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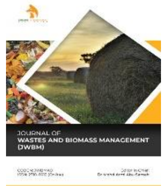

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RESEARCH ARTICLE

EFFECT OF DIFFERENT BIOCHAR CONCENTRATION ON THE GROWTH OF THREE AGRICULTURAL PLANTS IN AFGHANISTAN

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ABSTRACT

The present research was conducted in the laboratory of Badakhshan University, Afghanistan. The study was carried out for 40 days in 2021, to document the effect of biochar on the growth of three important agricultural plant species: radish, lettuce and spinach. The biochar was produced from the biomass of Oak tree under oxygen less condition. The study includes assessment of Petri plate bioassay and pot scale experiment to understand the effect of biochar under saline and non-saline conditions in order to mitigate the problem of salinity. Application of biochar has significantly increased the seed germination for all the seeds. The maximum seed germination was observed for radish > lettuce > spinach. NaCl has reduced the percentage of seed germination but a little increase was observed in case of radish as compared to lettuce and spinach. However, application of biochar has significantly increased its growth as compared to control plant. Both shoot and root of all three studied plants were increased with increase in BC concentrations. The maximum length of root was observed for radish > lettuce > spinach whereas for shoot it was lettuce > radish > spinach. At the same time application of 5 % biochar helped to improve the growth of both radish shoot and root. Lettuce was found sensitive to saline condition and application of biochar does not significantly improve their morphometric parameters. Spinach could be considered as moderately saline resistant species whose biomass can be improved by supplementing with biochar.

KEYWORDS

Biochar, salt stress, soil, seed germination, plant growth

1. INTRODUCTION

Rapid expansion of population and need to fulfil the food demand is increasing day-by-day, which is of primary concern for today's world. There is a 70 % increase in food production recommended by experts to fulfil the current and future demand. Despite such demand, the lower production of food is increasing which is mainly attributed to the various biotic (pathogens) and abiotic stresses (salinity, drought, temperature, precipitation) which hinder the crop productivity. Salinity is one the important abiotic factors which remain a problem for the globe. According to an estimate of FAO, over 6 % of the world's land is affected by salinity. Of the current 230 million hectares of irrigated land, 45 million hectares is salt-affected (19.5 %), and of the 1,500 million hectares under dry land agriculture, 32 million are salt-affected to varying degrees (2.1 %) (Parihar et al., 2015). Thus, salinity stress appears to be a major constraint to plant and crop productivity. Salinity of soil due to natural weathering of rocks is one of the major reasons for increase in chloride salts of Na, Ca and Mg content in soil thus resulting in sodicity. Secondly, deposition of oceanic salt carried in wind and rain. "Cyclic salts" are ocean salts carried inland by wind, deposited by rainfall, and are mainly sodium chloride. However, few anthropogenic factors such as excessive irrigation and fertilization are also responsible for such situations. Improper irrigations system, leaching fraction added with land clearing and deforestation have been marked as the major cause. Salinity is often recognized as excess of sodium ions (sodicity) that imparts life-threatening consequences in plant due to mal-textured soil hindered porosity and aeration leads to physiological water deficit. Mingling with other edaphic/environmental factors viz. flooding, temperature, precipitation, soil profile, water table exaggerates the catastrophe synergistically.

Biochar, the carbonaceous material produced during the thermochemical processing of biomass, showed it importance in decrease CO_2 emissions, enhancement plant productivity and mitigate climate change, improve soil quality, reduces drought and salinity stresses and toxic materials on plants (Biederman and Harpole, 2013; Diatta, 2016; Mukhtar and Khalid, 2018; Zameer et al., 2017). Biochar can help for enhance organic matter in soil, reduce influence of water deficit on plant growth (Branch et al., 2020). Combining biochar in the soil significantly helped in improving crop productivity in salt influenced soils (Akhtar et al., 2015a). In addition, they improve the physical, chemical and nutritional properties of biochar depends on the chemical composition of the feedstock applied, pyrolysis system and production conditions, including residence time and temperature. Significant quality of biochar are the high surface area and porosity, low bulk density, high stability, nutrient content, high cation exchange capacity (CEC), neutral to high pH and high carbon content (Pühringer, 2016). The biochar obtained from wood materials exhibited great carbon content and a high absorption character (Jindo et al., 2014). Biochar has potential to eliminate different pollutants from aqueous solutions (Li et al., 2020). The absorption capacity of the biochar produced at 600 °C was considerably higher than that of the biochar produced at 300 °C (Zhang et al., 2019). Biochar is regarded as a tool for the effective management of agricultural productivity and various environmental issues (Mansoor et al., 2021). The potential of biochar to increase plant biomass and crop yields has been demonstrated in a number of tropical agricultural studies, with a recent meta-analysis of published studies finding that biochar treatments increased crop yields by an average of 10

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%, with larger effects observed on acidic and coarse-textured soils, and at high addition rates (Jeffery et al., 2011).

The agricultural sector in Afghanistan supports the livings of nearly three-quarters of the total population and contributes nearly 28% to the Gross Domestic Product (GDP). Hence, agricultural growth is important for driving the country's economy and for ensuring national food security. Naturally low soil fertility, existence of saline and sodic soils, lack of water, high alkalinity, and soil compaction from usage of heavy equipment, pest and diseases attack, lack of farmer's understanding regarding soil management, and the harsh and dry climate affect Afghan agricultural production (Rahmani, 2014). The aim of the research was to study the effect of biochar on the growth of three important agricultural plant species: radish, lettuce and spinach. To investigate the influence of increasing biochar concentrations on the growth parameters of the studied agricultural plants under saline and non-saline conditions, this study has been done at Badakhshan University in Petri plate bioassay and pot scale experiment.

2. Materials and Methods

In the present research, three vegetable species namely radish (Raphanus raphanistrum subsp. sativus (L.) Domin), lettuce (Lactuca sativa L.) and spinach (Spinacia oleracea L.) were selected as a model plants to observe the effect of different doses of biochar on their biometric growth and development under normal and salt stressed conditions. The method used in the present study is divided into three major sections: 1) Biochar production and soil substrate preparation. 2) Petri-plate bioassay experiment. 3) Pot-scale experiment.

2.1 Biochar production and soil substrate preparation

Biochar was generated by thermochemical conversion of dry Oak (Quercus) biomass purchased from wood shops in Faizabad city, Afganisthan. Biochar was produced through pyrolysis process using a biochar production oven under limited oxygen conditions for 3 hours at a temperature between 300–400 ºC (Figure 1) (Sikder and Joardar, 2019). In order to achieve the same particle size as that of the soil the biochar was ground to small granules (Pühringer, 2016).

Figure 1: Biochar preparation via slow pyrolysis using a production oven

The contaminated soil was collected from the Botanical garden of the Badakhshan University, Afghanistan. The soil was sieved using 3 mm sieve and dried under natural sunlight. The solution of NaCl (25 mM) was added in the soil and left in the natural condition for equilibration for 10 days.

2.2 Petri plate bioassay

Three studied plants (radish, lettuce and spinach) were used to conduct the seed growth experiment to examine the effects of NaCl under different concentration of biochar (0, 2.5 and 5%). The seeds were procured from the local market of Fiazabad city of Afghanistan. A total of 20 seeds similar in size and shape were placed in petri plate fitted with sterile filter paper at the bottom. Each plate seeds including control were supplemented with 10 mL of 25 mM NaCl solution. A second control supplemented with similar quantity of sterile tap water was used to cross check the effect of NaCl on seedling growth and germination of seeds. The biochar were applied at the rate of 0, 2.5, and 5% in the Petri dishes. Plates with distillate water without BC and NaCl were considered as a control. The seeds were allowed to grow for 7 days with a photoperiod of 14:10 (day: night) at room temperature. The seed germination was recorded every day. Seeds with > 5 cm of radical were considered as germinated seed. The length of the root and shoot was measured and finally the fresh biomasses were recorded at the end of the experiment. The experiment was performed in 5 replicates.

2.3 Pot scale experiment

Pot experiments were conducted using 240 plastic pots (80 pots for each plant species) each with a diameter of 7 cm and height of 9.5 cm (200 mL). Each pot was filled with 500 g of soil thoroughly mixed. The experimental set included four variants: control (soil substrate), soil substrate with 1 % BC, with 2.5 % BC, and with 5 % BC. The experiment was carried out under irrigation with salt solution (25 mM NaCl) and tap water. Each pot was planted with 7 radish seeds, 10 lettuce seeds and 7 spinach seeds. Experiment was conducted in 10 replicates (10 pots) in completely randomized block design at natural conditions (Rome temperature ranging between 25 ± 2ºC) in the laboratory of Badakhshan University, Afghanistan. Plants were grown for 40 days.

2.4 Biometric plant growth assay

The seed germination rate was assessed by the number of emerging seedlings in each pot every 3–4 days for 18 days. The viability of the seed population was assessed by the final percentage of their germination. The equation to calculate of final germination percentage (GP) is (Al-Mudaris, 1998):

$$GP=\frac{\text{Total seeds germinated}}{\text{Total seeds in pot}} \times 100\% \tag{1}$$

The shoot height cm of studied species in each variant was measured every 4–6 days for 40 days of vegetation using a ruler (Kumar et al., 2017). The area of the leaf blade was measured on fully formed leaves in the middle (20 days of vegetation) and at the end of the experiment (40 days of vegetation) for each studied plant species in each variant in 5 replicates using the specialized program JMicrvision, version1.2.7. After 40 days, the root and shoot of lettuce, spinach and radish were harvested separately, and length and width of plants were measured and recorded (Ke et al., 2018).

2.5 Statistical analysis

All data were analyzed statistically following one-way ANOVA technique by using significant differences and comparisons among data to determine significant differences between all variants. The figures and tables presented the Mean ± standard error (SE). Different letters indicate significant difference at $p < 0.05$ according to Tukey's test. Other calculations and graphs were prepared by using SPSS 16.0 and Microsoft Excel 2010 and JMicroVision 1.2.7 software.

3. Result and Discussion

To examine the effect of biochar on seed germination and growth, three plant species: radish, lettuce and spinach under saline and non-saline conditions were used to conduct the soil-less Petri dish assay (Figure 2).

(a) (b) (c)

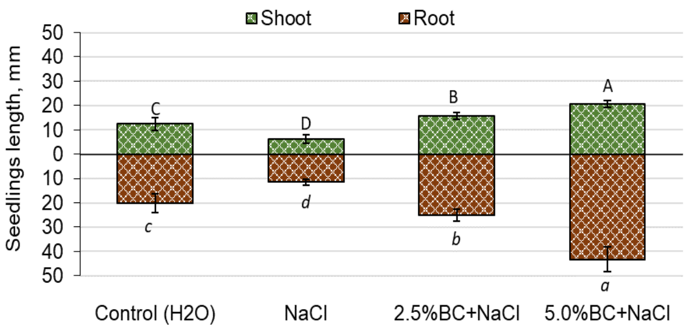
Figure 2: Seedling growth of radish (a), lettuce (b), and spinach (c) at different biochar treatments under saline and non-saline conditions (n = 5).

Application of biochar has significantly increased the seed germination for all the seeds. Moreover, the rate of application of biochar has also significantly affected the seed germination. Biochar generally increased the seed germination with increase in percentage and maximum seed germination was observed by 5.0 % followed by 2.5 % (see Table 1). The maximum seed germination was observed for radish > lettuce > spinach. NaCl has reduced the percentage of seed germination but a little increase was observed in case of radish as compared to lettuce and spinach.

Table 1: The seed germination percentage (GP) in Petri plate experiment (Mean ± SE; n = 5)

GP, %	Plant species		
	Radish	**Lettuce**	**Spinach**
Control (H_2O)	92.0 ± 4.5	83.0 ± 2.5	80.0 ± 8.5
NaCl	89.5 ± 8.5	75.5 ± 3.5	71.5 ± 4.0
2.5% BC+NaCl	93.5 ± 6.0	80.0 ± 2.5	82.5 ± 3.5
5.0% BC+NaCl	97.5 ± 3.5	87.0 ± 5.0	85.0 ± 4.0

It was found that presence of NaCl in the petri-plate has significantly reduced the growth of both root and shoot of all studied plants in compare to control (H_2O) treatment (see Figure 3). However, application of biochar has significantly increased its growth as compared to control plant. Because of the high absorption potential of Na^+ by BC, its transfer has been reduced which resulted in bigger biomass (Saifullah et al., 2018). Moreover, because of the high porosity, the water was available for longer time in the plates. Additionally, presence of high C with some K, Ca and Mg in BC could have helped to reduce the Na stress and provided essential macro-elements for the plant growth. Both shoot and root of all three studied plants were increased with increase in BC concentrations (see Figure 3) however, the maximum length of root was observed for radish > lettuce > spinach whereas for shoot it was lettuce > radish > spinach. Similar results were observed for biomass of seedling root and shoot with some exceptions. Since, radish is a rooted vegetable, it directly absorbs higher content of nutrients from BC and thus possibly showed best results for root growth than other studied plants.



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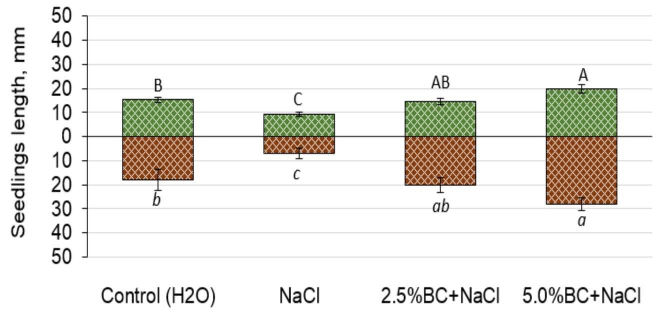

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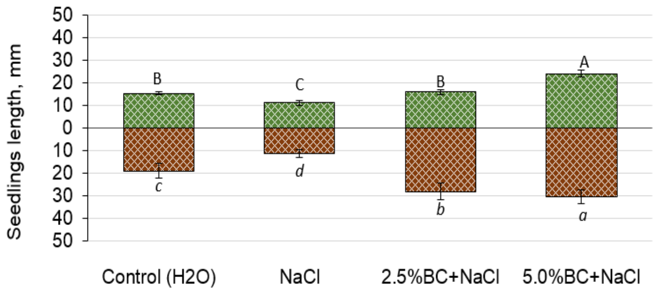

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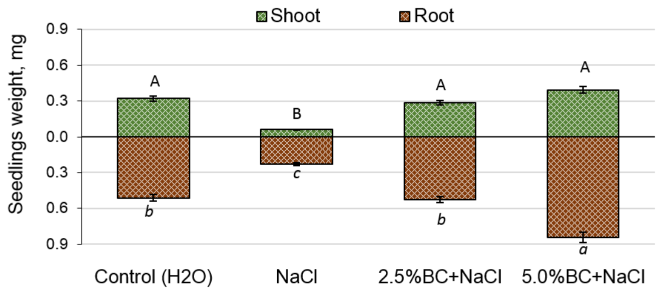
Figure 3: The length of shoot and root of radish (a), lettuce (b), and spinach (c) seedlings at different biochar treatments under saline and non-saline conditions. Different letters indicate significant difference at $p < 0.05$ according to Tukey's test (Mean ± SE, n = 60).



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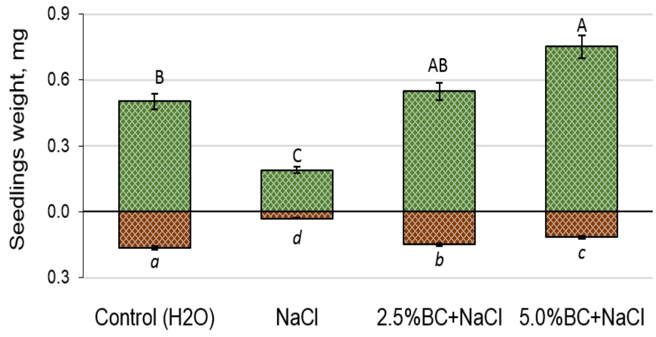

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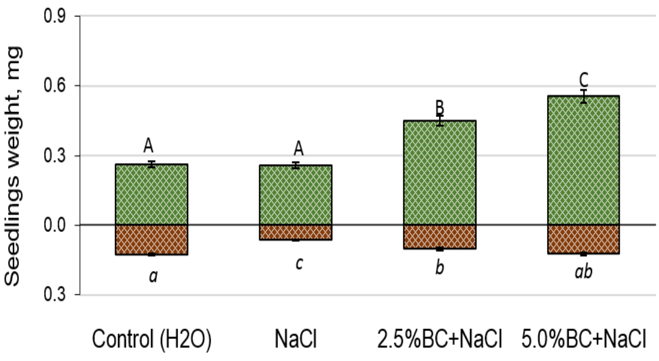
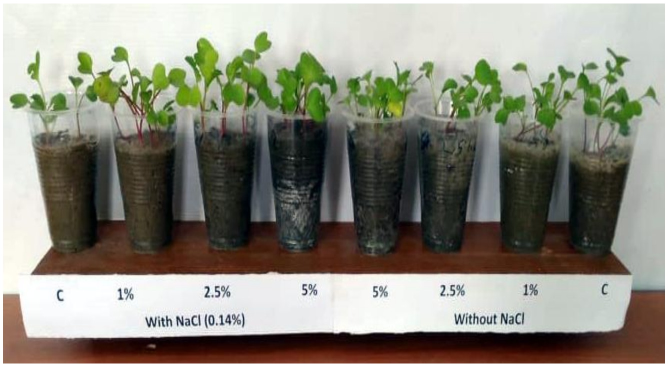


(c)

Figure 4: Fresh biomass of shoot and root of radish (a), lettuce (b), and spinach (c) seedlings at different biochar treatments under saline and non-saline conditions. Different letters indicate significant difference at $p < 0.05$ according to Tukey's test (Mean ± SD, n = 60)

A pot scale experiment was performed during 40 days of vegetation under saline and non-saline conditions with different doses of biochar (see Figure 5). Experiment carried out in Rome temperature (25 ± 2ºC) with indirect sunlight. Study was conducted by using 240 plastic pots (80 pots for each plant species). The experimental set included four variants: control, soil substrate with 1 % BC, with 2.5 % BC, and with 5 % BC. The experiment was carried out under irrigation with salt solution (25 mM NaCl) and tap water. Experiment was conducted in 10 replicates (10 pots) in completely randomized block design at natural conditions.



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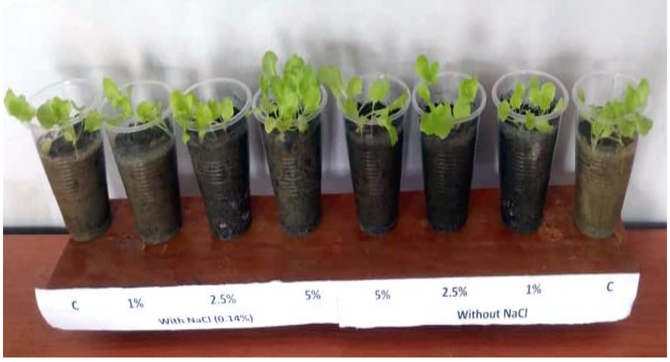

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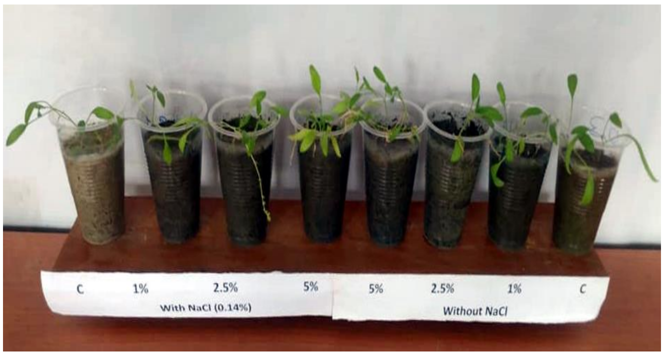

(c)

Figure 5: Pot scale experiment showing growth of radish (a), lettuce (b), and spinach (c) at different biochar treatments irrigated with 25 mM NaCl solution and tap water during 40 days of plant vegetation

Maximum germination of seeds was observed for radish followed by lettuce and spinach at 8th days of seedlings growth. With increase in time duration, the maximum number of seedlings emerged was found for radish in the variant with addition of 5 % BC that irrigated by 25 mM of NaCl for 18 days of growth period (see Figure 6).

Addition of BC increased the germination percentage of radish seedlings in NaCl treated soil however, increase in BC for lettuce and spinach reduced the germination, which was just opposite in case for tap water (see Table 2). The maximum seedling growth was observed in 5 % BC for radish. This indicates a higher resistance of radish to salinity in comparison with other tested plants. The use of BC increases seed germination percentage (see Table 2) and plant growth (see Figure 7) under saline conditions, as previously reported by other scientists (Saifullah et al., 2018).

Table 2: The seed germination percentage (GP) in pot scale experiment (Mean ± SE; n = 10)

GP, %	Plant species		
	Radish	**Lettuce**	**Spinach**
Irrigation with salt solution (25 mM NaCl)			
Control (soil)	51.4 ± 5.2	32.9 ± 2.8	27.5 ± 2.1
1.0% BC	67.1 ± 7.4	15.0 ± 1.8	20.0 ± 1.8
2.5% BC	69.2 ± 3.9	18.6 ± 1.6	17.1 ± 1.2
5.0% BC	91.4 ± 7.5	26.0 ± 2.0	22.5 ± 2.0
Irrigation with tape water			
Control (soil)	32.1 ± 2.7	14.0 ± 0.9	20.0 ± 1.8
1.0% BC	55.6 ± 4.9	17.5 ± 1.2	12.5 ± 1.5
2.5% BC	64.3 ± 5.5	32.5 ± 2.7	21.3 ± 2.0
5.0% BC	85.7 ± 7.8	35.0 ± 2.5	27.5 ± 1.8

It was observed that smaller concentration of BC (1.0 and 2.5%) had small effect to mitigate the NaCl problem while 5 % BC had significantly improved the results for most of the studied plants. However, the results for lettuce and spinach were good for control either with tap water or with NaCl solution. This suggests lower tolerance of these plants under NaCl stressed condition. Biochar just helps to improve the condition and higher percentage of BC could help to improve the results which need to be tested in future.

The leaf blade area of three studied plants was studied after 20 and 40 days of plant growth period (see Figure 7). At 20th days of growth period, non-significant difference in leaf area was observed between all the treatments for all the plants. With increase in time duration, it was observed that application of BC has significantly increased the leaf area and maximum growth was observed for 5 % BC treated plants however, non-significantly different leaf area was observed between 2.5 % BC and 5 % BC for radish. It is well reported that micro- and macronutrients, such as Ca, K, N, P and Zn, present in biochar is being slowly released into the soil and taken up by the plants and increases its productivity and yield thus the beneficial and actual effect of BC could be observed clearly in long term experiment (Thomas et al., 2013; Hammer et al., 2014; Drake et al., 2016; Kim et al., 2016).

It was found that the leaf area of radish was relatively higher than lettuce and spinach and minimum was noted for spinach. However, addition of BC at higher concentration not only promoted the growth of radish, which was found tolerant to NaCl, but it also helped to increase the leaf are of lettuce and spinach. In general, the minimum results were observed for control for all the studied plants either in tap water or in NaCl irrigated situation. Application of BC increased the root length of radish under both NaCl and non-saline water treated plants. However, in case of lettuce it was reduced even in the presence of higher percentage of BC whereas no effect was observed for non-saline water treated plants. In case of spinach, no effect was observed on root length either under saline or non-saline condition (see Figure 8 c, e).

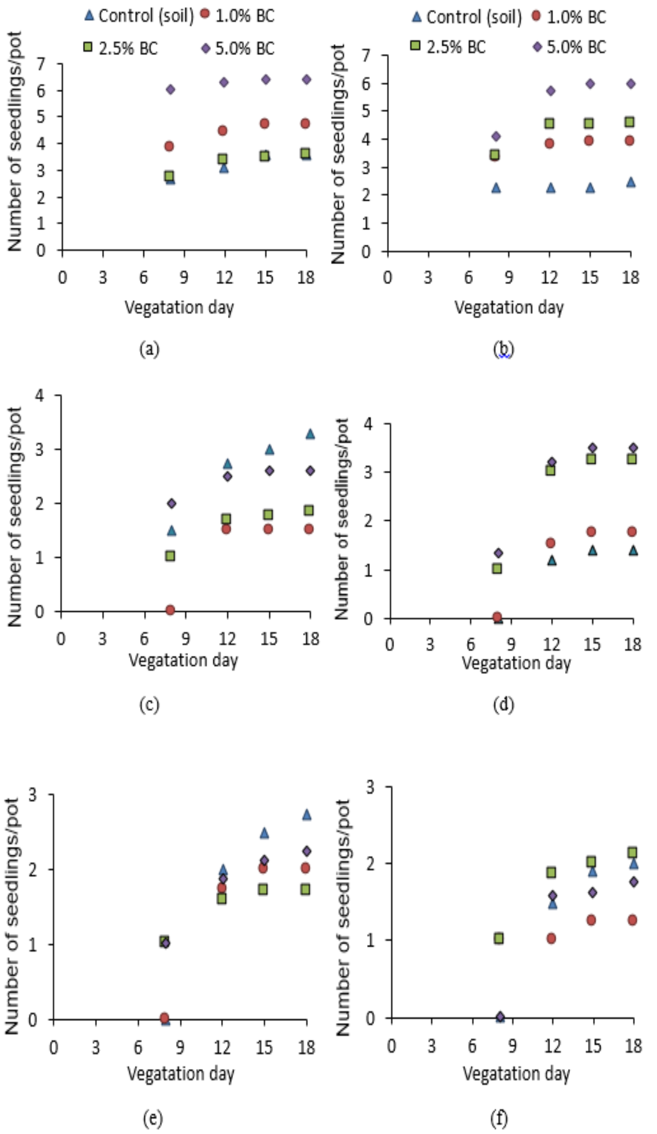

Figure 6: Number of seedlings per pot of radish (a, b), lettuce (c, d) and spinach (e, f) at dif-ferent biochar treatments irrigated with 25 mM NaCl (a, c, e) or tap water (b, d, f) for 18 days of plant vegetation

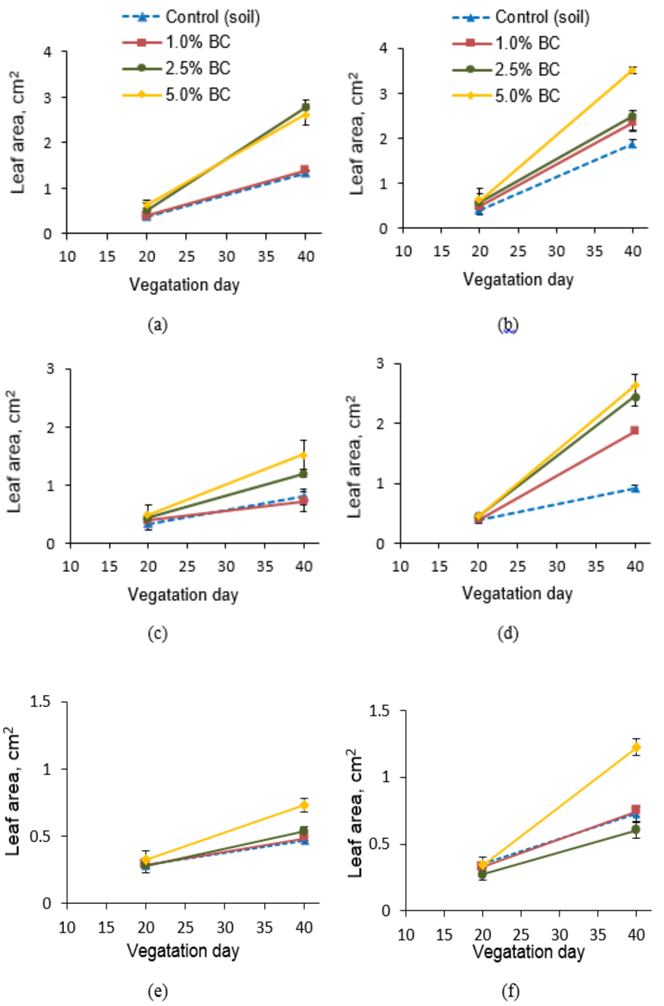

Figure 7: Leaf area of radish (a, b), lettuce (c, d), and spinach (e, f) at different biochar treatments irrigated with 25 mM NaCl (a, c, e) or tap water (b, d, f) for 40 days of plant vegetation (Mean ± SE, n = 5)

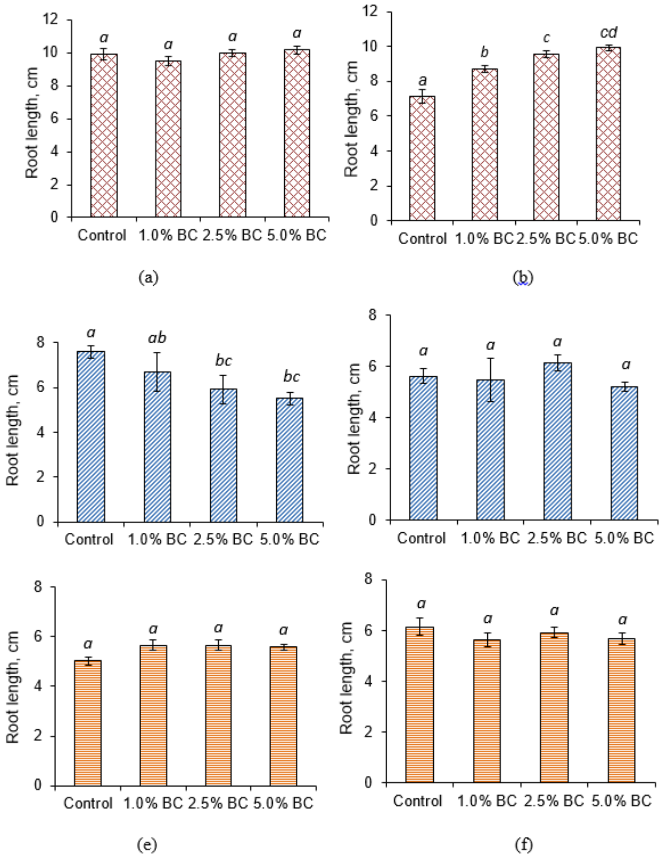

Figure 8: Root length of radish (a, b), lettuce (c, d), and spinach (e, f) at different biochar treatments irrigated with 25 mM NaCl (a, c, e) or tap water (b, d, f) for 40 days of plant vegetation (Mean ± SD, n = 30–40).

Generally, with increase in percentage of BC the root length increased, or no negative result was observed for all the plants. However, a contradictory result was observed in case of radish were application of NaCl does not negatively affect the root growth as compared to control, which suggests higher salinity tolerance of radish as compared to other tested plants. Maximum root length was observed for radish followed by lettuce and spinach. A group researcher also reported radish plant as moderate to low sensitive plant for saline condition (Saifullah et al., 2018). Since radish is a rooted vegetable it directly absorbs higher content of nutrients from BC and thus possibly showed best results for root growth than other studied plants.

The maximum shoot length was observed for radish > spinach > lettuce (see Figure 9). Application of NaCl has reduced the growth of all the plants (see Figure 3.8 a, c, e) however application of BC increased and mitigated the problem of salinity. Since, spinach is leafy vegetables, more transfer of nutrients could have transferred from the BC thus helped to increase in shoot length. Similarly, higher root length of radish may promoted higher shoot length. In the early stage of experiment, no significant difference was observed in the shoot length however with increase in time and release of nutrients from BC the shoot length improved. The best results were found in case of 5 % BC for radish and spinach under saline condition however a non-significant different between 2.5 % and 5 % was also observed in some cases.

Similar results were noted for the fresh biomass of studied plants (see Figures 10). Both radish and spinach showed high biomass of both root and shoot in 5 % BC treated soil followed by 2.5 % under saline condition however, no significant changes were observed between the treatments under non-saline condition for both roots and shoot. A slight decrease in shoot and root biomass was observed in case of NaCl treated plants, however, no significant difference was observed for radish, which again suggests better salinity tolerance than lettuce and spinach.

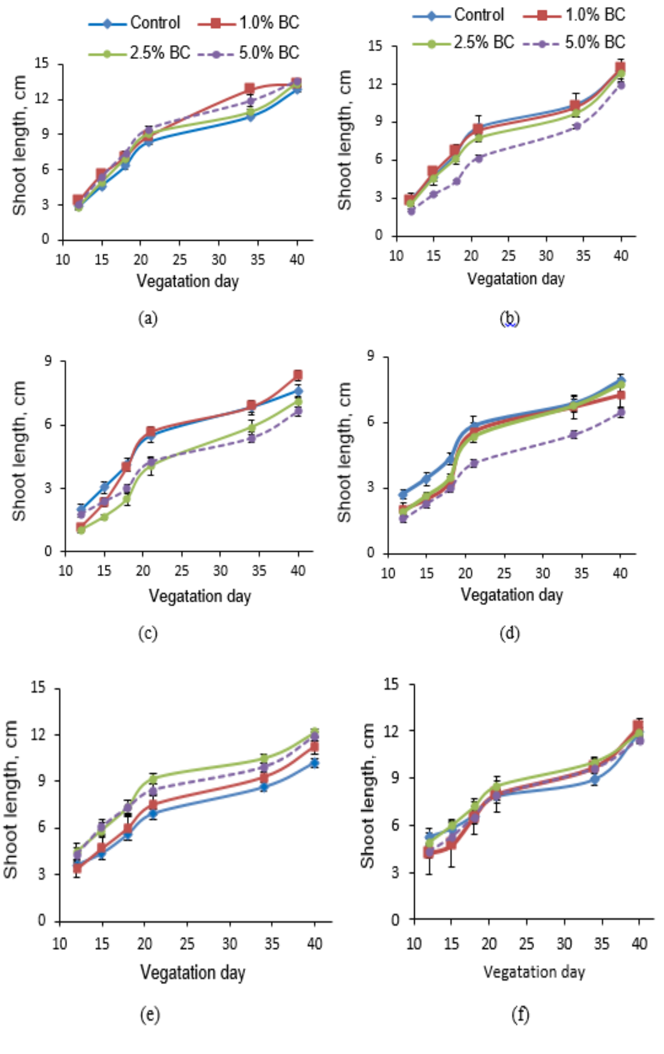

Figure 9: Shoot length of radish (a, b), lettuce (c, d), and spinach (e, f) at different biochar treatments irrigated with 25 mM NaCl (a, c, e) or tap

water (b, d, f) for 40 days of plant vegetation (Mean ± SD, n = 30–40)

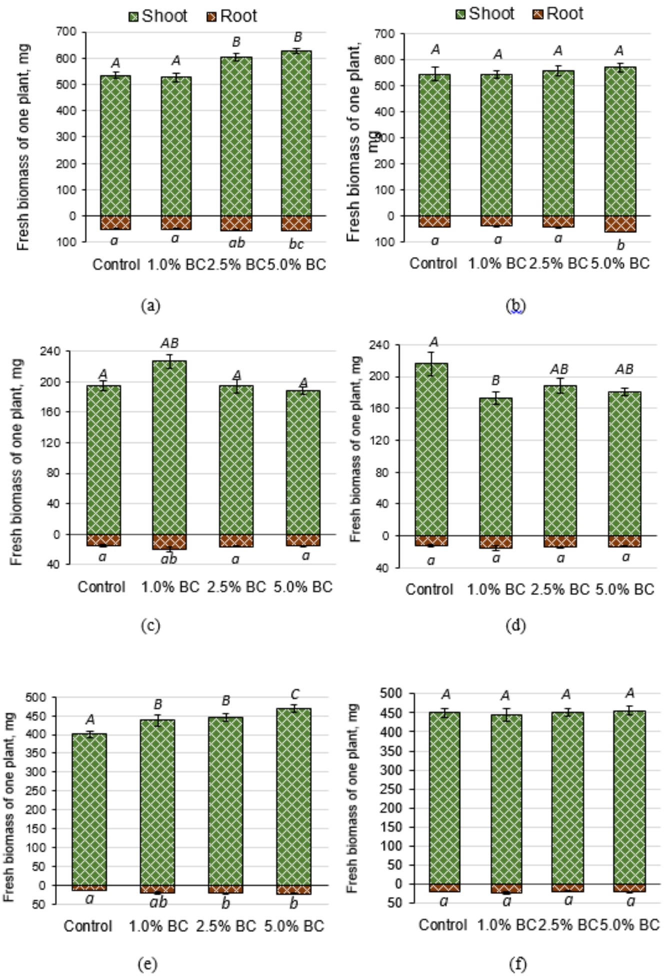

Figure 10: Shoot and root fresh biomass of radish (a, b), lettuce (c, d), and spinach (e, f) at different biochar treatments irrigated with 25 mM NaCl (a, c, e) or tap water (b, d, f) for 40 days of plant vegetation (Mean ± SD, n = 30–40)

Laboratory studies showed that addition of biochar to salt affected soils to a large extent alleviated salt stress and improved plant growth directly through the release of essential macro- and micronutrients such as Ca, K, N, P and Zn, to help offset the adverse impacts of salts. Reduction in osmotic stress occurs due to the increasing of water holding capacity and thus availability of water, improvement in stomatal density and conductance (Thomas et al., 2013; Abbas et al., 2017b; Akhtar et al., 2014; Ali et al., 2017; Rizwan et al., 2018). Perhaps biochar can stimulate growth and increase the biomass of radish and spinach by improving soil phosphorus, nitrogen and carbon, contents, and developing related microbial communities (Lebrun et al., 2020).

Similar study has been done in other countries, and in Afghanistan it is first time we are attempted to test effects of biochar on agricultural plants. Our results correspond with a research reported that Biochar and salt addition treatments had strong interactive influences on plant survivorship. Almost all plants (29 of 30) that did not receive salt additions survived until the end of the trial. For plants that did receive salt additions survivorship was strongly dependent on the amount of biochar added. Though, survivorship was high in the salt plus high biochar addition amount (Thomas et al., 2013). Another study documented that, biochar treatment, regardless of use rate, resulted in enhanced plant biomass by approximately 90%, as compared with the control treatment or other biochar treatments (Gonzaga; Hammer et al., 2014). Same results reported (Mansoor et al., 2021; Kim et al., 2016).

Indeed, Afghanistan has its varied geo-climatic condition, and possesses vast salinity lands, 12%, of the total land is suitable for agriculture practices. Of this, 5% is irrigated and the remaining 7% is rained (Rahmani, 2014). Thus, agricultural growth is vital for driving the country's economy and for ensuring national food security Wheat is the most important crop in Afghanistan, followed by rice, barley, and cotton. Unfortunately, because of the continuous war and conflicts, agricultural sector wasn't developed in all province of Afghanistan. BC influence on plant growth under salt stress with different plant species, especially because of uncertain interactions of biochar and plant species regarding reclamation and plant growth needs further field research. Currently, there are no previous reports about biochar effect on agricultural plants in Afghanistan. Therefore, these findings are of great importance for the agricultural growth and for future studies on agriculture.

4. Conclusions

The present study includes assessment of Petri plates and pot scale experiments to understand the effect of biochar on some agricultural plants under saline and non-saline condition in order to mitigate the problem of salinity. In order to reduce the salinity stress biochar was produced from the biomass of Oak tree under oxygen less condition, sieved, and homogenized to bring it to suitable size for its application. The Petri plate bioassay test for 7 days suggests that application of 25 mM of NaCl solution has reduced the length of root and shoot for lettuce and spinach however, no effect was observed for radish. Application of biochar has significantly enhanced the morphometric parameters i.e., root and shoot length and seed germination and thus helped in mitigating the saline stress for plant growth.

In order to validate the results, soil-based pot scale experiment was performed for 40 days, which suggests approximately similar results found in Petri plate bioassay. Application of biochar has significantly increased the leaf area, number of seedlings per pot, shoot and root length, and fresh biomass of all three studied plants. At the same time, maximum improvements in the morphometric parameters of plants were observed with 5 % of biochar treatment. The maximum growth of root and leaf was found for radish followed by spinach and lettuce. Altogether, it was can be concluded that radish showed better tolerance to 25 mM of NaCl application compare to other two vegetable species. However, application of 5 % biochar could help for better growth of both rooted and leafy vegetables under moderate saline stressed conditions.

Thus, both Petri plate bioassay and pot scale experiment suggest that irrigation with 25 mM NaCl has mildly affected the growth of radish plants and stimulated seed germination. At the same time application of 5 % biochar helped to improve the growth of both shoot and root. Lettuce was found sensitive to saline condition and application of biochar does not significantly improve their morphometric parameters. Spinach could be considered as moderately saline resistant species which biomass can be improved by supplementing with biochar.

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