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# Application of olive mill waste-based biochar for improving wheat response to salt stress

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**ABSTRACT**

The production of olive mill solid waste (OMSW) raises concerns due to its toxicity and negative environmental impact. However, by utilizing pyrolysis, OMSW can be converted into biochar, a carbon-rich material that detoxifies the waste and preserves its nutrient content. The OMSW-based biochar possesses alkaline properties (pH 9.6), low electrical conductivity (EC), high cation exchange capacity (CEC), a porous surface morphology, various surface functional groups, and high mineral content. This study assessed the influence of two concentrations (5% and 10%) of OMSW-based biochar on wheat plants' growth biomarkers and physiological characteristics subjected to salt stress conditions (150 mM NaCl). Findings of the study revealed that salt stress had deleterious effects on various parameters, including shoot height, fresh and dry weights of shoots and roots, relative water content (RWC**%**), membrane stability index (MSI**%**), photosynthetic pigments, and photosynthetic parameters such as the coefficient of the effective quantum yield of photochemical energy conversion of PSII (ØPSII), photochemical quenching (qP), and photochemical efficiency of PSII ($F_o$, Fm, Fv/$F_o$, and Fv/Fm). Furthermore, the levels of lipid peroxidation (MDA), hydrogen peroxide ($H_2O_2$), superoxide dismutase (SOD), and peroxidase (POD) activities significantly increased in stressed plants. On the other hand, applying both concentrations of OMSW-based biochar effectively improved the overall performance of wheat plants, irrespective of the presence of salinity. OMSW-based biochar is a promising strategy for promoting wheat growth in salt-stressed soil by improving various growth parameters and mitigating plant oxidative stress.

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## 1 Introduction

Abiotic stresses are a threat to the food security of the globe, causing a reduction of 50% in crop production (Sofy et al. 2020). Salt stress is the most brutal environmental factor that can cause harsh effects on many crops. Wheat is considered one of the imperative food crops whose production faces stiff competition due to salt stress. Salinity disturbs plant physiological characteristics such as enzymatic activities, photosynthesis, membrane integrity, hormonal balance, and the uptake of water and nutrients. It induces oxidative stress that increases the reactive oxygen species (ROS) levels, damaging membrane lipids, proteins, and nucleic acids. Plants developed different mechanisms for alleviating its effects via anatomical, physiological, and metabolic changes (Abou-Zeid et al. 2020; Soliman et al. 2023).

The olive oil industry produces huge amounts of polluted by-products, such as solid waste (OMSW) and wastewater. OMSW is a complex mixture comprising residual fractions of pulp, seeds, and olive skins. It is characterized by high levels of polyphenols and heavy metals, which contribute to its phytotoxicity and resistance to degradation (Leon-Camacho et al. 2003). The valorization of OMSW has emerged as a significant research area for environmental protection through effective waste management strategies (Batuecas et al. 2019). Pyrolysis of solid waste has been recently applied for OMSW-based biochar production, offering a sustainable waste management approach (Aly 2016). Numerous studies have accounted for biochar's potential role in soil carbon sequestration, contributing to climate change mitigation (Han et al. 2020; Haddad et al. 2021).

Biochar, a carbon-rich solid material, has emerged as an environmentally friendly approach to mitigate the detrimental effects of salinity on plants. It is produced through pyrolysis from large biomass of agricultural residues and wastes, industrial organic wastes, and organic municipal solid wastes (Rodriguez et al. 2021). Generally, it is alkaline with high cation-exchange capacity; it is often used in agricultural systems to improve soil's physiochemical properties, such as water holding capacity, pH, and electrical conductivity (Sun et al. 2021). Organic carbon from biochar enhances the mineral composition in soil and contributes to crops grown under harsh environments (Hannan et al. 2021). Applying biochar plays an essential role in the activity of the soil-biological factors and can modify plants' physiological metabolism. The utilization of OMSW-based biochar has been found to affect leaf carbon assimilation and photosynthesis positively. It exhibits a high adsorption potential, which helps minimize plants' uptake of Na+ ions. Additionally, OMSW-based biochar increases the content of $K^+$ ions in the xylem while reducing the $Na^+/K^+$ ratio. This leads to an improvement in the activities of antioxidant enzymes, thus reducing oxidative stress and enhancing the plants' resistance to salt stress (Akhtar et al. 2015; Iqbal et al. 2021; Soliman et al. 2023). However, high levels of biochar can contribute to an increase in soil salinity, as Thomas et al. (2013) reported. This study aimed to convert OMSW into biochar and evaluate the impact of incorporating OMSW-based biochar into the soil on wheat plants' growth biomarkers and physiological characteristics under both saline and non-saline conditions.

## 2 Materials and methods

### 2.1 Biochar production

OMSW was collected from an olive mill press in the Borg El-Arab region, Alexandria, Egypt, and olive pruning was air-dried before use. The biochar was produced through a slow pyrolysis process; the samples were prepared by placing them in crucibles with tightly sealed lids. Pyrolysis, an operation carried out under oxygen-limited conditions, was conducted in two stages. In the first stage, the pyrolysis temperature was gradually raised to 300 °C at a heating rate of 10 °C per minute using a muffle furnace; this temperature was maintained for 60 min. In the second stage, the biochar was allowed to cool overnight to room temperature; then, the pyrolysis temperature was raised to 500 °C at a heating rate of 10 °C $min^{-1}$ and held for 60 min. The OMSW-based biochar was allowed to cool to room temperature, then ground sieved through a ~2 mm mesh, and stored for subsequent analyses and treatments (Hassan et al. 2022).

### 2.2 Characterization of soil and biochar

The morphology and surface elemental analysis of the OMSW-based biochar was examined using an Energy Dispersive X-ray (EDX) spectrometer-Link ISIS analyzer integrated with a Scanning Electron Microscope (SEM). Fourier Transform Infrared (FTIR) spectroscopy was analyzed using a Perkin-Elmer Spectrum 1000 instrument to investigate the functional groups present. The soil and OMSW-based biochar were mixed with deionized water at a ratio of 1:5 and stirred for 24 hours. Subsequently, the mixture was centrifuged at 5000g for 10 min, and the resulting supernatant was used to analyze electrical conductivity (EC) and pH, following the method described by Novak et al. (2009). Soil and biochar organic matter were measured according to Sikora and Moore-Kucera (2014). After the ammonium acetate extraction method, the cation exchange capacity was detected for soil and OMSW-based biochar (Melo et al. 2013).

### 2.3 Plant material and growth conditions

*Triticum aestivum* L. (cv. Sakha 61) were acquired from the Agricultural Research Center, Giza, Egypt. The seeds were subjected to surface sterilization by immersing them in a 1% sodium hypochlorite solution for 30 seconds. Subsequently, the sterilized seeds were planted in pots with dimensions of 12 cm (diameter) and 17 cm (length) filled with a soil mixture of clay and sand in a ratio of 1:2 (W/W). The soil mixture was amended with biochar at 0%, 5%, and 10% concentrations. Plants were irrigated with distilled water at two-day intervals. The plants were subjected to alternate-day application of NaCl (150 mM) starting 10 days after sowing, with a solution at a concentration of 150 mM. The experiment employed a completely randomized block design, with each treatment replicated four times under natural environmental conditions. After 18 days, plants with similar growth characteristics were harvested, thoroughly washed, gently dried and separated into shoots and roots. These plant parts were preserved for subsequent growth measurements and chemical analyses.

**2.4 Estimation of growth biomarkers and photosynthetic pigments**

Shoot height and the fresh weight (FW) of shoots and roots were determined. Dry weight (DW) was considered in shoots and roots that oven-dried at 70 $^{\circ}$C for a duration of 72h. The quantification of photosynthetic pigments was performed using the method described by Moran (1982). The calculation of carotenoid content was carried out following the Wellburn procedure (1994).

**2.5 Measurement of photosynthesis parameters**

The chlorophyll fluorescence parameters were determined using the method described by Genty et al. (1989) and Van Kooten and Snel (1990). A chlorophyll fluorimeter OS-30P pulse modulated by Opti-sciences (Hudson, USA) was used, prior to the measurements, the leaf samples were subjected to a dark adaptation period of 30 min. The measurements were carried out using actinic light with an intensity of 400 mmol $m^{-2}$ $s^{-1}$ and a saturating pulse light of 8000 mmol $m^{-2}s^{-1}$. The parameters that were measured include the coefficient of the effective quantum yield of photochemical energy conversion of PSII (ØPSII), the photochemical quenching (qP), and the photochemical efficiency of PSII ($F_o$, Fm, Fv/$F_o$, and Fv/Fm).

**2.6 Estimation of leaf relative water content (RWC%)**

Leaf RWC% was assessed following the method described by Turner (1981). It was calculated using the formula: RWC (%) = (FW - DW) / (TW - DW) x 100, where FW represents the fresh weight of leaf discs, TW denotes the turgid weight measured after their immersing in water at room temperature for 24 h, and DW represents the weight of the oven-dry leaf discs at 80 $^{\circ}$C for 4 hours.

**2.7 Determination of membrane stability index (MSI%)**

Leaf discs weighing 0.1 g were utilized for the measurement of MSI%. The leaf discs were submerged in two test tubes containing 10 ml of double distilled water. One tube ($C_1$) was maintained at 40 $^{\circ}$C for 30 minutes, while the other (C2) was placed in a boiling water bath at 100 ºC for 15 minutes. The electrical conductivity of the solutions in both tubes was measured using a conductivity meter, as described by Deshmukh et al. (1991). MSI% = [1-($C_1$/$C_2$)] x 100.

**2.8 Estimation of malondialdehyde and hydrogen peroxide content**

Malondialdehyde (MDA) content was detected following the method of Wang et al. (2009) and calculated using the following formula:

MDA ($\mu$mol $g^{-1}$ FW) = [6.45($OD_{532}$-$OD_{600}$) - 0.56($OD_{450}$)×1000]/wt. Hydrogen peroxide ($H_2O_2$) was estimated according to Velikova and Loreto (2005) method.

**2.9 Extraction and determination of antioxidant enzymes activities**

Fresh (1g) shoots and roots were extracted in 50 mM phosphate buffer containing 0.5 mM EDTA (pH 7.5), centrifuged at 10,000g (4 $^{\circ}$C) for 10 min, the supernatant was kept for protein content determination (Hartree 1972), and assays of some enzymes activities. Superoxide dismutase (SOD, EC 1.15.1.1) activity was assayed following Giannopolitis and Ries (1977) method. Peroxidase (POD, 1.11.1.7) activity was estimated as described by Noreen and Ashraf (2009) via the record of the changes in absorbance at 470 nm every 20s in the reaction solution. The catalase activity (CAT, 1.11.1.6) was assessed according to Zhang et al. (2005) method following the decomposition of $H_2O_2$ at 240 nm.

**2.10 Statistical analysis**

Data were subjected to one-way analysis of variance (ANOVA) using the statistical software package SPSS (version 20). Duncan's multiple-range test was applied to identify means of significant differences. Statistical significance was defined at a level of $P \leq 0.05$.

**3 Results**

**3.1 Characteristics of biochar**

The physicochemical characteristics of OMSW-based biochar produced by slow pyrolysis are depicted in Table 1. There was a high variability of organic matter, EC, pH and CEC; the mean values for the OMSW-based biochar were higher than those recorded in the soil. Several peak intensities were detected in the FTIR spectra corresponding to the OMSW-based biochar, indicating the presence of several functional groups (Figure 1). For example, the peak at 754.976 $cm^{-1}$ showed the aromatics, 1$^{\circ}$, 2$^{\circ}$ amines, and bend alkenes function groups; the peak at 1566.769 $cm^{-1}$ was

Table 1 Basic properties of the studied soil and of OMSW-based biochar

| Parameter | Soil | Biochar |
|---|---|---|
| Organic matter (% ) | 74.45 | 94.7 |
| Electrical conductivity (dS $m^{-1}$) | 0.24 | 0.86 |
| pH | 6.68 | 9.61 |
| Cation exchange capacity (cmol $kg^{-1}$) | 18.88 | 35.85 |

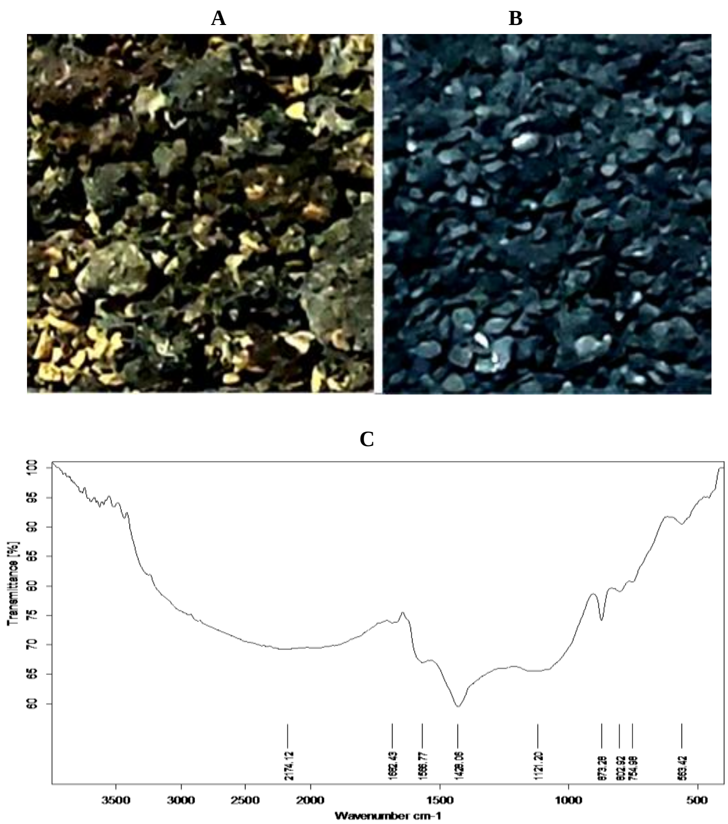


Figure 1 OMSW (A), OMSW-based biochar (B), and (C) the FTIR spectra of OMSW-based biochar

Table 2 FTIR-spectroscopy absorption peaks and their associated functional groups of OMSW-based biochar. Data was adopted from reference (Nandiyanto et al. 2019)

| Wave number ($cm^{-1}$) | Functional group/ Assignments |
|---|---|
| 2174.124 | –C≡C– stretch, C≡N stretch (alkynes, nitriles) |
| 1682.427 | –C=C–stretch, C=O stretch (alkenes, carbonyls) |
| 1566.769 | N–H bend (1° amines) |
| 1428.057 | C–C stretch (in ring- aromatics) |
| 1121.199 | C–O stretch, C–N stretch (alcohols, carboxylic acids, esters, ethers, aliphatic amines) |
| 873.282, 802.9145 | C–H "oop", N–H wag, =C–H (aromatics, 1°,2° amines, bend alkenes) |
| 754.976 | C–H "oop", C-Cl stretch,=C–H (aromatics, alkyl halides, bend alkenes) |
| 563.424 | C–Br stretch, C-Cl stretch (halides) |

attributed to N-H deformations of 1° amines group, while the peak intensity at 1121.199 $cm^{-1}$ showed the presence of a C–O stretch and C–N stretch chemical bond that may indicate the presence of aliphatic amines, carboxylic acids, alcohols, esters and ethers (Table 2 and Figure 1). Element percentages were measured using the energy dispersive spectroscopic (EDX) analysis of OMSW-based biochar; the average percent of carbon was 66.70±0.29, and the presence of macro- and micro-nutrients was detected, as mentioned in Table 3 and Figure 2.

Table 3 The elemental composition of OMSW-based biochar by EDX analysis

| Element | Mass% | Atom% |
|---|---|---|
| C | 66.70±0.29 | 75.15±0.29 |
| N | 0.90±0.03 | 0.77±0.06 |
| O | 20.30±0.48 | 17.05±0.38 |
| Na | 0.46±0.05 | 0.96±0.04 |
| Mg | 1.53±0.06 | 0.95±0.06 |
| Al | 0.42±0.04 | 0.21±0.02 |
| Si | 0.74±0.04 | 0.36±0.02 |
| P | 2.65±0.13 | 1.39±0.07 |
| S | 0.15±0.02 | 0.06±0.01 |
| Cl | 0.22±0.02 | 0.09±0.01 |
| K | 2.21±0.08 | 1.17±0.03 |
| Ca | 3.15±0.09 | 1.46±0.03 |
| Fe | 0.37±0.03 | 0.29±0.04 |
| Zn | 0.20±0.07 | 0.09±0.04 |

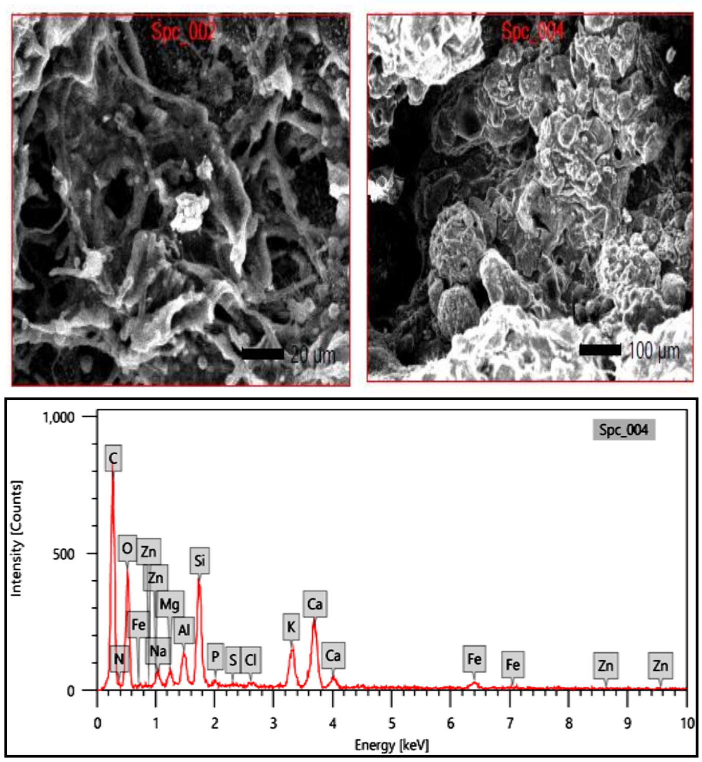


Figure 2 The energy dispersive spectroscopic (EDX) analysis of OMSW-based biochar

**3.2 Growth biomarkers, photosynthetic pigments, and photosynthetic parameters**

Figure 3 illustrates a significant reduction in growth biomarkers of wheat seedlings treated with 150 mM NaCl. The shoot height was reduced by approximately 37% compared to the control (Figure 3A). Similarly, the FW of shoots and roots showed reductions of about 54% and 61%, respectively, while the DW showed reductions of 49% for shoots and 60% for roots, all relative to their respective controls (Figure 3C and 3D). However, biochar provoked an increase in the aforementioned growth characteristics compared to the controls. Particularly, the DW of roots treated with 5% and 10% biochar showed an increase of approximately 25% and 38%, respectively, while the DW of shoots did not exhibit significant changes compared to their controls. Furthermore, biochar amendments at both 5% and 10% concentrations enhanced the growth characteristics of wheat seedlings under non-saline treatments (Figure 3). Salinity caused a reduction of approximately 28% in leaf RWC**%** compared to the control, which was effectively mitigated by biochar application (Figure 3B). Both concentrations of OMSW-based biochar (5% and 10%) resulted in a 10% increase in RWC under non-saline conditions compared to the control.

Using OMSW-based biochar significantly increased the content of leaf photosynthetic pigments in wheat plants grown under optimal conditions (Figure 4). However, irrigation with salt water (150 mM) led to a significant reduction of approximately 50% for Chl a, 31% for Chl b, and 45% for carotenoids, compared to the controls. OMSW-based biochar alleviated this negative effect on photosynthetic pigments under normal and saline conditions. Notably, the combined impact of 10% biochar and 150 mM NaCl resulted in a substantial increase in Chl a, Chl b, and Caroteniods contents, with approximately 36%, 34%, and 49% higher levels, respectively, compared to the control group. However, applying 5% biochar in the presence of salt didn't significantly affect Chl b and Caroteniod. Under salt-free conditions, applying OMSW-based biochar led to an increase in Chl a, Chl b, and Carotenoid contents, mainly when used at a concentration of 10%; the increase values reached about 52%, 45%, and 30%, respectively, compared to the control (Figure 4).

Salinity stress in this study resulted in alterations of the leaves-photosynthetic parameters (ØPSII, qP and Fv/Fm). They were significantly reduced by 46%, 40%, and 11%, respectively, compared to the unstressed plants (Figure 4). The application of OMSW-based biochar showed higher values for these photosynthetic parameters, with increases of approximately 12%, 32%, and 2% for 5% OMSW-based biochar and 28%, 38%, and 3% for 10% OMSW-based biochar, respectively, with respect of the controls. The amelioration of the decline was more pronounced in plants treated with 10% OMSW-based biochar under salinity stress conditions.

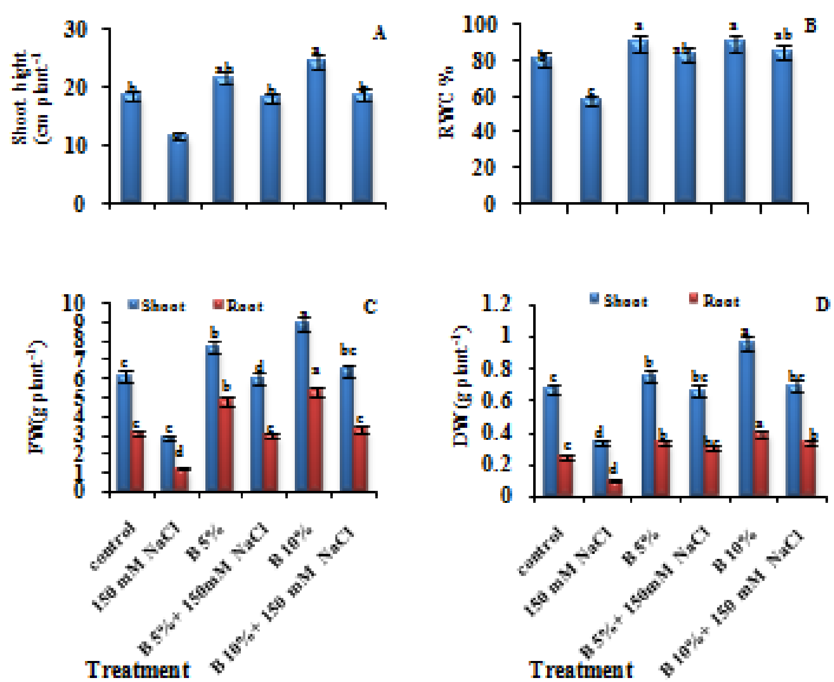


Figure 3 Effects of the interaction between salinity and OMSW-based biochar on growth biomarkers of 18-day-old wheat seedlings. A: shoot length, B: leaf relative water content (RWC), C: fresh weight and D: dry weight. Values are means ±SE (n= 4), bars followed by different letters indicate significant difference at $p \leq 0.05$ (Duncan's multiple range test)

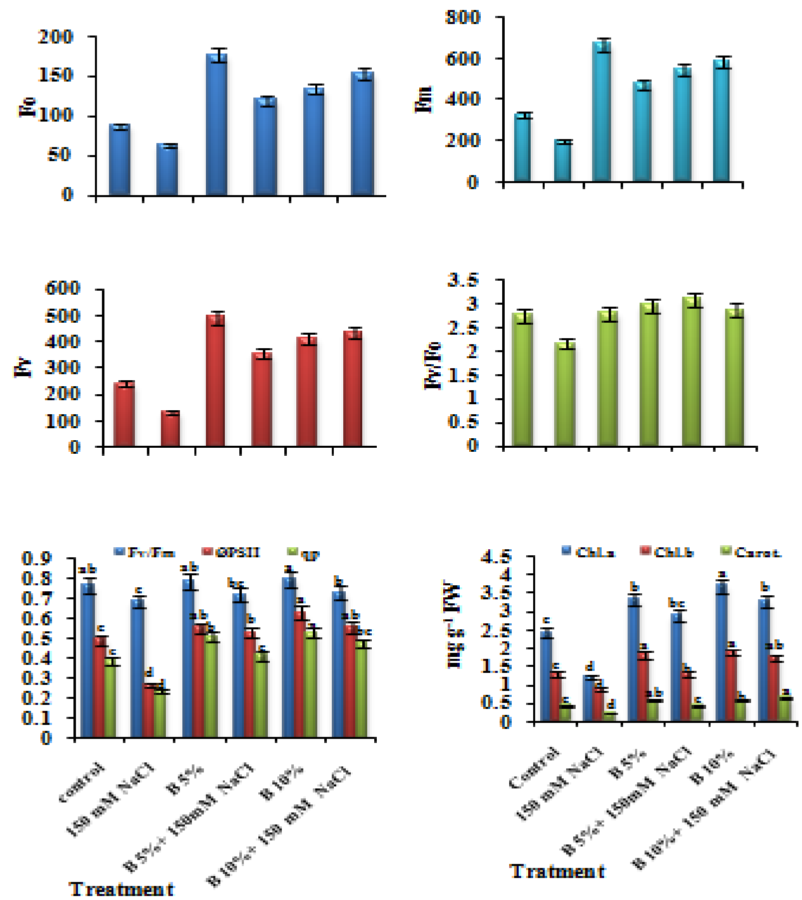


Figure 4 Effects of the interaction between salinity and OMSW-based biochar on photosynthetic efficiency and photosynthetic pigments of 18-day-old wheat leaves. Values are means ±SE (n= 4), bars followed by different letters indicate significant difference at $p \leq 0.05$ (Duncan's multiple range test)

### 3.3 Membrane stability index, malondialdehyde and hydrogen peroxide content

Figure 5A depicts the cell membrane stability index (MSI%), representing the percentage of membrane injury and damage. Notably, a significant decrease in MSI% was observed in response to 150 mM NaCl, reaching about 29%, indicating membrane destabilization, compared to plants grown under normal conditions ($p \leq 0.05$). However, applying OMSW-based biochar at concentrations of 5% and 10% increased MSI% by 7% and 9%, respectively, for plants grown without salinity. Similarly, under salt stress conditions, the presence of 10% OMSW-based biochar led to a 6% increase in MSI%. The results in Figures 5B and 5C indicated that MDA and $H_2O_2$ content significantly increased in the 150 mM NaCl-stressed plants. There was a considerable increase of 52% in MDA content in shoots and 35% in roots, while the corresponding values for $H_2O_2$ were 43% and 45% under salinity stress compared to normal conditions. A significant interaction effect of salinity and OMSW-based biochar was observed on MDA and $H_2O_2$. Under saline conditions, their content was significantly reduced by 15% and 11% in shoots and by 10% and 4% in roots with the application of 10% biochar compared to their controls.

In this experiment, salt stress led to a significant increase in the activity of SOD up to 39% and 32% in shoots and roots, respectively; the analogous values for POD were 43% and 33% compared to the controls (Figure 5E and 5F). The supplementation of 10% OMSW-based biochar effectively decreased SOD and POD activity in salt-stressed wheat plants, reaching approximately 32% and 16% for SOD and 8% and 24% for POD in shoots and roots, respectively. Conversely, the activity of CAT was significantly decreased under 150 mM salt stress, with reductions of 16% in shoots and 17% in roots compared to the controls. Otherwise, the impact of OMSW-biochar improved CAT activity by 32% and 14% in shoots and roots, respectively, under non-saline conditions at the 10% OMSW-based biochar concentration. Under saline conditions, the corresponding values were 24% and 8%, respectively.

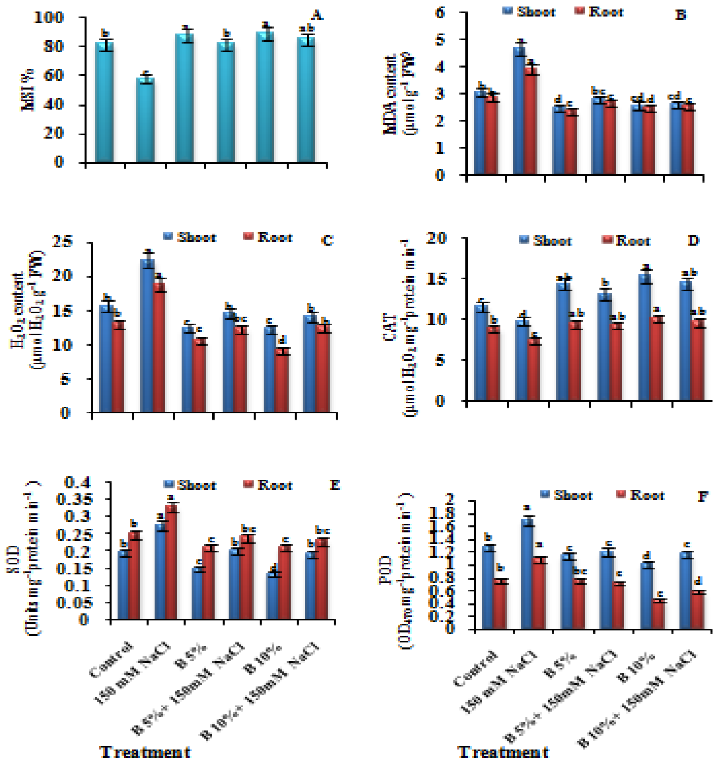


Figure 5 Effects of the interaction between salinity and OMSW-based biochar on A: membrane stability index (MSI%), B: malondialdehyde content (MDA), C: hydrogen peroxide content ($H_2O_2$), D: catalase activity, E: superoxide dismutase (SOD) activity and F: peroxidase (POD) activity of 18-day-old wheat seedlings. Values are means ±SE (n= 4), bars followed by different letters indicate significant difference at $p \leq 0.05$ (Duncan's multiple range test)

## 4 Discussion

Salt stress is a severe abiotic threat that restricts crop plant productivity and is negatively associated with reduced soil organic matter and C:N ratio. It also alters the soil microbial community by decreasing biomass and activity (Yan et al. 2015). Consistent with previous studies by Abou-Zeid et al. (2021) and Ekinci et al. (2022), the findings confirm the negative effects of 150 mM salt stress on wheat plants' growth and physiological characteristics. A significant reduction in growth biomarkers might be referred to the decrease in water availability that induced anomalous alterations in osmotic stress, nutritional imbalance, $Na^+$ toxicity, and variations in photosynthesis (Abou-Zeid et al. 2020). The results demonstrated decreased photosynthetic pigments and photosynthetic parameters (ØPSII, qP and Fv/Fm) under 150 mM salt stress (Figure 4). The osmotic effect of salt stress due to the accumulation of $Na^+$ and $Cl^-$ ions may lead to stomatal closure. At the same time, the disrupted activity of PSII could be attributed to disturbances in the ionic composition within the stroma caused by the influx of both ions, potentially resulting in alterations in the thylakoid structure (Ekinci et al. 2022). These effects may be associated with the inhibitory impact of salt on synthesis enzymes, the elevation of chlorophyllase activity, which is imitative of the damage of the photosynthetic apparatus, as well as the reduction in mineral availability required for chlorophyll biosynthesis (Sahin et al. 2018).

Furthermore, the damage to plant tissues consequently leads to excessive release of ROS, which causes lipid peroxidation, as MDA indicates, disrupting the MSI% (Yan et al. 2018). Numerous studies depicted that increased antioxidant enzyme activities are virtually associated with the salt tolerance of crops (Talaat and Shawky 2014; Azeem et al. 2023). In the current work, 150 mM salinity stress enhanced the activity of SOD and POD, which are involved in scavenging ROS and contribute to plant tolerance (Figure 5). In accordance with the current study, Kahrizi et al. (2012) reported that the induction of SOD, POD is the popular salt sensitivity mechanism for ROS production. Conversely, CAT activity is reduced, which is called the salt effect on the enzyme system (Farouk and AL-Huqail 2022).

The current work aimed to investigate the potential application of OMSW-based biochar in alleviating salinity stress on wheat plants. Biochar is a carbon-rich material produced from organic matter, it exhibits stability in soil, and it has been shown to improve its physicochemical and biological properties, such as physical structure, water holding capacity, and biological activity, ultimately creating favourable conditions for enhanced plant growth even under salt stress (Kapoor et al. 2022).

According to the present results, both OMSW-based biochar concentrations (5% and 10%) improved wheat plant growth, regardless of salinity stress. Li et al. (2022) previously reported that biochar amendment can reduce the entry of $Na^+$ ions into plant cells and ease the negative effects of salinity stress. This beneficial effect can be attributed to its porous structure and the high specific surface area. In this study, the pyrolysis temperature used to produce OMSW-based biochar resulted in a high nutrient content and increased nutrient bioavailability. Additionally, OMSW-based biochar exhibited a high pH value (9.61), which is important for soil pH neutralization and facilitates the accessibility of phosphorus and micronutrients in the soil (Rawat et al. 2019). Furthermore, the high cation exchange capacity of OMSW-based biochar designates its ability to retain and release nutrients, thereby increasing the availability of plant roots′ mineral absorption (Haider et al. 2022).

Corroborating our results with Farouk and AL-Huqail (2022) and Ekinci et al. (2022) findings have reported a reduction in oxidative biomarkers sustaining water relations, boosting redox homeostasis, and antioxidant aptitude, as well as regulating antioxidant enzyme activity and element content in the plant reflect on the affirmative effects of biochar application in mitigating the impact of salinity on borage and cabbage plants. EDX analysis proved that the direct wheat growth improvement of OMSW-based biochar appliance under normal or stressful conditions associated with increasing nutrients such as N, K, Ca, Mg, and Fe could be significant in reducing $Na^+$ via the increase of other elements in soil under salinity or because of the high potential of biochar for adsorption of $Na^+$ (Table 3 and Figure 2). Our results follow those of Ibrahim et al. (2020) and El-Gamal et al. (2021).

Applying OMSW-based biochar at concentrations of 5% and 10% significantly increased photosynthetic pigments in wheat plants under normal growth conditions and under 150 mM salt stress. The maximal mitigation of salinity arbitrated decline was detected in the photosynthetic parameters above the salt-stressed ones in plants treated with OMSW-based biochar. This might be due to OMSW-based biochar increased leaves-nitrogen level, enhanced photosynthetic rate by increasing the chlorophylls biosynthesis, oxidation of water at PSII, and Calvin cycle enzymes-activities besides the increase in the $CO_2$ absorption on account of the stomatal conductance and the nutrient uptake (Akhtar et al. 2015). Consistent with these findings, biochar application under saline conditions has been reported to improve plant growth and photosynthesis in maize and potato plants (Khan et al. 2022; Mahmoud et al. 2022). Biochar protects membrane stability, enhances water uptake, maintains nutrient balance, and reduces the production of ROS through the enhancement of antioxidant activities; consequently, it significantly improves the plant's tolerance to salt stress (Ndiate et al. 2022; Wu et al. 2023). Results of the current work showed an improvement in MSI% along with a reduction in MDA and $H_2O_2$ content (Figure 5B and 5C), as well as the activity of SOD, POD, and CAT in wheat plants treated with OMSW-based biochar under normal and saline irrigation. This observation is in accordance with previous studies by Zhang et al. (2020) and Cong et al. (2023) on soybean and maize plants, respectively. Contrarily, the findings of Ufkay et al. (2021) indicate that a heightened application rate of biochar may exacerbate the detrimental impacts of salinity on soil. This exacerbation is attributed to the cumulative levels of soil EC, which can cause a transition from beneficial to detrimental conditions for plant growth.

## Conclusion

The application of OMSW-based biochar in soil subjected to 150 mM salt stress demonstrated beneficial effects on wheat plant growth by mitigating the adverse impacts of salinity. The effectiveness of biochar was strongly influenced by its properties, including CEC, EC, porosity, functional groups, mineral content, and application rates. Salinity stress reduced growth, RWC%, MSI%, photosynthetic pigments, and photosynthetic parameters (ØPSII, qP, and Fv/Fm). Salinity induces oxidative stress, as evidenced by increased MDA, $H_2O_2$, and antioxidant enzyme activity levels. However, these detrimental effects were alleviated by supplementing OMSW-based biochar at 5% and 10% concentrations. The study's findings highlight the positive interactions between salinity stress and biochar, which positively influence wheat plants' growth biomarkers and physiological characteristics. The application of OMSW-based biochar holds significant potential for enhancing plant growth in salt-stressed soil.

## Conflict of Interest

The authors disclose that they have no conflicts of interest to declare.

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