MANOEL NELSON DE CASTRO FILHO

BIOCHAR AND FOLIAR APPLICATION OF POTASSIUM NITRATE ENHANCE PHOTOSYNTHESIS AND IMPROVE SOIL-PLANT RELATIONSHIP IN TOMATO CULTIVATION UNDER DEFICIT IRRIGATION CONDITIONS

Thesis submitted to the Plant Science Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of Doctor Scientiae.

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VIÇOSA - MINAS GERAIS
2024

Ficha catalográfica elaborada pela Biblioteca Central da Universidade Federal de Viçosa - Campus Viçosa

T

C355b
2024

Castro Filho, Manoel Nelson de, 1995-

Biochar and foliar application of potassium nitrate enhance photosynthesis and improve soil-plant relationship in tomato cultivation under deficit irrigation conditions / Manoel Nelson de Castro Filho. – Viçosa, MG, 2024.

1 tese eletrônica (134 f.)

Texto em inglês.

Orientador: Carlos Nick Gomes.

Tese (doutorado) - Universidade Federal de Viçosa, Departamento de Agronomia, 2024.

Inclui bibliografia.

DOI: https://doi.org/10.47328/ufvbbt.2024.391

Modo de acesso: World Wide Web.

1. Tomate - Irrigação. 2. Déficit hídrico. 3. Eficiência hídrica. 4. Tomate - Colheita. 5. Tomate - Qualidade. I. Gomes, Carlos Nick, 1979-. II. Universidade Federal de Viçosa. Departamento de Agronomia. Programa de Pós-Graduação em Fitotecnia. III. Título.

CDD 22. ed. 635.64287

Bibliotecário(a) responsável: Alice Regina Pinto Pires CRB-6/2523

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APPROVED: July 4, 2024.

Assent:

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Carlos Nick Gomes

Orientador

I dedicate this thesis to my parents
Manoel and Eleonora, my wife Edilane and my
son Christian Emanuel.

ACKNOWLEDGEMENTS

I would like to begin by expressing my gratitude to God, for providing me with strength during difficult moments throughout this journey and now allowing me to achieve the title of Ph.D. in Plant Science from the Federal University of Viçosa - UFV.

To my parents, Manoel Nelson and Eleonora Maria, for their love and encouragement throughout my journey. To my brothers Elton and Ésio for their friendship and words of comfort during difficult times. To my wife Edilane Cotrim, for her understanding, love, and assistance in the execution of this work, and to my son Christian Emanuel for all the inspiration.

I am immensely grateful to my advisor, Professor Dr. Carlos Nick, for his understanding and companionship when needed, for the teachings during these four years of collaboration, and for the significant contribution to my professional development. To my Co-advisor, Professor Leônidas Carrijo, for the teachings, commitment, and assistance with the research, and for the support during difficult times. I also want to thank my colleagues: José Ferreira, Ésio de Castro, Felipe de Oliveira, Jéssica Lino, and Rolando Corella, for their help and teachings in the development of this work. To the Federal University of Viçosa, for the opportunity to complete the postgraduate course.

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

To the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), for granting the scholarship.

ABSTRACT

De CASTRO FILHO, Manoel Nelson, D.Sc., Universidade Federal de Viçosa, July, 2024. **Biochar and foliar application of potassium nitrate enhance photosynthesis and improve soil-plant relationship in tomato cultivation under deficit irrigation conditions.** Advisor: Carlos Nick Gomes. Co-advisor: Leônidas Carrijo Azevedo Melo.

This thesis aimed to examine the effect of biochar as a soil conditioner and potassium source in tomato cultivation under different soil moisture levels, as well as to investigate the combined effects of biochar and foliar spray of KNO_3 to minimize damage caused by water deficit, maximize photosynthesis, and improve water use efficiency. The use of biochar derived from coffee husks (material rich in potassium) was employed to increase tomato crop productivity while preserving the environment through a circular economy approach. This approach involves returning agricultural waste material to the soil as a nutrient source, reducing waste disposal and the need for chemical fertilizer use. The study was divided into three chapters, with two conducted in a controlled environment and one in an open field. In the first chapter, we hypothesized that the application of biochar to the soil and foliar spraying with KNO_3, either alone or in combination, could positively influence the physiology, productivity, and quality of tomato fruits under different soil moisture conditions. In the second chapter, we evaluated the effects of these treatments on the physiology, biometric parameters of the aerial part and roots, and biomass accumulation in tomato plants. Finally, in the third chapter, we investigated the development and productivity of tomato plants in an open field with biochar application and varying irrigation levels, as well as the impact of biochar on the chemical and physical-hydrological properties of the soil. In all conducted trials, negative effects of water deficit on physiological and productive parameters of the tomato plants were observed. On the other hand, the use of biochar resulted in increased production, fruit quality, and improvements in the chemical and physical properties of the soil. Therefore, coffee husk biochar can be considered a good soil conditioner and good fertilizer that also provides stable carbon to the soil. Foliar spray of KNO_3 did not improve the productive aspects of tomato plants and did not have a synergistic effect with biochar.

Keywords: Deficit irrigation; Tomato; Water use efficiency; Harvest; Fruit quality.

RESUMO

De CASTRO FILHO, Manoel Nelson, D.Sc., Universidade Federal de Viçosa, julho de 2024. **Biochar e aplicação foliar de nitrato de potássio melhoram a fotossíntese e aperfeiçoam a relação solo-planta no cultivo de tomate sob condições de irrigação deficitária**. Orientador: Carlos Nick Gomes. Coorientador: Leônidas Carrijo Azevedo Melo.

Esta tese teve como objetivo examinar o efeito do biochar como condicionador de solo e fonte de potássio no cultivo de tomate sob diferentes níveis de umidade do solo, bem como, investigar seus efeitos em combinação com a pulverização foliar de KNO_3 para minimizar os danos provocados pelo deficit hidrico e maximizar a fotossíntese e a eficiência do uso da água. A utilização de biochar de cascas de café (material rico em potássio), foi empregada para aumentar a produtividade da cultura do tomateiro, ao mesmo tempo em que preserva o meio ambiente por meio de uma abordagem de economia circular, em que material proveniente de lavoura de cafés, retorna ao solo como fonte de nutrientes, reduzindo assim o descarte deste material e a necessidade de reposição de fertilizantes químicos. O estudo foi dividido em três capítulos, dos quais dois foram conduzidos em ambiente protegido e um em campo aberto. No primeiro capítulo, teve-se como hipótese de que a aplicação de biochar no solo e a pulverização foliar com KNO_3, isoladas ou em combinação, podem influenciar positivamente a fisiologia, produtividade e qualidade dos frutos de tomate sob diferentes condições de umidade do solo. No segundo capítulo, avaliamos a influência desses mesmos tratamentos na fisiologia, nos parâmetros biométricos da parte aérea e das raízes, e no acúmulo de biomassa em plantas de tomate. Finalmente, no terceiro capítulo, investigamos em campo aberto o desenvolvimento e a produtividade do tomateiro com a aplicação de biochar e diferentes níveis de irrigação, além disso, foi investigado o impacto do biochar nas características químicas e físicas-hídricas do solo. Em todos ensaios realizados, foi verificado efeitos negativos do deficit hídrico nos parâmetros fisiológicos e produtivos do tomateiro. Por outro lado, o uso de biochar resultou em aumento da produção, qualidade dos frutos e melhorias nas propriedades químicas e físicas do solo. Portanto, o biochar de cascas de café pode ser considerado um bom condicionador de solo e uma fonte eficiente de K. A pulverização foliar de KNO_3 não melhorou os aspectos produtivos das plantas de tomate, e não teve efeito sinérgico com o biochar.

Palavras-chave: Irrigação deficitária; Tomate; Eficiência no uso da água; Colheita; Qualidade do fruto.

SUMMARY

GENERAL INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetables globally due to its high level of production: 5.2 million hectares in 2021, with an average yield of 36.6 tons per hectare (FAOSTAT, 2023). Additionally, it is considered the second most cultivated and consumed vegetable worldwide (Dariva et al., 2020). Tomato fruits are rich in vitamin C, minerals, and antioxidants (especially lycopene) and are known to be highly beneficial for human health (Çolak et al., 2020; Jawad et al., 2020; Ahanger et al., 2021). To meet the growing global demand for food, and recognizing that commercial tomato cultivation requires irrigation to perform well and is impacted under severe water deficit conditions, studies are needed to improve crop performance under adverse conditions such as low soil water availability.

Water is one of the most limiting resources in agricultural production, and agriculture is the primary consumer of water (approximately 70 %) (Khapte et al., 2019). According to the OECD (2010), the major challenge faced by agriculture is meeting the projected food production demand for 2050, especially in the face of decreasing water availability for agriculture due to increasing demands from other sectors (Hatfield, 2015). Furthermore, water availability is expected to become even more critical as climate change presents one of the greatest global challenges, imposing new vulnerable situations (Dias, 2020). To address this crisis, investment in sustainable agricultural technologies and practices is necessary. Among agricultural practices related to improving productive performance under low water availability conditions, the use of biochar (Faloye et al., 2019; Streubel et al., 2011) and foliar spray of potassium nitrate (KNO_3) (Gimeno et al., 2014; Ávila et al., 2022) stand out.

Biochar is a fine-textured carbonaceous material with a high concentration of carbon in its organic composition. It is produced from the carbonization of biomass in the absence of oxygen (Lehmann and Joseph, 2015). Although discussions about the use of biochar began in agricultural books and scientific journals in the 19th century (Allen, 1849; Anonymous, 1851), interest in this material declined during the green revolution when synthetic fertilizers gained prominence, resulting in a reduction in studies about biochar (Santos et al., 2020). However, new studies began to emerge from the 1980s, such as the study by Iswaran et al. (1980), and, increasingly, studies have been showing the beneficial effects of biochar use (Macedo et al., 2019; Abbas et al., 2020; Mohawesh et al., 2021).

Biochar can be considered an effective component in agriculture, especially aimed at mitigating extreme drought events while promoting beneficial changes in soil properties

(Rashid et al., 2020; Murtaza et al., 2021). Moreover, the utilization of on-farm waste following a circular economy approach has become increasingly relevant in contemporary agriculture (Ormond, 2002; Rani, 2023). A concrete example of this concept is the use of biochar derived from coffee husks, as conducted in the present study. Coffee husks, after pyrolysis, return to the soil as a valuable source of nutrients (especially K), reducing the disposal of this material and the dependence on chemical fertilizers. In this context, there is a global trend to shift from intensive agriculture to more sustainable agriculture, termed green agriculture. This production system aims to reduce greenhouse gas emissions. In this sense, biochar emerges as an effective alternative, as it is a carbon source that can be incorporated into the soil, presenting a long duration (high half-life) (Qayyum et al., 2012). This duration time varies depending on the type of biochar (e.g., raw material type, pyrolysis temperature, pyrolysis time). Additionally, it improves soil fertility and nutrient cycling (Głąb et al., 2016; Foster et al., 2016) and enhances soil water retention capacity (Streubel et al., 2011), which can increase crop productivity and water use efficiency, especially important in arid and semi-arid regions (Diatta et al., 2020).

Foliar application of KNO_3 has can be a promising strategy in agricultural management under moderate water deficit conditions. Some studies have emphasized the positive effects of potassium (K) under water scarcity conditions for plants (Ahanger and Agarwal, 2016; Shehzad et al., 2020). Potassium plays a crucial role in adjusting osmotic pressure, maintaining membrane potential, activating enzymes, facilitating water absorption, and sustaining cell turgor (Adams and Shin, 2014; Shafiq et al., 2015). Nitrogen (N) is another essential element in plant life, being fundamental in the synthesis of various important plant substances, including chlorophyll, amino acids, proteins, and nucleic acids.

Due to the importance of tomatoes as one of the most globally cultivated and consumed vegetables, along with its significant nutritional value for human health, it is crucial to develop strategies that optimize its production, especially under conditions of limited water availability. With this purpose, the present study aimed to investigate the possibility of increasing tomato productivity and improving water use efficiency by exploring the effects of biochar application and foliar spray of KNO_3 at different soil moisture levels.

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CHAPTER 1. Improved tomato development by biochar soil amendment and foliar application of potassium under different available soil water contents

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* *Published in full in the magazine* ***Journal of Plant Nutrition (Impact Factor: 2.1; Qualis classification: A4)*** *DOI: https://doi.org/10.1080/01904167.2024.2354214.*

ABSTRACT

The use of carbonaceous materials such as biochar has been considered an innovative solution in agriculture, especially to mitigate dry events caused by climate change. Biochar can improve water holding capacity, physical and chemical properties of soil, and increase the productivity of agricultural systems. However, underlying physiological mechanisms remain poorly understood. Mineral supplementation with potassium nitrate (KNO_3) via foliar application is another promising strategy in agricultural management under irrigation deficit. Thus, we investigated the combined effects of biochar and foliar application of KNO_3 on growth, yield, and physiology of tomato plants under different irrigation regimes. Results did not indicate a synergistic effect of biochar and foliar application of KNO_3. Biochar application alleviated tomato plant stress under deficit irrigation, with plants showing better functioning of photosynthetic apparatus, higher yield, and better fruit quality, as well as increased water use efficiency. Coffee husk biochar (K-rich feedstock) fully met the tomato plant's demand for K and partially met the demand for some other elements (P, Mg, Fe, Zn, Mn, and Cu). Although positive effects of KNO_3 application was verified for some physiological and fruit quality components, overall, foliar application of KNO_3 did not improve tomato yield. It is concluded that biochar soil amendment can be a promising practice to increase yield and quality of tomato fruits under deficit irrigation. Therefore, biochar-based fertilizer can be an alternative K source that also provides stable carbon to soil and helps to mitigate stress caused by prolonged drought.

Keywords: Climate change; Drought tolerance; Pyrolysis; Vegetables.

Abbreviations: Optimum irrigation, OI; Deficit irrigation, DI; Total plant productivity, PY; Average fruit weight, PW; Fruit firmness, FF; Hydrogen potential, pH; Total Soluble Solids, TSS; Titratable acidity, TA; Lycopene content, Lyc ; Leaf temperature, LT; Leaf water potential in pre-dawn assessments, LWP_pd ; Leaf water potential in the evaluations at noon, LWP_m; Net photosynthesis, *An*; Stomatal conductance, g*s* , Intercellular CO_2 concentration, C*i* , Transpiration rate, *E*; Instant water use efficiency, WUE*i* ; Instantaneous efficiency in the carboxylation of Rubisco ($An\ Ci^{-1}$, CE*i*), Relative Chlorophyll Content, SPAD; Total electron flux from the antenna complexes of the PSII, LEF; Quantum yield for photosystem photochemistry II, Phi2; Total electrochromic shift, ECSt; Non-photochemical total extinction, NPQt; Non-photochemical photoprotective extinction, PhiNPQ.

1. INTRODUCTION

Population growth and climate change have given the agricultural sector the challenge of increasing food production sustainably. Furthermore, geopolitical conflicts have caused instability in the supply of fertilizers, bringing uncertainty to the growth of agriculture in several countries dependent on external inputs (Rabbi et al., 2023). Climate change, for example, has caused an increase in the frequency of drought events around the world, reducing the potential yield of the main crops (Jatav et al., 2014; Ahanger et al., 2014). Inadequate soil management has promoted excessive losses of nutrients, which increases fertilizer use (Bertol et al., 2007). Therefore, the sustainable use of water resources and fertilizers is important in future agricultural systems (Guo et al., 2021).

Irrigation can improve crop productivity and the number of harvests within the same year. Water resource is increasingly scarce, thus irrigation management practices must be optimized. Water deficit affects plants when available soil moisture is low and transpiration rate exceeds plant water uptake, which leads to lower photosynthetic rates (Rasheed et al., 2015; Avila et al., 2016), increased production of reactive oxygen species (ROS) (Hasanuzzaman et al., 2020; Rasheed et al., 2021), and severe oxidative stress that can damage lipids, proteins, and nucleic acids (Zhang et al., 2016).

Recent employment of carbonaceous materials such as biochar has been considered an innovative solution in agriculture, especially for the mitigation of extreme events and promotion of beneficial changes in soil property (Rashid et al., 2020; Murtaza et al., 2021). Biochar is a solid carbon-rich byproduct from several feedstocks such as crop straw, wood and manure, produced under relatively high temperatures (ranging from 300-700 °C) and limited oxygen conditions (Lehmann et al., 2015; Gonzaga et al., 2017). Feedstock type and pyrolysis conditions generates biochar with specific properties (Akhtar et al., 2014). Therefore, biochar effects on soil water retention, soil fertility and crop yields may vary widely (El-Naggar et al., 2019; Melo et al., 2022).

Biochar application improves soil fertility, nutrient cycling (Głąb et al., 2016; Foster et al., 2016), and soil water-holding capacity (Streubel et al., 2011), which can increase crop yield and water use efficiency (WUE), which is particularly important in arid regions (Diatta et al., 2020). Mineral supplementation of KNO_3 via foliar application is another promising strategy in agricultural management under deficit irrigation (Gimeno et al., 2014; Ávila et al., 2022). Beneficial effects of K application on plants under irrigation deficit conditions have been reported by several authors (Kanai et al., 2011; Ahanger and Agarwal, 2016; Shehzad et al., 2020). Potassium plays an essential role in osmotic adjustment, membrane potential, enzyme activation, water uptake, and cell turgor maintenance (Adams and Shin, 2014; Shafiq et al., 2015). Nitrogen (N) is a key element involved with the synthesis of several plant compounds, such as chlorophyll, amino acids, proteins, and nucleic acids. Sorghum plants cultivated under water deficient conditions and treated with KNO_3 in the pre-flowering phase showed higher levels of chlorophyll, photosynthetic rate, stomatal conductance, transpiration, carboxylation efficiency, and higher levels of P, K, Mg, S, Cu, and Fe, resulting in up to 32.2 % increase in grain yield (Ávila, 2022). Similarly, beneficial effects of foliar application of KNO_3 have been reported on wheat due to mitigation of terminal heat stress (Singh and Singh, 2020), and on barley by increasing tolerance to drought and salinity (Fayez and Bazaid, 2014).

Tomato is the second most grown and consumed vegetable worldwide (Dariva et al., 2020). It is rich in lycopene, carotene, and anthocyanins, which characterizes it as a natural antioxidant (Ahanger et al., 2021). Drought is the most limiting factor on tomato cultivation and studies with novel approaches on deficit irrigation are much needed. To the best of our knowledge, no studies have been carried out to assess the combined effects of biochar soil amendment and foliar supplementation of KNO_3 on the alleviation of negative effects caused by deficit irrigation on tomato. Thus, this research aims to evaluate the effects of a K-rich coffee husk biochar application as the source of K and a complementary foliar fertilization under optimum irrigation and deficit irrigation on the physiology, yield, and fruit quality of tomato plants.

2. MATERIAL AND METHODS

2.1. Biochar production and characterization

Coffee husk (husk + pulp + parchment) of *Coffea arabica* was used as feedstock to produce biochar. Coffee husk has large availability in Brazil and its biochar has shown favorable soil conditioning effects (Domingues et al., 2020). Coffee husk was obtained through dry bean pulping process at the Federal University of Viçosa (20° 45' 14" S, 42° 52' 53" W, 649 m of altitude). The material was previously oven-dried at 65 °C until constant weight, ground in a mill and then sieved with a 2 mm sieve. Biochar was produced in an adapted electric muffle with closed stainless-steel cylinders, equipped with a condenser that allows gas and bio-oil to be released (Lustosa Filho et al., 2017). Pyrolysis was carried out at 500 °C using a heating rate of 10 $°C\ min^{-1}$ and the material was kept inside the equipment during 12 h until reaching room temperature. Biochar was then removed from the cylinders, ground, and sieved (< 2 mm) to standardize particle size.

For biochar chemical characterization, representative samples were collected and processed in an agate mortar to 60 mesh (0.250 mm) and subsequently oven-dried at 65 ± 2 °C for 48 h to remove moisture. The following analysis were performed: (*i*) total CHN content in a Perkin Elmer Series II Elementary Analyzer 2400. Oxygen (O) was calculated by difference ($O = 100 - C - H - N -$ ash) (Fonseca et al., 2020). Ash content was determined following the ASTM D – 2866 standard method (ASTM D, 2007); (*ii*) electrical conductivity (EC) and pH were measured on water in a 1:20 solid to solution ratio, after 12 h of suspension agitation (Rajkovich et al., 2012); (*iii*) available nutrient contents were determined using a Mehlich-III solution as extractant in the proportion of 0.1 g mL^{-1} (Krounbi et al., 2021); (*iv*) total nutrient contents were determined according to a modified dry ashing method at 500 °C and wet digestion using HNO_3 and H_2O_2 (Enders and Lehmann, 2012); (*v*) identification of minerals in the crystalline phases was accomplished by X-ray powder diffractometry (XRD), using a PAN analytical device, model X` Pert Powder, with cobalt tube, nickel filter in the range from 4 to 50°2θ, and sweep speed of 10°2θ; (*vi*) identification of functional groups by molecular absorption spectrophotometry in the infrared region with Fourier transform (FTIR) (Jasco FTIR 4100), using spectra obtained with 60 scans, with wave number from 4000 to 400 cm^{-1} and resolution of 4 cm^{-1}. Cross-sectional biochar sections were prepared for observation in the scanning electron microscope (SEM) model Leo, 1430VP. The identification of biochar structures was done using reading between 10 and 100 µm.

2.2. Experimental setup and treatments

The experiment was carried out at a Research and Extension area at the Federal University of Viçosa, in Viçosa – Minas Gerais, Brazil (20° 45' 14" S; 42° 52' 53" W; 649 m of altitude), from July to December 2021. This research was a 2x2x2 factorial with treatments arranged in a randomized block design. Treatments included two biochar levels (1 % = +Biochar and 0 % = -Biochar), two foliar application regimes using KNO_3 (2 %= +KNO_3 and 0 % = -KNO_3), and two irrigation regimes (OI = optimum irrigation and DI = deficit irrigation), with four replications.

Meteorological data during the cultivation cycle was collected from the IRRIPLUS meteorological station, installed inside the greenhouse. The average temperature was 19.6 ºC, with a daily average of 22.7 ºC and a night average of 16.4 ºC, and the average relative humidity was 84.6 %.

2.3. Seedling preparation and experiment implementation

Hybrid tomato Vivacy® (Feltrin) seeds were sown in 128-cell expanded polystyrene trays containing a commercial substrate (MEC PLANT®). Transplanting was carried out 30 days after germination, when plants had 3-4 true leaves. One plant was transplanted to each pot in a greenhouse. Plants were grown in pots containing 10 kg of air-dried soil (~12 % moisture) collected from the B horizon of a loamy (sand: 30 %, silt: 12 %, clay: 58 %) Oxisol. The soil was previously sieved (<4 mm) and incubated with limestone during 30 days with adequate moisture to increase base saturation to 70 %. Soil properties before liming are shown in Table S1 and they were obtained following Brazilian standards (Teixeira et al., 2017).

The pots with biochar contained a mixture of soil and biochar at a concentration of 1% (m/m) (biochar:soil), i.e. 10 g of biochar per kg of soil. The concentration (1 %) was predetermined based on the K concentration in the biochar and the K demand of the tomato plant in a pot experiment (688 mg kg^{-1}). Foliar application of KNO_3 was performed using a manual sprayer every 15 days after the beginning of irrigation treatments. The KNO_3 solution was applied at 2 % (w/v) with volumes ranging from 10 to 40 mL per plant depending on the aerial development of the plant (e.g., in the first application, 10 mL per plant were used, in the second application, 20 mL per plant, and in the last application, 40 mL per plant). The volume was defined as the quantity necessary to cover the entire leaf area, preventing solution runoff. Deionized water was sprayed on plants that did not receive KNO_3 application as a control.

To supply plants with enough nutrient during the experimental period and considering the nutrient fraction provided by biochar, one nutrient solution was prepared for treatments with biochar and another one for treatment without biochar. In both situations, the amount of nutrients supplied was equivalent (Table S2). To prepare the nutrient solution for biochar treatments, we considered the total K content. For the other elements we considered the available contents. Preparation of nutrient solutions was based on recommendations for plant pot experiments (Novais et al., 1991) and specific recommendations for tomato plants (Alvarenga et al., 2013). Nutrient solutions were applied weekly.

2.4. Irrigation scheduling

The research study was carried out using a deficit irrigation treatment (DI), in which the soil water content was kept at 50 % available soil water (ASW); and an optimal irrigation treatment (OI), where the soil water content was kept at 90 % ASW. For this, a soil water retention curve was obtained based on the Van Genuchten equation (1980) using the SWRC Fit software (Seki, 2007).

All pots were filled to the same dry soil weight (W_{ds}). Water weight (W_{water}) at stress and control treatments in the first irrigation was determined by multiplying W_{ds} with the soil water content (kg kg^{-1}) equivalent to 50 and 90 % of ASW, respectively. To estimate water tension values corresponding to 50 and 90 % ASW, we assume that field capacity and wilting point were reached at matric soil water potentials of -33 and -1500 kPa, respectively (Bernardo, 2019). Soil water content was then monitored by weighing each pot daily. On the following irrigations, W_{water} applied was determined as the reference weight (W_{ref}) minus pot weight measured on the day.

Due to plants' increasing biomass over time, W_{ref} was adjusted every seven days. To this end, at the beginning of the day (6-7 am), when the plants were growing, the pots with plants were weighed and soil samples were collected and sent to the laboratory for analysis of moisture content. With data on the weight of the pot+plant and the moisture content obtained in the laboratory, the reference weight was adjusted based on the water retention curve. The collection was carried out using 25mm thick probes and the moisture analysis determined by the standard gravimetric method using an oven (Equation 1). After each evaluation, the collected soils were returned to the pots according to the respective treatment.

$$\theta_g = W_{ms} - W_{ds}/W_{ds} \qquad (1)$$

Where:

- θ_g = gravimetric humidity (kg kg^{-1});
- W_{ms} = wet soil weight;
- W_{ds} = dry soil weight.

Irrigation treatments was started ten days after transplanting to ensure adequate plant establishment. Water was provided once or twice a day.

2.5. Soil hydraulic parameters

Water retention curve before installing the study was determined using the Richards' pressure chamber at 10, 33, 100, 300, and 1500 kPa. The end of each applied pressure was defined when drainage ceased. After applying the stresses, the soil samples were dried in ovens at 105 °C for 48 h to determine dry soil-based water content. Then the water retention curve was fitted according to the model proposed by van Genuchten (1980), using the SWRC Fit software (Seki, 2007). After the end of the experiment, soil samples were collected from both biochar treatments (+Biochar and -Biochar) to determine moisture at field capacity (-33 kPa) and permanent wilting point (-1500 kPa).

2.6. Measured Variables

2.6.1. Harvest

Fully ripe tomato fruits were harvested during the experimental period. Yield parameters consisted of total plant yield (PY) (kg plant^{-1}), measured as the fresh weight of all fruits on each plant, and average fruit weight (FW) (g), measured as the average weight of 5 fully ripe fruits randomly picked from the third and fourth bunches.

2.6.2. Sensory quality attributes and fruit lycopene content

Fruit firmness (FF) was expressed in newton and measured using a digital penetrometer (model PDF-200, Soil control, USA). Fruits from each experimental unit were mixed together using a blender. The resulting mixture was used to determine pH, total soluble solids (TSS), total acidity (TA), and lycopene (Lyc) contents. pH values were determined using a benchtop pH meter (model pH 21, Hanna Instruments, Italy); TSS, expressed in ºBrix, was determined using a digital refractometer (model HI 96801, Hanna Instruments, Italy); TA was determined by titration using a standardized solution of 0.005 mol L^{-1} NaOH and 1 % phenolphthalein as an indicator (Dariva et al., 2021); fruit lycopene content was determined by spectrophotometry (model SP1105, Bel Photonics) (Rodriguez-Amaya, 2001); and pigment extraction was performed using acetone, followed by vacuum-filtering (Dariva et al., 2021).

2.6.3. Leaf temperature and leaf water potential (leaf Ψ)

Leaf temperature (LT) measurements were performed using an infrared thermometer on the upper third leaf during the afternoon (from 1 to 2 pm). Leaf water potential (Ψ_{leaf}) was measured using a pressure chamber (Scholander et al., 1965). Briefly, tomato leaves were excised and placed in the chamber within 20 s after collection, and evaluations were carried out in the early morning (from 4 to 5 am) (leaf water potential in pre-dawn assessments - LWP_pd) and midday (from 1 to 2 pm) (leaf water potential in midday assessments - LWP_m).

2.6.4. Gas exchange measurements

Net photosynthesis (*An*), stomatal conductance (g_s), intercellular CO_2 concentration (*Ci*), and transpiration rate (*E*) were measured using an Infrared Gas Analyzer (Irga LI-6400, LI-COR I-COR Biosciences Inc., Lincoln, Nebraska, USA). All measurements were performed between 8 and 10 am, 40 days after the beginning of the deficit irrigation treatment (at the fruit setting stage). Leaves were placed in the cuvette to stabilize for at least two minutes before the first recording. The internal conditions of the leaf chamber were adjusted to an average temperature of 22 °C, external CO_2 concentration of 400 μmol mol^{-1} of air, flow rate of 300 μmol s^{-1}, and saturation light of 1000 μmol photons m^{-2} s^{-1} with 10 % blue light.

Instantaneous water use efficiency ($An\ E^{-1}$, WUE*i*) and instantaneous carboxylation efficiency of Rubisco ($An\ Ci^{-1}$, CE*i*) were also determined. $An\ E^{-1}$ was estimated based.

2.6.5. Photosynthesis phenotyping

All chlorophyll fluorescence spectroscopic measurements were performed on intact, fully expanded leaves from the plant's upper third portion using a MultispeQ V 2.0 device (Kuhlgert et al., 2016). These measurements, including gas exchange measurements, were performed in the morning (from 8 to 10 am) 40 days after the beginning of the deficit irrigation treatment. The following parameters were measured: relative chlorophyll content (SPAD), total electron flux of the PSII antenna complexes (linear electron flow, LEF), quantum yield for photochemistry at photosystem II (Phi2, ΦII), total electrochromic shift (ECSt), total non-photochemical quenching (NPQt), and photoprotective non-photochemical quenching (PhiNPQ, ΦNQP).

2.6.6. Soil chemical and physical properties

At the end of the experimental period, soil samples were collected from the pots and characterized (Teixeira et al., 2017). pH was determined in H_2O in 1:2.5 soil:solution ratio using a digital bench pH meter. Available P and K were extracted using a Melich-1 extractor (HCl 0.05 mol L^{-1} + H_2SO_4 0.0125 mol L^{-1}), determined colorimetrically and by flame photometry, respectively. Exchangeable Ca^{2+} and Mg^{2+} were extracted with KCl 1.0 mol L^{-1} and determined by flame atomic absorption spectrometry (FAAS). Organic carbon content (C-org) was determined via oxidation with dichromate (0.167 mol L^{-1} of $K_2Cr_2O_7$) in an acid medium using an external heat source to maximize oxidation (Yeomans and Bremner, 1988).

2.7. Statistical analysis

A three-way analysis of variance (ANOVA) was performed to test treatments significance, i.e., biochar (+Biochar and -Biochar), foliar application of KNO_3 (+KNO_3 and -KNO_3), and irrigation levels (50 and 90 % of ASW). When differences were significant by the F test ($p < 0.05$), means were compared using the Tukey test at a significance level of 0.05. Statistical analyses were performed using R software (version 3.6.0) employing the ExpDes.pt package.

3. RESULTS

3.1. Biochar characterization

Biochar showed high total K content (68.8 g kg^{-1}), from which 45 % was available by Mehlich-3 (Table S3). Ash contents were equally high (225 g kg^{-1}) as well as the pH and EC, which are consistent with coffee husk as feedstock. Most relevant minerals found by XRD were: Calcite ($CaCO_3$) ($2\theta = 26$, 27, 36, and 40°), Astrophyllite [$(K, Na)_3 (Fe^{++}, Mn)_7 Ti_2 Si_8O_{24} (O, OH)_7$] ($2\theta = 10°$), Siderite ($FeCO_3$) ($2\theta = 43°$), Sylvite (KCl) ($2\theta = 33.5$ and 44.5°), and Hydroxylapatite [$Ca_{10}(PO_4)_6 (OH)_2$] ($2\theta = 37.5°$) (Figure S1). Sylvite represents an immediate K source to plants.

Biochar FTIR spectra shows three peaks, at 1560, 1530, and 1500 (Figure S2), associated with C=C, C=N, −COO vibrations, antisymmetric stretching of amino acids, C=O stretching vibration band of ketones, quinones, aldehydes, lactones and carboxylic groups as well as esters, and N−H band (Rodriguez et al., 2021; Taherymoosavi et al., 2017). Peaks at 906, 842, 760, and 720 cm^{-1} were assigned to P−O−P (Bekiaris et al., 2016; Lustosa Filho et al., 2017).

Biochar surface morphology is characterized by the presence of small and well-structured particles (Figure S3), possibly due to breakdown of different feedstock organic compounds, during pyrolysis. In addition, presence of C and O, and a relatively high K and Ca concentration were observed through EDS spectrum.

3.2. Total plant productivity (PY) and average fruit weight (FW)

There was a unique effect of irrigation levels and addition of biochar on PY (Figure 1a Table S4). PY under optimal irrigation was significantly higher (+60 %) than that of plants under deficit irrigation ($p<0.05$) (Figure 1a). PY was significantly increased ($p<0.05$) by biochar fertilization, with a 10 % increase when compared to the treatment without biochar. There was a significant interaction for FW between irrigation and biochar application (Table S4). Reduction in FW caused by deficit irrigation regime was observed in both with (+Biochar) and without (-Biochar) conditions. FW did not differ between biochar treatments at 50 % ASW ($p>0.05$), but it was significantly higher (+14 %) for tomato plants grown in biochar-treated soil when subjected to 90 % ASW treatment ($p<0.05$) (Figure 1b).

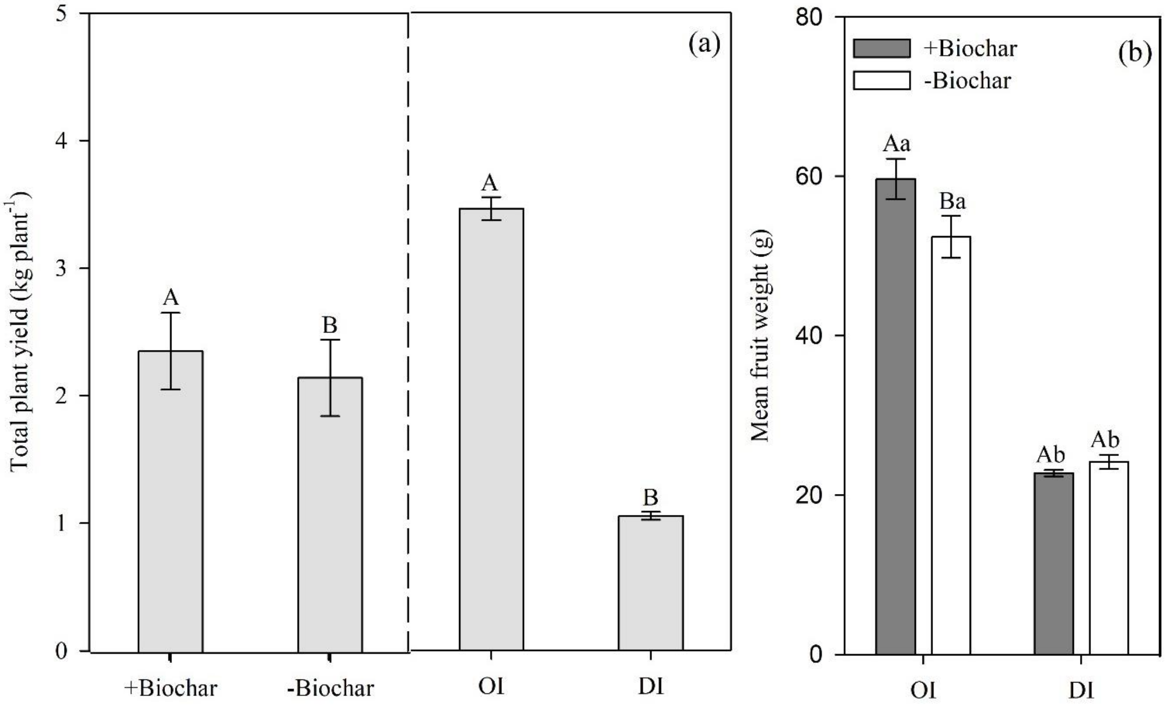

Figure 1. Total yield per plant (a) and average fruit weight (b) of tomato cultivated under different biochar levels (+Biochar and -Biochar), two foliar fertilization regimes with K (+KNO_3 and -KNO_3), and two irrigation regimes (OI and DI). Means followed by the same letter are not statistically different (Tukey's Test, $P<0.05$). In Figure 1a, uppercase letters compare +Biochar and -Biochar, and OI and DI. In Figure 1b, uppercase letters compare +Biochar and -Biochar within each irrigation regime, and lowercase letters compare biochar levels between irrigation regimes. The error bars show the standard error of the mean (a: $n = 20$ and b: $n = 10$). The optimal irrigation (OI) and irrigation deficit (DI).

3. 3. Sensory quality attributes and fruit lycopene content

The irrigation x biochar x KNO_3 interaction was significant ($p<0.05$) only for TSS and TSS/TA ratio (Table S4). Higher TSS levels were observed under DI regardless of any biochar x KNO_3 combination (Figure 2a). In addition, combined biochar and KNO_3 application significantly ($p<0.05$) improved TSS levels in plants grown under DI. Under OI conditions and without biochar application, KNO_3 application increased ($p<0.05$) TSS.

When biochar and KNO_3 were combined, no statistical difference in the TSS/TA ratio was found between OI and DI (Figure 2b). However, when these factors are considered separately, higher TSS/TA ratio values are observed in OI (Figure 2b). Furthermore, biochar application without KNO_3 significantly ($p<0.05$) increased TSS/TA ratio under OI treatment and when biochar was combined with KNO_3 in DI. KNO_3 application increased TSS/TA ratio only in the treatment without biochar under OI.

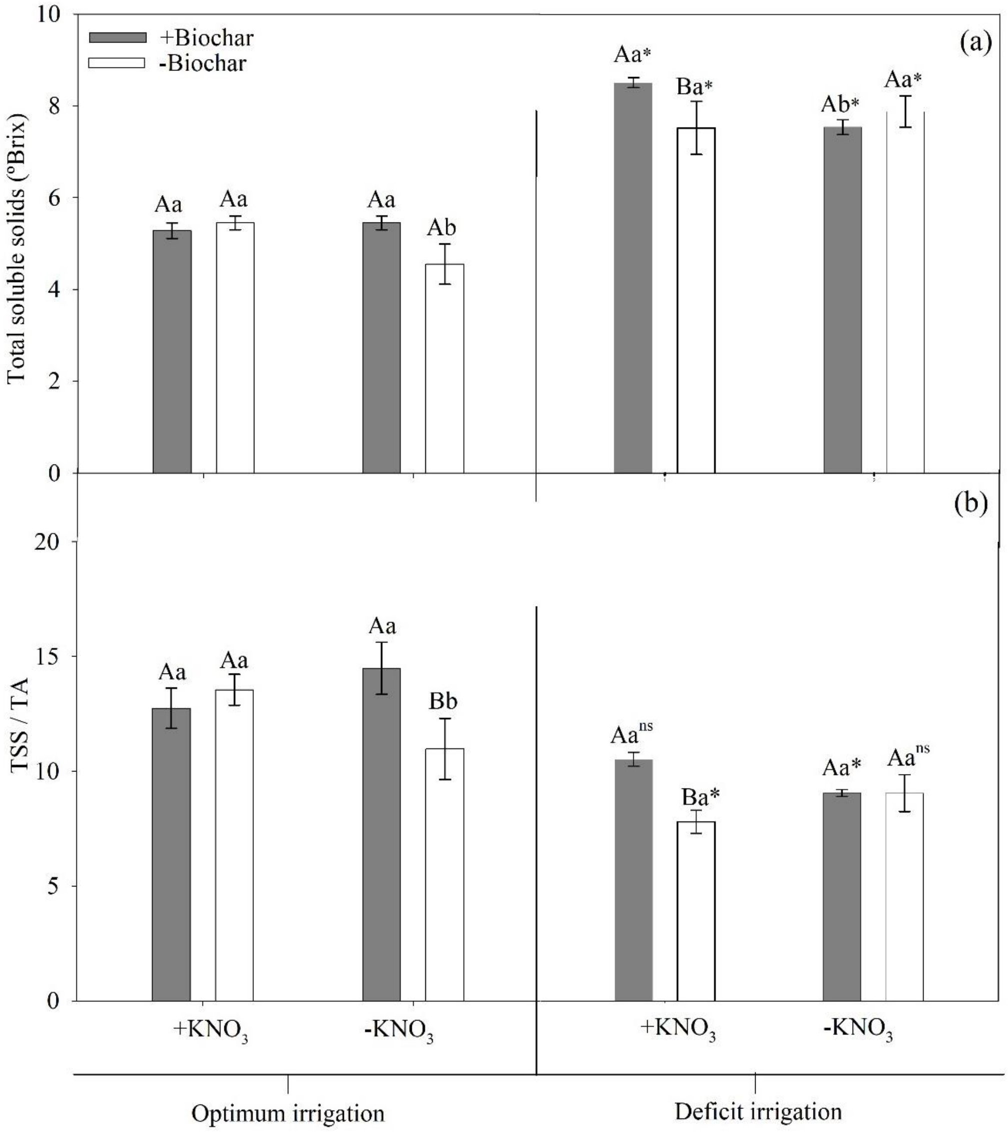

Figure 2. Soluble solids content (a) and relationship between soluble solids content and titratable acidity (b) of tomato cultivated under different biochar levels (+Biochar and -Biochar), two foliar spray regimes with K (+KNO_3 and -KNO_3) and two irrigation regimes (OI and DI). Lowercase letters compare with and without KNO_3 foliar application within each irrigation and biochar level. Capital letters compare with and without biochar within each irrigation level and KNO_3. *Shows the significance of irrigation treatments within each level of biochar and KNO_3. Error bars show the mean standard error (n=5). Data are expressed as mean ± standard error.

Irrigation x biochar interaction was significant ($p<0.05$) for total acidity (TA) (Table S4). TA of fruits under DI (0.8 % and 0.9 % citric acid, on average) was statistically higher

($p<0.05$) than that of fruits under OI (0.4 % and 0.4 % of citric acid, on average) (Figure 3a). TA was reduced by 12 % in fruits formed under DI conditions and in biochar treated soil.

In analyzing the individual irrigation effect, it was observed that deficit irrigation significantly affected pH and lycopene (Lyc) content ($p<0.05$) (Table S4). A decrease of 0.2 units in pH values was observed when fruits were formed under DI conditions (Figure 3b), while higher Lyc content ($p<0.05$) (59.8 μ g^{-1} fresh fruit weight) was observed under OI (Figure 3c). For both variables (pH and Lyc), the effect of soil biochar addition was also observed (Table S4), causing a slight reduction in fruit pH (pH = 4.0) and an increase (32 %) in Lyc content (52.3 μ g^{-1} fresh fruit weight) when compared to the treatment without biochar (Figure 3d-e, respectively). FF was lower ($p<0.05$) in fruits harvested from KNO_3-untreated plants (31 N) when compared to KNO_3-treated plants (37 N) (Figure 3f).

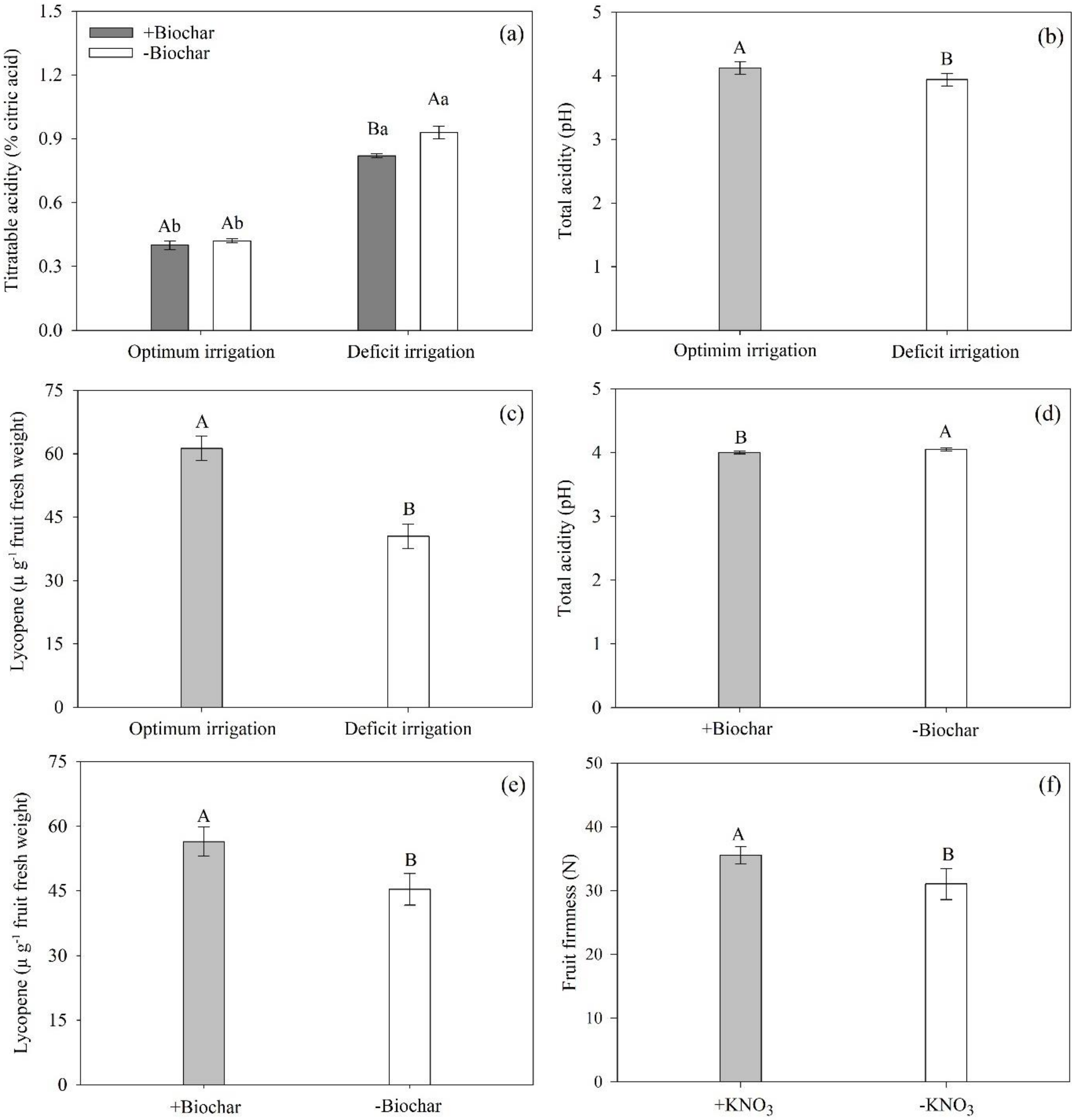

Figure 3. Titratable acidity (a); total acidity (b), lycopene (c), total acidity (d), and lycopene (e) and fruit firmness (f), in tomato plants cultivated under different biochar levels (+Biochar and -Biochar), two foliar spray regimes with KNO_3 (+KNO_3 and -KNO_3) and two irrigation regimes (OI and DI). Means followed by the same letter are not statistically different (Tukey's Test, $P<0.05$). In figure 3a, uppercase letters compare biochar treatments and lowercase letters compare irrigation treatments. Error bars show the mean standard error (a: n = 10 and bf: n = 20).

3.4. Deficit irrigation stress

Leaf temperature (LT) and leaf water potential (Ψ_{leaf}) mean values at pre-dawn (LWP_pd) and midday (LWP_m) are shown in Figure 4. For LT and LWP_m, a significant effect was observed ($p<0.05$) only for irrigation levels (Table S5), with LT 13 % higher in plants grown in DI ($p<0.05$) (Figure 4a), and LWP_m lower under DI (-2.0 MPa) than under OI (-0.7 MPa) (Figure 4b). For LWP_pd, a significant effect ($p<0.05$) was observed for irrigation levels x biochar interaction and individual effect of KNO_3 (Table S5). Without biochar, there is a reduction of 40 % in LWP_pd caused by deficit irrigation. Conversely, with biochar, no negative effect of deficit irrigation on LWP_pd was observed. KNO_3 application reduced LWP_pd by 16 % when compared to treatment without KNO_3 (Figure 4d)

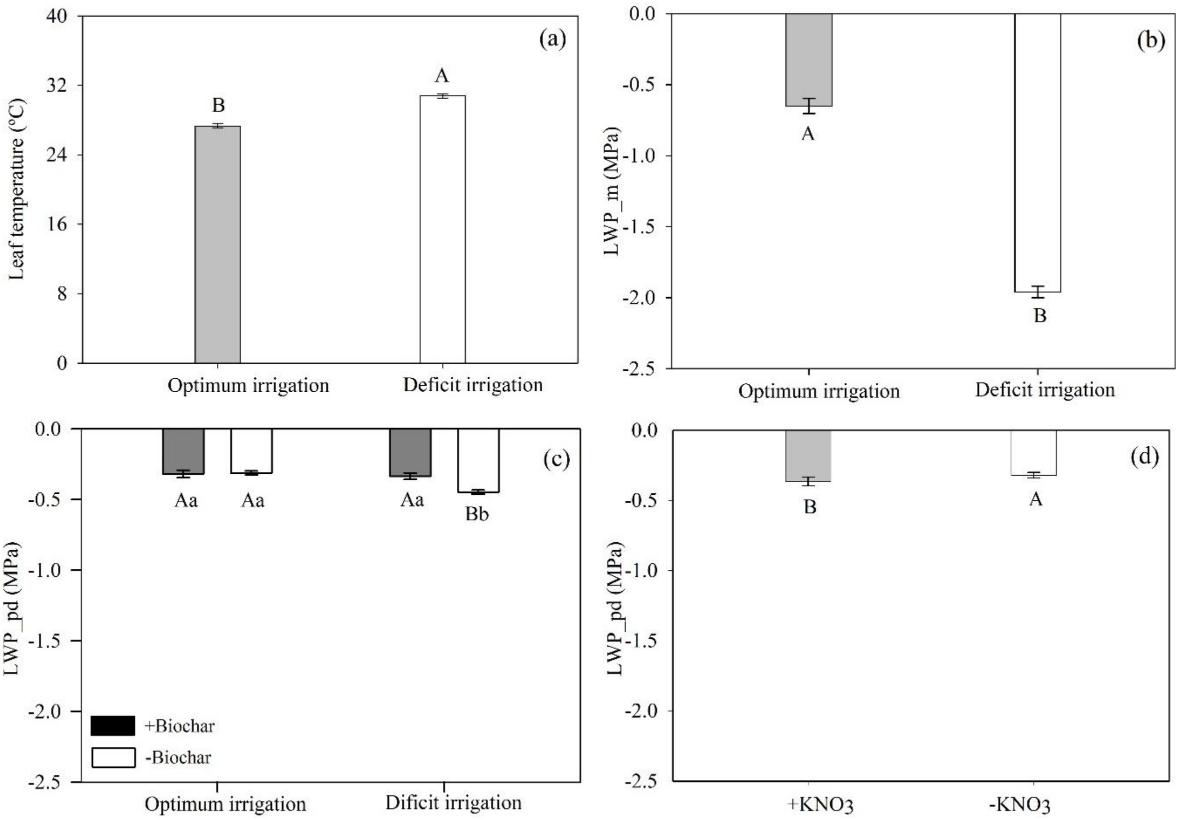

Figure 4. Leaf temperature (a), leaf water potential in midday evaluations (noon-1pm) (LWP_m) (b), leaf water potential in pre-dawn evaluations (3-5 am) (LWP_pd) (c) for *irrigation x biochar* interaction, leaf water potential in pre-dawn assessments (03-05 hours) (LWP_pd) (d) for KNO_3 levels, in tomato cultivated under different biochar levels (+Biochar and -Biochar), two K foliar spray regimes (+KNO_3 and -KNO_3) and two irrigation regimes (Optimum irrigation and Deficit irrigation). Means followed by the same letter are not statistically different (Tukey's Test, $P<0.05$). In figure 4c, uppercase letters compare biochar treatments (+Biochar and -Biochar) and lowercase letters compare irrigation treatments. Error bars show mean standard error (a, b and d: n = 20 and c: n = 10).

3.5. Leaf gas exchange

Interaction among three factors (biochar, foliar application of KNO_3, and irrigation regimes) was not significant ($p>0.05$) for gas exchange components (Table S5). However, irrigation regimes x biochar interaction was significant ($p<0.05$) for transpiration rate, where there was a significant effect ($p<0.05$) under DI for both biochar treatments. i.e., +Biochar and

-Biochar (Figure 5a). Nevertheless, *E* did not differ significantly ($p>0.05$) between biochar treatments in plants grown under OI. For other physiological attributes of gas exchange, only a significant effect of individual factors was observed (Table S5).

OI significantly and positively influenced ($p<0.05$) *Ci*, *gs*, *An*, and CE*i*, while lower means were observed under DI (Figure 5b-e). Biochar addition also significantly influenced ($p<0.05$) *gs, An,* and CE*i,* and WUE*i* in plants (Figure 5f-i), while foliar application of KNO_3 significantly influenced ($p<0.05$) *An* (11 %), CE*i* (11 %), and WUE*i* (16 %) (Figure 5j-l).

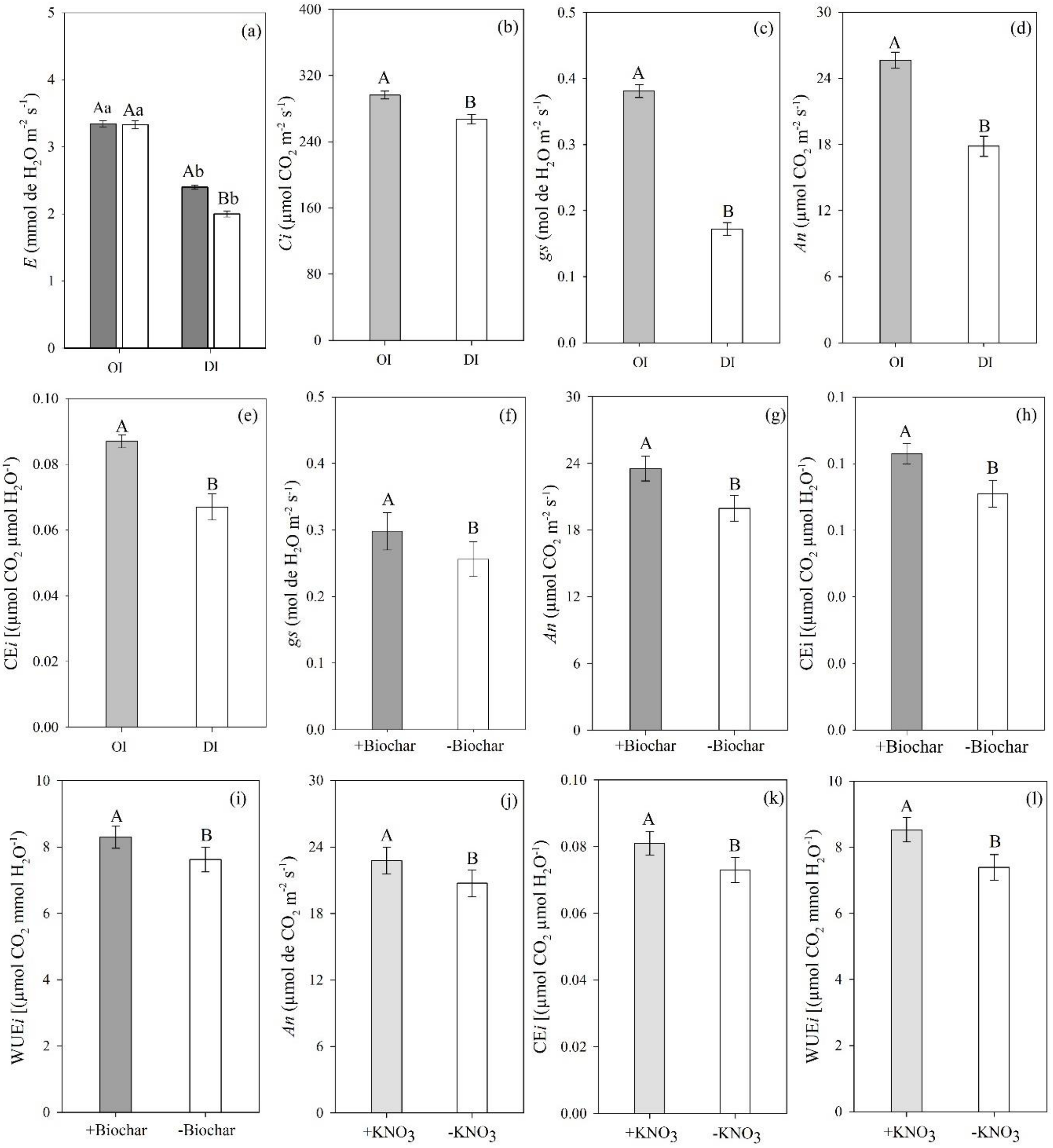

Figure 5. Transpiration rate (a) for irrigation x biochar interaction; internal CO_2 concentration (b), stomatal conductance (c), photosynthesis (d) and instantaneous carboxylation efficiency I in optimal irrigation and in deficit irrigation; stomatal conductance (f), photosynthesis (g),

instantaneous carboxylation efficiency (h) and instantaneous water use efficiency (i) for biochar levels; net photosynthesis (j), instantaneous carboxylation efficiency (k) and instantaneous water use efficiency (l) for KNO_3 levels, in tomato cultivated under different biochar levels (+Biochar and -Biochar), two KNO_3 foliar spray regimes (+KNO_3 and -KNO_3), and two irrigation regimes (OI and DI). Means followed by the same letter are not statistically different (Tukey's Test, $P<0.05$). In figure 5a, uppercase letters compare biochar treatments and lowercase letters compare irrigation treatments. Error bars show the mean standard error (a: n = 10 and bl: n = 20). Optimal irrigation (OI) and irrigation deficit (DI).

3.6. Spectroscopic measurements of chlorophyll fluorescence

The interaction among the three factors was not significant ($p>0.05$) for any of the variables evaluated by chlorophyll fluorescence spectroscopy (SPAD, LEF, ECSt, NPQt, Phi2, and PhiNPQ) (Table S5). The interaction between irrigation regimes x biochar was significant ($p<0.05$) for LEF, ECSt, NPQt, Phi2, and PhiNPQ. Biochar application increased ($p<0.05$) LEF (33 %) and ECSt (26 %) under OI (Figure 6a-b, respectively), and no significant effect was observed on NPQt and Phi2 under OI (Figure 6c-d, respectively). Significant ($p<0.05$) reductions were observed for LEF and ECSt (35 % and 27 %, respectively) under DI when biochar was added to the soil (Figure 6a-b), while ECSt and NPQt increased ($p<0.05$) under DI in the treatment without biochar (Figure 6b-c). In addition, biochar application reduced PhiNPQ by 7 % under OI and 44 % under DI (Figure 6e). Increase in PhiNPQ value under DI was verified only for biochar-untreated soil (Figure 6e). Considering irrigation regime only, it was observed that DI significantly ($p<0.05$) increased (23 %) SPAD values in plants, which reflects the chlorophyll content (Figure 6f). Interaction between biochar and foliar application of KNO_3 was significant only for ECSt, and in the absence of the foliar application of KNO_3, no effect was verified for both biochar (Figure 6g) and the opposite interaction, i.e., without biochar, no KNO_3 effect was observed. There was a 50 % increase in ECSt with KNO_3 and biochar applications, and 33 % decrease without biochar (Figure 6g). Foliar application of KNO_3 increased LEF by 14 % when compared to the treatment without KNO_3 (Figure 6h).

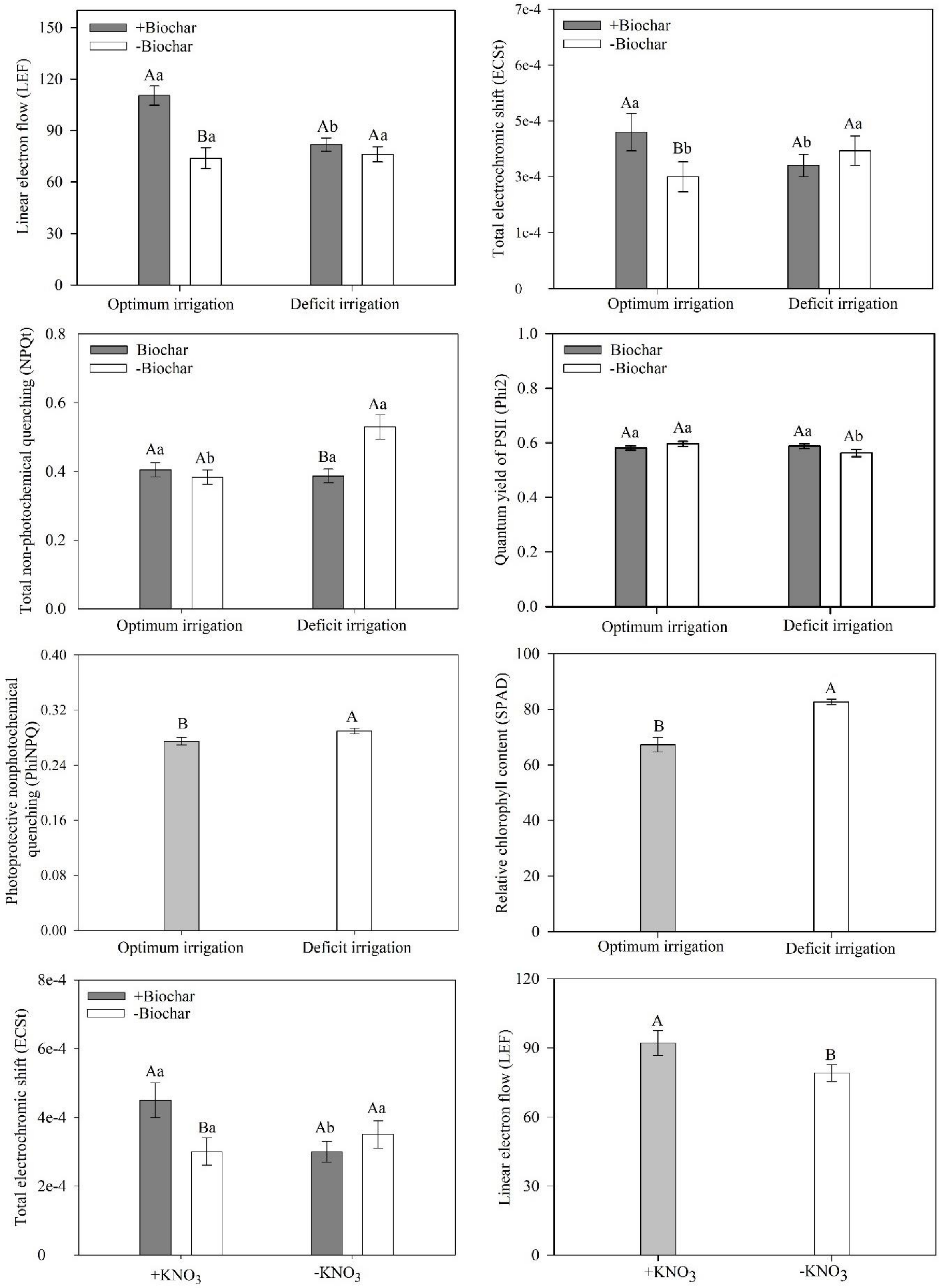

Figure 6. Total electron flux of PSII antenna complexes (a), total electrochromic shift (b), total non-photochemical quenching (c) and quantum yield for photochemistry of photosystem II (d) photoprotective non-photochemical quenching (e) for irrigation x biochar interaction; relative chlorophyll content (f) for irrigation levels; total electrochromic shift (g) for KNO_3 x biochar interaction and total electron flux from PSII antenna complexes (h) for KNO_3 levels, in tomato cultivated under different biochar levels (+Biochar and -Biochar), two foliar spray regimes with

KNO_3 (+KNO_3 and -KNO_3) and two irrigation regimes (OI and DI). Means followed by the same letter are not statistically different (Tukey's Test, $P<0.05$). In figures 6a-e uppercase letters compare biochar treatments and lowercase letters compare irrigation treatments. In figure 6g, uppercase letters compare biochar treatments and lowercase letters compare KNO_3 treatments. Error bars show the mean standard error (a-e and g: n = 10 and f and h: n = 20).

3.7. Soil chemical attributes after cultivation

Interaction of the three factors was significant ($p<0.05$) for phosphorus (P) contents (Table S6). Available P content was lower in treatments under OI when compared to DI, except for treatments combining both KNO_3 and biochar, which increased soil available P (Table 1). Conversely, no effect of biochar application was observed under DI conditions, regardless of the foliar application of KNO_3. Under OI, KNO_3 application promoted an increase in available P in biochar-amended soil compared to treatment -Biochar. On the other hand, under DI and -Biochar, KNO_3 application reduced available soil P when compared to treatment -KNO_3 and -Biochar.

Table 1. P contents in tomato cultivated under different levels of biochar (+Biochar and -Biochar), two foliar feeding regimes with K (+KNO_3 and -KNO_3) and two irrigation regimes (OI and DI)

Treatments	**P** (mg dm^{-3})			
	Optimum irrigation		Deficit irrigation	
	+KNO_3	-KNO_3	+KNO_3	-KNO_3
+Biochar	122.05 ± 4.88 Aa	66.83 ± 6.19 Bb	137.55 ± 1.70 Aa^{ns}	146.73 ± 10.85 Aa*
-Biochar	81.00 ± 4.16 Ba	90.15 ± 3.78 Aa	134.43 ± 7.74 Ab*	160.30 ± 5.89 Aa*

Lowercase letters compare with and without KNO_3 within each irrigation and biochar level. Uppercase letters compare with and without biochar within each irrigation level and KNO_3. ^{ns}non-significant for irrigation levels within each biochar and KNO_3 level. Data are expressed as mean plus standard error of mean (n=3).

The interaction between irrigation regimes x biochar was significant for K, Ca, Mg, and organic matter (OM) contents in soil. Lower K, Ca, Mg and OM values in treatments under OI were observed when compared with DI treatment in both +Biochar and -Biochar treatments (Table 2). Potassium content was lower ($p<0.05$) with biochar application under OI, while there was no difference under DI conditions ($p>0.05$) with or without biochar. Conversely, there was a reduction in Ca for the exchangeable Ca and Mg in biochar-treated soil, while soil OM was lower under OI and higher under DI in biochar-treated soil (Table 2).

Foliar application of KNO_3 reduced exchangeable Ca content in soil without biochar application while biochar application reduced soil Ca content without foliar application of KNO_3.

Table 2. Contents of K, Ca, Mg, OM, and pH, in tomato cultivated under different levels of biochar (+Biochar and -Biochar), two levels of foliar fertilization with KNO_3 (+KNO_3 and -KNO_3), and two irrigation regimes (OI and DI)

	K (mg dm^{-3})		
Biochar treatments	Optimum irrigation	Deficit irrigation	Biochar overall mean
+Biochar	132 ± 7 Bb	321 ± 19 Aa	227
-Biochar	193 ± 17 Ab	329 ± 15 Aa	261
Irrigation overall mean	163	325	
	K (mg dm^{-3})		
KNO_3 treatments	Optimum irrigation	Deficit irrigation	KNO_3 overall mean
+KNO_3	186 bA	352 aA	218
-KNO_3	139 bb	298 aB	269
Irrigation overall mean	163	325	
	Ca (mg dm^{-3})		
Biochar treatments	Optimum irrigation	Deficit irrigation	Biochar overall mean
+Biochar	3.56 ± 0.08 Ab	3.97 ± 0.05 Ba	3.77
-Biochar	3.36 ± 0.13 Ab	4.30 ± 0.10 Aa	3.83
Irrigation overall mean	3.46	4.14	
	Ca (mg dm^{-3})		
Biochar treatments	+KNO_3	-KNO_3	Biochar overall mean
+Biochar	3.82 ± 0.11 Aa	3.71 ± 0.12 Ba	3.76
-Biochar	3.64 ± 0.24 Ab	4.03 ± 0.21 Aa	3.84
KNO_3 overall mean	3.73	3.87	
	Mg (mg dm^{-3})		
Biochar treatments	Optimum irrigation	Deficit irrigation	Biochar overall mean
+Biochar	1.33 ± 0.04 Ab	1.57 ± 0.06 Ba	1.45
-Biochar	1.21 ± 0.05 Ab	1.75 ± 0.05 Aa	1.48
Irrigation overall mean	1.27	1.51	
	OM (%)		
biochar treatments	Optimum irrigation	Deficit irrigation	Biochar overall mean
+Biochar	2.49 ± 0.02 Ab	2.64 ± 0.03 Aa	2.57
-Biochar	2.44 ± 0.04 Aa	2.35 ± 0.07 Ba	2.40
Irrigation overall mean	2.47	2.50	

Biochar treatments	pH Optimum irrigation	Deficit irrigation	Biochar overall mean
+Biochar	6.27	6.11	6.19 A
-Biochar	6.06	5.86	5.96 B
Irrigation overall mean	6.16	5.98 b	

The same lowercase letters indicate that irrigation levels (OI and DI) or KNO_3 levels (+KNO_3 and - KNO_3) did not differ by F test or Tukey test (P>0.05). The same uppercase letters indicate that there was no statistical difference between the biochar levels (+Biochar and -Biochar) for the variable (p>0.05). Data are expressed as mean ± standard error.

4. DISCUSSION

Considering future projections for population growth, water scarcity and soil fertility degradation worldwide (Guo et al., 2021), strategies for sustainable soil and water use are urgently needed for tomato growers. Droughts reduce fruit size and yield, and hence profit (Petrović et al., 2019). Thus, sustainable water resource use and alternative fertilizer sources are essential for the design of future agricultural systems. Results from this research confirmed the biochar role as a soil conditioner and an effective K source for plants when using a K-rich feedstock (e.g., coffee husk). An improvement of the photosynthetic apparatus was observed with biochar addition to soil, improving yields and tomato fruit quality in response to different soil water regimes.

4.1. Biochar characteristic parameters

Biochar plays a vital role in improving soil chemical and physical properties, facilitating growth and increasing crop yields (Liu et al., 2014). This research study revealed that soil biochar addition increased soil water holding capacity (Θcc increased from 0.27 to 0.29 kg kg^{-1} after biochar application) and improved Ψ_{leaf} measured at pre-dawn under DI condition. This result of higher water storage capacity with biochar is due to biochar's porous structure, high surface area, and oxygen functional groups that help to form cohesion and adhesion forces (Suliman et al., 2017; Razzaghi et al., 2020), as observed by other researchers in soil amended with coffee husk biochar (Kiggundu and Sittamukyoto, 2019). The high surface area of biochar, as well as its high porosity, can retain water that runs off during an infiltration process (Uzoma et al., 2011). Furthermore, the increase in Θcc in clayey soil used in the present study may be related to small reductions in the soil's apparent density. Significant changes in soil water retention close to water saturation, also in clayey soil, were verified by other authors (Castellini et al., 2015). In addition, coffee husk biochar acts as a plant nutrient source, mainly as a K source (Table S3).

4.2. Biochar improves yield parameters of tomato plants grown under both deficit irrigation and well-watered conditions

Yield parameters (PY and FW) were significantly reduced under DI when compared with OI. This is due to the fact that tomato requires large amounts of water to grow well and is negatively affected by water stress (Klunklin and Savage, 2017), which impairs plant growth, photosynthesis, transpiration rate, and yield (Agbna et al., 2017).

Low soil water availability limits water plant uptake, thus causing a reduction in turgor pressure. This impacts cell division and inhibits plant growth and development (Dariva et al., 2021; Ahanger et al., 2021). Furthermore, deficit irrigation causes changes in absorption and translocation processes of nutrients (Ahanger et al., 2021), reduces photosynthetic rate (Rasheed et al., 2015; Avila et al., 2016), increases production of reactive oxygen species (Hasanuzzaman et al., 2020; Rasheed et al., 2021), and leads to severe oxidative stress that can damage lipids, proteins, and nucleic acids (Zhang et al., 2016). Thus, it is expected that reducing water application to tomato plants decreases fruit yield, as observed in this study, and in several other studies with tomato which have also reported yield losses due to limited soil water availability (Agbna et al., 2017; Medyouni et al., 2021; Klunklin and Savage, 2017).

Soil biochar addition improved total fresh fruit yield per plant in both water regimes (OI and DI), and also improved FW under OI. Similar results regarding the increase in tomato yield through the application of biochar were reported in other studies (Almaroai & Eissa, 2020). These positive effects on tomato yield are mainly due to biochar's physical structure that helps to form cohesive and adhesion forces (Suliman et al., 2017; Razzaghi et al., 2020), thereby improving soil water storage potential (Villagra-Mendoza and Horn, 2018). Furthermore, high CEC of coffee husk biochar can improve nutrient retention and soil fertility by increasing extractable nutrient contents such as K, Ca, and Mg (Laird et al., 2010). The application of biochar has been proven to boost plant growth by providing essential nutrients, improving water and nutrient retention capacity (Akhtar et al., 2014, Glazer et al., 2002), as well as increasing CEC, which in turn reduces the leaching of cationic nutrients. This combination of traits improves the soil-plant relationship and results in faster plant growth (Glaber et al., 2012; Laird et al., 2010). Moreover, biochar can increase fruit production by increasing leaf photosynthesis while reducing CO_2 losses due to increased activity of antioxidant enzymes, such as superoxide dismutase, peroxidase, catalase, and ascorbate peroxidase (Khan et al., 2021).

It was found that increased PY was more evident in OI (+11 %) than under DI (+6 %), but a positive effect on FW (+14 %) was only obtained under OI, indicating that despite the increased soil moisture caused by biochar, this was not the main reason for positive effects on PY. A smaller increase in PY and FW under DI conditions may have been caused by stress degree and duration (Chen et al., 2013). Moderate water stress at the vegetative stage has been shown to not affect tomato yields despite yield reductions when the stress occurs at flowering and fruiting stages (Ghannem et al., 2021). The deficit irrigation evaluated in this study can be considered severe and resulted in average reductions of 60 % and 57 % in PY and FW, respectively. High yield losses can be explained not only by stress degree, but also by the fact that plants spent their whole cycle under DI conditions. Similar results have been observed for maize, where biochar retained soil moisture under deficit irrigation conditions, but the increase in yield was greater in plants under optimum irrigation (Pandit et al., 2018).

4.3. Biochar under deficit irrigation improves tomato fruit quality and foliar application of KNO_3 improves fruit firmness

Despite losses in PY and FW, an improvement was observed in TSS and TA of fruits in plants under deficit irrigation. As observed in other studies, deficit irrigation seems to improve tomato quality attributes (Lu et al., 2019) due to a reduced water accumulation in fruits causing an increase in sugar and acid concentrations (Petrović et al., 2019). Unexpectedly, TSS/TA was lower under DI regime, which can be explained by the high increase in citric acid levels in fruits. Similar results were observed in tomato regarding citric acid content, with higher values under DI than under OI (Dariva et al., 2021). Despite lower TSS/TA values under DI conditions, it is observed that in both irrigation regimes TSS/TA values are within an ideal range (8-12) for tomatoes (Divéky-Ertsey et al., 2012).

Biochar application improved TSS/TA under OI without KNO_3 and under DI conditions with KNO_3 and reduced pH value. Biochar application also improved tomato fruit quality and flavor, as indicated by higher values of SS and TSS/TA (Agbna et al., 2017). It was also found that deficit irrigation caused a reduction in lycopene contents when compared to fruits of plants grown under OI. Lycopene is the main carotenoid present in tomato fruit, accounting for 80 % of total carotenoid content. Its accumulation occurs in the final ripening stage as an orange-red pigment (Klunklin and Savage, 2017) and has been shown to prevent cancer (Van Breemen and Pajkovic, 2008) and cardiovascular diseases (Kris-Etherton et al., 2002).

Soils amended with biochar increase fruit lycopene content, even under DI (Figure 3e). This result may be associated with better nutritional conditions provided by biochar presence in soil, especially related to increased K availability. An increase in lycopene content is directly related to balanced fertilization, especially K and Ca (Knee, 2002), which may be due to a gradual release of nutrients by biochar which acts as an efficient fertilizer alternative (Peng, 2015; Shrestha and Pandit, 2017; Carneiro et al., 2021) and positively impacts nutrition of tomato plants and, consequently, fruit lycopene concentrations, as observed when combining biochar and *Trichoderma* (Sani et al., 2020). In a study on the use of biochar in metal contaminated soils, it was observed that the application of 10 t ha^{-1} promoted significant increases in TA (33%), TSS (29%), vitamin C (39%) and lycopene (24%) in tomato juice, compared to the control treatment (Almaroai & Eissa, 2020). The authors indicated that this increase may be associated with the improvement in the availability and absorption of essential nutrients from the soil caused by biochar.

Fruit firmness is a key attribute to ensure a longer shelf life as well as greater tolerance to long-distance transport (Dariva et al., 2021). Consumers prefer fruits that are firm to the touch and do not deform easily (Ferreira, 2010). Foliar application of KNO_3 increased tomato fruit firmness and soluble sugars under OI (without biochar) and under DI (with biochar). Such results may be due to greater translocation of photoassimilates to the fruits caused by the foliar application of K, thus increasing soluble sugar content and fruit resistance, or even by the increase in cell turgidity related to the pressure potential of fruit tissue (Lester et al., 2006). In addition, K optimizes Ca assimilation by making a balance with N and P (Rees et al., 2012). Although K is not a structural element in plant tissues, it can increase fruit Ca content by increasing phloem transport, which helps to increase fruit firmness (Jifon and Lester, 2009).

4.4. Leaf temperature and leaf water potential

Despite Despite the fact that leaf temperatures (LT) found in this study were within the temperature range (10 and 35 °C) for tomato development (Alvarenga, 2013), it was observed that a reduction from 90 % (OI) to 50 % (DI) of available soil water caused approximately 4°C increase in LT (Figure 4a). Tomato plants grown in low soil moisture conditions are unable to obtain water from soil to meet the evapotranspiration demands. Excessively high LT first compromises stomatal opening and can subsequently compromise the efficiency of photosynthetic apparatus by damaging proteins with consequent reduction in photosynthesis and water use efficiency (Lu et al., 2017). Soil water deficit also negatively affected the Ψ_{leaf} at midday evaluation, which indicates a strong loss of leaf turgor.

An ability of plants grown in biochar-treated soil to preserve water under low soil moisture content was clearly demonstrated by their higher Ψ_{leaf} value (-0.3 MPa with biochar vs -0.4 MPa -Biochar). This greater leaf tissue hydration recorded in plants treated with biochar under deficit irrigation may be associated with better stomatal control of water loss, as well as improved soil moisture conditions. A drastic decrease in leaf water potential influences the Rubisco carboxylation efficiency and reduces internal CO_2 concentration (*Ci*) due to stomatal opening limitation (Cornic and Massagi, 1996), consequently reducing photosynthesis rate.

Similar results regarding increased leaf water status were also reported in rice cultivation in saline-sodic soil treated with biochar (Ran et al., 2021). The water retained in the porous biochar particles has the potential to stimulate the proliferation and development of roots, essential for maintaining leaf turgor pressure, stomatal density, and photosynthetic process. This becomes especially crucial during periods of water stress because roots represent the only transport route for water and nutrients to the plant (Khan et a., 2021).

4.5. Gas exchange parameters in response to biochar and KNO_3 under different irrigation regimes

Deficit irrigation negatively affected tomato plants gas exchange parameters, which is consistent with other study (Dariva et al., 2020). Water deficiency restricts stomatal opening, which decreases CO_2 fixation rates by reducing Rubisco synthesis and activity, thereby either reducing RuBP regeneration or ATP synthesis (Medrano, 2002). Decreased photosynthesis results in a decrease of metabolites available for plant development which affects plant performance and yield (Agbna et al., 2017).

In the present study, deficit irrigation was severe during the entire crop cycle (50 % ASW), and yet biochar soil amendment improved tomato plants photosynthetic performance, increasing stomatal conductance, net photosynthesis, instantaneous carboxylation efficiency, and instantaneous water use efficiency, all of which may indicate lower plant stress (better soil moisture condition). Water stored in porous biochar-soil particles can maintain leaf turgor pressure and photosynthesis, alleviating deficit irrigation's adverse effects (Khan et al., 2021). Biochar application also improved shoot biomass production of tomato seedlings, as well as supported water relations and leaf gas exchange rates under different irrigation levels (Guo et al., 2021). The water stored in the pores of biochar particles can increase the water retention capacity of plants, facilitating a continuous and timely supply of water for uptake. This increase in water uptake results in improved photosynthetic process and increased antioxidant activity that combats the accumulation of reactive oxygen species. Increases in the activities of the enzymes superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) were observed, reducing the levels of hydrogen peroxide (H_2O_2), electrolyte leakage (EL) and malondialdehyde

(MDA), indicating a system of robust defense in plants treated with biochar compared to those without biochar, especially under drought stress conditions (Khan et al., 2021). In another study, the application of biochar was also shown to improve the activity of antioxidant enzymes, reducing the overaccumulation of ROS in situations of water stress (Hafez et al., 2020).

Under water stress conditions, plants tend to synthesize and accumulate low molecular weight compounds such as free amino acids, free proline, non-structural carbohydrates, and quaternary ammonium compounds, so as to maintain the potential gradient between cell and external solution (Ahanger and Agarwal, 2016). Additionally, K ions contribute to maintaining the tissue water content by reducing stress susceptibility (Jatav et al., 2014). K application also increases hydraulic conductivity, which benefits the plant in maintaining cell turgor, stomatal conductance, and gas exchange (Oddo et al., 2012).

4.6. Chlorophyll fluorescence

Linear electron flow (LEF) is a photosynthesis indicator that reflects the amount of energy that is moving around the chloroplast after light exposure. Under OI conditions, biochar application promoted a higher LEF value, which corroborates the IRGA photosynthetic rate data (infrared gas analyzer). However, no significant biochar effect was observed under DI conditions. Similar behavior was observed for electrochromic shift (ECS) which reflects changes in electric field across the thylakoid membrane and influences accumulation of thylakoid proton motive force by photochemistry and its subsequent utilization by ATP synthesis (Cruz et al., 2001). This higher ECS value may indicate better internal CO_2 levels (Kanazawa and Kramer, 2002) and better plant health.

Under deficit irrigation conditions, plants grown in biochar-treated soil showed lower non-photochemical quenching (NPQt), which protects higher plants' photosynthetic apparatus from photodamage. NPQt is triggered by acidification of the thylakoid lumen as a result of light-induced proton pumping, which also drives ATP synthesis (Kanazawa and Kramer, 2002). An increase in NPQt in plants grown in soil without biochar under DI conditions may occur in response to increased water stress. NPQt increases when photosynthesis is limited by availability of oxidized nicotinamide-adenine dinucleotide phosphate ($NADP^+$) (Heber and Walker, 1992), In absence of NPQ modulation, the accumulation of reduced electron carriers would block LEF before the lumen is significantly acidified. Accumulation of excessive reducing power can result in the formation of reactive oxygen species, which damage photosynthetic apparatus components.

Lower ΦII values were observed in plants grown under DI without biochar application. ΦII represents the amount of light received by the leaf that goes to photosystem II, which is then converted into carbohydrates. Thus, this reinforces that plants submitted to deficit irrigation reduce carbohydrates formation, and biochar application can reduce such an effect.

PhiNPQ represents the amount of light dissipated as heat or other energy forms, thereby preventing leaves from being photodamaged. A higher PhiNPQ value was observed in plants grown in soil without biochar and under DI conditions, which was already expected. In conditions of low soil moisture, plants tend to close their stomata to reduce CO_2 concentration inside cells with a consequent reduction in CO_2 fixation rate. Thus, by reducing the CO_2 fixation rate due to reduced inner cell substrate, part of the intercepted irradiance is lost as heat. SPAD indicates the intensity of green, which is a direct representation of the relative chlorophyll content. Higher SPAD values were observed under DI, probably due to a concentration effect caused by reduced plant growth under DI conditions.

ECSt increased when combining biochar application in soil and foliar application of KNO_3, which reflects on better internal CO_2 levels and the accumulation of the photochemical thylakoid proton motive force for ATP synthesis. This positive effect of KNO_3 application on photosynthesis is also reinforced by a higher LEF value, which indicates photosynthesis improvement. A beneficial K effect under water deficit conditions has been reported by several authors (Kanai et al., 2011; Ahanger and Agarwal, 2016; Shehzad et al., 2020), and foliar application of KNO_3 has promoted significant gains in terms of productivity (Gimeno et al., 2014; Fayez and Bazaid, 2014). For instance, under drought conditions, sorghum plants fertilized with KNO_3 in the pre-flowering phase showed higher chlorophyll content, photosynthetic rate, stomatal conductance, transpiration, carboxylation efficiency, higher contents of P, K, Mg, S, Cu and Fe, in addition to a 32.2 % increase in grain yield (Ávila, 2022).

4.7. Soil nutrient availability

Reduced levels of available nutrients in soil might indicate greater extraction by plants since all treatments received equivalent nutrient amounts. Nutrient uptake was drastically affected under deficit irrigation treatment, which resulted in lower uptake and higher levels of available nutrients in soil at the end of the crop cycle. Low water availability in soil restricts plant photosynthetic processes with a consequent reduction in biomass accumulation. Due to reduced transpiration and impaired plant development, nutrient uptake is greatly affected. Lower N levels in forage species under water deficit has been observed regardless of the supplied N source (Kunrath et al., 2018). Also, reduced P uptake in soybean plants (*Glycine* *max* (L.) Merr.) cultivated under water deficit conditions has been reported (Gutiérrez-boem and Thomas, 1999).

Biochar application to soil also promoted greater nutrient uptake by tomato plants, especially K, Ca, Mg, and P when KNO_3 was not applied. Biochar seems to have synergistic effects with inorganic fertilizers on crop productivity, as observed for higher (+4 %) corn grain yield with 1.5 t ha^{-1} of biochar combined with NPK compared to NPK only (Peng et al., 2021), or a 26 % increase in corn yield for biochar-based fertilizer containing urea and additives in granulated form compared with conventional urea under tropical conditions (Puga et al., 2020). Thus, biochar can act as a matrix to increase fertilizer use efficiency to a greater extent than conventional fertilizer alone (Melo et al., 2022). In a study carried out on soil contaminated by heavy metals, it was found that the application of biochar at a rate of 10 t ha^{-1} significantly increased the availability of nitrogen (67%), phosphorus (54%) and potassium (43%), in addition to promoting an increase in the uptake of nitrogen (67%) and potassium (56%) compared to the control treatment (Almaroai & Eissa, 2020). This increased nutrient uptake from biochar may also be related, among other factors, to greater plant root growth. An increase in the growth of tomato plants grown in soil treated with biochar was observed, and the authors attributed these gains to the higher concentrations of NO_3, P, Ca^{2+} and Mg^{2+} present in the growing media.

Foliar application of KNO_3 promoted lower soil K uptake by tomato, which was already expected since the foliar application met some of the demand for K. Potassium uptake greatly influences crop productivity and is especially important for tomato crops as K is the most demanded nutrient (Fayad et al., 2002). In addition, foliar application of KNO_3 increased Ca extraction by tomato in treatments without biochar. Calcium is a structural component of cell walls and membranes, and in the ionic form (Ca^{2+}), it acts as second messenger, playing an important role in plant physiology regulation, specifically in post-harvest fruits and vegetables (Gao et al., 2019). Calcium deficiency in tomato plants can lead to a physiological disorder known as apical rot (Suzuki et al., 2003), characterized by water-soaked tissue that eventually turns dark brown due to an increase in membrane permeability, followed by plasmolysis and death.

5. CONCLUSION

Deficit irrigation had a negative effect on tomato's physiological and yield parameters, although improved fruit quality. Biochar application alleviated tomato stress under deficit irrigation, and plants showed better functioning of the photosynthetic apparatus, greater yield, and fruit quality, as well as greater water use efficiency.

Biochar from coffee husk (a K-rich feedstock) could fully meet the tomato plants' demand for K and partially meet the demand for other elements such as P, Mg, Fe, Zn, Mn, and Cu. Therefore, it can be considered a biochar-based fertilizer and alternative K source, which also provides stable carbon to soil and helps to mitigate stress caused by prolonged droughts as shown in this study.

KNO_3 did not improve the yield parameters of tomato plants, and neither had a synergistic effect with biochar.

Despite the beneficial effects of biochar on the physiological, qualitative and yield parameters of tomato plants, the effect is especially more pronounced under adequate soil moisture conditions (90 % ASW). Therefore, new further studies are necessary to better understand the mechanisms involved in stimulating tomato growth and quality promoted by biochar.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Departamento de Agronomia and Programa de Pós-graduação em Fitotecnia at Universidade Federal de Viçosa (UFV), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG).

Funding: This study was financed in part by the "Conselho Nacional de Desenvolvimento Científico e Tecnológico - Brasil (CNPq)".

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Supplementary material

Improvement of tomato growth by biochar and potassium foliar application under contrasting irrigation regimes

Table S1. Chemical soil attributes before applying amendments.

pH	**P**	**K**	**Ca^{2+}**	**mg^{2+}**	**Al^{3+}**	**H+Al**	**CEC**	**OM**	**Zn**	**Fe**	**Mn**	**Cu**	**Θcc**[1]	**Θcc**[2]
-H_2O-	mg dm^{-3}		------------- $Cmol_C\ dm^{-3}$ --------------					-%-	---------mg dm^{-3} --------				-----%-----	
5.1	1.1	57	1.16	0.39	0.3	4.95	6.6	2.15	0.2	34.9	18.0	2.1	27	29

Θcc[1] = Pot capacity measured before experiment installation; **Θcc**[2] = Pot capacity measured at the end of the experiment in pots treated with biochar.

Table S2. Nutrient solution for biochar-treated and -untreated soils for tomato cultivation

Nutrient	Nutrient solution – Treatment with biochar	Nutrient solution - Treatment without biochar
	mg/kg of soil	mg/kg of soil
N	400[1]	400[1]
P	296.98[3] + 3.02[2]	300[3]
K	0[1] + 688[2]	688[1]
S	40[1]	40[1]
B	0.81[1]	0.81[1]
Cu	1.325[1] + 0.0051[2]	1.33[1]
Fe	3.4[2]	3.4[1]
Mn	3.51[1] + 0.15[2]	3.66[1]
Mo	0.15[1]	0.15[1]
Zn	2.24[1] + 1.76[2]	4.00[1]

[1] Via reactant

[2] Via Biochar

[3] Application directly to the soil via triple superphosphate fertilizer (36% P_2O_5 and 10% Ca).

Table S3. Nutrient composition of coffee husk biochar.

Analysis	P	K	Ca^{2+}	Mg^{2+}	S	Al^{3+}	Zn	Fe	Mn	Cu
	……g kg $^{-1}$……						……mg/kg……			
Biochar (total nutrient content)	2.7 ± 0.1	68.8 ± 1.5	8.7 ± 0.3	3.8 ± 0.07	1.8 ± 0.02	6.5 ± 0.3	455.0 ± 56	4873.3 ± 210	199.0 ± 27	46.9 ± 1.3
Biochar (nutrient content available)	0.3 ± 0.0	30.9 ± 0.6	3.2 ± 0.1	0.44 ± 0.08	-	-	175.7 ± 7.2	340.5 ± 12.2	15.1 ± 0.6	0.5 ± 0.03
Physicochemical Attributes	yield g/kg	EC dS/cm	pH (H_2O)	C	H	N	O	ashes	Θ_{CC}	Θ_{PMP}
				……%……					……kPa……	
	341.2	5.1 ± 0.06	11.9 ± 0.02	37.5	2.4	0.4	31.5	28.2 ± 1.5	1.05	0.72

Phosphorus (P); potassium (K), calcium (Ca); magnesium (Mg); sulfur (S); aluminum (Al), zinc (Zn); iron (Fe); manganese (Mn); copper (Cu); productivity (Yield); electrical conductivity (EC); total acidity (pH); carbon (C); hydrogen (H); nitrogen (N); oxygen (O); field capacity (Θ_{CC}) and permanent wilting point (Θ_{PMP}).

Table S4. Analysis of variance of characteristics production and quality of fruit from tomato under different biochar levels (+Biochar and -Biochar), two foliar K fertilization regimes (+KNO_3 and -KNO_3) and two irrigation regimes (OI and DI)

SOURCE OF VARIATION	DF	Significance of effects							
		PY	FW	FF	SS	pH	AT	TSS/AT	Lyc
BLOCKS	4	ns	ns	ns	ns	ns	ns	ns	ns
IRRIGATION (IR)	1	**	**	ns	**	**	**	**	**
BIOCHAR (BIO)	1	*	ns	ns	ns	**	*	*	**
KNO_3	1	ns	ns	*	ns	ns	ns	ns	ns

IR × BIO	1	ns	*	ns	ns	ns	*	ns	ns
IR × KNO_3	1	ns	ns	ns	ns	ns	ns	ns	ns
BIO × KNO_3	1	ns	ns	ns	ns	ns	ns	ns	ns
IR × BIO × KNO_3	1	ns	ns	ns	*	ns	ns	**	ns
CV (%)		12.29	15.28	25.69	10.99	1.13	11.0	16.68	24.16

DF = Degrees of freedom; total plant productivity (PY); average fruit weight (FW); fruit firmness (FF); total solids soluble (TSS); titratable acidity (TA); lycopene (Lyc). ns = nonsignificant; ** and * = significant at 0.01 and 0.05 probability by the F test.

Table S5. Analysis of variance of characteristics physiological and potential leaf water of tomato under different biochar levels (+Biochar and -Biochar), two foliar K fertilization regimens (+KNO_3 and -KNO_3) and two irrigation regimens (OI and DI).

SOURCE OF VARIATION	GL	Significance of effects														
		LT	LWP_pd	LWP_m	*Ci*	*AND*	*gs*	*An*	CE*i*	WUE*i*	SPAD	LEF	ECst	NPQt	Phi2	PhiNPQ
BLOCKS	4	**	**	*	**	*	**	**	ns	**	ns	ns	**	*	ns	**
IRRIGATION (IR)	1	**	**	**	**	**	**	**	**	ns	*	**	ns	**	ns	**
BIOCHAR (BIO)	1	ns	*	ns	ns	*	**	**	**	*	ns	**	ns	*	ns	*
KNO $_3$	1	ns	*	ns	ns	ns	ns	*	*	**	ns	**	*	ns	ns	ns
IR × BIO	1	ns	*	ns	ns	*	ns	ns	ns	ns	ns	**	**	**	*	**
IR × KNO $_3$	1	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
BIO × KNO $_3$	1	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**	ns	ns	ns
IR × BIO × KNO $_3$	1	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
CV (%)		2.22	23.53	14.78	5.78	9.52	17.36	12.04	15.29	12.54	11.68	15.17	23.46	16.69	4.91	13.9

GL = Degrees of freedom; potential foliar fluid measured in the period pre-dawn (3:00-5:00pm) (LWP_pd); potential foliar fluid measured in the afternoon (noon−1:00 pm) (LWP_m); leaf temperature (LT); intracellular CO_2 concentration (*Ci*); transpiration rate (*E*); stomatal conductance (*gs*); total net photosynthesis (*An*); carboxylation efficiency (CE*i*); instantaneous water use efficiency (WUE_i); relative chlorophyll content (SPAD); total flow of electrons from the complexes PSII antennas (LEF); total electrochromic shift (ECSt); total non-photochemical quenching (NPQt); Yield quantum for photosystem II photochemistry (Phi2); photoprotective non-photochemical quenching (PhiNPQ); ns = nonsignificant ; ** and *= significant at 0.01 and 0.05 probability by the F test.

Table S6. Analysis of variance of characteristics soil chemicals cultivated with tomato under different biochar levels (+Biochar and -Biochar), two foliar K fertilization regimens (+KNO_3 and -KNO_3) and two irrigation regimens (OI and DI).

SOURCE OF VARIATION	GL	Significance of effects											
		pH	P	K	Ca	Mg	H	SB	t	T	V	OM	P-Rem
BLOCKS	4	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns
IRRIGATION (IR)	1	**	**	**	**	**	ns	**	**	**	**	ns	ns
BIOCHAR (BIO)	1	**	ns	**	ns	ns	**	ns	ns	**	**	**	ns
KNO_3	1	ns	ns	**	*	ns	*	ns	ns	ns	ns	ns	ns
IR × BIO	1	ns	ns	*	**	**	*	**	**	ns	**	*	ns
IR × KNO_3	1	ns	**	ns	ns	ns	ns	ns	ns	*	ns	ns	ns
BIO × KNO_3	1	ns	**	ns	**	ns	ns	*	*	ns	ns	ns	ns
IR × BIO × KNO_3	1	ns	*	ns	ns	ns	**	ns	ns	*	*	ns	*
CV (%)		1.28	9.01	9.47	4.23	7.94	5.56	3.98	3.95	2.66	4.42	4.64	7.00

GL = Degrees of freedom; total acidity (pH); phosphorus (P); potassium (K), calcium (Ca); magnesium (Mg); hydrogen (H); sum of bases (SB); effective CEC (t); potential CEC at pH 7.0 (T); saturation percentage by CEC bases at pH 7.0 (V); organic matter (OM); remaining phosphorus (P-REM). ns = nonsignificant; ** and * = significant at 0.01 and 0.05 probability by the F test.

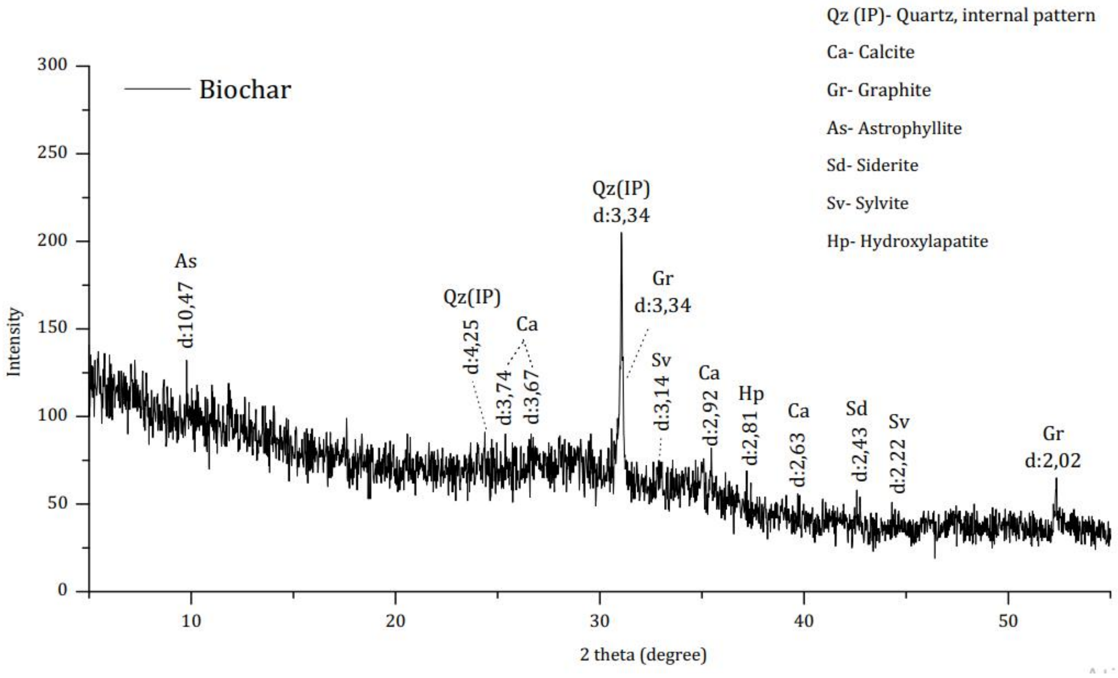


Figure S1. X-ray diffraction analysis of coffe husk-based biochar

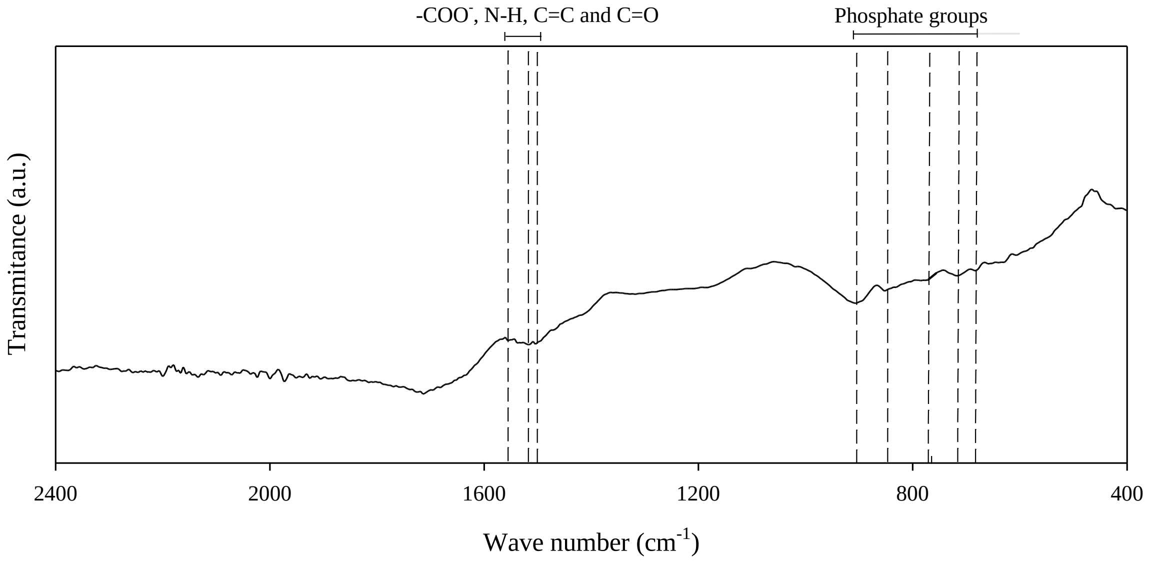
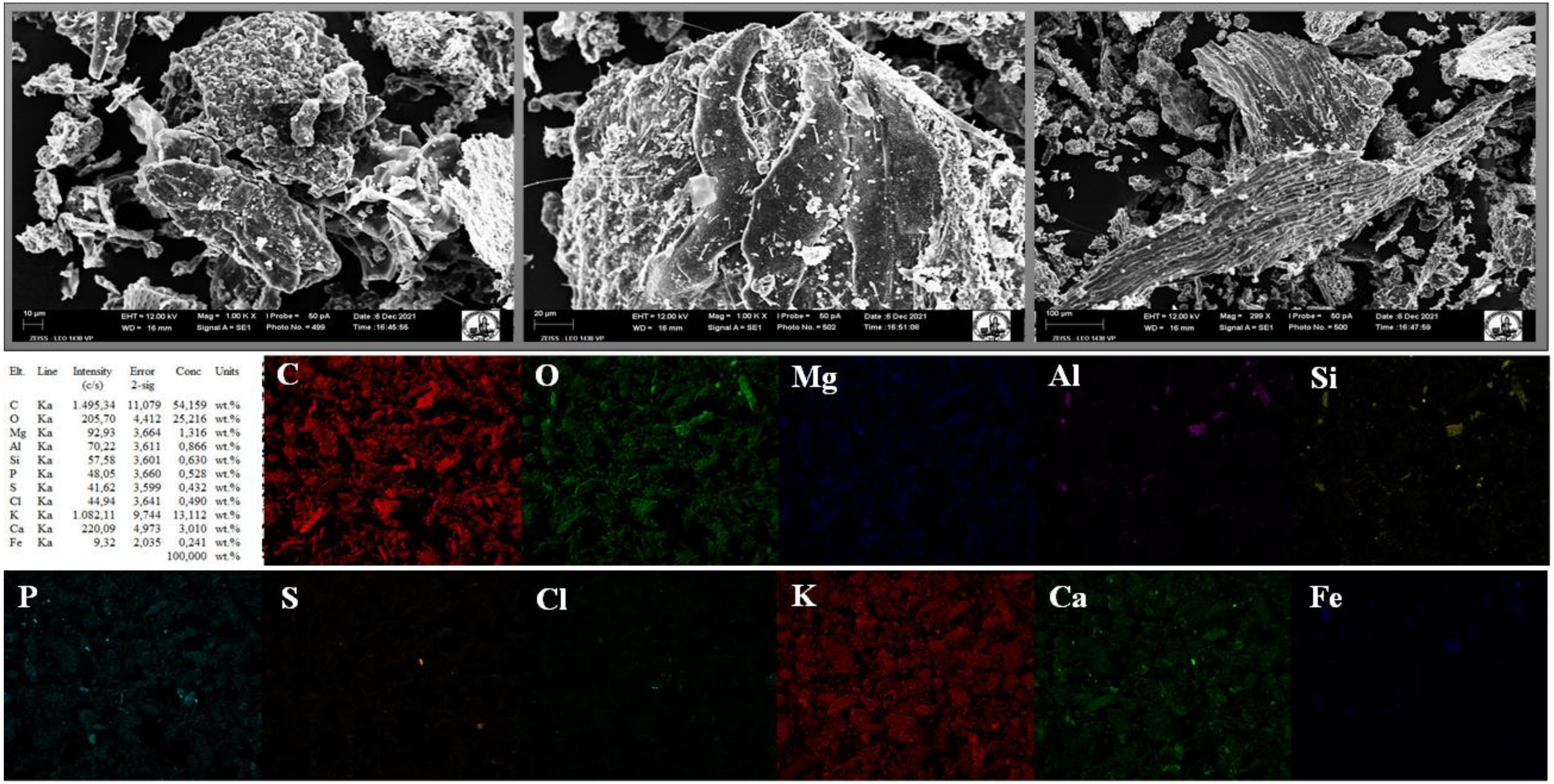


Figure S2. Fourier-transform infrared spectroscopy (FTIR) in biochar from coffee husks.



Elt.	Line	Intensity (c/s)	Error 2-sig	Conc	Units
C	Ka	1.495,34	11,079	54,159	wt.%
O	Ka	205,70	4,412	25,216	wt.%
Mg	Ka	92,93	3,664	1,316	wt.%
Al	Ka	70,22	3,611	0,866	wt.%
Si	Ka	57,58	3,601	0,630	wt.%
P	Ka	48,05	3,660	0,528	wt.%
S	Ka	41,62	3,599	0,432	wt.%
Cl	Ka	44,94	3,641	0,490	wt.%
K	Ka	1.082,11	9,744	13,112	wt.%
Ca	Ka	220,09	4,973	3,010	wt.%
Fe	Ka	9,32	2,035	0,241	wt.%
				100,000	wt.%

Figure S3. Images and chemical mapping of coffee husk-based biochar

CHAPTER 2. Impact of the addition of biochar and foliar KNO_3 on physiology, growth and root biometric parameters of tomato cultivated under different water regimes

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* *Published in full in the magazine* ***Scientia Horticulturae (Impact Factor: 4.3; Qualis classification: A1)*** *DOI: https://doi.org/10.1016/j.scienta.2024.113186*

ABSTRACT

Improving water use efficiency in agricultural production is mandatory either due to the high-water demand or due droughts caused by climate change. Looking for efficient strategies of soil and plant management is needed. Biochar is a carbon-rich material that has capacity to improves soil water retention and increase agricultural yields. Tomato is highly susceptible to water stress and the combination of biochar application in soil associated with foliar potassium nitrate (KNO_3) is a promising strategy to mitigate water stress. The present study aimed to investigate the effects of adding biochar and foliar KNO_3 application under different irrigation regimes (50 and 90% of available soil water - ASW) on physiology, growth, biomass accumulation, and root biometric parameters of tomato. The results of this study showed water deficit (50% of ASW) severely affected plant development. Conversely, beneficial effects on physiology, growth, biomass accumulation, and biometric parameters of root system were observed in plants grown in soil treated with biochar, regardless of the water level in soil. The study also showed foliar KNO_3 application did not alleviate the negative effects of water stress on tomato plant, nor did it have a synergistic effect with biochar.

Keywords: Climate Change; Drought tolerance; Pyrolysis; Vegetables; Root growth; Increase in plant biomass.

1. INTRODUCTION

One of the greatest challenges of modern agriculture is to increase food production by using water more efficiently (Gong et al., 2019; Khapte et al., 2019). Controlled deficit irrigation (DI) is a strategy that aims to reduce the amount of water applied without significant reductions in yields (Pereira et al., 2002). However, some crops, such as tomato, are sensitive to drought conditions, which significantly affects growth and yield (Khapte et al., 2019; Dariva et al., 2021). Therefore, research is needed to assess ways to increase crop productivity while improving water use efficiency.

Biochar is a solid carbon-rich organic material produced from biomass through thermal combustion in an oxygen-limited environment (Ahmad et al., 2014; Mohanty et al., 2018). Soil amendment with biochar has been reported to be effective in increasing soil water holding capacity by reducing percolation and evaporation losses, thereby improving water use efficiency (Abideen et al., 2020; Adhikari et al., 2022). Furthermore, biochar has been considered a chemically stable carbon pool associated with soil carbon content (Dodor et al., 2018). Several studies have showed beneficial effects of biochar in improving soil conditions and crop productivity (Akhtar et al., 2014; Faloye et al., 2019; Zhang et al., 2020). The beneficial effects of biochar include direct supply of nutrients, improved nutrient uptake by plants, and improved soil water conditions.

The use of biochar can favor growth of both aerial part and root system of plants due to improvements of soil chemical and physical conditions (dos Santos Matos et al., 2021). The biometric characteristics of roots play a vital role in the overall performance of plants, as factors such as biomass, length, surface area, root diameter and volume directly impact the ability of crops to acquire nutrients and water (Khan and Iqbal 2011). Moreover, in situations where water availability is limited, shallow-rooted vegetables are particularly vulnerable, with more substantial yield losses (Singh et al., 2019).

Foliar application of K can also alleviate drought stress (Fayez and Bazaid, 2014; Shehzad et al., 2020; Ávila et al., 2022). Drought conditions are recognized to cause a decline in CO_2 assimilation and degradation of photosynthetic pigments and plasma membranes (Ahmad et al., 2018). However, the complementary application of K has the potential to prevent such damages to chlorophylls and membranes, activate numerous enzymes, increase water uptake, and improve cellular hydration, especially under conditions of water deficit (Alam et al., 2011). Potassium is a key element in controlling crop productivity (Liu and Zhu, 1997). Foliar KNO_3 application was shown to increase chlorophyll contents, photosynthesis rate, stomatal conductance, transpiration, carboxylation efficiency and greater uptake of P, K, Mg, S, Cu and Fe in sorghum plants cultivated under water deficit conditions (Ávila et al., 2022).

While numerous studies have reported the beneficial effects of biochar on crop growth and yield (Atkinson et al., 2010; Lehmann et al., 2011; Faloye et al., 2019; Zhang et al., 2020) the combined effects of biochar and foliar K application to alleviate the detrimental effects of water deficit remain unexplored. We hypothesized that the combined application of biochar and foliar K application can delay the inhibition of transpiration and sustain high photosynthetic activity of plants subjected to low soil moisture conditions. The aim of this study was to assess the impact of the application of biochar derived from coffee husk (a material rich in K) and complementary foliar KNO_3 fertilization under both optimal and deficit irrigation on the growth and physiology of tomato plants.

2. MATERIAL AND METHODS

2.1. Production and characterization of biochar

Biochar was produced from coffee husk (husk + pulp + parchment) of the *Coffea arabica* species, obtained at the Federal University of Viçosa (20° 45' 14" S; 42° 52' 53" W; 648.74 m of altitude). The biochar was prepared in an electric muffle, adapted to a condenser through an iron tube to allow the release of gases and bio-oil, as described in Lustosa Filho et al. (2017). Pyrolysis was performed at a highest heating temperature of 500 ºC, using a heating rate of 10 °C min^{-1} and 1 h of holding time followed by slow cooling down to room temperature.

For chemical characterization of the biochar, the following analysis were performed: (*i*) total CHN content in a Perkin Elmer Series II Elementary Analyzer 2400. Oxygen (O) was calculated by difference ($O = 100 - C - H - N - \text{ash}$). The ash content was determined following the standard method ASTM D - 2866 (ASTM D, 2007); (*ii*) electrical conductivity (EC) and pH were measured in water in a 1:20 solid to solution ratio, after 12 h of suspension agitation (Rajkovich et al., 2012); (*iii*) available nutrient contents were determined using a Mehlich-III solution as extractant in the proportion of 0.1 g mL^{-1} (Krounbi et al., 2021); (*iv*) total nutrient contents were determined according to a modified dry-ashing method at 500 °C and wet digestion using HNO_3 and H_2O_2 (Enders and Lehmann, 2012); (*v*) identification of minerals in the crystalline phases by X -ray powder diffractometry (XRD), using a PAN analytical device, model X` Pert Powder, with cobalt tube, nickel filter in the range from 4 to 50°2θ, sweep speed of 10°2θ; (*vi*) identification of functional groups by molecular absorption spectrophotometry in the infrared region with Fourier transform (FTIR) (Jasco FTIR 4100), using spectra obtained with 60 scans, with wave number from 4000 to 400 cm^{-1} and resolution of 4 cm^{-1}. Cross-sectional sections of the biochar were prepared to be observed in the scanning electron microscope (SEM) model Leo, 1430VP. To identify the structures of the biochar, the resolution was set from 10 to 100 µm.

2.2. Experimental setup and treatments

The study was carried out in a greenhouse at the Federal University of Viçosa, Viçosa – MG, Brazil (20° 45' 14" S; 42° 52' 53" W; 648, 74 m altitude). The experiment was set up in a randomized block design, arranged in a 2×2×2 factorial. Treatments included with and without biochar (1% = +Biochar and 0% = -Biochar), with and without foliar spray of KNO_3 (2% = +KNO_3 and 0% = -KNO_3) and two irrigation regimes (OI = Optimum irrigation and DI = Deficit irrigation), with four replications (Table 1). Each treatment comprised an experimental unit containing two pots, each with one plant, resulting in 32 experimental units and a total of 64 pots.

Table 1. Summary of treatments

Treatments	OI (90% of Θ_{FC})	DI (50% of Θ_{PWP})	Foliar spray of KNO_3	Biochar
T1	+	-	-	-
T2	-	+	-	-
T3	+	-	+	-
T4	-	+	+	-
T5	+	-	-	+
T6	-	+	-	+
T7	+	-	+	+
T8	-	+	+	+

$^{+}$ Presence of treatment; $^{-}$ absence of treatment.

2.3. Seedling preparation and experiment implementation

Seedlings were transplanted at 30 days after germination. The seedlings had three to four true leaves, and one plant was transplanted per pot. The pots were placed inside a greenhouse to ensure plants only had access to the water provided. Plants were grown for 40 days after transplanting in pots containing 2 kg of air-dried soil (12% moisture). The soil was obtained from the B horizon and had a clayey texture (sand: 30%, silt: 12%, clay: 58%). It was previously sieved (4 mm) and corrected for acidity with limestone to increase base saturation to 70%. Chemical characteristics of the soil, before correction, are shown in Table S1.

The pots with biochar contained a mixture of soil and biochar at a concentration of 1% (w/w) (biochar:soil). The concentration (1%) was predetermined based on K concentration in biochar and on K demand of the tomato plant in a pot experiment. Foliar KNO_3 application was carried out using a manual sprayer with a capacity of 0.5 L. KNO_3 was applied twice, at 15 and 30 days after transplanting, at a concentration of 2% (w/v). Plants without KNO_3 application were sprayed with deionized water as a blank.

To ensure the supply of sufficient nutrients and considering that biochar provides nutrients to soil, two different nutrient solutions were prepared. The first solution was prepared for the treatment with biochar (+Biochar) and the second solution for the treatment without biochar (-Biochar), so that in both situations the total amount of nutrients supplied were equivalent (Table S2). The preparation of nutrient solutions was based on Novais et al. (1991) and according to tomato recommendations (Alvarenga et al., 2013). The nutrient solution was applied to plants weekly.

2.4. Irrigation management and calculation

The study was carried out using two different irrigation treatments: a deficit irrigation (DI) treatment, where soil water content was maintained at 50% of soil available water (ASW), and an optimal irrigation treatment (OI), where soil water content was maintained at 90% ASW. The selection of soil moisture levels was based on criteria involving critical and optimal levels for tomato plants. The aim was to investigate the effect of biochar under conditions of severe water scarcity (50% ASW), mirroring cultivation scenarios in water-limited regions. Additionally, an optimal moisture level was considered, allowing for the assessment of biochar effects without the constraining influence of water deficit. A soil sample was collected to estimate the parameters of soil water retention curve based on the Van Genuchten equation (1980) using the SWRC Fit software (Seki, 2007).

All pots were filled with the same weight of dry soil (W_{ds}). Water weight (W_{water}) in DI and OI treatments in the first irrigation event was determined by multiplying the W_{ds} by soil water content (kg/kg) at water potentials referring to 90 and 50% of ASW. In subsequent irrigations, W_{water} was determined as total weight of the reference pot (W_{total}), which varied depending on the irrigation treatment, minus the total weight of the pot measured on the day. The total weight of the reference pot was calculated as follows: $W_{total} = W_{pot} + W_{ds} + W_{water} + W_{plant}$, where: W_{pot} = pot weight, W_{ds} = dry soil weight, W_{water} = water weight, and W_{plant} = plant weight. W_{plant} was determined by weighing spare plants of the same age grown within the experiment. Every seven days, a spare plant by treatment was harvested and weighed to adjust W_{plant}. Water was provided to plants from two to four times a day depending on the demand.

2.5. Variables

All physiological, biometric and biomass evaluations were performed 40 days after transplantation as describe below.

2.5.1. Leaf water potential (leaf Ψ)

Leaf water potential measurements (Ψ_{leaf}) were performed according to the method by Scholander et al. (1965) using a pressure chamber. The evaluations were carried out in the morning (4-5 am) (leaf water potential in pre-dawn evaluations - LWP_pd) and in the afternoon (from 1-2 pm) (leaf water potential in afternoon evaluations - LWP_m).

2.5.2. physiological traits

Photosynthesis (*An*), stomatal conductance (g*s*), intercellular CO_2 concentration (*Ci*), transpiration rate (*E*), instantaneous carboxylation efficiency (*An/Ci,* CE*i*) and instantaneous water use efficiency (*An*/*E*, WUE*i*) were measured in the morning (8-10 am) using an Infrared Gas Analyzer (Irga LI-6400, LI-COR, USA). Leaves were placed in the cuvette to stabilize for at least two minutes before the first recording. The internal conditions of the leaf chamber were adjusted to an average temperature of 22 °C, external CO_2 concentration of 400 μmol mol^{-1} of air, flow rate of 300 μmol s^{-1} and saturation light of 1000 μmol photons m^{-2} s^{-1} with 10% blue light. The carboxylation efficiency (CE*i*) was expressed as *An/Ci* and the instantaneous water use efficiency (WUE*i*) was expressed as *An*/*gs*.

2.5.3. Biometric parameters of aerial part and root system

Plant height (PH), expressed in cm, was measured from the base to the apical meristem of the plant using a measuring tape. Number of leaves (NL) was determined by counting all leaves of the plant. Leaf area (LA), expressed in cm^2, was determined using a bench leaf area meter (LI-3100). Specific leaf area (SLA) was determined by the relationship between leaf area and leaf mass.

Morphometric evaluations of the root system were performed using WinRhizo computerized system (WinRhizo Pro, Regent Inc. Instr., Canada). Total root length (cm) (TRL), root volume (cm^3) (RV), root surface area (cm^3) (RSA), total length of very fine roots (cm) (diameter - Ø less than 0.5 mm) (VFR), total length of fine roots (cm) (> 0.5 Ø < 2.0 mm) (FR) and total length of thick roots (cm) (Ø > 2.0 mm) (TR) were measured (Magalhães et al., 2011)

2.5.4. Fresh and dry mass of aerial part and root system

Fresh mass (g) of both aerial part (leaves, branches, and stem) and root system was determined in the greenhouse using an analytical scale with 0.001 g precision. Then, the materials were packed separately in paper bags and placed in an oven at 65 °C ± 3 °C for 48 hours or until reaching a constant weight.

2.6. Statistical analysis

Three-way analysis of variance was performed to test treatment significance, i.e., biochar (+Biochar and - Biochar), foliar KNO_3 application (+KNO_3 and -KNO_3), and irrigation levels (50 and 90% of ASW). When differences were significant by the F test ($p < 0.05$), means were compared using the Tukey test at a significance level of 0.05. Statistical analyzes were performed on R software (version 3.6.0) employing the ExpDes.pt package.

3. RESULTS

3.1. Characteristics of biochar

The nutritional composition of coffee husk biochar, in terms of total nutrients, was: P = 2.7 ± 0.1 g kg^{-1}; K^+ = 68.8 ± 1.5 g kg^{-1}; Ca^{2+} 8.7 ± 0.3 g kg^{-1}; Mg^{3+} = 3.8 ± 0.07 g kg^{-1}; S = 1.8 ± 0.02 g kg^{-1}; Al^{3+} = 6.5 ± 0.3 g kg^{-1}; Zn = 455 ± 56 mg kg^{-1}; Fe = 4873.3 ± 210 mg kg^{-1}; Mn = 199 ± 27 mg kg^{-1} and Cu = 46.9 ± 1.3 mg kg^{-1}. For the content of available nutrients, the following values were found: P = 0.3 ± 0.0 g kg^{-1}; K^+ = 30.9 ± 0.6 g kg^{-1}; Ca^{2+} 3.2 ± 0.1 g kg^{-1}; Mg^{3+} = 0.44 ± 0.08 g kg^{-1}; Zn = 175.7 ± 7.2 mg kg^{-1}; Fe = 340.5 ± 12.2 mg kg^{-1}; Mn = 15.1 ± 0.6 mg kg^{-1} and Cu = 0.5 ± 0.03 mg kg^{-1}. The chemical attributes of the biochar were: yield = 341.2 g kg^{-1}; EC = 5.1 ± 0.06 dS cm^{-1}; pH (H_2O) = 11.9 ± 0.02; C = 37.5%; H = 2.4%; N = 0.4%; O = 37.2 %; Ashes = 22.5%; Θ_{FC} = 1.05 kPa and Θ_{PWP} = 0.72 kPa.

The biochar showed a high total K content (68.8 g kg^{-1}) with 45% available by Mehlich-3. Ash contents were also high (225 g kg^{-1}) as well as the pH and EC, which is consistent with coffee husk as feedstock. The most relevant mineral found with XRD were: Calcite ($CaCO_3$) (2θ = 26, 27, 36 and 40°), Astrophyllite [$(K, Na)_3 (Fe^{++}, Mn)_7 Ti_2 Si_8O_{24} (O, OH)_7$] (2θ = 10°), Siderite ($FeCO_3$) (2θ = 43°), Sylvite (KCl) (2θ = 33.5 and 44.5°), and Hydroxylapatite [$Ca_{10}(PO_4)_6 (OH)_2$] (2θ = 37.5°) (Fig. S1). Sylvite represents an immediate source of K to plants from coffee husk biochar.

Biochar FTIR spectra show three peaks at 1560, 1530 and 1500 (Fig. S2), associated with C=C, C=N, −COO vibrations, antisymmetric stretching of amino acids, C=O stretching vibrations in ketones, quinones, aldehydes, lactones and carboxylic groups and esters, and N−H band (Rodriguez et al., 2021; Taherymoosavi et al., 2017). Peaks at 906, 842, 760 and 720 cm^{-1} were assigned to P−O−P (Bekiaris et al., 2016; Lustrosa Filho et al., 2017).

The biochar surface morphology is characterized by the presence of small and well-structured particles (Fig. S3), possibly due to the breakdown of different organic compounds from the feedstock during pyrolysis. In addition, the presence of C and O and a relatively high concentration of K and Ca were observed through EDS spectrum.

3.2. Water potential and leaf gas exchange

Average values of leaf water potential (Ψ_{leaf}) measured in pre-dawn (LWP_pd) and at noon (LWP_m), and the photosynthesis parameters are presented in Fig. 1. The interaction of Irrigation x Biochar x KNO_3 was significant ($p<0.05$) only for the variables LWP_m and intrinsic Rubisco carboxylation efficiency (CE*i*) (Table S3). Regardless of any combination between biochar and KNO_3, more negative LWP_m values were observed under DI (Fig. 1a). Furthermore, combined application of biochar and KNO_3 significantly ($p<0.05$) improved LWP_m in plants grown under OI; however, they had no effect under DI conditions. CE*i* was reduced by DI, when KNO_3 and biochar were applied, and when neither KNO_3 nor biochar were applied. When biochar or KNO_3 were applied separately, no effect of DI was observed. No effect of biochar and KNO_3 was verified under OI conditions, however, under DI conditions, biochar increased CE*i* by 43%, when not applied KNO_3 (Fig. 1b).

The interactions of Irrigation x KNO_3 and Biochar x KNO_3 were significant ($p<0.05$) for net photosynthesis (*An*) (Table S3). Net photosynthesis decreased under DI conditions, regardless of leaf KNO_3 supply (Fig. 1c). The addition of biochar led to an improved *An* in plants without KNO_3 application. Conversely, the use of KNO_3 promoted a negative effect on *An*, even when using biochar (Fig. 1d).

As for the individual effect of irrigation, water deficit negatively affect ($p<0.05$) stomatal conductance (0.35 mol CO_2 m^{-2} s^{-1} under OI and 0.074 mol CO_2 m^{-2} s^{-1} under DI), transpiration (2.97 mmol H_2O m^{-2} s^{-1} under OI and 1.08 mmol H_2O m^{-2} s^{-1} under DI) and internal CO_2 concentration (471.5 µmol CO_2 m^{-2} s^{-1} under OI and 345.5 µmol CO_2 m^{-2} s^{-1} under DI) ($p<0.05$) (Fig. 1f-g, respectively). However, the individual effect of biochar caused a positive effect ($p<0.05$) on leaf water potential measured in the predawn (-3.15 MPa under +Biochar and -3.97 MPa under -Biochar), stomatal conductance (0.25 mol CO_2 m^{-2} s^{-1} under OI and 0.17 mol CO_2 m^{-2} s^{-1} under DI) and internal CO_2 concentration (416 µmol CO_2 m^{-2} s^{-1} under OI and 400 µmol CO_2 m^{-2} s^{-1} under DI) ($p<0, 05$) (Fig. 1h-j, respectively).

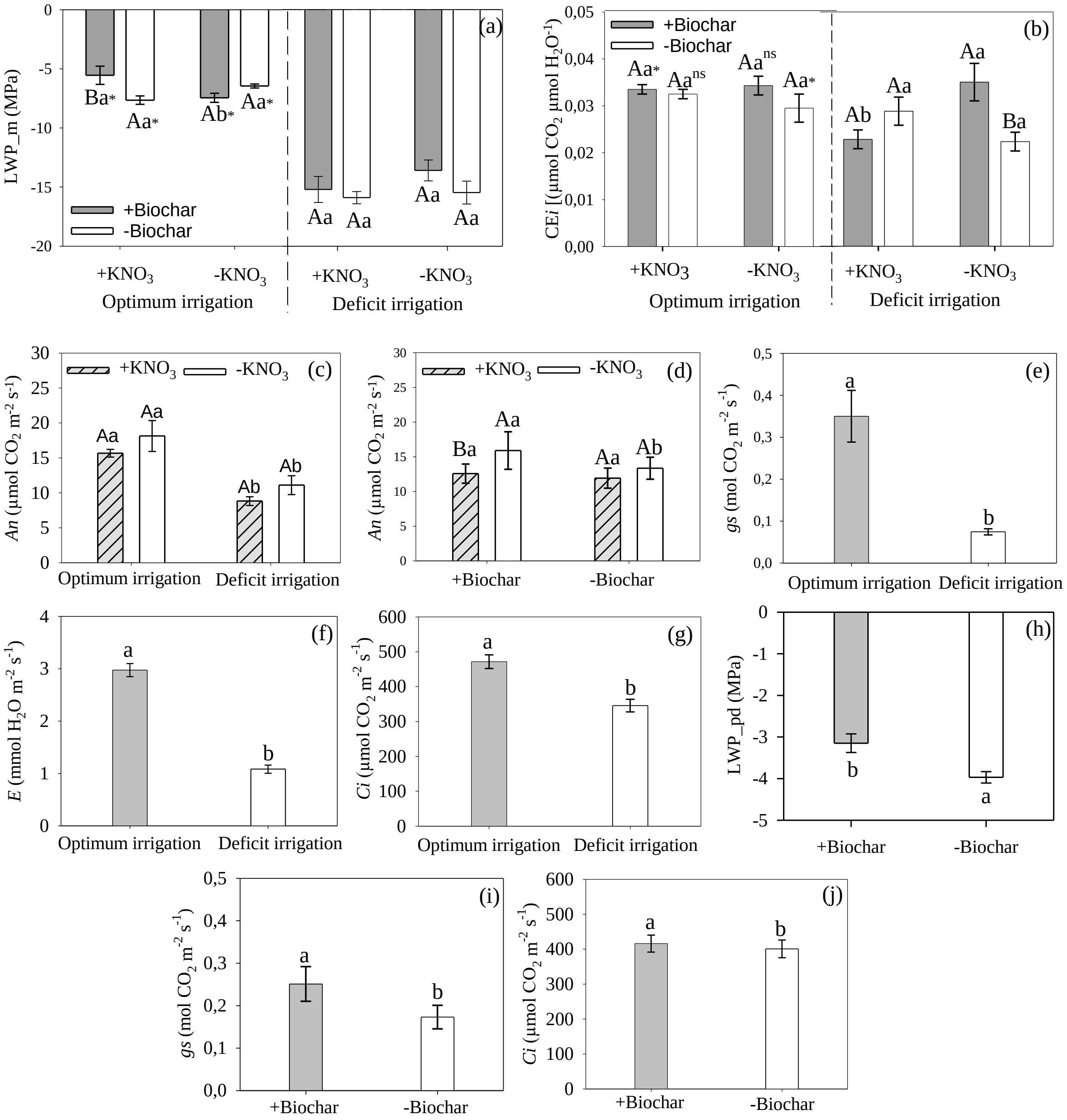

Fig. 1. Leaf water potential at noon (noon-1pm) (LWP_pd) (a), intrinsic Rubisco carboxylation efficiency (b), net photosynthesis as a function of *irrigation x KNO_3* interaction (c), net photosynthesis as a function of *biochar x KNO_3* interaction (d), stomatal conductance as a function of *irrigation* (e), transpiration as a function of *irrigation* (f), internal CO_2 concentration as a function of *irrigation* (g), leaf water potential in evaluations in the pre-dawn (LWP_pd) (3-5 am) as a function of *biochar levels* (h), stomatal conductance as a function of *biochar levels* (i) and internal CO_2 concentration as a function of *biochar levels* (j). For Fig. 'a' and 'b', compare lowercase letters with and without foliar KNO_3 application within each irrigation and biochar level. Uppercase letters compare with and without biochar within each irrigation level and KNO_3. *Shows the importance of irrigation treatments within each level of biochar and KNO_3 (Tukey Test, $p<0.05$). In Fig. 'c' and 'd', uppercase letters compare with and without KNO_3, and

lowercase letters compare irrigation (c) and biochar (d). For Fig. e-f, means followed by the same letter are not statistically different (Tukey test, $p<0.05$). For a and b; c and d; and e-j, the error bars show mean standard error (n = 4, 8 and 16 respectively)

3.3. Biometric parameters of shoot and root

The interaction among the three factors studied was not significant ($p>0.05$) for any of the biometric variables (Fig. S5). Plant height had a significant influence ($p<0.05$) on biochar and irrigation levels, separately. Water deficit reduced plant height by 31.5% when compared to OI (Fig. 2a). The application of biochar increased plant height by 12.4% (Fig. 2b). For the number of leaves, a significant effect ($p<0.05$) was observed for irrigation levels x KNO_3 interaction and for the single effect of biochar application. As for the interaction between irrigation levels x KNO_3, a reduction in the number of leaves was observed under DI conditions, under both KNO_3 treatments, but no effect of KNO_3 was observed. The application of biochar increased the number of leaves by 7% (Fig. 2d). Total leaf area was reduced under DI conditions and increased when foliar KNO_3 was applied under OI conditions (Fig. 2e). Furthermore, the application of biochar alone to soil increased leaf area by 3% (Fig. 2f).

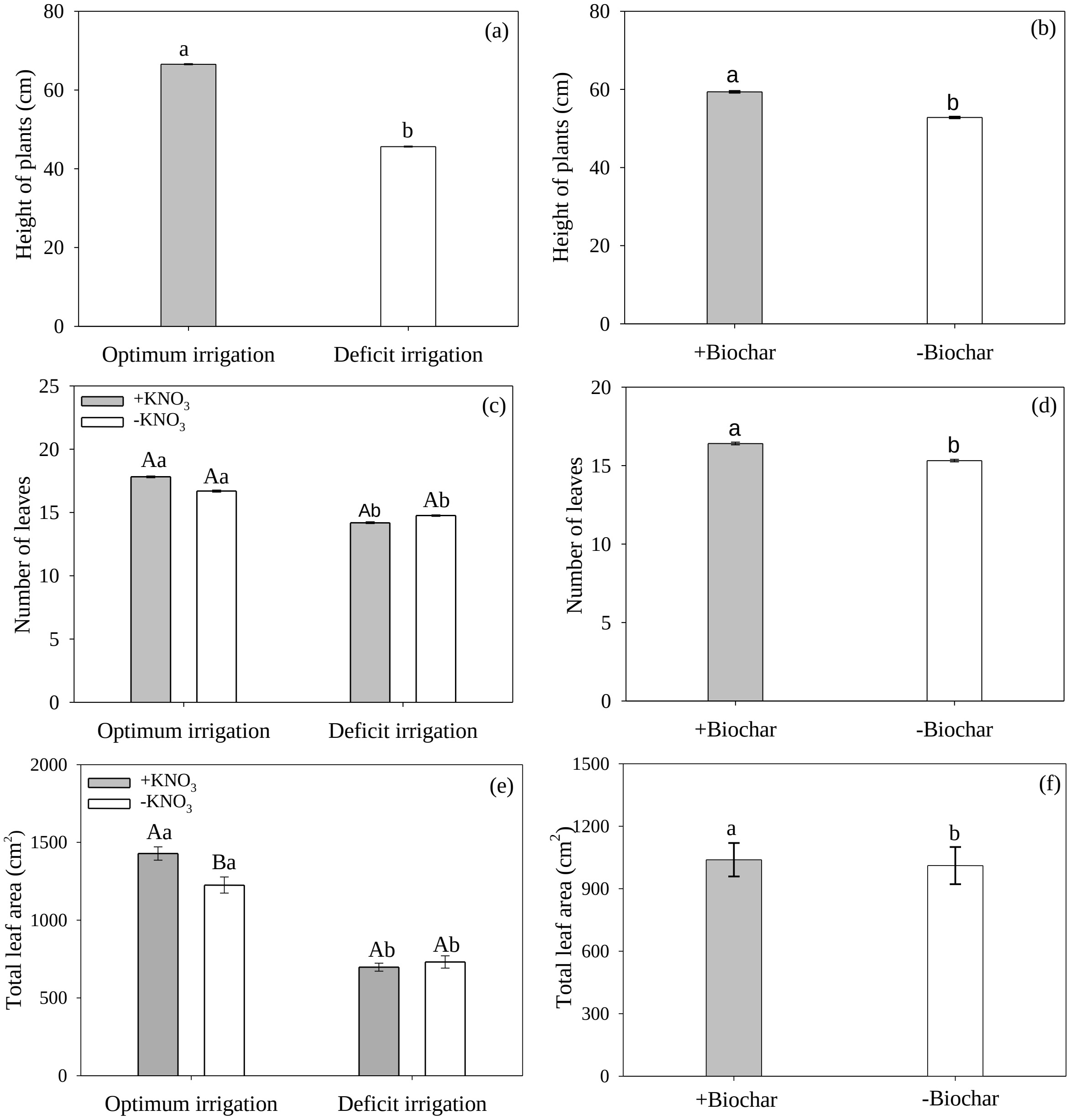

Fig. 2. Plant height as a function of irrigation (a), plant height as a function of biochar levels (b), number of leaves for irrigation levels x KNO_3 interaction (c), number of leaves for biochar levels (d), total leaf area for irrigation levels x KNO_3 interaction (e), total leaf area for biochar levels (f), in tomato cultivated under different levels of biochar (+Biochar and -Biochar), two regimes of K foliar application (+KNO $_3$ and -KNO_3) and two irrigation regimes (Optimal irrigation and Deficit irrigation). Means followed by the same letter are not statistically different (Tukey Test, $p<0.05$). Error bars show the mean standard error (a, b, c, and f: n = 16; e and g: n = 8).

The interaction between the three studied factors was not significant ($p>0.05$) for any of the root biometric variables evaluated (Total root length - TRL, root surface area - RSA, root volume - RV, total root length - very fine roots - VFR, total length of fine roots - FR and total length of thick roots - TR) (Table S4). There was a significant effect ($P>0.05$) for the isolated factors irrigation levels and biochar. For irrigation levels, we observed that at 50% ASW there was a reduction in TRL (42.6%), RSA (41.5%), RV (40.3%), VFR (42.5%), the FR (42.6%) and TR (45%) (Fig. 3a-f, respectively).

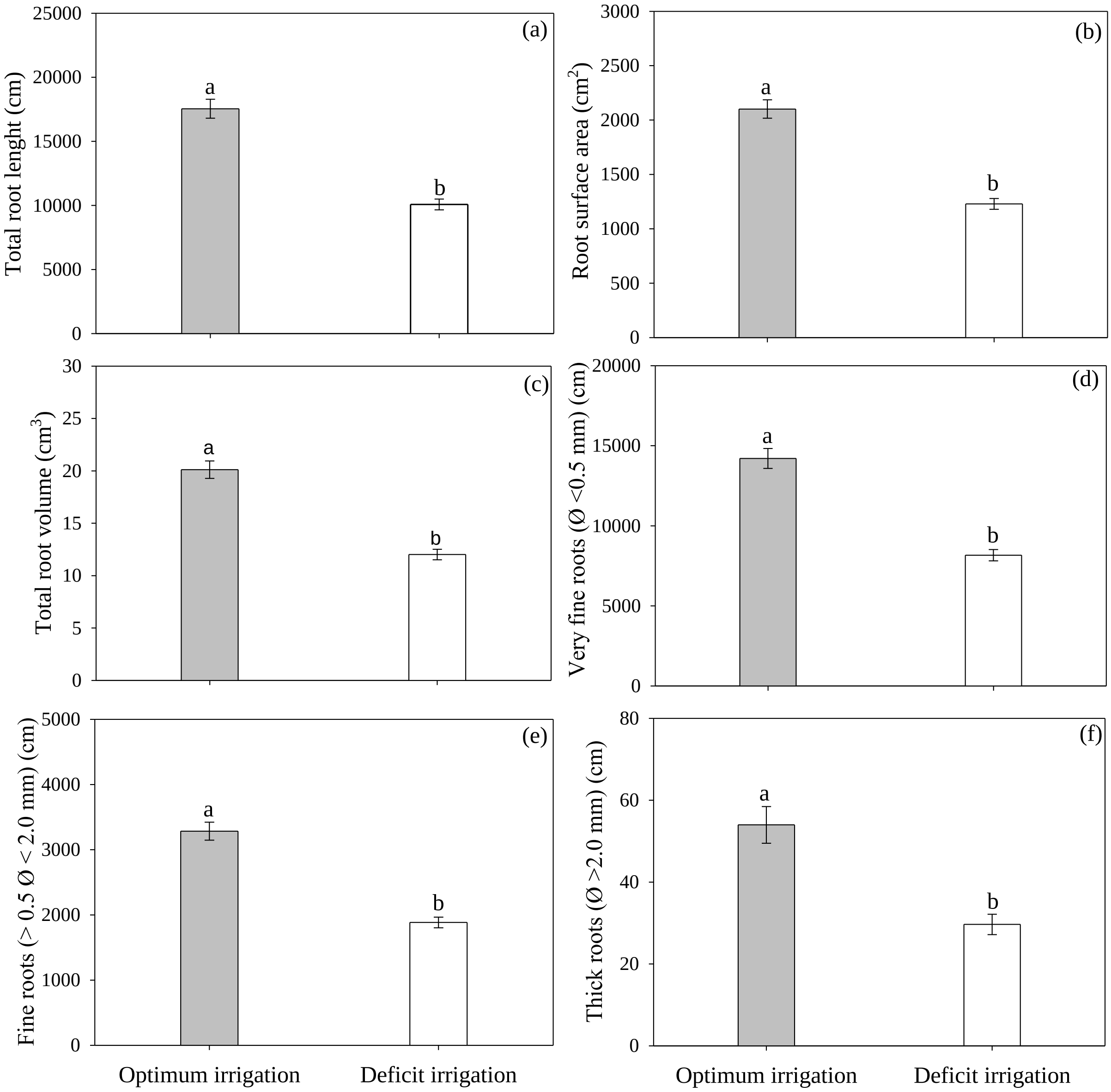

Fig. 3. Total root length (a), root surface area (b), total root volume (c), total length of roots less than 0.5 mm in diameter (d); total length of roots with a diameter greater than 0.5 and less than 2 mm (e) and total length of roots with a diameter greater than 2 mm (f), in tomato cultivated under different levels of biochar (+Biochar and -Biochar), two regimes of K foliar feeding (+KNO_3 and -KNO_3) and two irrigation regimes (Optimal irrigation and Deficit irrigation). Means followed by the same letter are not statistically different (Tukey Test, $p<0.05$). Error bars show mean standard error (n = 16).

Despite the reduction in biometric parameters of roots by reduced water supply, a positive effect ($p<0.05$) was verified by the application of biochar to soil causing an increase in

TRL (14.5%), RSA (14.6%), RV (14.8%), VFR (14.4%), and FR (14.7%). (Fig. 4a-e, respectively).

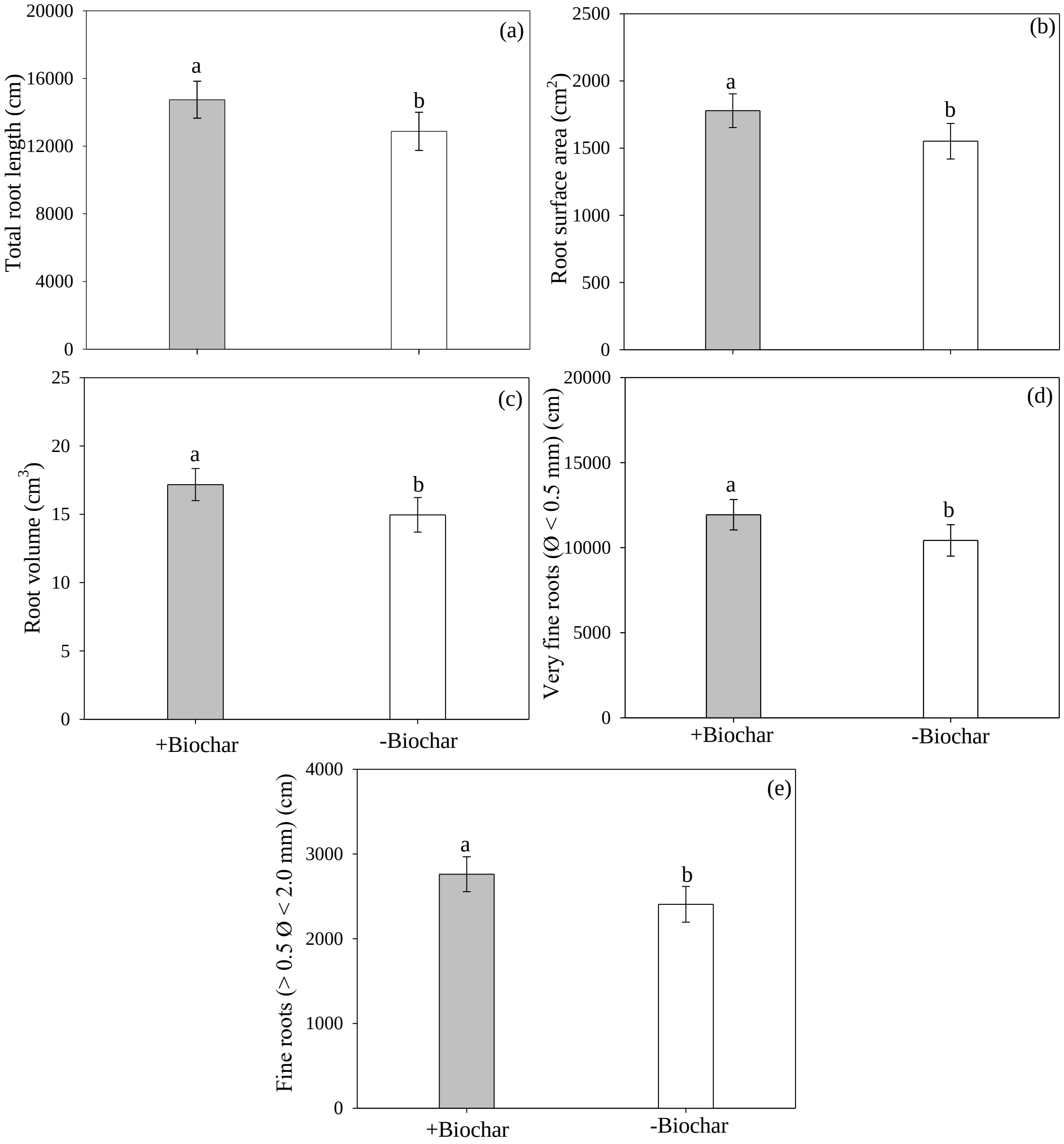

Fig. 4. Total root length (a), root-specific surface area (b), total root volume (c), total length of roots with a diameter of less than 0.5 mm (d) and total length of roots with diameter greater than 0.5 and less than 2 mm (e), in tomato plants cultivated under different levels of biochar (+Biochar and -Biochar), two regimes of K foliar application (+KNO_3 and -KNO_3) and two regimes of irrigation (Optimal Irrigation and Deficit Irrigation). Means followed by the same letter are not statistically different (Tukey Test, $p<0.05$). Error bars show mean standard error (n = 16).

3.4. Biochar effect on the accumulation of aerial and root biomass in tomato

The interaction of the three factors (Irrigation x Biochar x KNO_3) was significant ($p>0.05$) only for dry mass of leaves (LDW) and root dry mass (RDM) (Table S5). LDW was reduced when subjected to DI regardless of the combination of biochar and KNO_3 (Fig. 5a). In addition, a positive effect of biochar on roots under OI was verified when KNO_3 was not applied, and a negative effect was observed in plants treated with KNO_3. Negative DI effects were also observed for RDW, even when biochar and KNO_3 were applied (Fig. 5b). Increase in RDW was verified using biochar in OI, in the absence of foliar KNO_3 application. Likewise, an increase in RDW was verified using KNO_3 in OI, when biochar was not applied

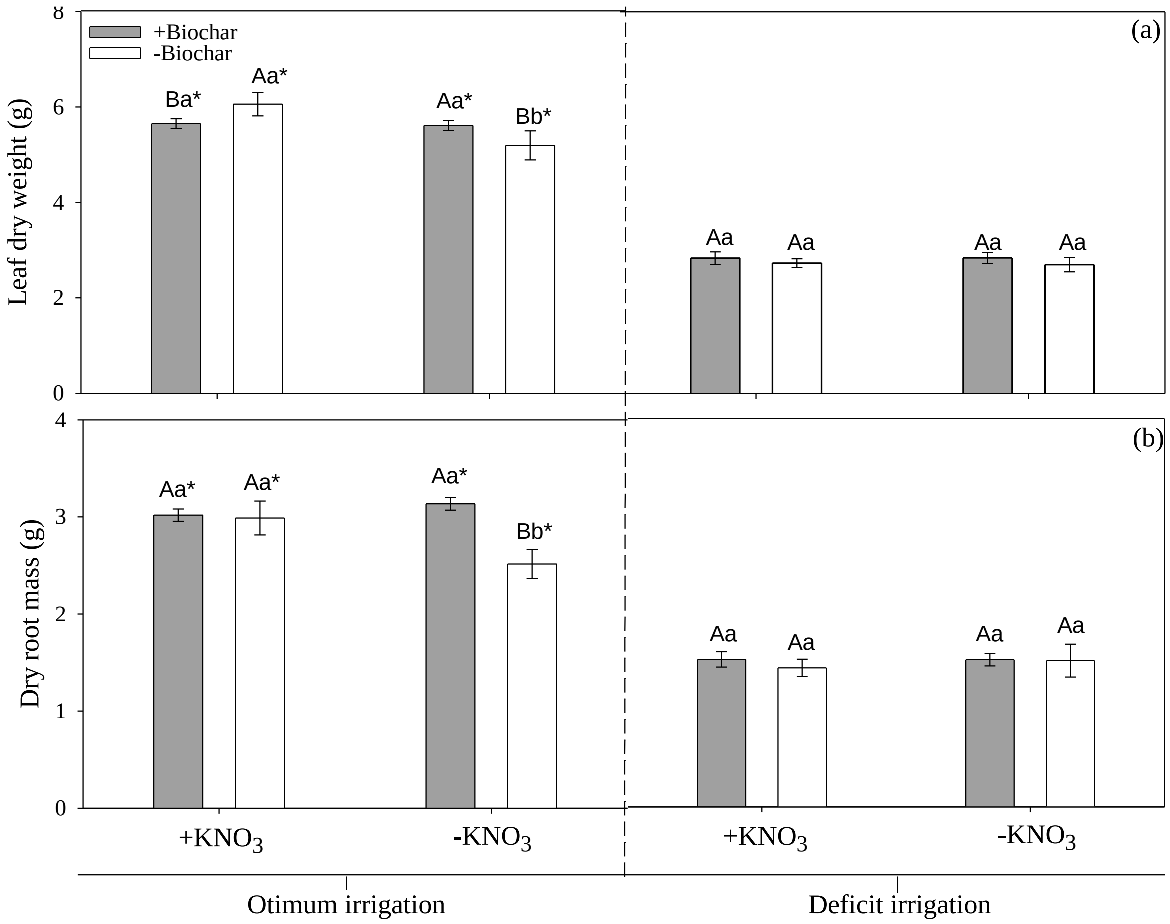

Fig. 5. Leaf dry weight (a) and root dry weight (b) of tomato cultivated under different levels of biochar (+Biochar and -Biochar), two foliar K fertilization regimes (+KNO_3 and -KNO_3) and two irrigation regimes (OI and DI). Lowercase letters compare with and without foliar KNO_3 application within each irrigation and biochar level. Uppercase letters compare with and without biochar within each irrigation level and KNO_3. *Shows the importance of irrigation treatments within each level of biochar and KNO_3. Error bars show mean standard error (n=4). Data are expressed as mean ± standard error.

The interaction of irrigation regimes x KNO_3 was significant ($p<0.05$) for stem dry weight (SDW) and leaf fresh weight (LFW) (Table S5). Foliar KNO_3 application increased

($p<0.05$) SDW (5%) and LFW (13%) under OI (Fig. 6a-b, respectively). DI promoted a reduction ($p<0.05$) in SDW (62% in $+KNO_3$ and 54% in $-KNO_3$) and LFW (54% in $+KNO_3$ and 46% in $-KNO_3$). Deficit irrigation drastically ($p<0.05$) reduced SFW (62%), FSW (58%), RFW (47%) and APDW (54%) (Fig. 6c-f, respectively).

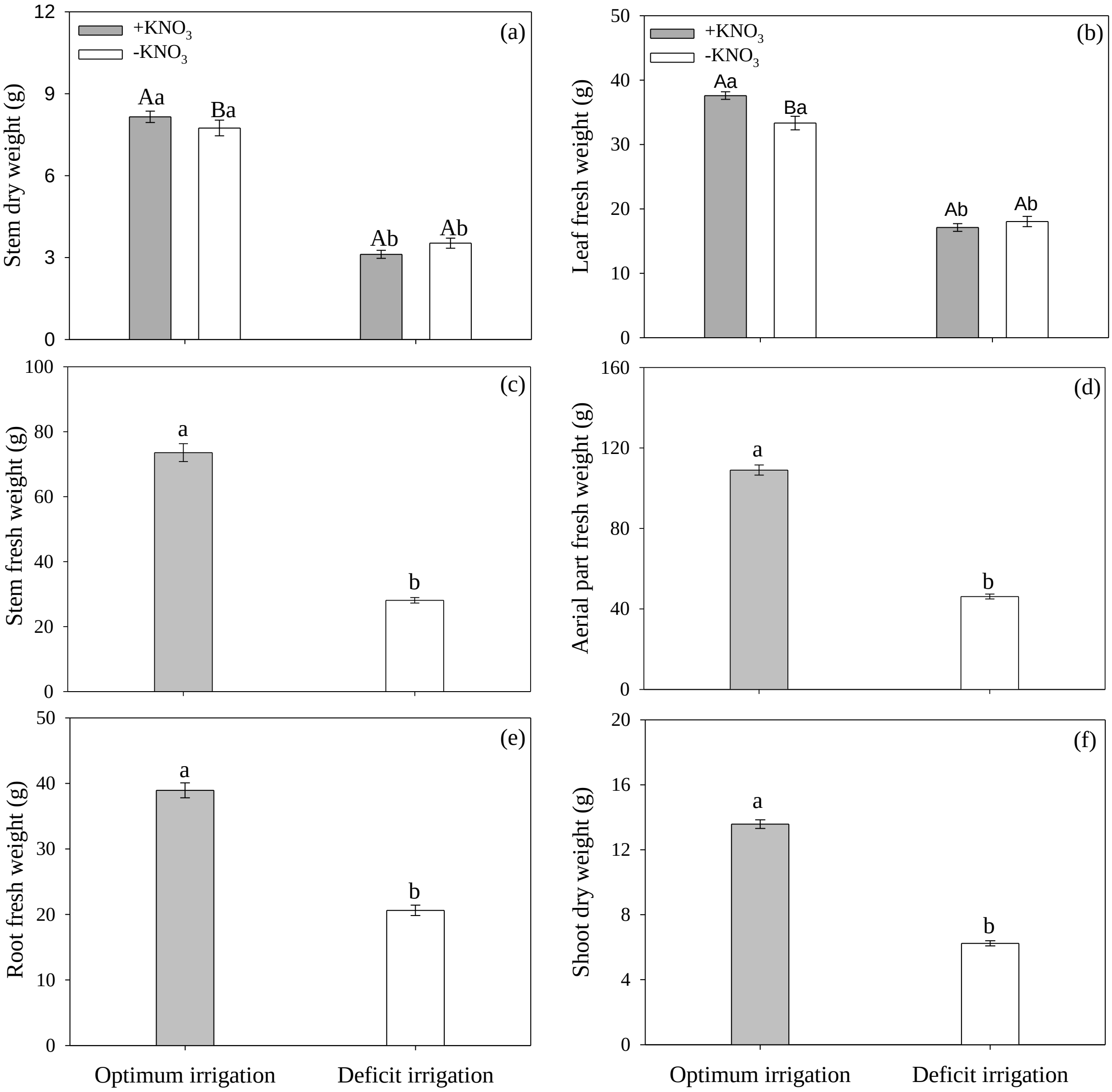

Fig. 6. Stem dry weight (a), leaf fresh weight (b), stem fresh weight (c), shoot fresh weight (d), root fresh weight (e), root dry weight (f), in tomato plants grown under different levels of biochar (+Biochar and -Biochar), two foliar K application regimes ($+KNO_3$ and $-KNO_3$) and two irrigation regimes (Optimal irrigation and Deficient irrigation). Uppercase letters compare KNO_3 treatments and lowercase letters compare irrigation treatments (Tukey Test, $p<0.05$). Error bars show the mean standard error (Fig. ab: n = 8; Fig. c-f: n = 16).

Biochar application caused a significant increase ($p<0.05$) for all the following variables: LFW (6%), SFW (8%), FSW (8%), RFW (13%), and APDW (9%) (Fig. 7a-e, respectively).

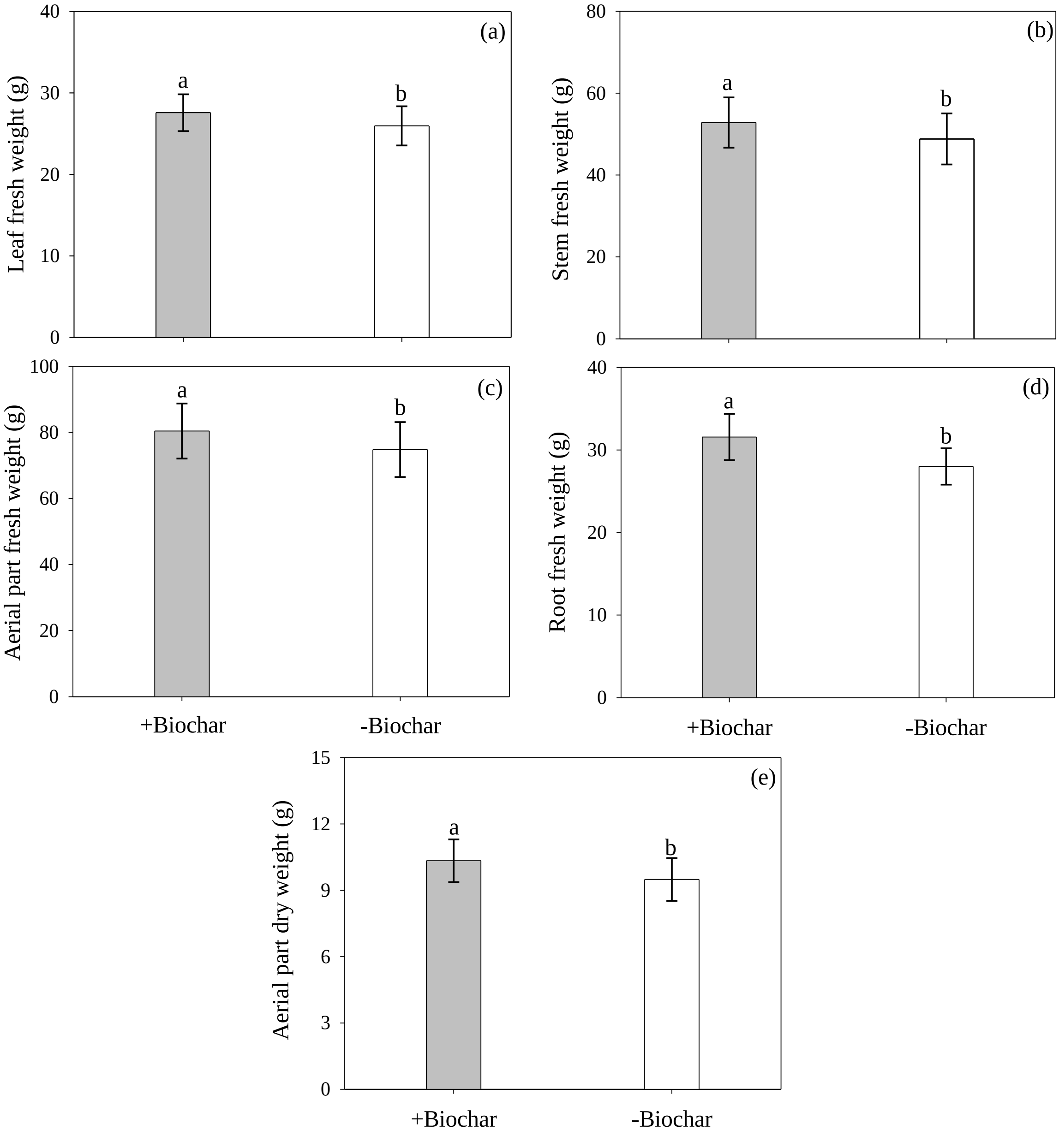

Fig. 7. Leaf fresh weight (a), stem fresh weight (b), shoot fresh weight (c), root fresh weight (d) and shoot dry weight (e), in tomato plants grown under different levels of biochar (+Biochar and -Biochar), two K foliar feeding regimes (+KNO_3 and -KNO_3) and two irrigation regimes (Optimal Irrigation and Deficit Irrigation). Means followed by the same letter are not statistically different (Tukey Test, $p<0.05$). Error bars show mean standard error ($n = 16$).

3.5. Principal component analysis (PCA)

PCA analysis showed two principal components describing 97.3% of the data, with 88.9% of the total variance described by the first principal component (Table 2).

Table 2. Eigenvalues and cumulative variance in principal component analysis for physiological and biometric attributes in tomato plants treated and not treated with biochar in two soil moisture conditions (50 and 90% ASW)

Component	**Principal component**	
	1	**2**
Eigenvalues	15.99	1.50
Proportion (%)	88.88	8.36
Accumulated proportion	88.88	97.25
Variables	**Factor loadings**	
	PC1	**PC2**
Ci (μmol CO_2 m^{-2} s^{-1})	0.93	-0.22
E (mmol H_2O m^{-2} s^{-1})	0.99	-0.07
gs (mol CO_2 m^{-2} s^{-1})	0.93	0.09
An (μmol CO_2 m^{-2} s^{-1})	0.99	0.10
An/ Ci (μmol CO_2 μmol H_2O^{-1})	0.96	0.23
An/E (μmol CO_2 mmol H_2O^{-1})	-0.76	0.62
AFT (cm^2)	0.98	-0.08
LDW (g)	0.97	-0.18
SDW (g)	0.99	-0.01
RDW (g)	0.98	-0.02
LWP_pd (MPa)	-0.38	-0.89
LWP_m (MPa)	-0.94	0.27
TRL (cm)	0.99	0.07
RSA (cm^2)	0.99	0.07
RV (cm^3)/	0.99	0.06
VFR (mm)	0.99	0.08
FR (mm)	0.99	0.05
TR (mm)	0.94	0.21

Internal CO_2 concentration (*Ci*), transpiration (*E*), net photosynthetic rate (*An*), stomatal conductance (*gs*), rubisco carboxylation efficiency (*An/Ci*), intrinsic water use efficiency (*An/E*), total leaf area (AFT), leaf dry weight (LDW), stem dry weight (SDW), root dry weight (RDW), predawn water potential (LWP_pd), midday water potential (LWP_m), total root length (TRL), root surface area (RSA), root volume (RV), length of very fine roots (VFR), length of fine roots (FR) and length of thick roots (TR).

In PC1, both physiological variables (*Ci, E, gs, An, An/Ci, An/E*, LWP_pd, and LWP_m) and biometric variables (LDW, SDW, RDW, TRL, RSA, RV, VFR, FR, and TR) contributed to the separation of biochar and irrigation treatments (Fig. S5). It is possible to observe that, with the exception of LWP_m and *An/E,* all the other variables had high positive scores. Based on these covariates, it was possible to separate treatments with and without biochar application under OI conditions. In PC2, the most important variable for separating treatments was LWP_pd, which was highly correlated with the absence of biochar and with DI.

3.6. Correlation between characteristics

Our study on the association between physiological and root biometric variables showed a total of 80 linear correlations in plants treated with biochar, 78 positive and two negative correlations. Furthermore, a total of 60 linear correlations were revealed in plants not fertilized with biochar, 59 positive and one negative ($p<0.05$ by t test) (Fig. S6a-b). Overall, biometric parameters of roots and biomass are strongly correlated with each other, regardless of biochar application. These results were already expected, as some biometric variables of roots (i.e., TRL, RV, VFR, FR and TR) and biomass (FSW, SDW and RDW) are directly influenced by each other. However, it is important to point out that for RDW, FR and TR, a greater number of linear correlations were obtained in the treatment with biochar. SLA had a negative linear correlation with SDW ($r = -0.71$) and TR ($r = -0.77$) in plants treated with biochar, while this association was not observed in the absence of biochar. The LWP_pd, on the other hand, seems to have had positive linear correlations with the root parameters (TRL, RSA, RV, VFR, and FR), biomass parameter (LA, FSW and SDW) and gs in plants grown without biochar (-Biochar).

Water use efficiency (WUE; *An*/*E*) showed a positive correlation ($r = 0.79$) with SLA in treatments cultivated with biochar. A similar pattern was observed for *gs*, which correlated ($p<0.05$) with *An/Ci* ($r = 0.77$), as well as LWP_m ($r = 0.90$), RSA ($r = 0.95$), RV ($r = 0.94$), FR ($r = 0.94$) and TR ($r = 0.73$) in the presence of biochar, but did not correlate with any of them in the absence of biochar ($p>0.05$). For *An*, a positive correlation with *An/Ci* ($r = 0.83$) and FR ($r = 0.71$) was observed only in the presence of biochar.

4. DISCUSSION

4.1. Biochar improves physiological parameters of tomato plants

Water potential measured in the morning can be used as an indicator of water availability in soil (Lopes et al., 1999; Paranychianakis et al., 2004). Biochar treatment increased Ψ_{leaf} measured in pre-dawn, and the Ψ_{leaf} measured at midday when combined with foliar KNO_3 application, which is a result of an improvement in soil water potential promoted by biochar. Biochar has low density and high porosity, and is able to improve chemical, physical and biological characteristics of soil (Lehmann et al., 2006; Shi et al., 2018; Singh et al., 2019). By reducing the bulk density of soil, biochar increases the total porosity, thus increasing water holding capacity (Hussein et al., 2022). In addition, the physical structure of biochar helps to form cohesive and adhesion forces (Razzaghi et al., 2020), which also improves soil water storage potential (Villagra-Mendoza and Horn, 2018). This improvement in water status for plants has a positive effect on the ability to accumulate biomass, as it is directly associated with stomatal opening and CO_2 assimilation for carbohydrate synthesis, as verified in this study by the increase in stomatal conductance, internal CO_2 concentration in the treatment with application of biochar. Increased photosynthesis results in a greater production of metabolites available for plant development, and consequently greater plant yield (Agbna et al., 2017). As verified in this study, biochar also increased net photosynthesis when applied alone and increased rubisco carboxylation efficiency under limiting water conditions.

Drought is the abiotic stress factor that most limits crop growth and yield (Ahmad et al., 2018), due to several physiological and morphological changes that occur in plants (Singh et al., 2019). The lack of water in soil causes the loss of cell turgor, which limits cell elongation and division, leading to reduced plant growth (Lawlor and Cornic, 2002), which translates into a smaller area of light interception, thus reducing photosynthetic activity (Singh et al., 2019). Drought cause a significant reduction in chlorophyll, water and different fluorescence parameters (Ahmad et al. 2017). However, the results observed in this study is in accordance with others showing improvements in the physiology of plants grown under water deficit with the application of biochar (Akhtar et al., 2014; Agbna et al., 2017; Youssef et al., 2018; Langeroodi et al., 2019).

Potassium (K) is an essential macronutrient needed for improved plant growth and yield, especially under water limiting conditions, as it plays an important role in osmotic adjustment, opening and closing of stomata, and enzyme activation, optimizing many physiological and biochemical processes that ultimately improve plant growth and yield (Ahmad et al., 2018). Despite the importance of K, we did not find significant beneficial effects of foliar KNO_3 application on the evaluated physiological characteristics. Thus, such foliar application might have a limited impact on tomato grown under water-stressed conditions.

4.2. Biochar improves growth and biomass accumulation in tomato plants under optimal and limited water conditions

Plant height, number of leaves and leaf area are important characteristics to assess in a study, with particular emphasis on the latter two due to their direct correlation with photosynthetic surface of a plant. Total leaf area, if divided by the area occupied by the plant, provides the leaf area index, a direct measurement of the photosynthetic capacity of plant's system (Monteith, 1977). With optimal water supply and the application of biochar, the plant exhibited an increase total leaf area and consequently improved net photosynthesis. In a greenhouse experiment carried out by Agbna et al. (2017) under water deficit conditions, it was observed that the supply of 25 t ha^{-1} of biochar resulted in significant improvements in various plant growth parameters, including plant height, number of leaves, fresh and dry weight of plants, as well as higher yield, when compared to the non-application of biochar. Remarkably, these positive outcomes were statistically similar to the control treatment without water stress. Similarly, increase in leaf area, plant height and dry weight of okra plants (*Abelmoschus esculentus* L. Moench) grown under water stress conditions with the addition of 1% (w/w) of biochar has also been reported (Batool et al., 2015).

Limited water availability severely reduced plant biomass accumulation in both shoot and root (Fig. S6). These results were anticipated, as the severe limitation of water in soil triggers stomatal closure, leading to reduced transpiration rates and limited entry of CO_2 into the substomatal chamber. Consequently, photosynthesis is hindered, resulting in reduced sugar synthesis and consequently lower biomass production (Agbna et al., 2017; Dariva et al., 2021). Although the literature suggests that soil water limitations can stimulate root growth, it is important to note that under severe stress, as tested in the present study (50% of ASW), has a detrimental effect. Severe water deficit can induce changes in nutrient uptake and translocation processes (Ahanger et al., 2021) while also promoting the generation of reactive oxygen species (Rasheed et al., 2021). Consequently, such conditions lead to severe oxidative stress, resulting in damage to membranes, proteins, and nucleic acids (Zhang et al., 2016).

In spite of the lower accumulation of biomass resulting from limited water supply, this study revealed the potential of soil biochar application to improve the production of both shoot and root biomass in tomatoes, regardless of the water regime (Fig. S6). Singh et al. (2019) have reported that biochar application to soil promotes positive effects on plant development, biomass production, and yield of various vegetables under water deficit conditions. For instance, Akhtar et al. (2014) observed that the addition of biochar derived from a mixture of rice husks and cotton seed husks at a rate of 5% (w/w) increased soil moisture levels under water deficit conditions, improving tomato physiology, yield and quality. In another study, an increase in wilting resistance was observed in tomato seedlings grown in pots with the application of 30% (v/v) of biochar derived from wood pellets (Mulcahy et al., 2013). This positive impact of biochar on plant development and its ability to alleviate the stress induced by the limited supply of water can be attributed to several factors, such as: *i)* improvement in the physical properties of soil by reducing the apparent density, increase in porosity, and increase in soil organic matter content; *ii)* improvement of chemical properties by increasing nutrient availability, cation exchange capacity and reducing leaching losses; and *iii)* improvement in the biological properties of soil, acting as a refuge for microorganisms (Blanco-Canqui, 2017; Ding et al., 2016; Tayyab, 2018). In addition to these effects, treatment with biochar seems to stimulate plant growth. According to Sun et al. (2017), application of biochar at a dose of 5% promoted a wide range of metabolic responses, including changes in metabolomic profile and metabolism of sugars and amino acids, which contributed to the stimulation of maize plant growth. Li et al. (2018) reported that 30 t ha^{-1} of biochar under water deficit can help save water without significantly impacting overall tomato productivity. However, in our study, using a lower application rate of biochar (1%, equivalent to 20 t ha^{-1}), we observed that the application of biochar only was insufficient to fully mitigate negative effects of water deficit, resulting in reduced plant growth. However, it is worth noting certain factors that may have influenced our results. Firstly, the volume of material applied in our study was lower compared to that used by Li et al. (2018). Additionally, the dynamics of field-grown tomatoes is different from those of pot-grown tomatoes. Furthermore, the water deficit imposed in our study, equivalent to 50% of available soil water, may have been too severe for tomato plants.

The application of foliar KNO_3 had a positive impact on the biomass production of the aerial part of the tomato plants (leaf and stem). Potassium is essential for plant nutrition as it plays a vital role in various physiological and biochemical processes that contribute to plant growth, crop quality and yield (Cakmak 2005). Furthermore, supplying K significantly increases leaf tissue water content (Ahmad et al., 2018). Irshad et al. (2022) have also indicated that foliar K application can increase plant resistance to abiotic stresses, as well as improve the physiological, biochemical and yield attributes of kabuli chickpeas grown under normal conditions. Despite the existing literature reporting positive results of foliar K application on physiological and productive characteristics of crops, limited benefits were observed with the application of foliar KNO_3 in this study. Furthermore, no synergistic effects were observed when combined with the application of biochar.

4.3. Biochar improves the plant root system either under limited and optimal water conditions

Root biometric parameters (TRL, RSA, RV, VFR, FR, TR) were significantly reduced under DI when compared to OI in our study. Tipically, under conditions of moderate water stress and depending on the stage of crop development when the stress occurs, there is an observed increase in root growth. Numerous studies have demonstrated that sustaining primary root elongation or promoting root hair formation serves as an adaptive response to low water potential (Wu et al., 1996; Yamaguchi & Sharp, 2010; Xu, et al., 2012; Sabir et al., 2020). Nevertheless, under conditions of severe water stress, as assessed in the present study, root development experiences limitations. This is due to substantial water demands required for the growth of tomato plants (Klunklin and Savage, 2017). Notably, similar findings regarding the adverse effects of water deficit-induced stress on root development were also observed in maize plants (Ma et al., 2010).

A novel aspect of this study was the discovery that soil biochar application positively influenced the biometric characteristics of tomato plant roots, irrespective of the water regime (optimal irrigation or deficit irrigation). These observed enhancement in root development can be primarily attributed to the physical modifications that biochar induces in the soil. The contrasting physical structures of soil and biochar lead to alterations in tensile strength, hydrodynamics, and gas movement within the soil matrix (Husseina et al., 2022). In soils comparable to the present study, i.e. those rich in clay, the tensile strength of clay surpasses that of biochar. Consequently, the addition of biochar in such soils results is a reduction in the overall tensile strength of soil (Husseina et al., 2022). This reduction in mechanical impedance favors the development of the root plant's system (Mertens et al., 2017; Nguyen et al., 2017). In addition to its impact on soil tensile strength, biochar also influences root growth and subsequently enhancing plant performance by directly controlling nutrient uptake by roots, serving as a source of nutrients, while indirectly modifying soil nutrient content (Prendergast et al., 2013). Bruun et al. (2014) observed positive results regarding the utilization of biochar in increasing water retention and root growth of barley (*Hordeum vulgare* cv. Anakin) in sandy soil. Additionally, the authors reported that the application of biochar at concentrations up to 2% significantly augmented root density at a depth of 40-80 cm.

4.4. Principal Component Analysis (PCA)

Principal Component Analysis (PCA) is a technique used to transform large datasets into independent variables known as principal components (PC), with the goal of reducing their dimensionality (Kul et al., 2021). The PCA provided a concise summary of the key findings in our study, as it effectively separate different groups of treatments based on biometric and physiological variables. The primary differentiation observed was associated with optimal irrigation (OI) treatment, where the majority of variables exhibited a positive correlation. Notably, the application of biochar under OI conditions further potentiated the positive effects on biometric and physiological variables of plants, as indicated by clustering of most of the variables around this treatment. This behavior underscores the beneficial impact of biochar application in enhancing plant performance under optimal irrigation conditions.

5. CONCLUSION

Overall, our research shows the potential of biochar as viable option for enhancing productive responses but also as a step towards sustainable agricultural practices under adverse weather and other environmental conditions. The results of this study clearly showed the negative effect of water deficit on the physiological parameters and biomass accumulation, both in shoots and root systems.

In-depth analysis of physiological analysis, growth patterns, biomass accumulation, and root system characteristics proved the benefits of biochar application in agricultural production systems. These benefits were shown not only under water-limited conditions but also in scenarios with optimal soil moisture levels. Based on our findings, it is recommended the incorporation of 1% (w/w) biochar at as soil amendment for pot-grown tomato.

Furthermore, our study reveals that foliar KNO_3 was unable to alleviate the negative effects of water stress on tomato, as it did not show any positive effects on the physiological and growth parameters of plants, nor did it exhibit synergistic effects when combined with biochar.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Departamento de Agronomia and Programa de Pós-graduação em Fitotecnia at Universidade Federal de Viçosa (UFV), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG).

Funding: This study was financed in part by the “Conselho Nacional de Desenvolvimento Científico e Tecnológico - Brasil (CNPq)”.

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Supplementary Material

Table S1. Chemical soil attributes before applying amendments

pH	**P**	**K**	**Ca $^{2+}$**	**mg $^{2+}$**	**Al $^{3+}$**	**H+Al**	**CEC**	**OM**	**Zn**	**Fe**	**Mn**	**Cu**	**Өcc^{1}**	**Өcc^{2}**
-H_2O-	mg dm $^{-3}$		------------ Cmol$_C$ dm $^{-3}$ -------------					-%-	--------mg dm $^{-3}$ --------				-----%-----	
5.1	1.1	57	1.16	0.39	0.3	4.95	6.6	2.15	0.2	34.9	18.0	2.1	27	29

Өcc^{1} = Pot capacity measured before experiment installation; **Өcc**^{2} = Pot capacity measured at the end of the experiment in pots treated with biochar.

Table S2. Nutrient solution for biochar-treated and -untreated soils for tomato cultivation

Nutrient	Nutrient solution – Treatment with biochar	Nutrient solution - Treatment without biochar
	mg/kg of soil	mg/kg of soil
N	150^{1}	150^{1}
P	$296.98^{1} + 3.02^{2}$	300^{1}
K	$0^{1} + 100^{2}$	100^{1}
S	40^{1}	40^{1}
B	0.81^{1}	0.81^{1}
Cu	$1.325^{1} + 0.0051^{2}$	1.33^{1}
Fe	3.4^{2}	3.4^{1}
Mn	$3.51^{1} + 0.15^{2}$	3.66^{1}
Mo	0.15^{1}	0.15^{1}
Zn	$2.24^{1} + 1.76^{2}$	4.00^{1}

[1] Via reactant

[2] Via Biochar

[3] Application directly to the soil via triple superphosphate fertilizer (36% P_2O_5 and 10% Ca).

Table S3. Significance of the effects of the variance analyses conducted for each physiological attribute in tomato plants cultivated under different levels of biochar (+Biochar and -Biochar), two foliar potassium feeding regimens (+KNO_3 and -KNO_3), and two irrigation regimes (Optimal Irrigation and Deficit Irrigation) ($p<0.05$)

FV	**GL**	**Quadrados médios**							
		LWP_pd	**LWP_m**	***Ci***	***E***	***gs***	***A***	***ECi***	***WUEi***
BLOCOS	3	**	ns	*	**	ns	*	ns	ns
IRRIGAÇÃO (IR)	1	ns	**	**	**	**	**	**	**
BIOCHAR (BIO)	1	**	*	*	ns	*	**	ns	ns
KNO_3	1	ns	ns	ns	ns	ns	ns	ns	ns
IR x BIO	1	ns	ns	ns	ns	ns	ns	ns	ns
IR x KNO_3	1	ns	ns	ns	ns	ns	*	ns	ns
BIO x KNO_3	1	ns	ns	ns	ns	ns	*	**	*
IR x BIO x KNO_3	1	ns	**	ns	ns	ns	ns	*	ns

Table S4. Significance of the effects of the variance analyses conducted for each biometric attribute in tomato plants cultivated under different levels of biochar (+Biochar and -Biochar), two foliar potassium feeding regimens (+KNO_3 and -KNO_3), and two irrigation regimes (Optimal Irrigation and Deficit Irrigation) ($p<0.05$)

FV	**GL**	**Quadrados médios**								
		HP (cm)	**NL**	**LA (cm)**	**RTL (cm)**	**RSA (cm^2)**	**RV (cm^3)**	**VFR (mm)**	**FR (mm)**	**TR (mm)**
BLOCOS	3	ns	ns	*	ns	ns	ns	ns	ns	ns
IRRIGAÇÃO (IR)	1	**	**	**	**	**	**	**	**	**
BIOCHAR (BIO)	1	**	*	**	*	*	*	*	*	ns
KNO_3	1	ns	ns	**	ns	ns	ns	ns	ns	ns
IR x BIO	1	ns	ns	ns	ns	ns	ns	ns	ns	ns
IR x KNO_3	1	ns	*	**	ns	ns	ns	ns	ns	ns
BIO x KNO_3	1	ns	ns	ns	ns	ns	ns	ns	ns	ns
IR x BIO x KNO_3	1	ns	ns	ns	ns	ns	ns	ns	ns	ns

Table S5. Significance of the effects of the variance analyses conducted for each biomass attribute in tomato plants cultivated under different levels of biochar (+Biochar and -Biochar), two foliar potassium feeding regimens (+KNO_3 and -KNO_3), and two irrigation regimes (Optimal Irrigation and Deficit Irrigation) ($p<0.05$)

FV	**GL**	**Quadrados médios**							
		MFF	**MFCA**	**MFPA**	**MFR**	**MSF**	**MSCA**	**MSPA**	**MSR**
BLOCOS	3	*	ns	ns	**	ns	ns	ns	ns
IRRIGAÇÃO (IR)	1	**	**	**	**	**	**	**	**
BIOCHAR (BIO)	1	**	*	**	**	ns	**	**	**
KNO_3	1	**	ns	ns	*	**	*	**	*
IR x BIO	1	ns	ns	ns	**	ns	*	ns	ns
IR x KNO_3	1	**	ns	ns	*	**	**	**	ns
BIO x KNO_3	1	ns	ns	ns	ns	**	ns	*	**
IR x BIO x KNO_3	1	ns	ns	ns	ns	*	ns	ns	*

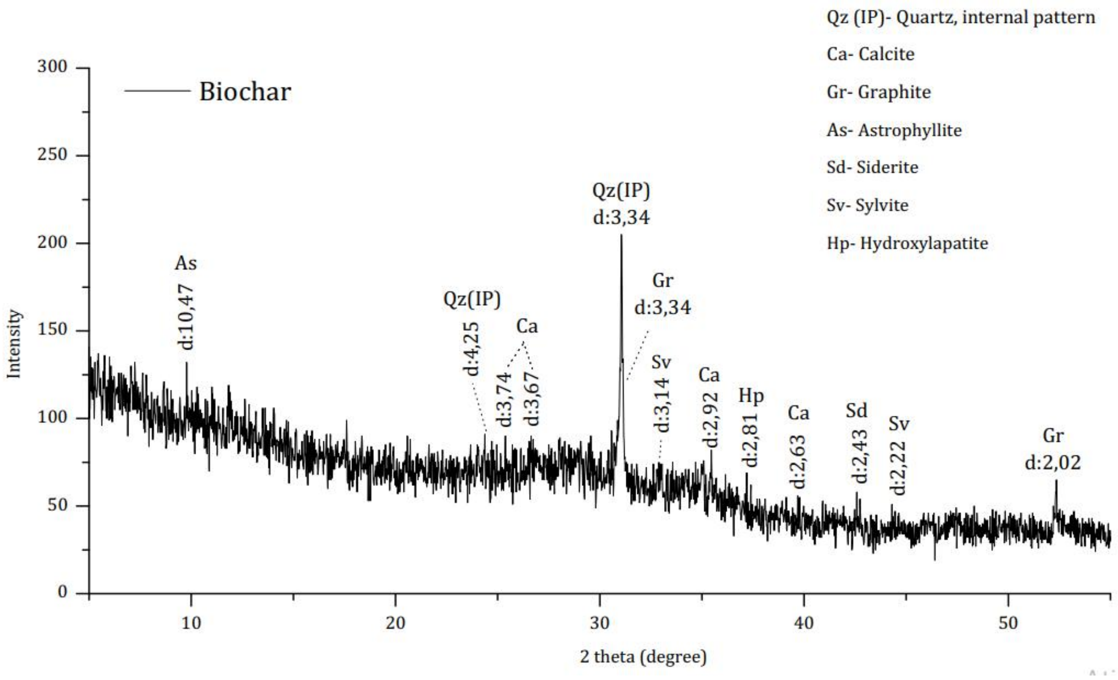


Fig. S1. X-ray diffraction analysis of coffe husk-based biochar

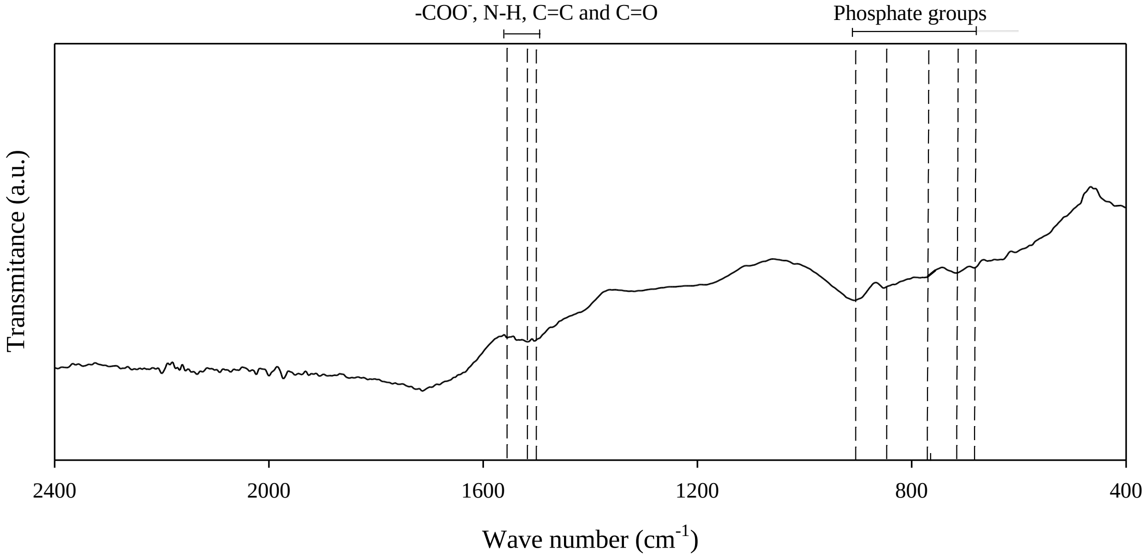
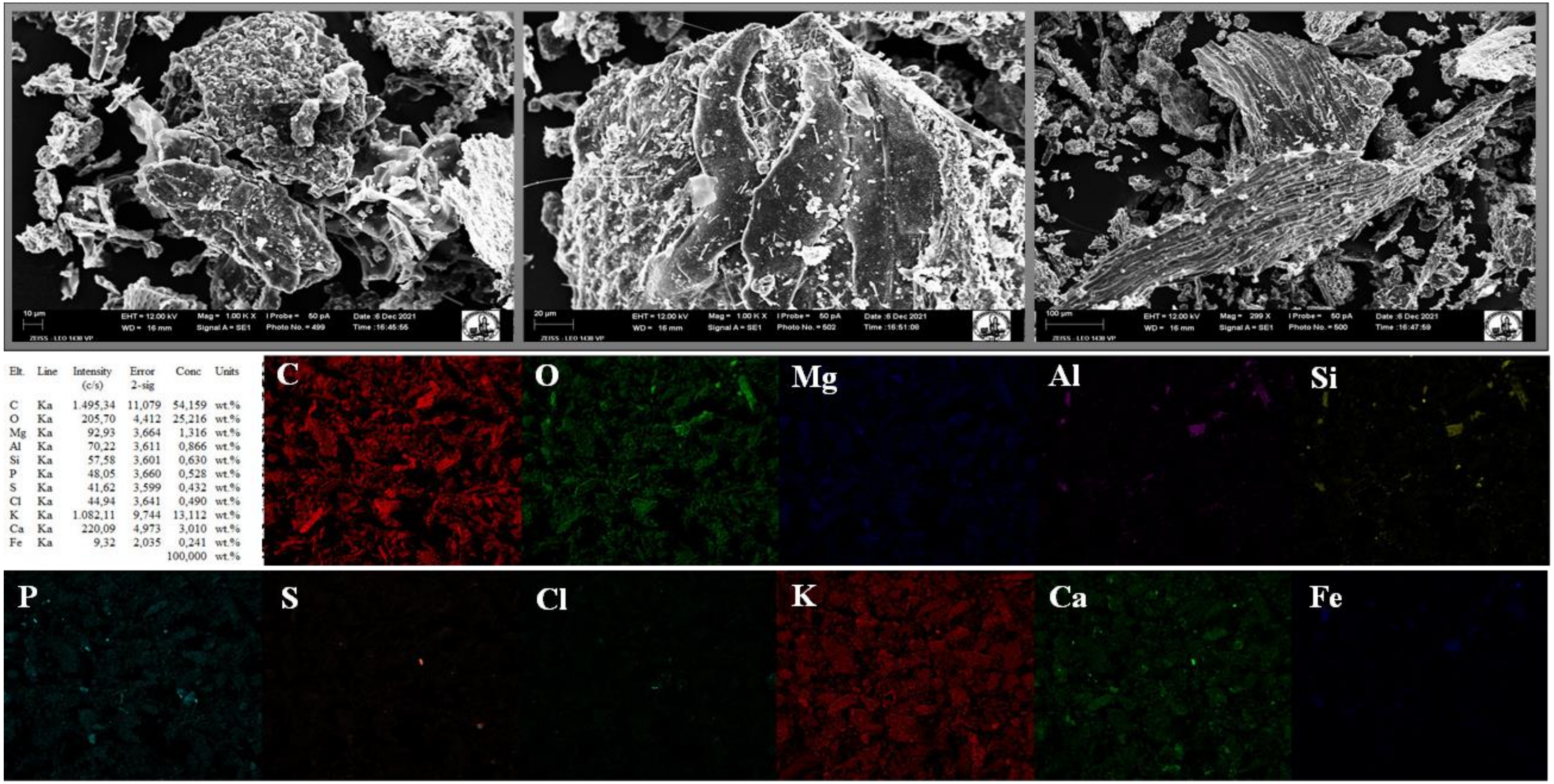


Fig.S2. Fourier-transform infrared spectroscopy (FTIR) in biochar from coffee husks



Elt.	Line	Intensity (c/s)	Error 2-sig	Conc	Units
C	Ka	1.495,34	11,079	54,159	wt.%
O	Ka	205,70	4,412	25,216	wt.%
Mg	Ka	92,93	3,664	1,316	wt.%
Al	Ka	70,22	3,611	0,866	wt.%
Si	Ka	57,58	3,601	0,630	wt.%
P	Ka	48,05	3,660	0,528	wt.%
S	Ka	41,62	3,599	0,432	wt.%
Cl	Ka	44,94	3,641	0,490	wt.%
K	Ka	1.082,11	9,744	13,112	wt.%
Ca	Ka	220,09	4,973	3,010	wt.%
Fe	Ka	9,32	2,035	0,241	wt.%
				100,000	wt.%

Fig.S3. Images and chemical mapping of coffee husk-based biochar

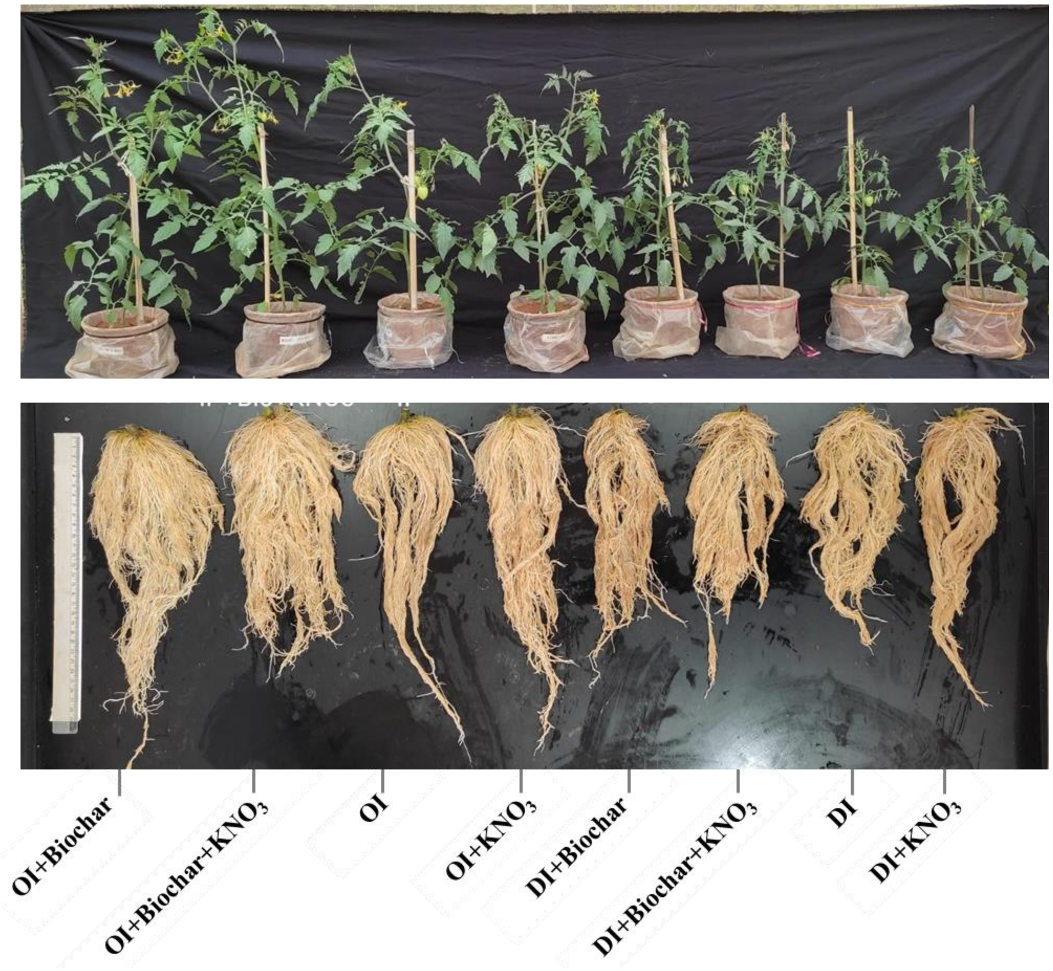


Fig. S4. Parte aérea e sistema radicular de tomate, 40 dias após transplante, sob diferentes níveis de biochar (+Biochar and -Biochar), dois regimes de fertilização foliar com KNO_3 (+KNO_3 and -KNO_3) e dois regimes de irrigação (OI e D)

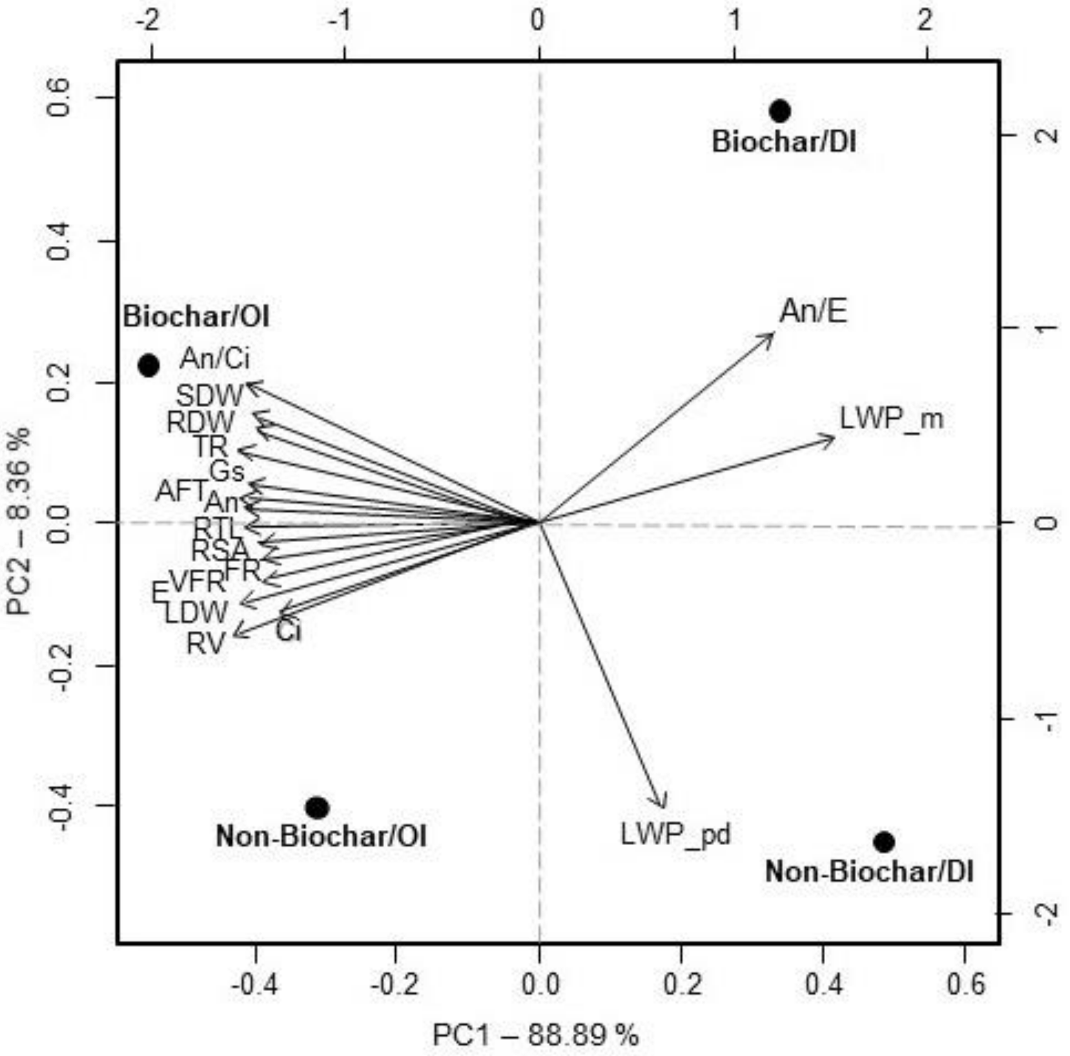


Fig. S5. Principal component analysis of the averages of physiological and biometric attributes in tomato plants under different levels of biochar (+Biochar and -Biochar) and two conditions of soil moisture (50 and 90% ASW). Internal CO_2 concentration (*Ci*), transpiration (*E*), net photosynthetic rate (*An*), stomatal conductance (*gs*), rubisco carboxylation efficiency (*An/Ci*), intrinsic water use efficiency (*An/E*), total leaf area (TLA), leaf dry weight (LDW), stem dry weight (LDW), root dry weight (RDW), predawn water potential (LWP_pd), midday water potential (LWP_m), total root length (TRL), root surface area (RSA), length of very fine roots (VFR), length of fine roots (FR), and length of thick roots (TR).

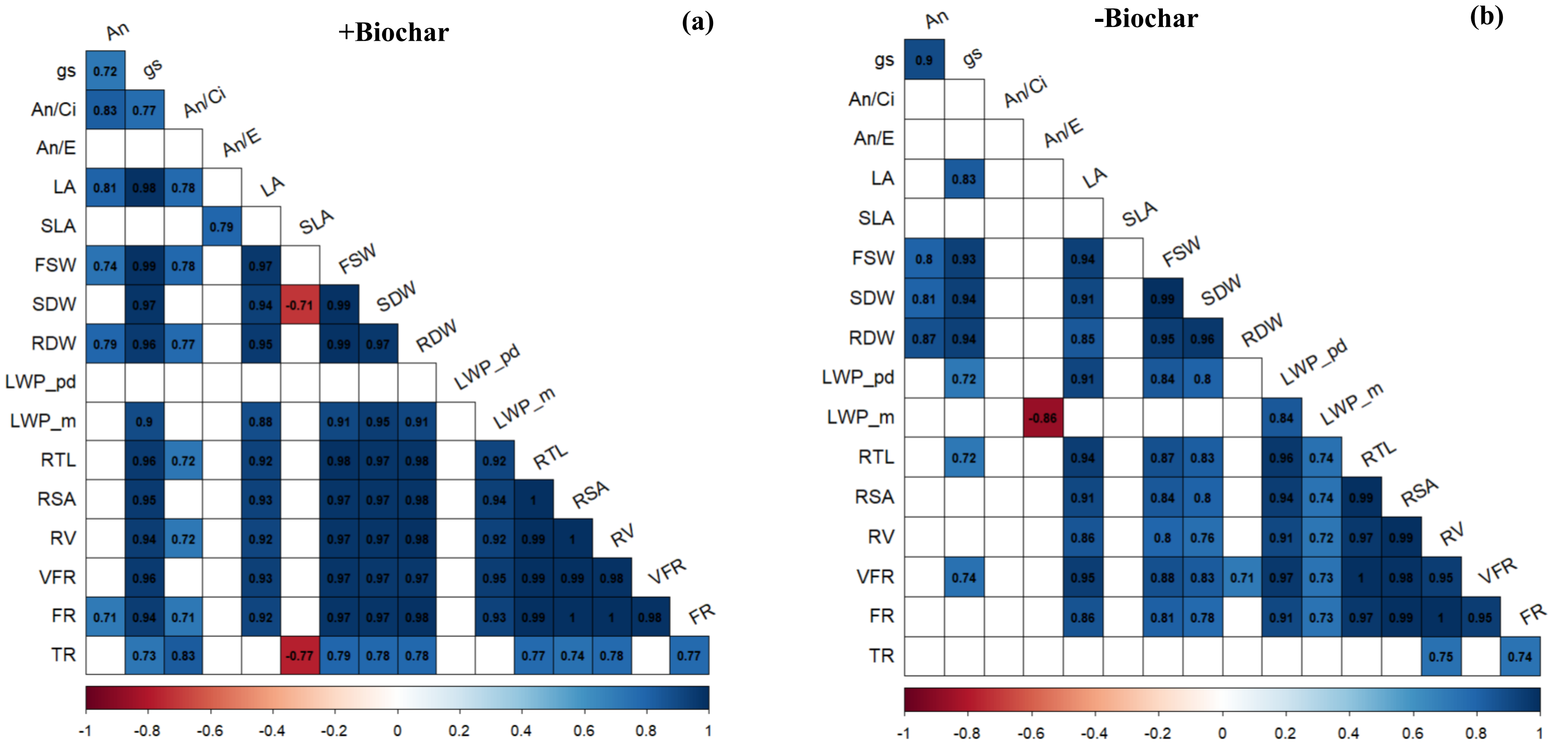


Fig. S6. Pearson's correlation matrix between all variables studied under conditions of Biochar application (a) and without Biochar application (b). Correlations were validated by the t-test at a significance level of 0.05. White squares mean that the correlation was not significant (p>0.05)

CHAPTER 3. Coffee husk biochar enhances soil chemical, physical, and hydraulic properties and increases tomato plant yield

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ABSTRACT

The depletion of soil fertility and freshwater reserves worldwide poses a threat to agricultural production and food security. Biochar bas been proposed as a soil amendment that improves both soil fertility and water retention, but there is a lack of studies under field conditions with varying soil moisture levels. This study aimed to assess the impact of coffee husk biochar under varied irrigation levels on soil characteristics, tomato yield, and water use efficiency. The field experiment employed a randomized complete block design with split-plot arrangements, with four irrigation levels in the main plots (50, 75, 100, and 125% of ETc), with and without biochar in the subplots (400 g plant^{-1} = +Biochar and no biochar = -Biochar). Results indicated that the highest fruit yield (179.6 t ha^{-1}) was observed with biochar application under the highest water level (125% of ETc). Conversely, the highest water use efficiency (104.8 kg m^{-3}) was found when no biochar was applied, and irrigation equivalent to 50 % of ETc. Soil moisture at field capacity under biochar application was increased by 9 % and 6 % at depths of 0-20 cm and 20-40 cm, respectively, while moisture at wilting point increased by 6 % at both depths. Moreover, available water in the 0-20 cm layer increased by 13 % under biochar treatment. Biochar application also led to notable enhancements in soil nutrient levels. The soil pH and the contents of K, P, and OM increased substantially in the 0-20 cm layer. There was also an increase in the levels of K, Mg^{2+}, and P in the 20-40 cm layer. In summary, coffee husk biochar was a beneficial soil conditioner and nutrient source. Besides enriching soil carbon content, it enhances water retention, optimizes nutrient availability, and increases tomato yields under optimal irrigation, but not under strong water deficit.

Keywords: *Solanum lycopersicum* L.; Yield; Sustainability

1. INTRODUCTION

The utilization of agricultural residues, a longstanding technique, is gaining renewed significance in modern agriculture under the concept of circular economy (Shinde et al., 2022; Yrjälä and Salo, 2022). This is primarily attributed to the enhancement in physical, chemical, and biological properties of soil, playing a fundamental role in sustainable agriculture (Ullah et al., 2020; Guo et al., 2021). Improper disposal of many of these residues can lead to environmental contamination (Chandra et al., 2005; Hasanudin et al., 2019); thus, the efficient utilization of agricultural residues not only enhances soil quality but also mitigates environmental pollution.

A wide range of agricultural residues are a valuable source for biochar production as a soil conditioner. For instance, coffee husk biochar is rich in potassium (K) and has a high cation exchange capacity, which can improve soil physical and chemical properties (Domingues et al., 2020). Biochar is a solid, carbon-rich product obtained through the pyrolysis of various organic feedstocks such as straw, wood, husks, and manures (Fonseca et al., 2020; Tao et al., 2023), which are thermally decomposed at high temperatures under limited oxygen conditions (Lehmann et al., 2015). The primary characteristic of biochar is its ability to slow down carbon turnover in terrestrial ecosystems, leading to increased carbon sequestration in soils (Joseph et al., 2020; Gross et al., 2021). Biochar releases nutrients in soil slowly while enhancing soil physical and hydraulic properties over longer periods (Mohan et al., 2018; Ponomarev et al., 2023). Therefore, biochar can influence the growth and development of crops by improving water and nutrient uptake (Wan et al., 2023; Singh et al., 2023).

Tomato is a popular, economically important vegetable crop produced and consumed worldwide (FAO, 2020). The plant is a warm-season crop that often faces periods of water shortage, especially during the most sensitive stages of fruit setting and filling (Di Stasio et al., 2020). At these stages, even a brief water shortage can lead to significant crop losses (Cui et al., 2019). Typically, plants respond to insufficient water supply through a series of physiological mechanisms, including stomatal closure, decreased photosynthesis, inhibition of cell growth and activation of antioxidant mechanisms, which generally result in reduced plant growth and yield (Tardieu et al., 2018).

Coffee husk biochar can be strategically used to mitigate water stress in tomato and to meet the plant’s demand for K because Brazil is the world's largest coffee producer, with production of over 50 million bags per year (Conab, 2022). Yet, Brazilian agriculture is highly dependent on K fertilizer imports and a circular economy using locally available K-rich resources should be encouraged. Coffee husk biochar has been shown to increase soil fertility and water use efficiency in maize grown on a sandy soil under greenhouse conditions (Lima et al., 2018). However, there is still a lack of evidence under field conditions. Therefore, we hypothesized that coffee husk biochar can effectively supply K to and improve water use efficiency of tomato plants under water stress conditions. The objective of the study was to evaluate the effect of coffee husk biochar application under field conditions on soil and chemical and physico-hydraulic characteristics, and how these soil changes impact fruit yield and water use efficiency of tomato plants.

2. METHODOLOGY

2.1 Study site

The study was conducted under field conditions in an Oxisol at the Horta Velha Research and Extension Area, at the Federal University of Viçosa, Viçosa, Minas Gerais state, Brazil (20° 45' 14" S; 42° 52' 53" W; 649 m above sea level) from June to October 2022. Prior to the field experiment, two greenhouse experiments were carried out (Castro Filho et al., 2024a; Castro Filho et al., 2024b) to evaluate the effect coffee husk biochar on the physiology, yield and fruit quality of tomato plants grown under two contrasting soil moisture levels (50 and 90 % of field capacity). The area where the test was conducted is dedicated to successive experimental crops. Before the implementation of the tomato plants, this area was used for pumpkin cultivation.

The local climate is Cwa type according to Köppen, with a humid mesothermal climate characterized by rainy summer and dry winter. The annual average precipitation, relative humidity and air temperature are 1,221 mm, 81 %, and 19.4 ºC, respectively (National Meteorology Department, 1992). Monthly data for rainfall (mm), temperature (ºC), and other climatic variables during the study period are shown in Figure S1.

2.2. Experimental design

The experiment was set up in a randomized complete block design with four replications in a split-plot arrangement. The irrigation levels (50, 75, 100, and 125 % of crop evapotranspiration - ETc) were assigned to the main plots, with and without biochar (400 g plant^{-1} = +Biochar and no biochar = -Biochar) in the subplots. The total area of the experiment was 273.6 m^2 (14.4 m x 19.0 m). The spacing was 0.4 m between plants and 1.2 m between rows. The blocks were spaced 1 meter apart. Each main plot consisted of three rows of 10 plants each, resulting in an area of 14.4 m^2. Each subplot was composed of 5 plants along a 2-m-long row, resulting in an area of 7.2 m^2. Observational units in the subplots consisted of 3 plants from the central row. Edge rows and the first and last plants of the central row were considered as border (Figure S2).

2.3. Biochar production and characterization

Biochar was produced using coffee husks (husk + pulp + parchment) from *Coffea arabica* obtained at the Federal University of Viçosa (20° 45' 14" S; 42° 52' 53" W; altitude of 649 m). The biochar was prepared in an electric muffle furnace, adapted with a condenser with pyrolysis carried out at 500 °C with a heating rate of 10 °C min^{-1} (Lustosa Filho et al., 2017).

Biochar was characterized as the following: (*i*) Carbon (C), hydrogen (H), and nitrogen (N) contents were measured using a Perkin Elmer Series II 2400 Elemental Analyzer. Oxygen (O) content was calculated by difference (O = 100 - C - H - N - ash). Ash content was determined following the ASTM D - 2866 standard method (ASTM D, 2007); (*ii*) Electrical conductivity (EC) and pH were measured in water using potentiometry in a 1:20 solution, after a 12-hour resting period and suspension agitation (Rajkovich et al., 2012); (*iii*) Available nutrient contents were determined using a Mehlich-III solution at a ratio of 1:10 (Krounbi et al., 2021); (*iv*) Total nutrient contents were determined using the dry ashing method at 500 °C and wet digestion using HNO_3 and H_2O_2 (Enders and Lehmann, 2012); (*v*) Identification of minerals in crystalline phases was conducted through powder X-ray diffraction (XRD) using a PAN analytical device, X' Pert Powder model, with a cobalt tube, nickel filter in the range of 4 to 50°2θ, and a scanning rate of 10°2θ; (*vi*) Identification of functional groups was performed through Fourier-transform infrared spectroscopy (FTIR) using a Jasco FTIR 4100 instrument, acquiring spectra with 60 scans, ranging from 4000 to 400 cm^{-1} wavenumbers, with a resolution of 4 cm^{-1}.

2.4. Chemical and physical-hydraulic properties of the soil

Soil chemical and physical-hydraulic properties were determined at two depths (0-20 and 20-40 cm) and are presented in Table S1. The soil was amended with limestone to raise the base saturation to 70 %. For this purpose, dolomitic limestone (neutralizing power of 76 %) was applied one month before cultivation. The experimental area was irrigated after the limestone application to accelerate the chemical reactions.

2.5. Field experiment setup

The experiment lasted from June 1, 2022 (transplant date) to October 16, 2022, when the final harvest took place. Tomato seedlings of the Hybrid 'Lampião' F1 variety (Topseed

Premium), belonging to the 'Saladette' group, were used. This variety has determinate growth, a medium cycle, and resistance to pathogens such as Fusarium 0-2, tomato mosaic virus (ToMV), tomato yellow leaf curl virus (TYLCV), and verticillium (Va and Vd).

The biochar was applied to the planting furrow at a depth of 20-30 cm, at a rate of 400 g per plant (8333), aiming to meet 100% of the K recommendation for tomato plants (573 kg ha^{-1}). The other available nutrients supplied by biochar were considered (Table 1) and discounted from the total amount applied to each treatment (+Biochar and -Biochar) to ensure that the total nutrient content provided was equivalent in both situations. Fertilization followed the recommendations for tomato (Alvarenga et al., 2013) and was applied weekly.

Table 1. Total nutrients supplied (kg ha^{-1}) in treatments +Biochar and -Biochar, regardless of the applied irrigation depth

Nutrient	+Biochar	-Biochar
N	396	396
P	425 (0,05 %)[1]	425
K	573 (100 %)[1]	573
S	400	400
Ca	340 (7.1 %)[1]	340
Mg	9.2 (35.9 %)[1]	9.2
Mn	2.9 (3.9 %)[1]	2.9
B	8.0	8.0
Zn	2.5 (52.7 %)[1]	2.5

[1] Percentage of nutrients supplied via Biochar.

The inorganic fertilizers used to meet these amounts of nutrients were: single superphosphate (00-18-00 + 16 % Ca + 10 % S), calcium nitrate (15-00-00 + 20 % Ca), urea (45-00-00), monoammonium phosphate (12-52-00), potassium chloride (00-00-60), potassium nitrate (12-00-46), potassium sulfate (00-00-50 + 18 % S), magnesium sulfate (9.5 % Mg and 12 % S), zinc sulfate (21 % Zn and 11 % S), manganese sulfate (15 % Mn and 14 % S), and boric acid (17 % B).

2.6. Irrigation management

Daily irrigation management was based on crop evapotranspiration (ETc) (Equation 1). This approach determines the irrigation level by considering the amount of water lost through plant transpiration and soil water evaporation (Ke). Therefore, irrigation water and precipitation were the water inputs in the water balance, and water output was ETc. The amount of irrigation water varied according to crop evapotranspiration (ETc), estimated using the dual crop coefficient (Kc) methodology (Allen et al., 2006), considering Kc values of 0.60 for the initial phase, 1.05 for the intermediate phase, and 0.80 for the final phase of the growth cycle (Allen et al., 2006).

$$ET_c = ET_o \times K_c \times K_l \tag{1}$$

Where:

-ET_c = crop evapotranspiration (mm d^{-1});
-ET_0 = reference evapotranspiration (mm d^{-1});
-K_c = crop coefficient (admissible);
-K_l = location coefficient (admissible).

The location coefficient (Kl) was calculated according to Keller & Bliesner (1990) (Equation 2).

$$K_l = 0.1\sqrt{PWA\ or\ PSA} \tag{2}$$

Where:

PWM = Percentage of Wetted Area (%);
PSA = Percentage of Shaded Area (%).

Initially, we considered the percentage of wetted area (PWA) resulting from the irrigation system; however, once the percentage of wetted area became lower than the shaded area percentage, the latter was used. The irrigation system operated with an efficiency of 97.5 % measured according to the determination of the Christiansen uniformity coefficient.

The irrigation system adopted was drip. This irrigation system was equipped with emitters spaced at 0.4 m, with lateral lines arranged at a spacing of 1.2 m. The system operated with an actual flow rate of 2.8 L h^{-1}. The amounts of water applied to the treatments were 50, 75, 100 and 125 % of ETc, always considering the system's efficiency.

2.7. Analyzed variables

2.7.1. Harvest

Fully ripe tomato fruits were harvested during the experimental period. Yield parameters consisted of the total fruit yield per hectare (TY) (kg ha^{-1}), measured as the fresh weight of all fruits collected from 3 plants and extrapolated to a density of 20,833 plants per hectare; and average fruit weight (FW) (g), measured as the average weight of 30 fruits from different harvests.

2.7.2. Determination of soil moisture and water use efficiency

Moisture values corresponding to field capacity and permanent wilting point, in each subplot and at depths of 0-20 and 20-40 cm, at the end of the experiment, were determined using the Richards pressure chamber at the pressures of 33 and 1,500 kPa. When drainage ceased, pressure application was stopped. After the pressures were applied, soil samples were dried in ovens at 105 °C for 48 hours to determine the water content based on dry soil.

The estimation of WUE was obtained based on the relationship between commercial yield of fruits and applied water volume, according to the equation 3, and was expressed in kg m^{-3}

$$WUE = \frac{TY}{WA} \tag{3}$$

Where:

WUE = Water use efficiency (kg m^{-3});

PY = Yield (kg ha^{-1});

V = volume of water applied (m^3 ha^{-1}).

2.7.3. Soil chemical properties

At the end of the cultivation period a 0–20 and 20–40 cm soil sample from each plot was collected and soil analyses were conducted following the methods described in Teixeira et al. (2017). Soil pH was determined in H_2O at a soil:solution ratio of 1:2.5, using a benchtop digital pH meter. Available P and K were extracted using the Melich-1 extractor (0.05 mol L^{-1} HCl + 0.0125 mol L^{-1} H_2SO_4), and determined colorimetrically and by flame photometry, respectively. Exchangeable Ca^{2+} and Mg^{2+} were extracted using 1.0 mol L^{-1} KCl and determined through flame atomic absorption spectrometry (FAAS). The content of organic carbon (C-org) was determined through oxidation with dichromate (0.167 mol L^{-1} $K_2Cr_2O_7$) under acidic conditions using an external heat source to maximize oxidation (Yeomans and Bremner, 1988).

2.8. Statistical analysis

A two-way analysis of variance (ANOVA) was conducted to test the significance of irrigation levels (50, 75, 100, and 125 % of ETc) and biochar (+Biochar and -Biochar). For qualitative data (biochar levels), when they were significant according to the F-test, means were compared using the Tukey test at $p<0.05$. For quantitative data (irrigation levels), regression analysis was employed. Regression models were defined based on significance ($p<0.05$), the highest coefficient of determination (R^2) value, and the biological response for each studied characteristic. All statistical analyses were performed using R software (version 3.6.0).

3. RESULTS

3.1. Biochar Characteristics

The biochar showed a high total K content (68.8 g kg^{-1}) with 45 % available by Mehlich-3 (Table S2). The ash content was also high (225 g kg^{-1}) due to the high level of inorganic material. The biochar pH and EC were 11.9 and 5.1, respectively, which align with values reported in the literature for coffee husk biochars (Domingues et al., 2017; Pouangam Ngalani et al., 2023.

The X-ray diffraction pattern of the biochar displayed a broad diffraction peak ($2\theta = 31°$), corresponding to quartz (Figure S3). The peaks at $2\theta = 31°$ and 52.5° could be attributed to graphite (C_n), which might have been crystallized during the pyrolysis process. Other identified minerals included calcite ($CaCO_3$) (2θ = 26, 27, 36, and 40°), astrophyllite [$(K, Na)_3 (Fe^{++}, Mn))7\ Ti_2Si_8O_{24}(O, OH)_7$] ($2\theta = 10°$), siderite ($FeCO_3$) ($2\theta = 43°$), sylvite (KCl) ($2\theta$ = 33.5 and 44.5°), and hydroxyapatite [$Ca10(PO_4)_6(OH)_2$] ($2\theta = 37.5°$).

The functional groups of the biochar showed three peaks at 1560, 1530, and 1500 (Figure S4) that were attributed to C=C, C=N vibrations, −COO, asymmetric stretching of amino acids, and stretching vibrations of C=O in ketones, quinones, aldehydes, lactones, carboxylic acids, and esters, as well as the N−H band (Rodriguez et al., 2021; Taherymoosavi et al., 2017). Peaks at 906, 842, 760, and 720 cm^{-1} were assigned to P–P–O vibrations (Bekiaris et al., 2016; Lustosa Filho et al., 2017).

The surface morphology of the biochar showed the presence of small, well-structured particles (Figure S5), likely due to the breakdown of different organic compounds from the raw material during the pyrolysis process. Additionally, the EDS spectrum indicated the presence of C and O, along with a relatively high concentration of K and Ca.

3.2. Total yield (PY) and average fruit weight (FW)

The results of crop yield and average fruit weight as related to biochar application and irrigation levels are presented in Figure 1. There was a significant interaction between the factors of biochar (+Biochar and -Biochar) and irrigation levels (50, 75, 100, and 125 % of ETc) on tomato yield. At the intermediate irrigation levels (75 and 100 % of ETc) there were no significant differences among the treatments. At the lowest irrigation level (50 % of ETc), the application of biochar resulted in a reduction of 20 % in crop yield; conversely, at the highest irrigation level (125 % of ETc), biochar application led to a significant increase of 6 % in crop yield (Figure 1A). When assessing the impact of irrigation levels in relation to different biochar, it was observed a linear increase in tomato crop yield as irrigation levels increased in both treatment groups (+Biochar and -Biochar). However, the growth response was more pronounced in the biochar-treated group (Figure 1B).

In terms of fruit weight average, no significant interaction was observed between biochar application and irrigation levels, suggesting that these factors act independently. The average fruit weight exhibited a quadratic trend in response to varying irrigation levels, with the maximum fruit weight occurring at an irrigation level of approximately 107 % ETc. At the maximum efficiency irrigation level, the average fruit weight reached approximately 130 g (Figure 1C).

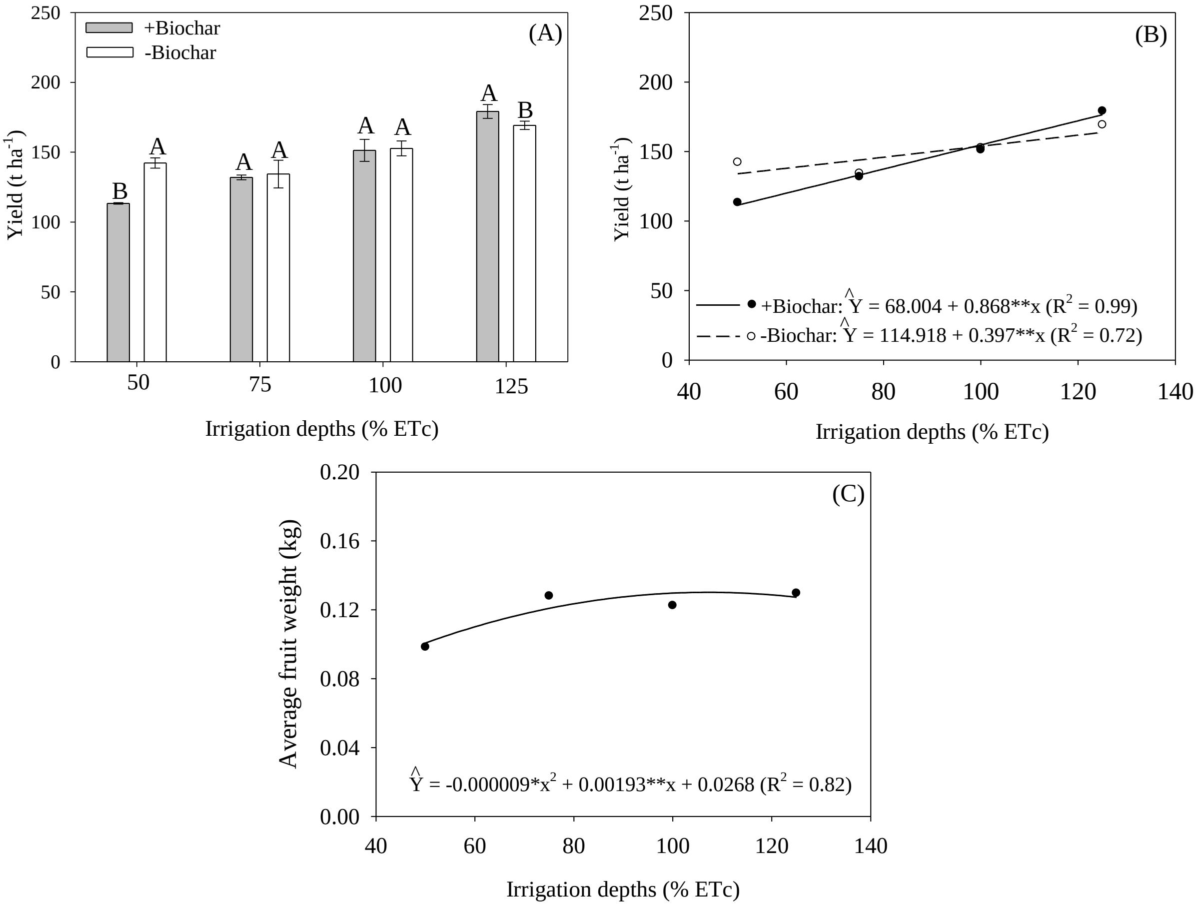

Figura 1. Tomato crop yield based on biochar treatments (+Biochar and -Biochar) at each irrigation level (A), tomato crop yield based on irrigation levels for each biochar level (+Biochar and -Biochar) (B), and average fruit weight based on irrigation levels (C).

3.3. Physical-hydraulic properties of soil

The application of biochar improved soil physical-hydraulic properties as evidenced by the increase of 9 % and 6 % in soil moisture content at field capacity at soil depths of 0-20 and 20-40 cm, respectively (Figures 2A and 2B). A similar trend was observed for soil moisture content at permanent wilting point, which increased by 6 % in the two soil depths 0-20 and 20-40 cm (Figures 2C and 2D). The biochar-treated soil showed higher available water (13 %) content only at the 0-20 cm depth (Figure 2E).

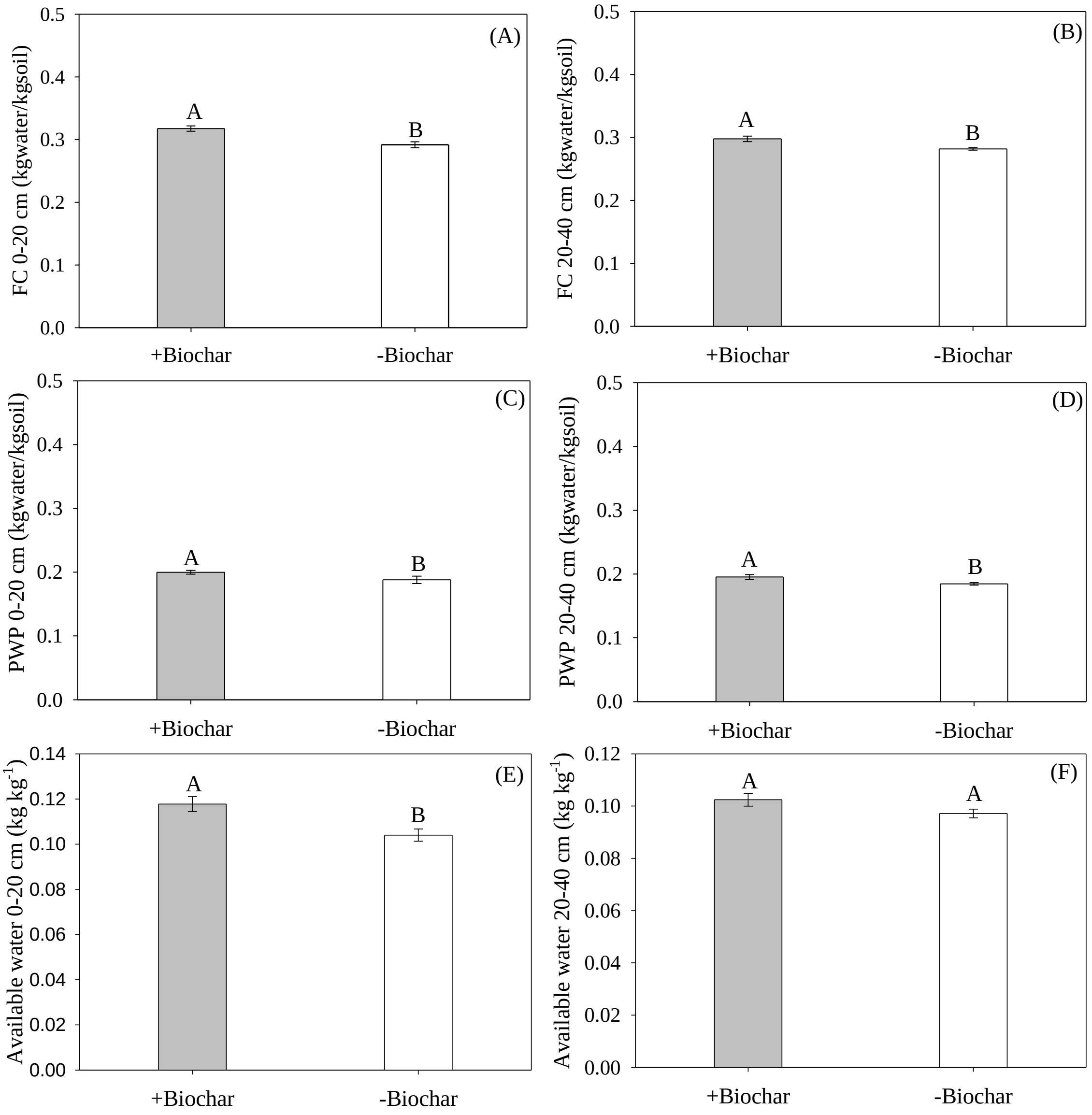

Figura 2. Field capacity 0-20 cm [FC 0-20] (A), field capacity 20-40 cm [FC 20-40] (B), permanent wilting point 0-20 cm [PWP 0-20] (C), permanent wilting point 20-40 cm [PWP 20-40] (D), available water 0-20 cm [AW 0-20] (E), and available water 20-40 cm [AW 10-40] (F).

3.4. Water use efficiency

There was a significant effect ($p<0.05$) for the biochar x irrigation levels interaction. When comparing the treatments with and without biochar (+Biochar and -Biochar), a significant difference was only observed at the 50 % ETc irrigation level. Specifically, in this scenario, the treatment without biochar application resulted in higher water use efficiency (Figure 3A). As for the estimation of water use efficiency based on irrigation levels, a decrease in WUE was observed as irrigation levels increased in both biochar treatments (+Biochar and -Biochar). However, this decline was less pronounced in the biochar treatment (26 % reduction in +Biochar versus 45 % reduction in -Biochar) (Figure 3B). This less pronounced reduction may be related to a more significant increase in yields of plants treated with biochar.

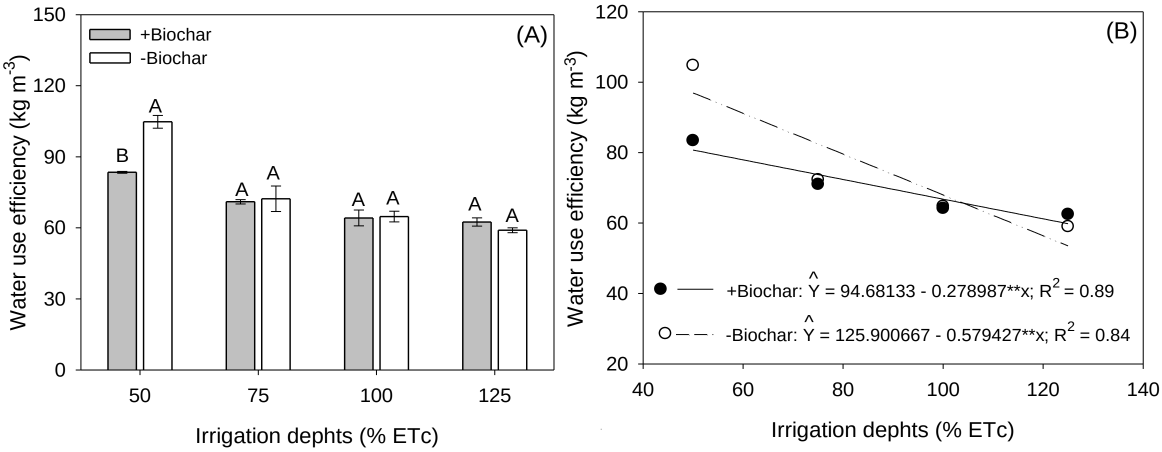

Figure 3. Water Use Efficiency in relation to biochar levels (+Biochar and -Biochar) at each applied irrigation level (A) and estimation of Water Use Efficiency based on irrigation levels for each biochar level (+Biochar and -Biochar) (B).

3.5. Soil chemical attributes

For chemical soil attributes, including pH, P, Ca^{2+}, cation exchange capacity (CEC), and organic matter (OM) at 0-20 cm depth, a significant interaction between biochar application and irrigation levels was observed. Conversely, for the attributes K, Al^{3+}, and H+Al isolated effects of irrigation levels and biochar application were observed in the soil. This indicates that these factors operate independently and exert separate influences on these variables (Figure 4).

When evaluating the individual effect of biochar on available K content in the soil, it becomes clear that the application of biochar led to higher concentrations (61 %) of this nutrient in the soil after tomato cultivation. Furthermore, the application of biochar resulted in a reduction of 63 % in exchangeable acidity (Al^{3+}) (Figure 4E) and 26 % in potential acidity (H+Al) (Figure 4F), which is favorable for better crop development. This reduction is important since Al^{3+} is toxic to most plant species, including tomato plants.

Higher levels of Ca^{2+} and cation exchange capacity (CEC) were observed when biochar (+Biochar) was applied at irrigation levels of 50 % and 125 % of the crop evapotranspiration (ETc). Conversely, the opposite behavior was observed at irrigation levels of 75 % and 100 % of the ETc, where Ca levels were higher in the treatment without biochar (-Biochar) (Figure 4D). As for P levels in the soil, biochar application enhanced in 196 % and 161 % P availability at the irrigation levels of 50 % and 125 % of the ETc, respectively (Figure 4B). Concerning the OM content in the soil, it is evident that the biochar application promoted an increase in content across various irrigation levels (44 % at 50 % ETc; 31 % at 75 % ETc and 96 % at 100 %), except at the 125 % ETc irrigation level, where no significant difference was observed (Figure 4G). On average, biochar application resulted in a 28 % increase in SOM.

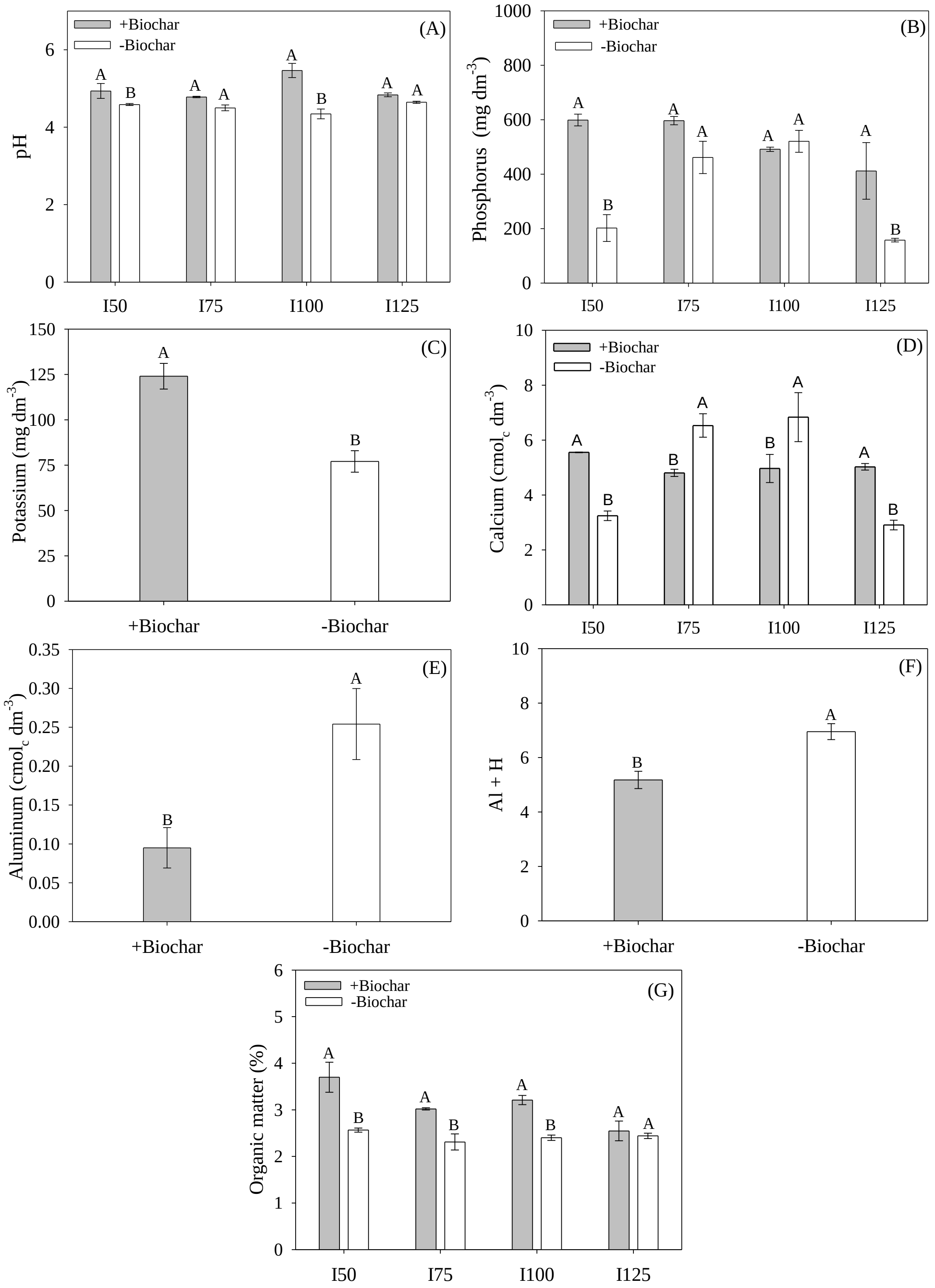

Figure 4. Soil pH 0-20 cm [pH 0-20] (A), Phosphorus 0-20 cm [P 0-20] (B), Potassium 0-20 cm [K 0-20] (C), Calcium 0-20 cm [Ca 0-20] (D), Aluminum 0-20 cm [Al 0-20] (E), Aluminum + Hydrogen 0-20 cm [Al + H 0-20] (F) and Organic Matter 0-20 cm [OM 0-20] (G), as a function of biochar levels (+Biochar and -Biochar) in tomato plants cultivated under different irrigation levels (50, 75, 100 and 125 % of the ETc).

When considering the individual effect of irrigation levels without biochar application in the soil, a quadratic behavior was observed for most of the evaluated variables (Figure 6), and the highest levels of P and Ca^{2+} occurred at irrigation levels of approximately 86 % and 77 % of the ETc, respectively (Figure 5A-B). While the OM content remained relatively constant when biochar was not applied to the soil (Figure 5C). Conversely, available P and OM content showed a linear decrease in the soil with increased irrigation levels with the application of biochar. For calcium and sum of bases content, a slight reduction is observed with increasing irrigation level under biochar-treated soil.

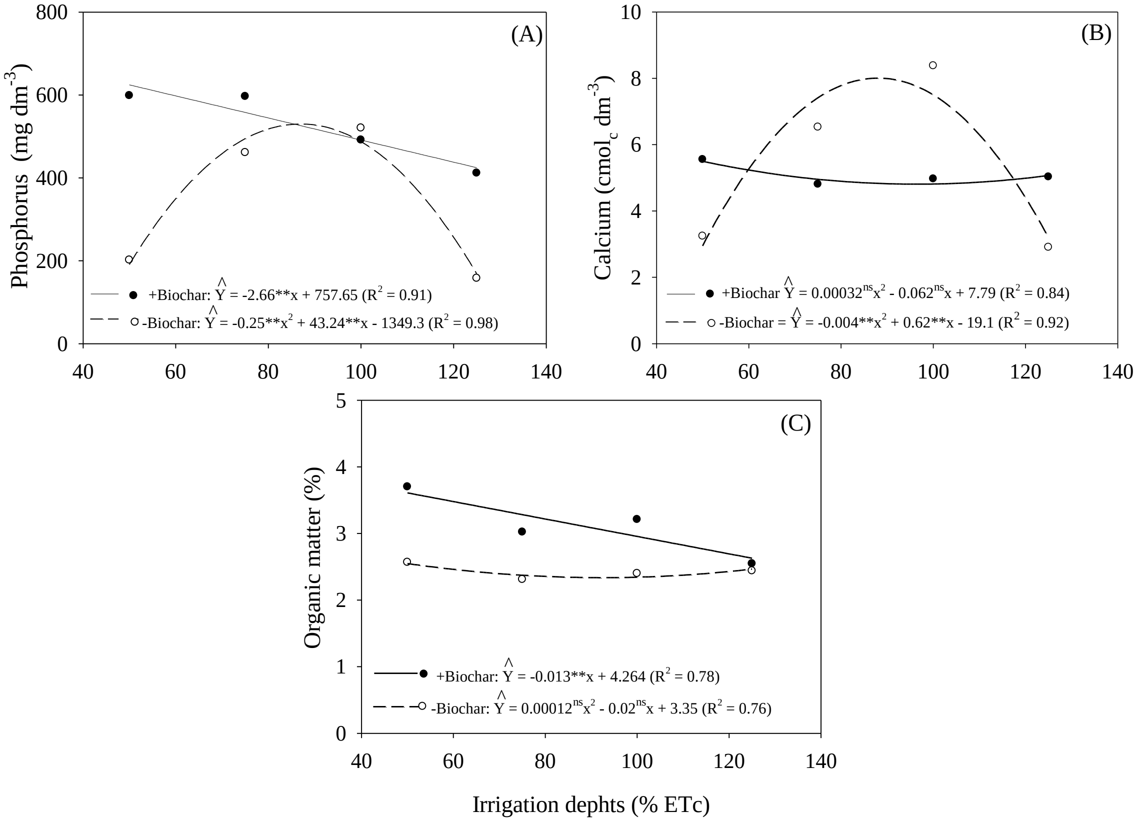

Figure 5. Phosphorus 0-20 cm [P 0-20] (A), Calcium 0-20 cm [Ca 0-20] (B) and Organic Matter 0-20 cm [OM 0-20] (C), as a function of different irrigation levels (50, 75, 100 and 125 % of the ETc) in tomato cultivation under two levels of biochar (+Biochar and -Biochar).

For P, SB, and Ca^{2+} at the depth of 20-40 cm, there was a significant interaction between the application of biochar and irrigation levels. However, for pH, Mg^{2+}, and K, only individual effects were observed (Figure 6), and biochar application led to higher levels of K and Mg^{2+} and reduced soil acidity. Under biochar application, soil subjected to lower irrigation levels showed higher available P content compared to that without biochar. However, at higher irrigation levels there was no significant difference among the treatments. Conversely, the application of biochar led to higher levels of Ca and sum of bases in soil under both lower and higher irrigation levels (50 % and 125 % of ETc). In the case of the 75 % of ETc irrigation level, there was no significant difference among treatments. In contrast, at the 100 % of ETc irrigation level, the available levels of Ca and base sum were lower in the treatment involving biochar application.

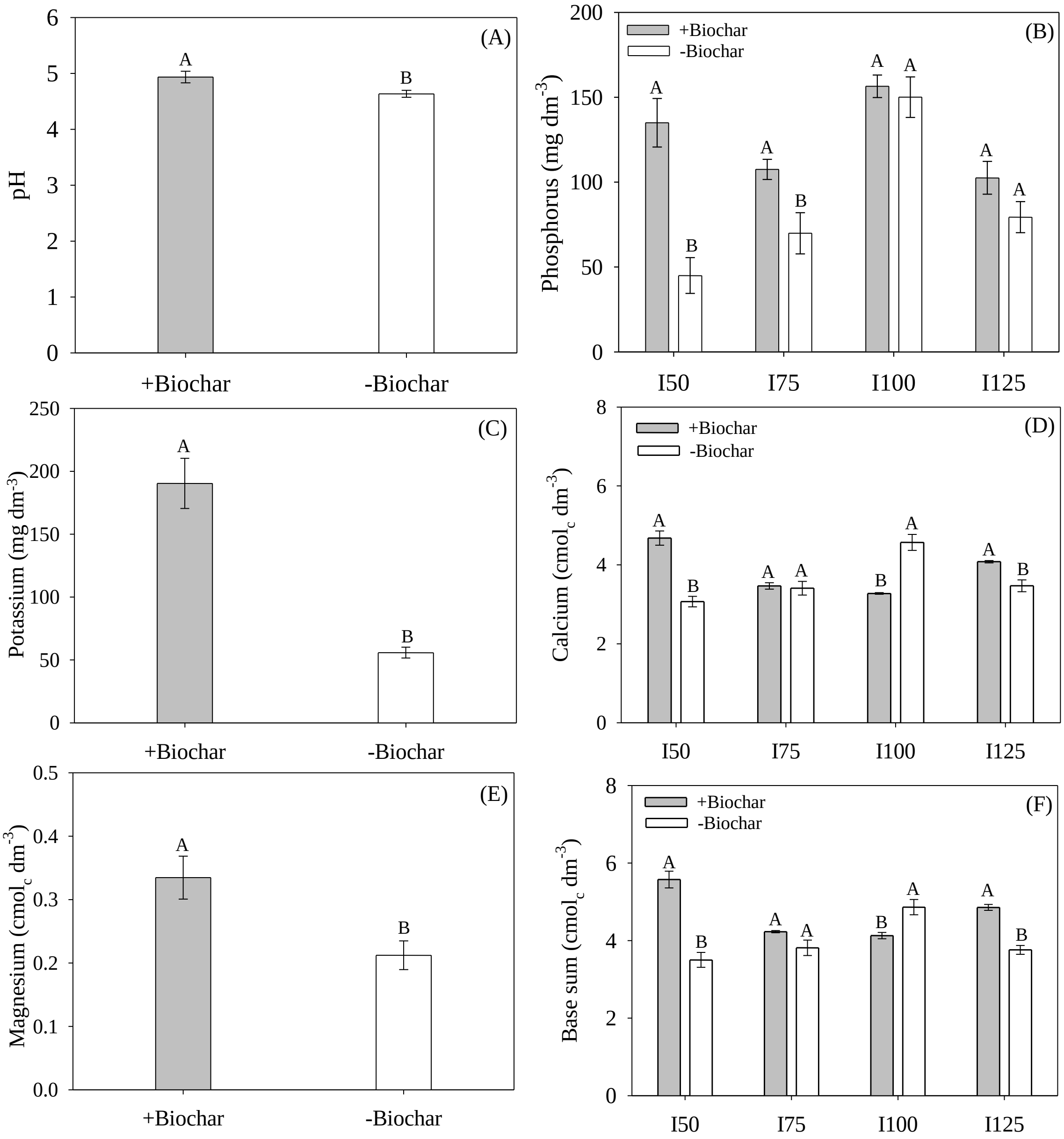

Figure 6. Soil pH 20-40 cm [pH 20-40] (A), Phosphorus 20-40 cm [P 20-40] (B), Potassium 20-40 cm [K 20-40] (C), Calcium 20-40 cm [Ca 20-40] (D), Magnesium 20-40 cm [Mg 20-40] (E), Sum of Bases 0-20 cm [SB 0-20] (F), based on levels of biochar (+Biochar and -Biochar) in tomato plants cultivated under different irrigation levels (50, 75, 100, and 125% of ETc).

For the individual effect of irrigation levels without biochar application, a quadratic behavior was observed for all evaluated variables with the highest levels of P, Ca^{2+}, and sum of bases occurring at irrigation levels of 98 %, 94 %, and 96 % of ETc, respectively (Figure 7).

However, for the individual effect of irrigation levels under soil biochar application, it was observed a quadratic behavior with a negative angular coefficient for Ca^{2+} and base sum, while for P no significant difference was observed.

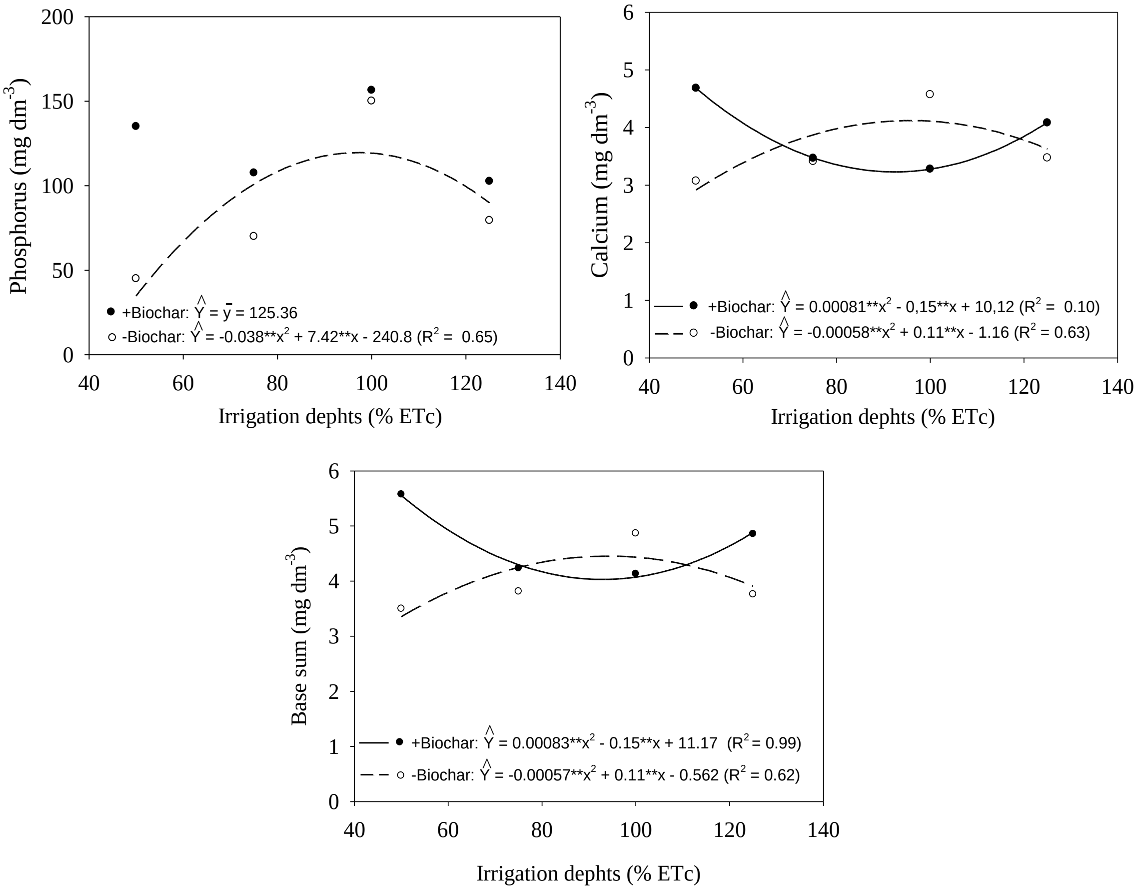

Figure 7. Phosphorus 20-40 cm [P 20-40] (A), Calcium 20-40 cm [Ca 20-40] (B), and Sum of Bases 20-40 cm [SB 20-40] (C), as a function of different irrigation depths (50, 75, 100 and 125 % of ETc) in tomato cultivation under two levels of biochar (+Biochar and -Biochar).

4. DISCUSSION

4.1. Coffee husks biochar has opposite effects on water use efficiency depending on soil water availability

We observed beneficial effects of coffee husks biochar application on tomato fruit productivity when irrigated considering 125 % of the crop evapotranspiration (ETc). This result can be attributed to improvements in the soil's physical-hydric characteristics (field capacity) and its nutrient retention capacity, preventing nutrient leaching. Furthermore, biochar application can alter soil hydraulic conductivity (Alghamdi, 2018), which would explain improved soil water dynamics under conditions of elevated irrigation water supply. Similar to the findings of Asai et al. (2009) in a clayey soil, where they checked the increase in hydraulic conductivity could reach up to 176 %. These results highlight the importance of also applying biochar to soils in high precipitation environments. Other researchers also observed improvement of chemical, physical, and biological soil conditions, as well as enhanced crop productivity under different conditions soil moisture and nitrogen (Ullah et al., 2020; Guo et al., 2021). In addition to the effects on the soil's physical and hydraulic properties, coffee husk biochar is nutritionally rich and, therefore, can contribute to the more efficient supply of nutrients to plants, as observed in our former study conducted under greenhouse conditions using coffee husk biochar at 1% (w/w) in tomato (Castro Filho et al., 2024b). Positive effects of using coffee pulp biochar on physical, chemical, and even biological quality of soil have been reported in a field experiment using coffee plants (Sánchez-Reinoso, 2023).

Contrary to our hypothesis, we did not observe an effect on tomato yield when biochar application was combined with irrigation levels of 75 % and 100 % of ETc, and when the irrigation was reduced to 50 % of ETc, there was a 20 % reduction in tomato yield. This could have occurred because the biochar elevated the permanent wilting point of this soil likely because of its water retention capacity that compete with available water for plant under severe drought conditions. This is the case of 50 % of ETc that represents a considerably low irrigation level, especially for tomato that require a large volume of water, this could have affected the access to readily available water for the crop, directly impacting crop productivity. The development and productivity of plants under water deficit conditions can be impaired based on the intensity and duration of the deficit as observed in tomato (Ghannem et al., 2021). However, some studies have pointed out the need for long-term research to better understand the effect of biochar on crop production, as its positive effects are often only observed over the long term due to the reactions that take place in biochar under soil environment (Zhang et al., 2016).

Water use efficiency (WUE) was reduced with the increase in the irrigation level, regardless of the use of biochar. This is possibly due to the low variation in productivity when compared to the different irrigation levels evaluated. A similar trend to the present study was observed in tomato plants subjected to different water levels (50, 75 and 100 % of Class A pan evaporation) and nitrogen in Northwest China, where the highest WUE was also associated with the lowest applied water level (Du et al., 2017). Furthermore, the WUE was lower in the biochar-treated group when provided with an irrigation level considering 50 % of ETc, possibly due to high biochar water retention that caused an increased moisture at the wilting point of this soil. This could hinder water absorption under conditions of low soil moisture, especially considering that the soil in the study is heavy-textured (clayey). Nevertheless, when a higher irrigation level was supplied (for example: 125 % of ETc), the biochar-treated group appears to have a tendency towards greater water use efficiency compared to the non-application of biochar. The effects of biochar application on water use efficiency are variable and dependent on experimental conditions, including specific soil properties, biochar parameters, and management factors (Gao et al., 2020). Our results indicate in a general trend that biochar reduces water use efficiency under low available water due to its high-water retention capacity and, conversely, increase water use efficiency under high levels of available water (e.g. 125 % of ETc) likely due to a slight reduction in easily water draining or evaporation. This brings important implications for water irrigation management in biochar-treated soils.

The moisture content at field capacity increased by 9% and 6% at depths of 0-20 cm and 20-40 cm, respectively. This may be attributed to the ability of certain types of biochar to alter the soil's pore network. However, further studies are needed to investigate the exact mechanism by which coffee husk biochar affects the water content at field capacity. Additionally, it raised moisture at the permanent wilting point by 6 % at both depths, 0-20 cm and 20-40 cm. Available water in the 0-20 cm layer was 13 % higher in the +Biochar treatment (0.12 kg kg^{-1} in +Biochar and 0.10 kg kg^{-1} in -Biochar). A similar result was also observed by other authors when coffee husk-derived biochar was applied (Lima et al., 2018; Kiggundu and Sittamukyoto, 2019). Increased moisture at the permanent wilting point may indicate that the fine pores of the biochar contributed to retaining more water near the dry end of the soil retention curve (Lima et al., 2018). This increase in available water could be attributed to the structural characteristics of biochar, such as internal surface area (Van Zwieten et al., 2009) and high porosity (Atkinson et al., 2010; Hina et al., 2010). Biochar can alter the physical and hydrological properties of the soil, including density, porosity, structure, aggregate stability, hydraulic conductivity, available water, and infiltration (Alghamdi, 2018). As a result, it can influence the growth and development of crops by affecting processes such as water and nutrient absorption and root respiration. Similar behaviors regarding increased soil water retention capacity, as observed in the present study, have also been reported in the literature corroborating our findings (Razzaghi et al., 2020).

The increase in field capacity due to the application of coffee husk biochar had a beneficial effect because of a significant reduction in drainage, thereby increasing water availability and storage (Lima et al., 2018). In contrast, the increase in moisture content at the wilting point affected the amount of available water under low water supply conditions for plants.

4.2. Biochar improves nutrient availability in soil

In the present study, biochar application significantly increased soil moisture at field capacity and available water content, and improved the chemical soil properties, particularly nutrient availability as observed in the literature for coffee husk biochar (Kiggundu and Sittamukyoto, 2019). Used as a K source, biochar was able to supply 100 % of this element to the plants while retaining the nutrient in the soil surface layer. As observed in the soil analysis, the levels of K in the 0-20 cm and 20-40 cm depth were higher in the soil treated with biochar (+Biochar). A similar result was observed in sandy soil when coffee husk-derived biochar was applied (Lima et al., 2018). Furthermore, an approximately 98% increase in soil K content was observed following the application of biochar produced from walnut shells (Novak et al., 2009). This increase in K concentrations could be attributed to the high electrical charge density of biochar, which retained the nutrient in layers 0-20 cm and 20-40 cm and reduced its leaching for deeper layers, since K as a monovalent element with a relatively large hydrated radius compared to other nutrients like Ca^{2+}, Mg^{2+} is more prone to leaching in soils (Huang, 2005). Additionally, the higher K content in soil could be linked to the alkaline nature of biochar with a high cation exchange capacity that possesses carboxyl and other functional groups on its surface (Mokolobate and Haynes, 2002). Similar behavior to the current study was found by Xia et al. (2020), who stated that the addition of biochar not only significantly increased available nutrients such as P and K, but also improved the physical and chemical properties of soil.

The levels of Ca^{2+} were also higher in soils amended with biochar, which is likely caused by an increase in soil CEC due to biochar addition as observed by Domingues et al. (2020) in soils treated with coffee husk biochar. Other studies also demonstrated an increase in nutrient levels in soils with biochar application due to an increase in the soils CEC (Mohan et al., 2018; Juriga and Šimansky, 2019). The CEC of soil is enhanced in the presence of biochar, due to the generation of negative charges resulting from the oxidation of functional groups present on the biochar surface, such as carboxylic and phenolic groups (Novak et al., 2009). Coffee husk biochar is rich in nutrients, mainly K, due to the characteristics of the original feedstock (Higashikawa et al., 2010; Domingues et al., 2017).

The higher levels of available P in soil amended with biochar may be related to the capacity to prevent adsorption onto iron and aluminum oxides present in soil. P becomes unavailable to plants due to the strong bond formed with oxides causing a fixation in the soil matrix (Oliveira et al., 2020). The high density of negative charges and functional groups present in biochar can form complexes with soil matrices, particularly iron and aluminum oxides, thereby blocking potential P adsorption sites (Fonseca et al., 2020). Furthermore, coffee husk biochar directly supplies P, and also through biochar oxidation in the soil, the P availability may increase in the soil (Fonseca et al., 2020; Lima et al., 2018).

The application of biochar also contributed to the reduction of exchangeable soil acidity, which is favorable for tomato development because Al^{3+} is a toxic element for most plant species (Borgo et al., 2020; Kopittke et al., 2015). This effect could be attributed to the increase in soil pH due to biochar liming effect (Mohan, et al., 2018), release of organic acids by the biochar, which participate in important soil reactions such as complexation and association with metals, altering their mobility and solubility, as well as adsorption and pH buffering (Mansoor et al., 2021; Liang et al., 2021). In the specific case of aluminum, the organic acids present in the biochar can form complexes with Al^{3+}, serving as a protective strategy for the extracellular root sites that are sensitive to aluminum (Meurer, 2015).

Despite the soil already containing a significant amount of carbon in the 0-20 cm layer (23 g of organic matter per kg of soil, equivalent to approximately 26.68 t of carbon), the organic matter content increased with the application of biochar. This increase is due to the direct incorporation of carbon from the biochar, owing to the high application rate of 8.3 t ha^{-1}, which added 3.11 t of carbon to the soil. An increase in soil organic carbon was observed by using coffee husk biochar in an acidic soil in West Cameroon (Pouangam Ngalani et al., 2023). Positive effects on tomato fruit yield and quality were also observed in soil treated with biochar under reduced irrigation conditions (Akhtar et al., 2014). In addition to increasing soil carbon content, it is important to emphasize that biochar is a carbon source with long residence times and low decomposition rates (Gross et al., 2021). This sets it apart from other organic materials such as animal manure, compost, and mulch, which contribute to soil carbon storage but may result in rapid decomposition of organic carbon and CO_2 emissions (Agegnehu et al., 2017; Paustian et al., 2016).

Despite biochar being a more stable carbon source compared to other sources such as vegetal and animal residues, a portion of its carbon can still undergo mineralization. The extent of mineralization depends on carbon stability, influenced by factors such as the type of material used in biochar preparation, pyrolysis time, and temperature. Additionally, other factors can affect the degree of mineralization of biochar's labile carbon, including soil moisture and the presence of microorganisms. This study observed an increase in organic matter mineralization with biochar application under increased soil moisture conditions (increased irrigation depth). It is important to note that in this study, the reduction in organic matter content is not solely due to the mineralization of organic matter provided by biochar, but rather to all organic matter present in the soil where biochar was applied. According to the literature, biochar carbon consists of at least two distinct pools, namely labile and stable organic carbon (Mandal et al., 2016; Zimmerman, 2010). Labile and stable organic carbon are characterized by low and high resistance to mineralization, respectively (Han et al., 2020). Up to 15–20% of biochar carbon can mineralize, with the mineralization rate decreasing over time (Han et al., 2020). This suggests that after the relatively rapid mineralization of labile organic carbon, the remaining stable organic carbon in biochar is mineralized very slowly by microorganisms.

5. CONCLUSION

The addition of biochar produced from coffee husks improved soil conditions for tomato growth and yield when irrigation exceeded 100 % of crop evapotranspiration (ETc). The main improvements included increased soil moisture at field capacity, available water content, and elevated levels of exchangeable cations.

Under conditions of low water availability, using coffee husk-derived biochar was detrimental to tomato crop productivity and water use efficiency. Conversely, in conditions of sufficient water availability, biochar proved beneficial, with plants under 125 % ETc having the highest tomato yield.

It can be concluded that the use of coffee husks biochar had a notably beneficial impact on the soil's physical-hydraulic and chemical conditions, and on the environmental footprint by increasing carbon content. Therefore, it can be considered a beneficial soil conditioner and a fertilizer based on sustainable organic materials, serving as a viable potassium source.

Ultimately, further studies are needed to assess the long-term effects of biochar application on soil and crop productivity.

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Supplementary Material

Table S1. Chemical and physical characteristics of the soil in the experimental area

	Variables	Measurement unit	Depth	
			0-20 cm	20-40 cm
Chemical analysis	pH	H_2O	5.7	5.7
	P	mg dm^{-3}	81.6	40.9
	K	mg dm^{-3}	130.0	96.0
	Ca^{2+}	cmol$_c$ dm^{-3}	4.1	3.3
	Mg^{2+}	cmol$_c$ dm^{-3}	0.5	0.3
	OM	%	2.3	1.3
Granulometry[1]	Sand	kg kg^{-1}	0.364	0.311
	Silt	kg kg^{-1}	0.095	0.124
	Clay	kg kg^{-1}	0.540	0.565
Soil water retention curve[2]	-10	kPa	0.395	0.398
	-30	kPa	0.337	0.345
	-100	kPa	0.309	0.320
	-300	kPa	0.253	0.268
	-1500	kPa	0.230	0.242

P, K available extracted with Mehlich-1; Ca, Mg, and Al extracted with 1 mol L^{-1} KCl; Potential acidity at pH 7.0 with calcium acetate obtained from 1 mol L^{-1}.

[1] Physical parameters of granulometry.

[2] Water tension at different potentials.

Table S2. Nutrient composition of coffee husk biochar

Analysis	P	K	Ca^{2+}	Mg^{2+}	S	Al^{3+}	Zn	Fe	Mn	Cu
	g kg $^{-1}$						mg/kg			
Biochar (total nutrient content)	2.7 ± 0.1	68.8 ± 1.5	8.7 ± 0.3	3.8 ± 0.07	1.8 ± 0.02	6.5 ± 0.3	455.0 ± 56	4873.3 ± 210	199.0 ± 27	46.9 ± 1.3
Biochar (nutrient content available)	0.3 ± 0.0	30.9 ± 0.6	3.2 ± 0.1	0.44 ± 0.08	-	-	175.7 ± 7.2	340.5 ± 12.2	15.1 ± 0.6	0.5 ± 0.03
Physicochemical Attributes	yield g/kg	EC dS/cm	pH (H_2O)	C	H	N	O	ashes	Θ_{CC}	Θ_{PMP}
				%					kPa	
	341.2	5.1 ± 0.06	11.9 ± 0.02	37.5	2.4	0.4	37.2	22.5	1.05	0.72

Phosphorus (P); potassium (K), calcium (Ca); magnesium (Mg); sulfur (S); aluminum (Al), zinc (Zn); iron (Fe); manganese (Mn); copper (Cu); productivity (Yield); electrical conductivity (EC); total acidity (pH); carbon (C); hydrogen (H); nitrogen (N); oxygen (O); field capacity (Θ_{CC}) and permanent wilting point (Θ_{PMP}).

Table S3. Significance of the effects of the analysis of variances conducted for the soil physico-hydric attributes and productive traits of tomato crops cultivated under two levels of biochar (+Biochar and -Biochar) and different irrigation levels (50, 75, 100, and 125% of ETc) ($p<0.05$)

SOURCE OF VARIATION	DF	Significance of effects							
		θ_{CC} 0-20	θ_{CC} 20-40	θ_{PMP} 0-20	θ_{PMP} 20-40	Available water 0-20	Available water 20-40	Yield	Average fruit weight
Blocks	2	ns	ns	ns	ns	ns	ns	ns	ns
Biochar (BIO)	1	**	*	**	*	*	ns	ns	ns
Irrigation levels (IR)	3	ns	*	ns	ns	ns	ns	**	**
BIO x IR	3	ns	ns	ns	ns	ns	ns	*	ns

DF = Degrees of freedom; field capacity at depth of 0-20 cm (Θ_{CC} 0-20); permanent wilting point at depth of 0-20 cm (Θ_{PMP} 0-20); field capacity at depth of 20-40 cm (Θ_{CC} 20-40); permanent wilting point at depth of 20-40 cm (Θ_{PMP} 20-40); ns = nonsignificant; ** and * = significant at 0.01 and 0.05 probability by the F test.

Table S5. Significance of the effects of the analysis of variances conducted for the soil chemical attributes, collected at the depth of 0-20 cm, cultivated under two levels of biochar (+Biochar and -Biochar) and different irrigation levels (50, 75, 100, and 125% of ETc) ($p<0.05$)

SOURCE OF VARIATION	DF	Significance of effects – Chemical Analysis 0-20 cm								
		PH	P	K^+	Ca^{2+}	Mg^{2+}	Al^{3+}	Al + H	SB	OM
Blocks	2	ns	ns	ns	ns	ns	ns	ns	ns	ns
Biochar (BIO)	1	**	**	**	ns	ns	**	**	ns	**
Irrigation levels (IR)	3	ns	**	*	**	ns	ns	ns	**	**
BIO x IR	3	**	**	ns	**	ns	ns	ns	**	*

DF = Degrees of freedom; total acidity (pH); phosphorus (P); potassium (K^+), calcium (Ca^{2+}); magnesium (Mg^{2+}); aluminum (Al^{3+}); aluminum + hydrogen (A + H); sum bases (SB); organic matter (OM); ns = nonsignificant; ** and * = significant at 0.01 and 0.05 probability by the F test.

Table S4: Significance of the effects of the analysis of variances conducted for the soil chemical attributes, collected at the depth of 20-40 cm, cultivated under two levels of biochar (+Biochar and -Biochar) and different irrigation levels (50, 75, 100, and 125% of ETc) ($p<0.05$)

SOURCE OF VARIATION	DF	Significance of effects - Chemical Analysis 20-40 cm								
		PH	P	K	Ca	Mg	Al	Al + H	SB	OM
Blocks	2	**	ns	ns	ns	ns	ns	ns	ns	ns
Biochar (BIO)	1	*	**	**	*	**	ns	ns	**	ns
Irrigation levels (IR)	3	ns	**	ns	*	ns	ns	ns	*	*
BIO x IR	3	ns	**	ns	**	ns	ns	ns	**	ns

DF = Degrees of freedom; total acidity (pH); phosphorus (P); potassium (K^+), calcium (Ca^{2+}); magnesium (Mg^{2+}); aluminum (Al^{3+}); aluminum + hydrogen (A + H); sum bases (SB); organic matter (OM); ns = nonsignificant; ** and * = significant at 0.01 and 0.05 probability by the F test.

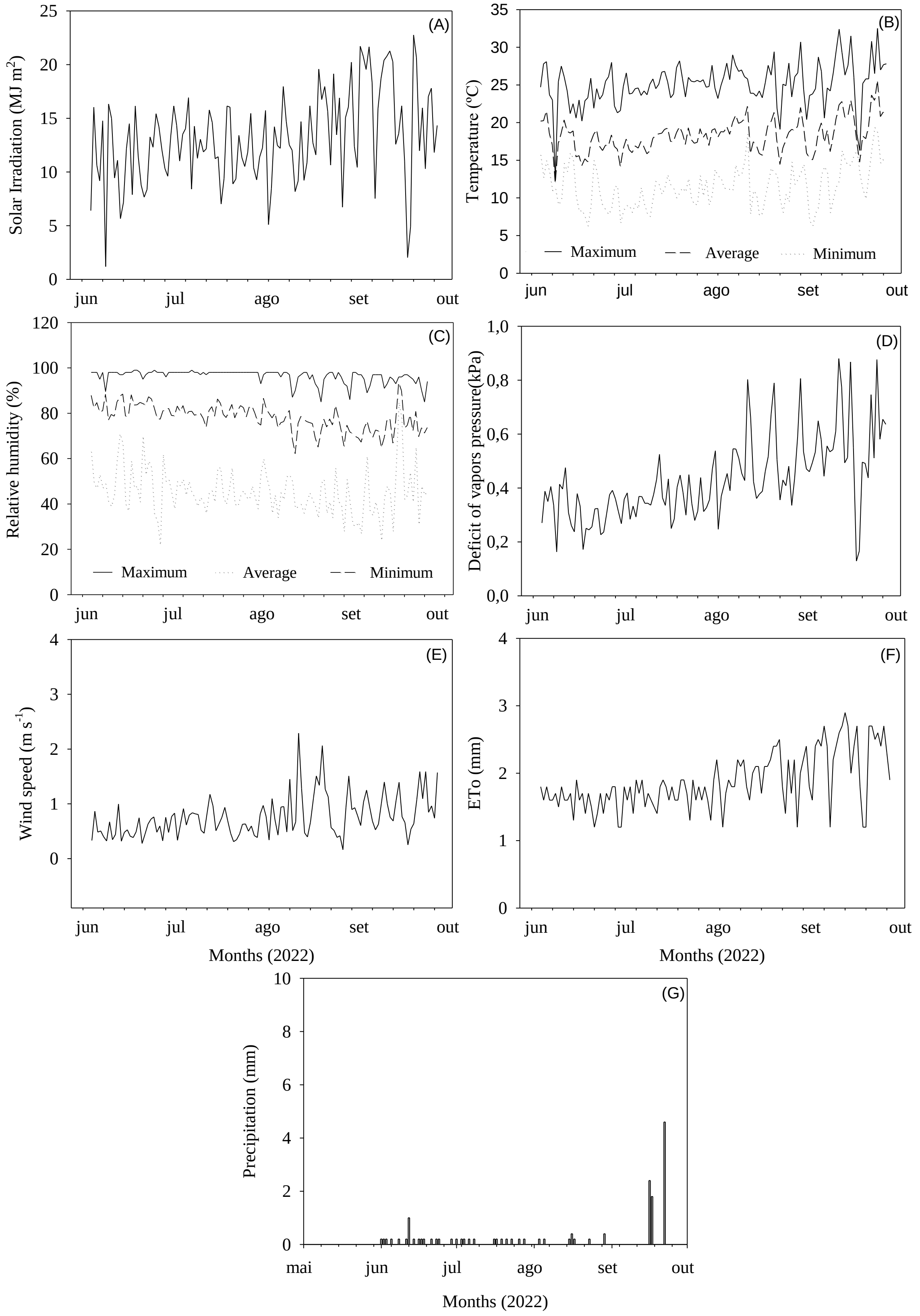


Figure S1. Solar radiation (a), maximum, minimum, and average temperatures (b), maximum, minimum, and average air relative humidity (c), vapor pressure deficit (d), wind speed (e), reference evapotranspiration (f), and precipitation (g). Viçosa-MG, 2022

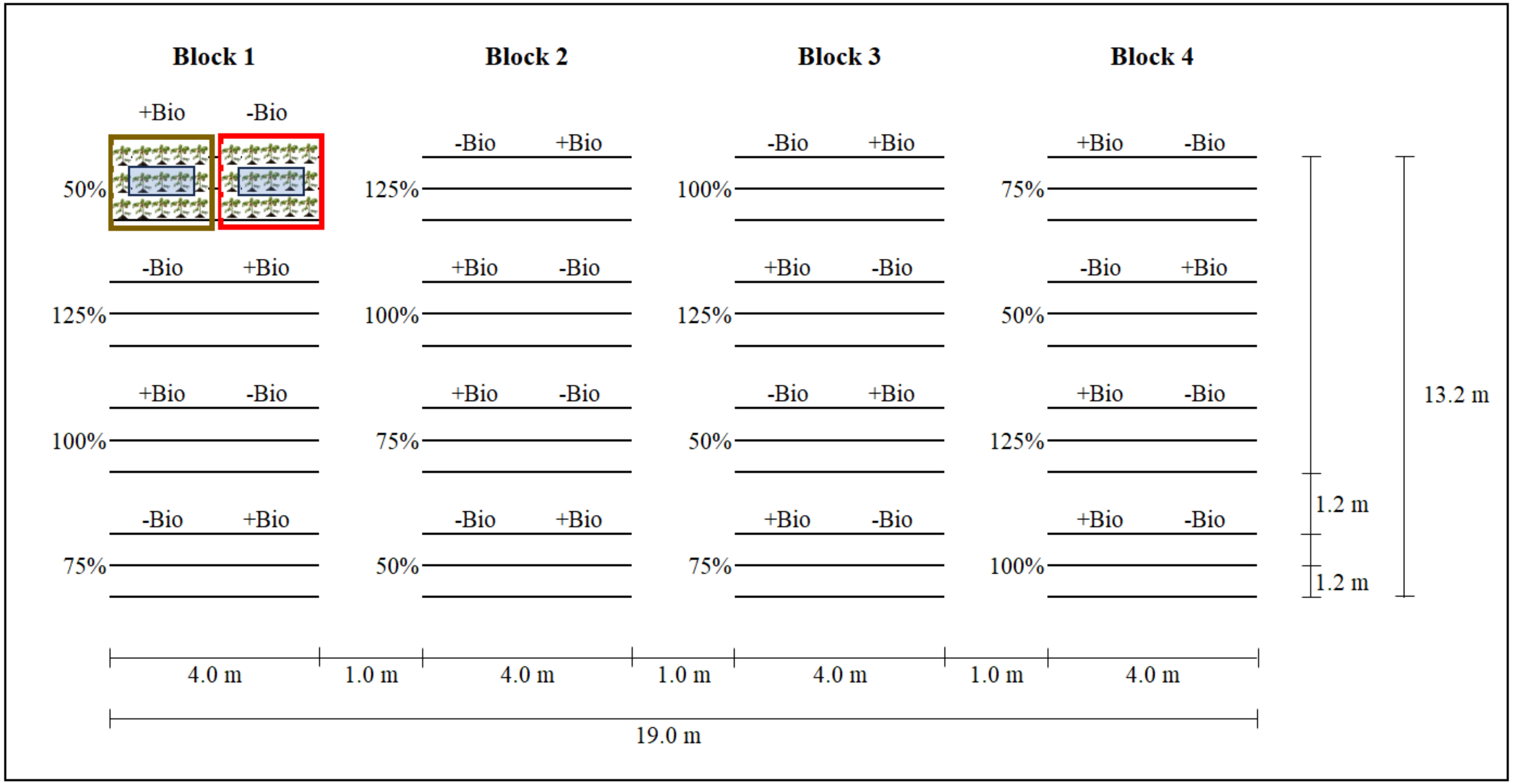


Figure S2. Distribution scheme of main plots and subplots on the field

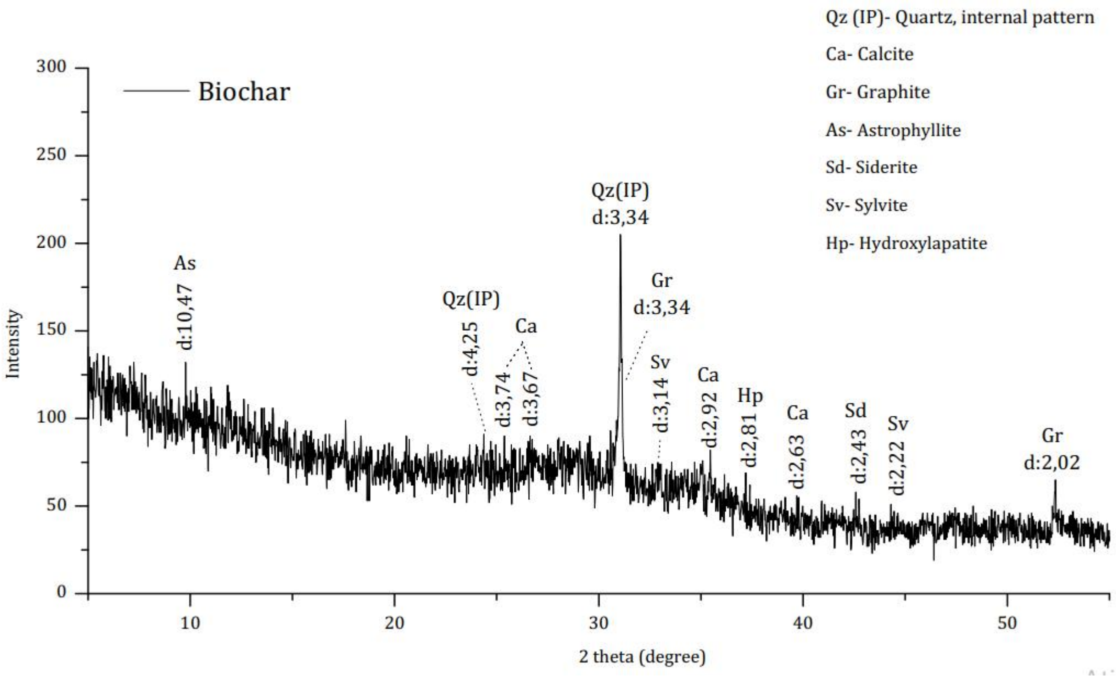


Figure S3 – X-ray diffraction analysis of coffe husk-based biochar

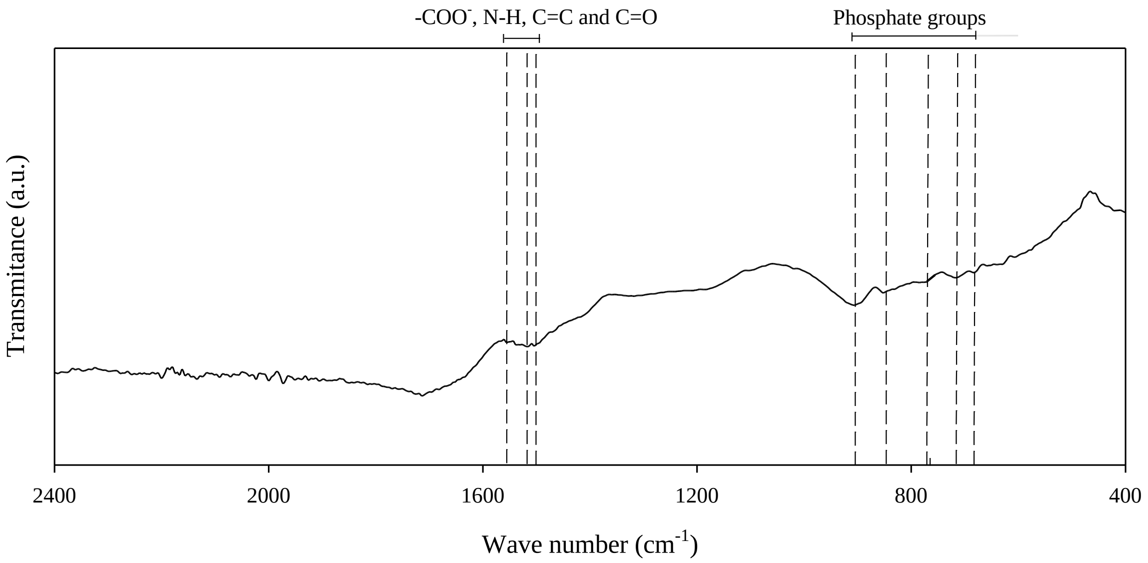
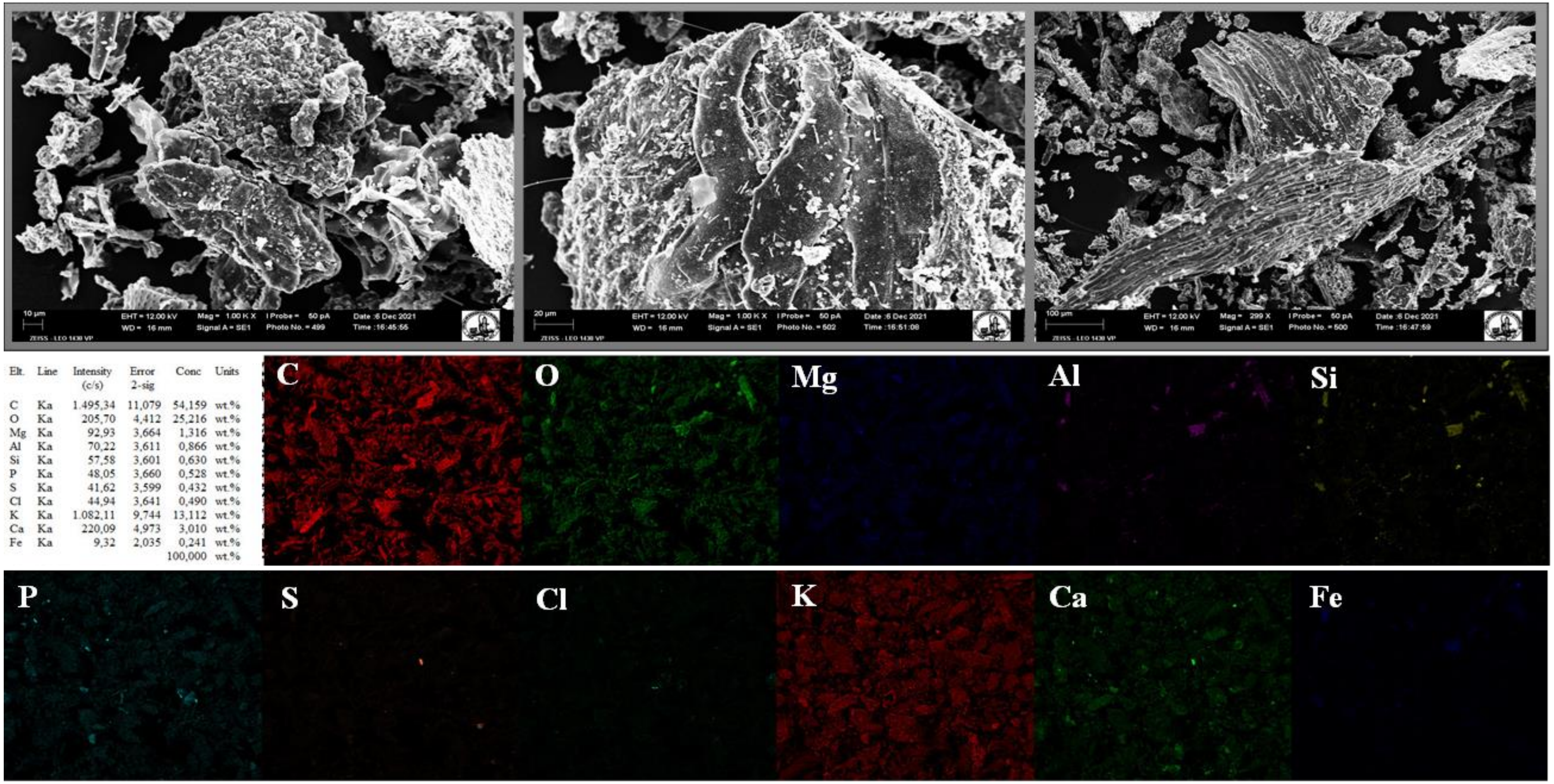


Figure S4 – Fourier-transform infrared spectroscopy (FTIR) in biochar from coffee husks



Elt.	Line	Intensity (c/s)	Error 2-sig	Conc	Units
C	Ka	1.495,34	11,079	54,159	wt.%
O	Ka	205,70	4,412	25,216	wt.%
Mg	Ka	92,93	3,664	1,316	wt.%
Al	Ka	70,22	3,611	0,866	wt.%
Si	Ka	57,58	3,601	0,630	wt.%
P	Ka	48,05	3,660	0,528	wt.%
S	Ka	41,62	3,599	0,432	wt.%
Cl	Ka	44,94	3,641	0,490	wt.%
K	Ka	1.082,11	9,744	13,112	wt.%
Ca	Ka	220,09	4,973	3,010	wt.%
Fe	Ka	9,32	2,035	0,241	wt.%
				100,000	wt.%

Figure S5 - Images and chemical mapping of coffee husk-based biochar

FINAL CONSIDERATIONS

The use of biochar derived from coffee husks has been shown to be an effective and sustainable tool for enhancing the physiological, productive, and quality performance of tomato plants under different soil moisture levels. Additionally, this practice has been beneficial in improving the chemical and physical properties of the soil. Although the study revealed that the effects of biochar were most notable under higher moisture conditions, the advantage of incorporating biochar into the soil was evident regardless of the water content present. In contrast, foliar application of KNO_3, either alone or in conjunction with biochar, yielded limited positive responses.

In this particular study, as it served as the sole source of K for the tomato plants and demonstrated positive improvements in physiological, productive, and quality variables, it is justifiable to conclude that biochar derived from coffee husks qualifies as a biochar-based fertilizer.

Finally, considering the notable advances resulting from biochar application, and taking into account its quality as an abundant source of stable carbon, capable of being integrated into the soil with the ability to persist for an extensive period, its adoption in tomato cultivation is highly recommended.