AMELIORATIVE EFFECT OF WOOD BIOCHAR FOR CADMIUM STRESS ON SOYBEAN (Glycine max L. Merrill) GROWTH AND YIELD

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ABSTRACT
Cadmium (Cd) is a heavy metal that affects plant growth and yield. Biochar can mitigate the detrimental effect of Cd on plants. To assess effectiveness of wood biochar (WB) in mitigating Cd stress, a pot experiment was conducted with six treatments in Soyabean crop viz., control (0% biochar and no Cd stress), 1.5% WB, 3% WB, 100 mg CdCl2 Kg⁻¹ soil, 100 mg CdCl2 Kg⁻¹ soil +1.5% WB, 100 mg CdCl2 Kg⁻¹ soil +3% WB. The impact of Cd on the growth, physiological status, yield and yield-contributing characters of Soybean were found to be significant (p<0.05). The application of 1.5% and 3% WB significantly (p < 0.05) alleviated Cd toxicity to Soybean under Cd stress conditions. There was no significant difference in the effect of two rates of wood biochar's (1.5% and 3% WB) on growth, physiological status and yield of Soybean. Therefore, it can be concluded that 1.5% WB is sufficient to reduce toxicity caused by Cd stress in Soybean.

Keywords: Biochar, Cadmium, Growth, Soybean, Yield

INTRODUCTION
Soybean (Glycine max L. Merrill) belongs to Fabaceae family native to East Asia and is an important oilseed crop over the world producing approximately 350 billion kg per year (Foreign Agricultural Service USDA, 2020). Soybean is a protein-rich food and feed as well as a significant source of nutraceutical compounds with many different medical benefits (Cober et al., 2009).

Cadmium (Cd) is an important toxic element in nature and harmful to most organisms. Cd is highly water soluble and readily taken up by plants, leading to decreased growth, chlorophyll content and seed production of the plants, thereby reducing their yield and nutritional quality, and posing a threat for human nutrition (McLaughlin et al., 1999; Xue et al., 2013). It has been reported that mean

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concentration of Cd ranges in plants from 0.013 to 0.22 mg kg\(^{-1}\) for cereal grains, 0.07 to 0.27 mg kg\(^{-1}\) for grasses, and 0.08 to 0.28 mg kg\(^{-1}\) for legumes (Kabata-Pendias and Pendias, 2001). At the whole plant level, Cd toxicity includes leaf chlorosis, a delay in the growth rate, and inhibition of respiration and photosynthesis (Navarro-Leon et al., 2019), increased oxidative damage, and decreased nutrient uptake ability (Mohamed et al., 2012).

Different biomasses are utilized to produce biochars; however, wood biochars (WB) are reported to be efficient in terms of their characteristics, are easily producible, cost-efficient. Modification of WB results in a 50-70% increase in heavy metal adsorption capacity as compared to pristine biochar (Boraah et al., 2022). Biochar has large specific surface area, rich porous structure, abundant surface functional groups, and high mineral content (Leng et al., 2019 and Kumar et al., 2018). Many studies have highlighted the benefit of using biochar in terms of mitigating global warming, soil amendment, enhancing crop yield and carbon storage (Whitman et al., 2011; Abit et al., 2012; Mao et al., 2012; Khare and Goyal, 2013; Verheijen et al., 2014). As a soil amendment, biochar has been applied to remediate organic and inorganic contaminants (Liang et al., 2021), alleviate the adverse effects of environmental stresses (Khan et al., 2021), enhanced soil water-holding capacity (Ndede et al., 2022) and improve crop yields (Knoblauch et al., 2021). Therefore, the present study was conducted to investigate the effects of wood biochar on growth, biomass production, SPAD chlorophyll content and yield of Soybean under Cd stress condition.

**MATERIALS AND METHODS**

**Test materials and growth conditions**

A pot experiment was conducted in the field laboratory of the School of Agriculture and Rural Development, Bangladesh Open University, Gazipur, Bangladesh. The experiment was carried out in a completely randomized design (CRD) with six treatments (control + recommended dose of fertilizer, RDF; biochar @ 1.5% + RDF; biochar @ 3% + RDF; 100 mg Cd Kg\(^{-1}\) soil + RDF; 100 mg Cd Kg\(^{-1}\) soil + Biochar @ 1.5% + RDF and 100 mg Cd Kg\(^{-1}\) soil + Biochar @ 1.5% + RDF) and three replications. The total pots were 18 and 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. The tested soil was collected from topsoil layer of the crop field in the Gazipur district, thoroughly mixed and filled in pots. 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\(_2\)O) salt of high purity (98%, Research-Lab fine chemical, India) and used to prepare desired Cd concentrations. Biochar was supplied by the Christian Commission for the Development of Bangladesh (CCDB). Biochar was produced from Mahogany (*Swietenia macrophylla*) 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. Five soybean (BARI Soybean-6) seeds per pot were sown. After germination, pots were hand-thinned keeping three plants per pot. Basic fertilizer was applied before planting consisting of 10 mg N kg⁻¹ (25 kg N ha⁻¹) soil as urea, 20 mg K kg⁻¹ (35 kg P ha⁻¹) as Triple super phosphate (TSP), 30 mg P kg⁻¹ (55 kg K ha⁻¹) as Muriate of potash (MoP) and 10 g S kg⁻¹ (18 kg S ha⁻¹) as 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. The pH was analyzed using pH meter (HACH HQ40d, U.S.A) in a 1:10 ratio. Soil organic carbon (OC) was determined by the dichromate wet oxidation method and dry combustion method for soil and biochar, respectively. Organic Matter (OM) was determined by the dichromate wet oxidation methods. Total N was determined by the micro Kjeldahl digestion method. Exchangeable Ca, Mg, K were extracted using ammonium acetate method. Available P, S, B, Cu, Fe, Mn and Zn were determined by Bray-1 extraction followed by molybdenum blue colorimetry. The basic properties of the soil and biochar are shown in Table 1.

Table 1. Basic properties of initial soil and biochar

<table>
<thead>
<tr>
<th>Properties</th>
<th>Soil</th>
<th>Biochar</th>
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<tbody>
<tr>
<td>pH</td>
<td>8.42</td>
<td>9.4</td>
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<tr>
<td>Organic carbon</td>
<td>0.728%</td>
<td>41.9%</td>
</tr>
<tr>
<td>Organic matter</td>
<td>1.25%</td>
<td>-</td>
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<tr>
<td>Total N</td>
<td>0.08%</td>
<td>1.40%</td>
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<tr>
<td>Exchangeable K (meq 100 g⁻¹ soil)</td>
<td>0.13</td>
<td>1.84</td>
</tr>
<tr>
<td>Exchangeable Ca (meq 100 g⁻¹ soil)</td>
<td>-</td>
<td>3.79</td>
</tr>
<tr>
<td>Exchangeable Mg (meq 100 g⁻¹ soil)</td>
<td>-</td>
<td>2.23</td>
</tr>
<tr>
<td>Available P (µg ml⁻¹)</td>
<td>0.092</td>
<td>0.15</td>
</tr>
<tr>
<td>Available S (µg ml⁻¹)</td>
<td>0.013</td>
<td>-</td>
</tr>
<tr>
<td>Available B (µg ml⁻¹)</td>
<td>0.006</td>
<td>-</td>
</tr>
<tr>
<td>Available Cu (µg ml⁻¹)</td>
<td>-</td>
<td>0.05</td>
</tr>
<tr>
<td>Available Fe (µg ml⁻¹)</td>
<td>-</td>
<td>0.08</td>
</tr>
<tr>
<td>Available Mn (µg ml⁻¹)</td>
<td>-</td>
<td>0.032</td>
</tr>
<tr>
<td>Available Zn (µg ml⁻¹)</td>
<td>-</td>
<td>0.012</td>
</tr>
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</table>

Measurement of leaf area

The leaf area was measured at the pod bearing stage with a digital leaf area meter (LICOR 3100).
Determination of SPAD value

SPAD 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. Mean value of SPAD was calculated from three readings.

Growth and yield parameters

The crop was harvested at maturity. The plant samples were collected 56 days after emergence. The height of Soybean plants was measured at harvesting. After plant harvest, yield contributing characters like pods per plant, seed per plant, and seed yield per plant were recorded.

Statistical analysis

The collected data was analyzed statistically using one way analysis of variance (ANOVA) with the Cropstat10 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 wood biochar on plant height, leaf area and leaf number of Soybean under Cd stress condition

Many studies have proven that Cd pollution has a variety of negative effects on plant growth (Zhao et al., 2021; Kaleem et al., 2022). Cadmium stress significantly ($P < 0.05$) decreased plant height and leaf area of Soybean by 17.65% and 47.71%, when compared with the control treatment. Application of wood biochar (WB1:1.5% and WB: 3%) significantly ($P < 0.05$) alleviated the Cd stress of Soybean. The addition of wood biochar treatment under Cd stress, which increased the height (23.48% and 35.73% at WB1:1.5% and WB:3%, respectively), leaf area (75.17% and 85.41% at WB1:1.5% and WB:3%, respectively) and leaf number (17.64% and 17.64% at WB1:1.5% and WB:3%, respectively) of Soybean than Cd-stressed plant. There was no significant ($P < 0.05$) difference in the plant height, leaf area and leaf number between 1.5% and 3% biochar treatments under Cd stress (Figs. 1, 2 and 3). Nagaraju et al. (2015) stated that heavy metals are carcinogenic in nature, and damage DNA, proteins and lipids that adversely affect plant growth. Dadasoglu et al. (2022) observed that Cd stress led to a decrease in seedling height and leaf area of the bean, whereas the biochar treatment could effectively increase plant height and leaf area in Cd-stressed bean plants. Further, the positive effects of biochar on plant growth performance under Cd contamination was similarly obtained in studies on wheat (Ali et al., 2019) and mint species (Jiang et al., 2022).
Figure 1. Effect of wood biochar on plant height of Soybean under Cd stress condition. (WB1: 1.5% wood biochar; WB2: 3% wood biochar; Cd: 100 mg cadmium Kg⁻¹ soil. Vertical bars represent LSD value at 5% level of significance. Means within the column followed by the same letter are not significantly different.)

Figure 2. Effect of wood biochar on leaf area (cm²) of Soybean under cadmium stress condition. (WB1: 1.5% wood biochar; WB2: 3% wood biochar; Cd: 100 mg cadmium Kg⁻¹ soil. Vertical bars represent LSD value at 5% level of significance. Means within the column followed by the same letter are not significantly different.)
Effects of wood biochar on biomass production of Soybean under Cd stress condition

Figure 4 shows that Cd stress treatment without biochar addition significantly (P < 0.05) reduced (by 36.47%) the biomass production in the Soybean plant compared to the control. The plant biomass also significantly (P < 0.05) increased by 66.33% and 82.17% after the addition of 1.5% and 3% biochar in Cd-stressed plant (Fig. 4), which indicated that the application of wood biochar could alleviate the inhibitory effect of Cd stress on the growth of Soybean. 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. Our results were similar to those reported in peanuts, in which biochar application also enhanced the biomass (Kang et al., 2022). Yao et al. (2021) indicated that with the application of biochar, the biomass of the roots, stems and leaves under Cd stress was markedly improved in all growth stages, with greater enhancements in response to the BC 2% application rates. This result can be attributed to the inactivation ability of biochar exerted on Cd.
EFFECT OF BIOCHAR ON GROWTH AND YIELD OF SOYBEAN

Effects of biochar on SPAD value of Soybean under Cd stress condition

Cd stress resulted in a significant (P < 0.05) decrease in the SPAD value by 27.25% in Soybean compared to the untreated control. When compared to the corresponding Cd-stressed plant, the application of 1.5% and 3% wood biochar treatment with Cd significantly (P < 0.05) increased the SPAD value by 34.94% and 38.67%, respectively (Fig.5). However, among the biochar’s treatment under Cd stress did not show statistically difference. Xue et al. (2013) reported decreased PS II activity and chlorophyll content in leaves of the Soybean seedlings under Cd treatments. These findings were consistent with the observations reported by Kang et al. (2022), who showed that adding biochar in Cd-contaminated soils increased the SPAD value of foxtail millet. Ijaz et al. (2020) also found that the chlorophyll SPAD value in wheat plants increased considerably with the addition of biochar in Cd contaminated soil. Higher chlorophyll levels often resulted in higher crop yields because chlorophylls participate in energy creation and conversion via photosynthesis for crop growth (Alam et al., 2019). Heavy metals may interfere with the structural components of chloroplasts, the synthesis of proteins thus resulting in the impairment of chlorophyll development (Keshan and Mukherji, 1992).
Effects of wood biochar on yield and yield contributing characters of Soybean under Cd stress condition

Cadmium directly has an adverse effect on grain yield but it can be mitigated by using an adequate amount of biochar. Fig. 6 show the results of the yield and yield components of Soybean under Cd stress. The pod per plant, seed per plant as well as seed yield of the Cd-stressed plant decreased by 16.70%, 16.36%, and 15.65%, respectively, compared to the control treatment. Pod number per plant and seed number per plant showed a significant (P < 0.05) difference between Cd stress plant and the addition of 1.5% and 3% biochar treatment under Cd stress condition. The seed yield per plant of the 1.5 % and 3% biochar have lessened the reduction by 20.61% and 35.05%, respectively in the Cd-stressed plant, compared to the Cd-stressed plant. However, there was no significant difference between biochar treatment under Cd stress conditions in pod per plant, seed per plant as well as seed yield per plant. Nagaraju et al. (2015) stated that heavy metals are carcinogenic in nature, damage DNA, proteins and lipids that adversely affect plant growth and yield. Further, Ramzani et al. (2016) cleared that enhanced plant growth and grain yield crop can be attributed to the positive effect of biochar. Maximum crop yield in contaminated soils might be due to furnishing the soil with nutrients such as carbon, nitrogen, and phosphorus by the amendment (Kumar et al., 2020). Islam et al. (2014) observed that flag leaf SPAD reading (Fig.4) showed a positive strong correlation with grain yield (Fig. 6C). Swain and Sandip (2010) also observed a significant and
positive correlation between flag leaf N content and SPAD value ($R^2 = 0.80$) and SPAD value at different growth stages and grain yield of rice.

**CONCLUSION**

This study concluded that the application of biochar could promote the growth and yield of Soybean in Cd stress condition. 1.5% and 3% wood biochar application alleviated the physiological stress of Cd on Soybean and improved SPAD value.
under Cd stress. The application of biochar is an effective technique to promote the growth and yield of Soybean under Cd stress condition and, helping to promote sustainable agriculture by optimizing the amount of biochar additives.

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REFERENCES


