

## BIOCHAR FROM DIFFERENT FEEDSTOCKS AS A SUSTAINABLE APPROACH TO ALLEVIATE WATER DEFICIT EFFECTS ON ZUCCHINI

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### Abstract

Rising temperatures and evapotranspiration (ET<sub>c</sub>) are causing drought in dryland regions, exacerbating water scarcity. Biochar has shown potential for water conservation and crop resilience under water deficit conditions. A study on zucchini plants grown under varying water regimes (100, 80, and 60% ET<sub>c</sub>) showed that date palm leaf midrib (DLMB) and maize bare cob (MBCB) biochar-amended media improved morpho-physio-biochemical traits. Zucchini plants grown in MBCB-amended media exhibited 9%, 10%, 7%, and 16% increase in plant height, plant spread, fruit weight, and yield compared to those grown in DLMB media. However, these attributes declined 39%, 28%, 35%, and 52% in sandy loam (SL) control media compared to MBCB media. Comparing water deficit conditions (80% and 60%), plants irrigated at 100% ET<sub>c</sub> showed significantly positive effects. The combined effect of both factors indicated that plants are grown at 100% ET<sub>c</sub> in MBCB- and DLMB-amended media surpass all other treatment combinations. However, impressively, zucchini plants grown in MBCB media at 80% and 60% ET<sub>c</sub>, and in DLMB media at 80% ET<sub>c</sub>, displayed growth, yield, and quality metrics comparable to those of plants grown in fully watered (100% ET<sub>c</sub>) SL soil. Water scarcity significantly suppressed plant growth, physiology, and fruit quality in SL media at 80% and 60% ET<sub>c</sub>. This concludes that incorporating MBCB and DLMB biochar can conserve 20–40% of irrigation water while maintaining growth and production of zucchini crop under greenhouse conditions. The findings of this study can provide practical guidance for applying biochar derived from empty maize cobs and date palm leaf midribs to conserve water resources particularly in the water scarce regions. Further research is needed to comprehend the long-term impacts of biochar on crop production, soil fertility, water quality, soil biology, nutrient cycling, and greenhouse gas emissions across various soil types.

**Key words:** Date palm, Maize, Drought, Zucchini, Soil amendments, Morphology, Physiology.

### Introduction

The increasing global population has led to a significant amount of agro-cellulosic biomass, which is inefficiently disposed of and managed. The worldwide production of biomass waste is projected to reach around 140 Gt annually, causing significant environmental pollution. Most biomass waste is either left to decompose in the fields or burned openly, leading to significant pollution (Tripathi *et al.*, 2019; Dessie *et al.*, 2020). Date palm biomass comprises dry leaves, seeds, stems, fruit bunches, and leaf sheaths, with Saudi Arabia producing over 200,000 tons of date palm biomass annually. Some of this biomass is used as fodder, compost, and raw material for the chipboard and paper industries (Mansur, 2010; Hussain *et al.*, 2014). Maize, the most widely grown crop worldwide, produces 5.87 tons ha<sup>-1</sup> of dry biomass used as fodder, biofuels, and digestate soil conditioner. However, bare maize cobs have no substantial use except for domestic fuel burning, discarded, or used as substrates for industrial enzymes (Patel & Kumar, 2010; Akinyemi *et al.*, 2016; Dziedzic *et al.*, 2021). The biomass of date palm and maize cobs contains low moisture content and high volatile solids, making them ideal for thermo-chemical conversion processes to recycle waste into useful products. Pyrolysis is one of the thermal processes commercially used to recycle biomass waste to produce biochar (Vaish *et al.*, 2019; Chong *et al.*, 2021; Adekanye *et al.*, 2022).

Biochar, a high-carbon material, is a long-term carbon sequester in soil, used in agriculture for water conservation

and improving soil quality, water holding capacity, organic carbon mineralization, and nutrient availability (Ghassemi-Golezani & Farhangi-Abriz, 2022). Studies showed that rice straw biochar significantly improves soil carbon balance, soil health, and plant growth (Farid *et al.*, 2022). Maize stalk biochar improves soil fertility by elevating organic matter, nitrogen use efficiency, and applied nitrogen agronomic efficiency (Amin & Eissa, 2017). Date palm biochar improves cucumber growth and yield (Munir *et al.*, 2020). Chitosan-modified biochar increases arsenic immobilization efficacy in contaminated soil (Mehmood *et al.*, 2021). Biochar also positively influences soil physical attributes, such as water content, porosity, bulk density, hydraulic conductivity, and soil water availability for plants (Toková *et al.*, 2020). Rice husk biochar increases potassium content, pH, organic carbon content, and electric conductivity, promoting a favorable environment for plant growth (Ghorbani & Amirahmadi, 2018). Biochar-amended soil boosts water usage efficiency and enhances specific surface area, nutrients, and water retention qualities, ultimately enhancing crop growth (Lima *et al.*, 2018).

Water stress or drought is a persistent problem in farming due to its unpredictable timing, intensity, and duration. Population growth, global warming, and climate change compound water constraints (Cisneros *et al.*, 2014; Seleiman *et al.*, 2021). These risks increase the demand for irrigation water, intensifying competition between agriculture and other sectors. Water stress affects leaf water potential, stomatal activity, transpiration, CO<sub>2</sub> absorption, and photosynthesis. Guard cells control turgor

pressure and stomatal closure, while ion and water transport systems regulate these fluctuations. Plants have evolved physiological, biochemical, and molecular pathways to regulate growth and development in response to water scarcity (Abdalla *et al.*, 2022; Mehtab *et al.*, 2022).

Zucchini squash (*Cucurbita pepo* L.), a popular crop worldwide, is grown in various environments and requires warm weather, sunshine, and moist soil. Harvested when unripe, it is intensively controlled with fertilizer and irrigation water (Ng'etich *et al.*, 2013). Zucchini squash is traditionally prepared as a savory entrée or side dish, with seeds roasted for snacking. It offers numerous nutritional and health benefits, including cholesterol-lowering benefits, cancer prevention, rheumatoid arthritis relief, vision improvement, heart disease risk reduction, and prevention neural tube defects during pregnancy. It is also a cholesterol-lowering diet alternative (Badiru & Moronkunbi, 2017). The rationale behind conducting the current research is based on the understanding that water availability and accessibility pose substantial risks to agricultural productivity, particularly in regions characterized by water scarcity. Addressing this issue is crucial for the long-term sustainability of farming practices in the agriculture. Moreover, different feedstocks can yield biochar with different properties, which can influence its effectiveness in alleviating water deficit effects. A limited literature is available to produce biochar using different crop feedstocks, however further research is needed to identify the most suitable ones for specific plant species grown in dryland regions. The hypothesis of current study is that the utilization of biochar derived from different feedstocks can serve as a sustainable approach to mitigate the negative effects of water deficit on zucchini plants. Hence, the primary objective of the current study was to explore the potential of biochar produced from maize and date palm wasted feedstocks and their impact on the growth, yield, and quality of zucchini crops under water deficit conditions. This study will improve our understanding to provide potential solutions to enhance agricultural productivity and resilience in water-scarce areas.

## Material and Methods

**Experiment site and biochar preparation:** A greenhouse experiment was conducted during 2021 and 2022 at the Research and Training Station, King Faisal University, Al-Ahsa, Kingdom of Saudi Arabia (latitude 25° 16' 24.4437" N and longitude 49° 42' 28.5934" E at 153 m altitude). Two types of crop species, specifically date palm (leaf midrib) and maize (bare cobs), were used as feedstocks for biochar production. The pyrolysis technique produced the biochar using waste materials from maize and date palms. To produce biochar from date palm, the wasted leaf midrib residue was dried in the sun for 24 hours and then cut into 10 cm pieces using a compact electric wood chipper. The chopped material was further dried for 24 hours in the oven at 60°C before pyrolysis. Subsequently, the dried material was placed in the muffle furnace at 350°C for three and a half hours. The black carbonized pieces were collected from the muffle furnace and left to cool at ambient temperature for 24 hours. The biochar was then crushed to a 2 mm particle size using a stainless steel handheld sieve and stored in plastic bags until use. Similarly, maize bare cobs were cut into 5 cm pieces, and biochar was produced

using the muffle furnace method, with the feedstock being pyrolyzed for 30 minutes. The biochar was derived from date palm leaf midrib and maize bare cobs labeled as DLMB and MBCB, respectively.

**Physicochemical properties of biochar and sand:** The DLMB contained 76.73% organic matter, 4.18 dS.m<sup>-1</sup> EC, 8.10 pH, 0.85% total nitrogen, 1.02% total phosphorus, 2.10% total potassium, 38.63 cmol kg<sup>-1</sup> CEC, 43.71% carbon, 0.89% calcium, 3.91% moisture content, 0.50 g cm<sup>-3</sup> bulk density, 0.81% porosity, and 39.71% water holding capacity. Similarly, MBCB contained 78.98% organic matter, 3.44 dS.m<sup>-1</sup> EC, 7.73 pH, 1.53% total nitrogen, 1.32% total phosphorus, 2.42% total potassium, 41.41 cmol kg<sup>-1</sup> CEC, 48.31% carbon, 1.33% calcium, 5.54% moisture content, 0.67 g cm<sup>-3</sup> bulk density, 0.72% porosity, and 42.69% water holding capacity. The sandy loam (SL) soil contained 80.91% sand, 13.21% clay, 5.88% silt, 3.27% moisture content, 0.31% organic matter, 1.43 g cm<sup>-3</sup> bulk density, 3.15 dS m<sup>-1</sup> EC, 7.41 pH, 5.18 cmol kg<sup>-1</sup> CEC, 0.21% total nitrogen, 188 ppm potassium, 1.57 ppm phosphorus, 2.15 mEq.L<sup>-1</sup> calcium, and 15.54% water holding capacity (Anon., 2005).

**Crop husbandry practices:** Certified summer squash zucchini cultivar Lorea seeds were obtained from S.E. Marshall and Co. Ltd., UK. The seeds were sterilized with 2% sodium hypochlorite disinfectant prior to sowing. They were sown into modular seed germination trays containing peat compost for seed germination. The seed trays were placed in an automated climate control chamber (Snijders Scientific B.V.). Tilburg, Holland) at 25 ± 2°C temperature, 16 h d<sup>-1</sup> photoperiod, and 60 ± 5% relative humidity. After two fully developed leaves had appeared, the seedlings were relocated into 30 L plastic pots and transferred to the greenhouse at a temperature of 25 ± 2 °C and relative humidity of 65 ± 5%. Standard SL soil media was used in all pots, alone or in combination with DLMB and MBCB (5%). Three seedlings were planted in each pot, with five replicated pots for each treatment. After one week, healthy seedlings were left to grow, while others were thinned out. The recommended doses of sulfate of potash (250 kg K<sub>2</sub>O ha<sup>-1</sup>) and single superphosphate (175 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) were applied during potting. However, urea was applied in two separate applications. The first dose (100 kg ha<sup>-1</sup>) was applied at the time of transplantation, while the second (75 kg ha<sup>-1</sup>) was applied after one month of transplantation. Other cultural practices were followed accordingly.

**Water regimes and experimental design:** The water deficit levels were estimated using the crop evapotranspiration (ET<sub>c</sub>) based Penman-Monteith model at 100, 80, and 60% ET<sub>c</sub>. The reference evapotranspiration was calculated using a CropWat 8.0 computer program following the FAO Penman-Monteith model, considering multiple meteorological factors such as air temperature, relative humidity, wind speed, and solar radiation. The microclimate data obtained from on-farm weather station were used in the Penman-Monteith model (Allen *et al.*, 1998; Mohammed *et al.*, 2021; Alnaim *et al.*, 2022). The experiment was laid out in a two-factorial, completely randomized design with five replications in each treatment.

The first factor was two types of biochar (DLMB and MBCB) and a sandy loam control (SL), whereas the second factor was three water deficit levels (100, 80, and 60% ETC). A total of forty-five pots were included in this study.

#### **Growth, yield, and physicochemical characteristics of fruits:**

The zucchini crop was harvested 70 days after sowing (DAS). Plant height, stem diameter, and number of leaves per plant were measured after the 50<sup>th</sup>, 60<sup>th</sup>, and 70<sup>th</sup> DAS. CI-202 leaf area meter was used to estimate leaf area, whereas the leaf area per unit of ground area recorded leaf area index (LAI). Specific leaf area (SLA) was calculated by the ratio of leaf area to leaf dry mass. Furthermore, the number of female and male flowers per plant was visually counted, and the female-to-male sex ratio was calculated. Fruit numbers per plant were counted manually. A Sartorius electronic balance was used to determine fruit weight. Measurements of fruit length and diameter were obtained using a digital Vernier caliper. The volume of zucchini fruits was calculated using the xylometric method, which involved immersing the fruits in a graduated container filled with water and measuring the displaced water. The yield per plant in grams was calculated, and the resulting measurements were converted to tons per hectare. Similarly, stem, leaf, and root dry weight were estimated by placing the respective biomass in an oven at 105 °C for 72 h until a constant weight. The root and shoot dry weight ratio was also calculated.

The chlorophyll content (*Chl*) was assessed a day before harvest using the SPAD-502 handheld chlorophyll meter (Konica Minolta Sensing Inc., Japan). The measurements of net photosynthesis (*Pn*), transpiration rate (*E*), stomatal conductance (*gs*), and intercellular CO<sub>2</sub> concentration (*Ci*) were conducted using a portable Li-6400XT, photosynthesis system. The water use efficiency (WUE) was calculated as the ratio of *Pn* to *E*, as described by Iqbal *et al.* (2022). The fruit color parameters were measured using a Hunter lab Color Quest -45/0 LAV color meter as described by Ahmed Mohammed *et al.*, (2020). Zucchini Fruit surface area (FSA) was calculated using the “ $2\pi(r+h)$ ” formula, where “ $\pi$ ” is equal to 3.14, “*r*” is the radius of the fruit, and “*h*” is the height of the fruit. Fruit firmness (FF) was determined using a K95590 Koehler penetrometer. Fruit water content (FWC) was determined by placing three fruits in an oven at 105°C for 72 hours. Fruit pH was recorded by a portable digital Hanna HI99121 pH meter, and a digital Hanna HI96801 refractometer was used to measure total soluble solids (TSS). To estimate titratable acidity (TA), the procedure described by Chang *et al.*, (1995) was used. The ratio between TSS and TA (TSSTAR) was also calculated. Similarly, the total sugar (TS) was determined as described by Pomares-Viciano *et al.*, (2018), Bates *et al.*, (1973) method was followed for leaf proline content (PC). The relative water content (RWC) was estimated as described by Khakwani *et al.*, (2012).

**Statistical analysis:** The data was analyzed using the two-way analysis of variance (ANOVA) method of GenStat, version 18 (VSN International Ltd., UK). The separation

of treatment means was determined using the Least Significant Difference (LSD) test at a 5% probability level ( $p < 0.05$ ). The correlation analysis was performed using R programming, version R 4.3.2. (R Core Development Team, The R Foundation, based in Vienna, Austria).

## **Results**

#### **Plant growth, dry matter partitioning, and yield parameters:**

The application of biochar amendments and water deficit levels significantly ( $p \leq 0.05$ ) influenced plant height, stem diameter, and leaf number per plant (Fig. 1). MBCB-amended media showed higher values for these characteristics compared to DLMB and SL media under all water deficit levels. MBCB-amended media showed a 4% increase in plant height and stem diameter and a 13% increase in leaf number per plant compared to DLMB-amended media under 100% ETC conditions. The increase was significantly higher when compared to SL media at 100% ETC, with a 19% increase in height, a 12% increase in stem diameter, and a 25% increase in leaf number per plant. SL media under 80% ETC had a 36% decrease in plant height, a 30% decrease in stem diameter, and a 32% decrease in leaf number per plant. MBCB- and DLMB-amended media performed better than SL media at all ETC levels, highlighting their potential for water conservation.

Leaf area (Fig. 2A), leaf area index (Fig. 2B), and specific leaf area (Fig. 2C) were improved in MBCB- and DLMB-amended media at 100% ETC, and both were statistically at par. However, the number of females (Fig. 2D) and male flowers (Fig. 2E) were highest in MBCB-amended media at 100% ETC, followed by DLMB-amended media. Except for the female-to-male sex ratio (Fig. 2F), all other parameters were significantly reduced in plants grown in SL media at 100% ETC. Plants grown in MBCB- and DLMB-amended media at 80% ETC showed promising results for leaf area, leaf area index, and specific leaf area, reducing them to 14%, 15%, and 11% compared to 100% ETC. These parameters were significantly better than SL media at 100% ETC. Similarly, 53%, 54%, 31%, 29%, and 56% reductions were observed in leaf area, leaf area index, specific leaf area, and the number of female and male flowers grown in SL media at 60% ETC.

The study found a significant ( $p \leq 0.05$ ) interaction between biochar feedstocks and water deficit levels in terms of plant spread (Fig. 3A), fruit number (Fig. 3B), weight (Fig. 3C), length (Fig. 3D), diameter (Fig. 3E), volume (Fig. 3F), yield per plant (Fig. 3G), and yield per hectare (Fig. 3H). Plants grown in media amended with MBCB and DLMB at 100% ETC had the maximum number of fruits per plant, higher fruit weight, larger fruit diameter, and increased fruit volume. However, plants grown in SL media at 100% ETC showed a 14%, 19%, 9%, and 23% reduction in these parameters. MBCB-amended media at 100% ETC had higher plant spread, fruit length, yield per plant, and yield per hectare. Plants grown in MBCB-amended media at 80% and 60% ETC and DLMB-amended media at 80% ETC performed best compared to plants in SL media at 80% and 60% ETC.

Plants subjected to water stress resulted in significant ( $p \leq 0.05$ ) changes in all dry matter partitioning parameters, causing reductions in most values (Fig. 4). Higher stem dry weight (Fig. 4A), leaf dry weight (Fig. 4B), and total dry weight (Fig. 4D) were recorded in plants grown in MBCB- and DLMB-amended media at 100% and 80% ET<sub>c</sub> and were statistically at par. However, root dry weight (Fig. 4C) was increased in plants grown in MBCB- and DLMB-amended media at 100% ET<sub>c</sub>. Similarly, the root shoot dry weight ratio (Fig. 4E) was highest in MBCB-amended media at 100% ET<sub>c</sub>. Plants grown in SL media at 80% and 60% ET<sub>c</sub> showed the lowest values of stem dry weight (40–52% reduction), leaf dry weight (21–32% reduction), root dry weight (40–50% reduction), total dry weight (26–37% reduction), and root shoot dry weight ratio (19–22% reduction). The dry matter partitioning characteristics were significantly higher when plants were grown in MBCB- and DLMB-amended media at 80% ET<sub>c</sub> compared to SL media at 100% ET<sub>c</sub>. It shows that these biochar-amended media have the potential to promote robust plant dry biomass even when water is scarce.

**Chlorophyll content and gas exchange parameters:** The imposition of water stress led to significant ( $p \leq 0.05$ ) changes in all physiological variables, resulting in most values being reduced (Fig. 5). Plants grown in MBCB- and DLMB-amended media and irrigated at 100% and 80% ET<sub>c</sub> had higher chlorophyll content (Fig. 5A), net photosynthesis (Fig. 5B), stomatal conductance (Fig. 5C), and transpiration rate (Fig. 5D). These treatment combinations were statistically at par. These parameters were significantly reduced by 20–26% (chlorophyll content), 41–58% (net photosynthesis), 27–39% (stomatal conductance), and 27–44% (transpiration rate) in plants grown in SL media at 80% and 60% ET<sub>c</sub>, respectively. However, maximum intercellular CO<sub>2</sub> concentration (Fig. 5E) and minimum WUE (Fig. 5F) were estimated in plants grown in SL media and received water at 80% and 60% ET<sub>c</sub>. The WUE was significantly higher in MBCB- and DLMB-amended media at 100%, 80%, and 60% ET<sub>c</sub> and SL media at 100% ET<sub>c</sub>. Statistically, these treatment combinations were at par.

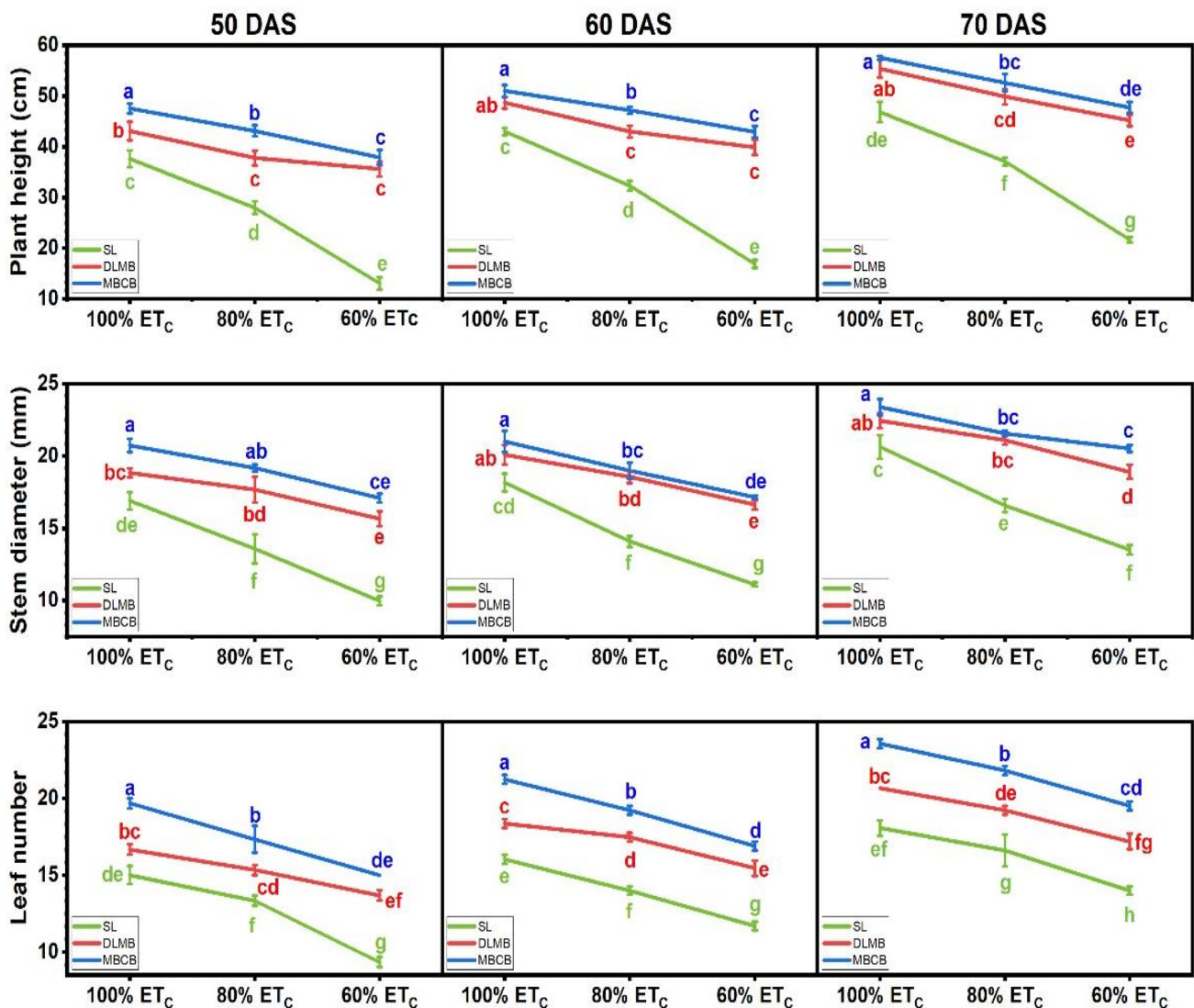


Fig. 1. Plant height, stem diameter, and leaf number response of zucchini grown in biochar-amended media viz. sandy loam control (SL), date palm leaf midrib biochar (DLMB), and maize bare cob biochar (MBCB) subjected to different water stress levels (100%, 80%, and 60% ET<sub>c</sub>). Y-bars represent the standard deviation ( $\pm$ ) among the treatment means, whereas different letters indicate significant variations among means at 5% significant level ( $p \leq 0.05$ ).

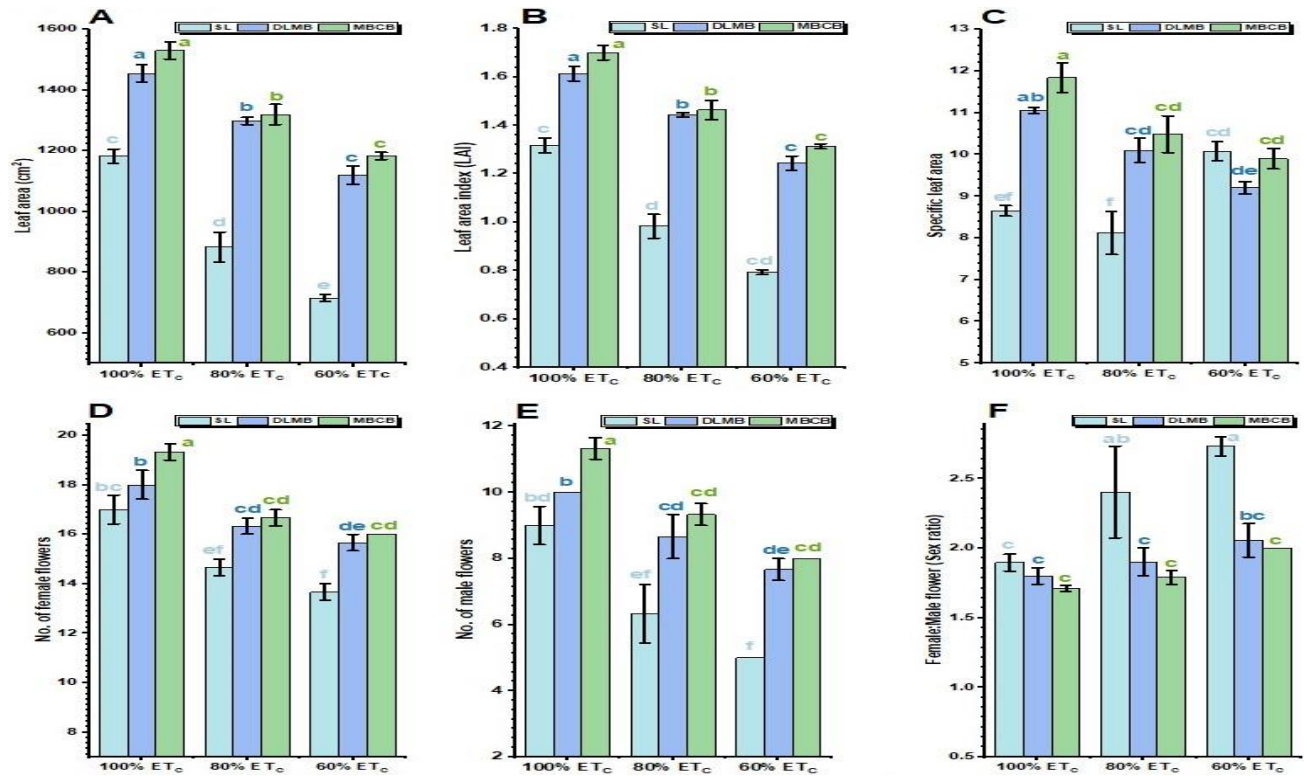


Fig. 2. (A) Leaf area, (B) leaf area index, (C) specific leaf area, (D) number of female flowers, (E) number of male flowers, and (F) female male flowers sex ratio response of zucchini grown in biochar-amended media viz. sandy loam control (SL), date palm leaf midrib biochar (DLMB), and maize bare cob biochar (MBCB) subjected to different water stress levels (100%, 80%, and 60% ET<sub>c</sub>). Y-bars represent the standard deviation (±) among the treatment means, whereas different letters indicate significant variations among means at 5% significant level ( $p \leq 0.05$ ).

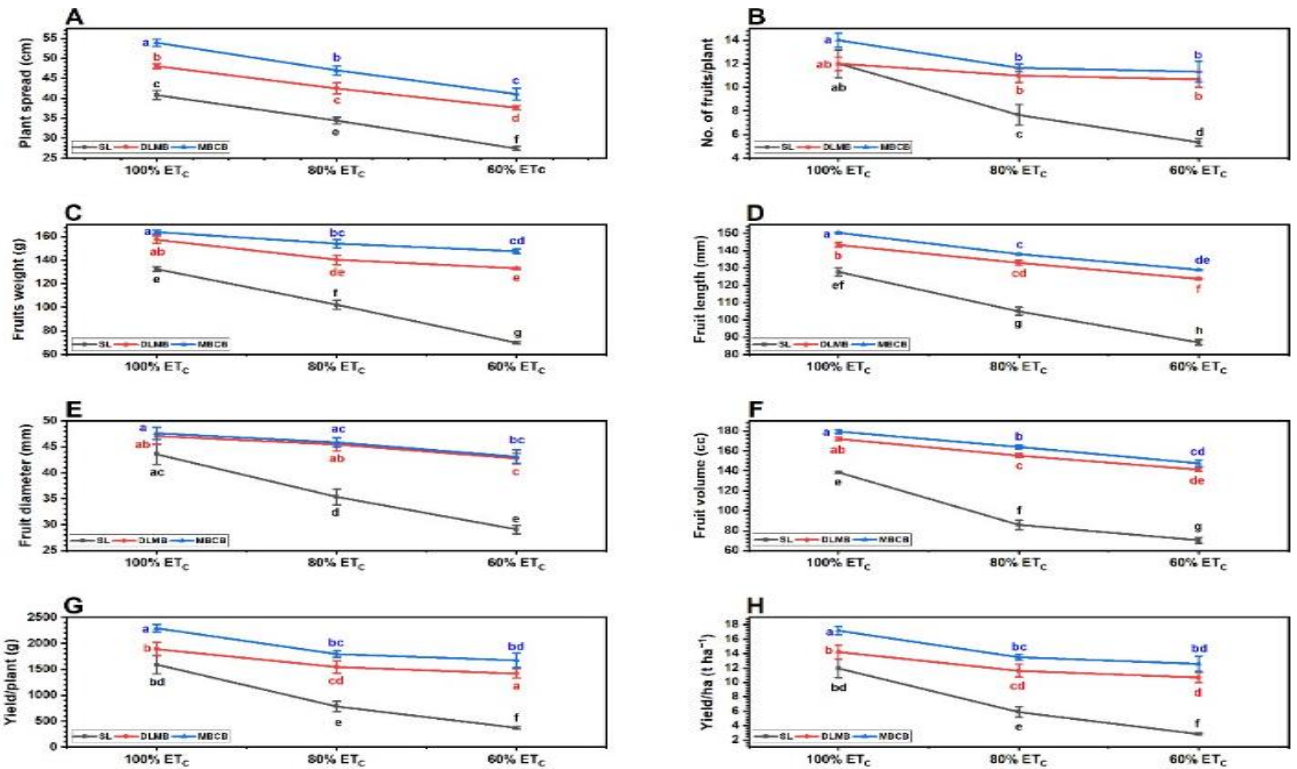


Fig. 3. (A) Plant spread, (B) number of fruits per plant, (C) fruit weight, (D) fruit length, (E) fruit diameter, (F) fruit volume, (G) yield per plant, and (H) yield per hectare response of zucchini grown in biochar-amended media viz. sandy loam control (SL), date palm leaf midrib biochar (DLMB), and maize bare cob biochar (MBCB) subjected to different water stress levels (100%, 80%, and 60% ET<sub>c</sub>). Y-bars represent the standard deviation (±) among the treatment means, whereas different letters indicate significant variations among means at 5% significant level ( $p \leq 0.05$ ).

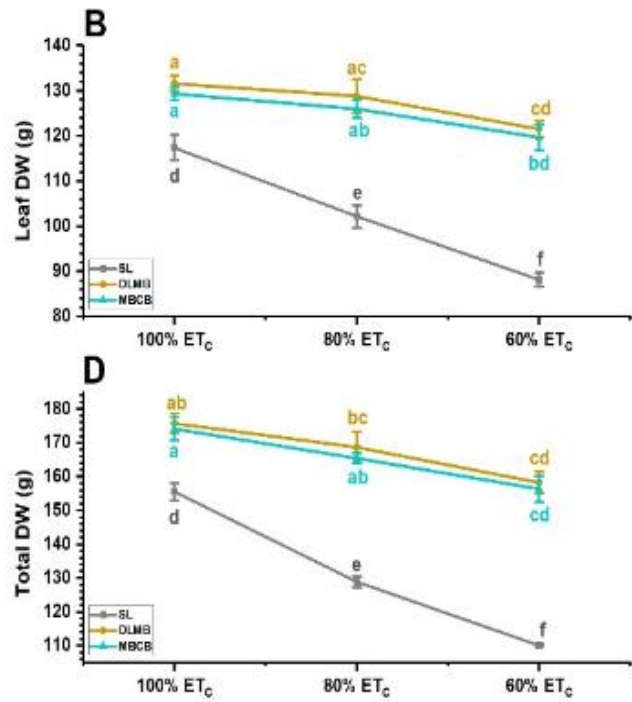
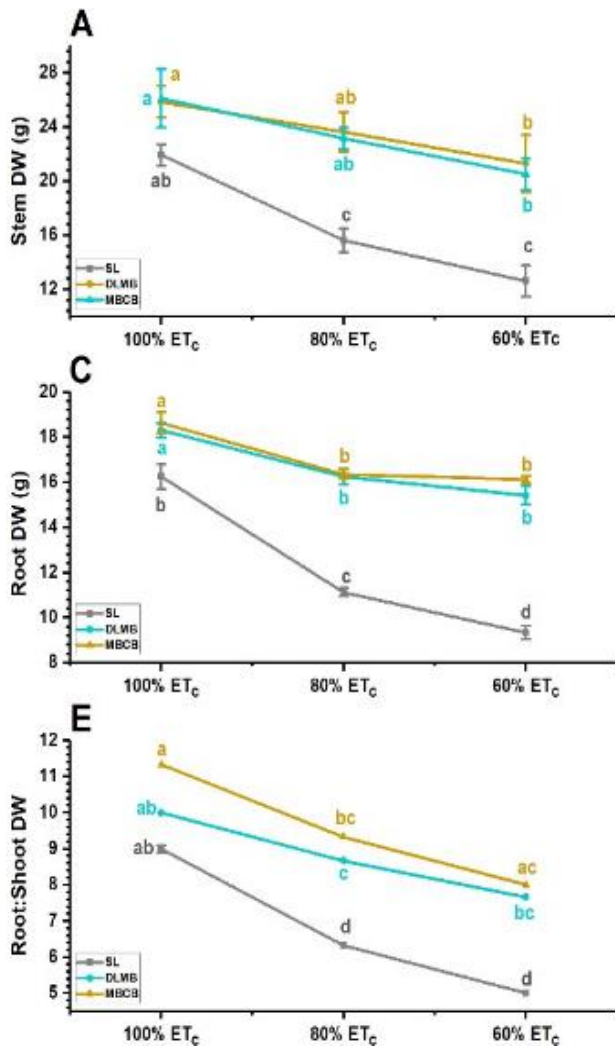


Fig. 4. (A) Stem dry weight, (B) leaf dry weight, (C) root dry weight, (D) total dry weight, and (E) root shoot dry weight ratio response of zucchini grown in biochar-amended media viz. sandy loam control (SL), date palm leaf midrib biochar (DLMB), and maize bare cob biochar (MBCB) subjected to different water stress levels (100%, 80%, and 60% ET<sub>c</sub>). Y-bars represent the standard deviation ( $\pm$ ) among the treatment means, whereas different letters indicate significant variations among means at 5% significant level ( $p \leq 0.05$ ).

**Physicochemical parameters of zucchini fruit:** The surface area of Zucchini fruit was significantly ( $p \leq 0.05$ ) increased in plants grown in MBCB- and DLMB-amended media that received a higher amount of water (100% ET<sub>c</sub>), which was 22% decreased in SL media growing plants irrigated at the same ET<sub>c</sub> (Fig. 7A). This reduction was 11–15% when plants were irrigated at 80% ET<sub>c</sub> level in MBCB- and DLMB-amended media, respectively. Fruits grown in SL media at 60% and 80% ET<sub>c</sub> had the highest fruit firmness compared to plants grown in MBCB- and DLMB-amended media at similar water deficit treatments (Fig. 7B). The fruit moisture content was higher in MBCB (100%, 80%, and 60% ET<sub>c</sub>), DLMB (100% and 80% ET<sub>c</sub>), and SL (100% ET<sub>c</sub>), indicating no significant differences between these treatments (Fig. 7C). Minimum TSS was determined in SL media at 60% and 80% ET<sub>c</sub>, whereas it was maximum in MBCB and DLMB media irrigated at 100%, 80%, and 60% ET<sub>c</sub> (Fig. 8A). Conversely, the highest titratable acidity was recorded in fruits from plants grown at water stressed levels, i.e., at 60% and 80% ET<sub>c</sub>, while it was lowest in fruits harvested from MBCB media at 100% ET<sub>c</sub> (Fig. 8B). Similarly, the TSS and titratable acidity ratio was higher but remained statistically similar across all other treatment combinations, except in plants grown in SL media at 60% and 80% ET<sub>c</sub>, which had the lowest values (Fig. 8C). Likewise, the total sugar was higher but statistically at par in zucchini fruits from

MBCB- and DLMB-amended media irrigated at 100%, 80%, and 60% ET<sub>c</sub>, whereas it was 41–52% lower in SL media plants irrigated at 80% and 60% ET<sub>c</sub>, respectively (Fig. 8D). Proline content exhibited a divergent pattern; it was highest in the leaves of SL media at 60% ET<sub>c</sub>. A similar trend was observed in all other stressed treatment combinations. Interestingly, plants grown in MBCB, DLMB, and SL media irrigated at 100% ET<sub>c</sub> exhibited statistically similar and had a minimum proline content (Fig. 8E). Finally, fruit pH was higher. However, statistically at par with all other treatment combinations, except MBCB media at 60% ET<sub>c</sub> showing a lower pH value (Fig. 8F).

**Color parameters of zucchini fruit:** Based on the data illustrating the interaction between the biochar feedstocks and water stress levels, the fruits produced by the plants grown in SL media and irrigated at 60% ET<sub>c</sub> had the highest values of *L*, followed by 80% ET<sub>c</sub> (Fig. 6A). Similarly, the highest values of *a* were measured in fruits harvested from SL, DLMB, and MBCB media at 60% ET<sub>c</sub> and DLMB media at 80% ET<sub>c</sub>, which were statistically alike (Fig. 6B). Likewise, the values of *b* (Fig. 6C), *C* (Fig. 6D), and *h<sup>o</sup>* (Fig. 6E) were maximum in fruits produced by the plants grown in MBCB media and irrigated at 100% ET<sub>c</sub>. However, the color difference values ( $\Delta E$ ) were higher in fruits produced by the plants grown in SL media and irrigated at 60% and 80% ET<sub>c</sub> (Fig. 6F).

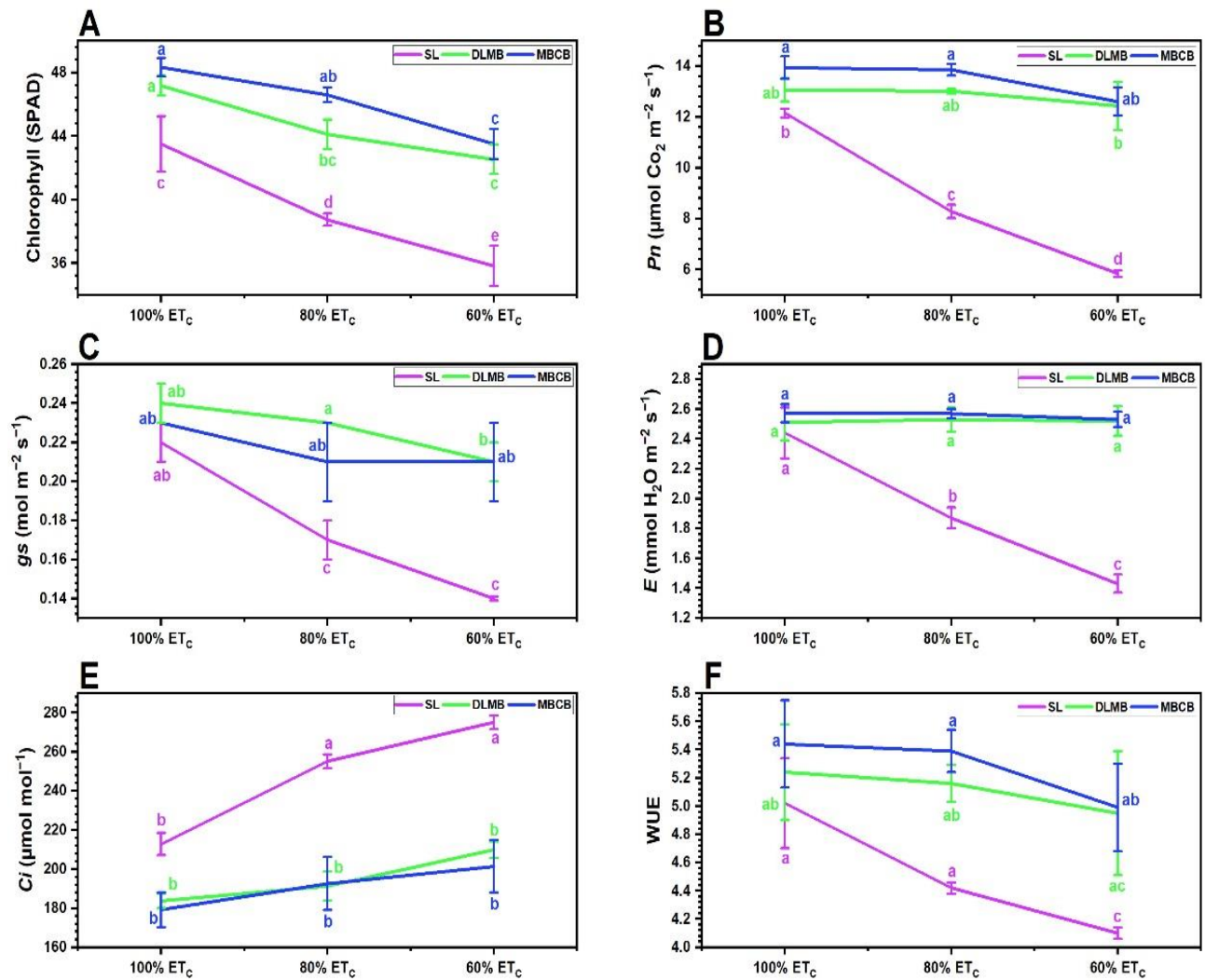


Fig. 5. (A) Chlorophyll, (B) net photosynthesis, (C) stomatal conductance, (D) transpiration, (E) intercellular CO<sub>2</sub> concentration, and (F) water use efficiency response of zucchini grown in biochar-amended media viz. sandy loam control (SL), date palm leaf midrib biochar (DLMB), and maize bare cob biochar (MBCB) subjected to different water stress levels (100%, 80%, and 60% ET<sub>c</sub>). Y-bars represent the standard deviation (±) among the treatment means, whereas different letters indicate significant variations among means at 5% significant level ( $p \leq 0.05$ ).

**Relative water content (RWC):** The RWC of the leaves in plants grown in biochar amended media increased significantly ( $p \leq 0.05$ ) in response to increasing ET<sub>c</sub> levels, as depicted in boxplot Fig. 7D. However, the average RWC showed a 36% and 30% decreasing trend in plants grown in SL media at 60% and 80% ET<sub>c</sub>, respectively. A significant increase in RWC was observed in plants grown in MBCB (100%, 80%, 60% ET<sub>c</sub>), DLMB (100%, 80% ET<sub>c</sub>), and SL (100% ET<sub>c</sub>) media. The response of these treatment combinations was statistically at par. A non-significant average RWC difference (4–6%) was recorded in plants subjected to water stress and grown in MBCB (80%, 60% ET<sub>c</sub>) and DLMB (80% ET<sub>c</sub>) media treatments, indicating plants resilience to water-limiting conditions.

**Correlation between the parameters:** The Pearson's correlation analysis for various attributes of zucchini were hierarchically clustered into two main groups (Fig. 9). The first cluster comprised LN, NFF, YPP, YPH, RSDWR, NMF, NFPP,  $C$ ,  $h^\circ$ ,  $b$ , PS, SLA, PH, FWC, FV, RWC,  $P_n$ ,

FSA, LA, LAI, FL,  $Chl$ , FW, SD, WUE, LDW,  $g_s$ , SDW, TDW, TSSTAR, RDW, TSS,  $E$ , FD, and TS variables, which were positively correlated to each other. On the other hand, the second cluster included  $a$ , pH, TA, FMSR,  $C_i$ , PC, FF,  $L$ , and  $\Delta E$  variables, and these were positively correlated to each other. However, a comparison between the two clusters revealed a negative correlation between the variables in the first and second clusters. The correlation coefficients among different variables revealed that the yield of zucchini (YPP, YPH) had a significant positive correlation with PS, SLA, NFF, NMF, NFPP, RSDWR,  $Chl$ , NFPP, and FW. The  $P_n$  had a significant positive correlation with  $g_s$ ,  $E$ , and WUE and a significant negative correlation with  $C_i$ . Color  $C$  had a significant positive association with  $b$  and  $h^\circ$  and a significant negative association with  $L$ ,  $a$ , and  $\Delta E$ . The RWC exhibited a significant positive correlation with PH, LA, LAI, RDW,  $P_n$ , FL, FD, FV, FSA, FWC, TS, and TSS. Among fruit quality parameters, the TS was significantly positive correlated with LA, LAI, RDW,  $E$ , FD, FV, and FSA.

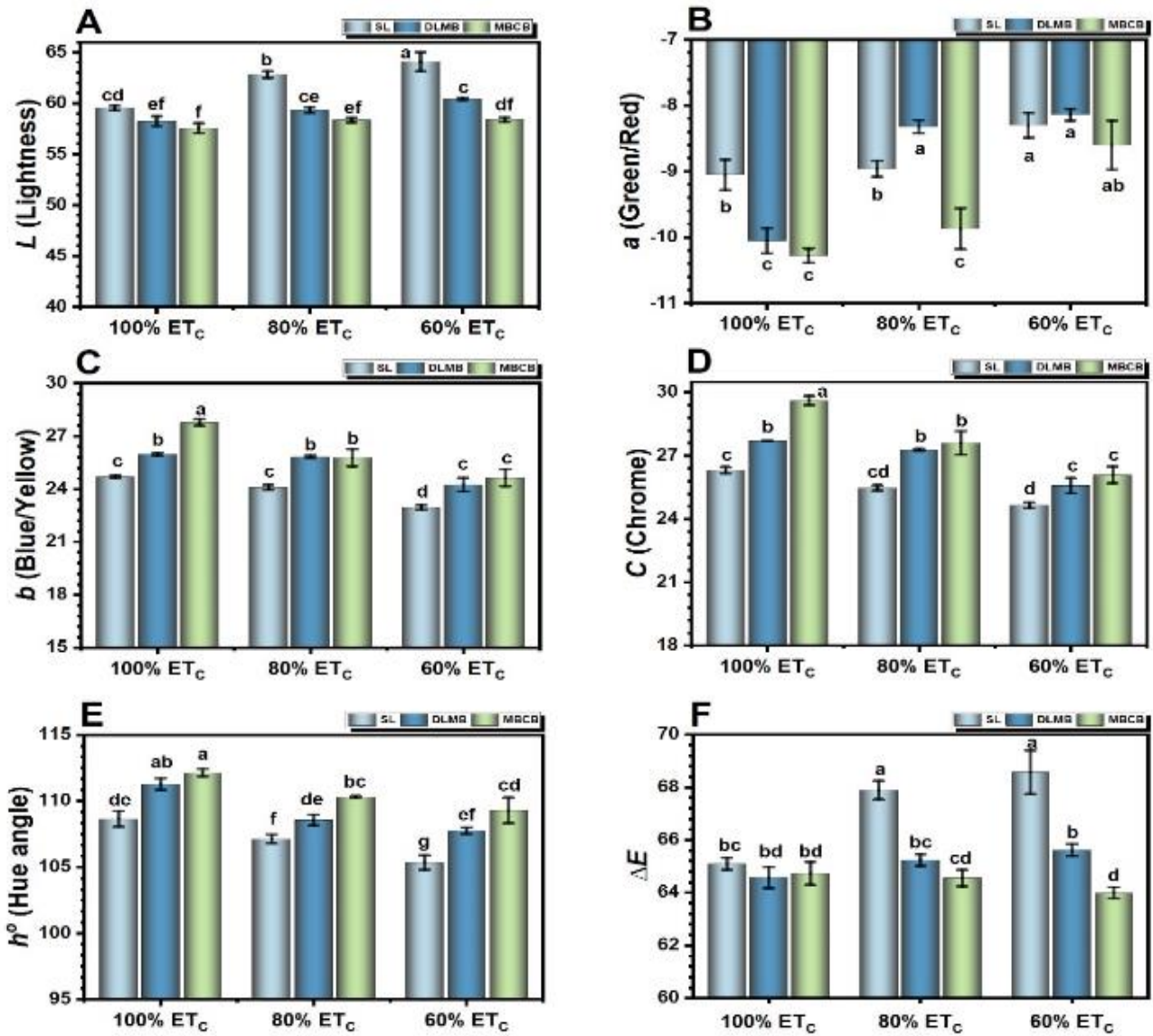


Fig. 6. (A) Lightness, (B) green/red color, (C) blue/yellow color, (D) chrome, (E) hue angle, and (F) color difference response of zucchini grown in biochar-amended media viz. sandy loam control (SL), date palm leaf midrib biochar (DLMB), and maize bare cob biochar (MBCB) subjected to different water stress levels (100%, 80%, and 60% ET<sub>c</sub>). Y-bars represent the standard deviation ( $\pm$ ) among the treatment means, whereas different letters indicate significant variations among means at 5% significant level ( $p \leq 0.05$ ).

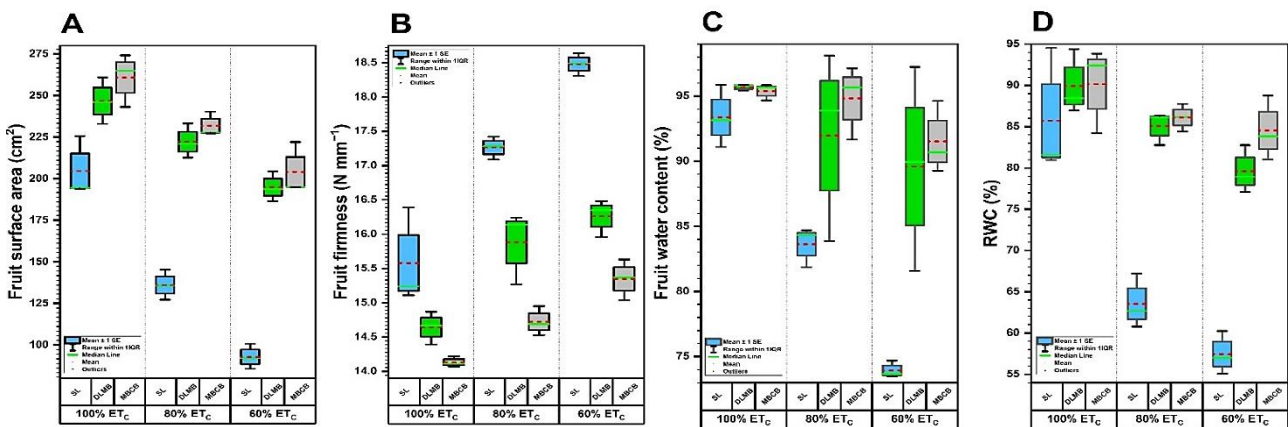


Fig. 7. (A) Fruit surface area, (B) fruit firmness, (C) fruit water content, and (D) relative water content response of zucchini grown in biochar-amended media viz. sandy loam control (SL), date palm leaf midrib biochar (DLMB), and maize bare cob biochar (MBCB) subjected to different water stress levels (100%, 80%, and 60% ET<sub>c</sub>). Y-bars represent the standard deviation ( $\pm$ ) among the treatment means, whereas different letters indicate significant variations among means at 5% significant level ( $p \leq 0.05$ ).



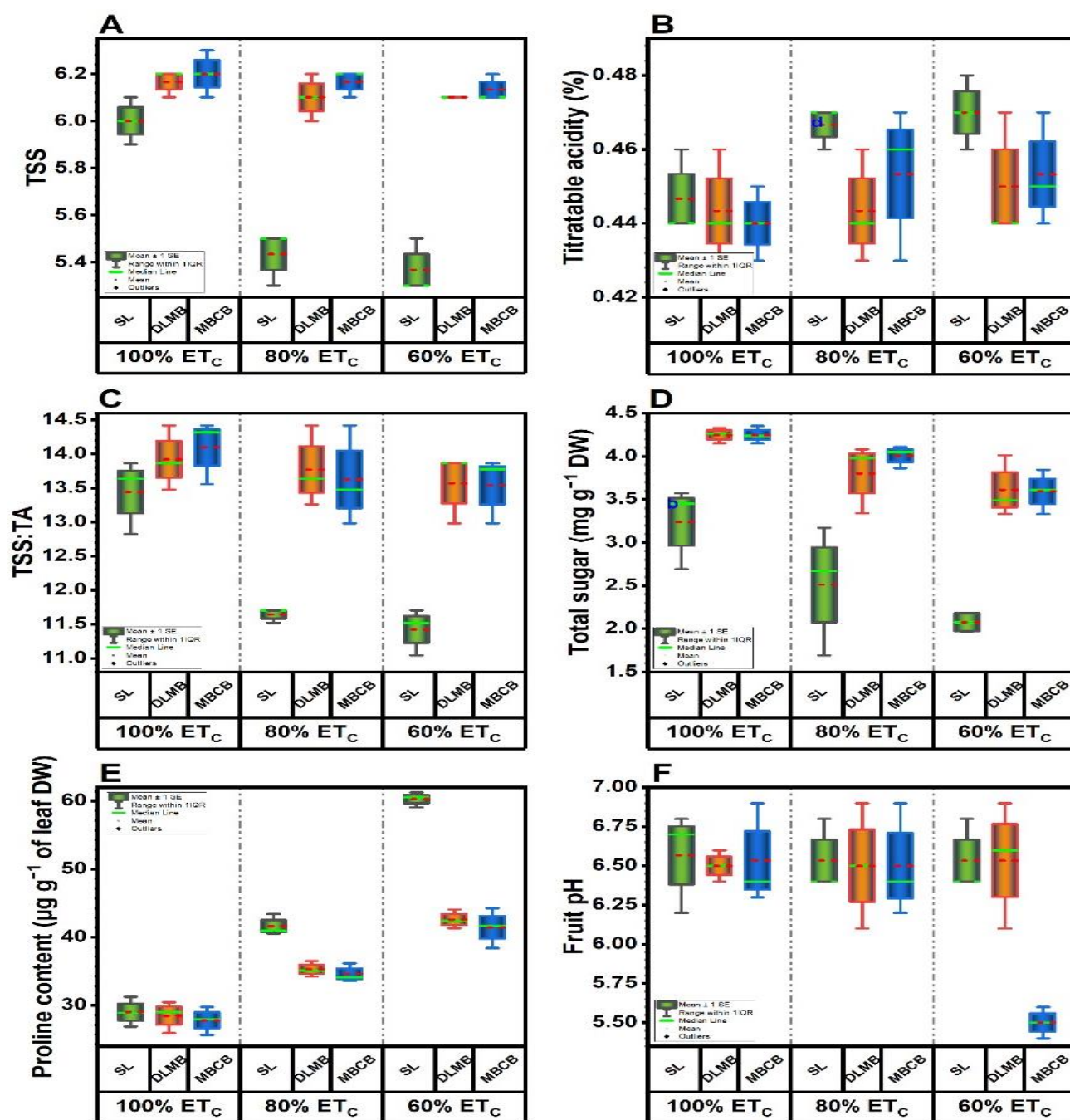


Fig. 8. (A) Total soluble solids, (B) titratable acidity, (C) total soluble solids and titratable acidity ratio, (D) total sugar, (E) proline content, and (F) fruit pH response of zucchinis grown in biochar-amended media viz. sandy loam control (SL), date palm leaf midrib biochar (DLMB), and maize bare cob biochar (MBCB) subjected to different water stress levels (100%, 80%, and 60% ET<sub>c</sub>). Y-bars represent the standard deviation ( $\pm$ ) among the treatment means, whereas different letters indicate significant variations among means at 5% significant level ( $p \leq 0.05$ ).

## Discussion

Water deficit stress is a significant challenge for plant growth and development in water-scarce regions. Optimal water content in the rhizosphere is crucial for better plant growth. Moisture-retaining products like biochar, hydrogel polymers, bentonite clay, vermiculite, perlite, peat moss, compost, and organic mulch are used to retain water in soil. The effectiveness of biochar depends on its chemical characteristics, which depend on the biomass feedstock and pyrolysis temperature. In the present study, using maize bare cobs

(MBCB) and date palm leaf midribs (DLMB) as feedstocks for biochar production found that all other growth, dry matter partitioning, and yield characteristics were significantly improved in MBCB-, followed by DLMB-amended media at 100% ET<sub>c</sub>. However, zucchinis plants grown in MBCB at 80% and 60% ET<sub>c</sub> and DLMB media at 80% ET<sub>c</sub> displayed growth, dry matter partitioning, and yield attributes similar to those in SL medium at 100% ET<sub>c</sub>. The study suggests that using MBCB and DLMB-amended biochar for zucchinis cultivation in water-scarce regions can save 20–40% of irrigation water.

Biochar application is more beneficial for degraded and sandy soils than for high-grade soils due to their low nutrient and water retention capacities. Mixing biochar and manure in zucchini cultivar, Izobilna improved crop growth and yield (Yordanova *et al.*, 2020). Adding compost, biochar, and composted biochar to sandy soil improved zucchini plant growth, dry weight, and soil characteristics (Farid *et al.*, 2022). Casuarina biochar helps immobilize heavy metals in soil, reduces uptake, and improves summer squash plant growth and dry weight (Ibrahim *et al.*, 2022). Wheat straw biochar improved tomato plant properties, and deficit irrigation and biochar enhanced tomato growth by improving water absorption, retention, soil porosity, and aeration (Agbna *et al.*, 2017). Deficit irrigation has been found to affect crop growth and productivity negatively. Studies show

that squash plants with 100% ETc and 85% ETc have the highest LAI, fruit weight, length, and yield (Abd El-Mageed & Semida, 2015). Water stress can decrease summer squash yield, while irrigation systems and growing seasons significantly affect fruit numbers, weight, and yield. Reduced irrigation water can also lead to declining summer squash yield (El-Dewiny, 2011). The 85% ETc irrigation strategy can save 15% of water in commercial squash production during the summer and fall seasons without affecting plant growth or yield (Abd El-Mageed *et al.*, 2016). Different irrigation strategies for zucchini cultivar Soraya have been found to increase yield (Salata & Stepaniuk, 2013), while cucumber cultivation in the fall season can save water through 80% and 60% ETc without affecting growth and yield (Abd El-Mageed *et al.*, 2018).

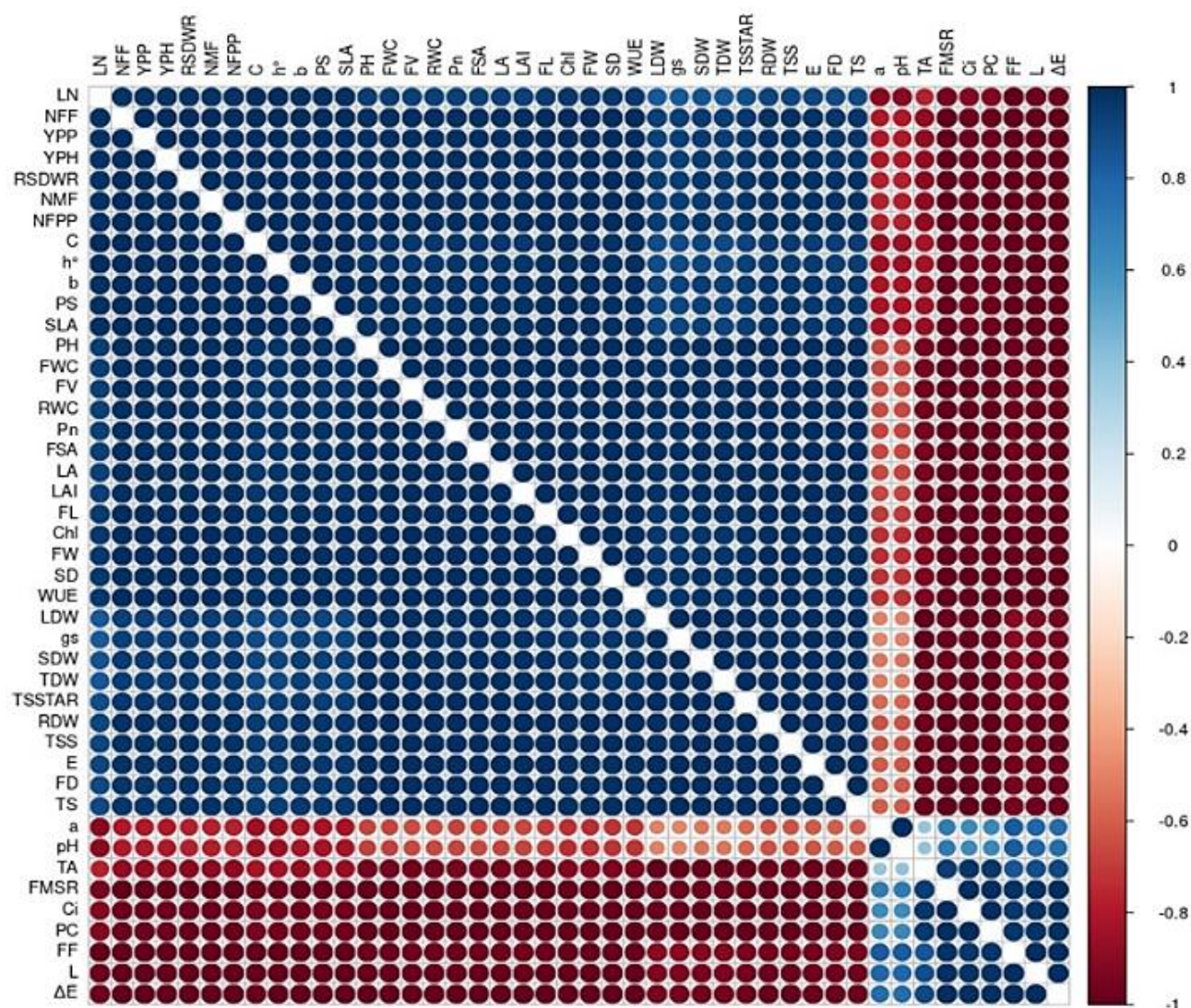


Fig. 9. Correlation analysis of morpho-physio-biochemical characteristics of zucchini plants grown in SL, MBCB- and DLMB-amended media and subjected to three water stress levels (100%, 80%, and 60% ETc). Abbreviations: LN: leaf number, NFF: number of female flowers, YPP: yield per plant, YPH: yield per hectare, RSDWR: root shoot dry weight ratio, NMF: number of male flowers, NFPP: number of fruits per plant, C: chrome color,  $h^\circ$ : hue angle,  $b$ : blue-yellow color, PS: plant spread, SLA: specific leaf area, PH: plant height, FWC: fruit water content, FV: fruit volume, RWC: relative water content,  $P_n$ : net photosynthesis, FSA: Fruit surface area, LA: leaf area, LAI: leaf area index, FL: fruit length,  $Chl$ : chlorophyll content, FW: fruit weight, SD: stem diameter, WUE: water use efficiency, LDW: leaf dry weight,  $g_s$ : stomatal conductance, SDW: stem dry weight, TDW: total dry weight, TSSTAR: total soluble solids and titratable acidity ratio, RDW: root dry weight, TSS: total soluble solids,  $E$ : transpiration rate, FD: fruit diameter, TS: total sugars,  $a$ : green-red color, pH: fruit pH, TA: titratable acidity, FMSR: female to male sex ratio response,  $C_i$ : intercellular  $CO_2$  concentration, PC: proline content, FF: fruit firmness,  $L$ : color lightness, and  $\Delta E$ : color difference.

Biochar application enhances plant chlorophyll content by improving soil fertility and nutrient availability, promoting chlorophyll synthesis, and producing higher chlorophyll content in plant tissues (Farid *et al.*, 2022). However, water stress reduces chlorophyll content, reducing its ability to absorb light energy and convert it into chemical energy (Queiroz *et al.*, 2017). This decline is due to reduced production or degradation of existing chlorophyll content, leading to photoinhibition and reactive oxygen species damage (Czarnocka *et al.*, 2018). The reduction in chlorophyll content is attributed to an increase in chlorophyllase enzyme, which degrades pigment molecules, reducing chlorophyll content biosynthesis and tissue differentiation in chloroplasts (Sousa *et al.*, 2021). Biochar enhances photosynthetic rate by improving nutrient availability, soil structure, and water-holding capacity (Tanure *et al.*, 2019). Water stress, however, usually decreases the photosynthetic rate due to reduced CO<sub>2</sub> uptake in plants' stomata, limiting the photosynthetic process (Siddique *et al.*, 2016). In this study, MBCB and DLMB amended media at 100% ETc and 80% ETc significantly improved *Pn*, *gs*, and *E*, which significantly declined in SL media at water stress levels. Biochar also enhances leaf photosynthetic and transpiration rates, enhancing plant water status and soil organic matter, thereby improving photosynthetic activity. This is reflected in the RWC, which indicates improved plant water status. An increase in transpiration rate elevates physiological metabolism, promoting the accumulation of photosynthetic products (Agbna *et al.*, 2017). In tomatoes, applying date fronds biochar significantly enhanced gas exchange parameters (Obadi *et al.*, 2023). Biochar also improved leaf gas exchange traits in tomatoes under drought and salinity stress conditions, retaining firm leaves under abiotic stresses (Zhang *et al.*, 2023). Zucchini under 100% ETc showed higher CO<sub>2</sub> assimilation rate, transpiration, and WUE, while 50% ETc increased internal carbon concentration despite increased salinity (Sousa *et al.*, 2022). These studies agree with the present study results as zucchini plants at 100% had maximum *Pn*, *gs*, *E*, and WUE, whereas plants subjected to water stress (80% and 60%) had maximum *Ci* concentration. The increase in *Ci* may be due to the stomatal closure during water stress restricting CO<sub>2</sub> diffusion into the leaf, but as photosynthesis continues, chloroplasts in mesophyll cells continue to consume CO<sub>2</sub>, leading to a buildup within the leaf and an increase in intercellular CO<sub>2</sub> concentration (Pirasteh-Anosheh *et al.*, 2016).

No research has been reported on the effects of biochar and water stress on the fruit color of the zucchini crop. However, in the current study, it was observed that zucchini plants grown in SL media at water stress conditions had higher fruit lightness, greenness, and color difference compared to fruit grown in MBCB and DLMB media at 100% ETc. This could be due to the limited irrigation, which decreased chlorophyll degradation during fruit ripening, resulting in higher green color retention. Therefore, insufficient irrigation might affect fruit pigment production during the ripening phase, causing fruit to

appear lighter and greener in color. Less green zucchini fruits are preferred by consumers, which is one of the fruit maturity indices. It is known that biochar enhances fruit color by improving soil fertility, nutrient availability, and plant health. It provides essential elements for pigment synthesis in fruits, leading to higher chrome values and more vibrant hues (Sharma *et al.*, 2022). Tomato fruit grown in biochar media has a darker red color with higher green-red values, linked to lycopene synthesis (Suthar *et al.*, 2018). Similarly, the fruits from tomato plants grown in water stress had higher values of green-red and chrome (Lipan *et al.*, 2021).

The economics of a vegetable enterprise heavily depend on the quality of fresh produce, particularly in the case of zucchini. It is revealed in the present study that the fruit quality attributes were improved when the plants were grown in MBCB- and DLMB-amended media and irrigated at 100% ETc. Zucchini fruits' strong nutrient absorption capacity also led to improved fruit quality parameters under moderate deficit irrigation (80% ETc). Biochar also enhances fruit quality by improving root development, retaining carbon and nitrogen, and promoting plant nutrient uptake (Agbna *et al.*, 2017; Sorrenti *et al.*, 2019). Similar results were reported in tomatoes, where biochar and compost application enhanced fruit quality traits (Hameeda *et al.*, 2019). Proline, an essential amino acid, is a key strategy for combating water stress in plants. In the current study, it was higher in the SL media at 80% and 60% ETc. The proline in plants grown in MBCB- and DLMB-amended media at 60% ETc was statistically equivalent to the proline in plants grown in SL media at 80% ETc. However, the fruit quality parameters were significantly improved due to biochar amendment. These results align with the application of biochar to tomato plants in water deficit conditions (Obadi *et al.*, 2023).

RWC is the water content in a plant, leaf, or tissue relative to its maximum capacity, assessing hydration status and water availability. It is crucial in identifying water stress, such as drought, water scarcity, or improper irrigation practices, enabling timely intervention to mitigate its effects (Pietragalla & Mullan, 2012). Biochar's porous structure and high surface area promote beneficial microorganism growth and soil structure improvement, enhancing soil aggregate formation and water infiltration and retention (Ababsa *et al.*, 2023). Water stress decreases leaf water content, leading to dehydration and a decline in RWC (Pietragalla & Mullan, 2012). Persistent water stress alters cell membranes, increasing penetrability but decreasing sustainability (Blokhina *et al.*, 2003). In tomatoes, adding 5% date fronds biochar at 100% ETc significantly enhanced leaf RWC (Obadi *et al.*, 2023). Applying biochar and chitosan to drought-stressed barley plants resulted in a significant increase in RWC (Hafez *et al.*, 2020). Eucalyptus bark biochar significantly improved RWC in maize plant (Tanure *et al.*, 2019). The application of biochar at 80, 60, and 40% field capacities improved RWC of soybean (Mannan *et al.*, 2021). In the present study, RWC was significantly higher in MBCB and DLMB media at 100% ETc, 80%, and 60% ETc, but significantly reduced in SL media at 80% and 60% ETc.

## Conclusions

This study unveiled compelling evidence for the transformative impact of biochar-amended media (MBCB or DLMB) for maximizing zucchini yields and quality under water stress levels (100%, 80%, and 60% ETc). Using MBCB and DLMB biochar amendments paired with optimal irrigation (100% ETc) maximized plant growth, yield, and fruit quality. Notably, these biochar amendments demonstrated remarkable resilience, enabling acceptable zucchini production even with reduced water (80% and 60% ETc), albeit with slightly lower yields and fruit quality. Conversely, relying solely on sandy loam (SL) media proved detrimental under water stress, highlighting the critical role of biochar in mitigating water scarcity impacts. Water stress in SL media significantly impacts fruit size and color traits, negatively impacting consumers' preferences. Our findings strongly recommend incorporating MBCB or DLMB biochar with SL media to save 20–40% of irrigation water while maintaining comparable outcomes. Future research is needed to understand the physiological and molecular pathways involved in mitigating water stress in biochar media. Future research could explore the genotype-specific response of different zucchini cultivars to biochar types under different water stress conditions, enhancing our understanding of the relationship between biochar, water accessibility, and zucchini crop growth characteristics.

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