



# Effects of biochar and chitosan on morpho-physiological, biochemical and yield traits of water-stressed tomato plants

Amrul Kayes<sup>1</sup>, Tafsin Araf<sup>1</sup>, Sika Mustaki<sup>1</sup>, Nazrul Islam<sup>1</sup> and Shormin Choudhury<sup>1\*</sup>

<sup>1</sup>Department of Horticulture, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka 1207, Bangladesh

\*Corresponding author: Shormin Choudhury, shormin2000@gmail.com

Received: 12 Feb 2024; Received in revised form: 25 Mar 2024; Accepted: 07 Apr 2024; Available online: 17 Apr 2024

©2024 The Author(s). Published by AI Publications. This is an open access article under the CC BY license

<https://creativecommons.org/licenses/by/4.0/>

**Abstract**— The application of biochar as an amendment can improve soil water retention, perhaps leading to higher crop yields. The experiment was conducted at Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during January to April 2022 to find out the effects of biochar and chitosan on physiological, and biochemical traits subjected to water stressed tomato plant. The treatments i.e. three water regimes i.e. i) Control (80% of field capacity) ii) 60% of field capacity (FC) and iii) 40% of field capacity (FC) and three drought mitigating agents i) chitosan (200  $\mu\text{L L}^{-1} \text{ha}^{-1}$ ), ii) rice husk biochar (20 t/ha) and iii) biochar + chitosan were used in this experiment. Twenty five days old, healthy and uniform seedlings were transplanted in plastic pot using biochar. Ten days after transplanting drought was imposed up to flowering stage. Ten days after transplantation, chitosan was administered by hand sprayer. The results showed that water stress dramatically lowered tomato morphological, physiological, and biochemical characteristics except lowering sugar. However, biochar and chitosan significantly reduced the effects of water stress on tomato plants. The use of biochar and chitosan resulted in large improvements in plant height, number of leaves, chlorophyll content, fruits per plant, and yield per plant, vitamin C, and total soluble solid content; however, these treatments resulted in significant declines in lowering sugar content in stressed plants. Therefore, combined effect of chitosan and biochar had a greater impact on growth, yield and biochemical parameters.

**Keywords**— Water stress, Biochar, Chitosan, Chlorophyll content, Sugar content

## I. INTRODUCTION

Tomatoes (*Lycopersicon esculentum* L.) are one of the most significant and commonly utilized agricultural vegetable crops, growing mostly in home and commercial gardens worldwide. There are numerous biotic and abiotic stress factors that influence plant growth and productivity. Significant reductions in growth parameters, such as leaf count, leaf area, and stem length, were seen in a variety of plants under drought stress (Abdelaal *et al.*, 2020). Drought stress, caused by insufficient water supply, induces physiological and biochemical responses in crops, reducing crop growth, development, and yield (Zhou *et al.*, 2017). Tomato plants are very prone to dry stress, especially during the flowering and fruit expansion stages (Jangid & Dwivedi,

2016). Drought stress has a major impact on crop yield and productivity in tomato plants (Chakma *et al.*, 2021).

The detrimental effects of several abiotic stresses on plants are linked to oxidative damage in the plant cells, which raises the levels of reactive oxygen species (ROS), lipid peroxidation, and electrolyte leakage (Sachdev *et al.*, 2021). Overuse of chemical fertilizers has recently had negative consequences on water quality, soil microbes, human health, and soil qualities. There are numerous techniques for reducing the detrimental effects of chemical fertilizers and mitigating the damaging impacts of various stresses; one of the safest and most successful is the use of natural chemicals such as biochar and chitosan.

Biochar is a solid, stable, and carbon-rich substance that is produced by thermochemical transformation in anaerobic or oxygen-limited environments. It alters the hydraulic characteristics and nutritional state of soils (Gao *et al.*, 2019). Using biochar as productivity and optimize nutrient and water usage. A soil amendment has been proposed as a way to boost long-term (Sarong and Orge, 2015). According to Xu *et al.* (2016), biochar produced notable growth characteristics and yield production under both stressful and natural environments. According to Zhang *et al.* (2023), adding biochar to plant physiology helps to mitigate the negative effects of drought stress on tomato seedling growth. By using biochar, plants were able to absorb more nutrients and had higher levels of nitrogen, phosphorus, and magnesium (Ch'ng *et al.*, 2016). In drought-stricken pumpkin plants, biochar application boosted nutrient absorption and chlorophyll concentrations at a rate of 20 t ha<sup>-1</sup> (Langeroodi *et al.*, 2019). Compared to traditional chemical fertilizers, biochar increased the amount of organic matter and macronutrients in the soil and enhanced its quality (Kizito *et al.*, 2019).

A naturally occurring biopolymer derived from sea crustaceans, chitosan promotes plant development and yield while also boosting the immune system of plants (Pongprayoon *et al.*, 2013; Sultana *et al.*, 2019). Chitosan foliar spray serves to lessen the effect of water stress on yield, which may be related to an increase in stomatal conductance under water stress and its involvement in reducing transpiration rate (Yan *et al.*, 2012). Recently, the antibacterial properties of chitosan, an organic polymer derived from the hard shells of aquatic animals like shrimp and crabs, have been studied (Elieh-Ali-Komi *et al.*, 2016). Certain tomato attributes, including plant height, leaf area, chlorophyll content, relative water content, and yield, are positively impacted by the foliar application of chitosan during drought conditions (Hassnain *et al.*, 2020). Chitosan is now used as a non-toxic, biodegradable, and environmentally friendly chemical to minimize and alleviate the impacts of various pressures, including drought stress (Dzung *et al.*, 2011). Many studies have shown that using chitosan improves plant yield and germination (Mahdavi *et al.*, 2014; Amiri *et al.*, 2015).

There is currently little evidence known about the effects of biochar and chitosan on the physiological and biochemical parameters of drought-stressed plants. Thus, the goal of our study is to determine the influence of biochar and chitosan on morpho-physiological, biochemical, and yield-contributing parameters in tomato plants exposed to various water stresses.

## II. MATERIALS AND METHODS

### 2.1. Plant materials and growing conditions

The experiment was conducted in a shed house at Sher-e-Bangla Agricultural University, Dhaka 1207, Bangladesh, under natural lighting conditions. Seeds of tomato variety (BARI tomato 8) were obtained from the Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh. Seeds were sowed in Poly vinyl chloride tanks (1.2×0.6×0.6 m) with soil mixture and slow-release fertilizers. Seedlings were transplanted to maintained pots (3 seedlings per pot), filled with soil, and fertilized as recommended.

### 2.2. Treatments and sample collection

The treatments were done as follows: plants received three water regimes i) Control (80% of field capacity), ii) 60% of field capacity (FC) and iii) 40% of field capacity (FC) were maintained from 30-35 days old seedling up to maturity. Plants also treated with chitosan (200 µL L<sup>-1</sup> ha<sup>-1</sup>), rice husk biochar (20 t/ha) and chitosan + biochar. Treatments in the experiment will be used as follows: 80% moisture, 80% moisture + chitosan, 80% moisture + biochar, 80% moisture + chitosan + biochar, 60% moisture, 60% moisture + chitosan, 60% moisture + biochar, 60% moisture + chitosan + biochar, 40% moisture, 40% moisture + chitosan, 40% moisture + biochar, 40% moisture + chitosan + biochar. Three replications will be applied for all the treatments. Various morphological, physiological and biochemical parameters will be assessed during flowering stage.

### 2.3. Measurement of plant height and leaf area

Plant height was measured three times, from the base of the plant to the tip of the main stem, in centimeters for each plant in each treatment. The mean value was then computed. Three plants from each treatment had their leaves removed, and the leaf area was measured. The maximum width (W) and length (L) of every leaf collected were measured using a ruler. The breadth was measured on the widest leaflet, while the length was measured from the distal end of the rachis to the insertion of the first leaflet.

### 2.4. Relative Water Content

Three leaves were combined for each replicate, and their fresh weights (FW) were calculated. After regaining their turgidity, the leaves were submerged in water for twelve hours at room temperature. The surplus water was then immediately blotted from the turgid tissue, and the turgid weights (TW) of the leaves were determined. To find the samples' dry weights (DW), they were subsequently dried for 24 hours at 65°C in an oven. The following formula was used to determine the RWC:

$$\text{RWC \%} = ((\text{FW}-\text{DW})/(\text{TW}-\text{DW})) * 100.$$

## 2.5. Photosynthetic pigment

The Porath (1980) method was used to detect photosynthetic pigments. One milliliter of 100% N, N-dimethylformamide (DMF) was used to homogenize 0.2 grams of leaf tissue that had been powdered using liquid nitrogen. The homogenized samples were centrifuged for 10 minutes at 10,000 rpm in order to collect the supernatant. Following another addition of 1 ml DMF, the samples were centrifuged. After discarding the supernatant, 1 milliliter of DMF was added. A spectrophotometer was used to record the absorbance at 663 and 645 nm. A 100% DMF blank was used for calibration. The following formulas were used to determine total chlorophyll, chlorophyll a, and chlorophyll b:

$$\text{Chlorophyll } a \text{ (mg g}^{-1} \text{ tissue)} = \frac{[12.7(OD663) - 2.69(OD645)] \times V}{1000} \times W$$

$$\text{Chlorophyll } b \text{ (mg g}^{-1} \text{ tissue)} = \frac{[22.9(OD645) - 4.68(OD663)] \times V}{1000} \times W$$

$$\text{Total Chlorophyll (mg g}^{-1} \text{ tissue)} = \frac{[8.02(OD663) + 20.20(OD645)] \times V}{1000} \times W$$

Where OD: Optical density at respective nm, V: Final volume of chlorophyll extract,

W: Fresh weight of the tissue extracted

## 2.6. Yield

The yield per plant was calculated using a per scale balance. The amount of fruit produced by each plant was counted separately during the time from fruit until the last harvest and the result was recorded in kilograms (kg).

## 2.7. Reducing sugar content

With a few changes to the assay volume and wavelength, reducing sugars were calculated using the phenol-sulphuric acid method (DuBois *et al.*, 1956). After homogenizing 0.2 grams of fresh leaf with deionized water, the extract was filtered. 0.4 milliliters of 5% phenol were combined with 2 milliliters of the solution. The liquid was then quickly mixed with 2 cc of 98% sulfuric acid. The test tubes were kept at room temperature for ten minutes before being submerged in a water bath set at thirty degrees Celsius for twenty minutes to allow the color to develop. Next, using the spectrophotometer, light absorption at 540 nm was measured. The same method was used to prepare the blank solution, which is distilled water. The reducing sugar content was given in mg/g FW.

## 2.8. Determination of proline content

The Bates *et al.* (1973) method was used to extract and assess the proline content of the leaf tissues. Liquid nitrogen was used in a mortar to grind fresh leaf materials weighing fifty milligrams. After combining the homogenate powder with 1 milliliter of aqueous sulfuric acid (3% w/v), it was filtered using Whatman #1 filter paper. After adding an equal amount of Glacial acetic acid and ninhydrin reagent (1.25 mg of ninhydrin to 30 mL of Glacial acetic acid and 20 ml of 6 M H<sub>3</sub>PO<sub>4</sub>) to the extracted solution, it was incubated for one hour at 95°C. Placing the reaction in an ice bath stopped it. Two milliliters of toluene were swiftly stirred into the reaction mixture. After warming to 25°C, the chromophore was detected at 520 nm. L-proline was employed as the standard.

## 2.9. Total soluble solids content (TSS)

TSS content in tomatoes was measured using a digital refractometer (MA871; Romania). Using a dropper, a drop of tomato juice was put to the refractometer prism. The refractometer value revealed the total soluble solids.

## 2.10. Ascorbic acid determination

The Oxidation-Reduction Titration Method (Tee *et al.*, 1988) was used to calculate the Ascorbic acid content of tomatoes. The fruit was mashed and then filtered through Whatman No. 1 filter paper. A volume of 100ml was produced using 5% oxalic acid. We used the dye solution 2, 6-dichlorophenol indophenol to carry out the titrations. Using L-ascorbic acid as the known sample, the mean observations showed how much dye was needed to oxidize an unknown concentration of a given amount of L-ascorbic acid solution. For every titration, 5 milliliters of the solution were utilized, and the pink color, which persisted for 10 seconds, indicated the end of the titration. Consequently, a burette reading was obtained and kept.

## 2.11. Data analysis

Data analysis was done with SPSS 20.0 software. When P < 0.05, the value was deemed statistically significant. The mean ± SE of the replicates was used to present all the results. Microsoft Excel was used to create the graphs.

## III. RESULTS AND DISCUSSION

### 3.1. Plant height (cm)

Plant height was markedly decreased by water stress in tomato plants. In case of different moisture level, the highest plant height (101.01 cm) was observed in plants kept under 80% moisture level and the lowest plant height (82.33 cm) was found in plants kept 40% moisture level. Water stress leads to increases in abscisic acid which causes an inhibition of the plant growth (Abdalla *et al.*, 2011). In 60% and 40% moisture levels, the highest plants height

(95.33cm) and (88.67cm) found in chitosan + biochar treatment respectively, which mitigated stress conditions corresponding to plants that did not employ chitosan and biochar. According to Haider *et al.* (2020), biochar significantly reduces the harmful effects of drought and improved plant height by increasing photosynthetic rate. Chitosan plays a major protective function against drought damage; there may be a reason for the rise in plant height observed in stressed plants treated with chitosan (Bistgani *et al.*, 2017).

**3.2. Leaf area (cm<sup>2</sup>)**

The highest leaf area (429.58 cm<sup>2</sup>) was observed in plants kept under 80% moisture level and the lowest leaf area (345.51 cm<sup>2</sup>) was found in plants maintained 40% moisture level. With the application of biochar and chitosan, plants mitigate drought stress and maintain plant leaf size under different water stressed conditions (Table 1). The highest leaf area (396.77 cm<sup>2</sup>) and (378.52 cm<sup>2</sup>) were noticed in plants treated with chitosan + biochar in both 60% and 40% moisture level respectively compared to drought stressed plants alone. This result of biochar application was similar to the obtained results of Wei *et al.* (2020). Ren *et al.* (2021) observed that biochar contributes in enhancement of leaf area by improving root growth which helps in delivering water and mineral nutrients to the leaf area. Biochar amendment can be contributed to improve nutrient status in the leaves (Vassilev *et al.*, 2013); therefore, biochar addition can alleviate the negative effects of drought on leaf area and ameliorate the photosynthetic rate which involves in enhancement of leaf area (Paneque *et al.*, 2016). Mondal *et al.* (2016) who reported that foliar application of chitosan at early growth stages increased leaf area of plant. Ke *et al.* (2001) reported that application of chitosan enhanced leaf area by increasing key enzymes activities of nitrogen metabolism.

**3.3. Relative water content (%)**

In case of different moisture levels, relative water content (82.02%) was observed higher in plants treated under 80%

moisture level and relative water content (65.01%) was noticed lower in plants under 40% moisture level. Due to application of biochar and chitosan, plants mitigate water stress conditions and contain higher relative water content compared to drought stressed plants alone (Table 1). In both 60% and 40% moisture level, the highest relative water content was detected in plants treated with chitosan + biochar compared to drought stressed plants alone. According to Lyu *et al.* (2016), applying biochar to leaves strengthens their defense mechanism against drought stress by enhancing the activity of protective enzymes and electron transfer, which reduces the damage that drought stress causes to RWC. By decreasing transpiration rate and increasing stomatal conductance during water stress, foliar application of chitosan can mitigate the negative effects of water stress on yield (Ibrahim *et al.*, 2023). RWC was lowered by deficit watering treatments, however plants sprayed with chitosan showed noticeably greater RWC values. RWC values drop when there is a prolonged water deficit, although these drops may be mitigated by chitosan spraying (Khalil *et al.*, 2021).

**3.4. Photosynthetic pigments**

In case of different moisture levels, the highest chlorophyll a (32.08 mg/g), chlorophyll b (34.71 mg/g) and total chlorophyll (22.15 mg/g) were observed in plants kept under 80% moisture level + chitosan and the lowest chlorophyll a (15.88 mg/g), chlorophyll b (21.26mg/g) and total chlorophyll (13.08 mg/g) were found in plants maintained 40% moisture level treated with no chemical. However, due to application of biochar and chitosan, plants mitigate drought stress and contain highest chlorophyll under different water stressed conditions compared to drought stressed plants alone (Table 1). In both 60% and 40% moisture level, the highest photosynthetic pigments were detected in plants treated with chitosan + biochar, which mitigated stress conditions corresponding to plants that did not employ chitosan and biochar (Table 1).

Table 1: Effect of biochar and chitosan with different water regimes on morphological, physiological and biochemical parameters of tomato

Treatments	Plant height (cm)	Leaf area (cm <sup>2</sup> )	Relative water content (%)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total chlorophyll (mg/g)
W <sub>1</sub> B <sub>0</sub>	93.01 cd	394.93 bc	78.33 ab	26.41 bc	25.50 b	17.63 b
W <sub>1</sub> B <sub>1</sub>	96.67 b	412.78 ab	82.02 a	32.08 a	34.71 a	22.15 a
W <sub>1</sub> B <sub>2</sub>	95.53 bc	413.13 ab	70.66 bcd	28.42 ab	21.66 bcd	13.42 cd
W <sub>1</sub> B <sub>3</sub>	101.01 a	429.58 a	79.22 ab	27.17 bc	25.03 bc	17.28 b
W <sub>2</sub> B <sub>0</sub>	92.01 d	372.00 def	78.67 ab	24.03 cd	17.27 d	12.34 cd
W <sub>2</sub> B <sub>1</sub>	92.67 cd	385.53 cd	77.33 ab	20.39 def	18.65 cd	12.23 bcd

W <sub>2</sub> B <sub>2</sub>	95.33 bc	383.26 cd	75.66 abc	20.90 def	17.21 d	15.36 bc
W <sub>2</sub> B <sub>3</sub>	95.33 bc	396.77 bc	76.04 abc	24.00 cd	21.51 bcd	15.17 bcd
W <sub>3</sub> B <sub>0</sub>	82.62 f	345.51g	65.01 d	15.88 g	21.26 bcd	13.08 cd
W <sub>3</sub> B <sub>1</sub>	82.33 f	349.37 fg	73.01 bcd	22.36 de	22.58 bc	11.83 cd
W <sub>3</sub> B <sub>2</sub>	83.67 f	358.73 efg	73.33 bcd	18.58 efg	19.65 cd	11.70 d
W <sub>3</sub> B <sub>3</sub>	88.67 e	378.52cde	68.00 cd	17.45 fg	21.81 bcd	13.37 cd
LSD <sub>0.05</sub>	3.12	22.71	8.39	4.00	5.02	3.54
CV%	10.27	11.57	11.81	10.22	13.34	14.08

Note. Means followed by same letter(s) in a column do not differ significantly at 5 % level of LSD. W<sub>1</sub>= 80% field capacity (control), W<sub>2</sub>= 60% field capacity, W<sub>3</sub>= 40% field capacity, B<sub>0</sub>= Control (No chemical), B<sub>1</sub>= 200 µL L<sup>-1</sup> chitosan, B<sub>2</sub>= Rice husk biochar @20 t/ha, B<sub>3</sub>= 200 µL L<sup>-1</sup> chitosan + Rice husk biochar @20 t/ha.

According to Cabillo *et al.* (2019), plants under drought stress had the least amount of chlorophyll in comparison to control plants; this led to decreased photosynthetic efficiency and plant development (Franzoni *et al.*, 2021). However, by raising chlorophyll levels in water-stressed plants and boosting photosynthesis, chitosan and glycine betaine treatments counteract these detrimental effects (Gurrieri *et al.*, 2020). By enhancing tomato plant chlorophyll synthesis, stomata conductance, and water use efficiency, biochar application reduced the negative effects of drought stress (Farooq *et al.*, 2021). According to recent reports, biochar treatment boosts photosynthetic rate and chlorophyll production, which in turn lowers ROS damage and boosts productivity (Gharred *et al.*, 2022).

### 3.5. Fruit yield and quality

Fruit yield was considerably lower in drought-stressed plants (60% and 40% moisture level) compared to non-stressed plants (80% moisture level). The use of biochar and chitosan boosted fruit yield in all water stressed plants. The highest fruit yield (1127 gm) and (552 gm) were noticed in plants treated with chitosan + biochar in both 60% and 40% moisture level respectively compared to drought stressed plants alone (Table 2). According to Bangar *et al.* (2019), a drought has a substantial impact on a variety of morpho-physiological and biochemical functions, which lowers yield. Enhancing plant development, increasing nutrient uptake, raising auxin and gibberellic acid concentrations, and ultimately boosting yield characteristics are all made possible by the use of biochar and chitosan (Langeroodi *et al.*, 2019).

The increased TSS content (5.23%) was observed at 40% moisture availability while the decreased TSS content (4.36%) was found in plant with 80% moisture level. Plants treated with biochar + chitosan in both 60% and 40% moisture availability showed the maximum TSS (5.07 %) and (5.23 %) respectively, compared to drought stressed plants alone (Table 2). The foliar spraying of chitosan to tomato plants resulted in fruits with increased total soluble solids (Reyes-Pérez *et al.*, 2020). According to Ávila *et al.* (2023), chitosan-treated plants accumulated higher TSS compared to controls. We hypothesize that drought-induced buildup is related to decreased saccharolytic enzyme activity. Biochar application had no significant effects on TSS (Akhtar *et al.*, 2014).

The highest ascorbic acid (25.92 mg/100g) was found over 40% field capacity. The lowest ascorbic acid reading (16.48 mg/100g) was measured in 80% field capacity (Table 2). At stressed conditions, the ascorbic acid content of tomato was the highest in biochar + chitosan treated plants with 60% (23.66 mg/100g) and 40% (25.92 mg/100g) moisture levels compared to drought stressed plants alone. According to Nahar *et al.* (2018), the improved fruit quality in tomatoes under water deficiency conditions could be attributed to ascorbic acid production. Biochar increases ascorbic acid concentration in tomato fruit without significantly altering TSS under various water stress conditions (Agbna *et al.*, 2017). Kamal *et al.* (2011) found that foliar application of chitosan increases ascorbic acid significantly.

Table 2: Effect of biochar and chitosan with different water regimes on yield and quality attributes of tomato

Treatments	Yield (g)	TSS (°B)	Ascorbic acid (mg/100g)
W <sub>1</sub> B <sub>0</sub>	1280.5 c	4.36 g	16.48 j
W <sub>1</sub> B <sub>1</sub>	1386.7 b	4.67 f	17.28 i
W <sub>1</sub> B <sub>2</sub>	1312.6 c	4.57 f	16.91 ij

W <sub>1</sub> B <sub>3</sub>	1489.8 a	4.80 e	18.587 h
W <sub>2</sub> B <sub>0</sub>	933.0 f	4.83 de	19.83 g
W <sub>2</sub> B <sub>1</sub>	1120.9 d	5.04 bc	22.41 e
W <sub>2</sub> B <sub>2</sub>	1037.3 e	4.93 cd	21.44 f
W <sub>2</sub> B <sub>3</sub>	1127.0 d	5.07 b	23.66 d
W <sub>3</sub> B <sub>0</sub>	196.0 i	4.93 cd	24.50 c
W <sub>3</sub> B <sub>1</sub>	504.7 g	5.13 ab	25.18 b
W <sub>3</sub> B <sub>2</sub>	389.0 h	5.07 b	24.96 bc
W <sub>3</sub> B <sub>3</sub>	552.0 g	5.23 a	25.92 a
CV%	3.50	1.57	1.86
LSD <sub>0.05</sub>	55.90	0.12	0.67

W<sub>1</sub>= 80% field capacity (control), W<sub>2</sub>= 60% field capacity, W<sub>3</sub>= 40% field capacity, B<sub>0</sub>= Control (No chemical), B<sub>1</sub>= 200 μL L<sup>-1</sup> chitosan, B<sub>2</sub>= Rice husk biochar @20 t/ha, B<sub>3</sub>= 200 μL L<sup>-1</sup> chitosan + Rice husk biochar @20 t/ha. Means followed by same letter(s) in a column do not differ significantly at 5 % level of LSD.

### 3.6. Biochemical Parameters

#### 3.6.1. Proline content (mg/g)

In case of different moisture levels, the maximum proline content (3.78 mg/g) was observed in plants kept 80% moisture level and the minimum (0.84 mg/g) was noticed in plants maintained 40% moisture level. However, due to the application of biochar and chitosan, plants mitigate water stress conditions and contain the maximum proline (Figure 1A). Under water stress, the maximum proline was observed in plants treated with chitosan + biochar in 60% (2.35 mg/g) and 40% (1.08 mg/g) moisture level, which mitigated stress conditions corresponding to plants that did not employ chitosan and biochar. Drought stress significantly reduces the accumulation of proline

compounds in plants (Furlan *et al.*, 2020). However, chitosan-treated plants store proline in plant tissue as a defense mechanism when there is a water scarcity (Sheikha *et al.*, 2015). Plants employ proline accumulation as one of their defense mechanisms against the damaging effects of water stress (Kijowska-Oberc *et al.*, 2023). Furthermore, the increased amount of proline in stressed plants caused by chitosan administration may indicate improved plant tolerance to water stress (Shafiq *et al.*, 2021). Proline buildup is thought to be one of plants' reactions to reducing damage under water shortages (Anjum *et al.*, 2011). This shows that using biochar can reduce the proline concentration of tomato leaves by enhancing soil moisture and assisting plants in adapting to abiotic stress.

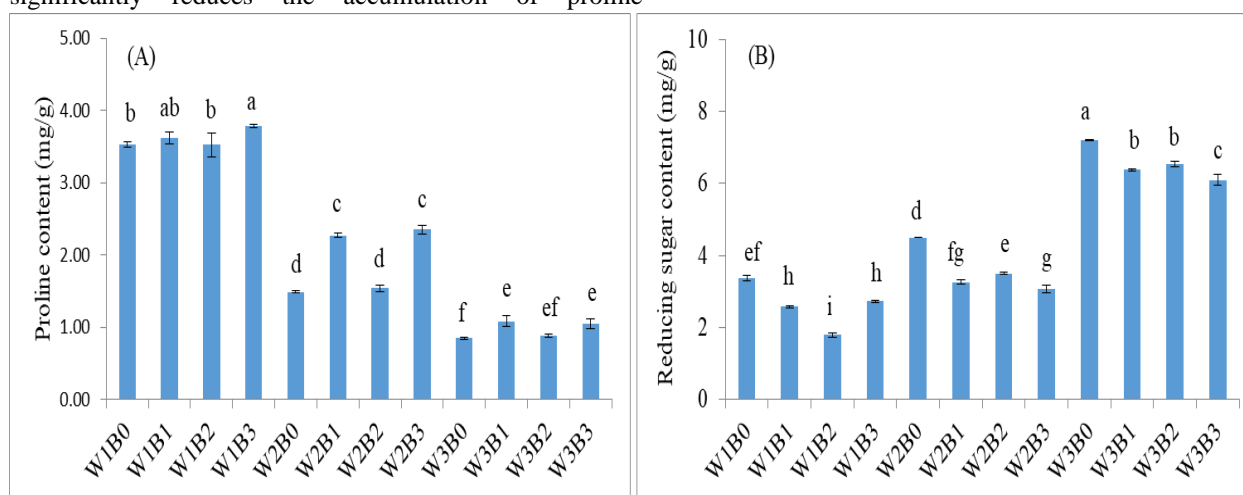


Fig.1. Effect of biochar and chitosan and different water regimes on proline and reducing sugar content of tomato

W<sub>1</sub>= 80% field capacity (control), W<sub>2</sub>= 60% field capacity, W<sub>3</sub>= 40% field capacity, B<sub>0</sub>= Control (No chemical), B<sub>1</sub>= 200 μL L<sup>-1</sup> chitosan, B<sub>2</sub>= Rice husk biochar @20 t/ha, B<sub>3</sub>= 200 μL L<sup>-1</sup> chitosan + Rice husk biochar @20 t/ha. Means followed by same letter(s) in a column do not differ significantly at 5 % level of LSD.

### 3.6.2. Reducing sugar content (mg/g)

In case of different moisture levels, the highest reducing sugar content (7.20 mg/g) was observed in plants kept under 40% moisture level and the lowest reducing sugar (1.79 mg/g) was found in plants maintained 80% moisture level (Figure 1B). Lowest reducing sugar was detected in plants with chitosan + biochar treatment in both 60% (3.08 mg/g) and 40% (6.10 mg/g) moisture level in soil, which mitigated stress conditions and resulted in lowest reducing sugar content. Drought stress causes decrease in growth parameters of tomato plants associated with increase reducing sugars contents, the increase was significant with increasing the drought stress (Mohammadi *et al.*, 2023). In comparison to control plants and stressed untreated plants, all applications of chitosan and biochar dramatically reduced the amounts of starch, sucrose, and soluble sugars in the stressed plants (Hafez *et al.*, 2020).

## IV. CONCLUSION AND RECOMMENDATIONS

Biochar and chitosan were highly efficient in reducing the adverse effects of drought stress on tomato plant. The use of Biochar and chitosan are maintained plant growth, biochemical traits from the adverse effects of drought stress. Chitosan was the most effective at mitigating the adverse effects of drought stress on tomato plants compared to biochar. However, combined chitosan and biochar had a greater impact on growth, yield and biochemical parameters. As a result, there is an urgent need for inducing chitosan and biochar to have the capability of reducing water stress and increasing crop production.

These findings can be applied to improve tomato production and productivity in drought-prone areas. However, there is a gap that must be bridged by future research: The study area's research should focus on replicating seasons and areas employing hybrid tomato varieties in drought-prone areas, as well as inducing biochar and chitosan to reduce water stress in tomato production and ease water stress problems.

## ACKNOWLEDGMENTS

The present work was financially supported by Ministry of Science and Technology (MOST), Bangladesh.

## REFERENCES

- [1] Abd El-Kader, A.A., Mohamedin A.A.M. and Al-Kady, K.A. 2007. Effect of nitrogen and micronutrients on growth, yield, nutrients uptake and some biochemical properties of onion (*Allium cepa* L.) plants under sandy soil. *Egypt J. Ap. Sci.*, 22(2): 767-778.
- [2] Abdalla, M. M. (2011). Beneficial effects of diatomite on the growth, the biochemical contents and polymorphic DNA in *Lupinus albus* plants grown under water stress. *Agriculture and Biology Journal of North America*, 2(2), 207-220.
- [3] Abdelaal, K.A., Attia, K.A., Alamer, S.F., El-Afry, M.M., Ghazy, A.I., Tantawy, D.S., Al-Doss, A.A., El-Shawy, E.S.E., M. Abu-Elsaoud, A. and Hafez, Y.M. (2020). Exogenous application of proline and salicylic acid can mitigate the injurious impacts of drought stress on barley plants associated with physiological and histological characters. *Sustainability*, 12(5), 1736.
- [4] Agbna, G. H., Dongli, S., Zhipeng, L., Elshaikh, N. A., Guangcheng, S. and Timm, L. C. (2017). Effects of deficit irrigation and biochar addition on the growth, yield, and quality of tomato. *Scientia Horticulturae*, 222, 90-101.
- [5] Akhtar, S. S., Li, G., Andersen, M. N., & Liu, F. (2014). Biochar enhances yield and quality of tomato under reduced irrigation. *Agricultural Water Management*, 138, 37-44.
- [6] Amiri, A., Sirousmehr, A. and Esmaeilzadeh, B. S. (2015). Effect of foliar application of salicylic acid and chitosan on yield of Safflower (*Carthamus tinctorius* L.). *Journal of Plant Research*, 28 (4), 712-725
- [7] Anjum, S. A., Xie, X., Wang, L., Saleem, M. F., Man, C. and Lei, W. (2011). Morphological, physiological and biochemical responses of plants to drought stress. *African journal of agricultural research*, 6(9), 2026-2032.
- [8] Ávila, R.G., Magalhães, P.C., Vitorino, L.C., Bessa, L.A., de Souza, K.R.D., Queiroz, R.B., Jakelaitis, A. and Teixeira, M.B. (2023). Chitosan induces sorghum tolerance to water deficits by positively regulating photosynthesis and the production of primary metabolites, osmoregulators, and antioxidants. *Journal of Soil Science and Plant Nutrition*, 23(1), 1156-1172.
- [9] Bangar, P., Chaudhury, A., Tiwari, B., Kumar, S., Kumari, R. and Bhat, K. V. (2019). Morphophysiological and biochemical response of mungbean [*Vigna radiata* (L.) Wilczek] varieties at different developmental stages under drought stress. *Turkish Journal of Biology*, 43(1), 58-69.
- [10] Bates, L. S., Waldren, R. P. A., & Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. *Plant and soil*, 39, 205-207.
- [11] Bistgani, Z. E., Siadat, S. A., Bakhshandeh, A., Pirbalouti, A. G. and Hashemi, M. (2017). Interactive effects of drought stress and chitosan application on physiological characteristics and essential oil yield of *Thymus daenensis* Celak. *The Crop Journal*, 5(5), 407-415.
- [12] Cabillo, C. M. (2019). Biomass production of lettuce (*Lactuca sativa* L.) under water stress. *International Journal of Scientific Engineering and Research*, 10(12), 928-935.
- [13] Chakma, R., Saekong, P., Biswas, A., Ullah, H. and Datta, A. (2021). Growth, fruit yield, quality, and water productivity of grape tomato as affected by seed priming and soil application of silicon under drought stress. *Agricultural Water Management*, 256, 107055.

- [14] Ch'ng, H. Y., Ahmed, O. H. and Majid, N. M. A. (2016). Improving phosphorus availability, nutrient uptake and dry matter production of *Zea mays* L. on a tropical acid soil using poultry manure biochar and pineapple leaves compost. *Experimental Agriculture*, 52(3), 447-465.
- [15] DuBois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. T. and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical chemistry*, 28(3), 350-356.
- [16] Dzung, N. A., Khanh, V. T. P. and Dzung, T. T. (2011). Research on impact of chitosan oligomers on biophysical characteristics, growth, development and drought resistance of coffee. *Carbohydrate polymers*, 84(2), 751-755.
- [17] Elieh-Ali-Komi, D. and Hamblin, M. R. (2016). Chitin and chitosan: production and application of versatile biomedical nanomaterials. *International journal of advanced research*, 4(3), 411.
- [18] Farooq, M., Almamari, S. A. D., Rehman, A., Al-Busaidi, W. M., Wahid, A. and Al-Ghamdi, S. S. (2021). Morphological, physiological and biochemical aspects of zinc seed priming-induced drought tolerance in faba bean. *Scientia Horticulturae*, 281, 109894.
- [19] Franzoni, G., Cocetta, G. and Ferrante, A. (2021). Effect of glutamic acid foliar applications on lettuce under water stress. *Physiology and Molecular Biology of Plants*, 27, 1059-1072.
- [20] Furlan, A. L., Bianucci, E., Giordano, W., Castro, S. and Becker, D. F. (2020). Proline metabolic dynamics and implications in drought tolerance of peanut plants. *Plant Physiology and Biochemistry*, 151, 566-578.
- [21] Gao, S., DeLuca, T. H. and Cleveland, C. C. (2019). Biochar additions alter phosphorus and nitrogen availability in agricultural ecosystems: A meta-analysis. *Science of the Total Environment*, 654, 463-472.
- [22] Gharred, J., Derbali, W., Derbali, I., Badri, M., Abdelly, C., Slama, I. and Koyro, H. W. (2022). Impact of biochar application at water shortage on biochemical and physiological processes in medicago ciliaris. *Plants*, 11(18), 2411.
- [23] Gurrieri, L., Merico, M., Trost, P., Forlani, G., & Sparla, F. (2020). Impact of drought on soluble sugars and free proline content in selected *Arabidopsis* mutants. *Biology*, 9(11), 367.
- [24] Hafez, Y., Attia, K., Alamery, S., Ghazy, A., Al-Doss, A., Ibrahim, E., Rashwan, E., El-Maghraby, L., Awad, A. and Abdelaal, K. 2020. Beneficial effects of biochar and chitosan on antioxidative capacity, osmolytes accumulation, and anatomical characters of water-stressed barley plants. *Agronomy*, 10(5), 630.
- [25] Haider, I., Raza, M.A.S., Iqbal, R., Aslam, M.U., Habib-ur-Rahman, M., Raja, S., Khan, M.T., Aslam, M.M., Waqas, M. and Ahmad, S. 2020. Potential effects of biochar application on mitigating the drought stress implications on wheat (*Triticum aestivum* L.) under various growth stages. *Journal of Saudi Chemical Society*, 24(12), 974-981.
- [26] Hassnain, Basit, A., Alam, M., Ahmad, I., Ullah, I., Alam, N., Ullah, I., Khalid, M.A. and Shair, M. 2020. Efficacy of chitosan on performance of tomato (*Lycopersicon esculentum* L.) plant under water stress condition. *Pakistan Journal of Agricultural Research*, 33, 27-41.
- [27] Ibrahim, E. A., Ebrahim, N. E. and Mohamed, G. Z. (2023). Effect of water stress and foliar application of chitosan and glycine betaine on lettuce. *Scientific Reports*, 13(1), 17274.
- [28] Jangid, K. K. and Dwivedi, P. (2016). Physiological responses of drought stress in tomato: a review. *International Journal of Agriculture, Environment and Biotechnology*, 9(1), 53-61.
- [29] Kamal, A. M. and Ghanem, K. M. (2011). Response of cape gooseberry plants (*Physalis peruviana* L.) to some organic amendments and foliar spray with chitosan. *Journal of Plant Production*, 2(12), 1741-1759.
- [30] Khalil, H. A. and Eldin, R. M. B. 2021. Chitosan improves morphological and physiological attributes of grapevines under deficit irrigation conditions. *Journal of Horticultural Research*, 29(1), 9-22.
- [31] Kijowska-Oberc, J., Dylewski, Ł. and Ratajczak, E. 2023. Proline concentrations in seedlings of woody plants change with drought stress duration and are mediated by seed characteristics: a meta-analysis. *Scientific Reports*, 13(1), 15157.
- [32] Kizito, S., Luo, H., Lu, J., Bah, H., Dong, R. and Wu, S. (2019). Role of nutrient-enriched biochar as a soil amendment during maize growth: Exploring practical alternatives to recycle agricultural residuals and to reduce chemical fertilizer demand. *Sustainability*, 11(11), 3211.
- [33] Langeroodi, A. R. S., Campiglia, E., Mancinelli, R. and Radicetti, E. (2019). Can biochar improve pumpkin productivity and its physiological characteristics under reduced irrigation regimes?. *Scientia Horticulturae*, 247, 195-204.
- [34] Li Ke, L. K., Lu XiangYang, L. X. and Peng LiSha, P. L. (2001). Effects of carboxymethyl chitosan on key enzymes activities of nitrogen metabolism and grain protein contents in rice.
- [35] Lyu, S., Du, G., Liu, Z., Zhao, L. and Lyu, D. (2016). Effects of biochar on photosystem function and activities of protective enzymes in *Pyrus ussuriensis* Maxim. under drought stress. *Acta Physiologiae Plantarum*, 38, 1-10.
- [36] Mahdavi, B., Modarres-Sanavy, S. A., Aghaalikhani, M., Sharifi, M. and Alavi Asl, S. A. (2014). Effect of foliar application of chitosan on growth and biochemical characteristics of safflower (*Carthamus tinctorius* L.) under water deficit stress. *Iranian Journal of Field Crops Research*, 12(2), 229-236.
- [37] Mohammadi Alagoz, S., Hadi, H., Toorchi, M., Pawłowski, T.A., Asgari Lajayer, B., Price, G.W., Farooq, M. and Astatkie, T. 2023. Morpho-physiological responses and growth indices of triticale to drought and salt stresses. *Scientific Reports*, 13(1), 8896.
- [38] Mondal, M., Puteh, A.B. and Dafader, N.C., 2016. Foliar application of chitosan improved morphophysiological attributes and yield in summer tomato (*Solanum lycopersicum*). *Pakistan Journal of Agricultural Sciences*, 53(2), 339-344.



- [39] Moran, R. & Porath, D. 1980. Chlorophyll determination in intact tissues using N, N-dimethylformamide. *Plant physiology*, 65(3), 478-479.
- [40] Nahar, K. and Ullah, S. M. (2018). Drought stress effects on plant water relations, growth, fruit quality and osmotic adjustment of tomato (*Solanum lycopersicum*) under subtropical condition. *Asian Journal of Agricultural and Horticultural Research*, 1(2), 1-14.
- [41] Paneque, M., José, M., Franco-Navarro, J. D., Colmenero-Flores, J. M. and Knicker, H. 2016. Effect of biochar amendment on morphology, productivity and water relations of sunflower plants under non-irrigation conditions. *Catena*, 147, 280-287.
- [42] Pongprayoon, W., Roytrakul, S., Pichayangkura, R. and Chadchawan, S. 2013. The role of hydrogen peroxide in chitosan-induced resistance to osmotic stress in rice (*Oryza sativa* L.). *Plant growth regulation*, 70, 159-173.
- [43] Ren, T., Wang, H., Yuan, Y., Feng, H., Wang, B., Kuang, G., Wei, Y., Gao, W., Shi, H. and Liu, G., 2021. Biochar increases tobacco yield by promoting root growth based on a three-year field application. *Scientific reports*, 11(1), p.21991.
- [44] Reyes-Pérez, J.J., Enríquez-Acosta, E.A., Ramírez-Arrebató, M.Á., Zúñiga Valenzuela, E., Lara-Capistrán, L. and Hernández-Montiel, L.G. 2020. Effect of chitosan on variables of tomato growth, yield and nutritional content. *Revista mexicana de ciencias agrícolas*, 11(3), 457-465.
- [45] Sachdev, S., Ansari, S.A., Ansari, M.I., Fujita, M. and Hasanuzzaman, M. 2021. Abiotic stress and reactive oxygen species: Generation, signaling, and defense mechanisms. *Antioxidants*, 10(2), 277.
- [46] Sarong, M. and Orge, R.F. 2015. Effect of rice hull biochar on the fertility and nutrient holding capacity of sandy soils. *OIDA International Journal of Sustainable Development*, 8(12), 33-44.
- [47] Shafiq, S., Akram, N. A., Ashraf, M., García-Caparrós, P., Ali, O. M. and Latef, A. A. H. A. 2021. Influence of glycine betaine (natural and synthetic) on growth, metabolism and yield production of drought-stressed maize (*Zea mays* L.) plants. *Plants*, 10(11), 2540.
- [48] Sheikha, S. A. A., & AL-Malki, F. M. (2015). Chitosan influence on the amino acids and proline content in the plants under drought stress. *Journal of Plant Production*, 6(4), 447-455.
- [49] Sultana, N., Zakir, H. M., Parvin, M. A., Sharmin, S. and Seal, H. P. (2019). Effect of chitosan coating on physiological responses and nutritional qualities of tomato fruits during postharvest storage. *Asian Journal of Advances in Agricultural Research*, 10(2), 1-11.
- [50] Tee, E. S., Young, S. I., Ho, S. K. and Mizura, S. S. (1988). Determination of vitamin C in fresh fruits and vegetables using the dye-titration and microfluorometric methods. *Pertanika*, 11(1), 39-44.
- [51] Vassilev, N., Martos, E., Mendes, G., Martos, V. and Vassileva, M. (2013). Biochar of animal origin: a sustainable solution to the global problem of high-grade rock phosphate scarcity?. *Journal of the Science of Food and Agriculture*, 93(8), 1799-1804.
- [52] Wei, W., Yang, H., Fan, M., Chen, H., Guo, D., Cao, J. and Kuzyakov, Y. (2020). Biochar effects on crop yields and nitrogen loss depending on fertilization. *Science of the Total Environment*, 702, 134423.
- [53] Xu, G., Zhang, Y., Sun, J. and Shao, H. (2016). Negative interactive effects between biochar and phosphorus fertilization on phosphorus availability and plant yield in saline sodic soil. *Science of the Total Environment*, 568, 910-915.
- [54] Yan, K., Chen, P., Shao, H., Zhao, S., Zhang, L., Zhang, L., Xu, G. and Sun, J. 2012. Responses of photosynthesis and photosystem II to higher temperature and salt stress in Sorghum. *Journal of Agronomy and Crop Science*, 198(3), 218-225.
- [55] Zhang, W., Wei, J., Guo, L., Fang, H., Liu, X., Liang, K., Niu, W., Liu, F. and Siddique, K.H., 2023. Effects of two biochar types on mitigating drought and salt stress in tomato seedlings. *Agronomy*, 13(4), p.1039.
- [56] Zhou, R., Yu, X., Ottosen, C.O., Rosenqvist, E., Zhao, L., Wang, Y., Yu, W., Zhao, T. and Wu, Z. 2017. Drought stress had a predominant effect over heat stress on three tomato cultivars subjected to combined stress. *BMC Plant Biology*, 17, 1-13.