

## Opposite tendency between yield and taste of organic tomato by increasing biochar doses in a slightly humous arenosol

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**Abstract.** The tomato is the edible berry of the plant *Solanum lycopersicum*. Tomato plants are widely grown in temperate climates worldwide and are mostly cultivated as annuals. The objective of this study was to understand the interrelation between fruit quality of tomato, some soil biological parameters, and the addition of increasing biochar (BC) soil amendment doses. BC is an industrial product, made from organic waste by pyrolysis. Its use in the soil is known to improve fertility and several soil functions. Among organic, ecological conditions, a field experiment was performed in a type of slightly humous arenosol soil. Effect of increasing doses of biochar (BC) (0.5-, 1.0-, 2.5-, 5.0, 10 m/m% and control) was studied. Nutrient content and Total Soluble Solid (TSS) of the fruits, the ripeness, and the marketable/non-marketable ratio of yield were assessed. The presence of some cultivable microbial physiological groups (fungi, bacteria) and the soil-dehydrogenase activity (DHA) was estimated. Results represented that the changes of fruit TSS content was not linear with the increasing doses of BC. The increased yield (+53%) had an inverse correlation with the TSS content of the berry's pulps, and the content was lowest at the highest BC dose. Optimum doses of BC were considered, like 1–2.5 m/m%, supported by the nutritive element content (+55% N, +76% P, +83% K) and enhanced microbial activities (+45% DHA). Grouping the parameters by Pearson Correlation Coefficient, the biochar amendment was a driving factor for tomato growth, with certain dose limits in the studied organic agricultural practice.

**Key words:** biochar, ecological farming, nutrient uptake, soil biology, tomato.

## INTRODUCTION

Recently, increasing consumer concerns about several issues, such as food quality, environmental safety, and soil conservation, have led to sustainable agricultural practices (Belák et al., 2014). Due to urbanization and globalization, the development and supply of organic fertilizers are becoming scarce (Ali et al., 2019). Finding alternative solutions may be the most critical task, particularly for horticulture and viticulture. Organic soil amendments are essential for nutrient supplementation of crops and for improving soil chemical, physical and biological properties (Ringer et al., 2021).

A possible way to improve soil characteristics and functions is to apply biochar (BC) products (Gao & De Luca, 2018; Gaffar et al., 2021). BC may offer direct and indirect benefits when applied to soils. Available research information supports the application of BC products in soils, particularly in low-quality or degraded soils. Among them, soils with relatively low soil organic matter (SOM) are mainly studied (El-Naggar et al., 2019). Most of the available results approve that BC might improve the physical, chemical, and even biological characteristics of amended soils. The effect of biochar on sustainable plant growth along with nutrient management (preventing the loss of essential nutrients from the soil-plant environment) is considered beneficial, as well (Lehmann et al., 2011). Currently, the platform of circular economy applications of BC is growing dramatically. Faced with the burning issue of global warming, the development and application of BC appear to be a way of removing carbon from the carbon cycle and sequestering it in the soil (Xu et al., 2021). BC in the soil is a promising option for mitigating climate change and improving soil fertility/soil quality (Vaccari et al., 2015). BC might be considered an intact industrial material produced from organic waste by reductive pyrolysis (Mohan et al., 2006). Many organic materials can be used to produce biochar, but it might be critical that the used substances come from environmentally and climate-friendly sources. Usually, BC is a 2–5 mm long black granulated material, which can be used similarly as a synthetic or inorganic soil amendment agent (Dencső et al., 2017). Previous studies showed that BC is a highly porous material with a high soil-aeration and water-storage potential (Ahmad et al., 2014). During production, the literature distinguishes two major groups in terms of the initial raw materials. The first group is made on a relatively low (450–550 °C) pyrolyzing temperature, using mainly high-carbon-content substances. The products are capable of long-term binding the groundwater and dissolved ions; usually are originating from plant residues, by-products, and/or animal manures. The second group has been produced from animal bones at high temperatures (> 600–650 °C) with a relatively high calcium phosphate content and significantly lower carbon contents. The metal sorption of BC is possible through electrostatic attraction, ion exchange, surface complexation and some precipitation process. Metal ions can heavily adsorb on active sites of biochar that comprise phenolic and carboxylic functional groups present on the surface (Sobik-Szołtysek et al., 2021). Heavy metals can become stabilized on BC surfaces by metal ion exchange with  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  or other cations; metal complexation by functional groups and inner complexation by hydroxyl groups; mechanisms based on electrostatic interactions or by physical adsorption and precipitation (Kocsis, 2018; Sobik-Szołtysek et al., 2021). Most of the microorganisms in soils are generally quite sensitive to drought-stressed conditions. BC application in soils might ensure the better survival of microbes (Głąb et al., 2016), control the degradation of soil (Abrol et al., 2016), maintain the

moisture content of the soil (Teixeira et al., 2021), and increase the bioavailable inorganic minerals in the soil (Lehmann et al., 2011). Literature suggests that biochar application increases the production of humic substances (Abd El-Rahim et al., 2021), which enhances microbial activity in the soil (Fekete et al., 2021). BC acts as an adsorption matrix and protects soil microorganisms due to its high porosity and large surface area (Ragályi et al., 2019). It has been reported on the other hand, that the application of a biochar and compost combination has a rather limited effect on the soil enzyme activities, in comparison to non-amended control plots (Gao & De Luca, 2018). In general, a change in enzymatic activity might respond to increased soil carbon content and the chemical composition after applying BC and/or compost (Dencső et al., 2017). Enzyme activity was shown to increase in sludge-biochar treated soils compared to untreated sewage sludge. This indicates that pyrolyzed organic matter may enhance biochemical properties. However, the effect on enzyme activities may be variable as a function on the soil and the type of enzymes (Joseph et al., 2021). It was also found that the hormone-like activity of solubilized humic substances might play a crucial role after the application of BC in drought-stressed conditions (Atik et al., 2013). In contrast, in temperate climates and alkaline soils, the application of BC often had a transient effect on crop yield and soil quality (Gaffar et al., 2021). Several studies have reported also, that the BC might somehow limit the crop production, even in very fertile soils (Xu et al., 2016; Liu et al., 2013). Based on the facts mentioned, the effect of BC highly depends on the certain site characteristics (soil and climate context) than on crop types or the type and preparation of BC.

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops, with global production estimated at 180 million tons of fresh fruit in 2020 (WPTC 2020). Tomatoes can optimally be grown in well-watered, loose-textured, nutrient-rich soil, where they require regular irrigation. Numerous studies and research have been carried out regarding the application of BC in soil on the quantity- and/or quality of tomato yields (Raave et al., 2020; Liao et al., 2021). It has been proven that biochar application significantly increases the carbon content in the soil, the capacity of soil cation exchange (CEC), and the availability of  $\text{NH}_4^+$ , P, and K in a tropical environment (Raave et al., 2020).

The present study aimed to measure tomato crop production, nutrient uptake, and soil microbiota in biochar treated soil (i.e. slightly humous, low-carbon content Arenosol). The study intends to examine crop production and some soil health parameters to identify critical issues at practical application of BC. Based on our hypothesis, the beneficial effects of increasing biochar doses might have some limitations on tomato nutrient uptake, amount of tomato yield and quality and some soil microbiological parameters.

## MATERIALS AND METHODS

### Study site and experimental setup

Field experiments were conducted at the Ecological Farm of the Hungarian University of Agriculture and Life Sciences, Soroksár, Hungary (47° 39' N; 19° 15' E); certified by Biokontroll Hungária Inspection and Certification Nonprofit Ltd. The landscape has an altitude under 200 meters, flat, with moderately hot and dry temperatures. The average hours of sunshine are less than 2000 in a year. The average annual temperature is 10–11 °C. The average annual precipitation is around 500–550 mm.

The soil-forming rock is glacial and alluvial sediment, while the soil type is sandy soil with low humus-content (Arenosol) (MEPAR, 2013). Regarding the field experiment, tomato (*Solanum lycopersicum* L. var. Mobil) was grown as a test plant, and increasing doses of biochar were used. The locally grown tomato seeds were germinated and pre-grown in a greenhouse. They were planted in the experimental plots at the age of eight weeks, then grown from May 1 to September 8 in 2016 without irrigation. The biochar used in this study was produced from mixed wood chips (oak and beech) at a temperature of 600 °C initially with 30% water content. The prepared biochar product was dried at a temperature of 60 °C until its constant weight before applying to the soil.

The characteristic of the applied biochar is represented in Table 1.

**Table 1.** Nutrient content and chemical properties of applied biochar in field experiment, Soroksár, Hungary

Characteristics (mg kg <sup>-1</sup> )	Biochar values
pH <sub>H2O</sub>	9.8
sulfate	445
Mg	33.7
Na	19.6
P <sub>2</sub> O <sub>5</sub>	1,210
K <sub>2</sub> O	10,100
N (%)	0.48
Cu	1480
Mn	10.6
Zn	3.9
salinity (m/m%)	< 0.02

The assessment were conducted by following the available standards for the studied soil characteristics, such as the pH: TS ISO 10390; water-soluble sulphate content: MSZ 14043-10; P<sub>2</sub>O<sub>5</sub>: MSZ EN 16170:2017; Mg-, K<sub>2</sub>O-, Cu-, Mn: MSZ EN 16170:2017; salinity: MSZ-08-0206-2:1978.

Each experimental plot was 4 m × 2.5 m (10 m<sup>2</sup>). The biochar was incorporated into the upper 20 cm layer of the soil. The dry weight was used to set the correct applied doses of biochar, as a percentage of 0.5%, 1%, 2.5%, 5%, 10%, and the control (without any treatment). The soil surface in the plots was covered with agro textile to prevent weeds and keep water in the soil. Soil samples were taken with a 20 mm diameter Pürckhauer 1175/1000 mm soil sampling auger (Bürkle GmbH, Switzerland). There were four plots per

replicates in all treatments. From each plot, soil samples were taken randomly and finally a composite sample was used. Thus, we worked on four soil treatment replicates for showing of one average mean results. Considering the physical-chemical soil characteristics, main parameters are shown in Table 2.

**Table 2.** Nutrient content and some physical-chemical properties of the slightly humous sandy soil in the field experiment (Soroksár, Hungary)

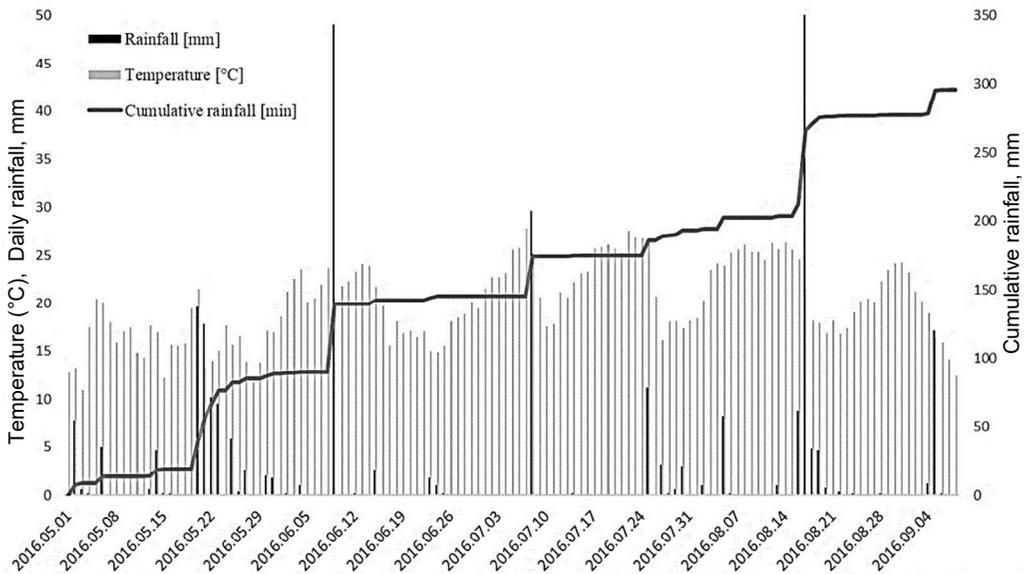
Characteristics (mg kg <sup>-1</sup> )	Experimental soil
pH <sub>H2O</sub>	6.8
sulfate	70.0
Mg	81.3
Nitrate-N	10.1
Nitrite-N	0.2
Na	77.7
P <sub>2</sub> O <sub>5</sub>	357.0
K <sub>2</sub> O	215.0
Cu (	2.4
Mn	98.3
Zn	2.9
CaCO <sub>3</sub> (m/m%)	0.7
SOM (m/m%)	1.6
salinity (m/m%)	30.0
Arany-type texture (KA)	< 0.02

The measurements were conducted based on the following standards: pH: TS ISO 10390; water-soluble sulfate content: MSZ 14043-10; Nitrate-N-, Nitrite-N: MSZ 20135:1999; P<sub>2</sub>O<sub>5</sub>: MSZ EN 16170:2017; Ca CO<sub>3</sub>: MSZ-08-1783-26:1985; Mg-, Na-, K<sub>2</sub>O-, Cu-, Mn-, Zn: MSZ EN 16170:2017; SOM: MSZ-08-0012-6:1987; salinity: MSZ-08-0206-2:1978; Arany-type texture: MSZ-08-0205:1978.

The spatial position of the tomato plants was 50×40 cm per plot. That means five plants in six rows. One week before planting the seedlings, a Viano [VIANO MIXPROF BIO1 9-3-3+2Ca] type granulated organic fertilizer, approved for organic farming, was applied, in 1.32 kg plot<sup>-1</sup> (660 kg ha<sup>-1</sup>) doses. From the inner rows, four plants were randomly selected for the study. Thus, we had sixteen plant samples (from four plots) per treatment. The fruit yield of the 16 plants in all treatments was measured, and the berries were classified into marketable/non-marketable groups, to get data on yield quality by the BC treatments. Non-marketable group was selected, as mechanically damaged or as contaminated by pathogens.

### Precipitation and temperature during the field experiment

Regarding the environmental conditions there were 298 mm total precipitation with three peaks; temperature values were between 1.9 °C, as a minimum, and 39.1 °C, as a maximum during the investigated periods (Fig. 1).



**Figure 1.** Measured average daily air temperature (°C) and precipitation data (mm) during the experiment at the research site (Soroksár, Hungary).

### Testing of soil-plant nutrient content and biochar contaminants

The macro-nutrients (N, P, K), meso-nutrients (Ca, Mg), and micro-nutrients (Mn, Zn) were estimated in the shoot (i.e., the leaves and stem residues of tomato). The 16 plants per treatment collected during sampling were averaged by quadruplicates; thus, four samples per treatment were prepared in a drying oven for 24 hours at 60 °C. For that purpose, 1.0 g of dried finely ground plant shoot sample was weighed and transferred to the digestion tube. The volume of 10 mL concentrated sulfuric acid (≥ 99%) containing 0.5% of Se (as metal) powder was added into it (Sahrawat et al., 2002). After adding the digestion mixture, the tubes were **transferred to a block digester and preheated to 400 °C. About 4 hours** were needed for completing the digestion until a clear and colourless digest solution was left. The extract was diluted to

50 mL with distilled water. The K, Ca, Mg, Mn, and Zn concentrations were measured by atomic absorption spectrophotometer (AAS, Trace Aurora AI-1200 type). The colorimetric determination of phosphorus in plant extracts was carried out with an ammonium vanadate - ammonium molybdate reagent ( $\geq 99\%$ ). Determination of ammonium nitrogen content in the digest was carried out by steam distillation with 40% sodium hydroxide to liberate ammonia and then titrated (Mohammad et al., 2004).

### **Fruit quality parameters**

The concentration of total soluble solids (TSS) can be determined from the smashed fruit juice using a hand-held refractometer (type Hanna HI 96801) (Cavalcanti et al., 2013). The fruits (skins and fruit-pulp) were crushed and homogenized with a blender and stored at a temperature of  $-25\text{ }^{\circ}\text{C}$  until analysis. From each fruit pulp sample, 2 g was diluted with 2 mL of deionized water. One mL of the mixture was pipetted out and filtered through a  $0.45\text{ }\mu\text{m}$  Syringe Driven Filter Unit. Colorimetric measurement of the fruit juice was also determined in the CIE (Commission International de l'Eclairage) chromaticity in tristimulus coordinate system  $L^*$  (lightness/darkness),  $a^*$  (red/green component), and  $b^*$  (yellow/blue component) by the CR-400 Chroma Meter. Before the sample measurements, the instrument was calibrated with a white reference plate (CR-A43). 1 mL of juice sample was spread out to the target plate, and the  $L^*$  value was measured at several points of each sample. The water content was calculated from the  $L^*$  value (Kocsis, 2018). The pH of the tomato juice samples was measured using a digital pH meter (Model 420A, Orion Benchtop pH meter, Allometrics Inc., Seabrook, USA). The device was calibrated with commercial buffer solutions at pH 7.0 and 4.0. Ten milliliters of the sample were placed in a beaker with a magnetic stirrer and measured at  $20 \pm 0.5\text{ }^{\circ}\text{C}$ . Measurements were taken at weekly intervals during the vegetation growth of the tomato.

### **Soil microbiological parameters**

Besides general physicochemical soil parameters, soil-microbiological status (aerobic/anaerobic bacteria and microscopic fungi) was assessed by the Most Probable Number (MPN) method, described by Downes & Ito (2001). Since the technique is based on statistical estimation, we used an average sample per treatment inoculated in 5 replicates. The essence of the method is that the microorganisms were grown in a liquid medium on 96-well microplate plates, and based on the number of (positive) wells showing microbial growth, we can statistically infer the number of microorganisms. A basic suspension was prepared from the soil samples to be examined, and a dilution series was designed from this on a decimal basis. Determination of the most probable live colony-forming unit count on microplate plates was performed by 5–5 parallel inoculations. The first step in the assessment is to determine the key number after five days of incubation. A key number is a five-digit number determined from the number of positive wells showing microbial growth at three consecutive dilution levels. We then retrieved the set point for the key number obtained from the Hoskins table and multiplied it by the dilution ratio for the first member of the key number. The value thus obtained was converted to normal to give the test result (Cochran, 1950).

Soil functioning, i.e., the specific enzyme analysis of soil microorganisms, was assessed by the dehydrogenase enzyme assay (DHA), based on the reduction of the 2,3,5-triphenyl-tetrazolium chloride ( $\geq 98\%$ ) (TTC) method (Veres et al., 2013).

A standard curve was plotted using a range of triphenyl-formazan ( $\geq 90\%$ ) (TPF) concentrations between 0 and 40  $\mu\text{g TPF mL}^{-1}$ . The DHA levels were expressed as  $\mu\text{g TPF g dry soil}^{-1} \text{ day}^{-1}$ . The soil microbiological measurements were made at the 14<sup>th</sup> week of tomato growth, the point of the highest microbial activity in the soil-plant system suggested by the literature (Dudás et al., 2017). Soil moisture content was given as a percentage by weight. The water content was calculated from the difference between the weight of the wet and dry soil samples (which was applied to the dried soil). Moisture content was calculated from the average of 5 subsamples per plot.

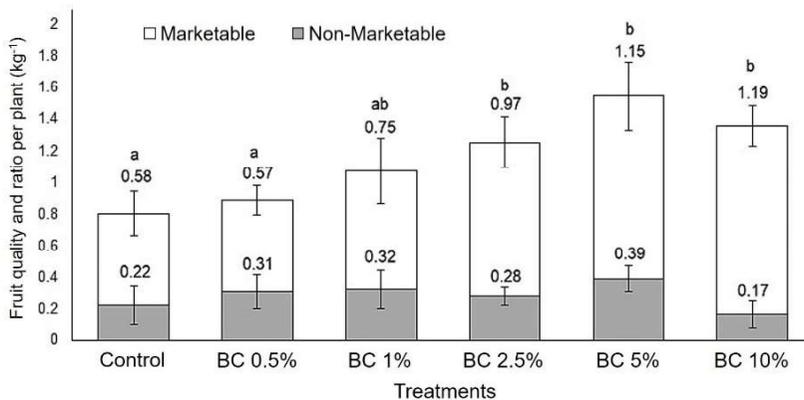
### Data analysis

One-way ANOVA was applied to the test result. The Kolmogorov-Smirnov test ( $p > 0.05$ ,  $p = 0.200$ ) or the Shapiro-Wilk test ( $p > 0.05$ ) were used to prove the assumption of normality, while the Levene's test ( $p > 0.05$ ) was used to prove the homogeneity of variances. If the data showed homogeneity of variance, Tukey's honestly significant difference (HSD) post hoc test was used. If the data were heterogeneous, Games-Howell's post hoc analysis was used. The statistical method of Cochran was applied to calculate MPN values. One-way ANOVA Tukey test with logMPN values and standard logMPN errors was used to determine whether MPN values of field experiment soil samples were significantly different at the 95% confidence level ( $> 0.05$ ). Pearson correlation analysis (2-tailed) was carried out to identify relationships between soil characteristics and plant components. Values of  $r^2$  for significant correlations (\*,  $p < 0.05$  or \*\*,  $p < 0.01$ ) and correlation trends ( $p < 0.1$ ) were reported.

## RESULTS AND DISCUSSION

### Yield and quality of tomato fruits under increasing doses of biochar

In the case of the yield parameters, biochar application did not result in any differences between the tomato fruit's intact and non-intact (marketable/non-marketable) ratio. On the other hand, significantly higher fruit weight was recorded at the highest (2.5%, 5%, 10%) BC doses-, compared to the untreated control (Fig. 2).

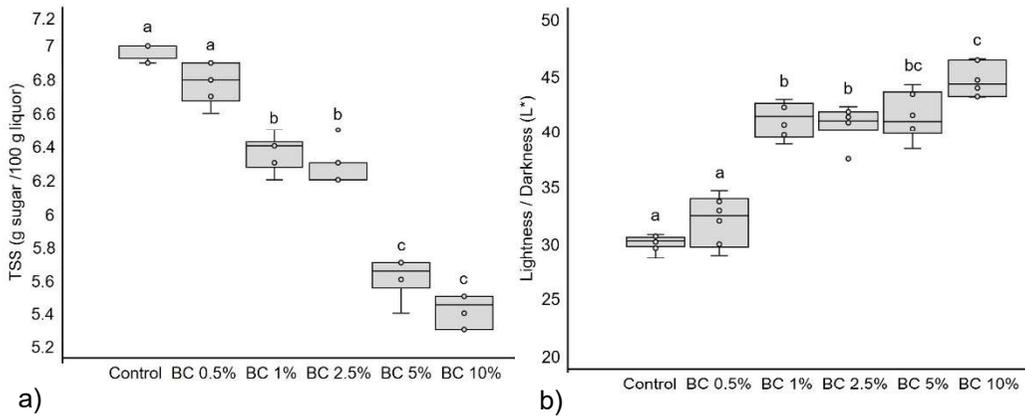


**Figure 2.** The average yield of tomato (*Solanum lycopersicum* var. Mobil) plants resulted from the increasing biochar doses (0-, 0.5-, 1-, 2.5-, 5-, 10%) of the upper soil layer (20 cm) in a slightly humous sandy soil ( $n = 16/$  treatment,  $P < 0.05$ ). Fruits were categorized as either marketable or non-marketable (small, misshapen, or diseased).

**As doses were increased, a significantly larger amount of marketable crop was harvested.** Referring to the annual precipitation (Fig. 1), biochar can retain the runoff rainwater that is determinative to the amount of yield. This result suggests that the water-absorbing capacity of biochar had a potentially greater effect on yield than its nutrient binding capacity. During the studied period, the average soil moisture content in the treatments was as follows: Control: 9.02%, BC 0.5%: 11.94%, BC 1%: 16.96%, BC 2.5%: 18.60%, BC 5%: 25.05%, BC 10%: 33.27%.

Hardie et al. (2014) had similar results when directly measuring the soil water content under different biochar treatments. Consequently, the application of biochar can increase the available water content of the soil through a direct contribution from the enlarged surfaces and its pores.

Besides the tomato yield, the nutritional properties were also examined. To determine the effect of various biochar doses on fruit quality, the TSS content and color intensity of the tomato fruits were also investigated. The increasing concentration of biochar resulted in a decrease in the TSS content of the fruit (Fig. 3, a). In contrast, the sample's  $L^*$  (water content) value was increased parallel with the BC doses (Fig. 3, b).



**Figure 3.** a) Total Soluble Solids (TSS) content-, b) Lightness attributes ( $L^*$ ) coloration of the mashed juice of tomato fruits, grown in slightly humous sandy soil with increasing biochar doses (0-, 0.5-, 1-, 2.5-, 5-, 10%) ( $n = 16$ / treatment,  $P < 0.05$ ).

During the experiment, the measured yield per plant significantly increased while the TSS content of the tomato fruits decreased concomitantly with the concentration of applied biochar. In summary, it is possible to increase the yield of tomatoes by increasing the available water content through the appropriate biochar addition. However, the synthesis of TSS might be a more complex process, resulting in higher yields with lower dry matter content. This negative correlation in the non-irrigated field experiment indicates the biochar's adequate water retention capacity, which becomes efficient and determinative for plant growth.

In general, the average pH levels of crushed tomato fruits ranged from pH = 4.27 to pH = 4.40 (within deviation) values. The color of the tomato fruit extracts was a continuously changing parameter along with the vegetation period and the ripening of

the tomato fruits. At the end of the maturation process, the colour of the samples became more intense and darker with maturity progression (Fig. 3, b).

### Nutrient uptake of plants from biochar treated soils

Regarding the mineral trace elements of plants, the optimal biochar dose significantly increased the nitrogen, phosphorus, and potassium content. The optimum concentration of these elements in plant shoots was recorded at the 1% dose (Table 3).

**Table 3.** Average nutrient content of the fresh biomass (leaves and stem residues) of tomato, grown in slightly humous sandy soil at increasing doses (0, 0.5, 1, 2.5, 5, and 10%) of biochar. (n = 4/treatment). Numbers are mean  $\pm$  Standard error. Means within a line followed by a common letter do not differ significantly ( $P < 0.05$ , Games-Howell test)

	Control	BC 0.5%	BC 1%	BC 2.5%	BC 5%	BC 10%
N (g kg <sup>-1</sup> )	<b>20.72<sup>a</sup></b> $\pm$ 0.98	<b>26.04<sup>b</sup></b> $\pm$ 0.29	<b>37.66<sup>c</sup></b> $\pm$ 0.69	<b>32.06<sup>d</sup></b> $\pm$ 1.18	<b>30.66<sup>de</sup></b> $\pm$ 0.59	<b>28.98<sup>e</sup></b> $\pm$ 1.97
P (g kg <sup>-1</sup> )	<b>0.26<sup>a</sup></b> $\pm$ 0.011	<b>0.27<sup>a</sup></b> $\pm$ 0.014	<b>0.34<sup>b</sup></b> $\pm$ 0.014	<b>0.29<sup>a</sup></b> $\pm$ 0.011	<b>0.27<sup>a</sup></b> $\pm$ 0.024	<b>0.26<sup>a</sup></b> $\pm$ 0.021
K (g kg <sup>-1</sup> )	<b>1.30<sup>bc</sup></b> $\pm$ 0.016	<b>1.36<sup>d</sup></b> $\pm$ 0.024	<b>1.56<sup>e</sup></b> $\pm$ 0.015	<b>1.35<sup>cd</sup></b> $\pm$ 0.018	<b>1.23<sup>a</sup></b> $\pm$ 0.031	<b>1.27<sup>ab</sup></b> $\pm$ 0.017
Ca (g kg <sup>-1</sup> )	<b>103.81<sup>ab</sup></b> $\pm$ 0.311	<b>105.86<sup>b</sup></b> $\pm$ 0.363	<b>91.39<sup>c</sup></b> $\pm$ 1.890	<b>101.06<sup>b</sup></b> $\pm$ 0.803	<b>89.62<sup>bd</sup></b> $\pm$ 1.400	<b>87.32<sup>d</sup></b> $\pm$ 1.776
Mg (g kg <sup>-1</sup> )	<b>9.65<sup>a</sup></b> $\pm$ 0.387	<b>10.51<sup>bc</sup></b> $\pm$ 0.091	<b>10.05<sup>ab</sup></b> $\pm$ 0.149	<b>10.01<sup>ab</sup></b> $\pm$ 0.175	<b>10.50<sup>bc</sup></b> $\pm$ 0.285	<b>10.75<sup>c</sup></b> $\pm$ 0.247
Mn (mg kg <sup>-1</sup> )	<b>85.34<sup>a</sup></b> $\pm$ 2.616	<b>90.04<sup>a</sup></b> $\pm$ 2.375	<b>116.00<sup>b</sup></b> $\pm$ 1.598	<b>54.58<sup>c</sup></b> $\pm$ 3.478	<b>49.66<sup>c</sup></b> $\pm$ 3.408	<b>31.24<sup>d</sup></b> $\pm$ 1.527
Zn (mg kg <sup>-1</sup> )	<b>97.52<sup>a</sup></b> $\pm$ 739	<b>129.28<sup>b</sup></b> $\pm$ 2.13	<b>121.12<sup>c</sup></b> $\pm$ 1.32	<b>126.26<sup>b</sup></b> $\pm$ 1.92	<b>104.48<sup>d</sup></b> $\pm$ 1.767	<b>47.70<sup>e</sup></b> $\pm$ 1.569

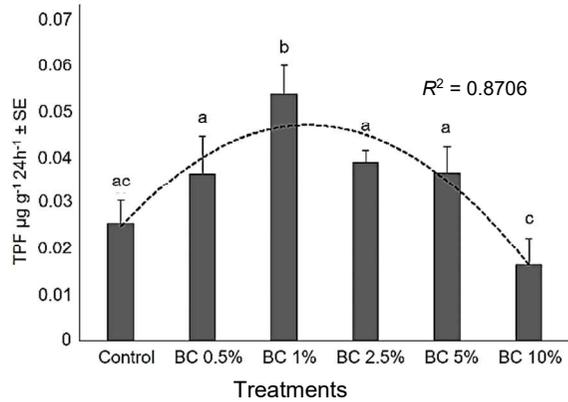
In the case of N (+55%), P (+76%), and K (+83%), the 1% dose resulted in the highest concentrations, but only for nitrogen did it yield significantly higher results than the control. One of the BC's beneficial effects is that due to its highly porous surface, the cations from water solutes can adsorb on it. Considering this mechanism, it is also advantageous that fewer nutrients are lost and leached into the subsoil water layers in biochar-rich soils. This binding effect was observed after the application of nitrogen-rich organic fertilizer. Biochar may retain nutrients directly through the negative charge that develops on its surface, and this negative charge offers a buffer in the soil. In this case, the relatively high biochar concentration could result in the unavailability of the bound nutrients, thus shifting the environmental conditions out of the plant tolerance limit (see in the column of BC10% dose). The increase in soil pH after the application of biochar due to its liming effects has been widely documented (Sheng & Zhu, 2018). Van Zwieten et al. (2021) observed an increase in soil pH after the application of biochar. On the other hand, the high soil pH value induced by biochar is coupled with its high carbonate content. It adversely affects plant growth at high application rates, which can be explained by nutrient deficiencies. Therefore, the application of the BC amendment must be based on the specific properties (especially the texture, water-holding capacity, CEC) of the soil, with particular attention to its effects on nutrient availability to plants and microorganisms.

### Soil microbial activity under increasing doses of biochar amendments

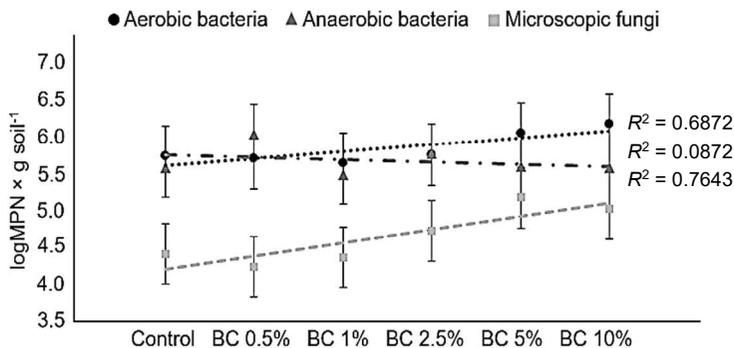
The MPN and DHA assessment methods were used to study microbial abundance and activities affected by BC addition. The hypothesis is that higher microbiological diversity and activity of the rhizosphere might result in an increased nutrient supply to the plants. There is also an optimum level of biochar dosage in terms of soil enzyme activity (Fig. 4).

The estimated highest activity occurred at 1% of biochar application, while a rate of 10% has reduced the activities below the actual rate in the studied soil. At the same time, the number of aerobic and anaerobic soil bacteria did not show such a trend.

On the other hand, the fungal counts showed an increased abundance parallel with the increasing biochar concentration. The highest microbial counts of fungi were measured at the two highest biochar doses, 5% and 10% (Fig. 5).



**Figure 4.** Dehydrogenase Enzyme Activity (DHA) of soils treated with increasing doses (0-, 0.5-, 1-, 2.5-, 5-, 10%) of biochar (BC). Significant differences are presented with the letters a, b, c, over the corresponding column of the graph. ( $P < 0.05$ , Tukey HSD test,  $n = 4/\text{treatment}$ ).



**Figure 5.** Most probable number (MPN) of aerobic/anaerobic bacteria and microscopic fungi in soils, treated with increasing doses (0-, 0.5-, 1-, 2.5-, 5-, 10%) of biochar (BC) ( $n = 5/\text{treatment}$ ;  $P < 0.05$ ).

Comparing the response of MPN and DHA assay to the biochar application, there was no statistical interrelation between the two measured parameters in the tested soil samples. Similar results were obtained in a mulching experiment by Kader et al. (2017). In the low-quality soil, reduced soil enzyme activities were frequently found because if any microbe is present, it is often in a dormant stage (waiting for a better soil-environmental condition). Regarding the used low humus sandy soil in our plots,

the surface increased by BC seems to be a critical issue of prospering soil functions. It can be assumed that biochar generally has a large sorption surface, which is vital for protecting microorganisms and preserving their activity in soil-plant systems. Still, attention must also be paid to other environmental stressors (SOM, texture, etc.). Extra high doses, for instance, can result in a negative trend in the measured parameters (yield of tomato; TSS content; nutrient content of the biomass; DHA activity). This can be justified because the large surfaces can bound the soil nutrients and, therefore, might reduce their availability for microorganisms and plants.

### Overall assessment of treatment effects

Biochar treatment has highlighted the system’s complexity that leads to successful crop production; studying the correlations between persistent biochar and crop production variables is therefore essential. Pearson product-moment correlation coefficient (PPMCC) was used to assess the correlation between the measured traits. The analysis showed significant relationships between tomato fruit parameters as well as the soil enzymatic activity and the nutrient uptake of plants. (Table 4).

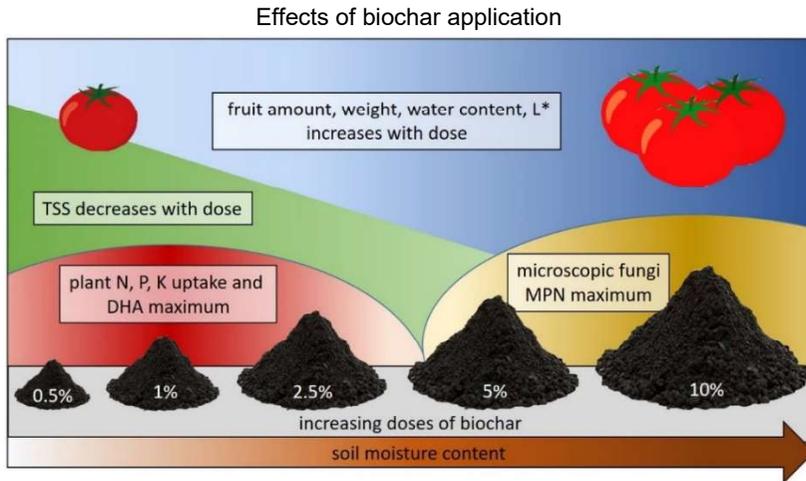
**Table 4.** Correlations among the different variables in the study

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1 TSS	1													
2 Lightness	-.95**	1												
3 Yield	-.93**	.90**	1											
4 Aerob	-.88**	.94**	.75*	1										
5 Anerob	.40	-.28	-.33	-.28	1									
6 Fungi	-.88**	.78*	.81*	.79*	-.65	1								
7 DHA	.27	-.48	-.07	-.67	.01	-.18	1							
8 Nitrogen	-.42	.19	.49	-.06	-.31	.35	.70	1						
9 Phosphorus	.11	-.36	-.05	-.56	-.30	-.04	.88*	.83*	1					
10 Potassium	.38	-.60	-.39	-.72	-.11	-.32	.78*	.61	.92*	1				
11 Ca	.88**	-.73	-.77*	-.68	.70	-.94*	.05	-.57	-.20	.05	1			
12 Mg	-.71	.67	.57	.70	.23	.53	-.28	.18	-.28	-.33	-.51	1		
13 Mn	.74*	-.91**	-.71	-.87*	.05	-.49	.69	.14	.64	.81*	.39	-.53	1	
14 Zn	.62	-.67	-.34	-.81*	.47	-.60	.80*	.23	.48	.49	.59	-.42	.63	1

Variables included were: N, P, K, Ca, Mn, Zn content of stem and leaf residues, L\*, TSS content of the fruits, and aerobic-, anaerobic-. microscopic fungi count, DHA activity in case of soil. (N = 86. \* $p < .05$ ; \*\* $p < .01$ ).

Table 4 shows the correlation results between the plants and the corresponding soil parameters. It was observed that the TSS content of the fruits was negatively significantly correlated with its water content (L\*) ( $p < 0.01$ ) and total fruit yield ( $p < 0.01$ ). A well-marked correlation was found between the anabolic soil processes (DHA) (caused by microbial activity and plant roots) and plant nutrient uptake. Phosphorus, potassium, and zinc concentrations of plant shoots were significantly correlated ( $p < 0.01$ ) with changes in DHA activity. Although significant correlations were found between the microbial values that could be counted and some plant parameters, the MPN method is not accurate enough to determine these correlations. In the case of the nitrogen uptake, no significant correlation was detected, which may reflect the abundant nitrogen application. Masood et al. (2020) reported that in tomatoes if the available nutrient content (local nitrogen and phosphorus) is adequate for the plant,

it does not promote rhizosphere colonization by the plant growth-promoting rhizobacteria (PGPR). The graphical summary of the scientific results and the potential tendencies of the used biochar doses is shown in Fig. 6.



**Figure 6.** The applied increasing biochar doses affected as tendencies on tomato food production and on some soil health parameters in a slightly humous sandy soil (Arenosol).

## CONCLUSIONS

The results highlight that biochar amendments, in general, can improve the growth of tomatoes, due to the improved adsorbing surface characteristics in the studied soil-plant system. Such characteristics of biochar can be beneficial for both plant nutrition and the assessed soil-biological activities. On one hand, the microbial activity in the soil was stimulated by the added biochar, on the other hand, negative effects were observed in some cases in sandy soils at extra high doses. It has been shown that the optimal dose of biochar may differ for crop production and soil ecosystem services. In favourable soil solubility conditions (improved by biochar), the nutrient elements would become ‘diluted’ in the bigger tomato berry, thus, total suspended solids (TSS) content in tomato will be decreased. High doses of biochar may result in a shift in the soil-environmental conditions due to their high alkaline chemistry, thus affecting the functionality of the microbial groups, investigated. Under arid environmental conditions, the balance between the nutrient absorption capacity and the water- and nutrient availability in the soil might be optimized by applying the right quantity of biochar. Such optimization experiments seems to be necessary to select the most appropriate type and dose of biochar for a certain soil-plant-microbe system.

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