

The role of wood-based biochar on growth, yield, and cadmium uptake in rice (Orvza sativa L.) grown under cadmium stress

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ABSTRACT

Background and Objective: Rice (Orvza sativa L.) is prone to absorb cadmium (Cd), which is a vital environmental pollutant and a harmful threat to food security and human health. In general, the application of biochar mitigates the detrimental effect of Cd stress on the growth, yield and quality of plants. To address this effect a pot experiment was conducted to investigate the effect of wood-based biochar on plant growth, yield, and physiological status in rice plants under Cd stress.

Methodology: The experiment was sequenced according to a completely randomized design with 3 replicates. Four different treatments namely control (0% biochar and no Cd stress), 25 ppm Cd stress, 2.5% wood-based biochar, and 25 ppm Cd stress + 2.5% wood-based biochar were used in the experiment. Data on plant height, tiller number, leaf area, and SPAD value were recorded 30 days after planting and analyzed statistically.

Main Results: Cadmium had a negative impact on the growth and yield of rice. The results exhibited that under Cd stress plant height (79.9 ± 2.00 cm), leaf area (47.09 ± 5.11 cm²), SPAD value (36.08 ± 0.37), grains per panicle (136 ± 9) and grain yield (51.37 ± 8.09 g hill-1) of rice significantly (P < 0.05) decreased. Cd-stressed plants significantly (P < 0.05) increased sterility percentage (35.55 ± 2.15) and grains Cd concentration (1.56 ± 0.065 mg kg⁻¹). In addition, soil pH was significantly (P < 0.05) increased (8.93 ± 0.24) in Cd-treated soil. The application of biochar in Cd-stressed plants significantly (P < 0.05) increased soil pH (9.15 ± 0.29), leaf area (54.83 ± 1.33 cm²), SPAD value (39.06 ± 1.50), grains per panicle (136 ± 9) and grain yield (70.78 ± 1.50 g hill⁻¹), whereas Cd concentration significantly (P < 0.05) decreased (1.26 ± 0.14 mg kg⁻¹).

Conclusions: These results suggest that mitigation of Cd toxicity by wood-based biochar results from a decrease in the uptake of Cd. indicating the role of wood-based biochar in enhancing plant growth and yield as well as reducing the potential risks to humans.

Keywords: Rice, wood-based biochar, cadmium, growth, yield

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INTRODUCTION

High heavy metal concentration becomes the main problem in the rice field receiving polluted irrigation. Heavy metal accumulation in crops

depends on several factors, including the plant type, soil characteristics, and the plant mechanism for heavy metal accumulation (Ahmad and Goni, 2010). The heavy metals could lessen crop growth and decrease crop production (Eid et al., 2020). Excessive heavy metal may lead to reduced crop productivity by interfering with photosynthesis. water, and mineral uptake in many plant species (Gallego et al., 2012). Cadmium (Cd) is an easily spread heavy metal toxicant in nature, which is absorbed and accumulated in plant tissues (Ali et al., 2013). This metal is highly bio-accumulated and toxic even at very low concentrations (Bashir et al., 2015), contaminates the food chains, and is then transferred to living organisms such as humans and animals (Rai et al., 2019). Cadmium toxicity resulted in harmful effects in many plant species including rice, such as growth inhibition. deficiency of photosynthesis pigments, leaf chlorosis. carbohydrate alteration, oxidative stress, imbalance of homeostasis, and lower crops yields (Mostofa et al., 2015; Rizwan et al., 2016). Thus, Cd stress adversely influences the growth, yield, and quality of rice (Ke et al., 2015).

Rice (Oryza sativa L.) is the staple food for over half of mankind. Cd is a well-known environmental pollutant in many areas in rice-growing regions of the world and ultimately poses a threat to human food (Williams et al., 2009; Zhuang et al., 2009). Cd enters plants through the root cortical tissues and travels into the xylem via a symplastic and/or apoplastic pathway before entering the xylem part of the roots (Lux et al., 2011). Rice root can absorb Cd, as a result, Cd is transported from stalks to the grain (Rizwan et al., 2017) causing toxicity to human beings who consume Cd-contaminated rice grains. Additionally, it has been reported that rice consumption was the top contributor to Cd intake for Asians (Kim et al., 2018). Studies conducted in Bangladesh showed that Cd dietary intake via rice consumption exceeds the FAO/WHO guideline limit (0.06 µg/kg/day), therefore, posed a serious public health risk in the country (Proshad et al., 2020).

Biochar is a solid charcoal product made from the pyrolysis process of biomass in the absence of oxygen. Various types of biomasses have been used on a commercial scale for biochar production successfully, including agricultural and forestry byproducts, industrial by-products, animal wastes, and sewage sludge (Mylavarapu *et al.*, 2013).

Converting residual biomass from the farm and food processing industry into biochar can help in achieving long-term carbon sequestration and other beneficial effects on soils and environmental properties (Parmar et al., 2014). Utilizing biochar in animal wastes also has the potential to adsorb pollutants, increase soil carbon sequestration, and improve plant growth (Onwuka and Nwangwu, 2016). Sludge biochar amendment promotes plant growth and improves certain soil health parameters (Junior and Guo, 2023). Biochar has a higher porosity, larger surface area, and ion exchange capacity, which provide great potential for plant-available nutrients (Lehmann and Joseph, 2015). Many studies have highlighted the benefit of using biochar in terms of mitigating global warming, soil amendment, enhancing crop vield and carbon storage (Whitman and Lehmann, 2011; Abit et al., 2012; Mao et al., 2012: Khare and Goval. 2013). However, wood biochars are reported to be efficient in terms of their characteristics, and are easily producible and cost-efficient. Modification of wood biochar results in a 50-70% increase in heavy metal adsorption capacity as compared to pristine biochar (Boraah et al., 2022). Biochar from wood sources shows more calorific value due to the presence of lignin, resin, pectin, and volatile materials (Gabhane et al., 2020). Wood-based biochar is a carbon-rich material, which is used as a soil ameliorant so that it can improve the water availability of soils (Lehmann and Joseph, 2015). However, it was not yet reported in Bangladesh whether wood biochar was more effective or not in controlling heavy metals in soils contaminated with heavy metals. The objective of the present study was to determine the effects of wood-based biochar on growth, yield, soil pH, and Cd accumulation in rice under Cd stress.

MATERIALS AND METHODS

Materials

Rice variety BRRI dhan93 was used as a planting material and seeds were collected from Bangladesh Rice Research Institute (BRRI), Gazipur, Bangladesh. BRRI dhan93 is a high-yielding variety of transplanted aman season and its growth duration



is 134 days. Biochar was supplied by the Christian Commission for the Development of Bangladesh (CCDB), Biochar was produced from Mahogany (Swietenia macrophyll) wood through a pyrolysis process using a biochar production stove. Krishi Bondhu Chula (KBC) under limited oxygen conditions for 90 min at a temperature between 300-700 °C. The tested soil was collected from a paddy field in the Gazipur district. Cadmium chloride (CdCl_a. H_oO) salt of high purity (98%) was purchased from Research-Lab Fine Chem Industries, India, and used to prepare desired cadmium concentration. The pH was analyzed using a pH meter (HACH HQ40d. USA) in a 1:10 ratio. Soil organic carbon (OC) was determined by the potassium dichromate wet oxidation method and dry combustion method for biochar, respectively. Organic matter (OM) was determined by the potassium dichromate wet oxidation methods. Total N was determined by the micro Kieldahl digestion method. Exchangeable Ca, Mg, and K were extracted using the ammonium acetate method. Available P was determined by Bray-1 extraction followed by molybdenum blue colorimetry method, available S and Zn were determined by turbidimetric method and DTPA extraction method, respectively. Othor nutrients (B, Cu, Fe, Zn, and Mn) for biochar were determined by dry ashing method. The basic properties of soil were: soil pH of 6.5, organic carbon (%) of 1.686, organic matter (%) of 2.908, total N (%) of 0.17, exchangeable K 0.27 meg/100 g soil, available P, S, and Zn respectively of 12.9, 25.01, and 9.07 ppm (Laboratory of Agronomy Division, BRRI). The basic properties of the mahogany wood biochar were pH

of 9.4, organic carbon (%) of 41.9, exchangeable Ca, Mg, and K respectively of 3.79, 2.23, and 1.84 meq/100 mL, total N (%) of 1.40, available P, Cu, Fe, Mn, and Zn respectively of 0.15, 0.05, 0.08, 0.032, and 0.012 µg/mL (BARI, 2016).

Experimental Design and Treatment Evaluation

The experiment was carried out in the net house of the School of Agriculture and Rural Development, Bangladesh Open University (90°38'N, 23°95′E). Gazipur. Bangladesh during the T. Aman season (July to November 2021), Containers of 29 cm in height and 30.5 cm in diameter were used for the pot trial. Each container contained 12 kg of air-dried soil and received 1.5 g N. 1.0 g P. 0.60 g K, 1.0 g S, and Zn 0.20 g, as Urea, TSP, MoP, Gypsum, and ZnSO₄ respectively. One cadmium stress level (25 ppm) and one biochar application rate 2.5% were set in a completely randomized design (CRD) and repeated three times. Biochar was weighed at the rate of 2.5% by soil weight and mixed thoroughly with soil before seedling transplanting. For heavy metal stress treatments, cadmium chloride (CdCl_a.H_aO) was mixed with the soil at 25 ppm concentration. Twenty-five-day-old seedlings were transplanted using two seedlings per hill on September 12, 2021. The full dose of P-K-S was applied during final soil preparation and N was applied in three splits (1/3rd at 15 days after transplanting (DAT), 1/3rd at 30 DAT, and 1/3rd at 45 DAT) following BRRI recommended dose. Regular irrigation and other management were adopted throughout the season. The following treatments were applied (Table 1).

Table 1 Treatments applied

SI. No.	Treatments
1	Control
2	Wood-based biochar @ 2.5%
3	25 ppm Cd
4	25 ppm Cd + Wood-based biochar @ 2.5%

Determination of SPAD Value

The concentration of leaf chlorophyll was recorded using a SPAD meter (Konica, Minolta SPAD-

502 Plus, Inc., Japan). Fully expanded leaves were used for the estimation of the SPAD values. The mean value of SPAD was calculated from three readings.

Measurement of Leaf Area

The leaf area of the plant was measured at the heading stage. The top 3 leaves from each treatment were used to measure leaf area with a digital leaf area meter (LICOR 3100).

Plant Sampling and Analysis

At 45 DAT, the total number of tillers hill-1 was counted from each pot. The height of rice plants was measured at harvesting. After plant harvest, the grain and shoots were separated for measuring panicle hill-1, grain panicles-1, 1,000-grain weight (q), sterility (%) as well as grain yield.

Sample Preparation for Heavy Metal Analysis

The rice grain and clean rice without husk are ground by a mini rice grinder machine. About 5 g of grinding grain was made and grinding grain was collected in a plastic box for chemical digestion and heavy metals analysis.

Determination of Cd Content

The concentrations of heavy metal were detected in rice using an atomic absorption spectrophotometer (AA-7000, Shimadzu, Japan). An air-acetylene flame was used to ensure maximum sensitivity during the instrument operation. The digestion and analysis of the collected samples were performed following the procedures described by Campbell and Plank (1992). Briefly, 0.5 g of the rice grain sample was transferred into a dry clean digestion 100 mL conical flask. Then 8 mL of 70% HNO3 was added to the conical flask and allowed to stand it overnight by covering the conical flask with a vapor recovery device. On the following day, the digestion conical flask was placed on a hot plate and was heated at a temperature slowly raised to 120 °C for 2 h. After cooling, 2 mL of 60% HCIO, was added to it and kept for a few minutes. Again, the conical flask was heated at 120 °C and continued until digestion, the content becomes like white sand. After digestion, the digest was cooled sufficiently. Milli Q water was added to the digested samples to make a final volume of 25 mL. The chemicals used for this analysis were of analytical grade and purchased from Merck (Germany). All the digested samples were then filtered using a Whatman filter paper No.1. Before analysis, all the consumables were soaked in diluted HNO_3 for 24 h and finally rinsed with distilled water. A calibration curve of Cd was made from standard known solution 0, 2.5, 5.0, 10, 20, 30, and 40 ppb and got the concentrations of 1.13, 1.94, 4.52, 9.82, 20.0, 30.1, and 40.1 respectively where r = 0.99932. Certified reference materials (Sigma Aldrich, USA) were used to ensure the good precision of the applied method.

Measurement of Soil pH

At the end of the experiment, soil samples were collected from the pots, and soil pH was measured by using a pH meter (HACH HQ40d, U.S.A), calibrated with pH 4 and 7 buffers. 10 g of soil sample was dissolved in 25 mL of distilled water and shaken for 30 min on a rotary shaker.

Statistical Analysis

The collected data was analyzed statistically using one-way analysis of variance (ANOVA) with the Cropstat 7.2 software. Treatment means were compared by the least significant difference (LSD) test at P < 0.05 level of significance.

RESULTS AND DISCUSSION

Meteorological Conditions

The highest rainfall prevailed in the study area during August (14.23 mm) followed by July (11.50 mm) and the mean maximum temperature was highest in September (34.1 °C) and October (33.9 °C) while the mean minimum temperature (18.9 °C) prevailed in November during the crop growth (Figure 1).

Effects of Wood-based Biochar on Soil pH under Cd Stress Condition

The soil pH played an important role in the contaminated soil mechanisms (Leibold and McPeek, 2006). The soil pH changes as shown in Figure 2 compared with no biochar application, the soil pH was significantly (P < 0.05) increased with



the sole application of biochar (2.5%) relative to control, while the soil pH was significantly (P < 0.05) changed in presence of Cd. The application of biochar significantly (P < 0.05) increased soil pH in Cd-stressed plants (Figure 2). Ijaz *et al.* (2020) observed that soil amendment with various types of biochar slightly increased soil pH in Cd-polluted soil. Ok *et al.* (2011) observed that due to alkaline nature of biochar enhanced soil pH in Cd-polluted

soil. The biochar has higher pH and cation-exchange capacity (Spokas *et al.*, 2011) and alkaline nature, containing CaCO₃, which dissociates that dissociate to Ca²⁺ and CO₃²⁻ subsequently, the reaction of CO₃²⁻ with water liberates OH⁻¹ ion, hence the pH of soil increased (Ok *et al.*, 2011; Yousaf *et al.*, 2016). Therefore, the addition of biochar under the Cd stress conditions is responsible for the significant increase in soil pH (Bashir *et al.*, 2019).

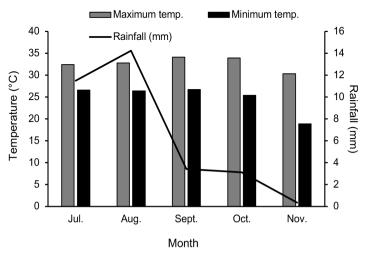


Figure 1 Temperature and rainfall status of Gazipur during the experimental period from July 2021 to November 2021 (Monthly average). Data retrieved from Plant Physiology Division (BRRI, 2021).

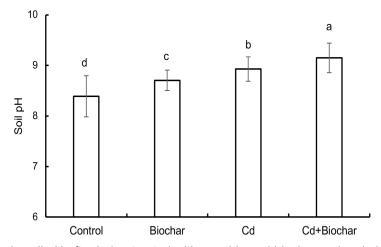


Figure 2 Change in soil pH after being treated with wood-based biochar and cadmium (Cd) at 2.5% and 25 ppm application levels, respectively. Vertical bars represent LSD value at a 5% level of significance. Different letters indicate significant differences between treatments at P < 0.05.

Effects of Wood-based Biochar on Growth Attribute under Cd Stress Condition

Cadmium toxicity induces growth inhibition in plants (Bhuyan *et al.*, 2020). The results showed that the sole application of biochar had no significant effect on plant height (94.66 \pm 4.25 cm), tiller number per hill (30.00 \pm 5.29), and leaf area (60.55 \pm 3.57 cm²) compared to the control. While Cd treatments

significantly (P < 0.05) decreased plant height, but tiller number was not influenced (P > 0.05) compared to the control. Furthermore, biochar treatments increased rice plant height and tiller number in Cd-stressed plants by 6.5% and 11.44%, respectively (Figures 3A and 3B). These results are consistent with previous studies, where biochar amendment increased plant growth under Cd stress (Qiu *et al.*, 2020).

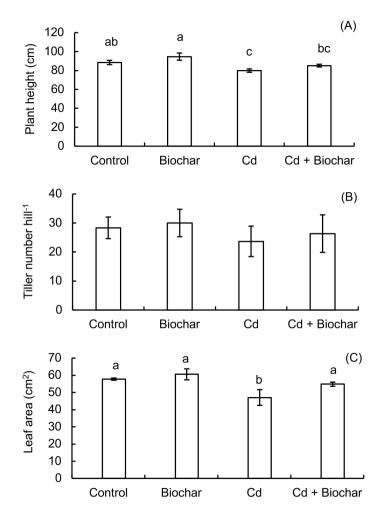


Figure 3 Effect of wood-based biochar on plant height (A), tiller number per hill (B) and leaf area (C) under cadmium (Cd) stress condition. Vertical bars represent LSD value at a 5% level of significance. Different letters indicate significant differences between treatments at P < 0.05.



The leaf area was affected by biochar treatment under Cd stress conditions (Figure 3C). Cd stress significantly (P < 0.05) decreased the leaf area compared to the control. At Cd stress, biochar significantly (P < 0.05) increased the leaf area compared to the respective Cd stress. This is in line with the recent finding (Majidi, 2022) observed that the application of biochar has significantly increased the leaf area in salt-stressed radish, lettuce, and spinach. Therefore, biochar can improve plant growth under Cd stress (Figure 3), suggesting that biochar has the potential to soil fertility through nutrient availability, increasing soil pH (Figure 2), and organic matter (Hague *et al.*, 2019).

Effects of Wood-based Biochar on SPAD Value under Cd Stress Condition

The result shows that SPAD values

significantly (P < 0.05) decreased in the Cd-stressed plants relative to non-stressed plants. However, the sole application of biochar had no significant effect on the SPAD value over their respective controls. The addition of wood-based biochar in the Cd-stressed plants significantly (P < 0.05) increases the SPAD chlorophyll content compared to the Cd-stressed plants (Figure 4), liaz et al. (2020) found that the chlorophyll SPAD value in wheat plants was considerably increased with the addition of biochar in Cd-contaminate soil. SPAD value can be used for predicting leaf N status in plants (Hou et al., 2021). There have been significant and positive relationships between SPAD value with N status and grain yield (Ramesh et al., 2002; Parvizi et al., 2004), indicating that biochar contributes to increasing N availability to the plants (Case et al., 2015).

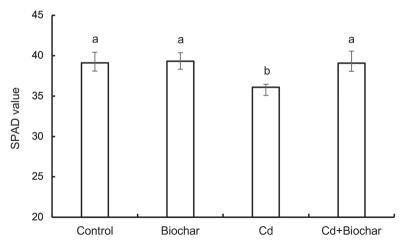


Figure 4 Effect of wood-based biochar on SPAD value of rice under cadmium (Cd) stress condition. Vertical bars represent LSD value at a 5% level of significance. Different letters indicate significant differences between treatments at P < 0.05.

Effects of Wood-based Biochar on Yield Components under Cd Stress Condition

From the perspective of rice yield components, the panicle number per hill (21.00 ± 3.78) and grains number per panicle (118 ± 6) of the Cd-stressed plant decreased by 12.5% and 7.81%, respectively, compared to the control treatment.

The rice grian sterility $(35.55 \pm 2.15\%)$ of the Cd treatment was higher than those of the control (Table 2). Therefore, the decrease in panicle number per hill and grains number per panicle, and the increase in grian sterility caused by the Cd stress was the main reason for the yield reduction (Figure 5) in rice under Cd stress conditions. The grains per panicle of

the wood-based biochar treatment was significantly (P < 0.05) increased in the Cd-stressed plant, compared to the Cd-stressed plant, while the rice grian sterility was significantly (P < 0.05) decreased than that of Cd-stressed plant, therefore biochar lessened the reduction of grain yield (Figure 5). Results indicate that wood-based biochar influenced the grain yield of rice

in Cd-stressed plants. However, biochar application did not affect 1,000-grain weight. Herath *et al.* (2014) reported that Cd-toxicity reduced yield and yield-related components in rice. Hussain *et al.* (2022) also reported that the application of biochar increased yield and yield traits in Cd-contaminated soil compared to the soil where biochar was not applied.

Table 2 Mean effect of wood-based biochar on yield contributing characters of BRRI dhan93 under Cd stress condition

Treatments	Panicles hill-1	Grains panicle ⁻¹	1,000-grain weight (g)	Sterility (%)
Control	24.00 ± 1.73	128 ± 11 ^{ab}	16.58 ± 0.37	33.35 ± 1.31 ^{ab}
Biochar	26.00 ± 3.00	127 ± 4 ^b	16.55 ± 0.32	31.62 ± 1.49 ^b
Cd	21.00 ± 3.78	118 ± 6 ^a	16.55 ± 0.24	35.55 ± 2.15 ^a
Cd + Biochar	26.00 ± 2.64	136 ± 9 ^b	16.38 ± 0.30	32.03 ± 1.47 ^b
CV (%)	12.3	3.2	2.2	3.8
LSD (0.05)	5.99	8.15	0.71	2.51

Note: Data are mean \pm standard deviation. Values with different letters in the same columns showed differ statistically among themselves (P < 0.05). Cd = cadmium.

Effects of Wood-based Biochar on Rice Grain Yield and Grain Cd Content under Cd Stress Condition

The rice grain yield of the Cd-stressed plant was significantly (P < 0.05) reduced compared to the control. The application of wood-based biochar significantly (P < 0.05) increased rice grain vield in Cd-stressed plant (Figure 5). Kanu et al. (2017) observed that Cd stress decreased the grain yield of rice plants. Chen et al. (2018) demonstrated that the two-year field experiment revealed that the application of biochar significantly improved rice yield in contaminated paddy soil. It is well reported that micro - and macronutrients (Ca, K, N, P, and Zn) available in biochar are being slowly released into the soil and taken up by the plants and increasing its productivity and yield thus the beneficial and actual effect of biochar could be observed clearly in long term experiment (Drake et al., 2016; Kim et al., 2016).

Cd is a toxic element that has a relatively high risk of transfer from paddy soil to rice grain. Reducing Cd accumulation in rice grain is important for food safety and human health. As shown in Figure 6, grain Cd concentration was significantly (P < 0.05) higher under 25 ppm Cd application in soil. The concentration of grain Cd significantly (P < 0.05) decreased in Cd-stressed plants under wood-based biochar treatments. This is similar to the recent finding of Nguyen et al. (2023) who demonstrated that the application of biochar in Cdcontaminated soil resulted in a decreased availability of Cd. These observations were consistent with the findings reported in rice (Rizwan et al., 2018). Also, the prepared biochar has a porous structure that is favorable for heavy metal immobilization by trapping in the porous structure, as stated by Lahori et al. (2017). In this study, rice grain Cd uptake decreased by 0.3 mg kg-1 with the application of 2.5% biochar (Figure 6). This result is similar to



that of He *et al.* (2017), who found that the brown rice Cd uptake decreased (0.09 mg kg⁻¹ for 0.5% biochar, 0.12 mg kg⁻¹ for 1% biochar, 0.28 mg kg⁻¹ for 2% biochar, and 0.41 mg kg⁻¹ for 4% biochar)

gradually with increasing biochar application. Biochar amendment at 40 t ha⁻¹ could even allow rice Cd levels to meet the guideline limit of 0.4 mg kg⁻¹ reported by the CAC (2005).

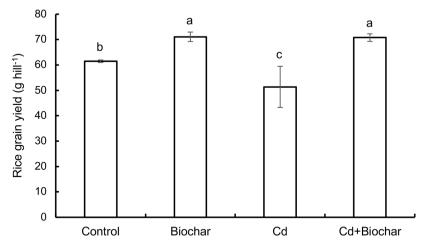


Figure 5 Change in rice yields with wood-based biochar treatment under cadmium (Cd) stress condition. Vertical bars represent LSD value at a 5% level of significance. Different letters indicate significant differences between treatments at P < 0.05.

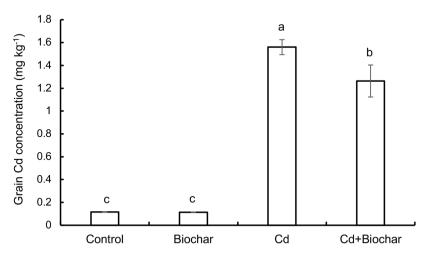


Figure 6 Change in grain cadmium (Cd) concentration with wood-based biochar treatment under Cd stress condition. Vertical bars represent LSD value at a 5% level of significance. Different letters indicate significant differences between treatments at P < 0.05.

CONCLUSION

The study revealed that Cd stress reduced the growth, chlorophyll content, yield. and vield components of rice. In addition, Cd stress increased soil pH and Cd content in grains of rice. However, wood-based biochar. lessened the reduction of its growth parameters as well as chlorophyll content, yield, and yield components of rice. The application of woodbased biochar caused more increase in soil pH

and reduced rice grain Cd concentration in Cdstressed plants. Therefore, wood-based biochar increases rice yield in Cd stress conditions by regulating grain ionic concentration and improving growth parameters.

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