

ECOPHYSIOLOGICAL RESPONSE OF EARLY STAGE *Eucalyptus camaldulensis* TO BIOCHAR AND OTHER ORGANIC AMENDMENTS UNDER SALT STRESS

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Salinity is a global issue and *Eucalyptus camaldulensis* Dehnh. is one of the most planted agroforestry tree species on saline soils in Pakistan. Organic amendments are reported to ameliorate plant growth in saline sodic soils. This study was designed to examine the effects of different organic amendments on the growth of *E. camaldulensis* grown in saline soil. A pot experiment was performed and seedlings of *E. camaldulensis* were grown in soil having pH (8.5) and EC(e) 20.5dS/m. Four types of organic amendments were used: farmyard manure, poultry manure, slurry and biochar. The maximum plant total biomass (132.12 g), root length (24.67 cm) and shoot length (174.33 cm) were recorded in the plants grown in soils amended with farmyard manure. The minimum plant total biomass (36.05 g), root length (9.44 cm) and shoot length (68.0 cm) were found in plants without any amendment. Plants treated with farmyard manure and biochar showed the best results for chlorophyll contents, photosynthetic rate and stomatal conductance. Sodium contents in the shoots and roots of plants treated with organic amendments were decreased whereas potassium contents were increased. Maximum sodium contents were found in the shoot (5.70 mg/g DW) and root (7.71 mg/g DW) of un-amended plants, whereas, maximum potassium contents were in the shoots (7.81 mg/g DW) and roots 6.33 mg/g DW) of plants treated with biochar. Biochar was found to be best amendment to improve physicochemical characteristics of soil by improving organic matter (%) and saturation (%) as well as decreasing EC and SAR values of saline soils. Findings of this experiment suggested that organic amendments are useful in enhancing the early stage growth of *E. camaldulensis* in saline soils.

Keywords: Salinity, agroforestry, tree growth, soil degradation, afforestation.

INTRODUCTION

Soil toxicity is a serious issue in the whole world (Amundson *et al.*, 2015; Gómez-Sagasti *et al.*, 2018). There are number of factors which can deteriorate soil and make it unsuitable for proper growth. Heavy metal accumulation (Adimalla, 2020), salinity (Kumar *et al.*, 2020), drought and water logging (Farkas *et al.*, 2020) are some of the major deteriorating factors which influence on growth of crops and are also responsible for creating the issues of food security. Among them, one of the most important factor for reducing crop growth is soil salinity (Akhtar *et al.*, 2015; Yu *et al.*, 2019).

Salinity is a worldwide problem. It has been estimated that almost 20% of total cultivated land area of the world is affected by salinity (Akhtar *et al.*, 2015). Problem of salinity is more sever in case of irrigated land area. Some studies have revealed that about 33% of irrigated agricultural land area is affected by salinity and more than 30% of the total soils of the world are saline or sodic (Minhas and Dagar, 2016). Many countries like Australia, India, Syria, Soviet Union, USA,

China and Pakistan are effected by some level of salinity (Daliakopoulos *et al.*, 2016). About 6% of total land of Asia Pacific zone is affected by salinity (Marcar, 2016). Pakistan is ranked at eighth position in world with regard to the land affected by salinity and the severity of the problem can be determined by the fact that around 25% of total irrigated area of Pakistan is affected by salinity (Qureshi, 2016). Plants face severe problems in saline soils like nutrient toxicity, nutrient deficiency, high pH, reduced redox potential, increased osmotic stress and poor root zone aeration. The results of these adverse effects are also very severe which includes the damaged and poor root growth, reduction in shoot growth, and the death of the plant (Marcar, 2016; Elshaikh *et al.*, 2018). In literature, there are several engineering, chemical and biological techniques that can be used to reclaim saline soils (Sun *et al.*, 2017; Cao *et al.*, 2018). Afforestation is known as the planting of trees. It is a vital practice to improve the quality of degraded soils like saline soil. Planting trees on degraded lands has countless benefits such as it improves the quality of soil, increased the biodiversity and enhance the conservation of the area (Meir *et*

al., 2014; Schirmer and Bull, 2014; Fleming *et al.*, 2019; Yasin *et al.*, 2019). Farmers are also able to get additional income from reforestation in the form of carbon credits and off set mechanisms (Nawaz *et al.*, 2018). Above all other benefits is that reforestation helps in the remediation and reclamation of unproductive lands while making them suitable for cultivation and ensuring food security (Shabir *et al.*, 2018).

Organic matter contents of salt affected soils are generally very low and these soils usually exhibit poor structural stability. A great number of researchers have suggested that addition of organic material could enhance the structural stability of soil to a considerable amount (Drake *et al.*, 2016). Among different types of organic amendments, farmyard manures, poultry manures, green manures, compost, agro-industrial by products and food processing wastes are some of the examples of organic amendments (Agegehu *et al.*, 2016; Horta *et al.*, 2018). These amendments are not only helpful in sustaining the soil fertility but also are responsible for enhancing it. The organic matter content of these amendments is obviously high, so, they can be potentially beneficial for ameliorating the salt affected soils (Sun *et al.*, 2017; Luna *et al.*, 2018; Maltas *et al.*, 2018). Organic matter is not only helpful in reclaiming saline soils but they have countless beneficial effects on the growth of plants and overall agricultural field (McGrath and Henry, 2016; Trivedi *et al.*, 2017; Yuan *et al.*, 2019).

Biochar is one of the very vital organic amendments amongst many others. The sources of the biochar production include the naturally produced organic substances such as manure, wood chips, and agricultural wastes. The pyrolysis of these substances occurs in a low oxygen environment. The end product is a high carbon product. This carbon rich product is known as biochar and can be applied to remediate degraded soil and increase its productivity (Xiao *et al.*, 2018).

Eucalyptus genus is considered to be one of the most important trees for industrial plantation. It is an important source for providing raw material for pulp and paper industry. It is native to Australian continent and it's neighboring countries (Tran *et al.*, 2018; Yang *et al.*, 2018). *E. camaldulensis* is commonly known as river red gum and distributed throughout the world. It is reported that almost 10,000 ha land of Pakistan is covered by *Eucalyptus* and it is part of almost every major afforestation program in country (Shah *et al.*, 2011; Bilal *et al.*, 2014). The above mentioned features of *Eucalyptus* make it very important for social for agroforestry, social forestry and carbon sequestration programs. Moreover, it is also grown in irrigated plantations to obtain timber.

Although biochar has showed very positive benefits on the production of some crops and herbs species, the knowledge about the effect of biochar on the growth of trees species in saline soils is very limited. So, this study has been designed to investigate the effect of different organic amendments on

the growth and eco-physiological characteristics of *E. camaldulensis* as well as the impact of *E. camaldulensis* and organic amendments on physicochemical characteristics of soil.

MATERIALS AND METHODS

Pot experiment was conducted in the research area of the Department of Forestry and Range Management (FRM), University of Agriculture Faisalabad (UAF) (31° 25'57" N, 73° 04' 21" E) during 2017. Climatic conditions during the pot experiment were collected from Agricultural Metrology Cell, UAF and described in Table 1.

Table 1. Climatic conditions data during Experiment

Month	Average Max. Temp. (° C)	Average Min. Temp. (° C)	Precipitation (mm)	Sunshine Duration (Hours)	ET _o (mm)
January	17.6	08.2	11.5	3.6	00.9
February	23.3	10.2	4.1	6.6	01.9
March	27.3	14.2	16.2	7.2	02.7
April	37.7	20.9	28.3	9.2	05.2
May	41.1	26.0	10.1	10.4	05.7
June	39.8	27.3	41.6	9.4	05.3
July	38.5	28.9	117.2	7.0	04.0
August	38.1	28.6	66.0	7.9	03.8

A detailed survey of degraded soils was carried out in district Faisalabad with special preference given to salt affected soils. Auger was used to collect samples from different sites. After collecting different samples, electrical conductivity (EC) of soil was determined. The field with poor fertility status and high EC was selected. Prior to experiment soil was air dried. Dried soil was grounded with the help of wooden roller. After that it was passed through 2 mm sieve and prepared for further use and analysis.

Table 2. Initial Physicochemical characteristics of soil

Characteristics	Values
Textural class	Sandy Loam
Sand (%)	60±1
Silt (%)	25±0.5
Clay (%)	15±0.3
Saturation (%)	28±1
pH	8.5±0.3
EC (dS/m)	20.5±9
TSS (mmol/L)	205±20
CO ₃ ²⁻ (mmol/L)	10±2
HCO ₃ ⁻ (mmol/L)	30±4
Cl ⁻ (mmol/L)	140±19
Ca ²⁺ + Mg ²⁺ (mmol/L)	12±4.5
Na ⁺ (mmol/L)	160±15.2
K ⁺ (mmol/L)	47±7
OM (%)	0.64±0.03

Different physicochemical characteristics of soil were determined according to the procedures described by McGeorge (1954) and Blume (1985). Initial physicochemical characteristics of soil are given in Table 2. About 10 kg of soil was used for each pot.

Uniform sized seedlings of these *E. camaldulensis* were collected from the Department of Forestry and Range management, UAF. Due consideration was given to fact that seedlings should be healthy and free of disease. Farmyard manure (FYM), poultry manure (PM) and slurry (SL) were collected from the farm area of the UAF. Biochar was prepared under slow pyrolysis at 450°C for the period of 3 hours in the Agro-climatology lab of Department of Agronomy, UAF. Farmyard manure in the form of dung cakes was used as feedstock material. Tap water was used to irrigate the plants. Characteristics of water used to irrigate the plants are given in table 3.

Table 3. Characteristics of tap water of nursery

Characteristics	Values
pH	7.29
EC (dS/m)	0.669
TSS (mg/L)	6.699
Carbonates	(-)
Bi Carbonates (mg/L)	4.8
Ca + Mg (mg/L)	3
Chlorides (mg/L)	2.5
RSC	1.8
Sodium (mg/L)	0.69

Completely Randomized Design (CRD) was used to perform this experiment with three replicates. Two months old seedlings of *E. camaldulensis* were moved in earthen pots from polythene bags. These pots were then kept in the ware house to keep them safe from any external disturbance. Four soil amendments excluding control at the rate of 6% (w/w) were applied: Control (T1), farmyard manure (T2), poultry manure (T3), slurry (T4) and biochar (T5).

After 6 months, chlorophyll contents (SPAD value) were measured by chlorophyll meter and other physiological parameters like photosynthetic rate and stomatal conductance were noted by Infra-Red Gas Analyzer according to the pattern described earlier (Yousaf *et al.*, 2019). Shoot height and collar diameter was measured before harvesting with measuring tape and Vernier caliper respectively. Then the plants were harvested and washed with distilled water before measuring root length. After that samples of branches, roots, shoots and leaves were oven dried at 70 °C and dry weight was measured. Na and K concentration in plant parts were measured after digesting them in accordance with the procedure explained previously (Abbas *et al.*, 2018; Rehman *et al.*, 2019). Data was analyzed by using analysis of variance technique (one-way ANOVA using computer-based software SPSS 25.0 for Windows. The significance of treatment difference was tested using Tukey's test.

RESULTS

Growth parameters: Figure 1 illustrates the findings of various growth parameters versus five different treatments. Shoot length, shoot diameter, root length and dry weight of root, shoot, leaves and branches were studied. The growth was enhanced by all the treatments to some extent. Farmyard manure (T2) showed the best results for total biomass of plants. Poultry manure (T3) and Biochar (T5) showed the minimum increase in total biomass and other growth characteristics like root and shoot length and shoot diameter among all the amendments. The minimum amount of biomass was observed in the plants of control (T1). Maximum root length and shoot length was calculated as 24.67 cm and 174.33 cm, whereas, minimum were 9.44 and 68.00 cm respectively. Maximum amount of above ground dry biomass (AGB) and below ground dry biomass (BGB) was calculated as 107.36 g and 24.75 g respectively whereas minimum amount of AGB and BGB was calculated as 25.73 g and 10.31 g, respectively. Following trend was observed for different treatments T2 > T4 > T5 ≥ T3 > T1.

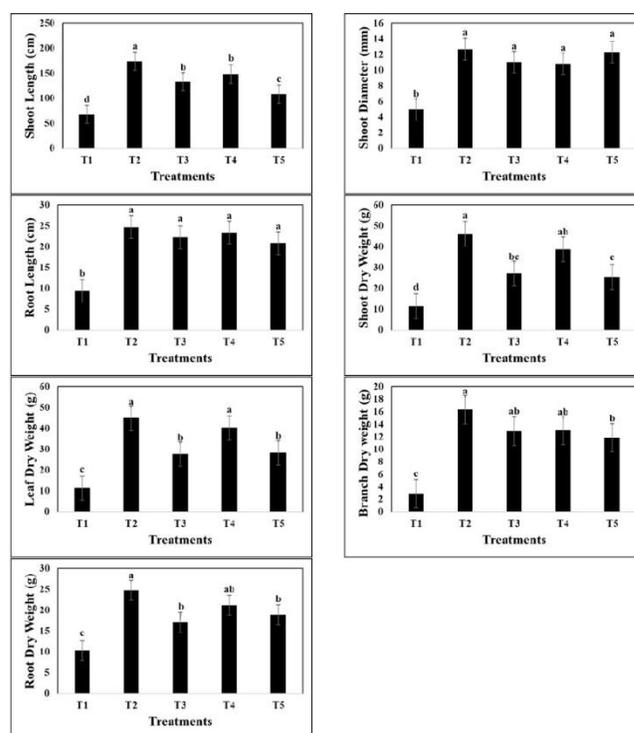


Figure 1. Growth parameters of plants. These values are means of three replicates and for each replication, there were three plants (n = 9). The difference between lower case letters is the indication that values are significantly ($p < 0.05$) different from each other. T1, Control; T2, Farmyard manure; T3, Poultry manure; T4, Slurry; T5, Biochar

Physiological parameters: Chlorophyll concentrations of plants were effected by all treatments with the plants of T5 and T2 treatments showing best results. These results are depicted in Figure 2. When compared with control, chlorophyll contents of *E. camaldulensis* plants increased by 65%, 62%, 54% and 70% in T2, T3, T4 and T5 respectively. Photosynthetic rate was also enhanced significantly in order of T2, T3, T4 and T5 by 44%, 44%, 34% and 53% respectively. The maximum stomatal conductance ($0.14 \text{ mol m}^{-2} \text{ s}^{-1}$), and sub-stomatal CO_2 ($318.33 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$) were shown by the plants treated with T2 and T5 respectively. The minimum stomatal conductance ($0.05 \text{ mol m}^{-2} \text{ s}^{-1}$) and sub-stomatal CO_2 ($270.11 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$) were shown the plants of T1. The results for physiological parameters are statistically different from control treatment but among different types of organic amendments results are not statistically significant.

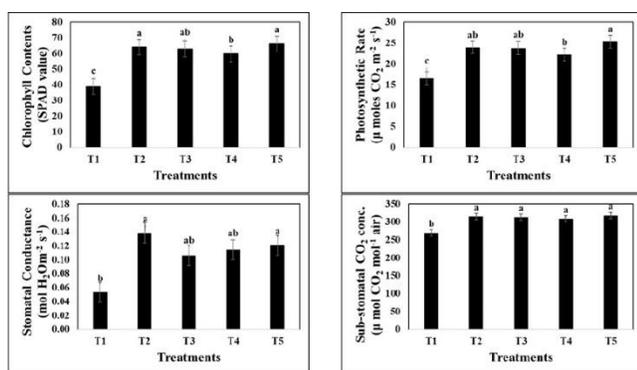


Figure 2. Chlorophyll contents (SPAD values) and gas exchange characteristics of plants. These values are means of three replicates and for each replication, there were three plants ($n = 9$). The difference between lower case letters is the indication that values are significantly ($p < 0.05$) different from each other. T1, control; T2, Farmyard manure; T3, Poultry manure; T4, Slurry; T5, Biochar.

Sodium (Na) and Potassium (K) contents in Plants: The concentrations of sodium and potassium were determined for shoot and root for all the treatments. The maximum concentration of Na in shoot (5.70 mg/g DW) and root (7.71 mg/g DW) was found in the plants of control treatment whereas the maximum concentration of potassium in shoot (7.81 mg/g DW) and root (6.33 mg/g DW) were present in the plants of T5 treatment. It was slightly higher than the concentration of potassium present in plants of T2 treatment. The minimum amount of Na was present in the plants of T5 whereas the minimum amount of potassium was present in untreated plants. The observed trend for Na concentration for different treatments was $T1 > T4 \geq T3 > T2 > T5$ whereas the observed trend for potassium was found to be $T5 \geq T2 > T4 > T3 > T1$. These results are illustrated in Figure 3.

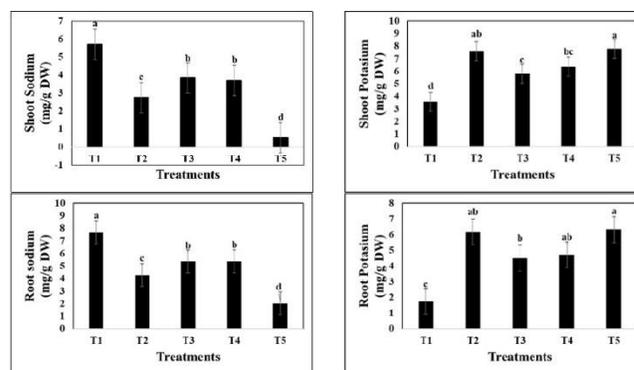


Figure 3. Concentration of Na and K in different plants' parts. These values are means of three replicates and for each replication, there were three plants ($n = 9$). The difference between lower case letters is the indication that values are significantly ($p < 0.05$) different from each other. T1, Control; T2, Farmyard manure; T3, Poultry manure; T4, Slurry; T5, Biochar

Post-harvest Soil Characteristics: Results of current study revealed that applied organic amendments and *E. camaldulensis* significantly affected post-harvest soil characteristics. Lowest pH was measured for plants of control treatment but there was no significant difference for pH among treatments. By applying organic amendments, the EC was reduced for all the treatments. Minimum EC along TSS, SAR was showed by the soils of farmyard manure biochar treated whereas maximum was shown by the soil of control. Organic matter and saturation (%) was also increased by use of organic amendments with biochar giving the best results. Organic amendments increased concentration of potassium and calcium as compared to sodium. Chlorides, carbonates and bi-carbonates were also reduced by using organic amendments. Data regarding different soil characteristics after harvesting is given in table 4. All treatments showed better results as compared to non-amended soil but among treatments biochar showed best results and plants of control showed poorest results as it can be seen in Table 4.

DISCUSSION

The findings of this study revealed that saline-sodic conditions have severe effects on the growth of *E. camaldulensis*. The plants of control which were not treated with any amendment showed the poorest results. Previous studies also supported these findings as described in following sentences. Salinity was responsible for the reduction in the yield of many plants (Elshaikh *et al.*, 2018). Hussain and Alshammery (2008) observed that the total biomass of four different agroforestry species *Acacia nilotica*, *Prosopis juliflora*, *Eucalyptus camaldulensis* and *Parkinsonia aculeate* decreased under increased salinity

Table 4. Effect of organic amendments on post-harvest soil characteristics

Parameters	Treatments				
	T1	T2	T3	T4	T5
pH	7.73a±0.04	7.81a±0.04	7.83a±0.04	7.82a±0.04	7.81a±0.04
EC (dS/m)	8.29a±0.35	3.77c±0.35	6.51c±0.35	3.88b±0.35	2.88c±0.35
TSS(mmol/L)	82.9a±3.53	37.6c±3.53	65.0b±3.53	38.8c±3.53	28.8c±3.53
CO ₃ ²⁻ (mmol/L)	(-)	(-)	(-)	(-)	(-)
HCO ₃ ⁻ (mmol/L)	4.20a±0.24	2.82b±0.24	3.03b±0.24	2.82b±0.24	2.42b±0.24
Cl ⁻ (mmol/L)	40.0a±0.9	16.0d±0.9	23.0b±0.9	19.67c±0.9	14.07d±0.9
Ca ²⁺ +Mg ²⁺ (mmol/L)	3.73b±0.55	6.67a±0.55	7.37a±0.55	6.07a±0.55	6.93a±0.55
Na ⁺ (mmol/L)	48.0a±1.19	17.0c±1.19	22.6bc±1.19	19.33c±1.19	14.37d±1.19
SAR	35.23a±0.91	9.37bc±0.91	11.82b±0.91	11.09b±0.91	7.73c±0.91
K ⁺ (mmol/L)	6.60b±0.50	10.87a±0.50	11.10a±0.50	11.20a±0.50	12.20a±0.50
OM (%)	0.51d±	0.70b±	0.66c±	0.67c±	0.87a±
TOC(%)	0.30d±	0.41b±	0.39c±	0.39c±	0.50a±
Saturation(%)	34.19d±0.32	39.40b±0.32	37.27c±0.32	38.40b±0.32	45.49a±0.32

*Mean value (n=3); ± Standard error; **Significant difference on the basis of p<0.05; The difference between lower case letters is the indication that values are significantly (p < 0.05) different from each other. T1, Control; T2, Farmyard manure; T3, Poultry manure; T4, Slurry; T5, Biochar

level. It is described that productivity of plants is decreased in saline soils due to lower water potential in root zone (Munns and Tester, 2008; Parida and Das, 2005). Salinity also reduces growth of plants by disturbing nutrient balance in plants (Porcel *et al.*, 2012). Severe saline conditions can lead to damaged and poor root growth, reduction in shoot growth, necrosis and even the death of the plant (Stiller, 2009; Marcar, 2016; Benjamin *et al.*, 2019). Plants of control showed poorest results as compared to treated plants in spite of *E. camaldulensis* has been reported to be the best among different species to tolerate salinity upto 28-36 dS m⁻¹ (Madsen and Mulligan, 2006).

In this study, almost all the amendments showed positive results in terms of trees growth but the best amendment was found to be farmyard manure. Biochar was found to be the best suited amendment to improve soil physico-chemical characteristics as compared to all other treatments. The findings are in accordance with previous studies as mentioned hereafter. It is reported that farmyard manure (FYM) has the ability to increase plant biomass to significant extent by providing them essential nutrients and increasing the quantities of nitrogen (N) (Liu *et al.*, 2007; Nawab *et al.*, 2018). In another study, farmyard manure was found to increase the grain yield and straw yield of wheat by 40.85% and 14.4 % respectively (Zoghdan and Ali, 2019). It is also reported that the growth and plant mineral uptake of alfalfa was increased when municipal solid waste and farmyard manure was applied at the rate of 40 mg ha⁻¹ under salt water irrigation (Mbarki *et al.*, 2020).

Results in figure 3 indicated that concentration of sodium in shoots and roots of *E. camaldulensis* plants reduced with organic amendments while the concentration of potassium enhanced with the usage of organic amendments. Among all

the amendments biochar showed the most profound results. The results are in accordance with the studies of previous results. It is reported that higher accumulation of Na⁺ ions is responsible for low plants growth as Akhtar *et al.* (2015) found that biochar improved the yield of potato crop by decreasing Na contents and increasing K contents. Lashari *et al.* (2015) observed that concentration K in leaf sap of maize significantly increased when treated with biochar. In another study, biochar application to salt affected soil reduced the bioavailability of Na ions and increased available potassium (Oram *et al.*, 2014). Abrishamkesh *et al.* (2016) found that the application of biochar increased the available K and biomass of lentils. It was reported that biochar was very helpful in removing toxic elements by the process of adsorption (Niazi *et al.*, 2018; Liew *et al.*, 2019).

Results of the current study revealed that unprocessed organic amendments showed better results as compared to biochar for morphological and growth parameters. The reason behind this is that biochar has the ability to release nutrients in slow fashion and supply it continuously which makes it better amendment than others. These results are supported by previous studies as it is reported that biochar incorporation results in enhanced nutrient cycling and affects plants performance (El-Naggar *et al.*, 2019; Purakayastha *et al.*, 2019). Large surface area and porous structure of biochar plays a very vital in slow release of biochar-bound nutrients (Xiao *et al.*, 2018). Structure of biochar helps in slow release of nutrient thus enhancing nutrient use efficiency and increasing nutrient bioavailability which in turns increase growth and yield of crop (Gwenzi *et al.*, 2017). In a study, it was suggested that chemical bonding to carbon molecules was another reason for this slow release of nutrients. In addition to that sorption capacity of some of the functional

groups of biochar is very high which allows to concentrate nutrients in problematic soils and releases it slowly through slow desorption process into aqueous phase for plants (Xiao *et al.*, 2018; Yu *et al.*, 2019).

Conclusion: Organic amendments have showed the positive effect on the growth of *Eucalyptus camaldulensis* under saline conditions. Unprocessed organic amendments showed better results as compared to biochar at early growth stages for eco-physiological characteristics of plants. Biochar is found to be most effective in improving physico-chemical characteristics of soil. Biochar lessened the Na concentration in plants and enhanced available K along with other organic amendments. Amending the saline soils with organic nature substances, that may be considered as environmental wastes, can help us in reclaiming saline soils by increasing plant growth and ameliorating soil fertility. A long term field study is further desired to examine the feasibility of soil amendments and to analyze the effects of biochar and other organic amendments on soil under natural field conditions.

Acknowledgement: We are highly acknowledged to Higher Education Commission of Pakistan for providing funding to Mr. Muhammad Talha Bin Yousaf, under the program of INDIGENOUS 5000 PHD FELLOWSHIP PROGRAM PIN NO. 213-60808-2AV2-166 (50025747).

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[Received 24 Aug 2020; Accepted 21 Dec. 2020; Published (online) 25 Jun 2021]