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# Alleviation of Cadmium Stress in Wheat through the Combined Application of Boron and Biochar via Regulating Morpho-Physiological and Antioxidant Defense Mechanisms

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**Abstract:** Cadmium (Cd) contamination in soil adversely affects crop productivity, grain quality, and human health. Applications of boron (B) and biochar are known to impart tolerance to crops against abiotic stresses. A pot experiment was performed to assess the effects of the sole and combined application of B and biochar on growth, physiological and antioxidant defense mechanisms, yield, and grain quality of wheat under Cd toxicity-induced stress. The treatments included control (0 mg $kg^{-1}$ and 0 g $kg^{-1}$), only Cd (15 mg $kg^{-1}$), only B (5 g $kg^{-1}$), only biochar (50 g $kg^{-1}$), B plus biochar, Cd plus B, Cd plus biochar, and Cd plus B plus biochar, which were applied at the time of sowing and were arranged using completely randomized design (CRD) with five replications. The individual Cd toxicity (15 mg $kg^{-1}$) significantly reduced chl a, chl b, and chl a+b, as well as primary metabolites (soluble protein, amino acids, total soluble sugar, and phenolic contents), while it increased the activities of enzymatic antioxidants like superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) in the leaves of wheat. In addition, Cd stress (15 mg $kg^{-1}$) increased lipid peroxidation in the form of malondialdehyde (MDA), and it enhanced the hydrogen peroxide ($H_2O_2$) content, electrolyte leakage (EL), and proline contents in the leaves. Furthermore, Cd (15 mg $kg^{-1}$) contamination reduced the grain yield and yield-related attributes relative to respective no-Cd treatments. Soil-applied B and biochar improved wheat grain yield by triggering the activities of enzymatic antioxidants. Individual or combined B and biochar applications improved proline contents and reduced $H_2O_2$ and MDA contents in plants. The combined application of B and biochar enhanced soluble sugars and total phenolic as compared to the control and Cd-contaminated plants. In conclusion, the combined application of B and biochar was found to be the best soil amendment strategy to improve the yield of wheat under Cd-contaminated soil.

**Keywords:** antioxidants; biochar; boron; cadmium toxicity; lipid peroxidation; productivity

## 1. Introduction

Cadmium (Cd) is one of the main hazardous elements and is generally considered the most toxic element in cereal crops [1]. For instance, Cd toxicity hinders the growth and development of plants, which ultimately reduces their morphological and yield attributes. Therefore, the application of Cd disrupts the development of plants and decreases their structure characteristics, morphological properties, and dry biomass production [2]. Cadmium (Cd) affects the physiochemical and biological processes in plants which ultimately disrupt plants vegetative growth and development, leading to decreased grain yields and lowered grain quality [3]. Moreover, cadmium (Cd) induced oxidative stress hinders antioxidant defense systems in crop plants [4]. Due to cadmium (Cd) stress in plants, reactive oxygen species (ROS) are produced, which stimulate antioxidant defense systems [5,6]. Antioxidants like catalase (CAT), ascorbate peroxidase (APX), superoxide dismutase (SOD), and peroxidase (POD) detoxified the adverse effects of reactive oxygen species in wheat grown under Cd-contaminated soil [7]. Severe oxidative damage was observed in crops due to enhanced ROS production [3]. Furthermore, antioxidant enzymatic processes were reduced due to cadmium (Cd) toxicity in wheat crops [8]. Globally, wheat (*Triticum aestivum* L.) is the leading cereal crop and is an important staple food. Hence, heavy metal contaminations, particularly that of Cd, create deleterious effects on human health because of their translocation and accumulation in cereal grains [9,10]. Similarly, the accumulation of Cd in plant cells impairs the plant's physiological and metabolic processes due to the possibility of imbalance in nutrient uptake, interrupting energy production [11].

Boron (B) is an important micronutrient necessary for crop growth and development, and it plays a significant role in improving plant's physiological attributes [12]. It plays a primary role in the physiological and biochemical activities of cell membranes, photosynthesis, cell division and elongation, nitrogen use efficiency, and in improving the sugar contents of plants [13]. B inadequacy influences different structural and functional activities of a plant's vascular bundles, for example, root initiation, translocation of sugar contents, and starch and nitrogen digestion [14]. B assumes a key part in a plant's cell wall, where 70–90% of the total copper, zinc, and Cd are situated in certain plants [15]. It enhances the probability of tweaking Cd accumulation and poisonousness. Hence, Boron (B) eases plant cadmium poisonousness due to retreating cadmium aggregation and relieving oxidative stress which was produced due to cadmium induction [16]. The cadmium adsorption in *Brassica napus* was improved due to the cell wall, which was an integral part, and it forestalls cadmium exit from the cell through the cell wall, and Cd chelates on the cell wall [17]. Likewise, boron is also engaged in the formation and cross-linking of different cell wall parts [18]. Therefore, it might be assumed that cadmium aggregation and toxicity management may be helpful for successful crop growth.

Yet, there is a lack of information to support the ability of boron to enable plants to survive under cadmium toxicity. In spite of the expanding considerations on the interaction of cadmium with different biochemical cycles in plants, less data exists on the association of cadmium with fundamental microelements. One more methodology for remediating metal-contaminated soils is immobilization; the utilization of biochar is an eco-accommodating and affordable immobilizer for substantial metal pollution [19]. The amendment of biochar added through soil facilitates heavy metal stabilization via different processes, such as surface complexation, adsorption, and metal exchange [20]. In rice crops, Cd immobilization into a more steady form diminishes metal substances and also diminishes bioavailable parts due to the application of biochar. Another study reported that the use of biochar treated with acid enhanced the growth, development, and gas exchange characteristics of quinoa by lowering the accumulation of heavy metals in various plant parts, such as the roots, shoots, and grains of quinoa [21].

Although there are various research articles on the use of boron [15] or biochar as an individual application [22] to lower the bioavailability of metals and enhance crop growth and development; however, there is no literature about the combined use of B and biochar to reduce the Cd toxicity in plants. Therefore, in this study, we planned to evaluate the

effects of B and soil-applied biochar, individually or combined, on the morphological, physiochemical, and biological traits of wheat grown under Cd toxicity.

## 2. Materials and Methods

### *2.1. Experimental Conditions and Design*

The current experiment was carried out in earthen pots in open conditions at Baha-Uddin Zakariya University, Layyah Campus, Pakistan during winter 2020 to assess the individual and combined effects of boron and biochar to mitigate the adversities of cadmium toxicity in wheat crop. Ten (10) healthy and uniform-sized seeds of the wheat variety Fakhar-e-Bhakhar-2019 were planted in each pot directly and filled with soil to avoid germination losses. After seven days of germination, thinning was done, and only five plants were kept in each pot to maintain optimum plant populations. Each earthen pot was 16 cm in diameter and 45 cm in height, and they were filled with 15 kg of sandy loam soil. For better crop growth and development, 0.08, 0.06, and 0.05 g N, P, and K, respectively, (in the form of urea 46% N, DAP 46% $P_2O_5$, and MOP 60% $K_2O$ $kg^{-1}$ of soil) were mixed in the soil at the time of sowing by the soil mass method. Treatments, comprised of the control, only Cd (15 mg $kg^{-1}$), only B (5 g $kg^{-1}$), only biochar (50 g $kg^{-1}$), B + biochar (5 g $kg^{-1}$ + 50 g $kg^{-1}$), Cd + B (10 mg $kg^{-1}$ + 5 g $kg^{-1}$), Cd + biochar (10 mg $kg^{-1}$ + 50 g $kg^{-1}$), and Cd + B + biochar (10 mg $kg^{-1}$ + 5 g $kg^{-1}$ + 50 g $kg^{-1}$ soil) were thoroughly mixed in sandy loam soil at the time of planting. These experimental treatments were arranged using completely randomized design (CRD) with five replications. There was a total 40 experimental units (8 treatments × 5 replications) in the experiment. Each replication contained three pots having five plants in each pot. Boron and biochar were mixed thoroughly, individually or combined, according to treatments. Cadmium stress was induced by spiking the soil using analytical grade cadmium chloride ($CdCl_2$).

### *2.2. Preparation of Rice Straw Biochar*

Biochar was prepared by using rice straw according to the method of Qayyum et al., 2015 [23]. Rice straw was put in the biochar machine for pyrolysis at 500 °C in anaerobic conditions. After 40 min, pyrolysed biochar was taken from the machines and passed through a sieve (2 mm) after crushing. Chemical properties of the rice straw biochar are given in Table S1.

### *2.3. Morphological and Yield-Related Attributes*

Seventy-five DAS days after sowing, five plants were randomly selected from each treatment. Leaf samples were collected from each plant and leaf area was measured with the help of a leaf area meter (CI-203CA Leaf Conveyor; 1554 NE 3rd Ave Camas, WA, USA). Upon reaching maturity, five plants were selected randomly, and plant height was measured from the ground to the tip of the plant using a meter rod. Spikes from the selected plants were separated with the help of scissors and spike length was measured with a measuring tape. The numbers of grains per spike were counted from five randomly selected spikes from each treatment after manually threshing the spike and grain yield and 100-grain weight were calculated. The 100 seeds were counted with a digital seed counter from each replication, then these seeds were weighed on an electric balance to calculate 100-grain weight and their average weight was calculated. For measuring the grain yield, seeds were cleaned from each replication, then weighed, and the average per plant was calculated.

### *2.4. Determination Photosynthetic Pigments*

Photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoid) were determined by using an extract of 0.5 g of fresh leaves. Leaf extract was obtained by keeping the leaves in 5 mL of 80% acetone for 24 h. Extracts were then centrifuged at 10,000× *g*. Chlorophyll a, chlorophyll b, and carotenoid contents were determined by taking the

absorbance reading of supernatant samples at 645, 663, and 480 nm, respectively, with a spectrophotometer [24].

### *2.5. Extraction of Enzymes*

For protein and antioxidant enzyme assays, frozen leaves were ground to a fine powder with liquid nitrogen and were extracted with ice-cold 0.1 M Tris-HCl buffer (pH 7.5) containing 5% (*w/v*) sucrose and 0.1% 2-mercaptoethanol (3:1 buffer volume/FW). The homogenate was centrifuged at 10,000 *g* for 20 min at 4 °C, and the supernatant was used for enzyme activity and protein determinations. Preparations for enzyme extraction and enzyme assay were carried out at 4 °C.

#### 2.5.1. Determination of Primary Metabolites

Total soluble proteins in the leaf sample were determined through Bradford [25] assay by using Coomassie Blue dye. The 100 µL leaf sample and 2 mL Bradford reagent were mixed in the test tube. The mixture was incubated for 20 min in the dark and the absorbance reading was determined at 595 nm. In 50 mM chilled potassium phosphate buffer (5 mL; pH 7.5), fresh leaf tissues (0.2 g) were homogenized and centrifuged for 20 min at 10,000× *g*. In a test tube, equal volumes of 10% pyridine and acid ninhydrin were homogenized with 1 mL of supernatant. After that, heat was given at 95 °C to the reaction mixture for 30 min using a water bath. Then, by using distilled water, the volume of the reaction mixture was raised up to 7.5 mL. Finally, at 570 nm, absorbance of the reaction mixture was observed [26].

In order to measure the total soluble sugars, 5 mL of aqueous ethanol (80%) and 0.2-g of fresh leaf tissues were homogenized, then centrifuged for 10 min at 3500 g. The sample extract (100 µL) obtained was then reacted with enthrone reagent (3 mL). Then, by using a water bath, the mixture was heated for 10 min at 95 °C. The mixture was then cooled and, using a spectrophotometer, absorbance was observed at 625 nm [27]. By using the method of Julkenen-Titto [28], the total phenolic contents of fresh leaf samples were determined. For this purpose, 1 mL of acetone (80%) and 0.2 g of fresh leaf tissues were homogenized. They were then centrifuged at 12,000 rpm for 15 min, then 20% $Na_2CO_3$ (2.5 mL), 0.5-mL Folin-Ciocalteu phenol re-agent, and supernatant (100 µL) were added to the test tube, and it was shaken. Then, in order to make a 5 mL final volume, distilled water was added. Lastly, absorbance was recorded at 750 nm after 20 min.

#### 2.5.2. Determination of Enzymatic Antioxidant Activities

SOD activity assay was based on the method of Dhindsa et al [29], which is based on the measurement of inhibition in the photochemical reduction of nitro blue tetrazolium (NBT) spectrophotometrically at 560 nm. The reaction mixture contained 50 mM K-phosphate buffer (pH 7.8), 13 mM methionine, 75 µM NBT, 0.1 µM EDTA, 4 µM riboflavin, and the required amount of enzyme extract. The reaction was started by adding riboflavin and placing the tubes under two 15 W fluorescent lamps for 15 min. A complete reaction mixture without enzyme, which gave the maximal colour, served as the control. A non-irradiated, complete reaction mixture served as a blank. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT as monitored at 560 nm, which was measured according to the method of Giannopolitis and Ries [30]. POD activity was determined at 436 nm by its ability to convert guaiacol to tetra guaiacol ($\varepsilon$ = 26.6 $mM^{-1}$ $cm^{-1}$), according to the method of Polle et al. [31]. The reaction mixture contained 100 mM K-phosphate buffer (pH 7.0), 20.1 mM guaiacol, 10 mM $H_2O_2$, and enzyme extract. The increase in absorbance was recorded by the addition of $H_2O_2$ at 436 nm for 5 min. CAT activity was determined by monitoring the disappearance of $H_2O_2$ at 240 nm ($\varepsilon$ = 40 $mM^{-1}$ $cm^{-1}$), according to the method of Aebi [32]. The reaction mixture contained 50 mM K-phosphate buffer (pH 7.0).

In order to determine $H_2O_2$ content, 1.5 mL of trichloroacetic acid (0.1%) and fresh leaf tissue (0.15 g) were homogenized. Then, centrifugation of the homogenate was done at

12,000× *g* for 15 min. Then, 0.5 mL of potassium sulfate buffer (10 mM, pH 7.0) and 1 mL of potassium iodide were added to supernatant. At 390 nm, using a spectrophotometer, absorbance of the reaction mixture and trichloroacetic acid (0.1%) was observed. By doing comparison of the absorbance with a standard curve, the amount of $H_2O_2$ content was measured [33].

### *2.6. MDA Determination*

Hodges et al. [34] described a method called the TBA method. Utilizing this method, the amount of MDA content was measured. For the purpose of analysis, fresh leaves (0.15 g) of wheat were ground with 5.0 mL of 5% (*w/v*) TCA. This was done in a mortar that was placed an ice bath, and then centrifugation was done. Then, by using a spectrophotometer, MDA content was measured at 600 nm and at 532 nm. For measuring the proline content, 5 mL sulpho-salicylic acid (3%) and fresh leaf tissue (0.1 g) were homogenized. Then, the homogenate was centrifuged. Then, ninhydrin and glacial acetic acid reagent were added to supernatant. By using a water bath, 1 h boiling of the reaction mixture was done. After boiling, the reaction mixture was cooled for 10 min in an ice bath. Using a spectrophotometer, absorbance was measured at 520 nm and proline was extracted from the mixture with toluene [35].

### *2.7. Determination of Electrolytic Leakage*

Using the method of Dionisio-Sese and Tobita [36], electrolyte leakage was measured. In a test tube, 10 mL distilled water and small and equal sized pieces of fresh leaf tissue (0.5 g) were added and stirred for 10 s. The test tubes were then left overnight, and electrical conductivity ($EC_1$) was calculated. For measuring the $EC_2$, test tubes were autoclaved at 100 °C for 1 h.

### *2.8. Data Analysis*

Statistical analysis of data was done with the help of Statistix 8.1 software (Analytical Software, Statistix; Tallahassee, FL, USA, 1985–2003) by using analysis of variance (ANOVA) techniques. For mean separation at the 5% probability level, Tukey's test was used. The graphical presentation of data was done by using Sigma Plot.

## 3. Results

### *3.1. Plant Height, Yield, and Yield Components*

Cadmium stress had a drastic effect on plant growth parameters, and it significantly reduced plant height by 16.57%, spike length by 15.58%, grains per spike by 16.43%, 100-grain weight by 14.54%, and grain yield per plant by 16.33%, as compared to the control (Figures 1 and 2). The applications of solely boron or biochar, and their combination, significantly affected the plants growth parameters. Under the cadmium stress condition, boron application enhanced plant height by 4.34%, spike length by 8.19%, grains per spike by 4.76%, 100-grain weight by 7.97%, and grain yield per plant by 10.10% and biochar application enhanced plant height by 3.01%, spike length by 5.86%, grains per spike by 10.77%, 100-grain weight by 12.50%, and grain yield per plant by 14.81% (Figures 1 and 2). Likewise, the combined application of boron and biochar enhanced plant height by 5.34%, spike length by 12.34%, grains per spike by 13.75%, 100-grain weight by 15.51%, and grain yield per plant by 16.83% under cadmium stress conditions (Figures 1 and 2). The maximum results were observed when the combined application of boron and biochar was done on wheat plants.

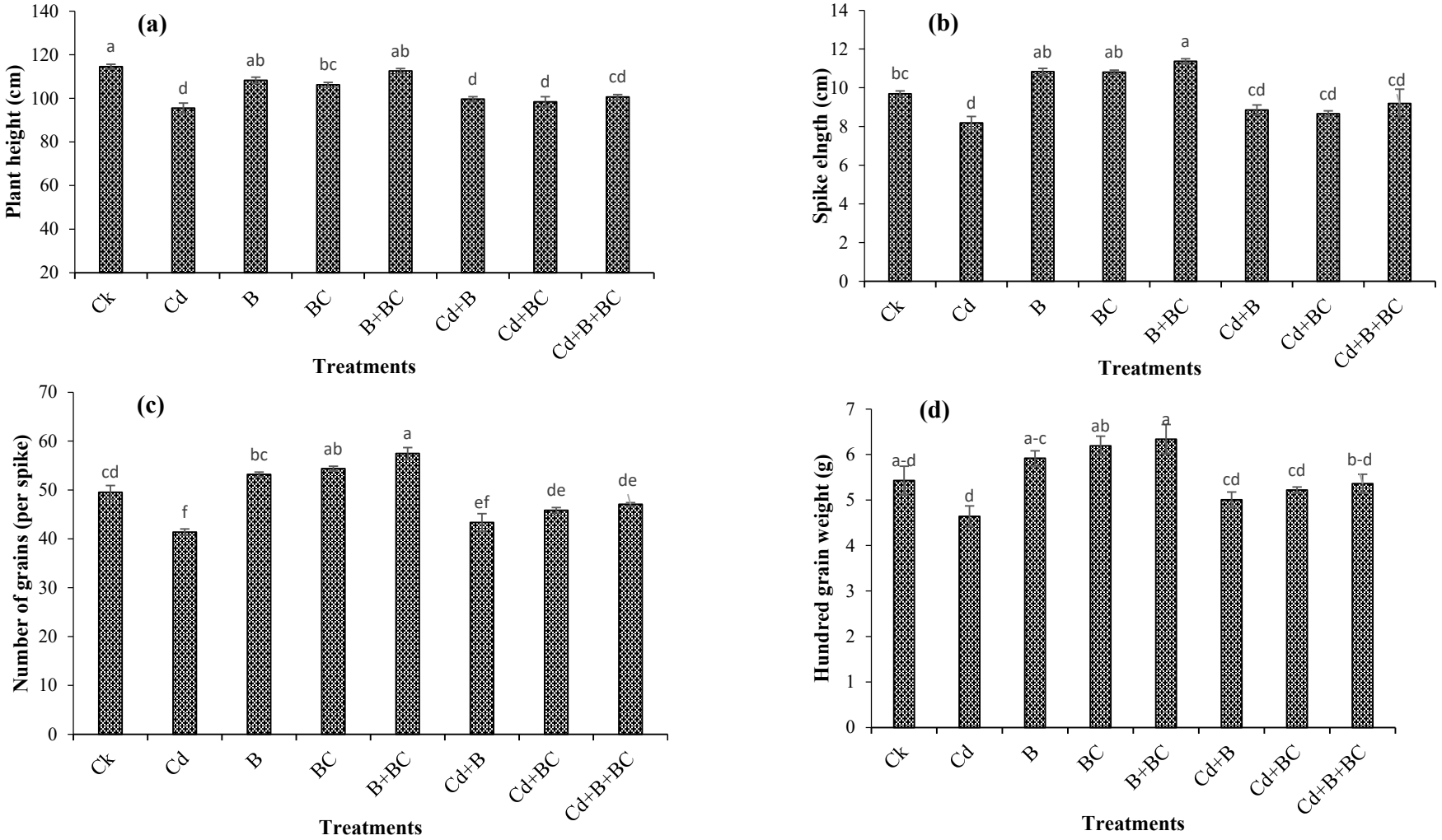


**Figure 1.** The influence of boron and biochar on plant height (**a**), spike length (**b**), number of grains (**c**), and 100-grain weight (**d**) of wheat under Cd stress, Ck control, Cd control stress, B boron, BC biochar, B+BC boron and biochar, Cd+B cadmium and Boron, Cd+BC cadmium and biochar and Cd+B+BC cadmium, boron and biochar. Different letters above the column indicate significance level among the treatments.

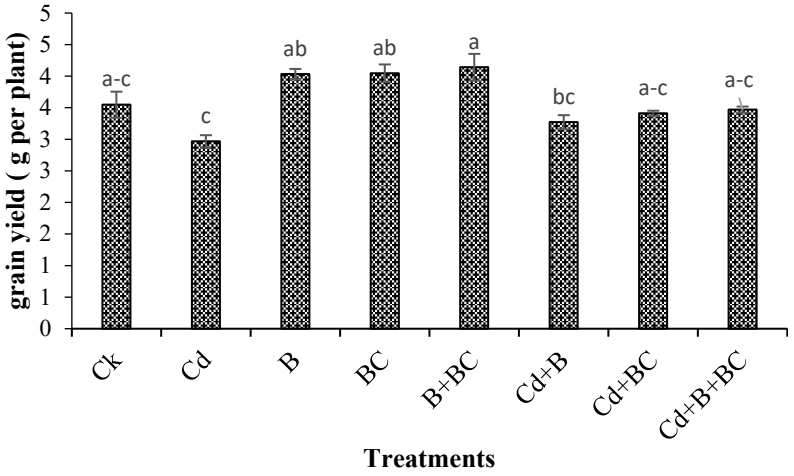


**Figure 2.** The influence of boron and biochar on the grain yield of wheat under Cd stress, Ck control, Cd control stress, B boron, BC biochar, B+BC boron and biochar, Cd+B cadmium and Boron, Cd+BC cadmium and biochar and Cd+B+BC cadmium, boron, and biochar. Different letters above the column indicate significance level among the treatments.

### *3.2. Photosynthetic Pigments*

Photosynthetic pigments (chl. a, chl. b, chl. a+b, chl. a/b, and carotenoid) of flag leaves were measured at the heading stage. In the absence of Cd stress, boron application enhanced 14.81% chl a, 18.51% chl b, 15.74% chl a+b, 3.98% chl a/b, and 17.64% carotenoid, while the biochar application enhanced 13.16% chl a, 6.17% chl b, 11.41% chl a+b, and 6.64% chl a/b as compared to individual Cd stress treatment. The sole application of biochar did not show any significant effect on carotenoid. However, the combination of both boron and biochar resulted in maximum activity and enhanced 22.22% chl a, 27.16% chl b 23.45% chl a+b, and 29.41% carotenoid as compared to the control, and chlorophyll a/b contents were reduced up to 4.65% when biochar was applied. However, when cadmium stress was imposed, it drastically decreased the content of photosynthetic pigments. It reduced 4.52% chl a, 25.92% chl b, 9.87% chl a+b, and 23.52% carotenoid content and chlorophyll a/b content were increased under Cd stress by 27.90%. The boron and biochar application on Cd-treated plants showed an almost similar effect with one another and enhanced 9.48% chl a, 20.0% chl b, 11.64% chl a+b, and 15.38% carotenoid as compared to Cd-treated plants. The combined application of biochar and boron on Cd-stressed plants enhanced 14.65% chl a, 23.33% chl b, 16.09% chl a+b, and 23.07% carotenoid as compared to Cd-stressed plants. There was a declined effect of boron and biochar and their combination on chlorophyll a/b under cadmium stress (Table 1).

### *3.3. Enzymatic Antioxidants Activities*

It was observed that application of only boron on wheat plants enhanced antioxidant activity as compared to the control condition. SOD, CAT, POD, and ascorbate peroxidase activities were improved by 17.03%, 20.57%, 13.53%, and 8.51%, respectively, as compared to the control. Biochar application enhanced 23.59% SOD, 9.59% CAT, 8.15% POD, and 10.63% ascorbate peroxidase as compared to control. However, when combined application of boron and biochar was done, it showed maximum results and increased 37.60% SOD, 13.25% CAT, 7.23% POD, and 19.14% ascorbate peroxidase as compared to control. In comparison, when cadmium stress was imposed, it boosted the antioxidant activity even much more than those plants where application of boron and biochar was done and increased 61.56% SOD, 60.12% CAT, 43.07% POD, and 90.42% ascorbate peroxidase as compared to the control. When boron was applied on Cd-treated plants, it enhanced 94.02% SOD, 8.78% CAT, 25.48% POD, and 22.90% ascorbate peroxidase activity as compared to control, and the application of biochar on Cd-treated plants resulted in 32.25%, 2.06%, 14.73%, and 7.82%

increases in SOD, CAT, POD, and ascorbate peroxidase activity, respectively as compared to individual application of Cd. The combination of boron and biochar showed maximum results and enhanced 51.57% SOD, 24.16% CAT, 32.58% POD, and 41.34% ascorbate peroxidase activity as compared to Cd-stressed plants (Table 2).

**Table 1.** The influence of the individual and combined applications of boron and biochar on the photosynthetic pigments of wheat leaves under cadmium stress.

| Treatments | Chlorophyll a (mg $g^{-1}$) | Chlorophyll b (mg $g^{-1}$) | Chlorophyll a+b (mg $g^{-1}$) | Chlorophyll a/b | Total Carotenoid (mg $g^{-1}$) |
|---|---|---|---|---|---|
| Control (ck) | 2.43 ± 0.02 e | 0.81 ± 0.017 d | 3.24 ± 0.037 e | 3.01 ± 0.039 cd | 0.17 ± 0.003 c |
| Cadmium (Cd) | 2.32 ± 0.01 f | 0.60 ± 0.020 g | 2.92 ± 0.015 f | 3.85 ± 0.013 a | 0.13 ± 0.004 e |
| Boron (B) | 2.79 ± 0.01 b | 0.96 ± 0.011 b | 3.75 ± 0.025 b | 2.89 ± 0.027 d | 0.20 ± 0.002 b |
| Biochar (Bc) | 2.75 ± 0.02 b | 0.86 ± 0.006 c | 3.61 ± 0.017 c | 3.21 ± 0.050 c | 0.18 ± 0.003 c |
| B+Bc | 2.97 ± 0.03 a | 1.03 ± 0.008 a | 4.00 ± 0.023 a | 2.87 ± 0.055 d | 0.22 ± 0.002 a |
| Cd+B | 2.54 ± 0.02 d | 0.72 ± 0.006 ef | 3.26 ± 0.026 e | 3.53 ± 0.015 b | 0.15 ± 0.001 d |
| Cd+Bc | 2.53 ± 0.02 d | 0.70 ± 0.008 f | 3.23 ± 0.027 e | 3.63 ± 0.027 b | 0.15 ± 0.001 d |
| Cd+B+Bc | 2.66 ± 0.01 c | 0.74 ± 0.004 e | 3.39 ± 0.011 d | 3.61 ± 0.030 b | 0.16 ± 0.002 d |
| LSD ≤ 0.01 | 0.079 | 0.038 | 0.090 | 0.203 | 0.010 |

Different small letters in the column indicated that the means are significantly different from each other at $p \leq 0.05$.

**Table 2.** Influence of the individual and combined applications of boron and biochar on the enzymatic antioxidants of wheat leaves under cadmium stress.

| Treatments | Superoxide Dismutase (Unit $mg^{-1}$ Protein) | Catalase (Unit $mg^{-1}$ Protein) | Peroxidase (Unit $mg^{-1}$ Protein) | Ascorbate Peroxidase (Unit $mg^{-1}$ Protein) |
|---|---|---|---|---|
| Control (ck) | 76.50 ± 1.28 g | 9.38 ± 0.17 d | 6.50 ± 0.117 d | 0.94 ± 0.051 d |
| Cadmium (Cd) | 123.60 ± 2.73 d | 15.02 ± 0.14 b | 9.30 ± 0.098 c | 1.79 ± 0.048 c |
| Boron (B) | 89.53 ± 2.45 f | 11.31 ± 0.44 c | 7.38 ± 0.061 d | 1.02 ± 0.024 d |
| Biochar (Bc) | 94.55 ± 1.57 f | 10.28 ± 0.57 cd | 7.03 ± 0.070 d | 1.04 ± 0.027 d |
| B+Bc | 105.27 ± 2.58 e | 10.67 ± 0.14 cd | 6.97 ± 0.052 d | 1.12 ± 0.046 d |
| Cd+B | 178.01 ± 2.29 b | 16.34 ± 0.31 b | 11.67 ± 0.149 a | 2.20 ± 0.069 b |
| Cd+Bc | 163.47 ± 3.03 c | 15.33 ± 0.72 b | 10.67 ± 0.167 b | 1.93 ± 0.023 c |
| Cd+B+Bc | 187.35 ± 1.23 a | 18.65 ± 0.21 a | 12.33 ± 0.147 a | 2.53 ± 0.028 a |
| LSD ≤ 0.01 | 7.73 | 1.44 | 0.891 | 0.199 |

Different small letters in the column indicated that the means are significantly different from each other at $p \leq 0.05$.

### *3.4. Lipid Peroxidation*

It was recorded that the application of only boron resulted in a 30.74% increase in $H_2O_2$ and 3.71% in soluble protein, with a 16.07% decline in malondialdehyde MDA content and 19.31% in electrolyte leakage as compared to the control conditions. Likewise, biochar application increased $H_2O_2$ by 20.24% and soluble protein by 16.64%, while it reduced malondialdehyde MDA content by 8.35% and electrolyte leakage by 14.39% as compared to control in wheat plants. When the combination of boron and biochar was applied, it also enhanced 37.25% $H_2O_2$ and soluble protein by 15.86% and reduced 4.25% malondialdehyde MDA content and 23.79% electrolyte leakage as compared to control. Although, when Cd stress was imposed, it increased 133.22% $H_2O_2$, 107.88% malondialdehyde MDA content, and 120.65% electrolyte leakage as compared to the control, but soluble protein content

was reduced by 76.94% as compared to control. When these Cd-treated plants were treated with boron, it decreased 18.68% $H_2O_2$, 29.87% malondialdehyde MDA content, and 26.98% electrolyte leakage as compared to individual application of Cd, while soluble protein content was increased by 90.24% as compared to individual application of Cd when boron was applied on Cd-treated plants. On the other hand, the application of biochar on Cd-treated plants resulted in 27.66%, 15.76%, and 21.27% decreases in $H_2O_2$, malondialdehyde MDA content, and electrolyte leakage, respectively as compared to individual application of Cd, while soluble protein content was increased by 100.0% as compared to individual application of Cd. However, the combination of boron and biochar decreased the 34.94% $H_2O_2$, 40.97% malondialdehyde contents MDA, and 38.51% electrolyte leakage as compared to individual application of Cd, the soluble protein content was increased by 169.26% as compared to individual application of Cd (Table 3).

**Table 3.** The influence of the individual and combined applications of boron and biochar on hydrogen peroxide, malondialdehyde content, electrolyte leakage, and soluble protein of wheat leaves under cadmium stress.

| Treatments | Hydrogen Peroxide ($\mu mol\ g^{-1}$) | Malondialdehyde Content ($\mu mol\ g^{-1}$) | Electrolyte Leakage (%) | Soluble Protein ($mgg^{-1}fw$) |
|---|---|---|---|---|
| Control (ck) | 21.34 ± 0.88 g | 12.69 ± 0.30 e | 35.77 ± 0.75 d | 8.89 ± 0.42 a |
| Cadmium (Cd) | 49.77 ± 0.83 a | 26.38 ± 0.68 a | 78.93 ± 0.89 a | 2.05 ± 0.05 c |
| Boron (B) | 27.90 ± 0.54 ef | 10.65 ± 0.65 f | 28.86 ± 1.97 e | 9.22 ± 0.65 a |
| Biochar (Bc) | 25.66 ± 0.29 f | 11.63 ± 0.27 ef | 30.62 ± 0.59 de | 10.37 ± 0.10 a |
| B+Bc | 29.29 ± 1.15 df | 12.15 ± 0.19 ef | 27.26 ± 1.10 e | 10.30 ± 0.68 a |
| Cd+B | 40.47 ± 0.79 b | 18.50 ± 0.41 c | 57.63 ± 2.02 b | 3.90 ± 0.27 b |
| Cd+Bc | 36.00 ± 0.57 c | 22.22 ± 0.33 b | 62.14 ± 2.19 b | 4.10 ± 0.08 b |
| Cd+B+Bc | 32.38 ± 0.64 d | 15.57 ± 0.40 d | 48.53 ± 0.63 c | 5.52 ± 0.12 b |
| LSD ≤ 0.01 | 3.24 | 1.85 | 5.89 | 1.72 |

Different small letters in the column indicated that the means are significantly different from each other at $p \leq 0.05$.

### *3.5. Osmo-Protectance*

Cadmium stress increased free proline by 37.95%, while it reduced soluble sugar by 56.25% and total phenolic by 64.13% in wheat, as compared to the control. However, when boron was applied to cadmium-stressed plants, it increased 19.83% total phenolic, 43.95% soluble sugar, and 36.17% total phenolic as compared to control, while biochar application on cadmium-stressed plants enhanced 9.26% total phenolic, 47.29% soluble sugar, and 27.64% total phenolic as compared to cadmium-stressed plants. The combination of boron and biochar enhanced 26.72% total phenolic, 53.95% soluble sugar, and 34.12% total phenolic as compared to cadmium-stressed plants (Table 4).

**Table 4.** The influence of individual and combined applications of boron and biochar on the osmo-protectants of wheat leaves under cadmium stress.

| Treatments | Free Proline ($mg\ g^{-1}$) | Soluble Sugar ($mg\ g^{-1}$) | Total Phenolic ($\mu mol\ g^{-1}$) |
|---|---|---|---|
| Control (ck) | 12.26 ± 0.052 d | 7.50 ± 0.45 cd | 4.79 ± 0.17 bc |
| Cadmium (Cd) | 19.76 ± 0.015 c | 4.80 ± 0.20 e | 2.93 ± 0.50 d |
| Boron (B) | 13.20 ± 0.258 d | 8.47 ± 0.37 bc | 5.64 ± 0.32 ab |
| Biochar (Bc) | 13.03 ± 0.619 d | 9.59 ± 0.11 ab | 6.28 ± 0.41 a |

**Table 4.** *Cont.*

| Treatments | Free Proline (mg $g^{-1}$) | Soluble Sugar (mg $g^{-1}$) | Total Phenolic (µmol $g^{-1}$) |
|---|---|---|---|
| B+Bc | 14.21 ± 0.648 d | 9.92 ± 0.30 a | 6.22 ± 0.55 a |
| Cd+B | 23.68 ± 0.787 ab | 6.91 ± 0.05 d | 3.99 ± 0.15 cd |
| Cd+Bc | 21.59 ± 0.799 bc | 7.07 ± 0.19 d | 3.74 ± 0.06 cd |
| Cd+B+Bc | 25.04 ± 0.272 a | 7.39 ± 0.56 cd | 3.93 ± 0.06 cd |
| LSD ≤ 0.01 | 2.30 | 1.25 | 1.15 |

Different small letters in the column indicated that the means are significantly different from each other at $p \leq 0.05$.

## 4. Discussion

The accumulation and toxicity of cadmium in crops can be reduced by lowering cadmium uptake in roots, as well as its translocation to above-ground plant parts.

### *4.1. Morphological and Yield Traits*

From the results of this study, it was revealed that after addition of biochar in Cd-contaminated soil, morphological and yield traits were improved as compared to the soil where biochar was not added (Figure 1). This enhancement in different morphological as well as yield traits might be accredited to the nourishing significance of biochar that enhances soil fertility and productivity. Another possible reason for this improvement is attributed to its internal capacity to enhance organic mineralization and increase crop growth, as well as yields [37,38]. Above all, biochar acts as a buffer and is a source of important plant nutrients in a sufficient quantity availability which markedly boosts crop's productivity [39]. These findings were established because of a noticeable reduction in the phytoavailability of cadmium in soil amended with biochar, hence hindering plant's uptake of Cd, which might have enhanced the ability of soil adsorption in biochar-amended soil. It has been reported that biochar has the ability to increase soil acidity and can improve the metal and basic nutrient availability from soil solutions that have an ability to reduce metal toxicity and, ultimately, improve crop growth and yield. Bashir et al. [40] reported in one previous study that the application of biochar in cadmium-polluted soils can proficiently overcome nutrient losses through leaching and volatilization, which is a vital factor in increasing soil fertility status and plant growth and yield.

The findings of our research depicted that the use of B application enhanced yield parameters due to its positive effects on plant growth (Figure 1). Lower yields of cadmium-stressed plants might be reasoned to the fast reduction in photosynthetic contents and assimilates. Hence, the movement of food assimilates from stem to grain is the main source, as well as a limiting factor for growth and development, and for reduction, in yields. Clemens et al. [41] showed that cadmium stress lowers plant growth first during the osmotic stress phase and, eventually, induces leaf senescence during the toxicity phase when more Cd is accumulated in leaves during transpiration. Ziaeyan and Rajaie [42] reported that the application of boron markedly enhanced plant biological yield, grain yield, 100-grain weight, number of grains per spike, and boron concentration in the tissues of plants grown under higher Cd-accumulated conditions. Boron, as an integral micronutrient, plays vital role in enhancing pollen grain germination and pollen tube enlargement, fruit set percentage, and, finally, yield. Boron is responsible for stimulating cell division, biosynthesis, and translocation of sugars, water, and nutrient uptake [43].

### *4.2. Photosynthetic Traits*

Photosynthetic activity is the basic functional process responsible for the growth and development of plants, but due to cadmium toxicity and cadmium stress, photosynthetic pigments are decreased in crop plants [44,45]. So, this cadmium stress leads to drastic effects on net photosynthetic processes. Presently, it was reported that the application of

exogenous cadmium lowered chlorophyll contents such as chl a, chl b, and carotenoid (Table 2). There was a decrease in carboxylase, ribose 1, phosphoenolpyruvate carboxylase, and 5-diphosphate carboxylase activities, which led to a reduction in photosynthetic pigments caused by cadmium toxicity. There was also an observation of the reduction in production of $H_2O_2$, which caused degradation of chlorophyll by an enzymatic action, hindered stomatal opening, and played a limiting function in the biochemical processes of crop plants, which ultimately lowered the accumulation of photosynthetic pigments [46]. From the present experiment's results, it was found that the use of biochar applications improved photosynthesis components and chlorophyll contents such as chl a, chl b, and carotenoid under cadmium toxicity or cadmium stress conditions (Table 2). This significant improvement was due to the reason that the application of biochar has a positive effect on the stabilization of heavy metals present in the soil, ameliorating the hindering impact of cadmium on photosynthetic processes and photosynthetic pigments, such as chlorophyll, production because of its porosity in structure, higher CEC, higher pH, and different surface complexities [47]. The results of this experiment depicted that the application of boron significantly improved wheat chlorophyll contents such as chlorophyll a, chlorophyll b, and carotenoid, even in the presence of cadmium stress (Table 2).

In many research article findings, boron is found to have ameliorating effects on the toxicity of heavy metals in various crop plants, though it is still unclear if boron can increase the resistance response of plants to heavy metals toxicity. Under cadmium toxicity, enhanced chlorophyll pigments might be due to B application because B is implicated in cell wall structure formation and cell wall functioning. B has the ability to connect borate complexes with several polyhydroxy compounds, including pectin assembly, glycosylinositol phosphoryl ceramides (GIPCs,) and rhamnogalacturonan-II (RG-II). Firstly, the cell wall of plants comes into contact with toxic heavy metals, being the outermost layer of a plant cell. The cell wall is the first respondent to heavy metal toxicity, as well as serving as a sink for toxic metals adsorption and accumulation [48]. Due to this active behavior and response of the cell wall, the accumulation of heavy metals is increased in the cell wall and the movement of heavy metals to protoplast is decreased. Different compounds present in the cell wall, such as certain carbohydrates and pectic sites, create immobilization of toxic metals by interconnecting heavy metal ions and hindering these compounds from entering into the cell's internal protoplasm. Cell walls can perform as a bio-sorbent for ameliorating the toxic impact of toxic heavy metals [48,49].

### *4.3. Antioxidant Enzyme Activities*

Presently, the results of this experiment showed that the application of exogenous cadmium caused stress and decreased the functions of various antioxidant enzymes, including SOD, CAT, $H_2O_2$, POD, and MDA, and electrolytic breakdown and leakage, as well as soluble proteins in wheat (Tables 3 and 4). This reduction was due to the reason that under cadmium stress, a greater number of ROS are formed. Those ROS can be significantly harmful to the functioning and metabolic processes of plants. Moreover, cadmium toxicity is also related to free radicals in membrane structures that lead to reductions in mobilization and the penetration of free ions in plant tissues, producing bigger quantities of MDA, and thus involving oxidative stress in plants, which eventually leads to a decline in antioxidant enzyme activities [50]. The use of biochar enhanced the processes of antioxidant enzymes and improved the response of plants against oxidative stress produced due to Cd [51].

Presently, in our results, the use of biochar improved the performance of antioxidant enzymes such as SOD, POD, and CAT, successfully ameliorating the adverse effects of Cd in wheat (Table 3). This positive effect is due to the reason that CAT activity is responsible for removing the impacts of toxicity of peroxides in plant cells, and superoxide dismutase (SOD) is the prime enzyme acting as a decomposer for superoxide radicals into $H_2O_2$. POD function in crop plants is to aid respiratory activities in plants and the changing of phenols to quinines for lowering the stress of heavy-metal-induced oxidative stress [52]. The plants can resist metal-induced stress by improving their health with the help of better

antioxidant defense mechanisms and can eliminate active oxygen with the addition of various amendments. Furthermore, a decrease of Cd concentration in various parts of plants likewise enhances anti-ROS safety activities in crop plants. The antioxidant enzymatic activities or antioxidant defense systems contribute key functioning in plants in relation to the response to heavy metal toxicity and stress. Enhanced levels of antioxidant enzymatic reactions can significantly diminish deposited ROS in crop plants. Several research findings have suggested that boron (B) diminishes cadmium stress by triggering antioxidant enzyme systems in crops [53]. In rice crops, improved performance of antioxidant enzymes played a key role in the mechanisms of resistance due to the addition of B against Cd toxicity [16]. As far as current experiment findings have determined, Cd occurrence leads to greater levels of MDA, $O^{-2}$, and $H_2O_2$, and increases the superoxide dismutase, catalase, and peroxidase functions to remove deposited reactive oxygen species in shoots. Surprisingly, the application of B under cadmium stress conditions worked to lower ROS deposition in shoots by triggering an antioxidant enzyme mechanism, relieving the lipid peroxidation of the cell membrane, and regulating the growth of wheat shoots (Table 3). This positive improvement in antioxidant enzyme activities might be due to the reason that boron, as a structural part, plays a role in building the cell wall structure and its components [18]. This cell wall act as the primary obstacle for plant cells exposed to contact with toxic heavy metals [54]. It performs an important function in enhancing plant's cadmium resistance through hindering Cd entry into plant cells and by adsorption of cadmium in cell wall components, which include cellulose, hemi cellulose, and pectin, and can chelate cadmium in the cell wall to lower the movement of cadmium into cells, and thus enhances antioxidant enzyme activities [55].

### *4.4. Biochemical Traits*

From our experiment findings, it was depicted that Cd toxicity reduced soluble sugar, total phenolic, and proline. Biochar application effectively increased proline, total phenolic, and soluble sugar contents in wheat under CD stress (Table 4). Various primary and secondary metabolic products such as soluble sugars and total proline and phenolic compounds play a vital role in cell osmotic adjustments. During stressful conditions, proline accumulation is higher in cells, as reported in many research studies. The findings of our experiment are in line with the results reported by [56]. The enhancement of the osmo-protectants, i.e., soluble sugars, proline, and total phenolic, could be attributed to biochar addition as a soil amendment in increasing the meristematic activity, which results in accelerated cell division and enlargement that ultimately improves the activity of osmo-protectants like proline, soluble sugar, and total phenolic [57]. From the current study, it was exhibited that biochar improved the osmo-protectants under Cd stress (Table 4). This improvement might be attributed to biochar application, which has the ability to hold water alongside increased nutrient absorption. Moreover, it has also been undeniably established that biochar can boost dry matter production (root and leaves) as a result of its positive effect on osmo-protectants under Cd stress [58].

## 5. Conclusions

It was concluded that B and biochar, when applied together at a 5 g $kg^{-1}$ and 50 g $kg^{-1}$ rate, significantly improved wheat plant's photosynthetic pigments, enzymatic activities, lipid peroxidation, osmo-protectants, and morphological and yield traits under both normal conditions and Cd toxicity. Therefore, it is suggested that wheat's photosynthetic pigments, enzymatic activities, lipid peroxidation, osmo-protectants, and morphological yield traits can be improved through the combined application of B and biochar under heavy metal (Cd) toxicity with a rate of 5 g $kg^{-1}$ and 50 g $kg^{-1}$, respectively.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/article/10.3390/agronomy12020434/s1, Table S1: The chemical composition of rice straw biochar.

**Author Contributions:** S.H. (Sajjad Hussain), J.Y., and A.S. designed the study and developed and drafted the manuscript. S.H. (Shabir Hussain), S.U. and M.C. gave technical support and helped in statistical analyses. S.H. (Sajjad Hussain), T.A. and F.N. performed the chemical analyses. A.A.-H., M.S.E., H.U.-R. and M.I. helped in draft-editing and grammatic correction. All authors have read and agreed to the published version of the manuscript.

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