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ORIGINAL RESEARCH

Counteracting negative effects of salinity on *Lepidium sativum* L. seedlings by prepared biochar

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Abstract

Purpose: Biochar is a carbon rich material that showed positive outcomes on plant growth and productivity enduring abiotic stresses. The objective of the present investigation is thus to determine the potential of biochar to mitigate the detrimental impacts of salinity in *Lepidium sativum*.

Method: Salinity stress was induced by NaC1 at different concentrations ranging from 0 to 5000 mg/L. Biochar was applied in two concentrations: 0.5 and 1%.. For biochar preparation, dry rice straw was heated at 400 ºC at certain pyrolysis conditions.

Results: The study established that salt medium significantly reduced seed germination and amylase activity, with the highest decrease of 63 and 50.6%, respectively, at 5000 mg/L. The relative permeability of the cell membrane was associated with substantial increases in lipid peroxidation and

hydrogen peroxide. The free radicle scavengers' total phenolic, flavonoid, and proline levels were also induced. The use of prepared biochar at 0.5 and 1% reduced the damaging effects of salt stress by enhancing the activity of the α-amylase enzyme, resulting in a significant rise in germination (95% at 5000 mg/L by 0.5% of biochar). In contrast, the application of 0.5% biochar at 5000 mg/L significantly decreased MDA and hydrogen peroxide concentrations to 24.4 mg/g f wt and 1.39 mM/g d wt, respectively, compared to 48.21 and 1.77 in the control. Positive relationships between the multiple data revealed the largest augmentation of germination, dry weight, and antioxidant chemicals in stressed seedlings with 0.5% biochar. Biochar alleviated the hazardous effects of NaCl on *L. sativum* by decreasing free radicle formation and lipid peroxidation, thereby enhancing germination and early growth.

Conclusion: The positive impact of biochar on salt stressed seedlings may underline its potential to have opposing NaCl consequences on development and sustain growth.

Keywords: Antioxidant compounds, Biochemical constituents, Oxidative stress, Proline, Tolerance

Introduction

In arid and semi-arid environments, numerous abiotic stressors affect field crops. Salt stress is one of the stressors that damages crops significantly and lowers their yield globally (El-Bially et al. 2022; Bakhoum et al. 2023; Semida et al. 2023). Numerous morphological, physiological, metabolic, and gene expression processes are impacted by salinity as an abiotic stressor (Sofy et al. 2021; Sadak and Dawood 2023). Salinity in the plant root zone causes osmotic, ionic, and oxidative stress in plants (Hussain et al. 2017; Sadak et al. 2023), which in turn reduces growth and yield of the plant (Rahman et al. 2016; Sadak 2023). Goharrizi et al. (2020) demonstrated that salinity stress may negatively impact the seed germination percentage, shoot and root lengths of various ecotypes. Osmotic stress also resulted in a decrease in the external water potential, a reduction in the capacity of plants to absorb water, and a variety of physiological changes (Sadak et al. 2022), including disruption of membranes, nutrient depletion, an impact on cell growth, stomata closure, a decrease in the plant's capacity to assimilate CO_2, a decrease in photosynthetic activity, and a reduction in the plant's capacity to detoxify reactive oxygen species (Quamruzzaman et al. 2021; Ragaey et al. 2022). Depending on the degree of stress, the stage of growth and the species' susceptibility, field crops are subject to a range of biotic and abiotic stresses that can have an impact on their metabolism, growth, and function (Makhlouf et al. 2022).

Reactive oxygen species (ROS) are produced when plants are exposed to a variety of abiotic stimuli, such as salinity and drought. So, normal plant growth is disrupted as a result of these stresses (Sharma et al. 2019; Abd El-Hameid and Sadak 2020). Hyperosmotic stress and ionic imbalance caused by salinity expose plants to oxidative damage by producing ROS such as hydrogen peroxide, superoxide, and hydroxyl radicals (Bose et al. 2013; Dawood et al. 2017; El- Bassiouny et al. 2020). These processes pose a serious threat to proteins, lipids, photosynthetic pigments, nucleic acids, and cell membranes (Ahmad et al. 2010; Sadak et al.,2016). Since hydrogen peroxide is a diffusible molecule, it has the potential to cross cell membranes and cause damage to the cells (Sadak 2016; Kordrostami et

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al. 2017). As well, a change in the activities of enzymatic antioxidant defense systems like APX, GPX, and SOD and non-enzymatic antioxidant defense systems such as the contents of total phenolic, flavnoids, and anthocyanin, total free amino acids and soluble carbohydrate occurred under salinity (Goharrizi et al. 2020).

Applying biochar under salt stress circumstances lessens salt-induced harms by reducing Na-uptake and enhancing nutritional uptake (Chganti and Crohn 2015). Yang et al. (2021) and Khanam et al. (2022) found that applying 10/tha of biochar boosted tropical rice output by enhancing the physical properties of the soil. Biochar can improve soil organic matter and lessen the impact of water stress on plant growth (Hou et al. 2023). Garden cress (*Lepidium sativum* L.) is regarded as a key therapeutic plant. According to Ramadan and Oraby (2020), glycosides, carbohydrates, proteins, minerals, and fiber of their seeds and leaves can be used for therapeutic purposes, such as treating inflammation, bronchitis, and due to their diuretic, aperient, and aphrodisiac properties, cress is regarded as a significant medicinal plant (Mali et al. 2007; Sharma and Agarwal 2011; Adera et al. 2022).

The type of biomass feedstock and the pyrolysis conditions determine the characterization of the biochar. The main characteristics of the biochar are its distinct surface area and physico-chemical properties, which prioritize its effectiveness in targeting pollutants in industrial wastewater treatment through adsorption, precipitation, and redox reaction with surfaces (He et al. 2022). Biochar is a suitable for effective remove water pollutants, recover nutrients (Wang et al. 2020), regulate urban runoff, and reduce industrial pollution (Chen et al. 2021; Shaheen et al. 2019). Moreover, it can also help achieve carbon neutrality via carbon sequestration (Yang et al. 2021). Hemp biochar significantly improved irrigation water by adsorbing Na and releasing Ca and Mg ions into solution. As the application rate of hemp biochar rose, the sodium adsorption increased (Awan et al. 2021).

Our research aimed to measure the effectiveness of prepared biochar to relieve the negative effects of salt stress during the germination and seedling growth phases. So, we hypothesize that biochar may adsorb sodium injurious ions, reclaiming the agriculture medium. That is because high quality irrigation water ensures high food production and yield. Recycling agricultural water may increase the concentration of water-soluble salts, particularly Na. So, many studies used biochar to reduce Na ions in irrigation water and agriculture soil. It was found that adding biochar (2.5%) to soil (w:w) and irrigating with water with an electrical conductivity of 5 dS m^{-1} increased faba bean output (Rezaie et al. 2019). Furthermore, when biochar of 5 and 10% was added to soil and watered with increasing NaCl concentrations (up to 7.0 dS m^{-1}), the okra yield was sustained at 10% biochar treatment (Elshaikh and She 2018). So, our hypothesis was to use the costless feedstock rice straw, preparing biochar and measuring its ability to adsorb Na from salted irrigated medium.

Materials and methods

Preparation of biochar

Rice straw was washed several times with de-ionized water to remove all dirt.. Filter paper was used to remove the excess water and then the straw rice was dried in the oven at 100 °C.. The dried rice straw was heated to 300 to 400 °C (in a porcelain cup) for three hours in a muffle furnace for slow pyrolysis, preparing biochar. The char materials were then sieved through a 0.5 mm sieve after being ground with

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a mortar and pestle (Sikder and Joardar 2019). Table 1 provides a description of the physico-chemical properties of prepared biochar (PB). Biochar was analyzed in Ecoloy Lab, Faculty of Science, Helwan University. Cairo, Egypt.

Shape 1. a- Rice straw b- Rice straw after grinding c- Biochar

Germination experiment

Lepidium sativum seeds were carefully chosen, sterilized for three minutes with a 2.5% sodium hypochlorite solution, and then completely rinsed with distilled water. For each treatment, a constant number of seeds (20 seeds) were transferred to a sterile petri-dish with one filter paper Whatman No. 1. The petri-dishes were divided into two sets, in the first set, salinity stress was induced by the addition 10 mL of NaC1solutions (0- 1000- 2000- 3000- 4000- 5000 mg/L). In the second set, the biochar was added to the NaCl in petri-dishes in two concentrations 0.5% and 1%. Seven days later, data concerning germination percentage and growth metrics (seedling fresh weight and dry weights (mg), plumule to radicle length ratio, and seedling vigor index) were recorded. A seed was considered to have germinated when the radicle and plumule attained a length of 2 mm (Chartzoulakis and Klapaki 2000). Seedlings were dried in an oven at 65 °C for 48 hours to determine their dry weight. The seedling vigor index was calculated according to Kulkarni et al. (2007). The current study was established at the Laboratory of Plant Physiology, Helwan University, Cairo, Egypt.

Chemical analysis

Chemical analysis of prepared biochar

pH and Ec were measured by pH meter (Jenway 3510) and EC meter (TDS3 TEMP), respectively. Potassium (K) and phosphorous (P) were determined by Microwave Plasma Atomic Emission Spec trometer technique (MPAES, Agilent, Santa Clara, CA 95051, United States). The biochar was digested by a mixture of 1:0.2 of HNO_3-H_2O_2 by volume. The mixture heated at temperature range 450-480 °C for 3 hrs to ensure digestion. Let samples overnight to cool, then the samples were filtered through a 0.45-µm nylon sealed filter membrane. The filtered sample was used for analysis of K and P.

α- Amylase enzyme assay

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Ddinitrosalicylic acid method was used to measure α-amylase activity quantitatively. 1 mL of 1% soluble starch dissolved in pH 5.6 sodium acetate buffer was combined with 1 mL of the enzyme extract. After 15 minutes of 40 °C incubation, the mixture was brought to a boil for 5 minutes with 2 milliliters of 3,5-dinitrosalicylic acid. Using a UV-vis spectrophotometer set at 540 nm, the developed color was measured (Miller 1959).

Total soluble sugars

A known weight of germinated seeds was grounded in 5 mL of 70% ethanol. The supernatant was completed to a known volume after centrifugation. One milliliter of the extract was added to two milliliters of anthrone reagent, and the mixture was kept in a bath of boiling water for three minutes (Umbreit 1959). After cooling the generated color was spectrophotometrically measured at 620 nm

Total soluble proteins

To measure total soluble proteins, one milliliter of freshly mixed (1:1 v/v) solutions of 2% sodium carbonate in 4% sodium hydroxide and 0.5% copper sulphate in 1% sodium potassium tartrate were added to a sample of the extract. The mixture was allowed to stand for 10 minutes before 0.1 mL of Folin was added. After 30 minutes, the mixture's optical density at 700 nm was determined (Lowery etal. 1951).

Proline content

Half gram of germinated seeds were homogenized in 10 mL of 3% aqueous sulfosalicylic acid, and the homogenate was subsequently filtered through Whatman No. 1 paper, in accordance with the procedure described by Bates et al. (1973) for estimating proline. A two-milliliter aliquot of filtrate was heated to 100 °C for one hour along with two milliliters of acid ninhydrin and glacial acetic acid. On ice, the reaction was stopped for fifteen minutes. The reaction mixture was then extracted with 4 mL of toluene and vortexed for 20 seconds. The absorbance of the upper phase at 520 nm was measured with a spectrophotometer.

Lipid peroxidation

Homogenize 0.2 g of sprouts in 3 mL of 50 mM phosphate buffer at a pH of 7.0 in order to quantify the content of lipid peroxidation. After centrifuging the homogenate for 20 minutes, 2.0 milliliters of 0.5% of 2-thiobarbituric acid in 20% trichloroacetic acid were added to a 1.0 milliliter aliquot of the supernatant. The combination was heated for 35 minutes to 96 °C in the water bath, and it was then chilled in an ice bath. The absorbance of the supernatant was measured at 532 nm wavelength following a 15-minute centrifugation period. Additionally, the absorbance measured at 532 nm was subtracted from the quantity of non-specific absorption reported at 600 nm (Heath and Packer 1968).

The Electrolyte Leakage

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The amount of electrolyte leakage was measured using the Hamed et al. (2007) method. 300 mg of plant samples were placed in plastic test tubes together with 15 mL of ultra-pure water. After three hours of incubation in a water bath at 25 °C, an EC meter was used to measure the basic electrical conductivity of these plastic test tubes. The plant samples were heated to 96 °C for 20 minutes, then cooled and the EC of the solution remeasured. The formula utilized to calculate the electrolyte leakage percentage was:

Electrolyte leakage = (EC elementary / EC final) × 100.

Total Phenolic Content

Plant extract and 0.5 mL of Folin-Ciocalteu reagent were combined, and the mixture was held at 25 °C for eight minutes. Following eight minutes, this solution was mixed with 2 mL of 7.5% sodium carbonate solution, and its volume was then increased to eight milliliters using water. The total phenolic content was measured at 725 nm wavelength after two hours (Chun et al. 2003).

Total Flavonoids

The total flavonoids were computed using quercetin as standard, which was created by (Vattem et al. 2004). One ml of extract was added to 0.30 mL of 5% $NaNO_2$, and 0.3 mL of 10% $AlCl_3$ was added after 5 minutes. After a 5-minute waiting period, 2 mL of 1 M NaOH was added, and 10 mL of distilled water was added to the mixture. The total flavonoids content was determined using a 510 nm wavelength (Zhishen et al. 1999).

H_2O_2 content

The H_2O_2 concentration was evaluated using a modified method developed by Christou et al., after 0.5 g of the germinated seeds were ground in 5 ml of 0.1% TCA. Supernatant was added to 1.5 mL of the assay solution, which was made up of 0.5 mL of 10 mM potassium phosphate buffer (pH 7.0) and 1 mL of 1 M KI. After the assay solution was gently mixed, the absorbance of the mixture was measured at 390 nm (Christou et al. 2013).

Statistical analyses

Data were analyzed by one-way ANOVA with SPSS. The ANOVA test will tell you whether there is a significant difference between the means of two levels (biochar treatment and salt treatment) of variables. The Dunkun post hoc test is conducted for multiple comparisons, shows how groups differed from each other with significant p value at $p < 0.05$.

Results and discussion

Chemical characteristics of Biochar

Prepared biochar analysis revealed that biochar pH is slightly alkaline. It has carbon organic matter and inorganic salt (Table 1). The highest percentage was for organic carbon 60.5%. Potassium and phosphorous content was 3.8 and 0.72 ppm, respectively. This result indicated by Ding et al. (2016) that biochar contains accessible N, P, and K. As a result, biochar may be used as fertilizer providing

minerals that absorbed by plants. The minerals in biochar provide nutrients to soil and plants via leaching. Many of the observed effects in agricultural applications have been connected to biochar's improved nutrient-use efficiency and supply (Laird et al. 2010). Despite its low accessible N concentration, biochar is important in controlling N availability in soil because it may affect several N forms and activities, either directly or indirectly. The increased availability of P in biochar is of special importance. *Lantana camara* biochar contains 0.64 and 711 mg/kg of phosphate and potassium, respectively (Masto et al. 2013).

Table 1. The physico-chemical properties of prepared biochar

The measured property or element	The value
pH	7.9
EC (ds m^{-1})	0.51
Ash content (%)	37.5
Carbon content (%)	60.5
Nitrogen (%)	1.03
K (ppm)	3.8
H (%)	1.84
P (ppm)	0.72

Moreover, biochar also had a high percentage of organic carbon which is effective in the adsorption of excess elements. Many studies have proved that biochar is low-cost adsorbent for the removal of different contaminants (Rosales et al. 2017). The production budget of biochar in relation to the type of feedstock biomass and production method was assessed for typical biochar around 0.076 $/kg which is nearly 3-6% of the price of other commercial carbon based adsorbent (Yoder et al. 2011). In the same way, biochar was effective in the current work to adsorb sodium ions, alleviating its deleterious effects on *Lepidium sativum* L.

Germination percentage and growth parameters

Seeds that were exposed to different levels of NaCl showed a significant ($P < 0.05$) decrease in their germination percentage. The germination percentage reached 34% at a concentration of 5000 mg/L of NaCl (Fig. 1). Seeds that were exposed to NaCl showed a significant ($P < 0.05$) decrease in their germination percentage, showing the lowest percent 34% at 5000 mg/L of NaCl (Fig. 1). Different levels of NaCl negatively affected all studied traits, including plumule and radicle lengths, fresh and dry weights, and SVI. For plumule length, it reached 1.68 cm at 5000 mg/L compared to control 3.15 cm (Fig. 2 and 3). The greatest reduction was in SVI at a high NaCl concentration of 5000 mg/L (Fig. 4). The water content in stressed plant tissues decreased to one-third of the control water content (Table 2). Among the salinity-causing water-soluble salts, Na^+ and Cl^- are considered the primary ions that contribute to soil salinity, while an excess of Na^+ among exchangeable cations increases sodicity (Foronda 2022). The negative effects of salt stress on plants can be seen in germination (impairing), morphology (slowed growth, chlorosis), physiology (photosynthesis inhibition and nutrient imbalance),

and biochemical properties (oxidative stress, electrolyte leakage, and membrane disturbance) (Ji et al. 2022).

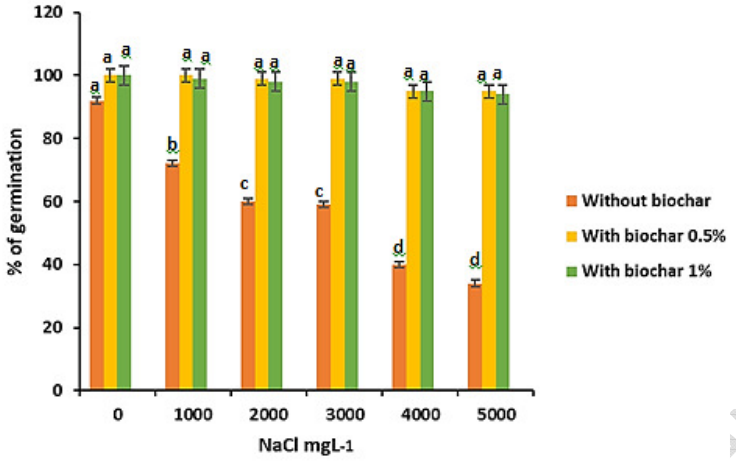

Figure 1. Effect of the prepared biochar application (5 and 10%) on germination of *Lepdium sativum* L. seeds grown under salinity stress conditions.

Similar letters indicate that means were not different significantly at 5%, probability based on Duncan's test.

The plant cell shrinks and dehydrates immediately under salt stress, so cell elongation and, to a lesser degree, cell division are impaired, resulting in slower rates of root and leaf development. Changes in the cell-water relationship caused by osmotic changes outside the root (the osmotic effect), trigger this reaction. The toxic effects of Na^+ led to the unviability of embryo in seeds, growth inhibition of leaves, roots, stems, bolls, and other organs and even mortality (Daszkowska-Golec 2011; Guo et al. 2015; Ren et al. 2021). Abiotic stressors inhibit plant growth by interfering with several physiological and biochemical processes, including photosynthesis, hormone signaling, and antioxidant systems (Sharma et al. 2016). Salt stress induced changes in the photosynthesis and metabolic profiles of one tolerant (Bonica) and one sensitive (Black beauty) eggplant cultivar (*Solanum melongena* L.) (Hannachi et al. 2022).

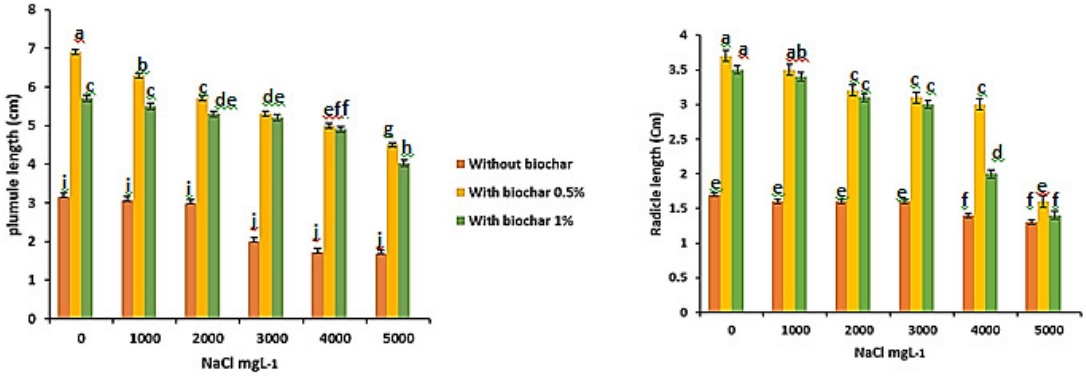

Figure 2. Effect of the prepared biochar application (5 and 10%) on plumule and radicle lengths of *Lepdium sativum* L. seedling grown under salinity stress conditions.

Similar letters indicate that means were not different significantly at 5%, probability based on Duncan's test.

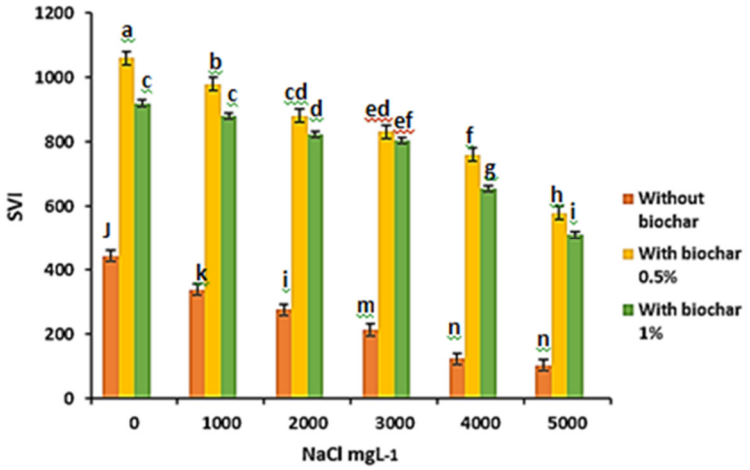

Figure 3. Effect of the prepared biochar application (5 and 10%) on seedling vigor index ratio of *Lepdium sativum* L. grown under salinity stress conditions.
Similar letters indicate that means were not different significantly at 5%, probability based on Duncan's test.

Total soluble sugars and proteins contents and α-Amylase enzyme activity

The gradual reduction of the amount of total soluble sugars and proteins as the concentration of NaCl increased in *Lepdium sativum* L. was shown in Fig. 4. We noted the lowest level at high concentrations of NaCl (4000 and 5000 mg/L). According to Plaut 2006 findings, the decline in total soluble protein content may be related to either a rise in protease activity or a fall in nitrate reductase activity, which affects the absorption of nitrogen. Such metabolites may also be reduced as a result of decreased carbon and nitrogen content, decreased synthesis, or increased protein breakdown (Monteiro et al. 2009). In *L. sativum* L. seedlings, there was an inhibition of the α-amylase hydrolytic enzyme activity by increasing NaCl from 1000 to 5000 mg/L, which inhibited the α-amylase hydrolytic enzyme activity (Fig. 5). The activity of α-amylase was slowed down by 50.62 percent when the concentration of NaCl was raised to 5000 mg/L. According to Adda et al. (2014), seed germination is affected when the activity of hydrolytic enzymes drops and food doesn't reach the embryo. Salt stress reduced the activity of α-amylase, leading to a decrease in the hydrolysis of starchy endosperm in seeds. The low water potential of the external medium influences the hydrolysis process. The mobilization of seed reserves is impacted by salinity, and this is because hydrolases, especially amylases, activate slowly (Ashraf et al. 2002; Oliveira-Neto et al. 1998). Amylase is the primary enzyme responsible for hydrolyzing starch, which promotes seedling establishment. Starch is a readily available source of energy and metabolites for seedling growth (Yu et al. 2015). Amylase activity is reduced as a result of a hormonal shift in the seeds that lowers gibberellin levels and, in turn, decreases the sugar content and prevents the use of reserves (Li et al. 2019).

Table 2. Effect of the prepared biochar application on seedling fresh and dry weight (mg) and water content of *Lepdium sativum* L. grown under salinity stress conditions.

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Treatments		seedling fresh weight	Seedling dry weight	Water content
Biochar %	NaCl conc mg/L			
0	0	33.7 ± 0.87^{a}	1.26 ± 0.04^{bcde}	32.44 ± 0.84^{a}
	1000	24 ± 0.62^{c}	0.98 ± 0.02^{fg}	23.02 ± 0.6^{d}
	2000	17 ± 0.44^{de}	0.78 ± 0.01^{gh}	16.22 ± 0.44^{f}
	3000	14 ± 0.37^{ef}	0.75 ± 0.01^{gh}	13.25 ± 0.42^{g}
	4000	13.8 ± 0.34^{ef}	0.72 ± 0.01^{h}	13.08 ± 0.4^{g}
	5000	12 ± 0.26^{f}	0.62 ± 0.01^{h}	11.38 ± 0.25^{h}
0.5	0	35 ± 0.9^{a}	1.53 ± 0.04^{a}	33.47 ± 0.87^{a}
	1000	32 ± 0.8^{b}	1.46 ± 0.04^{ab}	30.54 ± 0.71^{b}
	2000	30 ± 0.8^{b}	1.30 ± 0.04^{abcd}	28.99 ± 0.68^{b}
	3000	30 ± 0.8^{b}	1.26 ± 0.03^{cde}	26.55 ± 0.61^{b}
	4000	20 ± 0.6^{d}	1.19 ± 0.02^{ef}	18.81 ± 0.55^{e}
	5000	19.6 ± 0.5^{d}	1.10 ± 0.02^{cdef}	18.5 ± 0.49^{e}
1	0	34 ± 0.9^{a}	1.46 ± 0.04^{ab}	32.54 ± 0.87^{a}
	1000	27 ± 0.7^{cb}	1.40 ± 0.03^{abc}	25.6 ± 0.58^{c}
	2000	26.6 ± 0.6^{cb}	1.33 ± 0.02^{abcde}	25.27 ± 0.55^{c}
	3000	20 ± 0.6^{d}	1.16 ± 0.02^{def}	18.84 ± 0.49^{ef}
	4000	20 ± 0.6^{d}	1.13 ± 0.02^{def}	17.20 ± 0.46^{ef}
	5000	17.2 ± 0.4^{d}	1.00 ± 0.01^{f}	16.35 ± 0.44^{ef}
L.S.D at 5%		3.2	0.22	1.8

Data shown in the table represent the mean ± standard deviation, followed by a small letter; similar letters indicate that means were not different significantly at 5%, probability based on Duncan's test.

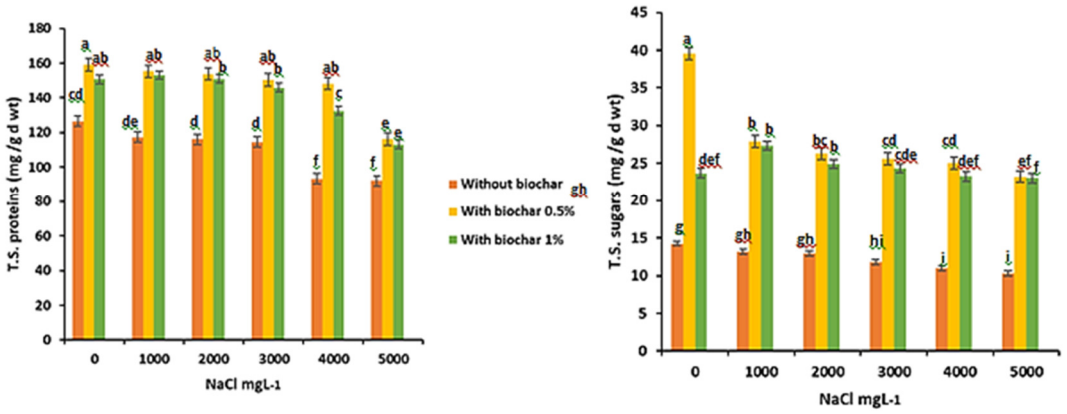

Figure 4. Effect of the prepared biochar application (5 and 10%) on total soluble proteins and sugars content in *Lepdium sativum* L. seedling grown under salinity stress conditions.

Similar letters indicate that means were not different significantly at 5%, probability based on Duncan's test.

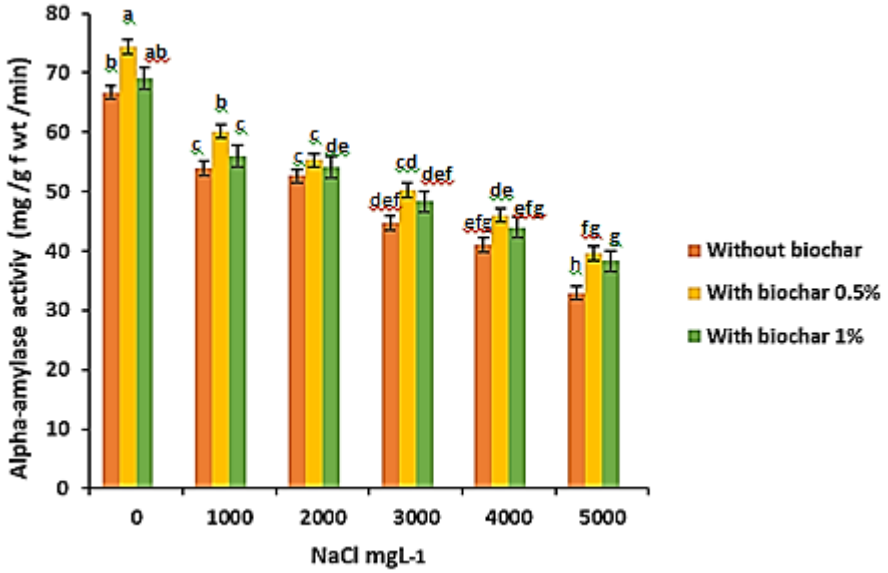

Figure 5. Effect of the biochar application (5 and 10%) on total α-amylase activity in *Lepdium sativum* L. seedling grown under salinity stress conditions.

Similar letters indicate that means were not different significantly at 5%, probability based on Duncan's test.

Electrolyte leakage, Lipid peroxidation levels and H_2O_2 content

Elevating NaCl concentrations significantly ($P < 0.05$) increased the H_2O_2 and MDA content (Fig. 6). The most notable increment occurring at 5000 mg/L of NaCl was 46.28% for hydrogen peroxide and 134.94% for lipid peroxidation relative to control. The electrolyte leakage showed the same trend of H_2O_2 and MDA content under different NaCl concentrations (Fig. 7). Plants under salinity stress face hyperosmotic stress, ionic imbalance, and reactive oxygen species such as superoxide, hydrogen peroxide and hydroxyl radicals, which can reveal oxidative damage (Bose et al. 2013). According to Ahmad et al. (2010), these reactions have the potential to seriously damage proteins, nucleic acids, lipids, photosynthetic pigments, and cell membranes. Diffusible molecules like hydrogen peroxide have the ability to pass through cell membranes and damage cells (Kordrostami et al. 2017). Salt stress disrupts a number of enzymatic processes, including photosynthesis, membrane structure, hormonal balance, water and nutrient uptake, and it can also cause oxidative stress, ionic toxicity and osmotic stress, as demonstrated by Ragaey et al. (2022). According to a number of studies such as Wang et al. (2016); Ahmad et al. (2016) (2017), (2018), (2019); Kaur et al. (2018), plants that are subjected to salinity stress exhibit significantly higher H_2O_2 contents, which is in agreement with the current study findings. These conditions include essential physiological and biochemical disruption as well as severe cellular damage from excessive reactive oxygen species generation (Ghafar et al. 2021; Perveen et al. 2021). The synthesis of more MDA as a result of cellular reactive oxygen species, especially hydrogen peroxide, encouraged lipid peroxidation of cellular membranes in salt stressed plant tissues (Kazemi et al. 2019).

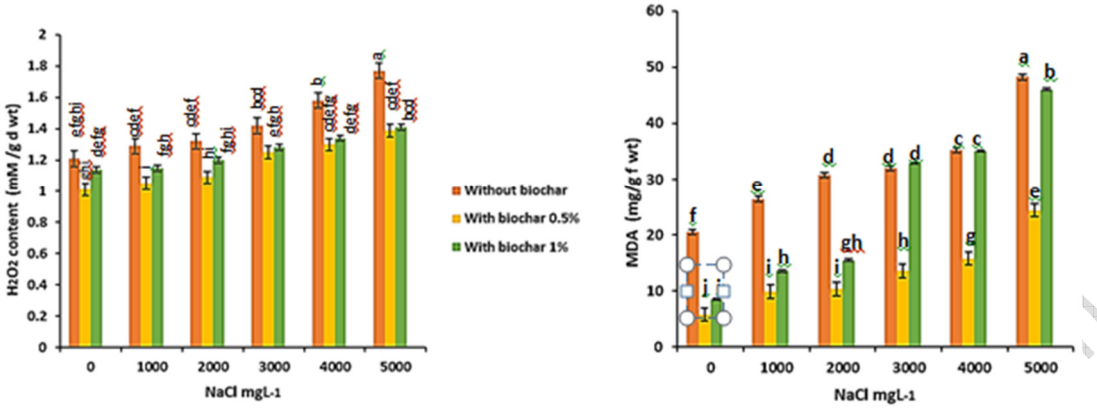

Figure 6. Effect of the prepared biochar application (5 and 10%) on hydrogen peroxide and MDA content in *Lepdium sativum* L. seedling grown under salinity stress conditions.

Similar letters indicate that means were not different significantly at 5%, probability based on Duncan's test.

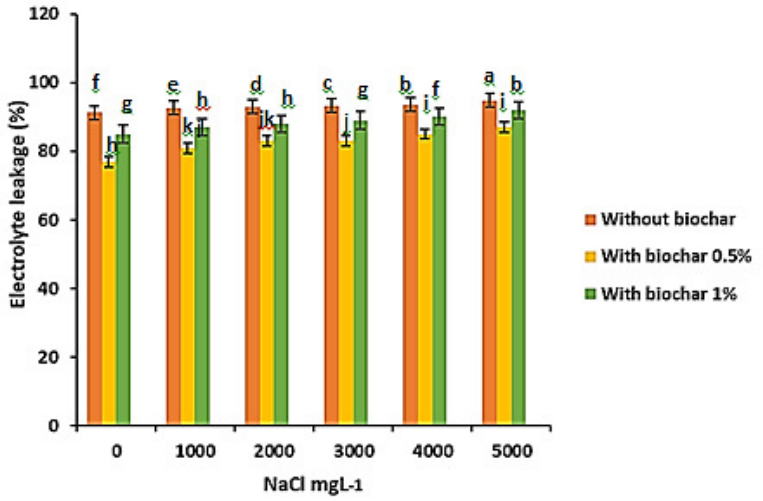

Figure 7. Effect of the biochar application (5 and 10%) on electrolyte leakage of *Lepdium sativum* L. seedling grown under salinity stress conditions.

Similar letters indicate that means were not different significantly at 5%, probability based on Duncan's test.

Flavonoids and phenolics content

In this study, the content of flavonoids and phenolics was assessed at various salt concentrations (Fig. 8). The results showed that the phenolic content increased by 100% and the flavonoid content increased by 131% mg/g dry wt at 5000 mg/L NaCl. The biochar treatment decreased phenolics and flavonoids content at 0.5 and 1.0%. The highest reduction was 76.6% at 0.5% biochar treatment compared to 15% in phenolics content compared to the control at 5000 mg/L. Owing to ionic stress during salinity, the biosynthesis pathway of total phenolic content may be built up. Plants produce more flavonoids under abiotic stress situations in order to combat the adverse stress circumstances (Sharma et al. 2019). Sharma et al. (2019) demonstrated that salinity stress can activate the pathways involved in the biosynthesis of phenolic compounds, ultimately leading to increased resistance to abiotic stress.

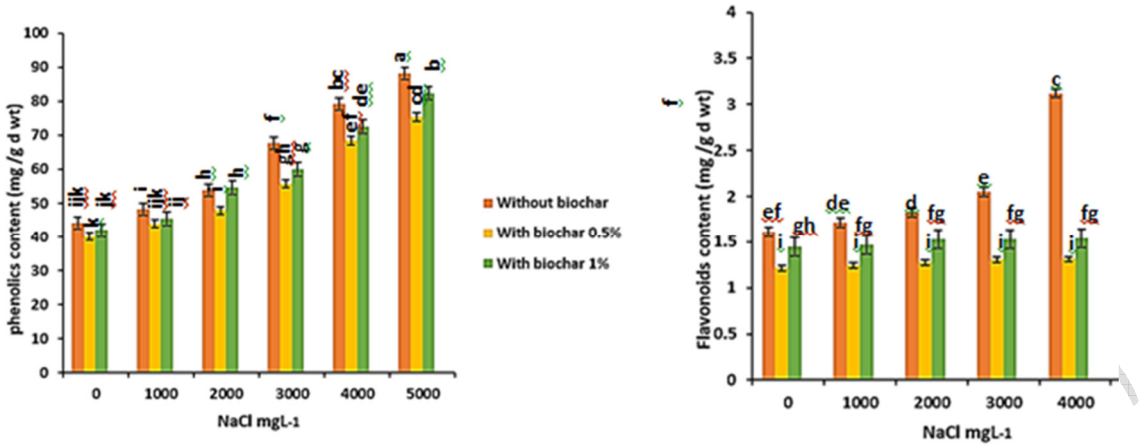

Figure 8. Effect of the prepared biochar application (5 and 10%) on antioxidant compounds (phenolics and flavonoids) content in *Lepdium sativum* L. seedling grown under salinity stress conditions**.**

Similar letters indicate that means were not different significantly at 5%, probability based on Duncan's test.

According to earlier studies, there is a considerable increase in total phenolic content as salinity concentrations rise (Akbari et al. 2018). The phenylpropanoid pathway, which produces phenolic chemicals in plants, can be stimulated in situations where there is salinity tension (Lim et al. 2012). The overexpression of the enzyme phenylalanine-ammonia-lyase (PAL) in response to the mechanical stress caused by the incision may be the cause of the rise in total phenols (Ciriello et al. 2021).

Proline content

The present study found a positive response of proline content in relation to NaCl dose. When salt increases, the concentration of proline in the plant tissue also increases. The proline content increased by 51.92% at 5000 mg/L compared to the control (Fig. 9). Proline is the most prevalent endogenous osmolyte accumulated under a variety of abiotic stresses, including salinity. Proline accumulation is a crucial metric for assessing the impact of stress on plants. It may be linked to a reduction in protein synthesis (Szabados and Savouré 2010; Slama et al. 2015). The findings of Zheng et al. (2016) that proline accumulation and salt tolerance have a negative connection are supported by these results. Thus, proline biosynthesis seemed to happen most likely as a result of a disruption in cell homoeostasis and/or an increase in the utilization of products from photosynthesis for proline biosynthesis at the expense of plant development (Silambarasan and Natarajan 2014). According to Jacobs et al. (2003) and Moradi and Ismail (2007), proline is a sign of salt stress injury rather than a resistance signal. Others have indicated that proline is a marker of poor performance and higher damage in response to salt stress. According to Yoshiba et al. (1997), proline may accumulate due to a process of stress tolerance whereby its synthesis is continuously increased while its catabolism is inhibited. Nonetheless, there is debate over its function in transferring stress resistance in salinized environments. Proline serves as an osmoticum, a scavenger of free radicals, protects cytoplasmic enzymes, stabilizes membranes, tampers with the machinery involved in protein synthesis, and serves as an energy sink to control redox potential, according to Younis et al. (2016).

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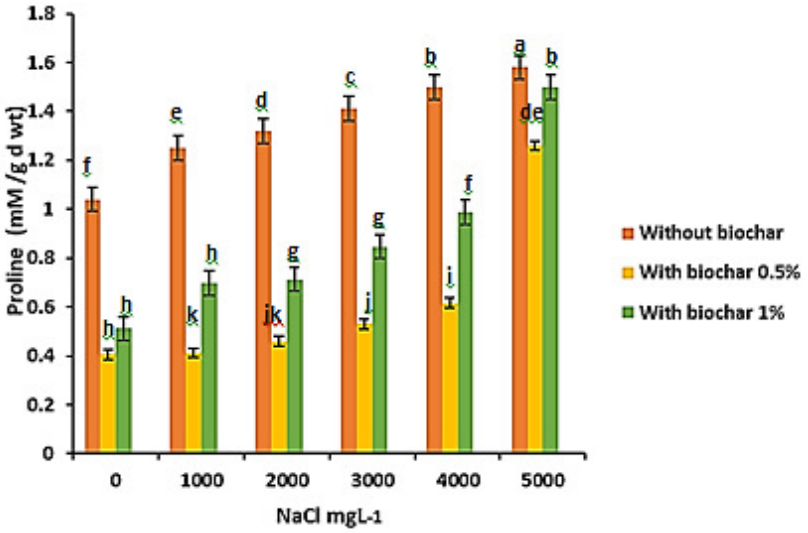

Figure 9. Effect of the prepared biochar application (5 and 10%) on proline content in *Lepdium sativum* L. seedling grown under salinity stress conditions.

Similar letters indicate that means were not different significantly at 5%, probability based on Duncan's test.

Biochar treatment

However the salt stress negatively impacted the germination, growth and metabolic activities of *L. sativum*, biochar treatment amended those negative influences under salt stress. Application of PB at 0.5 and 1% significantly ($P < 0.05$) increased the seed germination at all NaCl treatments, reaching 100% compared to control (Fig. 1). All measured growth criteria of *L. sativum* L. grown under control or saline conditions were improved by PB treatments (Fig. 2, 3, 4). The percentage change in the radicle length at 4000 mg/L NaCl was 81.6% and at 42.8% at 0.5 and 1.0% PB treatment, respectively, indicating more efficiency of 0.5 treatment than 1.0%. SVI, lengths, seedling weights (fresh and dry) and water content (Table 2) showed the same trend for the plumule and radicle. During germination, the addition of biochar, particularly 0.5%, caused the total soluble sugars and proteins to increase from 10.32 to 23.25 and from 92.09 to 116.23 mg/g dry wt, respectively, at 5000 mg/L of NaCl (Fig. 4). In Figure 5, it is clear that adding biochar (0.5%) improved α-amylase activity under control and salt stress conditions by 11.49% and 20.12%, respectively, at 5000 mg/L. However, using biochar combats salinity stress by lowering the levels of H_2O_2, lipid peroxidation, electrolyte leakage and proline content when compared to control values (Fig. 6, 7, 8 and 9). During NaCl salt stress, biochar adsorbed sodium ions lowers the concentration of sodium ions in the soil solution, which in turn reduces the amount of sodium ions that plants absorb (Hammer et al. 2015), reducing salinity stress (Hammer et al. 2015; Lashari et al. 2013; Lashari et al. 2015; Thomas et al. 2013). On the other hand, the addition of biochar improves soil physical and chemical characteristics and mitigates the impact of water scarcity on plant development. Biochar improved the water status and increased the soil's ability to hold water (Akhtar et al. 2015; Saifullah et al. 2018; Yang et al. 2020). Additionally, the biochar increased the amount of nutrients in the soil, including exchangeable potassium, which mitigates the detrimental effects of plant K^+ uptake imbalances caused by salty soils' (Munns 2002; Sarkar et al. 2018), enhancing the nutrient status of the plants (Wu et al. 2019). Biochar reduced bulk density, electrical

conductivity, exchangeable Na^+, and exchangeable Cl^-, which helps rice suffer less from salinity stress (Zhang et al. 2019). According to Atkinson et al. (2010), biochar's high cation exchange capacity, high porosity and high-water retention ability are caused by a few specific properties that also favor nutrient retention, prevent nutrient loss, and, depending on the form of the material, provide a direct source of nutrients.

The use of biochar may decrease the relative permeability of root membranes; considerably reduce lipid peroxidation and H_2O_2 levels. Wu et al. (2023) thought that the presence of biochar would increase membrane stability, with more cations exchanging capability, so a considerable reduction in electrolyte leakage as well as a reduction in the peroxidation of the membrane would improve barley plant conditions. Hafez et al. (2020) found that biochar and chitosan were used to combat drought stress because of their beneficial effects on lowering proline content, lipid peroxidation and electrolyte leakage. Biochar and chitosan may be particularly important in maintaining the stability of plasma membranes, controlling water pressure, increasing relative water content, and lowering oxidative stress. By raising the osmotic values of leaves, biochar improves the plant's water status and raises its resilience to future water stress circumstances, hence improving plant tolerance to a variety of abiotic stresses (Gonzalez et al. 2009; Kammann et al. 2011). By regulating antioxidant activity and osmolyte buildup, the biochar treatment proved to be the most successful soil amendment for preventing oxidation in rice plants (Glaser et al. 2002; Beesley et al. 2010). Those effects of biochar improve morphometric parameters such as seed germination and root and shoot length, reducing salinity stress during plant growth (Majidi 2022). Rice seedlings' salt stress was successfully reduced by biochar, according to Zhang et al.'s 2019 research. According to Yang et al. (2020), biochar also improved quinoa's growth and physiological traits when it was subjected to salt stress and drought, suggesting that it mitigated the effects of both conditions. According to Hou's 2023, salt stress decreased plant physiology and growth; it also decreased the yields of lint and seed cotton by 40.43-58.81% and 19.33-47.22%, respectively. On the other hand, the plant dry biomass allocation ratio increased and salt stress was lessened by the biochar addition. Yang et al. (2020) demonstrated that under conditions of salt stress and drought, biochar enhanced the physiological traits of *quinoa* development, suggesting that the use of biochar mitigated the effects of both conditions. Moreover, biochar treatment boosted grain yield and yield characteristics, according to Chen et al. (2021). According to Zhang et al. (2019), applying biochar improves the anatomical and ultra-structure of roots, which raises leaf mesophyll cell activity, photosynthetic capacity, and dry matter accumulation under salt stress conditions and lowers salt-induced damages.

Correlation between different measured parameters

The correlation between germination percentage, SVI, seedling dry weight and α-amylase activity at 5000 mg/L indicated the beneficial role of PB to alleviate the damaged salt stress effect on seed germination and the activity of α-amylase during germination (Fig. 10). So, we can propose that the biochar is able to improve salt tolerance in *L. sativum* recovering the correlated parameters. Ali et al. (2017) indicated the same result that the biochar increased plant salt tolerance. The figure also represented that the 0.5% PB treatment was superior compared to the control. In Fig. 11, the

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represented correlation indicated the highest accumulation of antioxidant compounds in stressed seedlings at 5000 mg/L (Fig. 11). In the same way, other studies such as Birhanie et al. (2022) study figured out that the phenolics with its high antioxidant properties in *H. cannabinus* extracts was highly related to the antioxidant capacity under salt stress

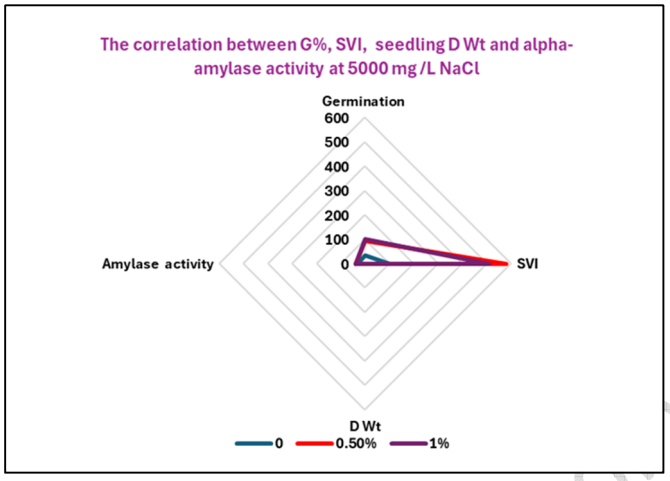

Figure 10. The correlation between G%, SVI, seedling D Wt, and α-amylase activity at 5000 mg/L of NaCl .

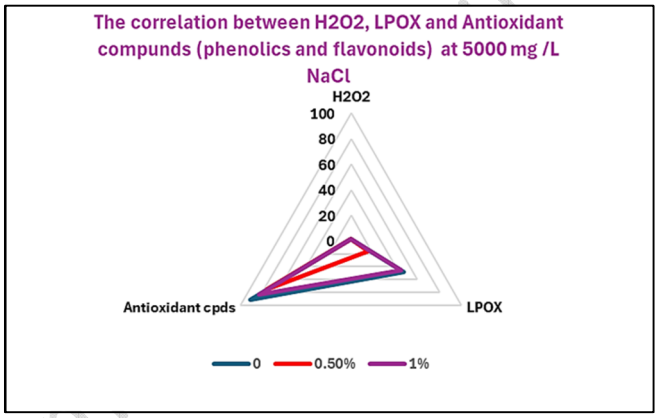

Figure 11. The correlation between H_2O_2 content, lipid peroxidation and antioxidant compounds content at 5000 mg/L of NaCl.

Conclusion

Based on the present results, it could be concluded that application of prepared biochar especially at 0.5% significantly increased α-amylase enzyme activity, soluble sugars and proteins and also alleviated the deleterious effect of salinity stress by significantly decreased proline, lipid peroxidation, hydrogen peroxide content, total phenolic and flavonoid content compared to control. So, the application of biochar is recommended to improve the tolerance of *Lepidium sativum* L. to salinity stress by providing seed nutrition, increasing the biochemical constituents which led to improvement in vegetative growth quality and quantity (see graphical abstract).

Author Contribution: Amira Mohamed Abd El-Sattar designed the study, performed the experiments, analysed the data and wrote the draft manuscript. Zeinab Ashour Shedeed helped perform the

experiments, helped in statistical analysis and wrote the final manuscript.All authors reviewed the final manuscript.

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