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Examining the Effects of Biochar in Spinach Cultivation with Increased Irrigation Water Salinity on Some Physical and Physiological Properties of the Plant

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Abstract – The inadequacy of fresh water resources in today's conditions has revealed the use of salty water as an alternative water source in agricultural irrigation but salinity, which is shown as the most important source of abiotic stress, increases osmotic stress and decreases the water intake of the plant, resulting in a physiological drought. For this reason, there is a need for applications to improve the decreasing efficiency in the use of salt water in irrigation through different practical and economic approaches. This study investigating the effects of biochar application into the soil against irrigation water salinity in spinach cultivation on some physical and physiological properties of the plant was carried out in five-liter pots in greenhouse conditions, in a completely randomized factorial experimental design with three replications, in three different irrigation water salinity levels with NaCl salt source [I0: tap water; 0.4 (control), I1: 2.5, I2: 5 dS m^{-1}] and three different biochar dosage based on weight [B0: 0 (control), B1: 1.5, B2: 3%] conditions. As a result of the study, plant height, leaf number and area, shoot wet weight from physical properties and chlorophyll and leaf relative water contents from physiological properties decreased with increasing irrigation water salinity levels but the membrane damage decreased, while the physical and physiological properties of spinach increased with the increasing mixing ratio of biochar to the soil, excluding membrane damage. It was found that the use of biochar against irrigation water salinity can be recommended considering that mixing biochar to the soil against irrigation water salinity in spinach cultivation regulates the physical and physiological properties of the plant but to obtain more comprehensive information on this subject, evaluation of different irrigation water salinity and biochar ratios in terms of field studies has emerged as a recommendable result.

Keywords –Biochar, Fresh Water, Physical and Physiological Properties, Salinity Of Irrigation Water, Spinach

I. Introduction

Although water is considered as a self-renewable resource in the hydrological cycle, it is actually a limited resource. Especially in recent years, the severity of the pressure on this limited resource has been increasing due to anthropogenic effects such as global warming, population growth, unplanned urbanization and industrialization, and the amount of this already limited resource is getting polluted and decreasing day by day [1]. For this reason, it is necessary to focus on studies on the effective management of water in all sectors, especially in the agriculture sector, which is the largest consumer of water [2] and to use alternative water sources instead of good quality water, which is expressed as a fresh water source in agriculture [3].

As an alternative water source, the use of salt water in irrigation can reduce the pressure on fresh water resources, but the use of salty water in irrigation negatively affects plant physiology, resulting in a stressful situation that reduces yield and quality in plant production. Salty conditions affect the physiological structure of the plant cellularly through osmotic and ionic stress and decrease the water potential of plant cells [4]. In addition, salinity causes the soil structure to be adversely affected and the soil microbial population to decrease [5]. For this reason, for the sustainability of fresh water resources, it is necessity to focus on materials for compensating the yield and quality losses of the plant, accompanied by applications that eliminate or reduce stress in the conditions where salt water is used as an irrigation water source.

Biochar, which emerges as a porous, stable and solid soil conditioning material as a result of thermochemical transformations in anaerobic or oxygen-limited conditions that generally improves the hydraulic properties and nutritional status of the soil [6] is accepted as a promising strategy in reducing the stress on plant physiology caused by the use of salt water in irrigation [7]. In addition to providing basic nutrients to the soil, biochar helps the plant to overcome stress conditions by increasing the uptake of nutrients such as Ca, Mg and K by limiting the plant's Na uptake in saline soils or under irrigation conditions with salty water [8]. With its high adsorption properties, biochar is defined as a good soil improvement product in soils exposed to salinity or irrigation with saline water [9]. In addition, biochar supports the increase of the yield and quality of the plant by improving the physical, chemical and biological properties of the soil [10].

Many studies have investigated the physical, physiological and yield-related properties of different cultivated plants of biochar application to soil against irrigation water salinity. However, considering the differences in the responses and stress thresholds of cultivated plants to salinity, and the complex and unique characteristics of plants' responses to salt stress, and also the limited number of studies in the literature to improve the physical and physiological properties of the plant by applying biochar to the soil of spinach grown in irrigation water salinity, in this study, the effects of mixing different ratios of biochar into the soil of spinach grown under increasing irrigation water salinity levels on plant height, leaf number and area, shoot wet weight, chlorophyll and leaf relative water contents and membrane damage were investigated. This study hypothesizes that increased irrigation water salinity levels would reduce the physical and physiological properties of spinach by stressing the plant but this stress effect could be alleviated by increasing the mixing ratio of biochar to the soil.

II. MATERIALS AND METHOD

The study, which was done in five-liter pots in greenhouse conditions, in a completely randomized factorial experimental design with three replications was carried out under the conditions of mixing the biochar to the soil in three different ratios on the basis of weight [B0: 0% (control), B1: 1.5%, B2: 3%], versus the irrigation of spinach with three different irrigation water salinity levels [I0: tap water; 0.4 dS m^{-1} (control), I1: 2.5 dS m^{-1}, I2: 5 dS m^{-1}]. Thus, the total number of pots in the study was 27 (3 replications × 3 biochar applications × 3 irrigation water salinity applications). In the study, irrigation water salinity levels were determined by considering the yield losses of spinach against irrigation water salinity according to Water Quality for Agriculture-Irrigation and Drainage Paper [11], while biochar doses were obtained by literature review as a result of previous studies.

After the sieved and air-dry the study soil, which has no salinity problem (0.34 dS m^{-1}) and has a medium alkaline (8.19) and low organic matter (1.19%) and total nitrogen (0.08%) content and sandy clay loam texture (sand: 45%, silt: 25%, clay: 30%), was mixed homogeneously with sawdust biochar with salinity, pH, organic matter and total nitrogen of 0.80 dS m^{-1}, 8.88, 45.2% and 0.20%, respectively, it was weighed and placed in pots with sand-gravel material at the bottom. The spinach seeds were sowed in the pots, and all the pots were completed to the field (pot) capacity with the same amount of irrigation water according to the pot capacity determined in the control application. During the process of spinach from seed to seedling, irrigation was carried out with tap water every day in order not to create any stress on the plant, and after this stage, irrigation applications were started with different irrigation water salinity levels. Irrigations were carried out at three-day intervals by applying equal irrigation to all applications, with the approach of completing the reduced moisture in the control application to the pot capacity. In the preparation of saline irrigation waters, 99.5% purity NaCl with high solubility was used because it is the most abundant salt source in nature and the salt that affects plant growth at the most important level, and different irrigation water salinity levels were dissolved in plastic water containers before each irrigation and prepared for irrigation.

Some measurements were made after the spinach plants were harvested. The shoot wet weight and plant height were determined by weighing the weight of the harvested spinach plant and measuring its height with the help of a ruler. The number of leaves was obtained by counting all the leaves of the spinach one by one. Leaf area was determined by measuring the leaves of the spinach with the Licor 3000C leaf area meter and dividing it by the number of leaves. Similarly, chlorophyll measurement was carried out by using SPAD measurements to determine the changing shade of green in randomly selected spinach leaves with the SPAD-502 chlorophyllmeter device. To determine the leaf relative water content, the fresh weights (Fw), the turgor weights (Tw) kept in pure water for 4 hours and the dry weights (Dw) kept in the 65ºC oven for 48 hours were determined and then [(Fw-Dw)/(Tw-Dw)] formulation was used. Disc samples taken from leaves were kept in distilled water for 24 hours at room temperature and their EC was measured, then the same samples were kept in a water bath at 95°C for 20 minutes and cooled to room temperature and their EC was measured, and finally, the membrane damage value was obtained by dividing the first EC value with the second EC value.

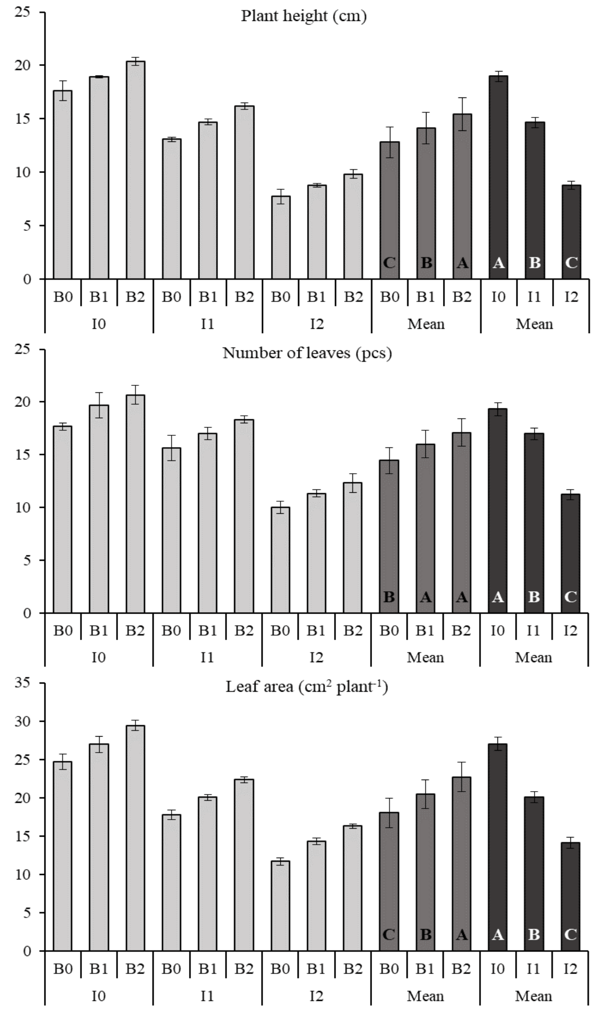
The data obtained as a result of the study were analyzed using the SPSS (Ver. 21) package program. After the data were analyzed with the General Linear Model, the significant averages were separated at the 5% probability level with the Duncan Multiple Range Test. In addition, Pearson correlation analysis was also used to determine the relationships among parameters through the same statistical package program.

III. Results and discussion

The effects of irrigation water salinity levels and biochar applications into the soil on plant height, leaf number and area, shoot wet weight, chlorophyll and leaf proportional contents and membrane damage were statistically significant at $p<0.01$ level (Table 1). While the physical and physiological properties of the spinach examined in this study were significantly adversely affected by increasing irrigation water salinity levels, these properties improved significantly with the increased mixing ratio of biochar to the soil (Fig. 1).

Table 1. The variance analysis results

Parameter	Source	df	Mean square	F	P
Shoot wet weight	**Irrigation (I)**	2	2 387.423	249.045	0.000
	Biochar (B)	2	228.836	23.871	0.000
	I × B	4	5.720	0.597	0.670
	Error	18	9.586		
Plant height	**Irrigation (I)**	2	234.834	370.142	0.000
	Biochar (B)	2	15.868	25.011	0.000
	I × B	4	0.199	0.313	0.865
	Error	18	0.634		
Number of leaves	**Irrigation (I)**	2	156.926	86.469	0.000
	Biochar (B)	2	16.148	8.898	0.002
	I × B	4	0.148	0.082	0.987
	Error	18	1.815		
Leaf area	**Irrigation (I)**	2	376.514	288.722	0.000
	Biochar (B)	2	48.788	37.412	0.000
	I × B	4	0.062	0.048	0.995
	Error	18	1.304		
Chlorophyll	**Irrigation (I)**	2	635.138	254.735	0.000
	Biochar (B)	2	48.429	19.423	0.000
	I × B	4	0.005	0.002	0.999
	Error	18	2.493		
Leaf relative water content	**Irrigation (I)**	2	1 632.450	447.474	0.000
	Biochar (B)	2	90.325	24.759	0.000
	I × B	4	0.238	0.065	0.992
	Error	18	3.648		
Membrane damage	**Irrigation (I)**	2	268.521	453.129	0.000
	Biochar (B)	2	15.501	26.158	0.000
	I × B	4	0.599	1.011	0.428
	Error	18	0.593		



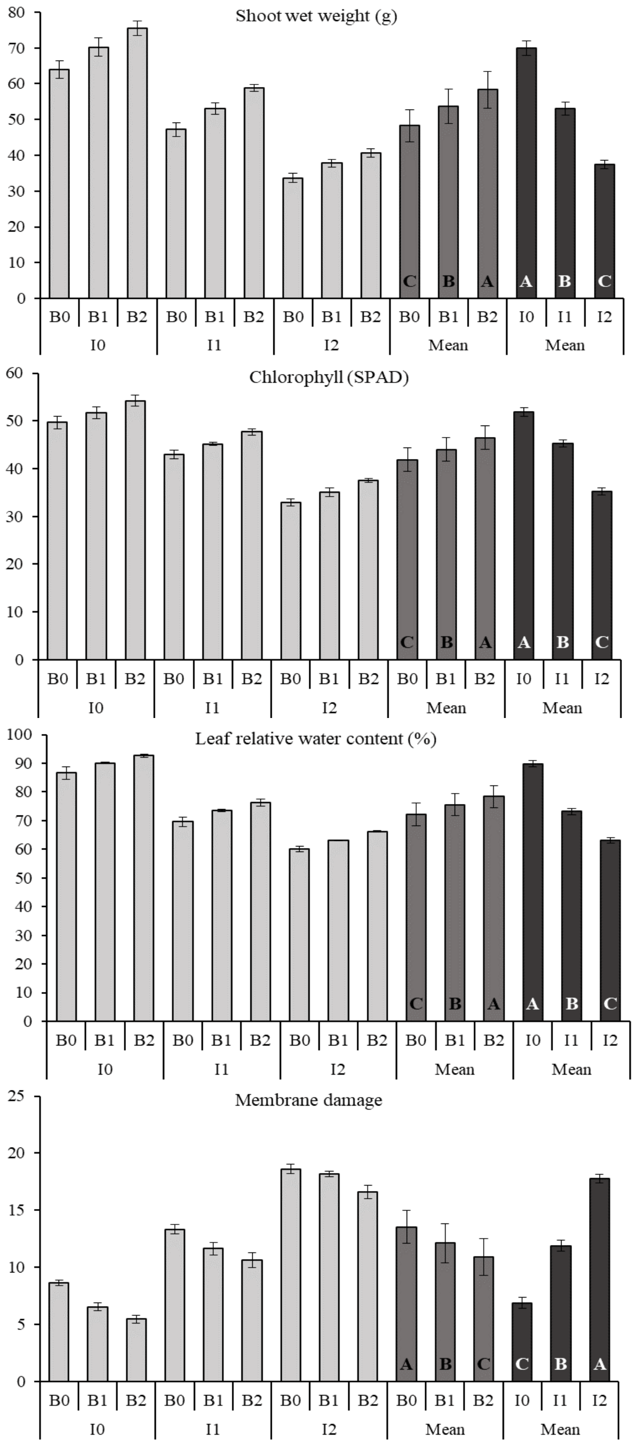


Fig. 1. Physical and physiological properties of spinach based on irrigation water salinity levels and biochar applications

The decrease in plant height, leaf number and area of spinach with increasing irrigation water salinity levels can be explained by the physical weakening of the plant as a result of the plant not getting enough water from soil under salt stress conditions. Salinity, which is an abiotic stress factor, limits the plant's water intake and thus the nutrient uptake also, and weakens the physical development of the plant by negatively affecting the physiological properties of the plant [12]. In addition, ion imbalance during water intake in salty conditions disrupts the physiological structure of the plant and reduces the development of the plant's physical properties [13]. The decrease in the height, number of leaves and area of the plant under salinity or drought stress is a mechanism that the plant resists to reduce stress in combating stress [14]. The first response of the plant exposed to salt stress is to reduce the leaf area and number and to limit its physical properties such as plant height to increase salt resistance [15]. Similarly, Ors and Suarez [16] reported that plant height, leaf number and area of spinach decreased with increasing irrigation water salinity levels. The decrease in the shoot wet weight of spinach with increasing irrigation water salinity can be explained by the decrease in plant height, leaf number and area of spinach under stress conditions. As a result of the decrease in plant height, leaf number and area with stress, the shoot wet weight also decreased. The significant ($p<0.01$) positive correlation relationships of shoot wet weight with plant height, leaf number and area also support this situation (Table 2). In addition, in many studies, it has been stated that with increasing irrigation water salinity levels, spinach's shoot wet weight and/or physical properties related to yield decrease [12, 16, 17].

Table 2. The correlation relationships among parameters

	PH	NL	LA	CH	LRWC	MD
SWW	0.958	0.917	0.981	0.953	0.969	-0.972
PH		0.954	0.958	0.977	0.957	-0.983
NL			0.915	0.944	0.873	-0.930
LA				0.956	0.966	-0.974
CH					0.951	-0.970
LRWC						-0.964

SWW: Shoot wet weight, PH: Plant height, NL: Number of leaves, LA: Leaf area CH: Chlorophyll, LRWC: Leaf relative water content, MD: Membrane damage. Statistical significance level of all parameters is $p<0.01$.

The decrease in the chlorophyll content of spinach with increasing irrigation water salinity levels can be explained by the accumulation of ions as a result of salt concentration and irregularities in the opening and closing of stomata. In addition, considering that chlorophyll is a clear indicator of water stress, the osmotic incompatibility experienced with salinity stress can be evaluated as a different explanation for decreased chlorophyll content of spinach with increasing irrigation water salinity. Ors and Ekinci [4] reported that the chlorophyll functions of the plant are damaged as a result of the plant's inability to provide sufficient water from the soil and the weakening of nutrient uptake under drought stress conditions. Deveci and Tugrul [18] stated that increasing salinity in two different spinach varieties decreased the chlorophyll content of the plant. The decrease in the leaf relative water content of the spinach with increasing irrigation water salinity can be evaluated due to drought stress caused by salinity and limited water uptake from the soil. Considering that the water consumption of the plant has a linear relationship with the leaf relative water content [19], it is expected effect that the the leaf relative water content of the spinach decreases with the increasing salinity stress. Basdic and Kabay [17] stated that increasing salinity in two different spinach varieties decreased the leaf relative water content of the plant. The increase in membrane damage of spinach with increasing irrigation water salinity can be explained by the decrease in the leaf relative water content under increased stress conditions. The significant ($p<0.01$) negative correlation relationship of membrane damage with leaf relative water content also supports this situation (Table 2). The increase in leaf temperature with the decrease in the water content of the leaf may cause damage to the cell membrane systems and increase the index of damage to the cell membrane systems as a result of the damage to the mesophyll cells [20]. Osmotic stress causes a decrease in the leaf relative water content, the closure of stomata, an increase in leaf temperature, damage to the membrane systems of the cell, and cell death at a later stage, respectively [21]. Deveci and Tugrul [18] stated that increasing salinity in two different spinach varieties increased the membrane damage of the plant.

The increase in plant height, leaf number and area, and shoot wet weight of spinach with the increased mixing ratio of biochar to the soil can be explained by the specific surface area feature of the biochar and its nutrient-providing contribution to the soil. The specific surface area of the biochar acts as a mechanism that provides nutrients to the soil, as well as the ability to use soil water and nutrients more efficiently [22]. Biochar adjusts the physical properties of the soil such as water holding capacity, aggregate stability, aeration and bulk density and chemical properties such as nutrient holding capacity, electrical conductivity, soil reaction and cation exchange capacity at a level where the plant can benefit from soil water and nutrients more effectively [23]. In addition, considering the regulating effect of organic matter in the soil water balance, it is possible that the plant height, leaf number and area and shoot wet weight of the spinach increased as a result of the increased mixing ratio of the biochar to the soil and the organic matter in the soil to manage the soil water more effectively and the spinach to benefit from the soil water more effectively. Xiang et al. [24] reported that instead of accumulating root biomass of plants grown in biochar-treated soil, they use the root profile to absorb soil water and nutrients more effectively and reflect this on their yields. In addition, the selective effect of biochar on some elements in the soil solution and removing soluble salts from the root zone also encourages the development of the physical properties of the plant [25]. Similar to the findings of this study, it has been stated in many studies that biochar application regulates the growth of the plant under drought and salt stress and improves the physical properties related to yield [25, 26, 27, 28].

The increase in the chlorophyll content of spinach with the increased mixing ratio of biochar to the soil can be evaluated due to both the biochar's effects on the soil providing nutrients such as N and Fe that encourage the development of chlorophyll, and on the protective effects of soil moisture. Younis et al. [29] stated that the chlorophyll content of spinach grown in biochar soil increased compared to spinach grown in soil without biochar, thanks to the nutrients provided by biochar to the soil and thus to the plant. Akhtar et al. [23] reported that chloroplast development increased as a result of the plant growing in biochar-treated soil accessing soil water more easily. Similarly, as a result of the effect of biochar facilitating the plant's access to water, in this study, it was observed that the leaf relative water content of the spinach increased with the increasing mixing ratio of biochar to the soil. In addition, as a result of the effect of biochar to increase the strength of the bond between soil aggregates, the increase in the leaf relative water content of the spinach with the increasing mixing ratio of biochar to the soil can be explained by the plant's more effective use of soil moisture. Kul et al. [25] stated that the increase in water storage of the plant as a result of the improvement of soil aggregate structure and the development of soil organic matter with the application of biochar increases the leaf relative water content. Similarly, Yildirim et al. [30] also reported that the leaf relative water content of the plant increased with the improvement of the moisture balance in the soil as a result of the increase in the soil organic matter of the biochar. Considering the significant ($p<0.01$) negative correlation relationship of leaf relative water content with membrane damage (Table 2), increasing leaf relative water content with the increased mixing ratio of biochar to the soil can explain the decreased membrane damage. Laghari et al. [8] stated that although biochar reduces the plant's Na uptake from the soil, it increases the uptake of nutrients such as Ca, Mg and K from the soil solution and reduces the damage to the membrane systems even under stress conditions. Similarly, Lyu et al. [31] also reported that the biochar application reduces the membrane damage of the plant under drought stress conditions. Similar to the findings of this study, it has been stated in many studies that biochar application regulates the growth of the plant under drought and salt stress and improves the physiological properties related to yield [22, 32, 33, 34].

IV. CONCLUSION

In this study, which investigated the effects of biochar application into the soil against irrigation water salinity in spinach cultivation on some physical and physiological properties of the plant, plant height, leaf number and area, shoot wet weight from physical properties of spinach and chlorophyll and leaf relative water contents from physiological properties of spinach decreased with increasing irrigation water salinity levels and the membrane damage decreased, while the physical and physiological properties of spinach increased with the increasing mixing ratio of biochar to the soil, excluding membrane damage. Since the mixing of biochar to the soil of spinach grown in irrigation water salinity conditions improves the physical and physiological properties of spinach, in this study, it was found that it can be recommended to mixing of biochar into the soil at a rate of 3% by weight in the cultivation of spinach in sandy clay loamy soil, in the use of salty water in irrigation. However, in order to obtain more comprehensive information on this subject, detailed field studies under different irrigation water salinity and biochar ratios have also emerged as a recommendable result.

REFERENCES

[1] TWB, (2023). The World Bank website. [Online]. Available: https://www.worldbank.org/en/topic/water-in-agriculture

[2] Mahant, M., Shukla, A., Dixit, S., & Patel, D. (2012). Uses of ICT in Agriculture. International Journal of Advanced Computer Research, 2(1), 46.

[3] Cosgrove, W. J., & Loucks, D. P. (2015). Water management: Current and future challenges and research directions. Water Resources Research, 51(6), 4823-4839.

[4] Ors, S., & Ekinci, M. (2015). Drought stress and plant physiology. Derim, 32(2), 237-250.

[5] Mbarki, S., Cerdà, A., Zivcak, M., Brestic, M., Rabhi, M., Mezni, M., Jedidi N., Abdelly C., & Pascual, J. A. (2018). Alfalfa crops amended with MSW compost can compensate the effect of salty water irrigation depending on the soil texture. Process Safety and Environmental Protection, 115, 8-16.

[6] Omondi, M. O., Xia, X., Nahayo, A., Liu, X., Korai, P. K., & Pan, G. (2016). Quantification of biochar effects on soil hydrological properties using meta-analysis of literature data. Geoderma, 274, 28-34.

[7] Hou, J., Zhang, J., Liu, X., Ma, Y., Wei, Z., Wan, H., & Liu, F. (2023). Effect of biochar addition and reduced irrigation regimes on growth, physiology and water use efficiency of cotton plants under salt stress. Industrial Crops and Products, 198, 116702.

[8] Laghari, M., Mirjat, M. S., Hu, Z., Fazal, S., Xiao, B., Hu, M., Chen, Z., & Guo, D. (2015). Effects of biochar application rate on sandy desert soil properties and sorghum growth. Catena, 135, 313-320.

[9] Thomas, S. C., Frye, S., Gale, N., Garmon, M., Launchbury, R., Machado, N., Melamed, S., Murray, J., Petroff, A., & Winsborough, C. (2013). Biochar mitigates negative effects of salt additions on two herbaceous plant species. Journal of Environmental Management, 129, 62-68.

[10] Mao, Q., Hu, B., Agathokleous, E., Wang, L., Koike, T., Ma, M., & Rennenberg, H. (2022). Biochar application improves karstic lime soil physicochemical properties and enzymes activity and enhances sweet tea seedlings physiological performance. Science of The Total Environment, 830, 154815.

[11] Ayers R.S., & Wescot D.W. (1989). Water Quality for Agriculture. Irrigation and Drainage Paper, Food and Agriculture Organization of the United Nations, 29, Rev.1, Rome 173.

[12] Erdem, F., & Celik, S. K. (2018). Effects of irrigation water with different salinity and leaching fraction on spinach (*Spinacia Oleracea* L.) growth, yield anddrainage water quality. Journal of Suleyman Demirel University Faculty of Agriculture, special issue, 73-82.

[13] Ors, S., & Suarez, D. L. (2016). Salt tolerance of spinach as related to seasonal climate. Horticultural Science, 43(1), 33-41.

[14] Blum, A., (2004). Sorghum physiology. In: Nguyen, H.T., Blum, A. (Eds.), Physiology and Biotechnology Integration for Plant Breeding. Marcel Dekker, New York, pp. 141–223

[15] Tardieu, F., Parent, B., Caldeira, C. F., & Welcker, C. (2014). Genetic and physiological controls of growth under water deficit. Plant Physiology, 164(4), 1628-1635.

[16] Ors, S., & Suarez, D. L. (2017). Spinach biomass yield and physiological response to interactive salinity and water stress. Agricultural Water Management, 190, 31-41.

[17] Basdic, M. A., & Kabay, T. (2022). The effects of vermicompost and algae on some physiological components in salt stressed spinach. Turkish Journal of Agricultural and Natural Sciences, 9(1), 188-194.

[18] Deveci, M., & Tugrul, B. (2017). The effect of salt stress on spinach leaf physiological characteristics. Academic Journal of Agriculture, 6, 89-98.

[19] Yazdic, M., & Degirmenci, H. (2018). Effect on leaf water potential and chlorophyll value of different irrigation levels in cotton. Kahramanmaras Sutcu Imam University Journal of Agriculture and Nature, 21(4), 511-519.

[20] Marcinska, I., Czyczyło-Mysza, I., Skrzypek, E., Filek, M., Grzesiak, S., Grzesiak, M. T., Janowiak, F., Hura, T., Dziurka, M., Dziurka, K., Nowakowska, A., & Quarrie, S. A. (2013). Impact of osmotic stress on physiological and biochemical characteristics in drought-susceptible and drought-resistant wheat genotypes. Acta Physiologiae Plantarum, 35, 451-461.

[21] Ozturk, N. Z. (2015). Literature review and new approaches on plant drought stress response. Turkish Journal Of Agriculture-Food Science and Technology, 3(5), 307-315.

[22] Ali, S., Rizwan, M., Qayyum, M. F., Ok, Y. S., Ibrahim, M., Riaz, M., Arif, M. S., Hafeez, F., Al-Wabel, M. I., & Shahzad, A. N. (2017). Biochar soil amendment on alleviation of drought and salt stress in plants: a critical review. Environmental Science and Pollution Research, 24, 12700-12712.

[23] Akhtar, S. S., Andersen, M. N., & Liu, F. (2015). Biochar mitigates salinity stress in potato. Journal of Agronomy and Crop Science, 201(5), 368-378.

[24] Xiang, Y., Deng, Q., Duan, H., & Guo, Y. (2017). Effects of biochar application on root traits: a meta-analysis. GCB Bioenergy, 9(10), 1563-1572.

[25] Kul, R., Arjumend, T., Ekinci, M., Yildirim, E., Turan, M., & Argin, S. (2021). Biochar as an organic soil conditioner for mitigating salinity stress in tomato. Soil Science and Plant Nutrition, 67(6), 693-706.

[26] Haider, G., Koyro, H. W., Azam, F., Steffens, D., Muller, C., & Kammann, C. (2015). Biochar but not humic acid product amendment affected maize yields via improving plant-soil moisture relations. Plant and Soil, 395, 141-157.

[27] Kim, H. S., Kim, K. R., Yang, J. E., Ok, Y. S., Owens, G., Nehls, T., Wessolek, G., & Kim, K. H. (2016). Effect of biochar on reclaimed tidal land soil properties and maize (*Zea mays* L.) response. Chemosphere, 142, 153-159.

[28] Gao, H., Bai, J., Deng, X., Lu, Q., & Ye, X. (2018). Short-term effects of tidal flooding on soil nitrogen mineralization in a Chinese tidal salt marsh. Physics and Chemistry of the Earth, 103, 3-10.

[29] Younis, U., Athar, M., Malik, S. A., Raza Shah, M. H., & Mahmood, S. (2015). Biochar impact on physiological and biochemical attributes of Spinach (*Spinacia oleracea* L.) in nickel contaminated soil. Global Journal of Environmental Science and Management, 1(3), 245-254.

[30] Yildirim, E., Ekinci, M., & Turan, M. (2021). Impact of biochar in mitigating the negative effect of drought stress on cabbage seedlings. Journal of Soil Science and Plant Nutrition, 21(3), 2297-2309.

[31] Lyu, S., Du, G., Liu, Z., Zhao, L., & Lyu, D. (2016). Effects of biochar on photosystem function and activities of protective enzymes in Pyrus ussuriensis Maxim. under drought stress. Acta Physiologiae Plantarum, 38, 1-10.

[32] Akhtar, S. S., Li, G., Andersen, M. N., & Liu, F. (2014). Biochar enhances yield and quality of tomato under reduced irrigation. Agricultural Water Management, 138, 37-44.

[33] Paneque, M., José, M., Franco-Navarro, J. D., Colmenero-Flores, J. M., & Knicker, H. (2016). Effect of biochar amendment on morphology, productivity and water relations of sunflower plants under non-irrigation conditions. Catena, 147, 280-287.

[34] Xiao, Q., Zhu, L. X., Shen, Y. F., & Li, S. Q. (2016). Sensitivity of soil water retention and availability to biochar addition in rainfed semi-arid farmland during a three-year field experiment. Field Crops Research, 196, 284-293.