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**Research Article**

# Biochar improves morpho-physiological growth, osmolyte accumulation, nutrients balance, anti-oxidative defense and oil productivity of *Brassica* under flooding stress

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**Abstract**

Soil flooding is a serious abiotic stress that can repress plant growth and yield. The intensity of flooding stress is continuously increasing owing to rapid climate change and changes in rain intensity and frequency. Biochar (BC) emerges as an important soil amendment to mitigate adverse impacts of abiotic stress. The role of BC against different stresses is well reported, however, its role under flooding stress is not determined yet. Thus, this experiment was conducted to determine the role of BC on the performance of brassica crops under flooding stress. The experiment was comprised of well-watered (WW) and flooding stress (FS) conditions and biochar application: control, 1% biochar, and 2.5% biochar. The results indicated that flooding stress stunted the plant growth and impaired the photosynthetic pigments, leaf water status, osmolytes yield and yield traits, and oil concentration and increased the electrolyte leakage (EL), hydrogen peroxide ($H_2O_2$) and malondialdehyde (MDA) concentration. The application of BC at the rate of 2.5% mitigated the adverse impacts of salinity and upgraded the antioxidant activities (~50-120%), reduced the oxidative stress markets (~70-300%), and increased the leaf water status, photosynthetic pigments, osmolyte accumulation, nutrient uptake, yield traits, and oil contents. In conclusion, BC application can improve the productivity and oil yield of *brassica* under drought stress by improving plant physiological and biochemical functioning. However, more in depth studies are direly needed to explore the mechanism of BC to induce flood tolerance.

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***Keywords:*** antioxidant; biochar; flooding stress; nutrient uptake; oil concentration

## Introduction

The climatic conditions around the globe are quickly changing and causing an imbalance in the environment and precipitation patterns which is leading to negative impacts on crops (Eigenbrod *et al*., 2015; Wang and Komatsu, 2022). The disturbed precipitation and glacier melting is causing flooding stress which negatively affects plant growth and development (Wang and Komatsu, 2022). The frequency of flooding is continuously increasing and it has been predicted that the frequency of flooding stress will be increased further under ongoing climate change (IPCC 2021; Zhao *et al*., 2023). Flooding refers to partial or complete submergence stress which has deleterious impacts on the germination, and vegetative and reproductive growth of plants (Chattopadhyay *et al*., 2021). Flooding stress also induces oxygen-deficient conditions which negatively affect plant growth stages (Zhong *et al*., 2021). The low oxygen conditions under flooding stress alter the plant metabolism from aerobic respiration to anaerobic fermentation which is considered to be an important cause of impairment of plant growth (Xu *et al*., 2014).

Flooding stress can affect plant processing ranging from morphology to physiology and biochemistry (Azeem *et al*., 2020; Jia *et al*., 2021). Flooding stress inhibits plant growth and alters biomass production in plants (Liu *et al*., 2014), reducing photosynthesis, leaf water potential, and stomata conductance (Miao *et al*., 2017). Flooding-induced root hypoxia decreases the hydraulic conductivity and water absorption by roots which further reduces the water transportation to leaves (Yuan *et al*., 2022). This decrease in water transport reduces the leaf water potential and consequently reduces photosynthesis (Yuan *et al*., 2022). Besides this flooding also reduces the absorption of nutrients which induces nutrient deficiency and decreases chlorophyll synthesis which further inhibits photosynthesis (Herrera, 2013). Flooding stress also affects the synthesis of potential osmolytes. For instance, soluble sugars and proline are accumulated in plants in response to flooding stress which serves as osmolytes to maintain the leaf turgor (Miao *et al*., 2017; Loreti *et al*., 2018; Wang *et al*., 2019). Besides this flooding stress also induces reactive oxygen species production that damages, proteins, lipids, membranes, and deoxyribonucleic acid (DNA) and causes a significant reduction in plant growth (Zhao *et al*., 2023).

One of the immediate impacts caused by flooding stress is the deficiency of oxygen (Voesenek and Bailey-Serres, 2015) which restricts plant growth and causes a reduction in crop yield (Nishiuchi *et al*., 2012). Further in flooding conditions growth of plants is also impeded owing to increase anaerobic respiration, however, plants have developed adventitious roots (ARs), aerenchyma, and radial oxygen (O2) loss (ROL) barriers to exchange the gas (Jia *et al*., 2021). Plants have evolved two strategies to resist the adversity of flooding: low oxygen escape syndrome (LOES) for partial submergence and low oxygen quiescence syndrome (LOQS) for complete submergence (Voesenek and Bailey-Serres, 2015). The plants with LOQS show restricted growth by keeping the minimum energy and carbon consumption to prolong their survival under flooding conditions (Loreti *et al*., 2016), and when flooding recedes these plants recover quickly. In LOES strategy plants maintain the upper leaves in the aerial environments for getting adequate carbon, light, and oxygen (Hattori *et al*., 2011; Jia *et al*., 2021).

To counter the toxic effects of flooding stress, it is urgently needed to develop innovative techniques to ensure crop productivity and global food security (Mehmood *et al*., 2023a). Amending soil with biochar (BC) is considered an effective practice to improve crop productivity and reduce the toxic effects of abiotic stresses (Fischer *et al*., 2019; Mehmood *et al*., 2018; Mehmood *et al*., 2022; Mehmood *et al*., 2023b). The application of BC improves soil texture, soil porosity, soil structure, and soil hydraulic functions (Faloye *et al*., 2019). Biochar application also increases the frequency of large pores, micro-pores, and soil optimum water content which in turn improves crop productivity (Liu *et al.*, 2017; Ni *et al.*, 2018; Ng *et al.*, 2022). Nonetheless, the effects of BC vary according to soil type, feedstock properties, production processes, and rate of application (Tomczyk *et al.*, 2020). However, many studies have been conducted to determine the effect of BC in reducing the toxic effects of different abiotic stresses like salinity, drought, heat stress, and heavy metals. Nonetheless, there is no information about the role of BC in mitigating the toxic effects of flooding stress. This is first-hand information regarding the role of BC in mitigating the toxic effect of flooding stress in *Brassica*. We hypothesized that BC can mitigate the toxicity of flooding stress by improving plant physiological and biochemical functioning and photosynthetic efficiency. Therefore, this study was determined with following objectives: i) to determine impact of BC on growth, physiological, physiological, and antioxidant activities of brassica ii) to determine impact of BC yield and oil productivity of brassica under flooding stress.

## Materials and Methods

### *Experimental details and treatments*

The present study was conducted to determine the impact of biochar application on the growth, physiological, and biochemical traits of brassica growing under flooding stress. The study was conducted at the Guangxi Hydraulic Research Institute-Qinzhou Irrigation Experimental Station, China. The soil used for filling pots was collected from the experiment field. The soil was collected from 0-20 cm soil with the help of spade. Thereafter, soil was sieved and all debris was removed. The tested soil had a pH (5.12), organic matter content (256 g $kg^{-1}$), total nitrogen content (0.81%), and available phosphorus and potassium 7.72 and 166 mg $kg^{-1}$ respectively.

The pots having a capacity of 10 kg were filled with soil and 10 seeds of brassica were sown in each pot after germination thinning was done to maintain five plants in each pot. The experiment was comprised of different treatments: well-watered (WW) and flooding stress (FS) and biochar application: control, 1% (22.4 t $ha^{-1}$) biochar, and 2.5% (56 t $ha^{-1}$) biochar. The biochar according to treatments was thoroughly mixed with the soil and pots were filled after that seeds were sown in each pot. The well water pots were irrigated to field capacity throughout the experiment. The flooding stress was initiated at the stem elongation stage by filling the pots with water 10-15 cm above the soil surface. The pots were visited regularly and all other management practices were kept constant to get a good stand establishment.

### *Measurement of growth traits*

Three plants from each pot were selected their heights were measured and the average was worked out. The plants were uprooted carefully and roots were removed from shoots and weighed to take the fresh weight and later oven dried (65 ◦C) for 48 hours to determine the dry weight.

### *Determination of photosynthetic pigments, leaf relative water content, and electrolyte leakage*

To determine chlorophyll and carotenoid contents, 0.5 g fresh leaf samples were taken and homogenized in 80% methanol solution to obtain the extract. Thereafter absorbance was noted at 645, 480, and 663 to determine the concentration of chlorophyll a, b, and carotenoid (Arnon, 1949). To access the leaf relative water contents (RWC), briefly leaf sample was taken and weighed (FW) and then this leaf sample was placed in water for 24 hours and weighed to determine turgid weight (TW). Later turgid leaf sample was oven-dried (65 ◦C) until constant weight and dry weight (DW) were taken and RWC was determined with the following procedure: RWC (%) = FW – DR/TW – DR × 100. In case of electrolyte leakage (EL): 0.5 g fresh leaf samples were taken and placed in water for 30 minutes first EC1 was taken and then samples for heated for 90 minutes and second EC2 was taken and finally EL concentration was determined with the following procedure: EL = EC1/EC2 × 100.

*Determination of osmolytes and oxidative stress markers*

To access the concentration of total soluble proteins (TSP), 0.5 g leaf sample was ground in phosphate buffer (5 ml), and then obtained extract was centrifuged for 15 minutes at 14000 and treated with Bradford reagent (2 ml) and allowed for 15-20 minutes at room temperature and absorbance was measured at 595 to determine TSP (Bradford, 1976). In the case of free amino acids (FAA): 1 ml crude extract was taken and placed in tubes containing 1 ml of pyridine and 1 ml of ninhydrin. Thereafter, tubes were placed in a water bath (90 ◦C) for 30 minutes and then allowed at room conditions for 20-25 minutes, and later absorbance was measured at 570 nm (Hamilton and Van-Slyke, 1943). For determination of $H_2O_2$; 0.5 g leaf samples were taken and ground with 0.5 ml tri-chloroacetic acid (TCA) to obtain supernatant and then 1 ml of each potassium iodide (KI: 1 M) and potassium phosphate buffer (PPB) was added in the supernatant and allowed for 30 minutes and absorbance was taken at 390 nm (Velikova *et al*., 2000). To determine the concentration of MDA, 0.5 g leaf samples were taken and homogenized by using 5 ml of TCA and centrifuged for 15 minutes at 12000 rpm. Thereafter obtained supernatant was added to 1 ml of thiobarbituric acid (TBA) and boiled for 30 minutes at 100 ◦C and then cooled quickly and absorbance was measured at 532 nm for MDA determination (Rao and Sresty, 2000).

*Determination of antioxidant activities*

The standard procedure suggested by Aebi (1984) was used to determine the activity of catalase (CAT). Briefly, 0.5 g fresh leaf samples were taken and blended with 5 ml of potassium phosphate buffer (PPB) and centrifuged for 15 minutes at 1000 rpm and absorbance was measured at 240 nm to determine activity of CAT.

To access the concentration of ascorbate peroxidase (APX); again 0.5 g fresh leaf sample was taken and homogenized by adding 5 ml of PPB (pH: 7.8). Therefore, this extract was centrifuged for 15 minutes at 10000 rpm and absorbance was recorded at 290 nm to determine APX activity (Nakano and Asada, 1987). For the determination of peroxidase (POD) activity, the standard procedures of Zhang *et al*. (1992) were used. Briefly, 0.5 g leaf samples were taken and homogenized using 5 ml of PPB and centrifuged for 15 minutes at 10000 rpm and absorbance was measured at 470 nm. In the case of SOD activity, a reaction mixture containing 400 µL $H_2O_2$, 25 mL buffer, 100 µL Triton, 50 µL sample, and 50 µL riboflavin was prepared thereafter absorbance was noted at 560 nm (Mukherjee and Choudhuri, 1983).

*Nutrient concentration*

The plant samples were taken and oven dried (70 °C) and ground to make powder. Then, grounded samples were digested by using a mixture of acids (HCl and $HNO_3$) at a ratio of 1:2. Thereafter, samples were filtered and diluted by using distilled water. The concentration of Ca, Mg, and K was determined by flame photometer, the concentration of P was determined by spectrophotometer, and N concentration in digested samples was measured by Kjeldahl procedure.

*Determination of yield traits and seed oil concentration*

The plants at physiological maturity were harvested to determine branches per plant, pods per plant, pod length, seeds per pot, and seed yield. The concentration of crude oil in seeds of brassica was determined by the Soxhlet apparatus as recommended by AOAC, 1990).

*Data analysis*

The recorded data on all the traits was subjected to a two-way analysis of variance (ANOVA) for flooding stress treatments, BC application rates, and their interactions. The difference among treatments was compared by using the least significant difference test (LSD) and sigma plot was used to prepare the figures and R studio was used to perform the principal component analysis (Steel *et al*., 1996).

## Results

### *Growth and morphological traits*

Flooding stress determined a significant (P > 0.05) in the growth and morphological traits of the brassica crop (Table 1). The taller plant (155 cm) was observed in well water conditions with an application of 2.5% BC and shorter pants (97 cm) were observed in flooding conditions without BC application (Table 4). The root length and fresh and dry biomass also showed relevant reduction under flooding conditions however; BC application mitigated the adverse impacts of flooding and appreciably increased the RL, root fresh, and dry biomass. The overall order of BC in improving the root length (RL), root fresh and dry mass under well-watered and flooding stress remained as: 2.5%BC>1%>control (Table 4). Flooding stress also induced a negative impact on branches per plant (BPP) and it reduced the BPP by 25.47% as compared to well water conditions. The BC addition showed a marked increase in the production of BPP under both well water and flooding conditions (Table 4).

**Table 1.** ANOVA sources, and significance in growth, photosynthetic pigments of *Brassica*

| Sources | DF | PH | RL | RFW | RDW | BPP | Chl-a | Chl-b | Cart. | RWC |
|---|---|---|---|---|---|---|---|---|---|---|
| **BC** | 2 | 583.72** | 66.50** | 125.05** | 42.66** | 2.72** | 0.068** | 0.038 | 0.415** | 170.89* |
| **FS** | 1 | 6013.39** | 660.05** | 982.72** | 382.72** | 8.00* | 0.372** | 0.259 | 5.194** | 2112.50* |
| **BC×FS** | 2 | 7.72* | 1.056* | 8.38** | 4.22** | 0.16* | 0.008** | 0.006 | 0.075** | 8.00* |

BC: biochar, FS: flooding stress, PH: plant height, RL: root length, RFW and RDW are root fresh and dry weight, BPP: branches per plant, Chl: chlorophyll, Cart: carotenoid, RWC: relative water content * and ** indicates significant at P ≤ 0.05 and P ≤ 0.01, respectively.

### *Photosynthetic pigments and leaf water status*

The flooding showed a significant positive impact on photosynthetic pigments and RWC (Table 2). Under flooding stress vs. well water conditions, chlorophyll a, chlorophyll b, and carotenoid contents were decreased by >30% however, BC application showed promising results and improved the photosynthetic pigments and leaf RWC (Table 5). The application of 2.5% BC increased chlorophyll a, chlorophyll b, and carotenoid by 21.21%, 15.25%, and 7.19% under well water conditions, while 2.5% BC increased chlorophyll a, chlorophyll b, and carotenoid by 21.21%, 15.25% and 7.19% under flooding stress 100%, 85.18% and 25.35% as compared to control (Table 5).

**Table 2.** ANOVA sources, and significance in osmolytes and antioxidant activities of *Brassica*

| Sources | DF | EL | MDA | $H_2O_2$ | TSP | FAA | Proline | APX | CAT | POD | SOD |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **BC** | 2 | 87.50** | 1.85** | 1.30** | 21.90* | 9.59* | 0.028* | 174.26** | 5.86** | 1.94** | 2.120* |
| **FS** | 1 | 5582.72** | 83.59** | 34.61** | 192.73* | 107.11* | 0.231* | 1193.98* | 40.47** | 17.66** | 20.05* |
| **BC×FS** | 2 | 15.39** | 0.719** | 0.102* | 0.067* | 0.42* | 0.003* | 13.91* | 1.65** | 0.08* | 0.068* |

BC: biochar, FS: flooding stress, EL: electrolyte leakage: MDA: malondialdehyde, $H_2O_2$: hydrogen peroxide, TSP: total soluble proteins, FAA: free amino acids, APX: ascorbate peroxidase, CAT: catalase, POD: peroxidase, SOD: superoxide dismutase, * and ** indicates significant at P ≤ 0.05 and P ≤ 0.01, respectively.

### *Oxidative stress markers, antioxidant activities, and osmolyte accumulation*

The three traits (EL, MDA, and $H_2O_2$) production was severely increased under flooding conditions (Table 3). The concentration of EL, MDA, and $H_2O_2$ was significantly increased by more than >100 times under flooding conditions (Figure 1). Nonetheless, the application of 2.5% BC particularly alleviated the strong damage to cell integrity by decreasing the production of aforementioned oxidative stress markets (EL, MDA, and $H_2O_2$). The activity of all the antioxidants was significantly increased under flooding conditions, which indicates the brassica plants activated an antioxidant defense system to counter the toxic effects of flooding (Figure 2). The application of BC increased the activity of all the tested antioxidants (APX, CAT, POD, and SOD); however, more promising results were seen under flooding conditions (Figure 1). The application biochar particularly, 2.5% BC increased the APX, CAT, POD, and SOD activities by 42.29%, 45.58%, 40.56%, and 36.67% respectively as compared to the control. Overall, BC application enhanced the antioxidant activity under normal and flooding stress according to the following ranking: 2.5% BC>1% BC> control (Figure 2).

**Table 3.** ANOVA sources, and significance in tissue nutrient concentration and yield traits of *Brassica*

| Sources | DF | N | Ca | Mg | K | PPP | PL | SPP | SYPP | Oil contents |
|---|---|---|---|---|---|---|---|---|---|---|
| BC | 2 | 25.30** | 147.13* | 75.53* | 46.45** | 1445.1* | 0.408** | 39.02** | 7.49* | 18.48** |
| FS | 1 | 245.01** | 1393.04* | 1014.75* | 286.40** | 20200.5* | 3.242* | 360.91** | 76.59* | 328.53** |
| BC×FS | 2 | 7.28** | 1.58* | 2.24** | 8.66** | 51.5* | 0.036* | 1.82** | 0.057* | 1.24* |

BC: biochar, FS: flooding stress. N: nitrogen, Ca: calcium, Mg, magnesium, K: potassium, PPP: pods per plant, PL: pod length, SPP: seeds per pod, SYPP: seed yield per pot. * and ** indicates significant at P ≤ 0.05 and P ≤ 0.01, respectively.

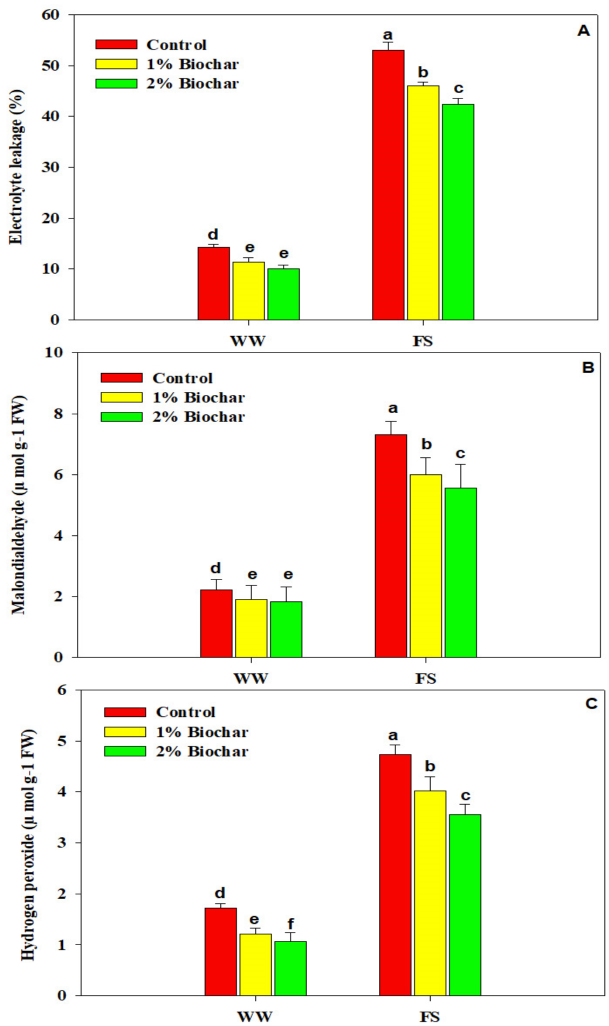



**Figure 1.** Effect of different rates of BC application on electrolyte leakage (a), malondialdehyde (b) and hydrogen peroxide (c) of brassica plants grown under normal and flooding stress conditions
The bars indicating the means of three replicates while different letters indicating the significance among treatments.

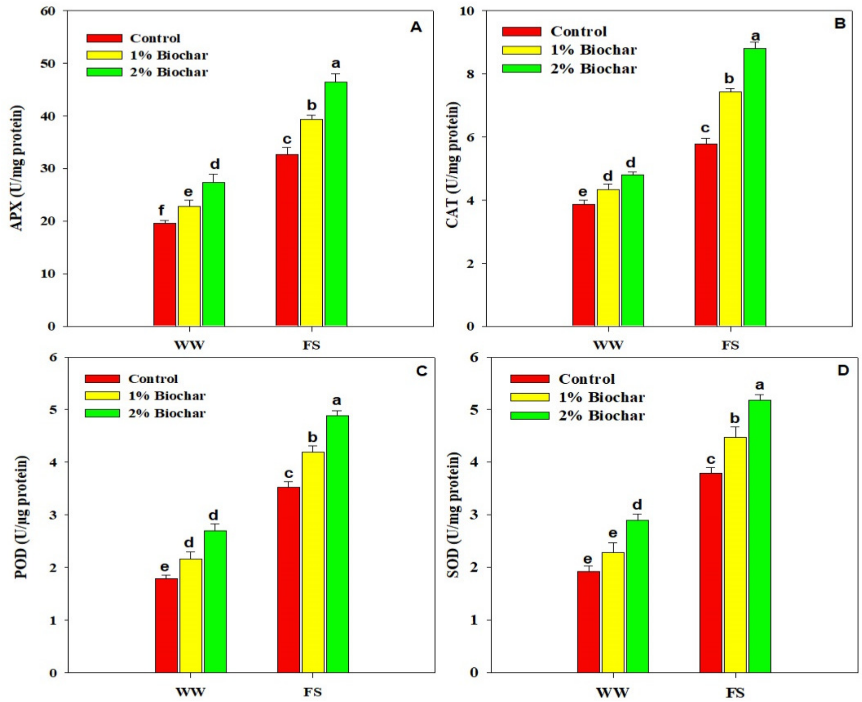



**Figure 2.** Effect of different rates of BC application on APX (a), CAT (b), POD (c) and SOD (d) activities of brassica plants grown under normal and flooding stress conditions
The bars indicating the means of three replicates while different letters indicating the significance among treatments

Flooding stress depressed the TSP and FAA, while concurrently boosting the proline synthesis (Table 3). Flooding stress decreased the TSP and FAA by 63.92% and 55.26% however; an increase of 34.37% in proline concentration was seen under flooding stress. This indicates that brassica plants increased the proline synthesis to counter the toxic effects of flooding stress (Figure 3).

The application of BC significantly increased the concentration of TSP, FAA, and proline under normal and stressful conditions and in this context application of 2.5% BC remained the top performer as compared to 1% BC application (Table 4).

**Table 4.** Effect of different rates of BC application on the growth traits of *Brassica* grown under normal and flooding stress conditions

| Treatments | Biochar | PH (cm) | RL (cm) | RFW (g) | RDW (g) | BPP |
|---|---|---|---|---|---|---|
| **Well water** | Control | 135c±3.09 | 35.22b±2.05 | 26.30c±1.23 | 13.30c±1.12 | 5.7bc±0.33 |
| | 1% BC | 145b±4.25 | 37.00b±2.98 | 33.00b±1.62 | 16.62b±0.98 | 6.7ab±0.53 |
| | 2.5%BC | 155a±5.23 | 42.21a±3.32 | 37.14a±2.76 | 20.31a±0.92 | 7.3a±0.42 |
| **Flooding stress** | Control | 97e±2.89 | 23.13d±1.89 | 13.38e±1.87 | 5.66f±0.56 | 4.3d±0.48 |
| | 1% BC | 111d±6.23 | 25.67d±2.26 | 18.67d±2.23 | 7.70e±0.48 | 5.3c±0.33 |
| | 2.5%BC | 116d±4.10 | 29.51c±2.91 | 20.29d±2.98 | 9.31d±0.89 | 5.7bc±0.52 |

BC: biochar, FS: flooding stress, PH: plant height, RL: root length, RFW and RDW are root fresh and dry weight, BPP: branches per plant. The values are means of three replicates with ± SE and different letters with values indicating significant differences among means.

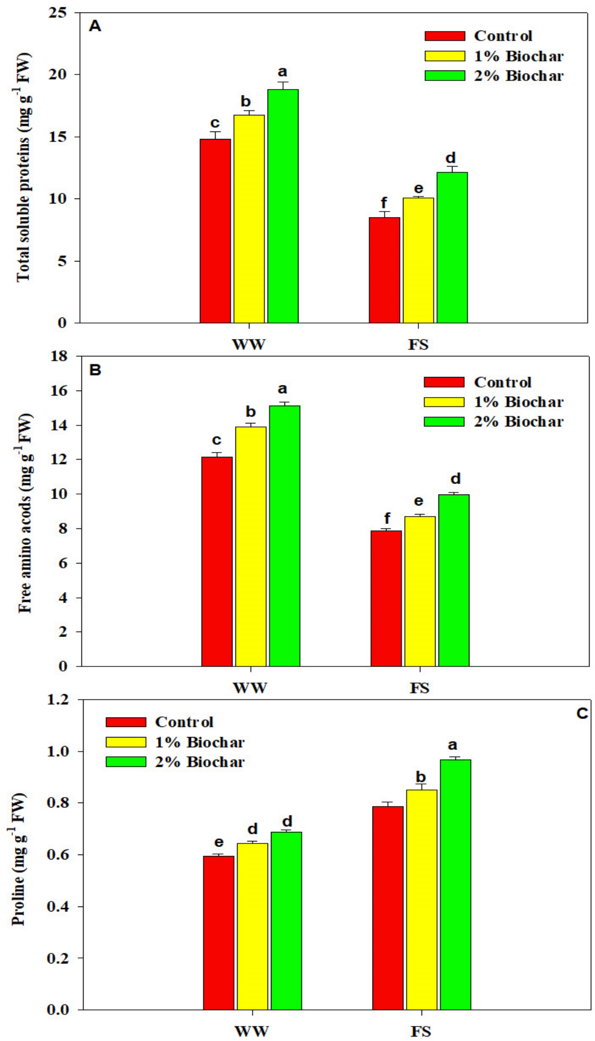



**Figure 3.** Effect of different rates of BC application on total soluble proteins (a), free amino acids (b) and proline (c) concentration of brassica plants grown under normal and flooding stress conditions
The bars indicating the means of three replicates while different letters indicating the significance among treatments.

*Nutrient concentration in plant tissues*

Flooding stress resulted in a strong decrease in the concentration of nitrogen, calcium, magnesium, and potassium. A relevant decrease in N and Ca concentration was observed under flooding conditions; similarly, magnesium and potassium also underwent a relevant decrease in their concentration under flooding stress (Table 6). Biochar application appreciably mitigated the above reduction in element concentrations and the application of 2.5% BC appreciably increased the element concentrations as compared to the application of 1% BC (Table 1). The usual ranking of BC in containment increase of aforesaid elements was observed as: 2.5% BC>1% BC> control (Table 6).

*Yield traits and oil content*

Brassica yield and yield traits at harvesting were significantly curbed by flooding stress (Table 3). The results indicated that under flooding vs. well water conditions, PPP, and PL were decreased by 27.27% and 19.14% respectively, while SPP and SYPP were reduced by 92.75% and 59.33% respectively (Table 7). The biochar application particularly 2.5% BC mitigated the adverse impacts of flooding stress and restored all the yield traits and the overall ranking was seen as: 2.5% BC>1% BC> control (Table 7). The results indicated that flooding stress also induced negative impacts on oil production and a decrease of 27.19% in oil content was recorded under flooding conditions as compared to normal conditions (Figure 4). The application of 1% and 2.5% BC improved the oil contents by 8.78% and 16.17% while the application of 1% and 2.5% BC improved the brassica oil contents by 5.48% and 14.46% respectively (Figure 4).

**Table 5.** Effect of different rates of BC application on photosynthetic pigments and relative water contents of *Brassica* grown under normal and flooding stress conditions

| Treatments | Biochar application | Chlorophyll a ($mg\ g^{-1}$ FW) | Chlorophyll b ($mg\ g^{-1}$ FW) | Carotenoids ($mg\ g^{-1}$ FW) | RWC (%) |
|---|---|---|---|---|---|
| **Well water** | Control | 0.66b±0.021 | 0.59c±0.015 | 4.17b±0.044 | 59.60c±1.99 |
| | 1% BC | 0.74a±0.016 | 0.64b±0.019 | 4.38a±0.052 | 65.33b±2.23 |
| | 2.5% BC | 0.80a±0.029 | 0.68a±0.033 | 4.47a±0.067 | 68.36a±3.22 |
| **Flooding stress** | Control | 0.29e±0.032 | 0.27f±0.029 | 2.84e±0.038 | 36.62f±2.24 |
| | 1% BC | 0.47d±0.012 | 0.42e±0.042 | 3.39d±0.042 | 42.50e±2.98 |
| | 2.5%BC | 0.58c±0.019 | 0.50d±0.018 | 3.56c±0.054 | 49.12d±2.05 |

BC: biochar, FS: flooding stress, RWC: relative water content. The values are means of three replicates with ± SE and different letters with values indicating significant differences among means.

**Table 6.** Effect of different rates of BC application on nutrient concentration in plant tissues of *Brassica* grown under normal and flooding stress conditions

| Treatments | Biochar application | Nitrogen | Calcium | Magnesium | Potassium |
|---|---|---|---|---|---|
| | $mg\ g^{-1}$ DW | | | | |
| **Well water** | Control | 12.80c±0.51 | 46.02c±0.56 | 38.53c±0.81 | 18.50c±0.98 |
| | 1% BC | 16.57b±0.72 | 54.07b±0.78 | 42.37b±1.27 | 23.30b±0.74 |
| | 2.5% | 19.03a±0.42 | 56.07a±1.02 | 46.82a±1.33 | 26.40a±0.98 |
| **Flooding stress** | Control | 7.88e±0.87 | 29.43f±0.65 | 24.83f±0.98 | 13.00e±0.56 |
| | 1% BC | 8.54e±0.65 | 35.43e±0.92 | 27.13e±0.97 | 15.17d±0.48 |
| | 2.5% BC | 9.85d±0.59 | 38.50d±1.16 | 30.70d±1.55 | 16.10d±0.76 |

BC: biochar, FS: flooding stress, RWC: relative water content. The values are means of three replicates with ± SE and different letters with values indicating significant differences among means.

**Table 7.** Effect of different rates of BC application on yield traits of brassica grown under normal and flooding stress conditions

| Treatments | Biochar application | Pods per plant | Pod length (cm) | Seeds per pod | Seed yield per pot (g) |
|---|---|---|---|---|---|
| **Well water** | **Control** | 169c±5.66 | 4.96c±0.12 | 15.70c±0.41 | 9.90b±0.22 |
| | **1% BC** | 193b±6.76 | 5.28b±0.19 | 18.70b±0.53 | 11.18a±0.25 |
| | **2.5% BC** | 204a±5.96 | 5.63a±0.22 | 21.66a±0.78 | 12.21a±0.33 |
| **Flooding stress** | **Control** | 109f±4.33 | 4.26e±0.10 | 7.67f±1.04 | 5.95e±0.19 |
| | **1% BC** | 122e±4.18 | 4.43e±0.25 | 9.67e±0.56 | 6.85d±0.38 |
| | **2.5% BC** | 135d±3.75 | 4.63d±0.21 | 11.67d±0.49 | 8.11c±0.91 |

BC: biochar, FS: flooding stress, RWC: relative water content. The values are means of three replicates with ± SE and different letters with values indicating significant differences among means.

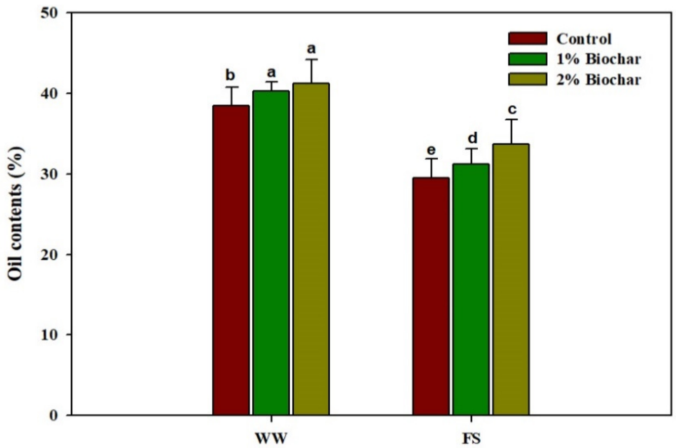
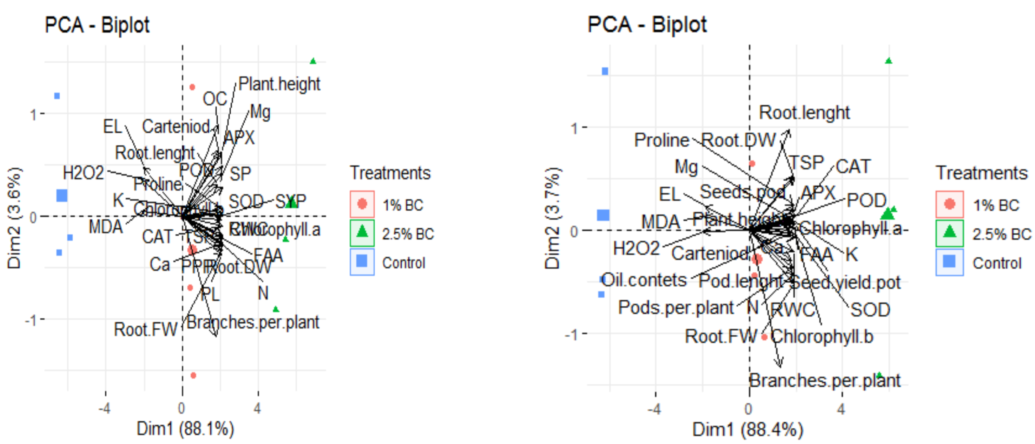



**Figure 4.** Effect of different rates of BC application on oil content of brassica grown under normal and flooding stress conditions

The bars indicating the means of three replicates while different letters indicating the significance among treatments.

*Principle component analysis*

The results indicate that two components (PC1 and PC2) showed 91.7% total variance. The results indicated BC application had a significant impact on growth, yield, and physiological and biochemical traits. The flooding stress showed negative impacts on the growth of physiological and biochemical traits and oil contents. Conversely, the application of BC offset the negative impacts of flooding stress and improved plant growth, yield, and oil contents as compared to the control (Figure 5).




(a) (b)

**Figure 5.** The scores on left and loading plots on right of principal component analysis (PCA) showing the effect of diverse treatments on studied traits under well water (a) and flooding stress (b) conditions

## Discussion

In the present study flooding stress significantly reduced the growth and biomass production of brassica plants (Table 1). The flooding stress leads to low oxygen concentration in the soil which makes plants vulnerable to damage from low oxygen stress and resulting in a significant reduction in plant growth and development (Qiu *et al.*, 2016; Zhou *et al.*, 2021). Besides this, flooding stress also induced oxidative damage increased the MDA and $H_2O_2$ production, and reduced the leaf water status, therefore resulting in a significant loss in growth traits of brassica (Liu *et al*., 2014; Yulianti and Sudrajat, 2016; Zuniga-Feest *et al*., 2017). We also observed the leaf yellowing after the imposition of flooding which might decreased the chlorophyll synthesis, photosynthetic efficiency, and dry matter production, thereby leading to a decrease in plant growth. The application of BC increased growth traits of brassica. This positive influence of BC in increasing the growth under flooding stress could be attributed to a substantial increase in osmolyte accumulation, antioxidant activities, nutrient uptake and reduction in ROS, and MDA production (Sun *et al*., 2017). The root is a key pathway that regulates the above-growth plant growth and in the present study, BC increases the root length and root growth which might improve the soil water status and increase the water consumption by plants. Besides this, BC also increased soil water holding capacity, soil structure, soil permeability, and nutrient availability which resulted in a significant increase in plant growth under stress conditions (Edeh *et al*., 2020; Gliniak *et al*., 2020; Ghorbani *et al*., 2022).

Chlorophyll is the main pigment for photosynthesis in plants (Yang *et al*., 2021), however, in the present study, flooding stress significantly reduced the chlorophyll content of brassica plants. This could be ascribed to flooding-induced oxidative that might damage the photosynthetic apparatus and increase the activity of chlorophyll degrading enzymes which reduced chlorophyll synthesis (Yulianti and Sudrajat, 2017; Yan *et al*., 2020; Creek *et al*., 2020). Under flooding stress leaf water status was also seriously decreased (Table 2). Flooding stress reduces the leaf water potential because water transport can be inhibited by root hypoxia which leads to a decrease in root hydraulic conductivity thus negatively affecting leaf water status in the above plant parts (Else *et al*., 2001; Yuan *et al*., 2022). We also noted that BC application increased chlorophyll synthesis which was linked with improved water uptake, antioxidant activities, and reduced oxidative damage (Lehmann *et al*., 2011; Habibi, 2012). In the present study, BC also showed a significant increase in leaf water status under both normal and flooding conditions. Biochar retains the water and improves root growth and root osmotic potential which favors an increase in water uptake and subsequently maintains better leaf water contents under stress conditions (Abd El-Mageed *et al*., 2019; Liao *et al*., 2019).

The cell membrane plays a regulatory role in controlling the exchange of substances inside and outside of cells (Yin *et al*., 2010). When the permeability of the plasma membrane is disrupted, there is extravasation of intracellular electrolytes, which is manifested as an increase in conductivity (Masoumi *et al*., 2012).

Flooding stress significantly increased the EL indicating that the cell membrane was severely damaged under flooding stress. This damage causes a large amount of ions extravasation from the leaves, contributing to increased electrolyte permeability and enhanced conductivity of the medium (Wang *et al*., 2021). Flooding stress also leads to the accumulation of MDA and $H_2O_2$ which is consistent with earlier findings of Mahmood *et al*. (2021). MDA is the final product of lipid peroxidation (Amnan *et al*., 2022) and flooding stressed plants produced more MDA over time. One of the possible reasons for this is the antioxidant enzymes might provide a lesser defense response against flooding stress and result in a significant increase in MDA production (Sharif *et al*., 2018). The flooding stress increased the ROS production which was accompanied by increased antioxidant activities. The brassica plants showed an increase in APX, CAT, POD, and SOD activities which alleviated the flooding-induced oxidative damages (Da-Silva and do Amarante, 2020; Mahmood *et al*., 2021; Xie *et al*., 2021). Plants produced different osmolytes in response to stress conditions, and in the present study flooding stress decreased the TSP and FAA however, proline (ROS scavenger) synthesis was significant increased under flooding stress. The increase in proline synthesis maintains membrane stability protects the plants from oxidative damage and improves the plant performance under stress conditions (Jia *et al*., 2019; Xiao *et al*., 2020).

The application of BC improves antioxidant activities and osmolyte accumulation, which lessens oxidative injuries by decreasing MDA and $H_2O_2$ production (Hafez *et al*., 2020). Biochar mediated in oxidative damage ensures the membrane stability and in return reduces the loss of important osmolytes (Hasanuzzaman *et al*., 2021). The application of BC eradicated the $H_2O_2$ production and improved the activation of antioxidant enzymes which permit the plants to stand with flooding stress. The enhancement in antioxidant activities with BC could result in improved water uptake, root growth, and soil properties (Ahmad *et al*., 2019). At the cellular level, BC may relieve oxidative injuries by using metabolic pathways more effectively in scavenging ROS, and leading to less membrane damage and improved plant performance (Hasanuzzaman *et al*., 2021; Salim *et al*., 2021).

Nitrogen, phosphorus, and potassium are the basic nutrients needed for plants and most of these nutrients taken by plants by roots from the soil depend on the water conditions of the soil (Da Silva *et al*., 2011). Flooding stress affects soil fertility and inhibits the nutrient absorption capacity of plants thus affecting plant nutrient status (Martínez-Alcántara *et al*., 2012). Different authors also found that flooding stress negatively affects the nutrient uptake and concentration in plants (Wang *et al*., 2017; Zhao *et al*., 2023). Biochar is a carbon-rich product and it directly improves the soil organic carbon (SOC) and soil physio-chemical and biological properties (Zhang *et al*., 2022). Biochar application increases the SOC owing to its stable structure that reduces surface SOC oxidation and degradation of microbial communities and prevents carbon mineralization (Yang *et al*., 2020). Consequently, a BC-mediated increase in SOC improves nutrient uptake and overall soil fertility (Zhang *et al*., 2022). BC application increased nutrient uptake which could be attributed to improved soil fertility, root growth, and an increase in nutrient concentration (Li *et al*., 2022).

Flooding stress progressively exerted a detrimental effect on root respiration and nutrient uptake and it also decreased the leaf water contents, and photosynthetic pigments and increased the MDA, $H_2O_2$, and ROS production thus resulting in a reduction in brassica yield and yield traits and oil concentration (Lin *et al*., 2020). However, BC application significantly yields trait and oil concentrations of brassica under normal and flooding stress. The use of BC increases water retention, microbial activities, photosynthetic activities, leaf water status, and antioxidant activities and reduces ROS production which in turn increases plant growth and yield (Li *et al*., 2022). Further, BC increases nutrient availability, uptake, and nutrient utilization efficiency, leading to higher photosynthetic and assimilated production which in turn increases the production and quality (Liu *et al*., 2016; Phares *et al*., 2020).

## Conclusions

Flooding stress significantly reduced the growth and yield of brassica plants by flooding-induced oxidative damages that impaired the leaf water status, and photosynthetic apparatus and altered the plant osmolyte synthesis, and nutrient uptake. However, under such circumstances, the application of biochar significantly improved physiological traits, leaf water status, potential osmolytes, antioxidant activities, and nutrient uptake and reduced oxidative stress markers. This, in turn, improved the growth, yield traits, and oil contents of brassica crops under normal and flooding stress conditions. Therefore, the application of BC could be an effective approach to mitigate the adverse impacts of flooding stress. This is the first study conducted to explore the mechanisms mediated by biochar to mitigate flooding stress. Therefore, transcriptomics, metabolomics, and genomic studies are needed to discover the mechanisms behind biochar-mediated decrease in flooding stress in plants.

## Authors' Contributions

Conceptualization: JS, FB, and ML. writing-original draft preparation: JS, FB, ML. writing-review and editing: RM, FR, WT, XL, MA, NA, AU, SAA and SA. All authors read and approved the final manuscript.

**Ethical approval** (for researches involving animals or humans)

Not applicable.

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## Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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