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Mitigating Salinity Stress in Barley (*Hordeum vulgare* L.) through Biochar and NPK Fertilizers: Impacts on Physio-Biochemical Behavior and Grain Yield

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Abstract: Increased soil salinity significantly inhibits crop production around the world. Over the last decade, biochar has been used in agriculture to improve plant productivity, soil quality, and as an alternative to plant amendment. This study was aimed to study the effect of biochar, NPK, and their combination on the growth, physio-biochemical traits, mineral contents, and grain yield of barley (*Hordeum vulgare* L.). Thus, a pot factorial experiment based on a completely randomized design with three replications was performed. Experimental treatments included four levels of biochar (0, 2, 5 and 10% of total pot mass), four different NaCl levels (0, 75, 125, and 200 mmol L^{-1}), and with or without NPK fertilizer. The results showed that a negative effect on gas exchange parameters, photosynthetic pigments, SPAD value, minerals contents, and grain yield of barley under salinity treatments. In addition, our funding showed the negative effect on biochemical traits such as proline, soluble sugars, individual sugar, and phenolic compounds. The use of biochar, combined with NPK fertilizers, considerably increases these parameters and especially improves barley grains yield under severe salinity conditions (200 mM) with a dose of 2% and 5% (394.1 and 280.61 g m^{-2}, respectively) of total pot mass. It is concluded that biochar amendment could be a promising practice to enhance barley growth under severe saline irrigation and NPK fertilization regimes.

Keywords: barley; salt stress; biochar; gas exchange parameters; phenolic profile; NPK fertilizer

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1. Introduction

Among various abiotic stresses, soil salinization poses a serious threat to future food production especially since FAO 2022 has stated that salinization will result in a major loss of agricultural productivity as the number of salt-affected croplands increases [1]. Primary soil salinization, which cannot be avoided, occurs due to the weathering of saline bedrock and sea level changes near the coast [2]. In this study, we focus on secondary salinization, which could be overcome by using sustainable and environmentally friendly farming methods. It is mainly caused by human activities such as irrigation with saline water, excessive use of mineral fertilizers, and other intensive monocultures [2–4].

Studies to improve crop tolerance to abiotic stress are critical due to current climate changes and variability, and the detrimental effects of conventional farming practices, especially on soil properties. Abiotic stress negatively affects the physical and biological conditions of soil, such as organic matter content and carbon/nitrogen ratio, microbial

community diversity and activity, soil aeration, bulk density, aggregate stability, and water holding capacity, resulting in significant yield losses [5]. In particular, the presence of salt in the soil negatively affects germination, growth, water uptake, transpiration, photosynthesis, enzyme activities and, consequently, crop yields. However, crop plants have various defense strategies to tolerate unfavorable conditions such as the accumulation of stress-related metabolites and the regulation of toxic ion balance [6,7].

Compared to wheat, maize, and rice, barley (*Hordeum vulgare* L.) is a more salt-tolerant plant; however, salt in the soil negatively affects the number of barley plantlets and their productivity [8–10]. To reduce the yield losses caused by soil salinity, the solutions often mentioned could be to use salt-resistant genotypes and/or to mitigate soil treatments in salt-affected croplands. Biochar is an organic amendment that has a positive effect on crops under stress conditions, mainly related to improved soil properties [5,11]. Biochar application can reduce soil bulk density and increase soil water and its storage capacity [12–14]. In addition, due to the adsorption capacity of biochar, plants reduce the uptake of toxic heavy metals from polluted soils and improve their growth and yield [15,16]. The application of biochar significantly affects soil quality and improves crop growth and yield due to its effect on microbial diversity and stimulation of their activities. It also improves organic carbon content and greenhouse gas storage [17–20].

In addition, the use of biochar has helped to improve the growth, biomass, and photosynthesis of plants, including barley, in salt-affected soils [5,11,21,22]. Moreover, it has been demonstrated that biochar application in saline soils can help crops tolerate adverse conditions by improving their nutrient uptake and accumulation [8–10]. Biochar is a good practice to reduce the harmful effects of salt because its application reduces toxic Na^+ and K^+ uptake and concentration in plants [8–10]. In addition, biochar can improve the gas exchange of plants under drought and salt stress [5,23,24]. Furthermore, the use of organic nutrient sources alone or in combination with inorganic fertilizers can help reduce the harmful effects of NPK on the environment without sacrificing grain yields [25]. It is also important to mention that the effect of biochar may vary depending on its physicochemical properties and the introduced concentration in the soil, as well as soil properties.

The objective of this study was to evaluate the effect of biochar alone and in combination with mineral fertilizer (NPK) under saline conditions on various physiological and agronomic traits such as photosynthetic pigments, gas exchange parameters, stress-related metabolites, phenolic compounds, leaf ion concentrations, and grain yield.

2. Materials and Methods

2.1. Preparation of Biochar (BC)

In this study, BC was prepared from pine (*Pinus halepensis* L.) under aerobic conditions (10 h at 450 °C) with the following characteristics (see Table 1). The SEM images (Figure 1A,B at 50 and 500 µm, respectively) illustrate the porous structure of biochar. The biochar produced has a higher content of nutrients such as phosphorus, sodium, calcium, zinc, etc. than the soil (Table 1).

Table 1. Physical-chemical characteristics of the Biochar and the soil.

Attribute	Unit	Biochar	Soil
Clay	%	-	2.17
Silt	%	-	4.92
Sand	%	-	92.64
Size	mm	0.2–2	-
pH	-	7.63	7.8
Cation exchangeable capacity (CEC)	meq 100 g^{-1}	54.6	4.5
Organic matter (OM)	%	81.2	0.6

Table 1. *Cont.*

Attribute	Unit	Biochar	Soil
Electrical conductivity (EC)	dS cm^{-1}	1.3	3.79
Total $CaCO_3$	%	-	7.22
Active $CaCO_3$	%	-	5.83
Total Nitrogen (N)	%	-	1.12
Phosphorus (P)	ppm	325.5	52.4
Sodium (Na)	mg kg^{-1}	27.9	13.6
Potassium (K)	mg kg^{-1}	58.7	60.9
Calcium (Ca)	mg kg^{-1}	1192.1	869
Magnesium (Mg)	mg kg^{-1}	9.5	13.9
Zinc (Zn)	mg kg^{-1}	0.392	0.035
Iron (Fe)	mg kg^{-1}	16.132	0.048
Manganese (Mn)	mg kg^{-1}	2.52	0.21

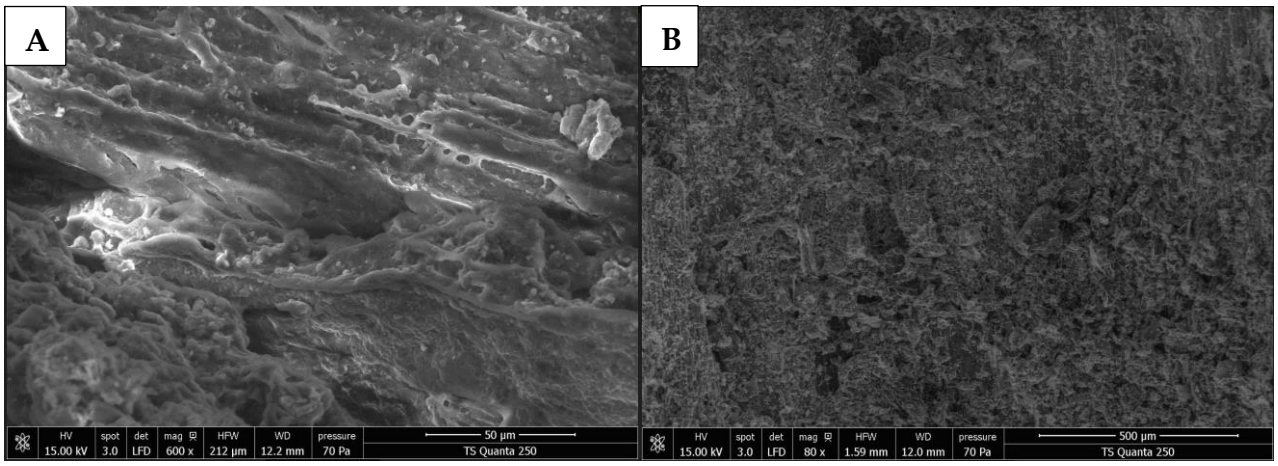

Figure 1. Scanning Electron Microscopy (SEM) images of the porous structure of biochar. (**A**): image of biochar enlarged to 50 µm; (**B**): image of biochar enlarged to 500 µm.

2.2. Experimental Setup and Growth Conditions

A pot experiment with barley (*Hordeum vulgare* L.) cultivar 'Ardhaoui' was factorially designed based on a randomized complete design with three replicates under greenhouse conditions at the Institute of Drylands of Medenine, Tunisia (10°38′30.34″ E, 33°29′53.23″ N, 106 m a.s.l.) during the 2021 harvest season. The average minimum temperature was 22 °C and the maximum temperature was 37 °C. Average relative humidity ranged from 32 to 55.5%. Treatments included biochar (BC) (0%, 2%, 5%, and 10% *w*/*w* in soil), irrigation water salinity (0, 75, 125, and 200 mM NaCl), and fertilized (F) and unfertilized (UF) NPK fertilizers [N (total nitrogen) = 1.41 g/pot; P (P_2O_5) = 1.76 g/pot; and K (K_2O) = 1.06 g/pot], the equivalent of 175 kg ha^{-1} of N, 100 kg ha^{-1} of P, and 125 kg ha^{-1} of K, according to field capacity (FC). Before sowing, the pots with a volume of 0.047 m^3 were irrigated with about 100% of the field capacity of fresh water to wash out the salts from the soil (sandy soil) layer at 0 to 25 cm depth by progressive accumulation and with seeding density equal to 20 g m^{-2}. All physiological and biochemical analysis was found at the tillering stage.

2.3. Analysis of Gas Exchange, Photosynthetic Pigments and Measurement of SPAD

Gas exchange parameters on flag leaves were measured using a portable gas exchange system (ADC BioScientific LC ProSystem serial number 3302, ADC Bioscientific Ltd., Hoddesdon, UK). Leaf temperature was maintained at 25 °C, light intensity was adjusted to 800 µmol photons m s^{-1} and CO_2 concentration to 400 µmol mol^{-1} using a red/blue light source. The distance between the leaf and air was maintained at 1 KPa. Chl 'a', 'b',

and carotenoid pigments were determined according to the Arnon method [26]. Leaf SPAD was measured using a standard chlorophyll meter (Minolta 1500, Osaka, Japan).

2.4. Analysis of Stress-Related Metabolites

Soluble sugars were quantified by the phenol-sulfuric acid method as described by [27]. An amount of 100 mg of the dry leaf was extracted in 80% (v/v) methanol. The extract was centrifuged at 5000 rpm for 10 min. The supernatant was used to estimate the concentrations of soluble sugars. The reaction mixture consisted of 0.5 mL of 5% phenol and 2.5 mL of 98% sulfuric acid. Once the extract was cooled, its absorbance was determined at 590 nm. The concentrations of fructose and glucose were determined analytically using LC-ESI-MS. The content of free proline was determined according to the method described by [28]. The leaf sample (1 g) was homogenized in sulfosalicylic acid (3%), and acid ninhydrin and glacial acetic acid were added to the filtrate. The mixture was heated in a water bath at 100 °C for one hour. The reaction was stopped in an ice bath and the mixture was extracted with toluene. The absorbance was measured spectrophotometrically at a wavelength of 520 nm. Malondialdehyde (MDA) content was determined as an indicator of lipid peroxidation of leaves according to [29]. Samples of dry leaves (100 mg) were homogenized in 5 mL of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 15,000× g for 20 min at 4 °C. A 1 mL aliquot of the supernatant was mixed with 3 mL of 0.5% thio-barbituric acid (TBA) prepared in 20% TCA and incubated at 90 °C for 20 min. After the reaction was stopped in the ice bath the samples were centrifuged at 10,000× g for 5 min. The absorbance of the supernatant at 532 nm was then measured. After subtracting the non-specific absorbance at 600 nm, the MDA concentration was determined using the extinction coefficient of 155 mM^{-1} cm^{-1} [30].

2.5. Ion Concentrations in the Leaf

Dried leaves were ground to a fine powder and incinerated in an oven at 550 °C for 6 h. Then, 2 N HCl was added to the cooled ash and the solution was filtered and analyzed after 15 min. The concentrations of Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, Zn^{2+} and Mn^{2+} were determined using an atomic absorption spectrometer (Thermo SCIENTIFIC iCE 3000 AA Spectrometer, Mundelein, IL, USA) and expressed as mg g^{-1} dry weight (DW).

2.6. Analysis of Phenolic Compounds by Analytical LC-ESI -MS

The methanolic leaf extract was analyzed using an LCMS-2020 mass spectrometer (Shimadzu, Kyoto, Japan). The LC System was equipped with an electrospray ionization (ESI) source. Spectra were recorded in negative ion mode, monitored, and processed using Shimadzu Lab Solutions LC–MS software 9050 (Kyoto, Japan). The LC-20AD XR binary pump system, the SIL-20AC XR auto-sampler, the CTO-20AC column oven, and the DGU-20AS degasser (Shimadzu, Kyoto, Japan) were the main elements of the LC system. For analysis, Thermo Electron (Dreieich, Germany) was equipped with an Aquasil C18 column (150 mm_3 mm, 3 mm) thermostatted to 40 °C, preceded by an Aquasil C18 guard column (10 mm_3 mm, 3 mm). The solvents were A (0.1% formic acid in H_2O, v/v) and B (0.1% formic acid in methanol, v/v). The elution gradient was 10–100% B for 0–45 min, 100% B over 45–55 min, and column equilibration lasted 5 min between runs. The mobile phase flow rate was 0.4 mL/min and the injection volume was 5 mL. High purity nitrogen was used as nebulizer and auxiliary gas. The ion spray voltage was set to −3.5 V in negative mode. The following settings were used: a nebulizer gas flow rate of 1.5 L/min, a dry gas flow rate of 12 L/min, a DL (dissolution line) temperature of 250 °C, a block source temperature of 400 °C, and a voltage detector of 1.2 V.

2.7. Grain Yield

At maturity, plants were harvested and grain yield data, including number of ears per plant, number of grains per ear, and 1000-grain weight, were recorded. The grain yield was

calculated based on pot area after that of the number of plants obtained per pot and finally number of ears per plant, number of grains per ear, and 1000-grain weight.

2.8. Statistical Analysis

The data were analyzed using the statistical program SPSS 25.0 (Chicago, IL, USA). The effects of biochar, NPK fertilizer, and salinity and their interactions on the physical-biochemical measurements of the plants were examined in three directions using ANOVA. Mean values of the traits were separated using Tukey's multiple range test at the 0.05 significance level.

3. Results

3.1. Effect of Biochar, NPK Fertilizers, and Salinity on Photosynthetic Pigments and Gas Exchange Parameters

Application of salinity with either F or UF treatments significantly affected the levels of chlorophyll a, b, carotenoids as shown in Table 2. Chlorophyll a (Chl a) was significantly decreased under saline conditions compared to the control. The mean values of Chl a reduced by 22.68% compared to 17.76% under treatments F and UF, respectively. The application of BC significantly increased Chl a concentration in all salt treatments. At the highest salt concentration (200 mM NaCl), Chl a concentration increased among BC treatments (0%, 2%, 5%, except 10%) and the mean values were 3.97, 5.05, 6.63 mg g^{-1} compared to 4.16, 4.43, 6.48 mg g^{-1} in UF and F treatments, respectively. For chl b, chl a + b, and the carotenoids, the evolution of the concentrations was similar to that of chl a. In addition, the values of SPAD decreased significantly under saline conditions compared with the control. The values of SPAD reduced by 9% compared with 2.28% under UF and F treatments, respectively. The application of BC significantly increased the mean values of SPAD with increasing BC treatment in all salinity treatments. For example, the SPAD mean values at 200 mM NaCl increased by 8.49% compared with 5.54% under UF and F treatments, respectively.

Table 2. Combined effect of salinity (NaCl) (mM NaCl), biochar (BC), and NPK fertilizers (NPK) on *Chl a* (mg g^{-1}), *Chl b* (mg g^{-1}), *Chl* (*a* + *b*) (mg g^{-1}), carotenoids (mg g^{-1}), and SPAD value of barley.

NPK	NaCl (mM)	(BC) %	*Chl a* (mg g^{-1})	*Chl b* (mg g^{-1})	*Chl* (*a* + *b*) (mg g^{-1})	Carotenoids (mg g^{-1})	SPAD
−NPK	0	0	6.82 ± 0.08 Aa	2.35 ± 0.04 Ab	9.18 ± 0.13 Aa	1.08 ± 0.01 Ba	42.36 ± 1.34 Ac
		2	8.87 ± 0.10 Aa	3.40 ± 0.21 Aa	12.28 ± 0.10 Aa	1.58 ± 0.01 Bb	42.71 ± 1.88 Abc
		5	6.32 ± 0.15 Aa	2.10 ± 0.01 Aab	8.42 ± 0.16 Aa	1.07 ± 0.03 Bb	43.35 ± 1.44 Ab
		10	3.91 ± 0.22 Ab	1.24 ± 0.07 Ac	5.16 ± 0.30 Ab	0.77 ± 0.02 Bc	45.70 ± 1.19 Aa
	75	0	7.61 ± 0.01 Ba	1.63 ± 0.02 Bb	9.25 ± 0.03 Ba	2.05 ± 0.00 Aa	40.50 ± 2.01 Ac
		2	4.95 ± 0.18 Ba	1.62 ± 0.02 Ba	6.57 ± 0.21 Ba	0.82 ± 0.13 Ab	41.90 ± 3.08 Abc
		5	5.86 ± 0.97 Ba	1.91 ± 0.38 Bab	7.77 ± 1.35 Ba	1.18 ± 0.15 Ab	42.16 ± 1.61 Ab
		10	5.40 ± 1.02 Bb	1.78 ± 0.42 Bc	7.18 ± 1.44 Bb	0.90 ± 0.12 Ac	44.96 ± 1.74 Aa
	125	0	5.41 ± 0.07 Ca	1.78 ± 0.01 Bb	7.19 ± 0.08 Ca	1.02 ± 0.07 Ca	37.73 ± 2.12 Bc
		2	4.83 ± 0.12 Ca	1.55 ± 0.00 Ba	6.38 ± 0.12 Ca	0.86 ± 0.16 Cb	39.95 ± 2.42 Bbc
		5	4.90 ± 0.19 Ca	1.63 ± 0.07 Bab	6.53 ± 0.27 Ca	0.79 ± 0.07 Cb	41.23 ± 1.60 Bb
		10	5.27 ± 0.54 Cb	1.70 ± 0.17 Bc	6.98 ± 0.72 Cb	0.94 ± 0.16 Cc	42.13 ± 1.36 Ba
	200	0	3.97 ± 0.09 Ca	1.35 ± 0.03 Bb	5.31 ± 0.12 Ca	0.67 ± 0.01 Ca	37.91 ± 2.91 Bc
		2	5.05 ± 0.02 Ca	1.64 ± 0.04 Ba	6.69 ± 0.02 Ca	0.90 ± 0.11 Cb	38.95 ± 2.65 Bbc
		5	6.63 ± 0.18 Ca	2.17 ± 0.01 Bab	8.80 ± 0.19 Ca	1.20 ± 0.01 Cb	40.31 ± 3.18 Bb
		10	4.40 ± 0.00 Cb	1.43 ± 0.01 Bc	5.83 ± 0.00 Cb	0.82 ± 0.01 Cc	41.13 ± 1.46 Ba
+NPK	0	0	7.73 ± 0.06 Aa	2.64 ± 0.01 Aab	10.38 ± 0.05 Aa	1.08 ± 0.01 Aa	43.73 ± 2.15 Ac
		2	5.96 ± 0.15 Ab	1.94 ± 0.12 Aab	7.91 ± 0.28 Ab	0.94 ± 0.08 Aa	43.90 ± 2 Abc
		5	5.43 ± 0.10 Ab	1.75 ± 0.04 Ac	7.18 ± 0.14 Ab	0.97 ± 0.02 Aa	44.35 ± 2.62 Aab
		10	8.13 ± 0.12 Aa	2.65 ± 0.05 Aa	10.78 ± 0.18 Aa	1.09 ± 0.29 Aa	46.70 ± 1.97 Aa
	75	0	7.94 ± 0.03 ABa	2.57 ± 0.18 Aab	10.52 ± 0.14 ABa	1.26 ± 0.10 Aa	42.76 ± 0.68 Ac
		2	6.61 ± 0.28 ABb	2.51 ± 0.42 Aab	9.13 ± 0.70 ABb	1.05 ± 0.00 Aa	44.21 ± 2.84 Abc
		5	6.16 ± 0.32 ABb	2.12 ± 0.09 Ac	8.28 ± 0.42 ABb	0.91 ± 0.09 Aa	46.23 ± 1.07 Aab
		10	5.15 ± 0.15 ABa	1.59 ± 0.13 Aa	6.75 ± 0.02 ABa	0.79 ± 0.00 Aa	46.43 ± 1.84 Aa
	125	0	5.81 ± 0.43 Ba	1.91 ± 0.14 ABab	7.73 ± 0.58 Ba	1.00 ± 0.13 Aa	42.83 ± 2.06 Ac
		2	6.59 ± 0.01 Bb	2.23 ± 0.03 ABab	8.83 ± 0.02 Bb	0.85 ± 0.01 Aa	43.08 ± 3.38 Abc
		5	5.73 ± 0.92 Bb	1.87 ± 0.32 ABc	7.61 ± 1.25 Bb	0.93 ± 0.10 Aa	44.73 ± 2.23 Aab
		10	6.64 ± 0.39 Ba	2.23 ± 0.17 ABa	8.88 ± 0.56 Ba	1.15 ± 0.01 Aa	45.38 ± 1.32 Aa

Table 2. *Cont.*

NPK	NaCl (mM)	(BC) %	*Chl a* (mg g^{-1})	*Chl b* (mg g^{-1})	*Chl* (*a* + *b*) (mg g^{-1})	Carotenoids (mg g^{-1})	SPAD
	200	0	4.16 ± 0.44 Ca	1.44 ± 0.13 Cab	5.60 ± 0.57 Ca	0.53 ± 0.10 Ba	42.36 ± 4.29 Ac
		2	4.43 ± 0.75 Cb	1.51 ± 0.16 Cab	5.94 ± 0.91 Cb	0.66 ± 0.20 Ba	43.38 ± 2 Abc
		5	6.48 ± 0.46 Cb	2.23 ± 0.22 Cc	8.71 ± 0.68 Cb	1.04 ± 0.04 Ba	44.15 ± 1.94 Aab
		10	7.33 ± 0.01 Ca	2.51 ± 0.07 Ca	9.84 ± 0.08 Ca	0.82 ± 0.11 Ba	44.71 ± 1.96 Aa
ANOVA		NaCl	***	***	***	***	***
		BC	**	*	***	***	***
		NPK	***	***	***	***	***
		NaCl × BC	***	***	***	***	ns
		NaCl × NPK	**	***	***	***	**
		BC × NPK	***	***	***	***	ns
		NaCl × BC × NPK	***	***	***	***	ns

The data values are mean ± SD (n = 3). Tukey was used for multiple comparisons. Different letters after the values indicate significant differences between treatments ($p < 0.05$). The uppercase and lowercase letters correspond to the effect of NaCl and biochar, respectively. *, **, *** correspond to the significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. ns: non-significant.

The effect of treatments were significant on transpiration (E), stomatal conductance (gs), photosynthetic rate (A), and instantaneous water use efficiency (iWUE) under treatments UF and F (Table 3).

Table 3. Analysis of variance of the effect of salinity (NaCl), biochar (BC), and their interaction (NaCl × BC) on some physiological [transpiration rate (E), stomatal conductance (gs), photosynthetic rate (A) and instantaneous water use efficiency (iWUE)], biochemical parameters, and grain yield in barley.

ANOVA	E	gs	A	iWUE	Fructose	Glucose	Proline	Soluble Sugar	MDA	Grain Yield
NaCl	***	***	***	**	***	***	***	***	***	***
Biochar (BC)	***	***	***	*	***	**	***	***	***	**
NPK	ns	**	***	***	*	ns	***	***	***	***
NaCl × BC	ns	ns	***	ns	***	***	***	***	ns	*
NaCl × NPK	***	**	***	ns	ns	ns	ns	**	**	**
BC × NPK	ns	ns	ns	ns	ns	***	ns	ns	*	ns
NaCl × BC × NPK	**	**	**	ns	***	**	ns	ns	ns	ns

*, **, *** correspond to the significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. ns: non-significant.

As shown in Figure 2A, transpiration rate (E) decreased significantly under saline conditions, and the mean values were 3.52, 2.63, 2, and 1.66 mmol H_2O m^{-2} s^{-1} compared with 2.96, 2.99, 2.39, and 2 mmol H_2O m^{-2} s^{-1} without and with NPK, respectively.

The application of biochar (0%, 2%, 5%, and 10%) increased the E value in each salt treatment. For example, at 200 mM, E values increased by 62.06%, 120.68%, and 182.75% compared to 17%, 87.23%, and 81.56% without and with NPK, respectively. Moreover, stomatal conductance (gs) significantly decreased under saline conditions and the mean values were 0.077, 0.055, and 0.042 mmol H_2O m^{-2} s^{-1} and 0.04 compared to 0.07, 0.07, 0.053, and 0.05 mmol H_2O m^{-2} s^{-1} for without and with NPK, respectively (Figure 2B). On the other hand, the application of BC treatments increased the gs values. At 200 mM, gs values increased by 40%, 72%, and 140% compared to 32.25%, 96.77%, and 112.90% for without and with NPK, respectively.

In addition, photosynthetic rate (A) decreased significantly under saline conditions and the mean values were 9.38, 7.37, 5.51 and 5.38 µmol CO_2 m^{-2} s^{-1} compared to 10.02, 9.41, 8.44, and 8 µmol CO_2 m^{-2} s^{-1} for without and with NPK, respectively (Figure 2C).

The application of BC treatments increased photosynthetic rate (A) values in all salinity treatments. For example, at 200 mM, values were increased by 89.83%, 160.67%, and 223.57% compared to 49.33%, 116.07%, and 149.33% without and with NPK, respectively.

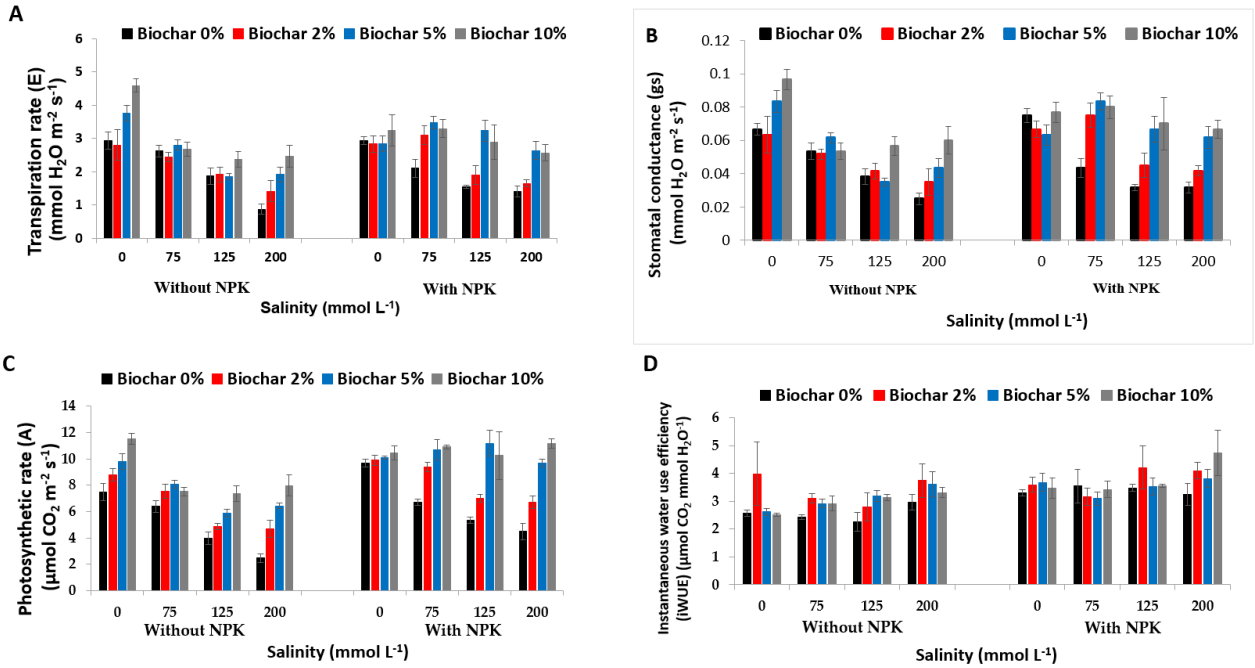

Figure 2. Combined effect of salinity (mmol L^{-1}), biochar (%), and NPK fertilizers on gas exchange parameters and iWUE in barley. (**A**): transpiration rate; (**B**): stomatal conductance; (**C**) photosynthetic rate; (**D**): instantaneous water use efficiency.

In addition, instantaneous water use efficiency (iWUE) increased significantly under salt treatments (Figure 2D). The application of BC treatments significantly varied iWUE in some salt treatments. At 200 mM, iWUE values were 2.97, 3.74, 3.60, and 3.30 µmol CO_2 mmol H_2O^{-1} compared to 3.24, 4.09, 3.82, and 4.74 µmol CO_2 mmol H_2O^{-1} for without and with NPK, respectively.

3.2. Effect of Biochar, NPK Fertilizers, and Salinity on Stress-Related Metabolites

As shown in Figure 3A, the proline content increased significantly under saline conditions. In fact, the mean values of proline were 97.33, 167.14, 174.77, and 381.84 µg g^{-1} compared to 89.33, 156.84, 166.09, and 354.21 µg g^{-1} under treatments UF and F. The application of BC treatments reduced the proline content in all salt treatments. At 200 mM NaCl, BC treatments (2%, 5%, and 10%) reduced proline mean values by 19.14%, 32.23%, and 63.14% compared to 18.92%, 35.98%, and 62.77% compared to control (0%) under UF and F treatments, respectively.

In addition, soluble sugars increased significantly under saline conditions (Figure 3B), and the mean values were 6.37, 6.82, 7.86, and 9.25 mg g^{-1} compared with 5.83, 6.34, 7.40, and 8.01 mg g^{-1} under UF and F treatments. The addition of BC treatments decreased the soluble sugar content in all treatments. At 200 mM, soluble sugar content decreased by 16.39%, 24.14%, and 31.54% compared to 10.77%, 26.47%, and 31.95% without and with NPK, respectively.

In addition, the individual sugars detected by LC-ESI-MS were significantly increased under saline conditions (Figure 3C,D). As shown in Figure 3, the mean values of fructose were 35.77, 40.11, 45.86, and 42.85 µg g^{-1} compared to 38.41, 41.15, 46.51, and 47 µg g^{-1} without and with NPK, respectively. The application of BC treatments increased the fructose concentrations in each salt treatment. At 200 mM, fructose concentrations without NPK were 44.08, 45.18, 47.49 µg g^{-1} compared to 43.66, 44.95 and 47.67 µg g^{-1} with NPK at 0%, 2% and 5% BC, respectively.

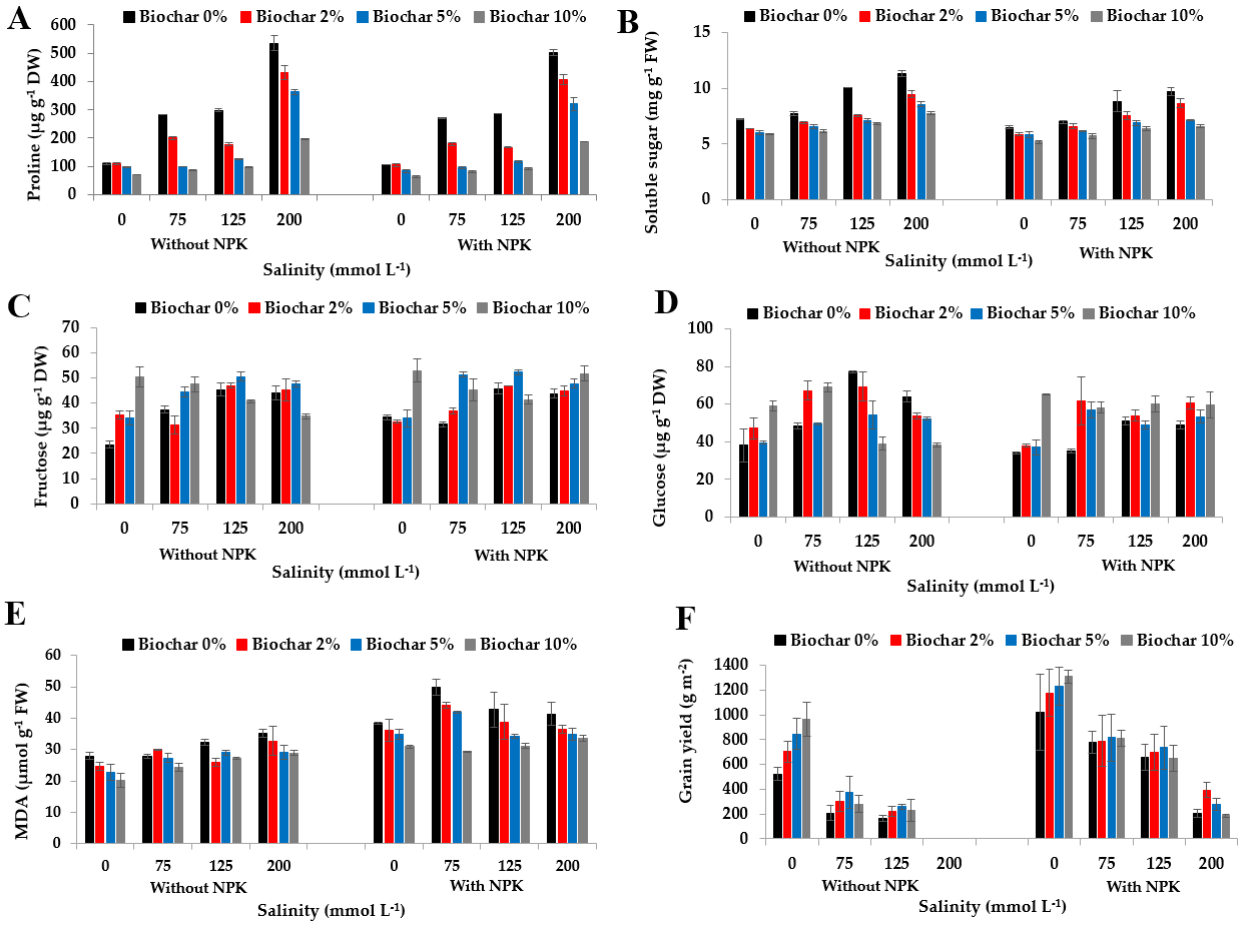

Figure 3. Combined effect of salinity, biochar, and NPK fertilizers on (**A**) proline, (**B**) soluble sugar, (**C**) fructose, (**D**) glucose, (**E**) MDA, and (**F**) grain yield in barley.

However, at 200 mM and 10% BC, the fructose concentration without NPK was lower and the value was 34.66 µg g^{-1} compared with that with NPK and the value was increased at 51.67 µg g^{-1}. Moreover, the change of glucose under saline conditions is similar to that of fructose (Figure 3C). The mean values were 45.90, 58.45, 59.88, and 51.92 µg g^{-1} compared to 43.52, 52.86, 53.50, and 55.58 µg g^{-1} without and with NPK, respectively.

Furthermore, salinity significantly increased MDA content in all treatments (Figure 3E) and the mean values without NPK were 23.96, 27.39, 28.66, and 31.51 µmol g^{-1} for 0, 75, 125, and 200 mM, respectively. With NPK fertilizer, the mean values of MDA increased at 75 mM and decreased progressively at the different salinity levels. The values were 35.15, 41.32, 36.74, and 36.56 µmol g^{-1} for 0, 75, 125, and 200 mM, respectively.

The application of BC treatments decreased the MDA content at all salt contents. For example, at 200 mM, MDA values were 35.13, 32.79, 29.17, and 28.98 µmol g^{-1} compared to 41.31, 36.50, 34.86, and 33.60 µmol g^{-1} for without and with NPK, respectively.

Also, salinity significantly decreased grain yield in all salinity treatments (Figure 3F). Without NPK fertilizer, the mean values of grain yield were 758.49, 291.14, 218.89, and 0 g m^{-2} for 0, 75, 125, and 200 mM, respectively. On the other hand, with NPK fertilizer the mean values of grain yield were 1182.89, 797.68, 684.44, and 267.23 g m^{-2} for 0, 75, 125, and 200 mM, respectively. Application of BC increased grain yield in all salinity treatments under both cases without or with NPK fertilizer. For example, at 125 mM, grain yield values were 166.71, 219.07, 258.81, and 230.98 g m^{-2} for 0%, 2%, 5%, and 10% BC, respectively, compared to 655.72, 698.41, 734.30, and 649.34 g m^{-2} for 0%, 2%, 5%, and 10% BC, respectively.

3.3. Effect of Biochar, NPK Fertilizers, and Salinity on Phenolic Compounds

A group of nine phenolic compounds was identified and quantified with LC-ESI-MS analytical (Table 4): five phenolic acids (quinic acid, 1,3-di-O-caffeoyquinic acid, gallic acid, p-coumaric acid, trans-ferulic acid) and four flavonoids (luteolin-7-o-glucoside, quercetin, apegenin, acacetin). Salinity significantly decreased the most important compounds. Indeed, quinic acid significantly decreased under salt treatments (0, 75, 125, and 200 mM) and the mean values were 1846.98, 1795, 1694.77, and 1620.09 μg g^{-1} compared to 1464.52, 1861.61, 1030.19, and 1051.52 μg g^{-1} without and with NPK, respectively. The application of BC treatments increases the concentration of quinic acid. At 200 mM and from 0% to 10% BC, the value of quinic acid was increased by 89.63% and 44.54% for without and with NPK, respectively. For 1,3-di-O-caffeoylic acid, gallic acid, p-coumaric acid and trans-ferulic acid, salinity also decreased the concentrations of these compounds, and the application of BC treatments caused a change in these compounds (Table 4). In addition, the salt treatments significantly decreased Lu-teolin-7-o-glucoside concentrations and the mean values were 26.64, 25.35, 13.83, and 25.73 μg g^{-1} compared to 19.94, 25.35, 13.83, and 12.88 μg g^{-1} without and with NPK, respectively.

At 200 mM and 0% to 10% BC, the value of luteolin-7-o-glucoside was increased by 30.61% and 42.88% without and with NPK, respectively. In addition, quercetin, apegenin, and acacetin significantly increase under 125 mM without NPK conditions and decrease at 200 mM compared with the control. The mean values for quercetin were 2.34, 2.31, 3.05, and 2.64 μg g^{-1} for 0, 75, 125, and 200 mM, respectively. The same was true for apegenin and acacetin as for quercetin (Table 4). Under NPK conditions, quercetin, apigenin, and acacetin significantly decrease and increase under salt treatments. When BC treatments (0%, 2%, 5%, and 10%) were applied and no NPK conditions were used, quercetin, apegenin, and acacetin decreased significantly and the values were 3.02, 2.78, 2.55, 2.22 μg g^{-1}, 0.86, 0.68, 0.57, 0.33 μg g^{-1}, 4.58, 1.04, 0.65, 0.53 μg g^{-1}, respectively. In contrast, BC treatments (0%, 2%, 5%, and 10%) under NPK conditions significantly increased quercetin, apegenin, and acacetin, respectively. The values were 2.29, 2.23, 2.09, 2.36 μg g^{-1}, 0.59, 0.62, 0.56, 1.34 μg g^{-1}, and 0.68, 1.70, 1.50, 3.12 μg g^{-1}, respectively.

Table 4. Combined effect of salinity (S) (mmol L^{-1} NaCl), biochar (BC), and NPK fertilizers (NPK) on phenolic compounds.

NPK	NaCl (mM)	BC (%)	Quinic Acid	1,3-di-O-caffeoyquinic Acid	Gallic Acid	p-coumaric Acid	Trans Ferulic Acid	Luteolin-7-o-glucoside	Quercetin	Apegenin	Acacetin
−NPK	0	0%	1769 ± 119 Ab	16.2 ± 1.6 Aa	10.2 ± 1.9 Aa	6.2 ± 0.2 Aa	6.9 ± 0.3 Aa	23.9 ± 0.8 Ab	3.2 ± 0.6 Ba	1.0 ± 0.1 Ba	1.4 ± 0.13 Bbc
		2%	1395 ± 78 Ab	15.3 ± 2.4 Aa	4.2 ± 0.2 Abb	2.9 ± 0.3 Ac	3.6 ± 0.4 Ab	25.9 ± 0.5 Ab	2.1 ± 0.0 Bb	0.48 ± 0.0 Bc	1.3 ± 0.04 Bc
		5%	1887 ± 31 Ab	12.9 ± 0.4 Aab	4.9 ± 1.5 Ab	4.4 ± 01 Ab	4.1 ± 0.2 Ab	26.7 ± 0.3 Aa	2.1 ± 01 Bb	0.55 ± 0.0 Bb	1.5 ± 0.02 Bb
		10%	2335 ± 18 Aa	12.3 ± 0.1 Ab	4.2 ± 0.1 Ab	4.2 ± 0.0 Ab	4.0 ± 0.1 Ab	30.0 ± 0.4 Aa	1.9 ± 0.1 Bb	0.54 ± 0.0 Bb	1.6 ± 0.03 Ba
	75	0%	1625 ± 37 Ab	11.7 ± 0.2 Ba	6.2 ± 1.3 Aa	3.9 ± 0.1 Bb	4.4 ± 0.1 ABb	20.2 ± 0.6 Ab	2.7 ± 0.3 Ba	0.59 ± 0.0 Bb	2.3 ± 0.32 Ba
		2%	1667 ± 55 Ab	10.8 ± 0.1 Bb	5.5 ± 0.6 Ab	3.4 ± 0.6 Bb	4.4 ± 0.3 ABb	21.6 ± 0.9 Ab	2.1 ± 0.1 Bb	0.60 ± 0.0 Bb	1.2 ± 0.14 Bbc
		5%	1817 ± 70 Ab	11.4 ± 1.0 Ba	3.4 ± 0.4 Ab	2.90 ± 0.2 Bc	4.4 ± 08 ABb	30.6 ± 1.1 Aa	2.5 ± 0.1 Bab	0.74 ± 0.0 Ba	1.03 ± 0.09 Bc
		10%	2069 ± 69 Aa	10.7 ± 0.3 Bb	3.9 ± 0.1 Ab	4.15 ± 0.0 Ba	5.0 ± 0.4 ABa	29.0 ± 0.9 Aa	1.9 ± 0.0 Bb	0.54 ± 0.0 Bc	1.59 ± 0.03 Bb
	125	0%	1892 ± 97 ABb	15.3 ± 0.6 ABa	4.8 ± 0.2 Ba	3.9 ± 0.0 Ca	4.8 ± 0.8 BCa	18.9 ± 1.7 Bb	4.1 ± 0.1 Aa	2.17 ± 0.1 Aa	3.04 ± 0.34 Ab
		2%	1558 ± 60 ABb	13.2 ± 0.3 ABb	2.1 ± 0.2 Bb	3.4 ± 0.1 Cb	3.7 ± 0.1 BCb	19.1 ± 1.6 Bb	2.1 ± 0.1 Ab	1.95 ± 0.0 Ab	3.09 ± 0.03 Ab
		5%	1319 ± 124 ABb	13.2 ± 1.1 ABb	2.0 ± 0.1 Bb	3.0 ± 0.0 Cb	3.8 ± 0.3 BCb	27.2 ± 1.3 Ba	2.5 ± 0.1 Ab	0.67 ± 0.1 Ac	3.01 ± 0.07 Ab
		10%	2009 ± 73 ABa	9.8 ± 0.9 ABc	1.7 ± 0.3 Bb	1.8 ± 0.1 Cc	3.6 ± 0.1 BCb	23.8 ± 1.3 Ba	3.6 ± 0.1 Ab	1.01 ± 0.0 Ab	3.62 ± 0.19 Aa
	200	0%	1340 ± 90 Bb	11.9 ± 0.8 Bab	4.7 ± 0.3 Ba	1.7 ± 0.1 Dc	5.7 ± 0.1 Ca	21.1 ± 0.9 Ab	3.0 ± 0.0 Ba	0.86 ± 0.1 Ba	4.58 ± 0.19 Ba
		2%	1462 ± 16 Bb	12.9 ± 0.9 Ba	2.8 ± 0.1 Bb	2.2 ± 0.0 Db	3.1 ± 0.0 Cb	27.8 ± 2.1 Aa	2.8 ± 0.1 Bb	0.68 ± 0.0 Bb	1.04 ± 0.03 Bb
		5%	1134 ± 21 Bb	12.4 ± 0.1 Ba	2.3 ± 0.3 Bb	3.4 ± 0.1 Da	3.3 ± 0.1 Cb	26.5 ± 0.2 Aa	2.5 ± 0.0 Bb	0.57 ± 0.0 Bb	0.65 ± 0.05 Bc
		10%	2542 ± 163 Ba	10.9 ± 0.5 Bb	2.6 ± 0.0 Bb	2.3 ± 0.1 Db	3.2 ± 0.2 Cb	27.6 ± 3.4 Aa	2.2 ± 0.1 Bb	0.33 ± 0.0 Bc	0.53 ± 0.00 Bc
+NPK	0	0%	1589 ± 206 Ba	12.9 ± 0.9 Bc	4.3 ± 0.3 Aa	4.1 ± 0.4 Aa	4.8 ± 0.6 Aa	11.7 ± 1.0 Ab	2.9 ± 0.3 Aa	1.22 ± 0.0 Ba	3.31 ± 1.21 Ab
		2%	1412 ± 84 Bb	13.3 ± 1.4 Bc	1.9 ± 0.0 Ac	2.8 ± 0.1 Ab	4.2 ± 0.6 Ab	25.2 ± 4.2 Aa	1.9 ± 0.1 Ab	1.05 ± 0.0 Bb	4.18 ± 0.10 Aa
		5%	1568 ± 28 Bab	16.3 ± 0.0 Ba	1.8 ± 0.1 Ac	2.5 ± 0.1 Ac	4.2 ± 0.1 Ab	22.5 ± 0.4 Aa	1.8 ± 0.1 Ab	0.43 ± 0.1 Bc	4.06 ± 0.07 Aa
		10%	1287 ± 75 Bc	15.4 ± 0.8 Bb	2.4 ± 0.2 Ab	2.8 ± 0.5 Ab	3.2 ± 0.2 Ac	20.4 ± 0.6 Aa	1.7 ± 0.1 Ab	0.45 ± 0.0 Bc	1.96 ± 0.39 Ac
	75	0%	2022 ± 181 Aab	16.7 ± 0.0 Ab	1.4 ± 0.1 Ca	2.4 ± 0.1 Ac	3.8 ± 0.1 Ba	9.5 ± 0.4 Bb	1.5 ± 0.0 Ac	0.50 ± 0.1 Bb	2.02 ± 0.02 Ab
		2%	1587 ± 49 Ac	17.5 ± 0.8 Aa	1.3 ± 0.0 Cb	3.7 ± 0.1 Aa	3.3 ± 0.1 Bb	17.7 ± 0.4 Ba	1.9 ± 0.0 Ab	0.45 ± 0.0 Bb	1.74 ± 0.26 Ab
		5%	1720 ± 34 Ab	16.7 ± 1.3 Ab	1.2 ± 0.0 Cc	2.8 ± 0.3 Ab	2.7 ± 0.1 Bc	17.8 ± 0.4 Ba	2.8 ± 0.1 Aa	1.13 ± 0.1 Ba	5.14 ± 0.26 Aa
		10%	2117 ± 98 Aa	14.1 ± 1.6 Ac	1.2 ± 0.1 Cc	2.9 ± 0.3 Ab	3.9 ± 0.5 Bab	18.7 ± 0.2 Ba	2.0 ± 0.0 Ab	1 ± 0.06 Ba	5.03 ± 0.22 Aa
	125	0%	965 ± 5 Cc	16.9 ± 0.2 Ab	2.1 ± 0.1 Ba	2.7 ± 0.1 Ba	3.3 ± 0.1 ABb	10.4 ± 0.6 Bc	2.1 ± 0.1 Ab	1.26 ± 0.0 Aa	1.16 ± 0.23 Bb
		2%	830 ± 125 Cc	17.6 ± 0. 8 Aa	1.9 ± 0.0 Bb	1.6 ± 0.1 Bb	4.3 ± 0.1 ABa	13.2 ± 1.6 Bb	2.4 ± 0.0 Aa	1.44 ± 0.1 Aa	1.86 ± 0.05 Ba
		5%	1032 ± 19 Cb	16.3 ± 0.4 Ab	2.1 ± 0.1 Ba	2.7 ± 0.5 Ba	3.4 ± 0.1 ABb	17.3 ± 4.0 Ba	2.4 ± 0.1 Aa	0.53 ± 0.1 Ab	0.77 ± 0.03 Bc
		10%	1294 ± 115 Ca	15.3 ± 1.4 Ac	1.9 ± 0.1 Bb	2.8 ± 0.2 Ba	4.3 ± 0.5 ABa	14.4 ± 0.3 Bb	2.1 ± 0.2 Ab	0.57 ± 0.0 Ab	0.78 ± 0.02 Bc
	200	0%	859 ± 46 Cc	17.8 ± 0.4 ABa	1.9 ± 0.1 Bc	2.9 ± 0.2 ABb	2.5 ± 0.1 Bc	9.8 ± 0.5 Bc	2.3 ± 0.2 Aab	0.59 ± 0.0 Bb	0.68 ± 0.01 Bc
		2%	939 ± 84 Cc	16.5 ± 0.4 ABb	1.8 ± 0.2 Bc	3.1 ± 0.1 ABa	3.4 ± 0.3 Bb	13.3 ± 1.1 Bb	2.2 ± 0.1 Ab	0.62 ± 0.0 Bb	1.70 ± 0.02 Bb
		5%	1166 ± 86 Cb	14.3 ± 1.1 ABc	2.1 ± 0.1 Bb	2.5 ± 0.1 ABc	3.3 ± 0.2 Bb	14.3 ± 0.6 Ba	2.1 ± 0.0 Ab	0.56 ± 0.0 Bb	1.50 ± 0.06 Bb
		10%	1242 ± 77 Ca	14.6 ± 0.4 ABc	2.6 ± 0.0 Ba	2.1 ± 0.0 ABc	3.9 ± 0.4 Ba	14.1 ± 0.8 Ba	2.4 ± 0.0 Aa	1.34 ± 0.1 Ba	3.12 ± 0.09 Ba
ANOVA		(S)	***	ns	***	***	***	***	***	***	***
		(BC)	***	***	***	***	***	***	***	***	ns
		NPK	***	***	***	***	***	***	***	ns	***
		S × BC	***	ns	***	***	***	*	***	***	***
		S × NPK	***	***	***	***	*	**	**	***	***
		BC × NPK	***	ns	***	**	***	*	***	***	***
		S × BC × NPK	***	**	ns	***	***	*	***	***	***

The data values are mean ± SD (n = 3). Tukey was used for multiple comparisons. Different letters after the values indicate significant differences between treatments ($p < 0.05$). The uppercase and lowercase letters correspond to the effect of NaCl and biochar, respectively. *, **, *** correspond to the significance at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. ns: non-significant.

3.4. Effect of Biochar, NPK Fertilizers, and Salinity on Leaf Ions Concentrations

Table 5 shows that Na^+ concentration was significantly increased under saline conditions, with mean values of 3.78, 3.87, 3.90, and 5.21 mg g^{-1}, compared with 3.75, 4.95, 5.65, and 5.84 mg g^{-1} for without and with NPK, respectively. With BC addition, Na^+ concentration decreased in all saline treatments. At 200 mM NaCl, Na^+ concentration without NPK was 9.14, 5.21, 3.51, and 2.97 mg g^{-1} for 0%, 2%, 5%, and 10% of BC, respectively. When compared with NPK fertilizers, Na^+ concentration was 6.42, 6.11, 5.50, and 5.32 mg g^{-1} for 0%, 2%, 5%, and 10% of BC, respectively.

Moreover, K^+ concentration was significantly decreased under saline conditions and the mean values were 36.60, 32.57, 31.70, and 27.75 mg g^{-1} compared to 32.12, 31.50, 31.39, and 31.37 mg g^{-1} for without and with NPK, respectively. The application of BC also decreased the K^+ concentration in all salt treatments. For example, at 200 mM, K^+ concentration decreased by 12.85%, 20.11%, and 35.80% compared to 14.56%, 17.26%, and 33.50% without and with NPK, respectively.

On the other hand, results showed that the concentrations of Ca^{2+}, Zn^{2+}, Fe^{2+}, and Mn^{2+} were significantly affected by salinity and BC treatments, except for Mg^{2+}. Ca^{2+} concentration was significantly reduced by salinity without NPK treatment, and the mean values were 106.44, 94.55, 91.99, and 80 mg g^{-1} for 0, 75, 125 and 200 mM, respectively. On the other hand, Ca^{2+} concentration increased under NPK conditions, and the mean values were 82.62, 83.81, 84.84, and 85.67 mg g^{-1} for 0, 75, 125, and 200 mM, respectively. The application of BC treatments increases Ca^{2+} concentration in the majority of salt treatments, both without and with NPK. Moreover, the variation of Zn^{2+}, Fe^{2+}, Mg^{2+}, and Mn^{2+} concentrations under saline and BC conditions follows the path of the saw tooth (Table 5).

3.5. Effect of Biochar, NPK Fertilizers, and Salinity on Grain Yield

As shown in Figure 3F, salinity treatments significantly reduced grain yield (GY). Mean values were 758.49, 291.14, 218.89, and 0 g m^{-2} compared to 1182.89, 797.67, 684.44, and 267.23 g m^{-2} for without and with NPK, respectively. At 0 mM (the control), the application of BC treatments (0%, 2%, 5%, and 10%) significantly increased GY and the values were 523.44, 703.47, 845.04, and 362 g m^{-2} compared to 1018.48, 1176.48, 1230.25, and 1306.71 g m^{-2} without and with NPK, respectively. At saline conditions (75 and 125 mM), BC treatments increased GY by only 2% and 5% BC, and decreased it by 10% BC. For example, at 125 mM, GY values were 166.71, 219.07, 258.81, and 230.98 g m^{-2} compared to 655.72, 698.41, 734.29, and 649.34 g m^{-2} without and with NPK, respectively.

Table 5. Combined effect of salinity (S), biochar (BC), and NPK fertilizer on minerals content (mg g^{-1}) of barley leaf.

NPK	NaCl (mM)	BC (%)	Na^+	K^+	Ca^{2+}	Mg^{2+}	Zn^{2+}	Fe^{2+}	Mn^{2+}
−NPK	0	0%	2.6 ± 0.02 Bc	52.17 ± 1.92 Aa	88.28 ± 9.41 Aab	3.27 ± 0.02 Ca	0.03 ± 0.001 Bb	0.15 ± 0.003 Abc	0.14 ± 0.003 Ac
		2%	2.71 ± 0.18 Bc	33.06 ± 0.90 Aa	79.11 ± 4.79 Aa	2.52 ± 0.26 Ca	0.02 ± 0.004 Bb	0.17 ± 0.004 Ac	0.12 ± 0.008 Ac
		5%	4.99 ± 1 Bb	31.73 ± 0.39 Aa	100.01 ± 15.82 Ab	2.69 ± 0.002 Ca	0.21 ± 0.01 Ba	0.16 ± 0.001 Aa	0.18 ± 0.003 Ab
		10%	4.82 ± 0.25 Ba	29.43 ± 1.10 Aa	158.37 ± 9.54 Aab	2.80 ± 0.02 Ca	0.02 ± 0.001 Bb	0.15 ± 0.00 Aab	0.15 ± 0.003 Aa
	75	0%	5.53 ± 0.05 Ba	34.09 ± 1.13 ABa	95.91 ± 6.04 Bab	3.17 ± 0.11 ABa	0.04 ± 0.003 Db	0.14 ± 0.007 Abc	0.10 ± 0.01 Bc
		2%	3.85 ± 0.21 Bb	32.57 ± 0.27 ABa	138.29 ± 4.18 Ba	5.05 ± 0.15 ABa	0.05 ± 0.006 Db	0.07 ± 0.002 Ac	0.13 ± 0.007 Bc
		5%	3.14 ± 0.29 Bc	32.12 ± 0.68 ABa	73.66 ± 0.96 Bb	2.83 ± 0.15 ABa	0.03 ± 0.005 Da	0.25 ± 0.09 Aa	0.16 ± 0.007 Bb
		10%	2.98 ± 0.21 Bc	31.51 ± 1.06 ABa	70.33 ± 0.92 Bab	2.83 ± 0.24 ABa	0.03 ± 0.004 Db	0.22 ± 0.05 Aab	0.15 ± 0.002 Ba
	125	0%	5.20 ± 0.30 Bb	36.63 ± 0.78 BCa	94.57 ± 1.36 Bab	3.22 ± 0.02 Aa	0.07 ± 0.01 Cb	0.14 ± 0.003 Abc	0.09 ± 0.006 Cc
		2%	4.27 ± 0.05 Ba	33.51 ± 3.61 BCa	94.23 ± 6.20 Ba	2.89 ± 0.08 Aa	0.04 ± 0.01 Cb	0.16 ± 0.02 Ac	0.08 ± 0.00 Cc
		5%	3.83 ± 0.25 Bc	28.63 ± 0.51 BCa	99.47 ± 1.86 Bb	4.23 ± 2.01 Aa	0.06 ± 0.01 Ca	0.18 ± 0.02 Aa	0.09 ± 0.02 Cb
		10%	2.32 ± 0.11 Bc	28.05 ± 0.46 BCa	79.69 ± 0.38 Bab	5.68 ± 0.17 Aa	0.04 ± 0.003 Cb	0.16 ± 0.01 Aab	0.13 ± 0.01 Ca
	200	0%	9.14 ± 0.42 Aa	33.51 ± 1.85 Ca	85.33 ± 2.75 Cab	3.23 ± 0.13 BCa	0.07 ± 0.01 Ab	0.16 ± 0.02 Abc	0.08 ± 0.006 Cc
		2%	5.21 ± 0.91 Ab	31.51 ± 2.79 Ca	78.64 ± 5.33 Ca	3.21 ± 0.32 BCa	0.07 ± 0.03 Ab	0.14 ± 0.02 Ac	0.08 ± 0.007 Cc
		5%	3.51 ± 0.13 Ac	29.20 ± 0.30 Ca	88.05 ± 1.66 Cb	3.16 ± 0.13 BCa	0.19 ± 0.01 Aa	0.15 ± 0.004 Aa	0.11 ± 0.004 Cb
		10%	2.97 ± 0.60 Ac	26.77 ± 1.01 Ca	67.99 ± 3.19 Cab	3.42 ± 0.03 BCa	0.06 ± 0.008 Ab	0.15 ± 0.002 Aab	0.15 ± 0.006 Ca
+NPK	0	0%	5.48 ± 0.66 Ca	36.43 ± 1.83 Aa	88.80 ± 8.27 Aa	3.06 ± 0.28 Aa	0.11 ± 0.03 Aa	0.19 ± 0.03 Aa	0.07 ± 0.006 ABab
		2%	3.92 ± 0.32 Cb	32.26 ± 2.83 Ab	77.48 ± 1.36 Aa	8.70 ± 6.10 Aa	0.12 ± 0.03 Aa	0.14 ± 0.01 Ab	0.04 ± 0.00 ABb
		5%	3.25 ± 0.07 Cc	31.55 ± 1.67 Ab	77.35 ± 50 Aa	2.75 ± 0.05 Aa	0.08 ± 0.05 Aa	0.24 ± 0.07 Aa	0.05 ± 0.005 ABab
		10%	2.36 ± 0.03 Cc	28.23 ± 2.73 Ab	86.86 ± 1.18 Aa	3.08 ± 0.13 Aa	0.12 ± 0.07 Aa	0.16 ± 0.02 Ab	0.08 ± 0.02 ABa
	75	0%	5.37 ± 0.25 Bb	36.52 ± 0.82 Aa	82.06 ± 2.36 Aa	2.83 ± 0.08 Aa	0.11 ± 0.01 Aa	0.21 ± 0.01 ABa	0.05 ± 0.004 Bab
		2%	5.17 ± 0.25 Bc	30.85 ± 0.45 Ab	78.15 ± 3.35 Aa	3.07 ± 0.00 Aa	0.08 ± 0.00 Aa	0.12 ± 0.004 ABb	0.04 ± 0.007 Bb
		5%	4.65 ± 0.18 Bc	30.11 ± 0.36 Ab	83.88 ± 2.34 Aa	2.82 ± 0.23 Aa	0.09 ± 0.05 Aa	0.14 ± 0.003 ABa	0.05 ± 0.004 Bab
		10%	4.62 ± 0.12 Ba	28.51 ± 5.66 Ab	91.16 ± 12.16 Aa	14.30 ± 11.45 Aa	0.13 ± 0.09 Aa	0.14 ± 0.003 ABb	0.06 ± 0.007 Ba
	125	0%	6.21 ± 0.03 Aa	32.38 ± 3.76 Ab	83.88 ± 1.58 Aa	2.92 ± 0.07 Aa	0.07 ± 0.04 Aa	0.14 ± 0.006 Ba	0.06 ± 0.01 Bab
		2%	6.18 ± 0.38 Ab	31.70 ± 2.05 Ab	88.33 ± 1.13 Aa	2.92 ± 0.32 Aa	0.11 ± 0.005 Aa	0.10 ± 0.008 Bb	0.06 ± 0.00 Bb
		5%	5.13 ± 0.16 Ac	30.85 ± 3.35 Aa	92.55 ± 8.40 Aa	3.43 ± 0.06 Aa	0.11 ± 0.07 Aa	0.15 ± 0.07 Ba	0.06 ± 0.003 Bab
		10%	5.08 ± 0.28 Ac	30.63 ± 4.88 Ab	74.60 ± 2.92 Aa	2.78 ± 0.48 Aa	0.03 ± 0.002 Aa	0.11 ± 0.01 Bb	0.05 ± 0.002 Ba
	200	0%	6.42 ± 0.15 Aa	37.50 ± 0.67 Aa	90.42 ± 1.22 Aa	3.10 ± 0.02 Aa	0.12 ± 0.01 Aa	0.16 ± 0.01 Ba	0.06 ± 0.004 Aab
		2%	6.11 ± 0.26 Ac	32.03 ± 1.68 Ab	92.73 ± 16.16 Aa	2.87 ± 0.02 Aa	0.07 ± 0.02 Aa	0.15 ± 0.002 Bb	0.07 ± 0.00 Ab
		5%	5.50 ± 0.22 Ab	31.02 ± 1.20 Ab	73.91 ± 0.71 Aa	3.53 ± 0.18 Aa	0.06 ± 0.03 Aa	0.15 ± 0.04 Ba	0.07 ± 0.01 Aab
		10%	5.32 ± 0.12 Ac	24.93 ± 9.83 Ab	85.61 ± 21.13 Aa	2.75 ± 0.05 Aa	0.09 ± 0.005 Aa	0.12 ± 0.00 Bb	0.07 ± 0.01 Aa
ANOVA		NaCl	***	**	***	ns	*	**	***
		BC	***	*	ns	ns	**	***	***
		NPK	***	ns	***	ns	***	ns	***
		NaCl × BC	***	***	***	*	*	**	***
		NaCl × NPK	***	**	***	ns	**	*	***
		BC × NPK	***	**	ns	ns	***	**	***
		NaCl × BC × NPK	***	***	***	***	***	***	***

The data values are mean ± SD (n = 3). Tukey was used for multiple comparisons. Different letters after the values indicate significant differences between treatments ($p < 0.05$). The uppercase and lowercase letters correspond to the effect of NaCl and biochar, respectively. *, **, *** correspond to the significance at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. ns: non-significant.

4. Discussion

This study evaluates the comparative effects of biochar and NPK fertilizer on barley (*Hordeum vulgare* L.) grown under saline conditions. Chlorophyll a, b and carotenoids of barley decreased under the salt treatments. The decrease in chlorophyll content in salt-stressed plants is a typical symptom of salt stress [31,32] and has been attributed to the inhibition of chlorophyll synthesis along with the activation of its degradation by the enzyme chlorophyllase [33]. In our study, application of BC increased chlorophyll content in both cases, with and without NPK fertilizer. Similar results in wheat [34] and bell pepper [35] showed that biochar application under salt stress increased chlorophyll content. According to [36], biochar application increased the photosynthetic rate in wheat, which is an indication of increased chlorophyll content.

Moreover, the contents of chlorophyll a, b and carotenoids increased in barley in BC without and with NPK fertilizer, as found by [37]. The high chlorophyll content of barley might be related to the improved physical and chemical properties of the soil, which facilitate the plant's uptake of nutrients, especially nitrogen, and increase light absorption to enhance photosynthesis [37,38]. Moreover, the levels of SPAD were decreased under saline conditions and increased with BC application. Similar to our results, Ref. [39] reported that the chlorophyll content in basil decreased with an increase in salinity and explained that this might be due to the inhibition of photosynthesis, the activity of chlorophyllase enzyme, the production of ROS, and the instability of protein complication of pigments. Moreover, Ref. [40] reported a similar observation in *S. hortensis* under saline conditions. Consequently, gas exchange parameters decreased under saline conditions in our study. The decrease in gas exchange parameters in plants under salt stress is primarily due to the decrease in water availability. Photosynthesis is also inhibited when high Na^+ and/or Cl^- concentrations accumulate in chloroplasts, and chlorophyll is an important photosynthetic material that directly correlates with plant development [41,42]. Our results showed that biochar application improved gas exchange of barley without and with NPK fertilizer, which was consistent with the previous results of [25,36,43]. The content of glucose, fructose, soluble sugars, proline, and MDA, which are very important under hydric stress conditions, is increased by their synthesis and accumulation in the cell, acting as indicators to oxidative stress [44]. Similarly, Ref. [45] reported that same biochar application in durum wheat under drought stress. In addition, it has been reported that proline, soluble sugars, and MDA contents of plants in biochar-treated soil have decreased [46]. Biochar application decreased the accumulation of proline, soluble sugars, and phenolic compounds in barley without and with NPK fertilizer, indicating a reduced deleterious effect of salt stress as also reported by [24,47,48]. The application of biochar changes phenolic compound concentration in barley. Our results are in agreement with those obtained by [49] in cowpea. In addition, Refs. [50,51] found that the addition of biochar can also impact on phenolic compounds in wheat and in *Viola cornuta* flowers, respectively. The simultaneous application of NPK and biochar had a positive effect on barley performance under control conditions, while the response of plants varied depending on the salt concentration and the amount of biochar in the soil.

5. Conclusions

The use of biochar mitigates the deleterious effects of salt and improves barley plant tolerance to stress conditions. Simultaneous application of NPK fertilizer and biochar can help plants reduce the negative effects of salt on yield parameters while reducing excessive use of mineral fertilizer. Our study revealed that the growth, physio-biochemical parameters, and grain yield was higher under biochar (2% and 5% of total pot mass), therefore, it is necessary to recommend these concentrations. Further research studies are needed to evaluate the agronomic and environmental benefits of biochar combined with compost with different treatments, especially under field conditions.

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