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**Biochar Improves the Growth and Physiological Traits of Alfalfa and Maize Grown under Salt Stress**

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**Keywords:** Salt stress, biochar, alfalfa, amaranth, maize, total root length, root growth, chlorophyll content.

31 **Abstract**

32 **Purpose:**Salinity is a main factor in decreasing seed germination, plant growth and yi  
33 stress is a major problem for economic crops, as it can reduce crop yields and quality. S  
34 occurs when the soil or water in which a crop is grown has a high salt content.

35 **Methods:**We studied the impact of biochar on plant growth and the physiological prope  
36 alfa, amaranth and maize under salt stress conditions.

37 **Results:**The results showed that the maize, Alfa-alfa, and amaranth under biocha  
38 significantly enhanced the plant height and root morphological traits over the control. Th  
39 significantly increased the total root length, root diameter, and root volume. Compared to  
40 the biochar significantly increased the chlorophyll a, b content, total chlorophyll and  
41 content under salt stress. Furthermore, the biochar significantly increased enzyme activi  
42 under salt stress in the three crops

43 **Conclusions:**Biochar treatments promote plant growth and physiological traits of alfalfa  
44 and maize under salt stress condition. Overall, biochar is an effective way to mitigate sal  
45 in crops. It can help to reduce the amount of salt in the soil, improve the soil structure,  
46 the availability of essential nutrients, which can all help to improve crop yields.

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## 59 Introduction

60 Soil degradation in the Republic of Uzbekistan is mainly caused by salinity;  
61 irrigated lands of Uzbekistan are subject to varying degrees of salinity; this salinity is es  
62 in the Republic of Karakalpakstan, Khorezm, Bukhara, Syrdarya and Jizzakh regions (l  
63 al., 2018). Among soil degradation in the world, soil salinity plays a major role; the o  
64 salinity affects the amount of carbon (C), nitrogen (N), carbon emission, bacterial gene c  
65 actinobacteria, thermophilic and betaproteobacteria in soil (Chao et al., 2021). Soil salin  
66 to soil physical properties; the effects of wind erosion on salts in soil were studied. These  
67 conducted on samples treated with salts in an arid region, and wind tunnel tests and a st  
68 18 m/c were conducted under the influence; according to the results,  $\text{Na}_2\text{SO}_4$ ,  $\text{MgSO}_4$   
69 formed high emissive surfaces,  $\text{Na}_2\text{SO}_4$  and  $\text{Na}_2\text{CO}_3$  crystals appear in sharp form, deh  
70 aggregates for  $\text{MgSO}_4$  were the primary source of dust (Jiadong et al., 2022). Soil  
71 negatively affects soil processes, including carbon-nitrogen ratio, phosphatase, cellul  
72 Deleted: has a negative impact on activity, and microbiological diversity (Shuai et al., 2021). Salinity harms the nutrition  
73 the soil; the use of rhizobacteria with compost in such soils reduces the amount of sodi  
74 the soil, and affects the activity of soil enzymes resulting in the improvement of plant nu  
75 et al., 2022).

76 The drying up of the Aral Sea was caused by global climate change and the irre  
77 water resources since 1960, the poor functioning of drainage collectors and the expansion  
78 agriculture in the region, resulting in a decline in the water level and an enhance in salin  
79 1988). Also, the transition from hydromorphic to automorphic accelerated, and de  
80 became more active. The Aral Sea's drying up, and the soil formation process began in th  
81 dunes (Assouline et al., 2015). Aral Sea, the amount of water-soluble salts in the soil inc

82 Deleted: amount 04-05 g/l to 71,3 g/l; according to studies, the number of microorganisms decreased with  
83 of salts in the soil (Hongchen et al., 2021). A decrease in plant life has been observe  
84 South Aral Sea. Therefore, monitoring and increasing plant life ensure ecologi  
85 (Kochkarova, 2019). The increase of plants in the dry bottom of the island leads to the i  
86 of soil properties; in this regard, the concentration of cations in the soil ( $\text{Ca}^{2+}$ ,  $\text{K}^+$ ,  $\text{Mg}$   
87 Deleted: phosphatase cation exchange capacity, rN environment, enzyme activity (phosphatase, b-glucod  
88 acetylglucosaminidase) in the 0-10 cm layer, when planting plants decreased the conc  
89 basic cations, and electrical conductivity, the activity of enzymes and the number of mic  
90 have increased (Jiae et al., 2020), the widespread introduction of phytoremediation o

94 prevents sand flying (Issanova et al., 2015). In the dry regions of the Aral Sea, the sal  
95 soils is chloride-sulfate; the amount of salts is from 2,09 to 4,21%, the amount of chl  
96 0,59 to 0,82%, sulfate is from 0,68 to 2,24%, sodium is 0,67 from 1,08%, black saxovol  
97 in the studies, its phytomass (58,7 t/ha), 669 16-year-old seedlings per 1 ha, phytomas  
98 1682 saxovol per hectare the seedling is correct (Shakhmatov et al., 2016).

99         Aral Sea, where the study was conducted, does not have complete soil form  
100 moment because if we take into account the participation of 6 factors in the formatio  
101 assume that 1 cm of soil is formed on average in 200 years if it is considered that it has b  
102 since the drying of the island until now, there is still a 1 cm layer in the dry bottom c  
103 There is no soil, and the process of soil formation has started in some areas (Jabbarov et  
104 From this point of view, it can be said that scientists and experts who plan to condu  
105 practical and innovative research in the dry bottom of the Aral Sea should take into  
106 absence of soil cover.

107         Biochar application to soil plays an essential role in promoting nutrient dy  
108 modifying soil pollutants and microbial functions (Muhammad et al., 2018). Biochar co  
109 used in agriculture, promoting soil properties and improving productivity (Day et al.,  
110 effect of biochar is either directly by supplying nutrients to plants or indirectly by im  
111 properties, resulting in increased plant nutrient utilization efficiency (Keeney, 198  
112 improves soil quality and crop productivity; its effect is realized through greater soil mic  
113 enzyme activity, including microbial biomass depending on soil types, urease, alkaline  
114 and dehydrogenase enzyme activity, respectively 21,7%, 23,1%, 25,4% increased to 19,8  
115 et al., 2020), 5 years after the application of biochar, the carbon in the soil increased by  
116 the amount of oxygen increased by 2,81% (Dong et al., 2017).

117         Biochar affects the changes in the physical properties of soils, including improved t  
118 moisture retention properties, enhanced CO<sub>2</sub>, N<sub>2</sub>O and CH<sub>4</sub> gas formation, reduced g  
119 composition when applied to heavy soils, reduced density, increased porosity, increased  
120 use (Masiello, 2015, Kinney et al, 2012, Van Gestel 1992, Zhang 2012). Several studies  
121 that salt stress decreased seed germination (Ashraf and McNeilly, 2004) and plant grow  
122 al., 2015; Menezes et al., 2017; Jabborova et al., 2020; Jabborova et al., 2021a; Kumar et  
123 Jabborova et al., 2021b). Moreover, on the root morphological traits (Wang et al., 2008;  
124 2016; Zeeshan et al., 2020) and physiological properties (Azari et al., 2012; Jabborova et

125 Saddiq et al., 2021; Jabborova et al., 2021e) in plants. Biochar's beneficial impact on pl  
126 (Jabborova et al., 2021c Jabborova et al., 2022) and enzyme activities in soil (Jabborova  
127 Song et al., 2022) were investigated in various plants under normal and stress condition  
128 Rashidabad et al. (2009) investigated that biochar improvesmungbean's physiological and  
129 traits under salt stress. The present study evaluated the beneficial impact of biocha  
130 amaranth and maize growth and physiological characteristics under salt stress conditions.

## 131 **Materials and Methods**

### 132 **Biochar, soil, and seed**

133 The municipal solid waste biochar was obtained from the Soil Sciences department  
134 faculty, NUU. Pyrolysis of municipal solid waste biochar was carried out at 500 °C for  
135 municipal solid waste biochar traits are shown in Table 1.

136 Sand samples were collected from the dry bottom of the southern part of th  
137 Analysis of the sand's physicochemical properties and the salinity level of the sands in th  
138 of the Aral Sea are shown in Table 2 and Table 3.

139 Alfa-alfa (*Medicago sativa* L.), amaranth (*Amaranthus caudatus* L.) and maize (*Zea m  
140 were usedfor field experiments.*

### 141 **Experimental design**

142 The Experimental work was conducted on the dried bottom of the Aral Sea. The exp  
143 conducted to study the effect of biochar on the growth, root morphological and physiolog  
144 alfalfa, amaranth and maize. The experiment was carried out in randomized block desig  
145 replications experiments in the dried bottom of the Aral Sea. Experimental treatments in  
146 Alfa-alfa (Control), T2 - Alfa-alfa (Biochar), T3 - Amaranth (Control), T4 - Amaranth (

47 - Maize-(Control), and T6 - Maize (Biochar). After forty days, plants were harveste ,  
148 height, shoot and root fresh weights were measured.

### 149 **Measurement of root parameters**

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151 The roots of alfalfa, amaranth, and maize were cleaned with water with extreme caution.  
152 of the root system was dissected using a scanning system, and the results were evalua  
153 help of the Win RHIZO program.

#### 154 **Measurement of physiological traits**

155 Photosynthetic pigments in alfa-alfa, amaranth and maize were measured spectrophotor  
156 the method of Hiscox and Israelstam (1979). The relative water content of leaves  
157 amaranth and maize was analyzed by the method of Barrs and Weatherly (1962).

#### 158 **Analysis of soil nutrients**

159 The agrochemical parameters of the soil were analyzed after cultivation. The carbon (C)  
160 humus content were analyzed using the modified method by Tyurin (Soils, 2003) The P  
161 N contents in soil were analyzed by the method (Soils, 2002; Soils, 2005).

#### 162 **Analysis of soil enzymes**

163 Urease activity of soil was determined using the method by Pancu and Gautheyrou,  
Deleted: sample 64 samples (2.5 g) were added with toluene (0.5) mL for 15 min. After mixing, urea (2.5 mL  
165 buffer were addedto 5 mL. Incubator at 38°C for 24 h. The urease activity was determ  
166 spectrophotometer. The soil enzyme activities (Invertase and catalase) were assayed  
167 according to the method by Xaziev (2005).

#### 168 **Statistical analyses**

169 Experimental data were analyzed SPSS version 29 for Mac OS. Data were described  
170 mean and standard deviations. The influence of Biochar and different plants w  
171 statistically using two-and one-way analysis of variance (ANOVA) and MANOVA.  
Deleted: Heatmaps 72 comparisons between groups. Duncan's Multiple Range Test was applied at 0.05 level. F  
173 generated using PAST statistical software. The magnitude of the F-value determined the  
174 of the effect of treatment ( $p < 0.05$ ,  $< 0.01$ , and  $< 0.001$ ).

#### 175 **Results**

178 The results in Table 4 show the effect of biochar on morphological traits of alfa-alfa, a  
179 maize under salt stress. Morphological traits of alfalfa, amaranth and maize were  
180 decreased by salt stress.

181 Alfa-alfa (biochar) treatment significantly increases the plant height by 28%,  
182 weight by 50% and roots' fresh weight by 33% as compared to control under salt s  
183 conditions, amaranth (biochar) treatment significantly enhanced the plant height by 24%  
184 weight by 54% and root fresh weight by 43% than the control. The additions of biochar  
185 caused significantly enhanced the plant height by 48%, shoot fresh weight by 25% ar  
186 weight by 50% in maize under salt stress.

187 Data revealed that biochar treatments significantly improved root parameters as well a  
188 control (Table 5.). Compared to the control, Alfa-alfa (biochar) treatment significantly i  
189 root projected area by 39% and the root surface area by 31% under salt stress. The total  
190 diameter and volume were sharply enhanced by Alfa-alfa (biochar) treatment, which  
191 increased by 46%, 47% and 82%, respectively, to the control. The root surface area, pr  
192 and diameter were improved with the amaranth (biochar) by 24%, 25% and 27% to the c  
193 stress. In salt stress, amaranth (biochar) treatment significantly increased the root volume  
194 the total root length by 50%. The highest values of root surface area (63%) were obs  
195 treatment of maize (biochar) than in the control. In salt stress conditions, mai  
196 significantly increases the total root length by 24%, root projected area by 32%, root vol  
197 and root diameter by 43% more than the control.

198 Data revealed that biochar treatments increased photosynthetic pigments of alfa  
199 and maize as compared with the control under salt stress (Figure 1.). Alfa-alfa (biocha  
200 significantly enhanced the chlorophyll a content by 8%, chlorophyll b content by  
201 chlorophyll content by 9% and carotenoid content by 25% than the control under salt s  
202 salt stress, amaranth (biochar) treatment significantly enhanced chlorophyll b content b  
203 chlorophyll content by 21% and carotenoid content by 12% than the control. The high  
204 chlorophyll b content was observed in maize (biochar) treatment recording a significan  
205 64% over the control under salt stress.

206 Data in Figure 2 indicated that salinity decreased the relative water content of le  
207 amaranth and maize. Compared to the control, all biochar treatments improved the re

210 content of leaf in alfalfa, amaranth and maize. However, amaranth's maximum relative v  
211 was detected in amaranth (biochar) treatment, respectively. The results in Table 6 show  
212 impact of biochar on soil nutrients under salt stress.

213

214 The soil nutrients of N, P, K and humus contents were promoted by apply  
215 treatments. The Alfa-alfa (biochar) treatment significantly increased N content by 18%,  
216 16%, K content by 17% and humus by 55%, respectively, than the control. The P, N, K  
217 contents increased by 15%, 17%, 17% and 20% when inoculated with amaranth (biocha

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18 However, the highest P and K contents were observed with maize (biochar) treati  
219 (biochar) treatment had a greater effect on increasing P content (24%), K content (26%  
220 content (28%).

221 The impact of biochar treatments on enzyme activities in salt stress is given in T  
222 alfa (biochar) treatment significantly enhanced the catalase by 30%, invertase by 40%, a  
223 25% in soil under saline conditions. The catalase and urease activities were improved  
224 17%, respectively, when the soil was amended by amaranth (biochar) over the control i  
225 Under salt stress, the catalase and urease activities increased by 13% and 24% in mai  
226 treatment.

227 The overall impact of biochar treatments on plant growth, physiology and enzym  
228 under salt stress is given in Table 8 in terms of multivariate analysis of variance pr  
229 impact of biochar treatment, different plants, and the interaction between plants and bi  
230 alfa (biochar). An overall highly significant effect of biochar was recognized in most o  
231 variables, including; Plant height, SFW, RFW, Total Length, Total root ProjArea, Total  
232 Volume, Diameter, Chl-a, Chl-b, total chlorophyll, Carotenoid, Catalase, Invertase, Ure  
233 and Humus. However, biomass allocation and relative water contents showed a no  
234 impact of biochar treatment. The difference on three plant species was significant in  
235 parameters as revealed by MANOVA. The interaction between biochar treatment and i  
236 was significant in plant height, shoot fresh weight, total root length, total root proj. area,  
237 area, root volume, total chlorophyll, carotenoids, urease activities, P, K, and humus.

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38 The interrelationship between study variables in terms of Pearson's correlati  
239 plotted as a heatmap presented in Figure (3) using PAST software. The blue boxes indic



Deleted: , 42 correlation between variables; the red boxes indicate a negative correlation, and the grey boxes indicate  
243 for significant correlations. Accordingly, Biochar treatments had a positive correlation with  
244 the growth parameters measured, and significantly in plant height, diameter, urease, K  
Deleted: was 45 (%). Moreover, N and P were significantly and positively correlated to pigment concentrations.

246

## 249 Discussion

250 Salt stress is one of the focal contrary environmental factors that restrict plant growth in  
251 can cause a marked reduction in plant height, root length, plant fresh weight, dry we  
252 productivity of many crops (Huang et al., 2022; Ibrahim et al., 2020, 2021; Kumar et  
253 Moreover, chlorophyll a and total chlorophyll contents of tomato plants significantly de  
254 active photosynthetic process in leaves was also damaged,  
255 tochlorosis and early leaf senescence under salinity conations. Integrating biochar in  
256 study could enhance the morphological characteristics of the chosen plants under salinity  
257 finding is consistent with the report of Hussein et al. (2022), who observed biochar impr  
258 length and plant growth in spinach (*Spinacia oleracea* L.) under salt stress. The prospec  
259 of biochar on leaf area index, photosynthetic potential, transpiration rates and chlorop  
260 significantly helped the leaf photosynthesis and net assimilation rate of rice growth and  
261 (Huang et al., 2022; Piao et al., 2022). In the current study, salt stress reduced ph  
262 pigments such as the total chlorophyll content and carotenoid content in alfalfa, amarant  
263 Applying biochar greatly improved the salt tolerance of cabbage seedlings and  
264 increased chlorophyll a, b and total chlorophyll while lowering sucrose, proline and H<sub>2</sub>C  
265 Zahra et al. (2020) observed a reduction in chlorophyll content and carotenoid conte  
266 genotypes under salt stress. Photosynthetic pigments and plant nutrients in winter and s  
267 showed a sharp decrease under salt stress (Saddiq et al., 2021). Also, numerous rese  
268 reported that biochar increased nutrient uptake (Ndiate et al., 2021) and plant physiologic  
269 (Zhang et al., 2020; Kul et al., 2021) in various plants under salinity stress. It was reveale  
270 et al. (2021) that the addition of biochar under salinity stress had a substantial impact  
271 ability to absorb nutrients and maintain water status, and biochar could reduce osmotic st  
272 increasing soil water content and releasing mineral nutrients in the soil solution and plant  
273 transient sodium ion binding due to its high adsorption ability. Biochar could attract ad  
274 into the soil under salinity conditions, releasing nutrients and lowering osmotic stress b  
275 water holding capacity and CO<sub>2</sub> absorption, which ultimately led to a noticeable imp  
276 photosynthetic, stomatal conductance and transcription rates (Ibrahim et al., 2021).  
277 biochar was able to reduce Na/K ratio and the amount of Na in many plants, which  
278 negative impact of salts on plants (Lashari et al., 2015; Ali, 2017; Huang et al., 2022).

279 Biochar application could directly improve the nitrogen status of salt-affected soils. Also,  
280 impacted the quantity and activity of bacteria, which could drive N transformation and  
281 nitrogen release (Yao et al., 2022). Moreover, a considerable rise in soil nitrogen was noted,  
282 this increase was closely correlated with the applied dose of biochar. These findings confirm the  
283 ability of biochar to hold the nutrients. The amount of soil organic matter was dramatically increased  
284 due to biochar application. In severely saline-sodic soil, marked increases in soil organic matter and  
285 nutrients after biochar addition. Numerous studies suggested that biochar could directly improve  
286 source and indirectly improve soil texture to boost P status in salt-affected soils (Saifullah et al. 2018). Similar findings were also informed by Alfadil et al. (2021); biochar improved  
287 (Saifullah et al. 2018). Similar findings were also informed by Alfadil et al. (2021); biochar improved  
288 nutrient contents in the soil under salt stress. Premalatha (2022) reported that biochar increased  
289 organic C and available N, P and K under salt stress. Similarly, Yao et al. (2022) have shown that  
290 biochar promoted the availability of soil total N, P and K under saline conditions. Similar findings  
291 have been informed by Huang et al. (2022). The biochar improved soil organic matter, total N, P and K  
292 contents under salt stress. Gunarathne et al. (2020) reported that biochar promoted soil's  
293 alkaline phosphatase activity in salt stress conditions.

294 Soil microbial activity has been exemplified by enzyme activity, which is susceptible to changes in  
295 the soil environment (Elzobair et al., 2016; Huang et al., 2017; Khademand Raiesi, 2019). In addition, soil enzyme activities were critical for soil organic matter degradation and  
296 nutrient cycling (Song et al., 2019; Yao et al., 2022) and are considered important indicators of soil  
297 quality. However, few studies focused on biochar's effect on enzyme activities in saline soils. Previous  
298 results showed that adding biochar positively affected enzyme activity in saline soils. The increase in  
299 of soil organic nitrogen into usable inorganic nitrogen was related to urease. Invertase was increased  
300 increasing the amount of soluble nutrients in the soil, which gave soil organisms energy for growth.

301 Catalase was a major factor in the oxidation of organic matter and humus production, and it was  
302 the level of biological redox and microbial activity in the soil. Compared to control, biochar application  
303 biochar application had a significant effect on the activities of catalase, invertase, and urease. In the  
304 current investigation (Yao et al., 2022). The use of biochar could enhance enzyme activity, thereby  
305 enhancing SOM, microbial activity, and microbial biomass or by placing the enzymes close to the  
306 and allowing them to interact with the biochar surface (He et al., 2020; Qu et al., 2020; Resi, 2021).

## 309 Conclusion

Biochar treatments have more stimulation impact on most of the root morphological traits and growth parameters of alfalfa, amaranth and maize compared to the control in salt stress. biochar application could improve photosynthetic pigments viz: the total chlorophyll, chlorophyll a and chlorophyll b. The application of biochar treatments positively improved the contents of N, P, K and humus contents in the soil in salt stress. The stimulation impact of biochar on increasing soil enzyme activities such as catalase, invertase and urease and enhanced nutrient availability can reduce the application of the chemical fertilizers in salt stress.

**Conflicts of Interest:** The authors declare no conflict of interest.

#### Author Contributions

Conceptualization, D.J., T.A., and Z.J.; methodology, S.M., D.J., A.E, M.E, A.A, I.M, S. K.S.; software, D.J., Z.J., A.E, M.E, A.A, I.M, A.N., and S.M.; validation, D.J., Z.J., A.E, I.M, A.N. ; investigation, D.J., T.A., A.E, M.E, A.A, I.M, Z.J.; writing—original draft, D.J.; writing—review and editing, D.J., T.A. A.E, M.E, A.A, I.M and Z.J. All authors have read and agreed to the published version of the manuscript.

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