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Research Article

Soil Amendment with Biochar Reduces the Magnitude of Drought Stress in Soybean

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ABSTRACT

Drought stress severely affects plant growth and causes huge economic losses worldwide. The beneficial effect of biochar to improve soil properties and plant growth is well discussed nowadays. To investigate biochar's potential for drought stress mitigation, a two-factorial pot experiment was conducted. Factor one was drought stress (100% Field capacity (FC), 70% FC, and 40% FC) and another was biochar treatment. Cow dung biochar (CDB) and poultry manure biochar (PMB) was applied at three rates (0, 4 and 8 g pot^{-1}). The experiment was designed following a completely randomized design with four replicates. The results showed that drought deteriorated all the studied parameters whereas biochar application significantly improved all the parameters compared to control treatment. The chlorophyll concentration was maximum at the application of CDB as 8 g pot^{-1} followed by PMB as 8 g pot^{-1} at both drought conditions (70% FC and 40% FC). Biochar application significantly improved plant height at 28 and 42 days after sowing and the highest plant height was found at the application CDB as 8 g pot^{-1} followed by PMB as 8 g pot^{-1}. The highest leaflet number was found at the application of CDB at a rate of 8 g pot^{-1}. On average, the root length and biomass at 70% and 40% FC was the highest at the application of 8 g CDB pot^{-1} which was 28.66% and 61.59% higher, respectively over control. The application of CDB at a rate of 8 g pot^{-1} increased the shoot biomass by 45.35 and 69.52% compared to control at 70% FC and 40% FC, respectively. Therefore, application of biochar could be an option to reduce drought stress in plants.

Introduction

Abiotic stresses are seriously threatening global food security and crop productivity. The severity of abiotic stresses such as drought, salinity, heat, and heavy metal contamination is steadily rising, resulting in detrimental impacts on crop productivity (Hassan et al., 2021). Across the world, a serious threat is being posed by drought on ecosystems and very big havoc. Approximately 65% of the Earth's geographical area is comprised of arid regions (Wu et al., 2023). Drought is one of the severe abiotic stresses, leading to substantial declines in crop productivity (Latif et al., 2022). It induces leaf degradation, stomatal closure, and chlorophyll breakdown. These effects diminish the plant's ability to harness light, while the disruption of electron flow diminishes photosynthesis, impeding the generation and storage of energy resources (Wu et al., 2023). Under drought stress, plants generate a notable quantity of reactive oxygen species (ROS), triggering an upsurge in oxidation and activating mechanisms of oxidative damage such as lipid peroxidation in membranes (Bhattacharjee, 2005).

Soybean (*Glycine max* L.) is a vital legume crop renowned for its nutritional richness, making it highly sought after in developing countries due to its nutritional benefits and it contains ample amounts of protein, oils, and fibers (Specht et al., 2014; Novikova et al., 2022; Razgonova et al., 2022). Soybean cultivation spans the globe, recognized as a staple in cuisine for its abundant nutrient profile. Despite significant advancements in production, environmental stress continues to pose a persistent threat to the soybean crop. The cultivation of soybean in Bangladesh as a cash crop is increasing day by day, especially in the southern part of Bangladesh and there is huge scope to cultivate and increase soybean yield in Bangladesh due to

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favorable environmental conditions (Islam et al., 2022). However, soybeans are vulnerable to the detrimental effects of drought stress (Basal and Szabó 2020), and research has shown that drought stress results in a notable decrease in seed yield, ranging from 24 to 50% (Dong et al., 2019; Zhang et al., 2019). So, there is an urgent need to adopt eco-friendly and efficient management strategies that will mitigate drought stress in soybean.

The application of biochar has proven to be effective in enhancing water use efficiency in various crops when faced with limited water conditions. It is the organic byproduct of biomass derived through a pyrolysis process conducted in the minimal or absence of oxygen conditions (Maia et al., 2011). Biochar possesses the ability to retain water for extended durations, enabling plants to efficiently carry out their normal functions even when water is limited (Mannan et al., 2016). Incorporating biochar into the soils of arid regions, where water scarcity is a significant concern, has the potential to stimulate crop growth and ultimately boost farmers' income (Manzoor et al., 2022). The substantial enhancement of physical, biological, and chemical soil properties resulting from biochar amendment facilitates sustainable cultivation practices and leads to increased production (Kookana et al., 2011). Biochar improves soil properties by enhancing soil aeration and reducing soil bulk density (Tayyab et al., 2018). The application of biochar increases the population of microorganisms in soil by enhancing its chemical and physical attributes, leading to improved soil and crop yields (Zhang et al., 2022). Again, the application of biochar significantly boosts the activity of enzymes such as dehydrogenase, acid phosphatase, alkaline phosphomonoesters, protease, alkaline phosphatase, chymotrypsin, phosphohydrolase, trypsin, lipase-esterase, and esterase (Jabborova et al., 2021a). Alotaibi et al. (2023) reported that application of AMF-biochar increased spinach (*Spinacia oleracea* L.) shoot fresh weight, shoot dry weight, shoot length, root fresh weight, root dry weight, and root length by 20.34, 21.23, 3.37, 16.10, 15.51, and 38.03%, respectively over control under drought condition. Similarly, Zhang et al. (2023) reported biochar application to mitigate drought stress and increase seedling growth and physiology of tomato (*Lycopersicon esculentum* L.) seedlings.

So based on plenty of evidence this experiment was undertaken to observe the effects two biochar (cow dung and poultry manure) on the mitigation potential of drought stress in soybean growth grown under different imparted drought stress conditions.

Materials and Methods

Description of the study area

A pot experiment was conducted in the net house during the *Kharif I* (March to April) season in 2024 at the Department of Agricultural Chemistry, Bangladesh Agricultural University, Mymensingh, Bangladesh. The place of the experiment is physically situated 20 meters above sea level at latitude 24° 43' 18" N and longitude 90° 26' 32" E. The climate of the study area was under the sub-tropical climatic zone, which was characterized by moderate to high temperature, heavy rainfall, high humidity, and relatively long days during *Kharif* (April to September) and scanty rainfall, low humidity, low temperature and short-day period during *Rabi* season (October to March).

Soil collection, processing and pot filling

The soil was brought from the 0-15 cm soil layer of the Agronomy Field Laboratory of Bangladesh Agricultural University. The area is under the AEZ-9 and regular cultivation practices. The soil is non-calcareous dark gray flood plain soil. The collected soil was made free from plant residues and other extraneous materials, air-dried, ground, and sieved through a 2 mm sieve. Approximately 500 g of sieved soil was preserved in a polythene bag for physical and chemical analysis following the standard method of analysis (Page et al., 1982; Tandon, 2005). The soil was characterized as silty loam in texture, having bulk density, particle density, and moisture content of 1.26, 2.59 g cm^{-3}, and 27.24%, respectively. Chemical parameters such as pH, organic matter (%), total N (%), P (mg kg^{-1}), K (meq 100 g^{-1}), S (mg kg^{-1}), Zn (mg kg^{-1}), B (mg kg^{-1}), Ca (meq 100 g^{-1}), and Mg (meq 100 g^{-1}) were 6.56, 0.97, 0.062, 3.02, 0.14, 4.06, 1.71, 0.064, 3.36, and 0.74, respectively. About 11 kg of processed soil was taken in each plastic pot having 28 cm height and 25 cm average diameter. The pot was loaded with soil leaving 4 cm from the peak and labeled by using a tag. During pot filling the whole amount of biochar, urea (2 g pot^{-1}), triple super phosphate (5.40 g pot^{-1}), muriate of potash (4.40 g pot^{-1}), gypsum (3.60 g pot^{-1}), solubor (0.40 g pot^{-1}), and $ZnSO_4.2H_2O$ (0.60 g pot^{-1}) was added to the soil according to Fertilizer Recommendation Guide–2018 (FRG, 2018). BINA soybean 7 was used as planting materials in this experiment.

Treatment details

This experiment comprised two factors: one was drought and another was biochar treatment. Two droughts along with one control and four biochar rates along with one control treatment were used. Biochar was collected from the Department of Physics, Bangladesh Agricultural University, Mymensingh having the physico chemical properties—Moisture= 3.8%, C=

78.4%, H=2.03%, N=1.47%, Ash=4.3%, pH=7.3, CEC=33.5 cmolkg^{-1}. The whole experiment was laid out following a completely randomized design (CRD) with four replications. The details of treatments are presented in Table 1. The artificial drought condition was created at the emergence of trifoliate (16 DAS) of soybean by maintaining soil field capacity (FC) at 70% and 40%, respectively (Cassel and Nielsen, 1986). To determine FC a 50 cm soil column was prepared using UPVC pipe and oven dried soil. The column was completely wet by adding water and then allowed to drain out access water for 72 hours. The evaporation loss of water stopped by sealing the top of the column with a piece of polythene. Then the moisture content was measured and this moisture content considered as 100% FC. Finally, 70% and 40% FC was maintained by monitoring moisture content using a moisture meter and water was added to the pot when moisture content falls below 5% of 100%, 70% and 40% FC, respectively to obtain the determined FC in this experiment.

Table 1. Treatment details of the experiment

Treatment identity		Treatment details
Drought	Biochar	
100% FC (normal condition)	B_0	No biochar
	B_1	4 g CDB pot^{-1}/ 7.27 t ha^{-1}
	B_2	8 g CDB pot^{-1}/ 14.55 t ha^{-1}
	B_3	4 g PMB pot^{-1}/ 7.27 t ha^{-1}
	B_4	8 g PMB pot^{-1}/ 14.55 t ha^{-1}
70% FC	B_0	No biochar
	B_1	4 g CDB pot^{-1}/ 7.27 t ha^{-1}
	B_2	8 g CDB pot^{-1}/ 14.55 t ha^{-1}
	B_3	4 g PMB pot^{-1}/ 7.27 t ha^{-1}
	B_4	8 g PMB pot^{-1}/ 14.55 t ha^{-1}
40% FC	B_0	No biochar
	B_1	4 g CDB pot^{-1}/ 7.27 t ha^{-1}
	B_2	8 g CDB pot^{-1}/ 14.55 t ha^{-1}
	B_3	4 g PMB pot^{-1}/ 7.27 t ha^{-1}
	B_4	8 g PMB pot^{-1}/ 14.55 t ha^{-1}

Note: CDB= Cow dung biochar, PMB= Poultry manure biochar

Data collection

Chlorophyll a, (Ch a) b (Ch b), and total chlorophyll (T Ch) concentration were determined following the method described by Arnon, (1949). Upper leaf samples of soybean plants from each pot were collected. Exactly, 0.5 g leaf sample was pasted in a mortar with 80% acetone, extracted, and poured into a falcon tube by making a final volume of 15 mL. After filtration, the absorbance reading was taken at 663 nm for Ch a and 645 nm for Ch b. Plant height was recorded at 14, 28, and 42 days after sowing (DAS) using a scale from bottom to top of the plants. After harvesting leaflet number was counted and fresh biomass was recorded by using a weighing balance. The root was carefully separated from the soil by breaking the soil clod and washed with water. Immediately after washing fresh biomass of root and root length were recorded by removing the adjacent water using blotting papers.

Statistical analysis

The normality and linearity of the whole data were first confirmed using Kolmogorov Smirnov and modified Levene's test. Two-way analysis of variance (two-way ANOVA) and mean comparison was performed using the statistical software package Minitab 21 (Minitab Inc., USA). For mean comparison, pairwise Tukey's test was used at 5% level of significance.

Results

Effect of drought and biochar treatment on chlorophyll (Ch) concentration

The chlorophyll concentration of soybean leaf was significantly ($p < 0.05$) influenced by single and interaction effect drought and biochar treatment except for the interaction effect of Ch b concentration (Figure 1). Drought significantly reduced the Ch a, Ch b, and TCh concentration of soybean (Figure 1). On the contrary biochar application to soil significantly increased the chlorophyll concentration of soybean under drought stress (Figure 1). Though there was no drought at 100% FC but biochar application substantially increased the Ch a concentration than control treatment (Figure 1A). The highest increase over control was found in B_2 (10.91%) treatment and the lowest was in B_3 (0.61%) treatment, respectively. At 70% FC a significantly higher Ch a concentration was at B_2 (21.99%) over control and the lowest was at B_3. At 40% FC drought severely reduced the Ch a concentration but biochar amelioration significantly increased the Ch a concentration in all the treatments except B_3 over control. The Ch b concentration was significantly higher in B_2 (32.31%) treatment over control at 100% FC which was identical to B_1 and B_4 treatments, respectively (Figure 1B). A significant variation of Ch b concentration was found both in 70% FC and 40% FC where B_2 treatment showed a higher concentration of Ch b over control treatment. The overall increment of Ch b concentration due to biochar application was about 28.43% at 70% FC and 49.09% at 40% FC, respectively. The TCh content was significantly varied at 100%, 70%, and 40% FC (Figure 1C). At 100% FC B_2 treatment showed a significantly higher TCh concentration over control which was identical to all other biochar treatments. Under 70% FC, both B_2 and B_4 showed a significantly higher TCh content than the control where the increment was 29.74% and 25.36% at B_2 and B_4 treatments, respectively. Again, except B_3 all the biochar treatments showed a significantly higher TCh concentration than the control treatment under 40% FC. The highest increase over control was at B_2 (80.43%) treatment.

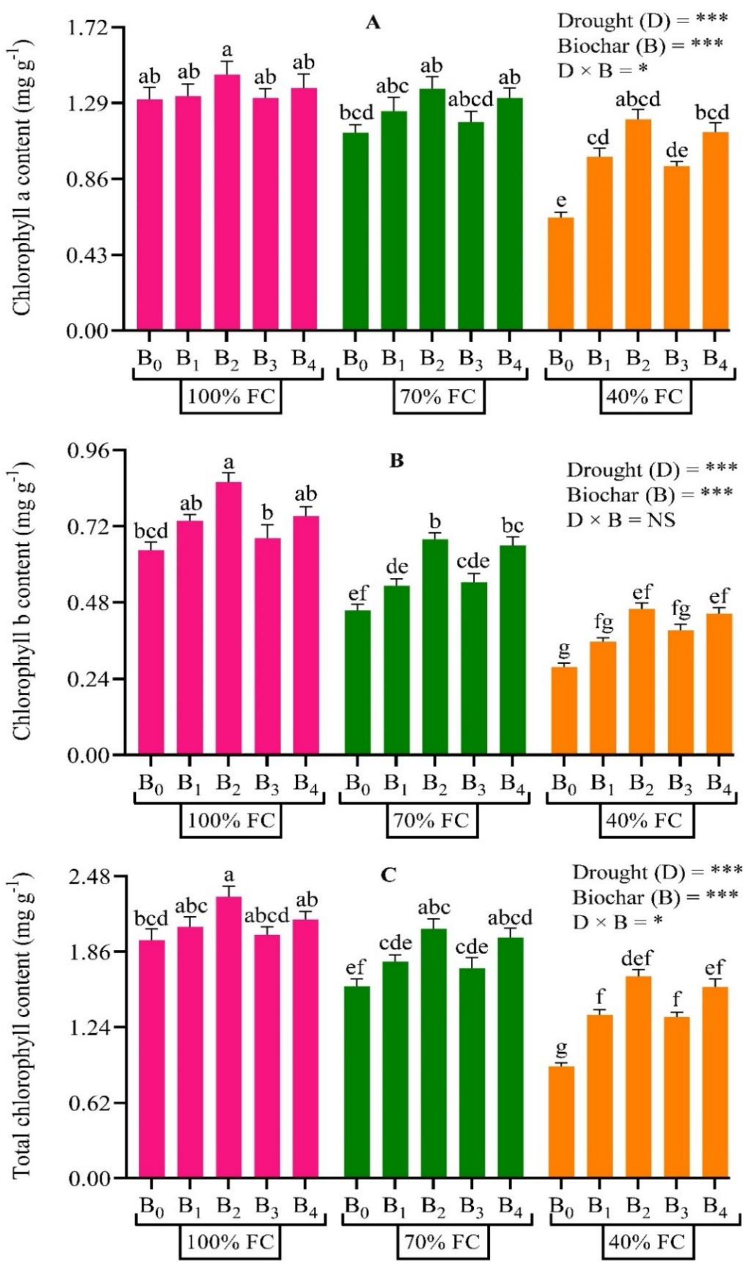

Figure 1. Effect of different biochar treatments on chlorophyll a (A), chlorophyll b (B), and total chlorophyll (C) content under drought stress. Bars are made with mean ± SEM. Bars with the same letter (s) are statistically identical among them according to Tukey's test at 5% level of significance. * Indicate significant at 5% and *** indicate significant at 5%, 1%, and 0.1% level of significance. NS indicate non-significant.

Effect of drought and biochar treatment on plant height

At 14 DAS there was no drought condition as it was created from 16 DAS to harvest. So, the effect of drought was non-significant at this stage. However, biochar application to soil showed a significant effect on plant height over control treatment (Table 2). Application of biochar showed a variable degree of increase of plant height where more increase was found at the higher dose of biochar (8 g pot^{-1}) than at the lower dose (4 g pot^{-1}). At 28 and 42 DAS drought significantly reduced the plant height of soybean plants whereas the application of biochar to the soil under drought stress showed a significant effect on the increase of plant height (Table 2). However, the interaction of drought and biochar treatment didn't show any significant effect on the plant height of soybean (Table 2). Treatment B_2 showed the highest plant height both in 28 and 42 DAS in all three conditions (100%, 70%, and 40% FC). On average, plant height increased by 8.11, 21.01, and 29.55% in B_2 treatment at 100%, 70%, and 40% FC, respectively.

Effect of drought and biochar treatment on leaflet number and root length

Drought and biochar treatment showed a significant (< 0.05) effect on leaflet number and root length of soybean (Figure 2). Drought significantly reduced the leaflet number and root length whereas biochar treatment significantly increased the leaflet number and root length of soybean (Figure 1). The interaction of drought and biochar treatment didn't show any significant effect on the leaflet number and root length (Figure 1). At normal conditions (100% FC), biochar application showed a significant improvement in leaflet number at B_2 treatment over control which was identical to all other biochar treatments. Similarly, a significantly higher leaflet number was also found in the B_2 treatment which was identical to the B_1 and B_4 treatment, respectively in both of the 70% and 40% FC. Again, root length didn't show any significant variation in all the 100%, 70%, and 40% FC. However, in all three conditions, there was a substantial increase in root length found under biochar treatments compared to control treatment.

Table 2. Effect of drought and biochar treatment on plant height of soybean. Values in the column contain mean ± SEM. Values with the same letter (s) are statistically similar among them according to Tukey's test at 5% level of significance

Treatment		Plant height (cm)		
Drought	Biochar	14 DAS	28 DAS	42 DAS
100% FC	B_0	17.54±1.54 ab	32.92±1.40 abc	49.38±2.10 abc
	B_1	17.33±1.56 ab	33.79±1.68 abc	50.69±2.52 abc
	B_2	18.08±1.07 ab	35.59±2.03 a	54.13±3.47 a
	B_3	20.67±1.28 a	33.50±1.76 abc	50.25±2.63 abc
	B_4	17.67±0.56 ab	34.75±1.44 ab	52.88±2.77 ab
70% FC	B_0	15.08±0.55 b	28.75±1.38 abcd	45.13±2.13 abcd
	B_1	17.79±1.47 ab	31.81±2.18 abcd	47.71±3.27 abcd
	B_2	15.67±0.83 ab	34.79±1.95 ab	51.94±2.94 abc
	B_3	19.58±0.63 ab	30.83±1.42 abcd	46.25±2.14 abcd
	B_4	17.75±1.13 ab	33.25±2.10 abc	50.63±3.20 abc
40% FC	B_0	16.17±0.65 ab	24.06±1.33 d	35.71±1.67 d
	B_1	18.71±1.24 ab	26.75±1.44 bcd	40.63±2.56 bcd
	B_2	20.25±0.88 ab	31.17±2.20 abcd	47.88±3.09 abcd
	B_3	16.63±1.03 ab	25.75±1.44 cd	39.13±2.12 cd
	B_4	18.17±1.26 ab	29.92±1.87 abcd	45.13±2.43 abcd
Drought (D)		NS	***	***
Biochar (B)		*	***	***
Interaction (D × B)		NS	NS	NS

- Represents significance at 5% level of significance. ***represents significant at 5%, 1%, and 0.1% level of significance. NS indicates non-significant.

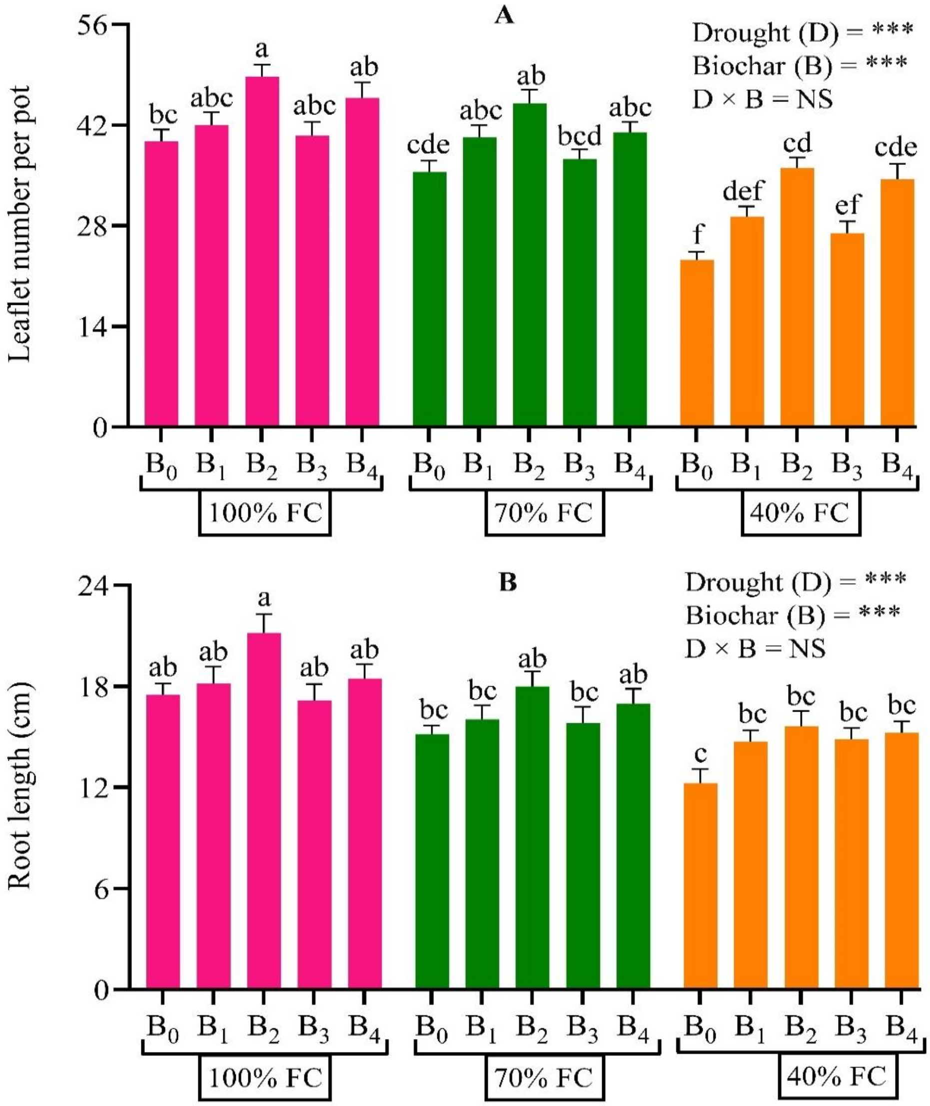

Figure 2. Effect of different biochar treatments on leaflet number per pot (A) and root length (B) under drought stress. Bars are made with mean ± SEM. Bars with the same letter (s) are statistically identical among them according to Tukey's test at 5% level of significance. * Indicate significant at 5% and *** indicate significant at 5%, 1%, and 0.1% level of significance. NS indicate non-significant.

Effect of drought and biochar treatment on biomass yield

The effect of drought significantly ($p < 0.05$) reduced the shoot, root, and total biomass yield of soybean. While application of biochar significantly ($p < 0.05$) increased the shoot, root, and total biomass yield of soybean under drought conditions (Figure 3). Again, the interaction of drought and biochar also showed a significant effect on the biomass yield of soybean (Figure 3). At 100% FC, biochar application showed no significant improvement in shoot biomass yield over the control treatment (Figure 3A). However, shoot biomass yields significantly increased over control treatment for both the 70% and 40% FC. The highest shoot fresh biomass was found at B_2 (61.43% higher over control) which was identical to all other biochar treatments, respectively. The root biomass was significantly increased at B_2 treatment in the case of 100, 70 and 40% FC over control treatment which was identical to B_4 treatment only (Figure 3B). The total biomass of soybean was significantly higher at the B_2 treatment than at the control treatment and showed statistical similarity with the B_1 and B_4 treatments, respectively for all the drought stress conditions (Figure 3C).

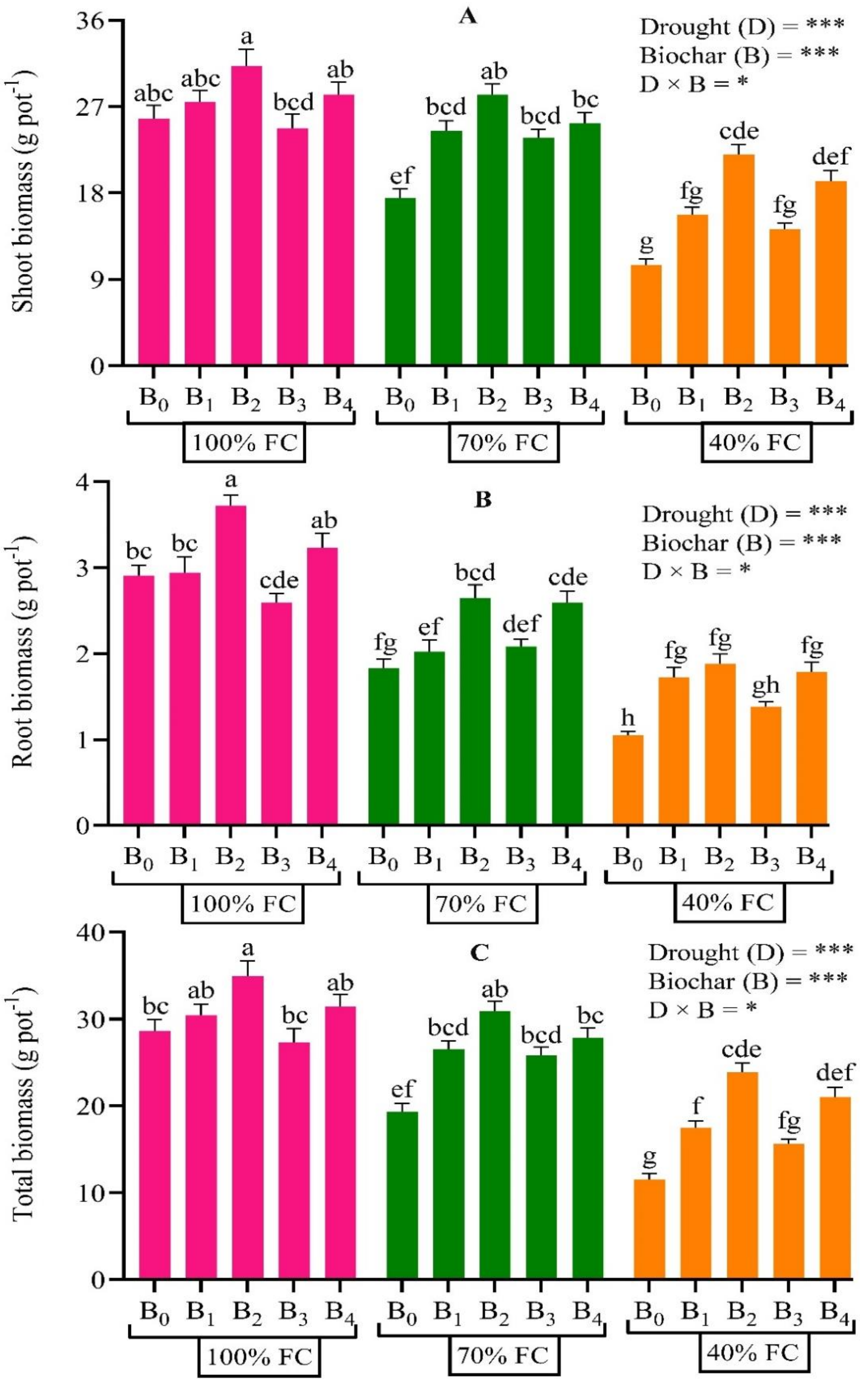

Figure 3. Effect of different biochar treatments on shoot biomass (A), root biomass (B), and total biomass (C) under drought stress. Bars are made with mean ± SEM. Bars with the same letter (s) are statistically identical among them according to Tukey's test at 5% level of significance. * Indicate significant at 5% and *** indicate significant at 5%, 1%, and 0.1% level of significance. NS indicate non-significant.

Discussion

As a carbon-rich substance, biochar can be applied as a soil conditioner to enhance soil quality and soil carbon sequestration (Rajakumar and Sankar, 2016). It is an effective, affordable, and environmentally friendly source of soil conditioner due to its many distinctive and specific qualities (Oliveira et al., 2017). Because of the numerous pores in biochar's porous structure, soil microorganisms (bacteria, actinomycetes, and AMF) can thrive and proliferate there, improving the health of the soil and the performance of plants (Kapoor et al., 2022). In addition, biochar increases the soil's capacity to contain nutrients, its efficiency in absorbing water, its organic matter content, and its physiochemical and biological characteristics, all of which promote the growth of plants (Ohtsuka et al., 2021; Kapoor et al., 2022; Qu et al., 2022).

In this experiment, biochar application to soil successfully mitigates drought stress in soybean plants and increases growth. Another important finding was also notable that under 100% FC (normal condition) biochar positively influences all the parameters than control (no biochar application) condition. The chlorophyll concentration was decreased under drought stress and severe reduction was found at 40% FC. The devastating effect of drought stress is mainly responsible for the reduction due to reactive oxygen species (ROS) generation and enzyme breakdown (Wu et al., 2023). However, biochar application greatly mitigates drought stress and increases chlorophyll concentration where a significantly notable effect was found at 40% FC rather than 70% FC (Figure 1). This beneficial effect may be imparted from the increased production of antioxidant enzymes and growth-regulating substances under biochar application (Wu et al., 2023) that helps the plants to carry out successful metabolisms and results increase in chlorophyll concentration. In a previous experiment, Farooq et al. (2021) reported that biochar application successfully mitigated the drought stress on okra, tomato, and cowpea plants and increased chlorophyll concentration.

The plant height was significantly reduced at 28 and 42 DAS due to drought stress conditions but biochar application showed a significant effect on the increase of plant height even at 14 DAS where there was no drought condition. Again, leaflet number and root length were also significantly increased due to biochar application under drought stress. The water retention by biochar itself and overall improvement of soil physicochemical properties e.g., decreasing bulk density, increasing water holding capacity, and improving nutrient availability (Mansoor et al., 2021) may result from more water and nutrient uptake by plants and finally plant growth increased and the consequence was increasing plant height, leaflet number, and root length. In line with our findings, Yildirim et al. (2021) reported that BC application reduced the drought stress and increased the growth of cabbage seedlings. Again, Sun et al. (2017) reported that biochar application under drought stress increases the root length and root density of maize.

Finally, improvement of soil physicochemical properties and increasing water availability, decreasing stress reduction due to antioxidant defense help to maintain proper growth and metabolic activity of soybean plants under biochar application resulting in higher above-ground, belowground, and total biomass (Figure 3). This finding is consistent with the findings of Alotaibi et al. (2023) who found that AMF-biochar application increased shoot fresh weight by 20.34% and root fresh weight by 16.10% under drought stress over control treatment.

Conclusion

The adverse effect of drought seriously deteriorates plant growth due to oxidative damage and reduces water availability to plants. In this experiment biochar efficiently mitigates drought stress in soybean plants and improves the growth and physiological attributes. The chlorophyll concentration, plant height, leaflet numbers, root length, shoot, root, and total fresh weight significantly improved due to biochar application under drought stress. The best comparable results were found at 40% FC rather than 70% FC. The higher dose of biochar (8 g pot^{-1}) showed better performance than the lower dose (4 g pot^{-1}). Overall cow dung biochar showed better performance than poultry manure biochar. Therefore, application cow dung and poultry manure biochar may be a promising option for the mitigation of drought stress and successful cultivation of soybean in drought prone areas of Bangladesh.

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