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Title: Growth Performance of Soybean (*Glycine max* L. Merrill) Treated with Biochar under Cadmium Stress

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**ABSTRACT**

Cadmium (Cd) is a non-essential heavy metal that adversely affects plant growth. Biochar is predicted to facilitate plant growth. Under cadmium stress condition the effects of biochar on Soybean seedling growth, biomass production and SPAD value were poorly understood. In the current pot study Soybean was grown under different biochar and Cd combinations: 0% biochar and no Cd (control), 1.5% biochar, Cd (100 mg $kg^{-1}$ of soil), Cd (100 mg $kg^{-1}$ of soil) + 1.5% biochar. The results exhibited that under Cd stress shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), root dry weight (RDW), shoot length (SL), root length (RL), whole plant biomass, stress tolerance index (%) and SPAD value decreased in Soybean, while root and shoot length ratio increased in comparison to control. Application of biochar solely or in addition of Cd enhanced SFW (14.07% with solely and 126.44% with Cd); RFW, SDW, RDW, SL and RL (156.66%, 55.89%, 61.29%, 21.14% and 16.62% with Cd, respectively), whole plant biomass (8.41% with sole biochar and 137.77% with Cd) and SPAD value (34.94% with Cd) in Cd-stressed Soybean. The sole application of biochar or Cd increased stress tolerance index (%) (SFSTI, RFSTI, SDSTI, RDSTI, SLSTI and RLSTI as recorded 114.04%, 92.25%, 104.47%, 105.78%, 100.73% and 99.22%, respectively; SFSTI, RFSTI, SDSTI, RDSTI, SLSTI and RLSTI value by 125.90%, 156.67%, 55.79%, 61.31%, 30.16% and 16.62% with Cd, respectively) in Cd-stressed Soybean. These results demonstrate that soil amendment with 1.5% biochar enhanced growth, biomass production and SPAD value of Soybean under Cd stress. It is therefore recommended that biochar can be used as effective tool to mitigate the detrimental effect of Cd stress on Soybean seedlings.

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## INTRODUCTION

Soybean (*Glycine max* L. Merrill) is an important leguminous crop that provides 30% oil and 69% protein in worldwide food production (Lam et al., 2010). Due to its high protein and oil content, Soybean is an important cash crop for the human diet, animal feeds, and biodiesel. Therefore, the demand for Soybean is ever-increasing worldwide. This is the main reason Soybean is now being established not only on traditional arable lands, but also on marginal soils. However, its cultivation faces difficulties in lands with contaminated or stressed soils.

Cadmium (Cd) is one of the most harmful heavy metals, widespread in soils, and recognized as an extremely dangerous pollutant due to its high solubility in water and is known as the fourth most toxic to vascular plants (Ghosh and Singh, 2005). Excessive $Cd^{2+}$ accumulation in plant tissue has the potential to result in numerous morphological, physiological, and biochemical toxic effects (Lutts and Lefèvre, 2015; Wali et al., 2014, 2015 and 2016). The toxicity of $Cd^{2+}$ in plants is generally associated with disruption of water relations, reduced plant growth and respiration, damage to photosynthetic machinery in chloroplasts and stomatal closure (Wali et al., 2016; Souza et al., 2011). Organic amendment of such soils with the application of biochar would extend the cultivation of Soybean.

Biochar is a carbon-rich byproduct that arises from pyrolyzing organic compounds under high temperature and low or zero oxygen concentrations (Lehmann and Joseph, 2009; Sohi et al., 2010). The amendment of biochar to soil has widely shown positive changes in plant growth and crop productivity (Liu et al., 2013). Such effect was addressed with the manipulation of plant metabolic processes (Abbas et al., 2017; Sun et al., 2017), stimulation of plant development (Rawat et al., 2019), and root morphology (Xiang et al., 2017), improvement nutrient availability (DeLuca et al., 2015) as well as with the increase of chlorophyll content and net photosynthesis rate of plants (Abideen et al., 2020). Previous studies have been carried out on the impact of biochar on Soybean growth (Zhu et al., 2018; Yooyen et al., 2015; Głodowska et al., 2017). However, very few studies have focused on the impact of biochar on Cd-stressed Soybean (Haider et al., 2021). The present study was conducted to investigate the effects of biochar on seedling growth, biomass production and SPAD chlorophyll content of Soybean under Cd stress.

## MATERIALS AND METHODS

The pot experiment was conducted in the field laboratory of the School of Agriculture and Rural Development, Bangladesh Open University, Gazipur in January to February, 2022. Each plastic pot (height of 21 cm and top and bottom diameter of 22 cm and 13 cm, respectively) was filled with 4.5 kg of soil. Soybean variety namely BARI Soybean-6 was collected from Bangladesh Agricultural Research Institute (BARI), Gazipur and used in this study. Cadmium chloride ($CdCl_2.H_2O$) salt of high purity (98%, Research-Lab fine chemical, India) and used to prepare desired Cd concentrations. Basic properties of soil before the experiment were: soil pH of 8.42, organic carbon of 0.728%, organic matter of 1.25%, total N of 0.08%, exchangeable K 0.13 meq 100 $g^{-1}$ soil, available P, S and Zn, respectively, of 9.20, 13.02 and 6.2 mg $kg^{-1}$ soil. Biochar was supplied by the Christian Commission for the Development of Bangladesh (CCDB). Biochar was produced from Mahogany (*Swietenia macrophyll***)** wood through pyrolysis process using a biochar production stove, Krishi Bondhu Chula (KBC) under limited oxygen conditions for 1 hour 30 minutes at a temperature between 300–700°C. Basic properties of the biochar were: pH of 9.4, organic carbon of 41.9%, total Nof 1.40%, exchangeable Ca, Mg and K respectively of 3.79, 2.23 and 1.84 meq/100 ml, available P, Cu, Fe, Mn and Zn respectively of 0.15, 0.05, 0.08, 0.032 and 0.012 µg/ml.

The experiment was carried out in a completely randomized design (CRD) with four treatments (control + recommended dose of fertilizer, RDF; biochar @ 1.5% + RDF; 100 mg Cd $Kg^{-1}$ soil + RDF and 100 mg Cd $Kg^{-1}$ soil + biochar @ 1.5% + RDF) and three replications (1 replication = 4 pots) and the treatment combination are 12. Five Soybean (BARI Soybean-6) seeds per pot were sown. After germination, pots were hand-thinned keeping three plants per pot. Each pot was fertilized NPKS @ 25:35:55:18 kg $ha^{-1}$, respectively from Urea, Triple super phosphate (TSP), Muriate of potash (MoP) and Gypsum (BARI, 2020). Total dose of fertilizer was applied to the soil of the pot before Soybean sowing. The pots were irrigated regularly to maintain optimum soil moisture content. Weeds were removed from pots manually.

Thirty days old seedlings were harvested, and then plants were separated into roots and shoots. The shoot and root lengths (cm) were recorded using a ruler. Shoot fresh weight (g) of each pot was recorded by using a weighing balance, and samples were put in a forced-draft oven (70 °C temperature) until the constant weight of the shoot dry weight (g). The roots from each pot were washed separately and carefully with tap water followed by distilled water, then the fresh weight of the root was taken and after that, the root was placed in the oven for the dry weight. Before oven drying, the root or shoot length ratio (average root length: shoot height) of the seedlings was determined.

***Stress tolerance index***

Stress tolerance indices (STI) for different growth parameters were calculated using following formulae (Wilkins, 1957):

$$\text{Shoot fresh weight STI} = \frac{\text{Shoot fresh weight of stress plant}}{\text{Shoot fresh weight of control plant}} \text{X100}$$

$$\text{Root fresh weight STI} = \frac{\text{Root fresh weight of stress plant}}{\text{Root fresh weight of control plant}} \text{X100}$$

$$\text{Shoot dry weight STI} = \frac{\text{Shoot dry weight of stress plant}}{\text{Shoot dry weight of control plant}} \text{X100}$$

$$\text{Root dry weight STI} = \frac{\text{Root dry weight of stress plant}}{\text{Root dry weight of control plant}} \text{X100}$$

$$\text{Shoot length STI} = \frac{\text{Shoot length of stress plant}}{\text{Shoot length of control plant}} \text{X100}$$

$$\text{Root length STI} = \frac{\text{Root length of stress plant}}{\text{Root length of control plant}} \text{X100}$$

***Determination of SPAD Value***

The concentration of leaf chlorophyll was recorded using a SPAD meter (Konica, Minolta SPAD-502 Plus, Inc., Japan) following the procedure described by Ling et al. (2011). Fully expanded leaves were used for the estimation of the SPAD values. Mean value of SPAD was calculated from three readings.

***Statistical analysis***

The collected data was analyzed the statistically using one way analysis of variance (ANOVA) with the Cropstat7.2 software. Treatment means were compared by the least significant difference (LSD) test at $P \leq 0.05$ level of significance.

## RESULTS AND DISCUSSION

***Effects of biochar on shoot and root fresh weight, dry weight and whole plant biomass under Cd stress condition***

The result of the current study shows that Cd stress significantly ($P \leq 0.05$) decreased the fresh weight of the shoot and root by 64.51% and 72.52%, respectively, when compared with that of the control. On the other hand, exogenous application of biochar significantly increased the shoot and root fresh weight of the Cd-stressed plants by 126.44% and 156.66%, respectively. It also increased shoot fresh weight of the non-stressed plants, by 14.07%, however root fresh weight did not change by biochar application in non-stressed plant (Fig. 1). Dad et al., (2021a) found that Cd stress significantly reduces biomass accumulation. Tanveer et al. (2022) found that shoot and root fresh weight of spinach was reduced in cadmium-contaminated soil. Current findings are similar to Bashir et al. (2021), who reported that the addition of various kinds of 2% biochar's in wastewater irrigated soil improved sunflower fresh and dry shoot root biomass.

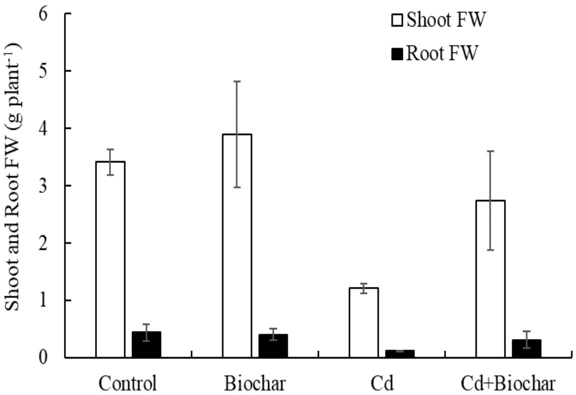


**Figure 1. Effect of biochar on the fresh weight (FW) of shoot and root of Soybean seedling under Cd stress condition. Vertical bars represent LSD value at 5% level of significance.**

Cd stress significantly reduced shoot and root biomass (Fig. 2). In respect of control, the Cd stress decreased shoot dry weight by 54.16% and root dry weight by 57.93%. Compared with no biochar application under Cd stress, the application of Cd-biochar minimized the toxic effect of Cd on biomass accumulation by improving shoot and root dry weight as 55.89% and 61.29% respectively. However, exogenous biochar did not increase shoot and root dry weight of the non-stressed plants (Fig. 2). Dad et al. (2021a) reported that the 0.75 mg $Kg^{-1}$ Cd stress decreased root dry weight by 85% and shoot dry weight by 30%, relative to control. According to them, Fe-Biochar minimized the toxic effect of Cd on biomass accumulation by improving root dry weight and shoot dry weight up to 21-fold and three-fold, respectively, compared with no Fe-Biochar application under Cd stress.

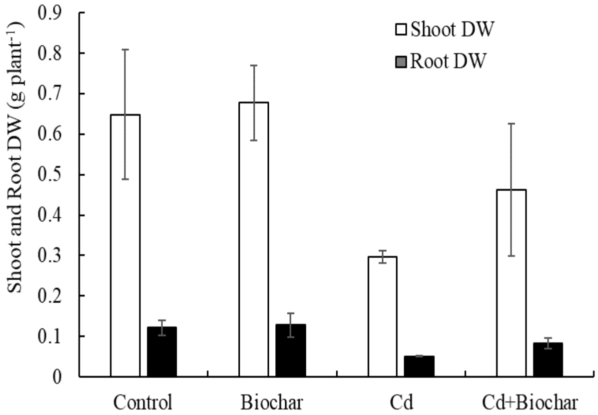


**Figure 2. Effect of biochar on the dry weight (DW) of shoot and root of Soybean seedling under Cd stress condition. Vertical bars represent LSD value at 5% level of significance.**

Whole plant biomass decreased considerably (68.31%) in the Cd stress condition. The whole plant biomass also increased by 137.77% after the addition of biochar in Cd-stressed plant (Fig. 3).

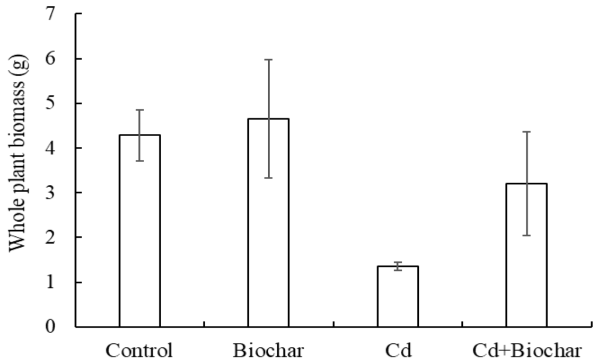


**Figure 3. Effect of biochar on whole plant biomass of Soybean seedling under Cd stress condition. Vertical bars represent LSD value at 5% level of significance.**

However, biochar amendment slightly increased (8.41%) whole plant biomass of the control plants (Fig. 3). Kukier and Chaney (2004) reported that Cd disrupts nutrient absorption and photosynthesis which ultimately reduces biomass production. Similar results were reported by Ullah et al. (2020) who observed that the toxicity of Cd reduced the whole plant biomass in Chickpea cultivars. Tian et al. (2017) indicated that added biochar significantly increased plant biomass in both the Asian Lotus (*Nelumbo nucifera*) and Chinese Sage (*Salvia miltiorrhiza*) while they were grown in the artificially Cd-polluted condition in containers.

### *Effects of biochar on shoot and root length under Cd stress condition*

Root and shoot length is the most sensitive morphology of plants and are directly correlated to the effect of heavy metal (Ahmad et al., 2008; Jun-yu et al., 2008). Figure 4 indicated that Cd stress decreased shoot and root length, by 33.38% and 22.55%, respectively, when compared with control.

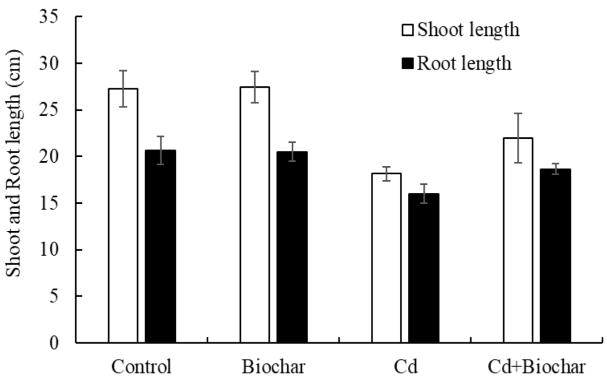


**Figure 4. Effect of biochar on the shoot and root length of Soybean seedling under Cd stress condition. Vertical bars represent LSD value at 5% level of significance.**

Comparatively, the combined application of biochar and Cd increased shoot and root length, by 21.14% and 16.62% respectively, when compared with Cd-stressed plant. According to the Rascio et al. (2008), root length declined under Cd stress because it changes the morphogenesis of roots. Tanveer et al. (2022) also reported decreased root and shoot length in *Spinacia oleracea* grown under Cd stress. Bashir et al. (2021) found an increase in shoot-root length of sunflower after the addition of 2% biochar in wastewater irrigated soil containing Cd. Dad et al. (2021b) also found that biochar significantly reduced the negative impact of cadmium on the root and shoot length of the tomato plant.

### *Effects of biochar on root/shoot length ratio under Cd stress condition*

The root/shoot length ratio was affected by Cd stress and biochar applications (Table 1). The Cd stress increased root/shoot ratio, by 15.93%, relative to control. Sole application of biochar decreased the root/shoot ratio by 1.32% compared to that of control whereas application of biochar to the Cd-stressed plant decreased root/shoot ratio by 2.63% compared to that of Cd-stressed plant. These results are supported by Lu et al. (2013) who suggested that root/shoot ratio of peanut cultivar Xvhua 13 increased in the presence of Cd while in the other cultivars (Haihua 1), it decreased or remained constant, indicating the influence of Cd on root/shoot ratio is cultivar specific. Recent studies (Zhu et al., 2018) indicated that biochar addition reduced the root/shoot ratio of Soybeans.

**Table 1. Mean effect of the biochar on the root and shoot length ratio of Soybean seedlings under Cd stress condition.**

| Treatments | Root and shoo length ratio |
|---|---|
| Control | 0.753 |
| Biochar @ 1.5% | 0.743 |
| 100 mg Cd/Kg soil | 0.873 |
| 100 mg Cd/Kg soil + Biochar @ 1.5% | 0.850 |
| LSD (0.05) | 0.98 |
| CV (%) | 6.8 |

### *Effects of biochar on stress tolerance index under Cd stress condition*

Figure 5 shows that seedlings grown with biochar have the highest value of stress tolerance index (STI %) and it was lowest in Cd-stressed plants. STI (%) was calculated from the ratio of stressed plants and control plants. Sole application of biochar resulted in the highest value of SFSTI, RFSTI, SDSTI, RDSTI, SLSTI and RLSTIas recorded 114.04%, 92.25%, and 104.47%, 105.78%, 100.73% and 99.22% respectively. While, Cd treatment reduced the SFSTI, RFSTI, SDSTI, RDSTI, SLSTI and RLSTI value by 35.63%, 27.33%, 45.83%, 42.06%, 66.61% and 77.44% respectively. Biochar application lessened the reduction of the SFSTI, RFSTI, SDSTI, RDSTI, SLSTI and RLSTIvalue by 125.90%, 156.67%, 55.79%, 61.31%, 30.16% and 16.62% respectively. Amirahmadi et al. (2020) found that the plant tolerance index for the highest Cd rate of 50 mg $kg^{-1}$ increased significantly by 40.9%, 56%, and 60.6% with rice husk biochar application in oak seedlings contaminated with Cd at the rates of 1%, 3%, and 5%, respectively. Zhang et al. (2015) also concluded that addition of biochar enhances soil nutrient status and the presence of major growth promoting nutrients in biochar help the plants to cope with water stress.

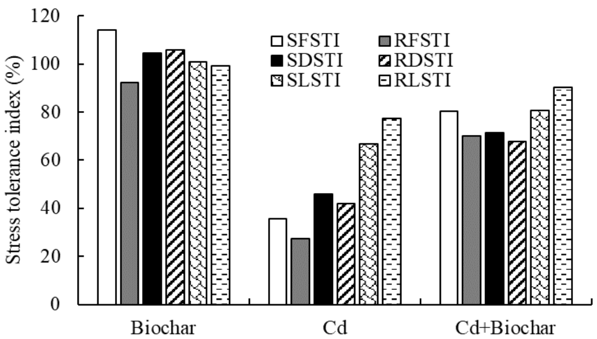


**Figure 5. Stress tolerance index (%) for shoot fresh weight (SFSTI), root fresh weight (RFSTI), shoot dry weight (SDSTI), root dry weight (RDSTI), shoot length (SLSTI) and root length (RLSTI) of Soybean seedling under Cd stress.**

### *Effects of biochar on SPAD value under Cd stress condition*

SPAD values can reflect leaf chlorophyll concentrations.Chlorophyll is the source for biomass production. The significantly reduction in the SPAD value (by 29.47%) was observed in plants treated with Cd compared to the untreated control. When compared to the corresponding Cd-stressed plant, biochar supplementation of plants treated with Cd increased the SPAD value by 34.94% (Fig.6). Xue et al. (2013) reported decreased PS II activity and Chlorophyll content in leaves of the Soybean seedlings under Cd treatments. Ijaz et al. (2020) found that the chlorophyll SPAD value in wheat plants increased considerably with the addition of biochar in Cd contaminated soil.

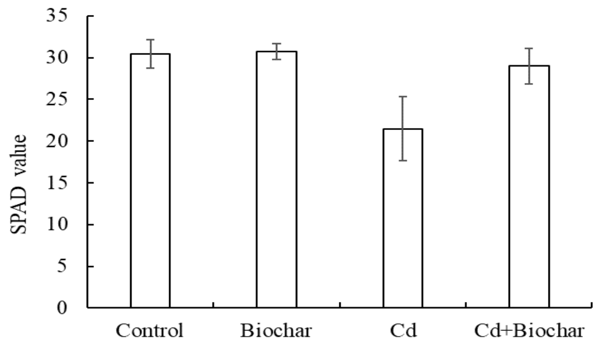


**Figure 6. Effect of biochar on SPAD value of Soybean seedling under cadmium stress condition. Vertical bars represent LSD value at 5% level of significance.**

## CONCLUSION

In this study, the effects of biochar were evaluated in Cd-amended pot culture of Soybean. The treatments of the Soybean seedlings with Cd resulted in an inhibition of shoot and root fresh weight, shoot and root dry weight, shoot and root length, whole plant biomass, tolerance index (%) and SPAD value. Added biochar (1.5% in soil mix) lessened the inhibition of shoot and root fresh weight, shoot and root dry weight, shoot and root length, whole plant biomass, stress tolerance index (%) and SPAD value under Cd stress condition. Therefore, it could be concluded that as a valuable soil amendment, biochar has a huge potential in green agriculture production due to its positive effects of improving plant growth in Cd-contaminated soil.

## CONFLICT OF INTEREST

The author declares that there is no conflict of interest regarding the publication of this article.

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