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# EFFECT OF BIOCHAR AMENDMENT ON SPINACH (*Spinacia oleracea* L.) GROWTH UNDER SALT STRESS CONDITIONS

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ABSTRACT

Salinity is considered a top challenge facing food security, globally. The effects of different amounts of date palm-derived biochar on spinach (*Spinacia oleracea* cv. Balady) affected by saline water irrigation were studied in a pot experiment carried out on October and November during the winter season of 2021. Results showed that salt stress negatively affected the morphological and physiological parameters of spinach plants such as the shoot system (4%, 13.3% at salinities of 5 and 10 $dSm^{-1}$, respectively). The addition of biochar significantly increased the root length under all salt stress conditions. The results showed that the addition of biochar improved biomass weight at 2.5 and 5 $dSm^{-1}$ salinity at all levels. The number of leaves increased by only 3.08% at 2.5 $dSm^{-1}$ salinity, while it decreased by 13.8 and 35.2% at the next two salinities; 5.00 and 10.0 $dSm^{-1}$, respectively. The overall reduction values were achieved corresponding to the higher saltwater concentration (10 $dSm^{-1}$) compared to the control plants. It seems that the addition of biochar up to 1% also showed promising results in all observations. The results obtained from measuring membrane stability index (MSI) and electrolyte leakage (EL) showed that all stressed plants had increased MSI values and decreased EL values compared to the control. Sodium, soluble sugars, and proline levels significantly increased corresponding to saline water. Soil amendments utilizing biochar resulted in a significant improvement in plant growth and alleviated salt stress.

## INTRODUCTION

Recently, there are too many challenges that affects agricultural productivity such as progressive declining in soil condition and poor nutrient use efficiencies by which food insecurity issues were raised **(Singh *et al.*, 2022**). Crops suffering from drought and salinity reflect threats to food security. Organic-derived biochar, once applied to the soil, has the potential to improve soil and mitigate salinity and drought stress **(Yang *et al.*, 2020**). There are several techniques which are widely investigated in many regions of the world related to water-saving irrigation **(Chai *et al.*, 2016**). Soil supplemented with salt is the main stress on agricultural land and becomes obstacles to the productive use of agricultural land for vigorous plant growth. The global use of natural resources is increasing day by day and the population is growing, which greatly affects agriculture and various factors that contribute to poor soil conditions and create a saline condition **(Ahmed *et al.*, 2020**). Plant growth is affected by ion toxicity, particularly the action of Cl and Na ions, once the soil become under saline stress. High concentrations of Na and chloride are often synonymous with high salinity **(de Oliveira *et al.*, 2013**).

Many researchers have proven that organic-derived biochar is effective in increasing plant growth and physiological characteristics when applied to saline soil **(Akhtar *et al.*, 2015; Amini *et al.*, 2016;**

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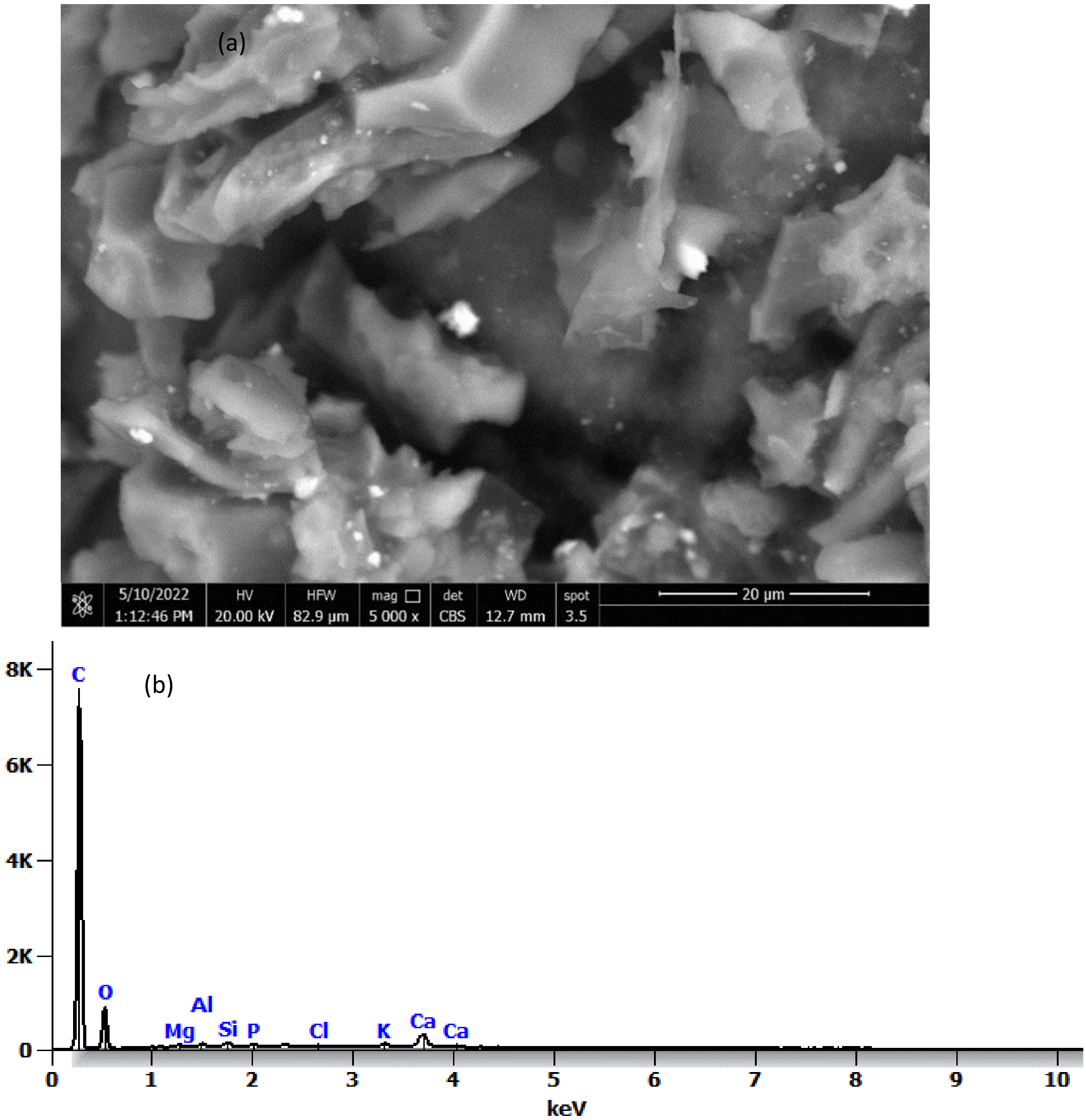
**Bogusz and Oleszczuk, 2018**). However, it has been shown that biochar with a high level of addition may increase soil salinity. Also, there is no sufficient published data in terms of biochar application in salt-affected soil in long term (**Abdelrasheed *et al.*, 2021**). The mechanism of biochar to reduce the influence of saline's negative impacts on soil is related to improving the physicochemical and biological properties of soil, particularly decreasing sodium-related reactions (**Dahlawi *et al.*, 2018**). The benefit of using biochar as soil fertilizer is 10 -12 % increase in plant productivity as a result of increased crop growth biomass (**Jabborova *et al.*, 2021**). The effect of biochar is mainly depending on its source which may negatively or positively affect the soil hens crop productivity. (**Liu *et al.*, 2015; Solaiman *et al.*, 2020**). The specific surface area of biochar is considered the most important factor that let such material gain high adsorption capacity (**Wu and Wu, 2019**).

The nutritional improvement effects of biochar in the soil through supplying basic cations and elemental nutrients by improving water and soil fertility quality have been studied in literature and gained favour in this concern. Due to its chemical characteristics, biochar is known to reduce soil contamination and produce sustainable productivity through increasing plant productivity (**Wu and Wu, 2019; Singh *et al.*, 2022**). Increasing crop biomass by biochar application could be explained by two actions: one for plants and the second for soil. It immediately affects crop biomass through elemental nutrients transported from soil to plant and indirectly improves plant growth as a result of improving the biogeochemical properties of soil (**Kocsis *et al.*, 2022**). Organic-derived biochar behaves as a source of elemental nutrients that improve and increase soil fertility (**Hadroug *et al.*, 2021**). Hence, we assumed that the negative effect of ion toxicity due to saline conditions might be mitigated by organic-derived biochar application generated from local organic residual in North Sinai area, Egypt. The main purpose of the current work is to study the effects of biochar manufactured from local organic residual (date-palm seeds) in North Sinai, Egypt on mitigating the negative effects of irrigation with saline water on spinach plant growth parameters.

## MATERIALS AND METHODS

### Preparation and Characterization of Biochar from Date-Palm

A full ripened date seed was used. The seed was treated and washed manually to remove any unwanted organic or inorganic debris and then oven-dried for 24 hr at 100℃. The date seed was then crushed and placed in a carefully covered crucible and heated in an electrical muffle furnace for 2 hr at 350℃ to synthesize biochar under a limited amount of oxygen as described by **Mahdi *et al.* (2017**). The produced biochar was then cooled down for 3 hr in the furnace and then immediately stored inside a desiccator for further use. The morphological features and elemental weight percentage of the produced biochar were investigated by scanning electron microscope coupled with an EDX unit (SEM-EDX; Quanta 450 FEG-ESEM, FEI Company). Fig. 1 showed smooth surfaces with different porosity sizes. The pore sizes were not uniform and were in the range of different micro-meters. Produced biochar was chemically characterized; biochar pH was measured using a pH meter (Model pH 209, HANNA Instruments, UK) in water: solid ratio of 1: 2.5. Total metal concentrations were measured in acid digested biochar (3:1 concentrated HCl: $HNO_3$) using atomic absorption spectrophotometry (SHIMADZU AA-6800). Biochar organic matter was estimated using the wet chemistry traditional method (**Walkley and Black, 1934**). Table 1 summarizes the chemical characteristics of date palm-derived biochar.




| | C | O | Mg | Al | Si | P | Cl | K | Ca |
|---|---|---|---|---|---|---|---|---|---|
| **EDX** Weight % | 58.28 | 32.00 | 0.45 | 0.47 | 0.99 | 0.34 | 0.34 | 1.23 | 5.90 |

**Fig. 1. Scanning electron micrograph of biochar produced at 350 ºC (a) with associated EDX spectrum and value results**

**Table 1. Summary of chemical characteristics of date palm seed-derived biochar**

| pH | EC | OM | Zn | Fe | Na | Mg | K | Ca |
|---|---|---|---|---|---|---|---|---|
| **1:2.5** | **$dSm^{-1}$** | **(%)** | | | **g/kg** | | | |
| 8.84 | 0.163 | 65.5 | 0.032 | 0.022 | 0.056 | 2.63 | 9.22 | 25.1 |

## Growing Spinach Plant with Biochar and Salinity Level Treatments

Spinach (*Spinacia oleracea*) seeds were purchased from Agriculture Research Centre, Giza, Egypt (ARC). Seeds were immersed in 0.01% $HgCl_2$ for 2 to 5 min for sterilizing then directly germinated in 5 kg soil-occupied pots (30 cm diameter) according to the procedures described by **Hashem *et al.* (2016**). Biochar was added at 4 different rates (0.00, 0.50, 1.00, 1.50%). The germination processes were monitored and irrigation with tap water was used for 20 days to avoid the stress of saline water on plant germination rate. The transition from tap water to saline water was conducted gradually; for three days before being fully treated with the exact concentration of saline water. Irrigation was undertaken daily as 1.25 of soil filed capacity amount. After 35 days old (after the first 20 days in the germination process), plants were carefully uprooted and analyzed for the different parameters. The soil used in the current study was loamy sandy soil (top 0-30 cm) collected from Experimental Farm of Faculty of Environmental Agricultural Sciences, Arish University, North Sinai, Egypt. The experiment was carried out on October and November during the winter season of 2021. Physicochemical properties of the soil used in the current study presents in Table 2 according to the standard methods of **Sadzawka *et al.* (2006)**.

Pots were divided into four groups with triplicates and irrigated with four different salinity water: (i) tap water irrigation, (ii) saline water irrigation with 2.5 $dSm^{-1}$,(iii) saline water irrigation with 5 $dSm^{-1}$, and (iv) saline water irrigation with 10 $dSm^{-1}$. The saline water was prepared by dilution from natural saline water obtained from a water-well with an initially salinity concentration of 15 $dSm^{-1}$.

## Data Collection and Plant Sample Analysis

### Plant growth parameters

Harvested plants (shoot with root systems) were transferred to the laboratory for characterization. Plants were cleaned from soil particles by washing and air-dried for recording the fresh weight (FW) of the plant shoot and root system. The number of leaves (as average; mean) and the shoot and root length (cm) of the plants were recorded. Plant dry weight was recorded after oven-dried at 70 °C for 24 hr (g).

### Chemical characterization of plant samples

Total chlorophyll was estimated using a Soil Plant Analysis Development (SPAD) chlorophyll meter at the end of the experiment. Soluble protein content was measured according to the method described by **Lowry *et al.* (1951**). Proline content was measured in DW samples according to the procedures obtained from **Bates *et al.* (1973**). Briefly, an amount of DW plant samples (0.5 g) were soaked in 3% sulfosalicylic acid for 10 min and the supernatant was separated by centrifugation at 3500 rpm for 10 min. The supernatant was treated by ninhydrin reagent and the absorbance was monitored at a wavelength of 520 nm. The soluble sugar content was measured related to the method summarized by **Irigoyen *et al.* (1992**) using anthrone reagent at a wavelength of 625 nm. Membrane stability index (MSI) was determined with fresh leaf samples by which small disc pieces of leaves were boiled twice and electrical conductivity (EC) was measured; EC (C1) at 25 ◦C after the first boiling time and EC (C2) at 120 ˚C after 20 min from second boiling time. The MSI was calculated as MSI (%) =[1-(C1/C2)] ×100. Electrolyte leakage (EL) was determined according to the approach described by **Sullivan (1979).** Briefly**,** similar to MSI EC was measured after a serial

**Table 2. Physicochemical properties of the soil used in the current experiment**

| Soil property | Analytical value |
|---|---|
| **Sand (%)** | 86.3 |
| **Silt (%)** | 1.00 |
| **Clay (%)** | 12.7 |
| **Texture** | Loamy sand |
| **pH (in 1:2.5 soil water suspension)** | 7.93 |
| **EC ds/m** | 0.40 |
| **Available nitrogen (mg N kg$^{-1}$)** | 0.393 |
| **Available phosphorus (mg kg$^{-1}$)** | 1.232 |
| **Exchangeable K (g kg$^{-1}$)** | 37.83 |
| **Exchangeable Na (%)** | 5.308 |

process of boiling in 10 ml of deionized water; (EC1 at 100◦C, EC2 at 55°C, and EC3 at 100°C) where EL was calculated as EL % = [(EC2- EC1)/EC3] ×100. Acid-digested plant samples were used to determine the elemental plant. Nitrogen was measured using the popular micro-Kjeldahl method described by **Bremner (1960**). Phosphorous, potassium, and sodium were measured according to the method described by **Sen Tran *et al.* (1988**) and **Wolf (1982**).

### Statistical analyses

The mean value of triplicates ± standard error (SE) was presented. Parried t-test and Pearson correlation were tested, if necessary, to compare the obtained results between groups. Moreover, compare 95% confidence intervals using Tukey's test with two-way ANOVA analysis with two factors (Biochar additions and salinity levels of irrigation water) using Minitab ® statistical software V. 17.1.0 **(Minitab, LLC, 2021)**.

## RESULTS AND DISCUSSION

### Characterization of Plant Growth

Results in Table 3 show the influence of different rates of biochar on the plant growth properties of spinach. The presented results in Table 3 reveal that all rates of biochar significantly increased shoot length at salinity level of 2.5 dSm$^{-1}$ by 10.0% while decreased shoot length at 5.00 and 10.0 dSm$^{-1}$ salinity level by 4.0% and 13.3%, respectively. However, root length increased at different rates of biochar as a result of biochar additions, root length increased under salt stress by 11.8, 4.0%, and 5.6 % for 2.5, 5.0, and 10 dSm$^{-1}$ saline levels, respectively. The fresh weight of the shoot system was significantly increased at salinity level of 2.5 and 5.0 dSm$^{-1}$ by 50.4% and 30.1%, respectively while declined by 2.48% under 10 Sdm$^{-1}$ salinity levels. The same trend was observed for the dry weight of the shoot system; the increments at 2.5 and 5.0 dSm$^{-1}$ increased DW by 59.9% and 23.5%, respectively while it declined DW by 3.68% under 10 dSm$^{-1}$ salinity level. Fresh and dry weights of the root system showed the same trend as fresh and dry weights of shoot system with a significant decrease at the highest salinity level (34.0% for fresh root weight and 45.1% for dry root weight). It seems that the biochar addition at all levels improved biomass weight of root at 2.5 and 5 dSm$^{-1}$ salinity levels, it was about (15.0 - 12.7%) by adding 1% biochar. The number of leaves increased only by 3.08% at 2.5 dSm$^{-1}$ salinity level while it decreased by 13.8 and 35.2% under the next two salinity levels (5.0 and 10.0 dSm$^{-1}$). It seems also that the addition of biochar up to 1% showed promising results

**Table 3. Effect of different rates of biochar (%) on plant growth characterization under different salinity levels (dSm-1). Shoot and root length (cm), FW and DW of shoot and root system(g)**

| Treatment | | Shoot length (cm) | Root length (cm) | FW of shoot (g) | DW of shoot (g) | FW of root (g) | DW of root (g) | No of leaves |
|---|---|---|---|---|---|---|---|---|
| TABE WATER | B0 | 14.73 ± 1.57cd | 6.53 ±0.64 ef | 6.9±1.25 ef | 3.53±0.68 ef | 0.6 ± 0.10 d | 0.09 ±0.01de | 62.67± 7.05 cdefg |
| | B.5 | 18.96 ± 3.05 abcd | 7.83 ±0.78cdef | 15.73±2.9 def | 9.97±2.78 bcdef | 1.07 ± 0.17cd | 0.5 ±0.25bcde | 84.33 ± 6.00 abs |
| | B1 | 20.33 ± 3.43 abc | 9.67 ±1.07abc | 24.67±11.2 abcd | 15.23±7.58 abc | 2.00 ± 0.90abc | 1.27 ±0.48ab | 78.33± 19.59 bcde |
| | B1.5 | 19.93 ± 3.29 abc | 9.73 ±1.04abc | 14.43±0.9 def | 8.17±1.17 cdef | 1.13 ± 0.19bcd | 0.87 ±0.15abcd | 77.67± 11.25 bcde |
| ds 2.5 | B0 | 16.53 ± 1.87 bcd | 8.07 ±0.66bcdef | 17±2.08 cdef | 10.61±1.67 bcdef | 0.73 ± 0.15d | 0.3 ±0.15cde | 74.00± 4.00 cdef |
| | B.5 | 21.73 ± 1.10 ab | 10.53 ±1.09ab | 27.67±2.85 abc | 16±2 abc | 1.37 ± 0.32abcd | 0.97 ±0.12abc | 102.33± 9.61 a |
| | B1 | 24.00 ± 1.44 a | 9.73 ±0.68abc | 29±3.21 ab | 19.33±1.67 a | 1.50 ± 0.40abcd | 1.03 ±0.24abc | 80.00 ± 9.81 abcd |
| | B1.5 | 19.13 ± 1.35 abc | 9.4 ±1abcd | 19.13±2.2 bcd | 13.05±1.97 abcd | 1.37 ± 0.23 abcd | 0.97 ±0.15abc | 56.00 ± 3.21 efgh |
| ds 5 | B0 | 16.66 ± 2.27 bcd | 6.00 ±0.35f | 13±3.06 def | 5.17±0.78 def | 0.73 ±0.28d | 0.27 ±0.21cde | 47.00 ± 2.08 gh |
| | B.5 | 18.20 ± 2.05 abcd | 7.00 ±0.3def | 17.33±4.49 f | 11.83±2.46 abcde | 1.13 ±0.23bcd | 0.67 ±0.28bcde | 52.67± 8.68 fgh |
| | B1 | 20.60 ± 3.43 abc | 10.33 ±0.18abc | 31.67±0.33 a | 17.67±0.88 ab | 2.20 ±0.49 ab | 1.5 ±0.36a | 100.33± 3.67 ab |
| | B1.5 | 15.46 ± 1.28 bcd | 11.8 ±1.14a | 18.33±3.84 bcde | 10.9±3.66 abcdef | 2.27 ±0.69a | 1.5 ±0.58a | 61.00± 2.51 cdefg |
| ds 10 | B0 | 12.33 ± 1.69 d | 9.92 ±1.54abc | 5.87±0.98 | 2.67±0.44 f | 0.43 ±0.09d | 0.05 ±0.03e | 33.67± 6.12 h |
| | B.5 | 16.60 ± 0.87 bcd | 9.87 ±0.87abc | 14.67±3.18 def | 8.37±2.47 cdef | 0.90 ±0.31d | 0.43 ±0.28cde | 58.00 ± 8.02 defg |
| | B1 | 17.13 ± 2.94 bcd | 6.73 ±1.5ef | 23.33±5.93 adcd | 16±5.51 abc | 1.27 ±0.03abcd | 0.7 ±0.06bcde | 56.67± 7.85 defgh |
| | B1.5 | 18.07 ± 2.98 abcd | 9.13 ±0.44bcde | 16.27±2.9 cdef | 8.47±2.32 cdef | 0.57 ±0.29d | 0.32 ±0.24cde | 48.00 ± 3.51 gh |
| **Significant** | | ** | ** | ** | ** | ** | ** | ** |
| **Treatment(T)** | | NS | NS | * | * | * | * | *** |
| **Biochar(b)** | | * | ** | *** | *** | ** | *** | *** |
| **Interaction (T*B)** | | NS | ** | NS | NS | NS | NS | ** |

Each value is a mean (±SE) of three replicates, and different letters on column indicate a significant range at p-value ≤ 0.05. * and ** indicate a difference at a p-value < 0.05 or 0.01, respectively based on two-way ANOVA.

for all observations while the reduction of plant productivity was corresponding to the higher concentration of saline water irrigation (10.0 $dSm^{-1}$) compared with control.

It is noted that the best positive results of biochar for different measurements were at a rate of 0.5%, followed by a rate of 1.0%, except for the fresh weight, where the effect was negative. As for the rate of adding biochar to 1.5%, it had a negative effect in all measurements except for the length of the root, where it had a positive effect. It has been reported that biochar had several potential positive effects on both plant and soil features. It improves crop biomass and enhances soil's physical and chemical properties including water use efficiency, nutrient content, biotic activities, and soil pH **(Hasan *et al.*, 2020)**. The beneficial effect of biochar could be attributed to the availability of carbon and mineral nutrients, as well as phytohormones such as gibberellin and indoleacetic acid, which improve morphological properties. The availability of water and nutrient defiantly improved the plant root system (**Mohamed *et al.*, 2016**). According to the results shown in Figure 1 and Table 1, the nutrient burden of biochar used in the current study is considered the main reason for improving the root and shoot length of plants. Our findings were corresponding with the results obtained by **Abiven *et al.* (2015**). Availability of nutrients in soil produced from biochar addition was reflected in increasing plant nutrient elemental uptake **(Abiven *et al.*, 2015**) or increasing water field capacity for plant growth **(Hasan *et al.*, 2020**). Biochar can also decrease the specific effect of Na toxicity in corresponding to high salinity level applied to the soil. The protective effect of saline water on plant enhancement could be related to excessive cell osmotic potential, ion toxicity, leaf stomata closure that decreases carbon dioxide absorption and limits the photosynthetic apparatus and impeding the release of crop improved biomass agents such as cytokinin **(Kamran *et al.*, 2019**). The substitution of ions accountable for salinity could be considered worthy of improving soils under saline conditions by applying organic-based biochar **(Naeem *et al.*, 2020**).

## Membrane Stability Index (MSI) and Electrolyte Leakage (EL) Measurements

Figs. 2 and 3 show the results obtained from MSI and El measurements. The results showed that biochar and the interactions of biochar and salinity levels had a significant effect on MSI values. It is relatively seen from Figure 2 that MSI values increased with increasing salinity levels. However, it significantly decreased at control with high addition of biochar (21.5%). The highest value of MSI was observed at 1.5% biochar and 10 dSm-1 salinity level (46.2%). The results showed that all stressed plants had an increased value of MSI compared with control, except for the value of MSI at salinity of 0.5, it was greater in the control than the rest of the values when adding biochar. Maintaining significant amounts of relative water in the leaves is a practical approach to achieve optimal plant growth through better cell division and more nutrient uptake, as well as higher photosynthetic capacity in saline conditions **(Kumar *et al.*, 2020**)

Interesting observations have been recorded when stressed plant treated with biochar regarding EL values. Results shown in Fig. 3 reveal that the existence of salt stress derived from irrigation with saline water promotes osmotic salt conditions for spinach which significantly increased EL value. The effects of reduction of EL values were significantly attributed to both levels of biochar and salt additions ($p < 0.001$). These results agreed with those published by **Sofy *et al.*(2021a**). One of the most important practices that can mitigate the salt stress on the grown plant is to maintain sufficient amounts of water in the plant shoot system to accomplish a good quality cell division process with relatively high nutrients taken up by the plant **(Kumar *et al.*, 2020**).

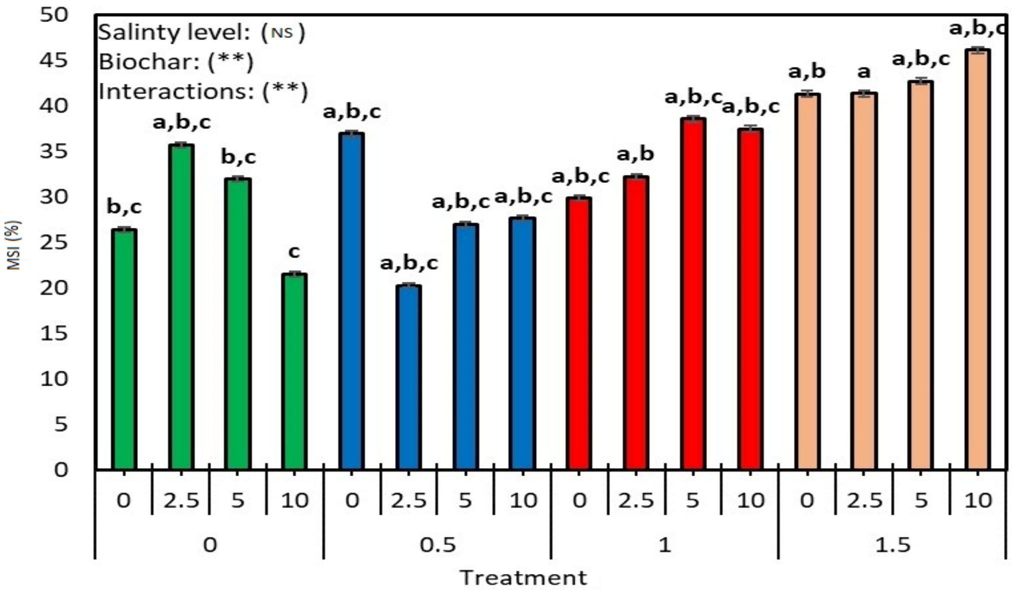



**Fig. 2. Effect of biochar (0, 0.5, 1 and 1.5%) on the membrane stability index (MSI) (%) values of spinach plant under different salinity levels (0, 2.5, 5 and 10 dSm$^{-1}$). Error bars represent (±SE) of triplicates while means that do not share a letter are significantly different according to Tukey statistical method at 95% confidence. The sign of * and ** represent a significant difference at 95% and 99% confidence, respectively according to ANOVA test**

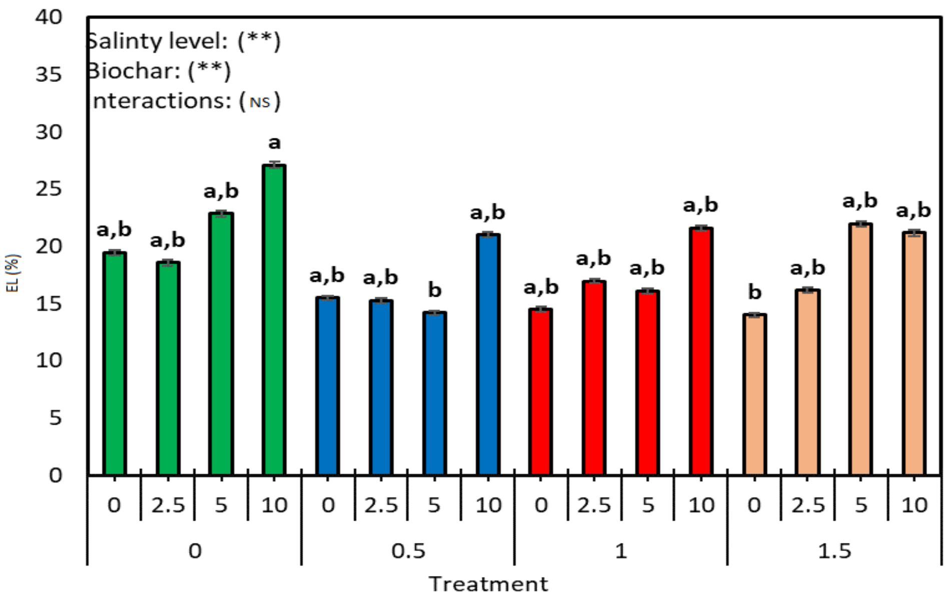



**Fig. 3. Effect of biochar (0, 0.5, 1 and 1.5%) on the electrolyte leakage (EL) (%) values of spinach plant under different salinity levels (0, 2.5, 5 and 10 dSm$^{-1}$). Error bars represent (±SE) of triplicates while means that do not share a letter are significantly different according to Tukey statistical method at 95% confidence. The sign of * and ** represent a significant difference at 95% and 99% confidence, respectively according to ANOVA test**

## Chlorophyll (Chl) Plant Content

Chlorophyll (Chl) pigment as total pigment content on salt-stressed spinach, is seen in Fig. 4. Compared with control, the total Chl increased when irrigated with salt water. It seems that biochar addition has a significant positive effect on increasing photosynthetic pigment in spinach plants. Also, the interaction between salinity and biochar treatments showed a significant effect on increasing Chl in plants. However, our findings proved that biochar addition to the salt-stressed plant could be used to mitigate the effect of negative effects of saline water used for irrigation. This suggests that all total chlorophyll content has been increased as a result of biochar treatments to salted affected soil (**Sofy *et al.*, 2021b**).

Some researchers reported that plant grown under salt conditions showed a decrease photosynthetic proses due to decreasing Chl content (**Muhammad *et al.*, 2022**). It seems that biochar may gain a favour in this concern due to different proposed mechanism which are not the scope of the current paper. Salt stress decrease plant Chl content due to various reasons such (i) physiological inhabitation of metabolic enzyme insufficient level of minerals which considered a primary component in producing Chl pigments such as iron, zinc, and manganese. (iii) blocking the leave stomata that prevent carbon dioxide to enter plant tissue (**Ahmad *et al.*, 2015**). Adding biochar indicated that the improvement in performance in the photosynthetic rate and efficiency is solely due to higher build-up of biosynthetic pathway intermediates and metabolites necessary for chlorophyll formation under salinity stress (**Farooq *et al.*, 2020**). Enhancing chlorophyll biosynthesis by stimulating plant growth regulators can also improve the performance of pigments (**Sharma *et al.*, 2020**).

## Mineral Ion Contents

The results in Figs. 5, 6, 7, and 8 show the total concentrations of N, P, K, and Na, respectively in spinach plant tissue. In general, in the case of N content, biochar had a significant effect on increasing the amount of N in plant tissue while the rest of the elements (with P exception) have been significantly affected by salinity (ANOVA results, (see Figs. 5 -8). N content was increased with increasing biochar addition. On the other hand, there is no significant difference in terms of elemental composition between different treatments. However, the addition of biochar decreased the amount of Na in plants. Due to its high sorption capacity, biochar can immobilize $Na^+$ from the soil solution. Therefore, it can reduce Na+ uptake and support ion balance by releasing mineral nutrients that directly improve plant growth and physiology in saline conditions. Biochar and Trichoderma can remove a large amount of $Ca^{2+}$ and $Mg^{2+}$ and replace $Na^+$ at the exchange site. In addition, higher $Na^+$ leaching and lower soil salinity improve the stability and properties of soil structures. Na ions could be prevented from being adsorbed by plant due to the competition with high sorption capability of applied biochar. Therefore, biochar can play an important role in reducing Na ions and lowering its toxicity to plants (**Hashem *et al.*, 2016; Naveed *et al.*, 2020**).

## Proline, Total Soluble Sugar, and Protein Contents

Figs. 9, 10, and 11 show the results obtained from measuring proline (Pr), total soluble sugar (SS), and protein content in plant tissue, respectively. The results showed that biochar significantly increased Pr content in plant tissue, and this was associated with salt stress. All treatments with interactions had a significant effect on this issue (Fig. 9). However, the highest level of SS was observed in the high level of salt stress (Fig. 10). Also, biochar had a

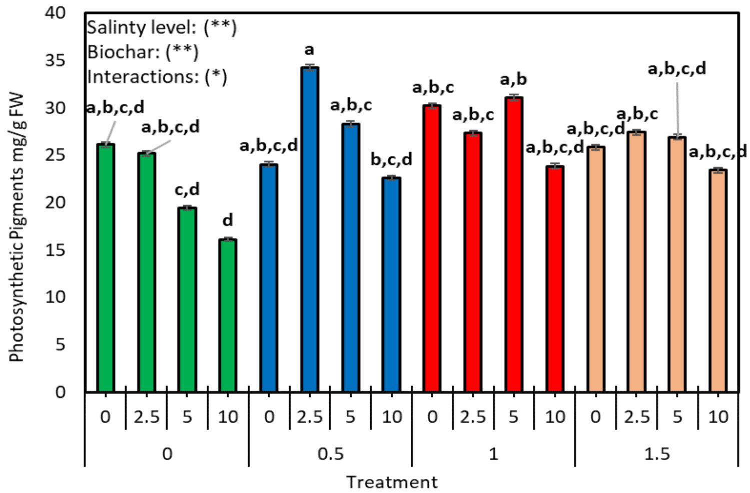



**Fig. 4. Effect of biochar (0, 0.5, 1 and 1.5%) on photosynthetic pigment (mg/g FW) values of spinach plant under different salinity levels (0, 2.5, 5 and 10 dSm$^{-1}$). Error bars represent (±SE) of triplicates while means that do not share a letter are significantly different according to Tukey statistical method at 95% confidence. The sign of * and ** represent a significant difference at 95% and 99% confidence, respectively according to ANOVA test**

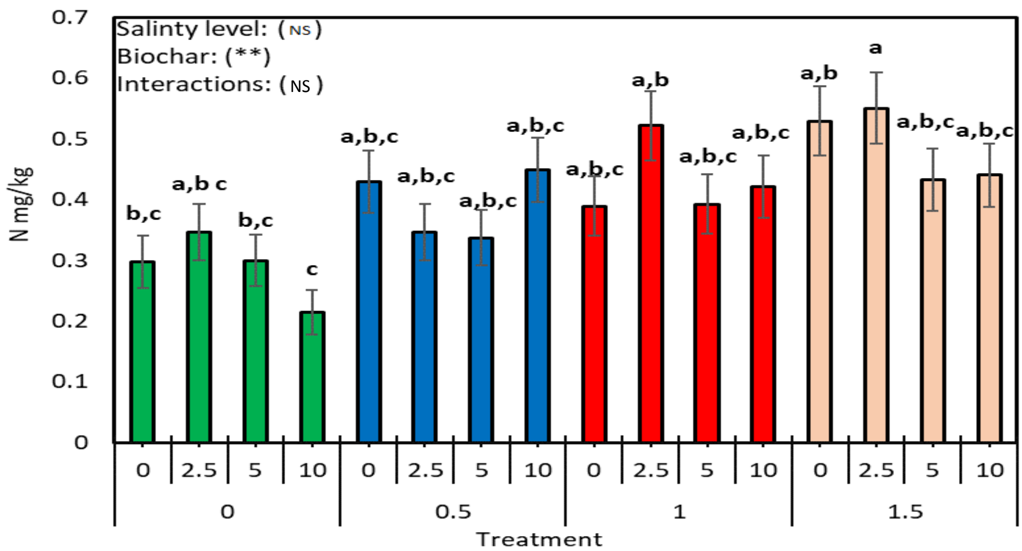



**Fig. 5. Effect of biochar (0, 0.5, 1 and 1.5%) on N (mg/kg) values of spinach plant under different salinity levels (0, 2.5, 5 and 10 dSm$^{-1}$). Error bars represent (±SE) of triplicates while means that do not share a letter are significantly different according to Tukey statistical method at 95% confidence. The sign of * and ** represent a significant difference at 95% and 99% confidence, respectively according to ANOVA test**

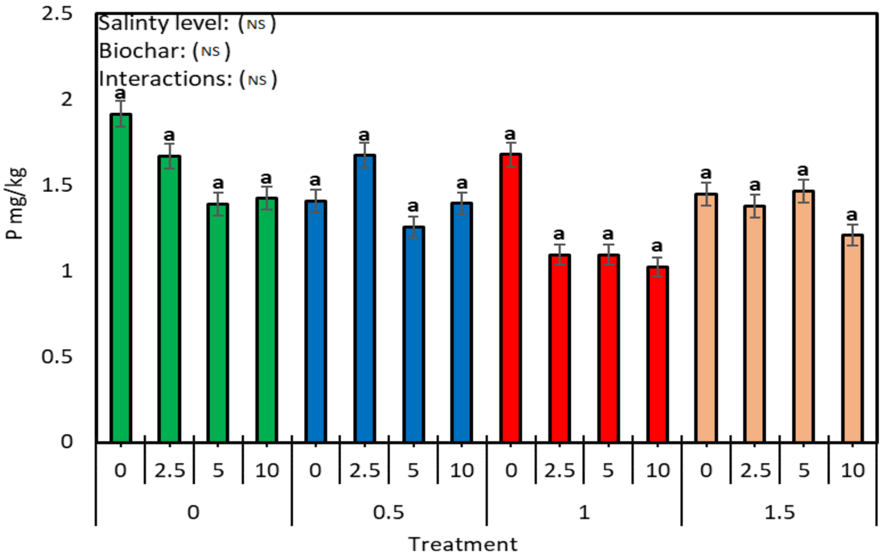



**Fig.6. Effect of biochar (0, 0.5, 1 and 1.5%) on P (mg/kg) values of spinach plant under different salinity levels (0, 2.5, 5 and 10 dSm$^{-1}$). Error bars represent (±SE) of triplicates while means that do not share a letter are significantly different according to Tukey statistical method at 95% confidence. The sign of * and ** represent a significant difference at 95% and 99% confidence, respectively according to ANOVA test**

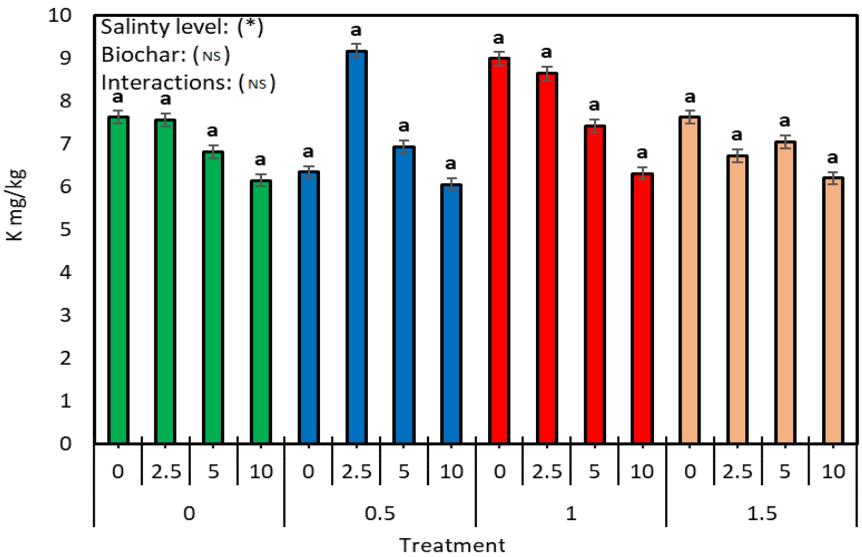



**Fig. 7. Effect of biochar (0, 0.5, 1 and 1.5%) on K (mg/kg) values of spinach plant under different salinity levels (0, 2.5, 5 and 10 dSm$^{-1}$). Error bars represent (±SE) of triplicates while means that do not share a letter are significantly different according to Tukey statistical method at 95% confidence. The sign of * and ** represent a significant difference at 95% and 99% confidence, respectively according to ANOVA test**

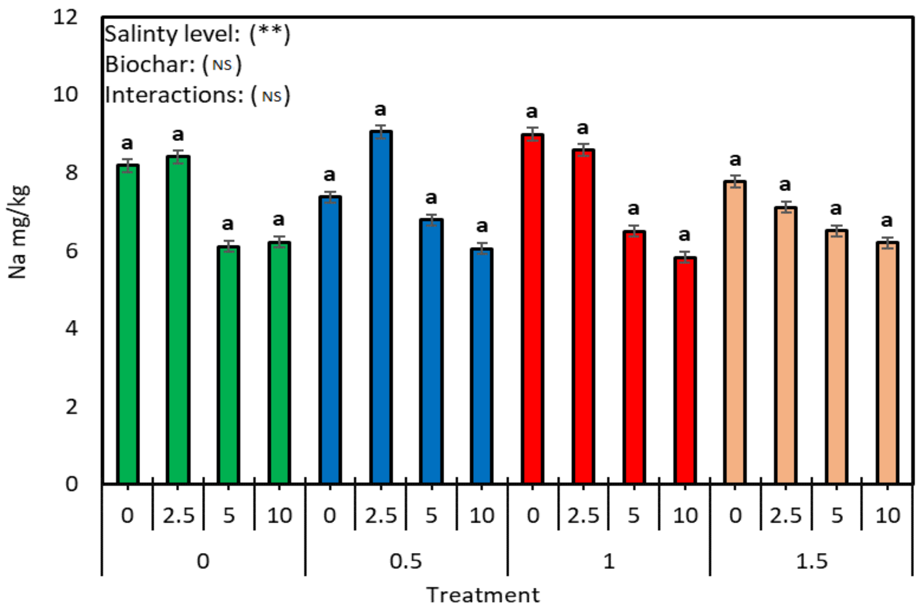



**Fig. 8. Effect of biochar (0, 0.5, 1 and 1.5%) on Na (mg/kg) values of spinach plant under different salinity levels (0, 2.5, 5 and 10 dSm$^{-1}$). Error bars represent (±SE) of triplicates while means that do not share a letter are significantly different according to Tukey statistical method at 95% confidence. The sign of * and ** represent a significant difference at 95% and 99% confidence, respectively according to ANOVA test**

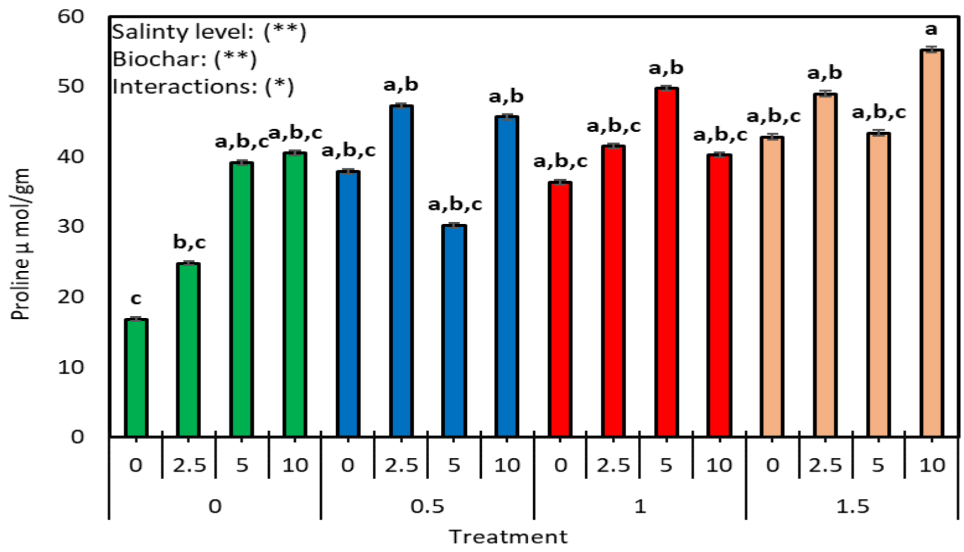



**Fig. 9. Effect of biochar (0, 0.5, 1 and 1.5%) on proline (µMo/gm) values of spinach plant under different salinity levels (0, 2.5, 5 and 10 dSm$^{-1}$). Error bars represent (±SE) of triplicates while means that do not share a letter are significantly different according to Tukey statistical method at 95% confidence. The sign of * and ** represent a significant difference at 95% and 99% confidence, respectively according to ANOVA test**

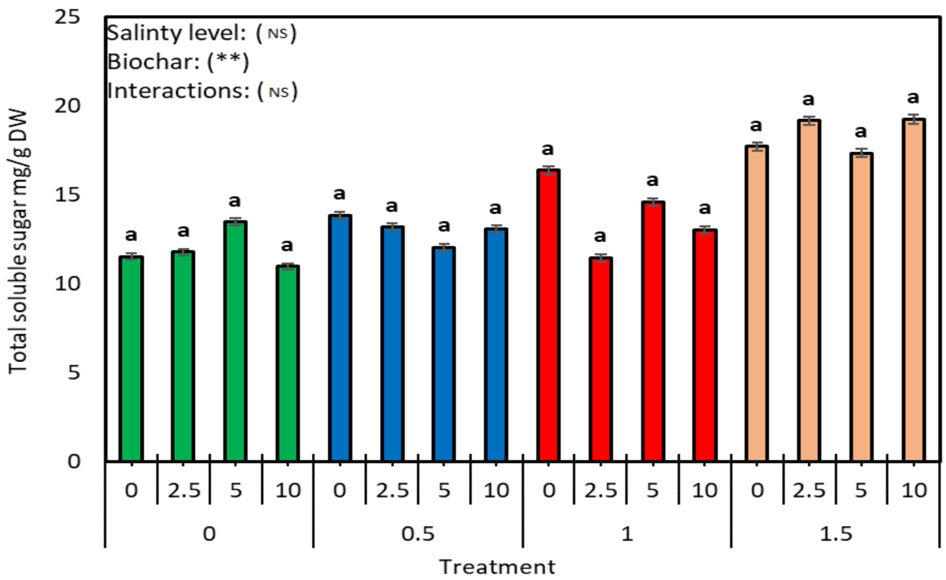



**Fig.10. Effect of biochar (0, 0.5, 1 and 1.5%) on total soluble sugar (mg/g DW) values of spinach plant under different salinity levels (0, 2.5, 5 and 10 dSm$^{-1}$). Error bars represent (±SE) of triplicates while means that do not share a letter are significantly different according to Tukey statistical method at 95% confidence. The sign of * and ** represent a significant difference at 95% and 99% confidence, respectively according to ANOVA test**

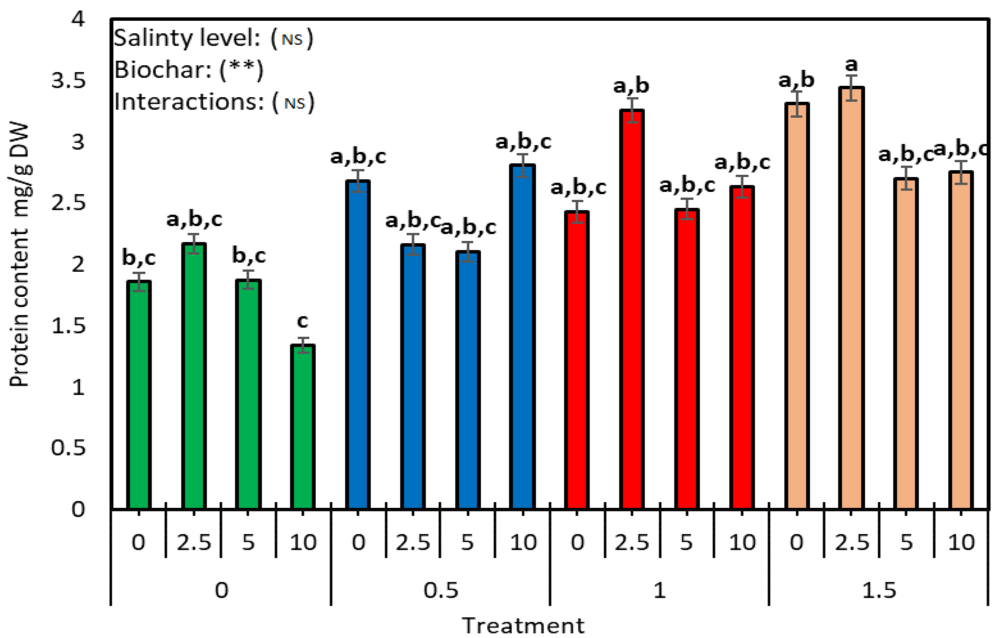



**Fig. 11. Effect of biochar (0, 0.5, 1 and 1.5%) on protein content (mg/g DW) values of spinach plant under different salinity levels (0, 2.5, 5 and 10 dSm$^{-1}$). Error bars represent (±SE) of triplicates while means that do not share a letter are significantly different according to Tukey statistical method at 95% confidence. The sign of * and ** represent a significant difference at 95% and 99% confidence, respectively according to ANOVA test**

significant effect on increasing protein content under salt stress conditions (Fig. 11). Soluble sugar and proline were used as a biomarker for the plant under salt conditions. Both measurements were increased significantly in the salt-stressed plant compared with the control. The present evidence reveals that the increase in these salt biomarkers was correlated with increasing salinity levels (Figs. 9 and 10).

The stress-induced accumulation of salt markers has two potential physiological reactions: (i) decline of the cell's osmotic capacity; and strength of cell membrane **(Yang and Guo, 2018; El-Beltagi *et al.*, 2020**). The excellent benefits of biochar have been linked to improved protein and salt biomarker levels. **Solaiman *et al.* (2020**) reported that biochar addition to the plant improved their growth by developing compatible solutes and proteins in the plant cell system.

Generally, in stress conditions, proline contributes significantly to the stability of membranes and other cellular structures under stressful conditions by producing reactive oxygen species. Additionally, it preserves the pH and turgor of the cell. Under stressful circumstances, an elevated sugar level supports physiological functions like photosynthesis, nutrition mobilisation, and exportation, whereas a low sugar level promotes the storage of carbohydrates and senescence. Biochar incubation increased the soluble sugar and proline content of stress-exposed plants as compared to control one **(Desoky *et al*., 2021)**.

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## الملخص العربي

# أثر محسنات البيوشار على نمو السبانخ تحت ظروف الاجهاد الملحي

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الملوحة من أكبر التهديدات التي تعوق الأمن الغذائي العالمي. تمت دراسة تأثير كميات مختلفة من البيوشار المشتقة من نوى نخيل التمر على نباتات السبانخ (*Spinacia oleracea* cv. Balady) النامية في ظل ظروف الإجهاد الملحي وذلك من خلال تجربة أصص أجريت في شهري أكتوبر ونوفمبر خلال موسم شتاء 2021 أكدت نتائجنا أن الإجهاد الملحي أثر سلبا على الظواهر المورفولوجية والفسيولوجية لنباتات السبانخ مثل المجموع الجذري (4%، 13.3% عند الملوحة من 5 و10 ديسي سيمنزم$^{-1}$، على التوالي). كما حسنت إضافة البيوشار بشكل كبير من طول الجذر تحت جميع مستويات الإجهاد الملحي. وأظهرت النتائج أن إضافة البيوشار أدى إلى تحسين وزن الكتلة الحيوية عند 2.5 و5 dSm1الملوحة على جميع المستويات. وزاد عدد الأوراق بنسبة 3.08% فقط عند ملوحة 2.5 ديسي سيمنزم$^{-1}$، في حين انخفض بنسبة 13.8 و35.2% في درجات الملوحة التالية (5 و10 ديسي سيمنزم$^{-1}$)، على التوالي. تم تحقيق قيم التخفيض الإجمالية المقابلة لتركيز المياه المالحة الأعلى (10 ديسي سيمنزم$^{-1}$) مقارنة بمعاملات المقارنه. يبدو أن إضافة البيوشار بنسبة تصل إلى 1% أظهرت أيضا نتائج واعدة في جميع الملاحظات. أظهرت النتائج التي تم الحصول عليها من قياس مؤشر استقرار الغشاء (MSI) وتسرب المنحل بالكهرباء (EL) أن جميع النباتات المجهدة قد زادت من قيم MSI وانخفضت قيم LE مقارنة بالكنترول. الصوديوم والسكريات القابلة للذوبان ومستويات البرولين زادت بشكل كبير استجابة للإجهاد الملحي. أدى استخدام البيوشار إلى تحسن كبير في نمو النبات وتم استخدامه بنجاح لتخفيف الإجهاد الملحي أثارة.

**الكلمات الاسترشادية**: الإجهاد الملحي، بيوشار، السبانخ، الري، التربة الرملية.

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