



Article

Effect of Biochar Application to Fertile Soil on Tomato Crop Production under Saline Irrigation Regime

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Abstract: Biochar application is a promising sustainable strategy for enhancing soil properties thus crop production. However, biochar application to soil certainly alters its biological and physical properties, and could require extra costs. Therefore, biochar suitability to agroecosystems must be proactively estimated. The advantage of biochar addition to poor fertile or weathered soils has been well studied, however, its feasibility to fertile soil under low quality (saline) irrigation water was not frequently studied. Consequently, this work investigates the hypothesis of whether the application of biochar at a rate of 4.8 tons/ha to fertile soil (Nile Valley, Giza, Egypt) would ameliorate the negative effects of saline irrigation regime (3000 ppm) on tomato crop and soil. The results of two seasons experiments showed that saline irrigation significantly reduced tomato crop yield by an average reduction ratio of 51%, and biochar addition could not compensate such reduction. Furthermore, biochar did not reduce accumulated Na⁺ in fruits or roots. Tomato fruits produced from biochar-added soil were lower in TSS levels (41.7% reduction ratio) yet larger in diameter by approximately 1.5-fold increase. Interestingly, biochar addition into soil greatly promotes the length of stem-borne lateral roots and elevates the expression of LeNR (encodes nitrate reductase enzyme) in leaves yet under fresh irrigation regime. For soil properties, biochar application enhanced the soil properties under either saline or fresh water irrigation conditions. Collectively, it is assumed that biochar application to fertile soil in Nile Valley of Egypt could not alleviate tomato fruits yield reduction affected by applied saline irrigation regime.

Keywords: soil amendment; salinity; cash crop; yield; Na⁺ content; roots; gene expression



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1. Introduction

Biochar is a high carbon content and fine-grained organic material synthesized by the thermal decomposition (300–600 °C) of biomass organic materials in the complete or the partial absence of oxygen, a process known as pyrolysis [1]. Although not completely uniform, the application of biochar to agricultural ecosystems has received considerable attention during the last decades because of its potential benefits to crop productivity and the environment [2,3].

Biochar can provide agronomic benefits at the level of soil quality/fertility improvement and can enhance crop productivity and mitigate climate changes by sequestering

carbon. The properties of soil such as pH, bulk density (BD), water-holding capacity (WHC), and cation-exchange capacity (CEC) could be relatively improved upon by biochar efficient addition [4,5]. The central factors behind the positive effect of biochar on soil properties thus crop productivity could be briefly arranged as follows: (i) increasing cation-exchange capacity (CEC) of soil due to increased negative surface charged area by carboxyl groups; enhanced CEC soils absorb more nutrients and subsequently enhance nutrient availability for plants, in particular, that of N [6], (ii) enhancing crop tolerance to drought by elevating soil water holding capacity (WHC) [7], (iii) the potentiality to enhance microbial activity which could be beneficial to plant growth and development, and in addition, enhance plants' defenses against several diseases [8], and (iv) reducing the bioavailability of heavy metals, sodium ions, and organic pollutants in the soil by adsorbing them through biochar-extended charged surface areas [9].

Biochar is not a simple carbon material with uniform physical and chemical properties [10]. Therefore, considering the source of biochar, synthesis conditions, type of soil, and even the type of the crop are essential factors that should be collectively considered for achieving better sustainable agricultural production without posing undesirable physical or biological alternations to utilized agroecosystems [2,11]. Biochar effect is soil type-dependent; for example, for decreasing sandy soil bulk density, at least 1% of biochar addition is required, whereas it was at least 5% for sand-loam soil [12,13]. Indeed, it is frequently reported that the application of biochar to soil for ameliorating its properties and enhancing crop productivity is more successful with well-weathered soils (degraded), low fertility soils, and acidic soils, than with fertile healthy soils [11,14–16].

Despite it being widely accepted as a value-added product of biomass waste and beneficial soil amendment [17,18], it is highly recommended that the sustainability of biochar use in agriculture be carefully assessed [19]. Several concerns were raised about biochar application, in particular to fertile high-producing soil agroecosystems with optimal pH, low salt levels, and sensitive balanced microbial or microfauna (protists and nematodes) communities [20,21]. The high-dose application of biochar to clay soil was reported to reduce water content [21] whereas pH increase and soil salinization are two negative consequences that biochar application produces, yet both are associated with feedstock type (ex: poultry litter) from which biochar was synthesized [22]. At the level of the microbial community, biochar was confirmed to alter the balance between bacteria and fungi in the soil, but in a contradictory manner. There is no tangible answer to whether biochar enhances or diminishes soil microorganisms, as both effects were reported with biochar-added soil [13,21,23].

Salinity stress is one of the most destructive environmental challenges for global agriculture; more than 20% of the irrigated land of the world has been affected by salinity [24]. The unprecedented levels of elevated soil salinity were, to a great extent, driven by the climate changes in terms of elevated atmospheric temperature, high levels of evapotranspiration, and expected sea level rise [25]. In addition to climate changes, human activities also significantly induced surface soil salinity, particularly in arid and semi-arid regions such as Egypt where 30% of arable land is already salinized [26,27]. This is mainly due to poorly planned irrigation regimes that use moderate to high saline agriculture drainage water (≈ 2000 ppm) thus elevating salinity levels in highly fertile clay Nile Delta topsoil (0–60 cm) by 26% [28,29]. The highly fertile land of Egypt (i.e., the Nile Delta and Nile Valley) represents a key agricultural area and is the food basket of Egypt, thus encouraging adaptive investments by developing a new bio-saline agriculture ecosystem (either in open-field or greenhouse conditions), which in these regions requires a well-designed sustainable system that combines enhancing water/soil properties and crop type for maximized crop yield and healthy soil structure [26,29,30].

Tomato (*Lycopersicon esculentum* Mill.) is the most geographically dispersed horticultural crop in Egypt and worldwide with moderate tolerance to salinity stress, although it is sensitive to excessive stress. It is one of the most significant vegetable crops in Egypt for local utilization and exportation [31,32]. Globally, Egypt is the fifth top country in

tomato production where the top four are: China (64,768,158 tons), India (20,573,000 tons), Turkey (13,204,015 tons), and USA (12,227,402 tons). The total harvested area in Egypt is 170,862 hectares with a production amount of 6,731,220 tons (4% of global contribution in tomato production). In growing season 2020, Egypt exported 79,700 tons of fresh tomato generating US \$40,415,000 [33,34]. In addition, the tomato is a model crop of Solanaceous Plants; thus, the acquired fundamental knowledge obtained from experimental studies on tomato could be beneficial for other high-value cash crops such as pepper, eggplant, and potato [34,35]. Nevertheless, tomato and other vegetable crops in Egypt are vulnerable to the negative impact of climatic changes including heat, water stress, and salinity with an expected accumulative reduction ratio of 11% in 2050 [30].

In parallel to time-consuming, yet indispensable, tomato breeding programs, adaptive investment using sustainable agricultural practices including applying soil amendments as biochar for enhancing current production is a required strategy for mitigating climate changes impact on agricultural production. In addition to previously mentioned ecological concerns about biochar–soil interaction, the economic feasibility of biochar application should be advertently considered. Biochar application, especially with large amounts, certainly comes at a financial cost. Due to the fact that there is no single standard for synthesizing biochar (ex: multiple feedstocks), the costs could vary between US \$0.18 to \$18 per kg [36]. One possible strategy for overcoming the potentially high cost of biochar is the cultivation of high economic benefit cash crops such as tomato or strawberry, ideally with low fertile land and/or with low quality (saline) irrigation water. Collectively, before producing agricultural extension protocols regarding biochar application, ecological and economic aspects must be definitely examined.

Biochar is used for mitigating salinity stress damage on crop yield in degraded or low fertility sandy soil, the soil types that commonly exist in newly reclaimed agricultural land in Egypt [36–39]. To our knowledge, the suitability of biochar to fertile soil under a saline irrigation regime was not extensively investigated, in particular with perishable cash crops such as tomatoes. It is hypothesized that the addition of biochar would not be necessary for bio-saline agriculture in fertile soil. Therefore, the present study investigates several agronomic, physiological, phenotypical, and molecular traits of a tomato crop (hybrid 448) in response to saline water irrigation (3000 ppm NaCl) under the auspice of biochar with an application rate of 4.8 tons/hectare to the fertile soil of the Nile Delta, Egypt. Soil properties also have been assessed in the presence and absence of biochar. Collectively, the work could be beneficial for establishing sustainable adaptive practices using soil amendments with different types of soil under challenging environmental conditions such as salinity or drought.

2. Materials and Methods

2.1. Experimental Layout and Plant Materials

Two net house experiments were conducted in the winters of 2019–2020 and 2020–2021 at the greenhouse facilities of the Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center (ARC), Giza, Egypt. The experiment's location latitude was 30°01'27'' N, and the longitude was 31°12'11.3'' E. The soil was clay loam in texture showing low saline content (EC ranged from 1.8–2.3 ds·m^{−1}) and was slightly alkaline (pH ≈ 7.5).

The seeds of the commercial greenhouse tomato (*Lycopersicon esculentum* Mill.) cv. hybrid 448 were sown in seedling trays under controlled greenhouse conditions in the middle of September 2019 and 2020. After 5 weeks of seed sowing, healthy seedlings with uniform size growth having five true leaves were transplanted into rows of 20 m length and 1 m width. The distance between plants was 20 cm. Fertilization and other cultural practices were applied as commonly recommended in commercial tomato production [40]. Four treatments were applied in this study during the two seasons: (i) fresh tap water irrigation without biochar (fresh no BC), (ii) fresh tap water irrigation with biochar (fresh with BC), (iii) saline water irrigation (3000 ppm NaCl) without biochar (saline no BC), and

(iv) saline water irrigation (3000 ppm NaCl) with biochar (saline with BC). Biochar was applied to the top 20 cm of the soil on a scale of 4.8 tons/ha as recommended by [37].

2.2. Plant Measurements

2.2.1. Plant Growth Parameters

At the early budding stage (2 months after transplanting), several vegetative traits were recorded for treated tomato plants: plant height (Ph.), number of branches (Br.), number of leaves, number of initial inflorescences (Inf.), and number of flowers. For yield, fruits were harvested and yield was estimated per plant and per hectare. Fruit diameter (dm) was also measured.

2.2.2. Total Soluble Solids (TSS)

The digital refractometer (DR-101-60, Krüss, Germany) was used to quantify total soluble solids (TSS) of fresh filtered fruit juice using Whatman filter paper No. 1. One drop was pounded to the prism and TSS was measured in Brix percentage according to [41].

2.2.3. Na⁺ and K⁺ Content in Fruits and Stem-Borne Lateral Roots

Roots (stem-borne lateral roots) and fresh fruits were harvested and washed several times with double distilled water, then dried in an oven set at 80 °C for 3 days. Subsequently, all tissues were subjected to maceration under liquid nitrogen resulting in a fine powder, which was then delivered to the Regional Center for Food and Feed (RCFF), Agricultural Research Center (ARC), Giza, Egypt for quantifying the sodium and potassium content using atomic absorption protocol.

2.2.4. Stem-Borne Lateral Root Length

After harvesting fruits, plants were allowed to dry while still anchored in the soil by preventing irrigation for 3 weeks. Subsequently, stem-borne root systems were manually extracted from the topsoil and then washed with water to remove soil residues. Roots were then allowed to be air-dried for 1 week. Root samples were imaged by using a high-resolution camera (d7500, Nikon, Japan), then decent images were analyzed using ImageJ software for determining the length of lateral roots ramified from the below ground tomato stem.

2.2.5. Quantitative Gene Expression of Salt Stress-Related Genes

Total RNA and cDNA Synthesis

Tomato plants were cultivated in pots containing the same rate of biochar and irrigated with the same amount of NaCl as in the net-house experiment. After one month of treatment, the total RNA from the leaves was isolated using Spectrum™ Plant Total RNA Kit (Sigma-Aldrich, St. Louis, MO, USA). cDNA was synthesized from 1000 ng of total RNA using the RevertAid First Strand cDNA synthesis kit (Thermo Scientific, Vilnius, Lithuania) according to the supplied protocol. The cDNA was diluted by a 1:5 ratio with nuclease-free water and used for subsequent gene expression analysis.

Real-Time PCR Assay

Real-time PCR was performed using Stratagene M × 3000 P (Stratagene, Houston, TX, USA). The reaction was performed in a final volume of 10 µL containing 1.5 µL of the cDNA, 0.5 µL of each forward primer and reverse primers (10 µM each), 5 µL 2× JumpStart Taq Ready Mix, and 2.5 µL RNase-free water. The mixture was gently pipetted and briefly centrifuged, then subjected to the following thermal cycling program: 94 °C for 2 min, and 40 cycles (94 °C for 15 s and 66 °C for 1 min). The primer sequences for the target genes in addition to the reference gene are listed in Table 1. Quantification of relative expression values was calculated according to [42]. The gene expression of two target genes, LeNHX4 (encodes Na⁺/H⁺ antiporter protein) and LeNR (encodes nitrate reductase enzyme), relative to the reference gene elongation factor leEF1, was quantified.

Table 1. The sequence of forward and reverse primers investigated in this study.

Gene Name	Forward Primer 5' to 3'	Reverse Primer 5' to 3'
LeNHX4	TGGTGGGCAGGTTTGATGAGAG	TGTGGTGGCAGCAGGAGACTTA
LeNR	GGTTCATCACTCCCGTACCACTT	TCTGCTTCACCATATTCTGCTCT
LeEF1	TTGCTTGCTTTCACCCTTGG	TTGGCACCAGTTGGGTCCTT

2.3. Soil Analysis and Biochar Addition

The biochar was synthesized from cob corn residues [37]. Before planting, biochar was added to the soil at a rate of 4.8 tons/ha. The biochar is high in carbon (60.3%) and oxygen (32.9%), but poor in nitrogen (0.5%), phosphorus (1.6%), and potassium (0.5%). Biochar also has a high BET (Brunauer-Emmett-Teller) surface area and CEC, which improves nutrient and cation adsorption on its surface. The pH of the biochar is alkaline, and the EC value is low.

In addition, soil samples (0–30 cm depth) representing all of the treatments from the experimental site were taken after soil preparation and before fertilization, as well as after harvest time, to determine the effect of salinity stress on soil chemical properties under different experimental conditions. The materials were air-dried before being sieved through 2 mm holes. The OM (organic matter) content of soil samples was determined by the Walkley and Black approach and pH was tested in a soil/water suspension (1:2.5), whereas EC, main cations, and anions were determined in the saturated soil paste extract [43,44].

2.4. Statistical Analysis

SPSS software (SPSS 20.0, IBM Corp., Armonk, NY, USA), was used for one-way ANOVA analysis followed by Duncan's multiple range test (DMRT) at significance level of $p \leq 0.05$. Microsoft Excel 2019 was used for plotting. Plant response data were combined over the two growing seasons 2019/2020 and 2020/2021. The experiment plots were arranged in a randomized complete block design. The number of biological replications and the number of plants per replication used for each type of measurement were described in corresponding figure legends.

3. Results

3.1. Analysis of Variance

Fifteen (15) phenotypical and physiological traits representing the response of tomato plants, cultivated in the fertile soil of the Nile Valley, to fresh and saline irrigation regimes were investigated in the presence and absence of biochar. As shown in Table 2, the analysis of variance indicates the significant effect of the saline water irrigation regime with “no BC”-treated soil on all investigated traits except Ph. (plant height) and K^+ content in fruits (K^+ fruit). On the other hand, salinity stress with BC-added soil (with BC) caused a significant alteration in the following traits: number of flowers, Na^+ content roots and fruits, Na^+/K^+ ratio in roots and fruits, and stem-borne lateral root length. Furthermore, Table 2 indicates the significant effect of biochar under a fresh irrigation regime on the number of branches, number of leaves, number of flowers, yield, fruit diameter, and K^+ content in stem-borne lateral root length. The effect of biochar addition to saline water-irrigated soil causes a significant alteration in the number of leaves, number of flowers, fruit diameter, and total soluble solids (TSS).

Table 2. Analysis of variance indicating the effect of season, biochar addition, saline irrigation regime, and the corresponding interactions on tomato crop plants' traits and yield.

	Ph.	Br.	Leaves	Inflo.	Flowers	Yield	Fruit dm	TSS	Na ⁺ Root	K ⁺ Root	Na ⁺ /K ⁺ Root	Na ⁺ Fruit	K ⁺ Fruit	Na ⁺ /K ⁺ Fruit	Root Length
season	0.314	0.729	0.849	0.033	1.435	0.48	0.95	0.817	0.213	3.53	0.16	4.5	1.62	2	2.3
saline (no BC)	0.104	11.146 **	29.79 **	7.03 *	5.229 *	297.192 **	49.85 **	7.3 *	228.09 **	10.42 *	68.174 **	32 **	4.91	18 **	18.84 **
season x saline (no BC)	0.594	0.054	0.045	0.075	0.12	0.405	3.87	0.084	5.88*	6.28*	5.64*	2	1.26	0	2.86
season	0.033	0.568	0.008	3.648	1.435	0	0.002	0.1	0.505	1.259	2.722	0.333	11.28 *	0.33	3.347
saline (with BC)	0.11	0.104	0.008	1.861	5.229 *	4.85	0.347	4.04	67.39 **	0.026	25.817 **	6.259 *	1.116	8.33 *	8.293 *
season x saline (with BC)	0.761	0.29	2.229	1.508	0.012	0.4	0.574	0.13	3.98	1.117	0.004	0.037	11.38*	0.33	5.441*
season	0.152	0.172	0	2.875	0.715	0.371	0.821	0.321	11.19 *	1.75	15.45 **	2	1.199	0.33	2.447
biochar (fresh)	1.952	11.59 **	5.78 *	0.059	44.12 **	7.2 *	5.67 *	2.5	0.445	7.08 *	3.99	0.22	0.607	0.33	6.77 *
season x biochar (fresh)	0.007	0.062	1.143	1.188	0.13	0.009	0.021	0.33	3	1.47	3	0.889	1.182	0.33	6.56 *
season	1.18	1.57	1.87	0.038	0.281	0.303	3.11	0.289	1.79	1.251	0.174	2	4.1	0	0.623
biochar (saline)	0.223	0.233	15.28 **	2.751	10.125 **	5.87	25.75 **	66.51 **	1.708	0.117	0.616	0.22	2.05	0	15.1
season x biochar (saline)	0.287	0.009	0.153	0.086	1.125	0.352	0.99	0.093	0	1.846	1.55	0.889	0.001	0	0.958

Values shown are F values, and *p* values are indicated by * *p* < 0.05 and ** *p* < 0.01.

3.2. Biochar-Enhanced Number of Leaves, Number of Flowers, and Fruit Diameter under Salinity Stress

The effect of salinity stress varied among investigated traits and biochar addition. As shown in Figure 1a, the height of tomato plants was not affected by salinity stress in the presence or absence of biochar. Biochar addition reduced both the number of branches and leaves under fresh water but raised the number of leaves under salinity compared with corresponding no BC conditions (Figure 1b–d). Furthermore, the number of initial inflorescences was enhanced in response to saline irrigation in plants cultivated in soil without biochar addition (no BC). Biochar addition to soil enhanced the number of flowers under saline irrigation compared with no BC soil. Nevertheless, biochar application significantly diminished the number of flowers by a reduction ratio of 45.45% biochar in plants irrigated with fresh water (Figure 1e).

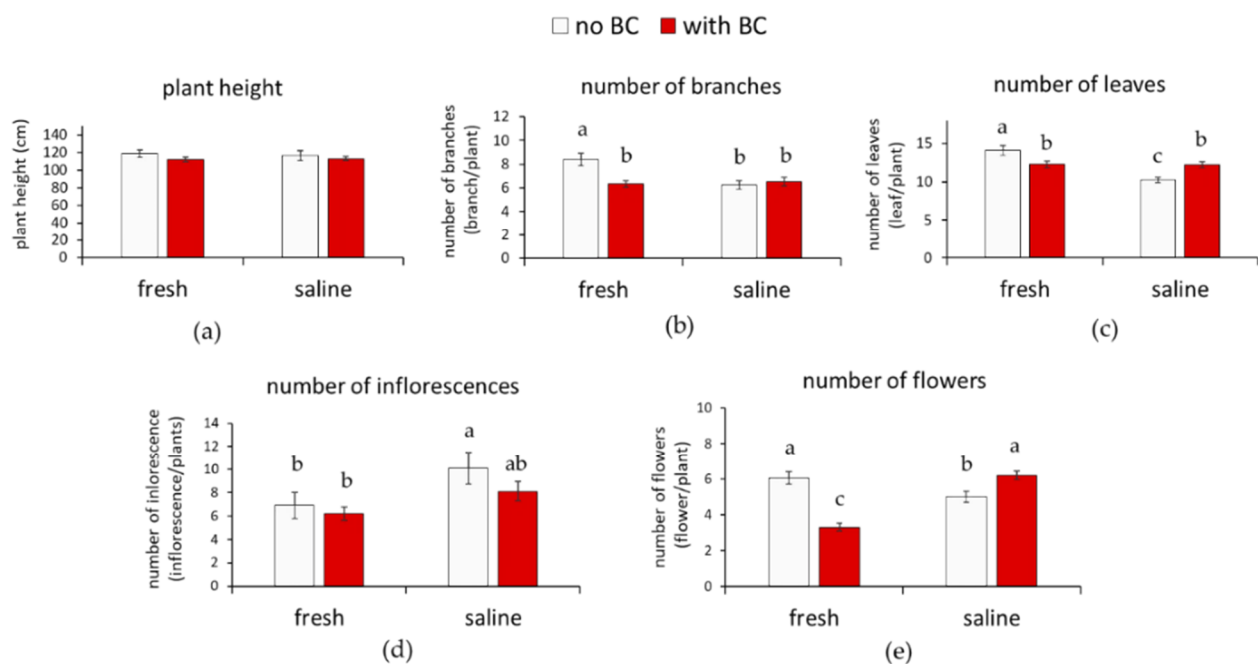


Figure 1. Effect of biochar addition on tomato plant response to salinity stress in terms of the following traits: (a) plant height, (b) number of branches, (c) number of leaves, (d) number of inflorescences, and (e) number of flowers. Values represented the means of five biological replications (three plants each) \pm SE ($n = 120$, two seasons). Means with the same letters are not significantly different according to Duncan's multiple range test (DMRT) ($p \leq 0.05$).

Applied salinity treatment (3000 ppm) led to a reduction in fruit yield by an average ratio of 50.15% (Figure 2a,b), nevertheless, the yield of fruits was reduced with BC-added soil by a ratio of 26.44% under fresh water irrigation, compared with no BC soil treatment. For fruit quality, the addition of biochar (with BC) to the soil, on the other hand, significantly enhanced fruit diameter by 1.48-fold increase compared with no BC soil (Figure 2c). Figure 2d shows the estimated level of total soluble solids (TSS). Biochar application did not significantly alter fruits' TSS levels when plants were irrigated with fresh water. Salinity stress, on the other hand, significantly elevated TSS levels in mature tomato fruits in the absence of biochar, yet was significantly reduced with biochar-added soil compared with no BC plants by an approximated reduction ratio of 44%.

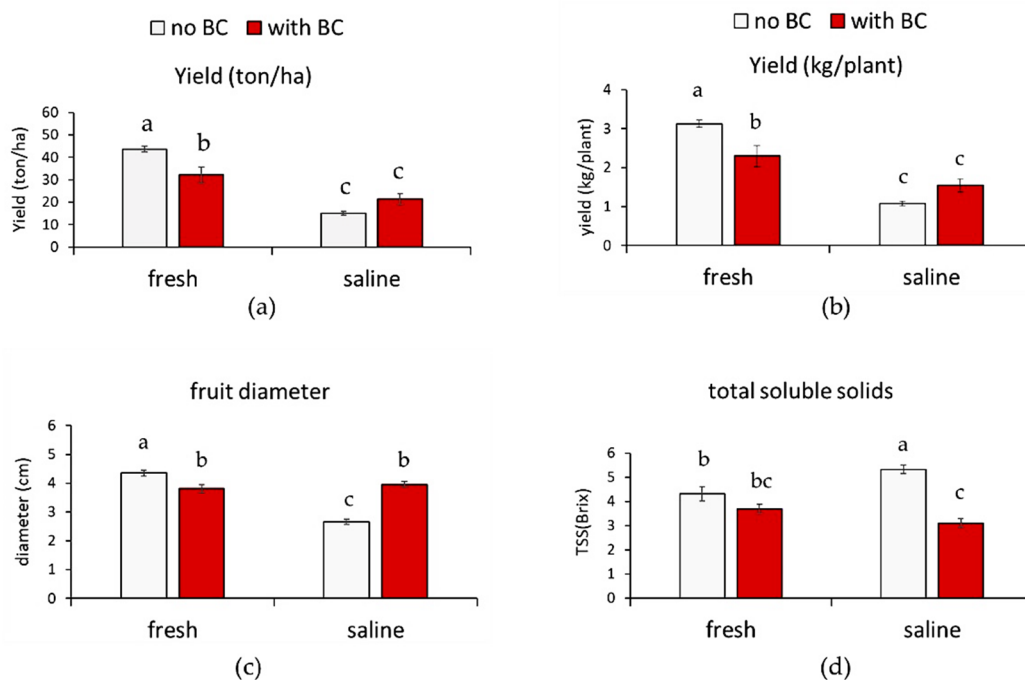


Figure 2. Effect of biochar addition on tomato plant response to salinity stress in terms of the following traits: (a) crop yield in ton/ha, (b) crop yield in kg/plant, (c) fruit diameter, and (d) fruit total soluble solids (TSS). Values represented the means of five biological replications (three plants each) \pm SE ($n = 120$, two seasons). Means with the same letters are not significantly different according to Duncan's multiple range test (DMRT) ($p \leq 0.05$).

3.3. Biochar Did Not Alter Sodium and Potassium Ions Content in Roots or Fruits

Sodium ions (Na^+) content was elevated in saline-stressed fruits in the presence and absence of biochar. The sodium ions (Na^+) content in fruit measured in the presence of biochar was 1.43 times greater compared with fruit irrigated with fresh water (Figure 3a). Likewise, stem-borne roots (topsoil underground adventitious roots) accumulate 2.89 times more sodium compared with fresh irrigated fruits (Figure 3b). In either case, i.e., the presence and absence of biochar, there was no significant difference in sodium ions content (Figure 3a,b). It is worth mentioning that sodium ions content in roots was greater than in tomato fruits: 0.15% and 0.5% (relative to dry weight), respectively. Regarding potassium ions content, in either fruits or roots, salinity stress did not alter its content in the case of "no BC" and "with BC" treatment (Figure 3c,d, respectively). Figure 3e,f shows that Na^+/K^+ ratios were significantly elevated in saline irrigation compared with fresh water-irrigated tomato plants; again, biochar addition did not alter this ratio in either tissue.

3.4. Biochar Addition Enhances Stem-Borne Lateral Root Length

Stem-borne lateral root length was investigated in this work (Figure 4a,b). It is noted that in biochar-added soil, the length of stem-borne lateral roots was increased under fresh irrigation conditions by 1.77-fold compared with the "fresh no BC" addition (Figure 4a). Additionally, under saline irrigation, stem-borne lateral root length was significantly diminished in the case of biochar-added soil by a reduction ratio of 48% relative to fresh with BC treatment. Likewise, there was no significant differences between no BC and with BC treatments in saline irrigated lands in terms of lateral root length ramified from underground-stem.

3.5. The Expression of Gene *LeNR* Was Altered after Biochar Addition under Fresh Water Irrigation

We have investigated the quantitative gene expression of two salt stress-related genes: *LeNHX4* (encodes vacuolar Na^+/H^+ antiporter) and *LeNR* (encodes nitrate reductase

enzyme). As shown in Figure 5a, applied salinity stress (3000 ppm NaCl) caused a clear elevation in the expression of LeNHX4 by an average fold increase of 5.75 times. Nevertheless, there was no significant difference in LeNHX4 expression in the absence or the presence of biochar. Unlike LeNHX4, the expression of LeNR varied between biochar treatments (Figure 5b). Salinity stress inhibited LeNR expression in tomato leaves in the presence and absence of biochar. However, the relative expression ratio was significantly higher in the presence of biochar compared with plants cultivated in “no BC” soil with a fresh irrigation regime.

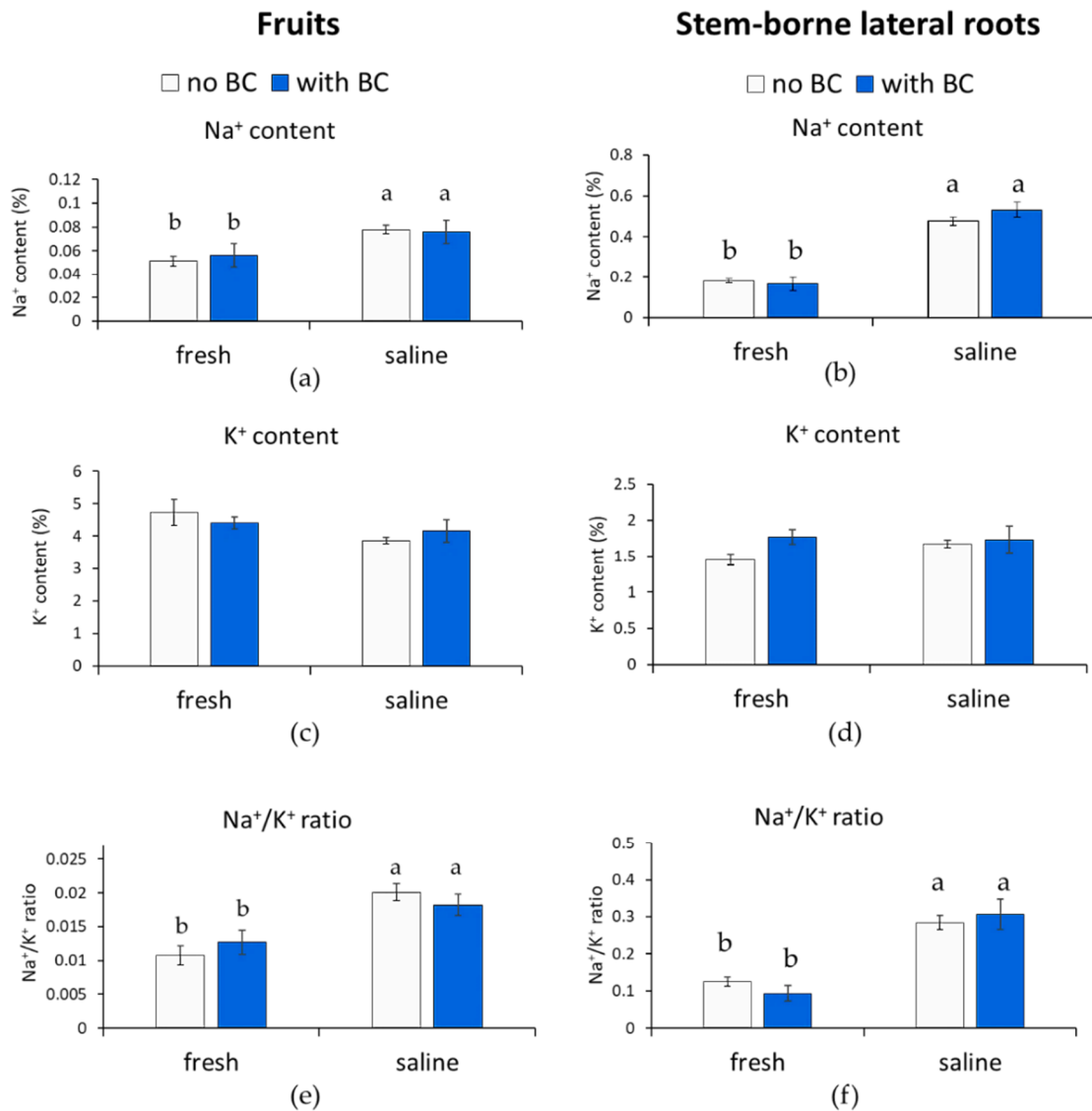


Figure 3. Effect of biochar addition on accumulated Na⁺ content in fruits (a), K⁺ content in fruits (c), Na⁺/K⁺ ratio in fruits (e), Na⁺ content in stem-borne lateral roots (b), K⁺ content in stem-borne lateral roots (d), and Na⁺/K⁺ ratio in stem-borne lateral roots (f). Values represented the means of four biological replications (three plants each) ± SE (n = 96, two seasons). Means with the same letters are not significantly different according to Duncan’s multiple range test (DMRT) ($p \leq 0.05$).

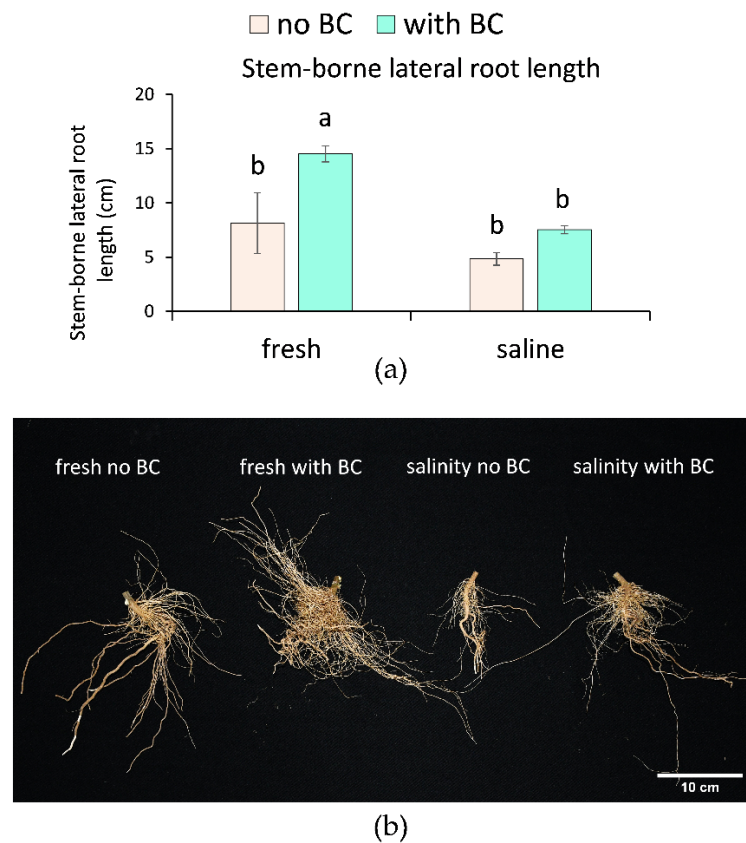


Figure 4. Effect of biochar addition on stem-borne lateral root length under fresh and saline irrigation regime: (a) stem-borne lateral root length. Values represented the means of five biological replications (three plants each) \pm SE ($n = 120$, two seasons). Means with the same letters are not significantly different according to Duncan's multiple range test (DMRT) ($p \leq 0.05$). (b) a representative image of the topsoil 10 cm of the stem-borne lateral root extracted from top soil, scale bar equals 10 cm.

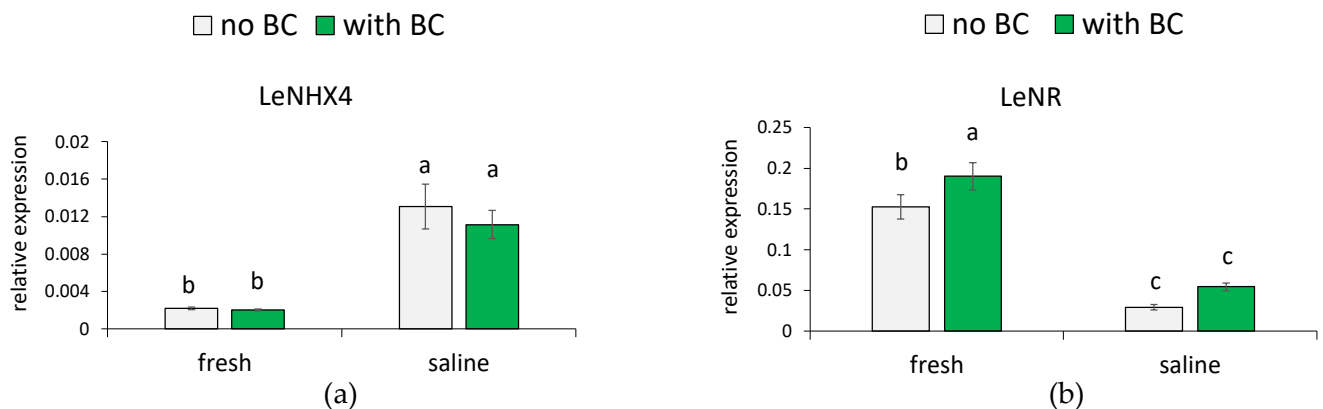


Figure 5. Alternation in transcripts accumulation for (a) LeNHX4 (encodes Na^+/H^+ antiporter), and (b) LeNR (encodes nitrate reductase) in tomato leaves under saline irrigation regime in the absence (no BC) and presence of biochar (with BC). Values represented the means of three biological replications (two plants each) \pm SE ($n = 48$, two seasons). Means with the same letters are not significantly different according to Duncan's multiple range test (DMRT) ($p \leq 0.05$).

3.6. Soil Properties

Some soil parameters were investigated over the seasons as mentioned in Table 3 to study the influence of biochar on decreasing the detrimental impact of using salty water

as an alternative water supply on the soil. The soil texture was a clay loam (35.8% clay, 33.7% silt, and 29.9% sand), low in organic matter, and containing 4.2% CaCO_3 . The soil was classified as very slightly saline. The results illustrate that saline water has a highly significant and significant effect on EC, major cations, and anions concentration whereas it has an insignificant effect on pH and soil organic matter. Furthermore, biochar has the ability to improve the chemical characteristics of the soil. Biochar has a large and considerable impact on soil organic matter as well as EC, whereas biochar has an insignificant effect on pH.

Table 3. Some properties of soil samples under study seasons 2019/2020 and 2020/2021.

	Samples	pH (1:2.5)	% OM	ds·m ⁻¹ EC	meq·L ⁻¹						
					Ca ²⁺	Mg ²⁺	Na ⁺	K ⁺	HCO ₃ ⁻	Cl ⁻	SO ₄ ²⁻
Season 2019/2020	Control	7.80	0.36	1.80	3.80	3.20	10.30	0.60	4.2	9.7	3.1
	Fresh	7.82	0.79	1.87	3.85	3.25	10.62	0.71	4.21	9.80	3.25
	Saline	7.91	0.78	2.17	4.00	3.30	13.35	0.85	4.90	12.75	4.00
	F test 0.05	NS	NS	**	*	*	**	*	**	**	**
	No BC	7.85	0.35	2.08	3.95	3.31	12.55	0.80	4.80	11.85	3.70
	BC	7.88	1.22	1.95	3.91	3.22	11.42	0.76	4.31	10.70	3.55
	F test 0.05	NS	**	*	NS	*	**	NS	**	*	*
	WN B0	7.80	0.36	1.86	3.80	3.30	10.60	0.70	4.20	9.80	3.20
	WN B1	7.85	1.22	1.88	3.90	3.20	10.64	0.72	4.22	9.80	3.30
	WS B0	7.90	0.34	2.30	4.10	3.32	14.50	0.90	5.40	13.90	4.20
	WS B1	7.92	1.23	2.05	3.92	3.25	12.20	0.80	4.40	11.60	3.80
	F test 0.05	NS	*	**	*	NS	**	NS	**	*	*
Season 2020/2021	Fresh	7.85	0.9	1.95	3.94	3.27	11.57	0.77	4.55	10.35	3.75
	Saline	8.00	0.89	2.35	4.20	3.55	14.35	1.00	5.20	13.75	4.00
	F test 0.05	NS	*	**	**	*	**	**	**	**	*
	No BC	7.95	0.43	2.20	4.16	3.52	13.25	0.92	4.90	12.50	3.90
	BC	7.92	1.35	2.10	3.98	3.30	12.65	0.85	4.85	11.60	3.85
	F test 0.05	NS	**	*	*	*	**	*	NS	*	*
	WN B0	7.80	0.45	1.90	3.92	3.25	11.35	0.75	4.50	10.30	3.70
	WN B1	7.90	1.35	2.01	3.96	3.30	11.80	0.80	4.60	10.40	3.80
	WS B0	8.10	0.42	2.50	4.40	3.80	15.20	1.10	5.30	14.70	4.10
	WS B1	7.95	1.36	2.20	4.00	3.30	13.50	0.90	5.10	12.80	3.90
	F test 0.05	NS	*	**	*	NS	**	*	**	*	*

Values shown are F values, and p values are indicated by * $p < 0.05$ and ** $p < 0.01$, NS is for not significant.

4. Discussion

4.1. Tomato Plant Response to Saline Irrigation Water in “No BC” Soil

The saline irrigation regime challenges crop plants with two types of stresses; (i) sodium ions toxicity, and (ii) physiological dehydration (drought). Both challenges eventually cause severe plant growth inhibition and yield reduction [45]. In this study, applied progressive salinity stress (3000 ppm NaCl) significantly reduced the number of branches, number of leaves, number of flowers, yield, and fruit diameter in the absence of biochar (Table 2 and Figures 1 and 2) [46,47]. Interestingly, the applied saline irrigation regime in no BC soil elevated the number of initial inflorescences compared with fresh water irrigation (Figure 1d). This might be related to the drought escape (DE) strategy which is a well-known adaptive mechanism against dehydration. In particular terminal drought episodes, DE aids plants by shortening their life cycles and producing seeds before experiencing severe water shortage courses [48,49]. Furthermore, salinity stress might relatively enhance

tomato fruit yield quality by elevating total soluble solids (TSS) and sugar. This might be due to the stress-induced reallocation of sugars into other tissues such as fruits [38,50].

The accumulation of sodium ions in saline-treated plants is a basic saline-related physiological trait for determining salt-sensitive vs. salt-tolerant plants [51]. Na^+ content was increased in roots and fruits by a ratio of 2.94 and 1.45, respectively, as compared with fresh water-irrigated plants (Figure 3a,b). It is reported that sodium ions could accumulate in inflorescences, pericarp, and even in tomato seeds under salinity stress, however, in higher amounts compared with data produced in this study [52,53]. Although fruits (the marketable part of the tomato) accumulated more sodium ions under a saline irrigation regime of 3000 ppm NaCl (no BC, 77.8 mg/100 gm), the sodium level in dried fruits was below the risk threshold according to USDA health standards [54].

Roots are the main plant organs responsible for water and nutrient uptake; thus, their traits are well associated with enhancing crop performance under different soil-borne environmental stresses such as drought or salinity [55]. The tomato plant readily generates stem-borne roots during its normal developmental stages and is considered a model plant for studying root architectural traits [56]. As indicated in Figure 4a, the applied biochar did not alter stem-borne lateral root length relative to no BC treatment under salinity stress (Figure 4a). Plant response to abiotic stress as salinity seemed to be greatly genotype-dependent, i.e., varied among investigated cultivars [57,58]. It is important to continue studying root traits in tomato crops using a wide range of lines and genotypes with different levels of salinity tolerance for more conclusive results.

The response of tomato leaves to salinity stress was also evaluated at the level of the molecular aspect by quantifying the expression of two well-acknowledged salt stress-related genes: LeNHX4 (encodes Na^+/H^+ antiporter) and LeNR (encodes the enzyme nitrate reductase) (Figure 5). As expected, the level of LeNHX4 expression was significantly elevated in response to salt stress which is indicative of elevated sodium ions accumulation in leaves (Figure 5a) [59]. LeNR encodes the NO_3 metabolizing enzyme nitrate reductase, a key limiting enzyme in nitrogen assimilation. It is confirmed that the absorption of nitrogen from soil is negatively affected by salinity stress [45,60]. Indeed, accumulative doses of NaCl in saline irrigation water led to a significant reduction in LeNR expression by a reduction ratio of 82.16% (Figure 5b). LeNR gene expression was elevated in biochar-added soil plants compared with no BC-treated plants under a fresh irrigation regime. This observation could be related to enhanced root branching in biochar-treated plants under fresh water indicating a better nitrogen uptake [40].

4.2. Biochar Addition Did Not Alleviate Tomato Fruit Yield Reduction under Salinity Stress

In this work, biochar was added to fertile clay soil (Giza, Egypt), and then salinity stress was triggered by applying a 3000 ppm NaCl solution. The results demonstrate that biochar addition could significantly enhance several agronomic traits such as the number of leaves, number of flowers, and tomato fruit diameter (Figures 1 and 2). This might be attributed to biochar's ability to maintain water repellency and thus attenuate the negative effect of salinity-related physiological drought on plants, in particular with low fertility (ex: sandy) soil [61,62]. Biochar has the potential to immobilize sodium ions and thus hinder their influx into plant roots [37]. However, according to our results, the added amount of biochar (4.8 tons/ha) was not fully functional in restoring a salinity-induced reduction of fruit yield or in reducing accumulated sodium ions content in either fruits or roots (Figures 2 and 3a,b). Recently, [63] reported that biochar addition with a rate of 12 tons/ha reduced sodium content in wheat grains cultivated in the low fertile/saline-affected soil in the Yellow River Delta. Although not investigated in this work, the application of a higher amount of biochar could enhance a tomato plant's adaptive response to saline water in terms of minimizing sodium influx into plant tissue [38,64]. Nevertheless, the addition of biochar to clay soil seemed to be applied with extreme caution as any unoptimized high addition might cause a disturbance in the balance between the liquid and gaseous phases and thus diminish available water in the rhizosphere [65]. Therefore, we speculate

that the biochar mitigating effect against salinity stress (double stressors environmental stress imposes both physiological drought and sodium ion toxicity) needs to be finetuned through extensive comparative studies.

The predictable effect of biochar on crop yield in fertile soil is unclear, and in many cases, contradictory [15]. Our data revealed that biochar addition to fertile soil could enhance some vegetative growth traits (Figure 1) or root growth (Figure 4) but did not alleviate the negative impact of salt stress on tomato fruit yield. The yield was even reduced under the fresh irrigation regime with tomato plants cultivated in biochar-added soil. It was reported by [66] that biochar addition to fertile soil under regular standard conditions could enhance a tomato plant's vegetative growth but not necessarily promote fruit yield. This could be related to the highly expressed nitrate reductase gene which is a key enzyme in nitrogen assimilation in leaves (Figure 4) [60]. Biochar addition was more effective with sandy loam soil than clay loam soil in terms of N availability and uptake which eventually altered plant growth traits. On the other hand, certain types of biochar could in some cases immobilize nitrogen and thus inhibit plant growth [67]. Several studies have highlighted a reduction in crop yield upon biochar addition to nutrient-rich soils, and the level of reduction was increased with applied biochar amounts [68–70]. Biochar addition was reported to reduce the number of branches, number of leaves, number of flowers, yield, and fruit diameter under a fresh water irrigation regime (Figures 1 and 2). This observation might be associated with the negative interaction between phosphorous (P) and biochar in saline-sodic soil due to excessive phosphate sorption and/or precipitation which eventually reduced P availability and thus plant yield [71]. Further research is required in this area with particular consideration of biochar type/amount and cultivated plant/soil.

4.3. Effect of Saline Water and Biochar on Soil Properties

The use of saline water in irrigation has an influence on soil qualities, even when drip irrigation is used, since salt builds up in the soil [72]. Excess salt, particularly sodium, has a negative impact on soil's physical and chemical characteristics, microbiological processes, and as a result, crop development and production. The results show that the utilized soil was slightly saline according to saline soil guidelines [73], and utilizing saline water in irrigation raised EC and salt concentrations across the two seasons.

As a soil amendment, biochar improves soil quality. As shown in Table 3, biochar treatment has a considerable influence on soil properties and reduces saline water's negative impact on the soil. The results refer to the increase of the soil organic matter percentage that enhances the soil–water holding capacity [65], increases cation-exchange capacity (CEC), decreases the bulk density, and minimizes the loss of nutrients and other agricultural chemicals in soil run-off [74]. The favorable effect of biochar on soil chemical properties may occur by two mechanisms: (i) cations sorption; and (ii) control of contaminated organic and inorganic compounds in soil. Thus, biochar reduces the negative effects of saline water on soil productivity through its ability to adsorb soil ions and its impact on nutrient availability, including N, K, and P.

4.4. Practical Implications of This Study

Soil salinity increase is mainly owed to improper irrigation practices and limited fresh water irrigation [75]. Many farmers have had to use mixed saline water for irrigating their crops cultivated in the fertile soil of the Nile Delta and Valley, practices that imposed negative effects on both soil properties and crop productivity. Unfortunately, expectations indicate a much more complicated situation by 2050. In light of this, using soil amendments is one of the most required sustainable adaptive investment strategies to ameliorate the negative impact of progressive saline irrigation on crop production. In addition, biochar might impose significant cost burdens, in particular with large amounts. Therefore, the careful assessment of the interaction between biochar and the utilized agroecosystem is essential for obtaining better results.

Expectedly, a wide range of studies was interested in examining biochar's effect on plant production in low fertility soil and could develop useful knowledge in this regard. However, the effect of biochar application on fertile soil is unclear and could even be contradictory. This study investigates the hypothesis of whether biochar would be beneficial to fertile soil against a saline irrigation regime, in regards to mitigating crop yield loss and enhancing soil properties. It is strongly suggested that further studies dealing with the long-term application of biochar in fertile soil fields considering different types/doses of biochar are needed to verify whether biochar could be safely involved in sustainable agricultural practices.

5. Conclusions

The scientific question posed in this research was whether biochar addition to fertile clay soil in Egypt might mitigate the negative impact of saline irrigation on tomato crop productivity. In conclusion, the addition of biochar at a rate of 4.8 tons/ha relatively enhanced several agronomic traits including the number of leaves, number of flowers, and fruit diameter but was not significantly effective in compensating for fruit yield reduction under salinity stress. In the same context, biochar did not reduce sodium ions accumulated in roots or fruits. The results of this study argue that adding biochar to fertile soil with a rate of 4.8 tons/ha could not produce the desired effect, i.e., mitigating salinity's negative effect on tomato crop yield. Extensive comparative field studies should be designed and conducted to investigate this hypothesis and the long-term applications of biochar in fertile soil fields.

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