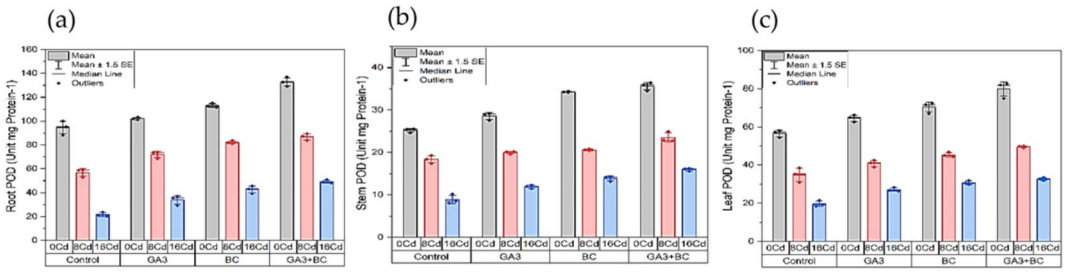

Fig. 5. Effect of the BC-GA3 intervention for enhancing Cadmium tolerance in maize growth parameters: (**a**): root POD (Unit mg $Protein^{-1}$), (**b**): stem POD (Unit mg $Protein^{-1}$), (**c**): leaf POD (Unit mg $Protein^{-1}$). (GA3 = Gibberellic acid; BC = Biochar).

by 56.58%, decreasing to 34.88% under 8Cd and further to 19.67% under 16Cd stress. GA3 treatment without stress raised leaf POD by 64.54%, and with 8Cd stress, it reached 40.83%, while with 16Cd stress, it increased by 27.11%. BC treatment without stress significantly enhanced leaf POD to 70.33%, and under 8Cd and 16Cd stress, it reached 45.41% and 30.75%, respectively. Combining GA3 + BC treatment during 0Cd stress resulted in a substantial 79.83% increase in leaf POD, while under 8Cd and 16Cd stress, it measured 49.60% and 32.69%, respectively.

Effect of gibberellic acid (GA3) and Biochar (BC) on maize catalase (CAT) activity of root, stem and leaves under Cd stress

The results indicate distinct responses in CAT activity based on different stress and treatment conditions. In the root, exposure to 0Cd stress led to a substantial 51.96% increase in CAT activity compared to the control, whereas under 8Cd stress, a decrease to 35.38% was observed, further dropping to 12.91% under intense 16Cd stress (Fig. 4).

Treatment with GA3 alone, particularly under 0Cd stress, significantly elevated root CAT activity by 10.64%, while the combined GA3 and 8Cd stress increased it by 18.71%.The addition of BC without stress (0Cd) remarkably enhanced root CAT activity by 20.07%, and under 8Cd stress, it marked a substantial 32.33% increase. The GA3 + BC treatment during 0Cd stress resulted in a considerable 31.23% rise, and under 8Cd stress, it showcased a notable 40.89% increase. High CdS (16Cd) with GA3 + BC treatment displayed a significant 192.25% rise over the control. Similar patterns of response were observed in the stem and leaves, highlighting the varied impact of GA3 and BC on maize CAT activity under different Cd stress conditions.

Effect of gibberellic acid (GA3) and Biochar (BC) on maize Ascorbate peroxidase (APX) activity of root, stem, and leaves under Cd stress

The results reveal distinct patterns in APX activity based on various treatments. Notably, under 0Cd stress, root APX exhibited a significant increase while GA3 treatment without stress further augmented this activity by 11.43%. In contrast, BC treatment without stress remarkably enhanced root APX by 23.44%. Interestingly, combining GA3 + BC treatment during 0Cd stress resulted in a substantial 38.93% increase in root APX. Similar trends were observed in stem and leaf APX activities, highlighting the nuanced responses to different treatments and Cd stress levels (Fig. 5).

Discussion

Under stress, H_2O_2 has a significant signaling role, but it can also negatively affect plant cells. According to recent research, the treatment of jasmonic acid and gibberelline in chickpea plants under Cd stress lowered H_2O_2 levels[41]. Cu and Ni stress increased H_2O_2 levels in all cultivars, with maize showing the most increase

in H_2O_2 with Ni stress, no matter the cultivars, using *Trichoderma asperellum* and BC together reduced the oxidative stress brought on by Cu poisoning[42]. The study found that treating the ML-2056 variety with Cd increased H_2O_2 levels, while exogenous GA_3 reduced ROS generation, thus mitigating Cd-induced oxidative damage. Similar effects were observed in fenugreek[43]. Recent research showed decreased H_2O_2 in GA-treated *Solanum lycopersicum* (tomato)[44]. Under Ni stress, maize had the highest H_2O_2 increase. Application of *T. asperellum* and BC combination reduced Cu-induced oxidative stress, regardless of cultivars[45]. Thiourea and BC together effectively decreased H_2O_2 and EL levels by 26%, 20%, and 21% in comparison to the control group[46]. High concentrations of heavy metals enhance the generation of ROS, such as hydrogen peroxide, hydroxyl radicals, and superoxide radicals, which disrupt metabolic activities in plants[47]. Despite H_2O_2's role in signaling during stress, CdS induced higher H_2O_2 production in chickpea plants[48]. However, a previous study found that Chickpea plants under Cd stress were given JA and GA_3 treatments exhibited lower H_2O_2 and MDA levels, indicating defense against Cd stress-induced lipid peroxidation; jasmonic acid has a reputation for protecting cell membrane components[49].

Previous research revealed that the application of Bio + Asa's combo application had a significant positive influence. Further studies found that different kinds of BC significantly increased wheat grain yield while also reducing Cd bioavailability[50]. P and GA_3 administration resulted in a reduction in MDA, H_2O_2, EL, and MDA was found in the roots and leaves of *C. capsularis*, and plants grown under different levels of Cu stress showed an increase in the enzyme activity of SOD, POD, CAT, and APX. SOD, CAT, GR, and APX enzymatic antioxidant activity increased in plants subjected to growth regulators (GA_3 and Db-H)[51]. Current findings suggest that BC has a more pronounced effect in enhancing stem SOD activity, the antioxidant defense system in maize plants under CdS is considerably strengthened by the combination with GA_3. The mechanisms behind the synergistic action of GA_3 and BC in improving plant tolerance to heavy metal toxicity require further study. Without regard to cultivars, the combination application of *T. asperellum* and BC increased the concentration of SOD and APX under Cu toxicity. In seedlings subjected to Cd stress, SOD, CAT, and POX activities were higher than in controls, and they grew in proportion to the amount of metal present in the medium. The presence of BC considerably lowered the Cd levels[52]. Low GA_3 concentrations (3–5 mg L^{-1}) significantly increased the activities of antioxidant enzymes (SOD, POD, CAT, and APX) and biomass. These elements may be the root of this phenomenon: According to Kohli, (i) low GA_3 concentration can minimize the oxidative damage to plants brought on by high Cu concentration, and hence lessen the harm brought on by Cu[53].

The presence of HMs in the water interfered with the important enzyme activities for plant absorption; this interference was prevented by the administration of GA_3. Our findings align with a previous study, that investigated mung beans[54]. Increased antioxidant enzyme activities (APX, GPX, GST), better Gly enzyme (Gly I and II) activities, improved nutritional uptake, and efficient ROS detoxification were all produced when JA and GA_3 were applied to chickpea plants under Cd stress. Furthermore, the favorable characteristics of BC, including its porous structure, high surface charge density, and extensive surface area, enhance its ability to adsorb inorganic contaminants[55]. Previous studies have demonstrated that GA increases antioxidant enzyme activity, which may promote plant growth, and reduces the negative impacts of a variety of abiotic stimuli, as was seen in our work. The APX activities significantly changed because bamboo charcoal was added to the soil. There was no discernible difference between the impacts of the ZnO NPs alone and the bamboo BC on the APX activities in maize, according to a recent study[56]. Spring corn's leaf APX concentration was substantially lower when exposed to Ni toxicity than the control. However, regardless of the cultivars, *Trichoderma asperellum* and BC applied together improved the APX concentration under Cu toxicity. According to earlier research, the administration of Fe-BC under Cd stress inhibited the activities of CAT and APX. P and GA_3 administration decreased MDA, H_2O_2, and EL in *C. capsularis* roots and leaves while increasing SOD, POD, CAT, and APX activity[57].

By applying exogenous foliar Thiourea and BC incorporation to the soil, CAT activities in maize were markedly improved. In seedlings subjected to the concurrent treatment of GA_3 and Cd, a significant rise in the enzymatic activity was also observed[58]. Previous studies have demonstrated that GA increases the activity of antioxidant enzymes, reducing the negative effects of a variety of abiotic stressors, which, as demonstrated by our research, may accelerate plant growth. According to previous research, several forms of BC wheat grain showed a significant increase and Cd bioavailability was reduced[59]. The agricultural area that has high levels of Cd contamination has an impact on plant growth. To reduce the toxicity of Cd in plants, Cd uptake and accumulation must be restricted. Cd poisoning harmed the development of maize plants. P and GA_3 administration resulted in a reduction in MDA, H_2O_2, EL, and MDA *C.* Capsular roots and leaves, while plants grown under varied levels of Cu stress showed an increase in the activities of SOD, POD, CAT, and APX[60]. However, regardless of the cultivars, the combination application of *Trichoderma asperellum* and BC increased the CAT concentration under Cu toxicity. CAT activities were increased in maize leaves after GA_3 treatment, which may help to lower the H_2O_2 level. In comparison to controls, soybean leaves with higher Cd toxicity produced less SOD and CAT and more POD[61]. In comparison to controls, soybean leaves with higher Cd toxicity produced less SOD and CAT and more POD. BC treatment boosted SOD and CAT production while decreasing POD formation in soil with low and high Cd spiking levels[62]. By reducing oxidative stress and enhancing antioxidant defenses, gibberellins (GA) can protect plants from the damaging effects of trace metals. POD activity rises as a result of BC enhancement of the stem's antioxidant defense mechanism. According to earlier studies, treatment with BC and thiourea decreased the negative effects of Cd on lipid peroxidation and cell membrane permeability. Neelam and desi makai, however, displayed the maximum development at 200 m Cadmium chloride and 0.25 mg GA[63]. The POD concentration under Cu toxicity was increased by the combination treatment of *T. asperellum* and BC, regardless of the cultivars, these are consistent with our findings. Recent studies have shown that plants under Cd stress treated with uniconazole and GA_3 have higher POD activity than the plants in the control group. POD

activities were increased in maize leaves after GA_3 treatment, which may help to lower the H_2O_2 level, similar to the current study[64].

For future investigations, it is recommended to delve deeper into the combined application of BC with other soil amendments to understand their synergistic effects on plant growth and stress tolerance. Long-term field trials are essential to assess the persistent impacts of these amendments on soil properties and crop productivity over multiple seasons. Exploring the influence of BC and GA3 on soil microbial communities can provide insights into their role in enhancing soil fertility and ecosystem functioning. Additionally, practical field application strategies need to be optimized to ensure the effective delivery of these amendments to plant roots while minimizing environmental risks. Crop-specific responses to BC and GA3 under heavy metal stress conditions should be investigated to tailor management practices for different agricultural systems. Furthermore, assessing the ecological impacts and scaling up studies to larger agricultural landscapes can validate the feasibility and sustainability of these interventions for mitigating heavy metal stress in agriculture.

Conclusion

This study focused on addressing the environmental challenge posed by cadmium (Cd), a prevalent contaminant due to widespread industrial use. The stress induced by Cd significantly disrupts essential physiological and metabolic functions in plants, emphasizing the need for effective mitigation strategies. Through a comprehensive investigation, this research explored the combined impacts of BC and GA3 in alleviating CdS, specifically in maize. The results revealed that the application of GA3 + BC significantly enhanced growth parameters, including root, stem, leaf H_2O_2, SOD, APX, CAT, and POD compared to the control under different Cd levels. The findings suggest the potential for the sustainable mitigation of heavy metal-induced stress in crop cultivation, offering valuable insights into environmental and agricultural practices. Detailed molecular studies can unravel the specific pathways influenced by GA3 + BC treatment, shedding light on how these components interact at the cellular and genetic levels.

Data availability

The author confirms that all data generated or analyzed during this study are included in this published article.

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Author contributions
TA: Methodology, supervision, Writing and drafting, and research design; HQ: Experimentation, validation, Software, and data Curation; MJ: writing, Investigation, drafting, statistical analysis; and validation; EHS: writing, Software, Resource, research design, validation; WZ: validation, data collection, drafting, statistical analysis; SAA, MJA: writing, statistical analysis, Resource, software, validation. All authors have read and approved the final manuscript and declare that they have no competitive interest.

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Declarations

Competing interests
The authors declare no competing interests.

Ethics approval and consent to participate
We all declare that manuscript reporting studies do not involve any human participants, human data, or human tissue. So, it is not applicable.

Consent for publication
Not applicable.

Study protocol must comply with relevant institutional, national, and international guidelines and legislation
Our experiment follows the relevant institutional, national, and international guidelines and legislation.

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