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Research Article

Effects of Biochar and Chitosan on Growth and Yield of Rice (*Oryza sativa* L.) Under Salt Stress

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Abstract

Salinity is one of the leading abiotic stresses that hindering growth and yield of rice. Biochar (BC) and Chitosan (CHT) has been shown to promote growth and yield of plants. However, it still unexplored whether the use of soil amendment BC and exogenous CHT can alleviate the detrimental effects of salt stress on rice. Hence, the current study explored the effect of BC and CHT to rice variety BRRI dhan100 grown under 80 mM NaCl stress conditions. The experiment was sequenced according to a Completely Randomized Design with three replicates. Six different treatments namely control (control, neither salt nor biochar or chitosan), BC (5%), CHT (200 ppm), NaCl (80 mM), NaCl plus biochar (NaCl+BC, 80 mM NaCl plus 5% biochar) and NaCl plus chitosan (NaCl+CHT, 80 mM NaCl plus 200ppm chitosan) were used in the experiment. The results confirmed that salt stress negatively affected plant height, number of tillers, leaf area, total chlorophyll concentrations, SPAD value, yield and yield contributing characters of rice plants. However, MDA content and Na^+/K^+ ratio significantly increased under salt stress. The use of biochar and chitosan led to significant increases in plant height, leaf area, total chlorophyll concentrations, SPAD value, yield as well as yield contributing characters of salt-stressed BRRI dhan100 plants; however these treatments cause significant decreases in MDA content and Na^+/K^+ ratio in the salt-stressed rice plants. The results demonstrated the significance of biochar and chitosan in mitigating the detrimental impacts of salt on growth and yield of BRRI dhan100 plants.

Keywords

Rice, Biochar, Chitosan, Growth, Yield and Salt

1. Introduction

Biochar (BC) is a carbon-rich solid product that generated by the thermal pyrolysis (between 300 and 1000 ℃) or gasification of biomass (waste-based, animal-based, or plant-based) in the absence of oxygen or under oxygen-limited conditions. [1]. BC has a higher porosity, larger surface area, and ion exchange capacity, which provide great potential for plant-available nutrients [2]. Many studies have emphasize the advantages of using BC in respects of mitigating global warming and salt stress, en-hancing crop growth and yield and, carbon storage [3-5]. A number of studies mentioned that the uses of BC maintained ion homeostasis by decreasing the Na^+/K^+ ratio in plants [6, 7]. Moreover, the application of BC enhances osmotic balance through improving water holding capacity in soil and CO_2 absorption that lead to improved photosynthetic activity [8, 9].

Chitosan (poly[1,4]-2-amino-2-deoxy-D-glucose; CHT) is

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a natural biopolymer derived *via* the deacetylation of biodegradable chitin from sea crustaceans [10]. Since the discovery of natural, environmentally friendly and low-toxic chemical CHT by Rouget in 1859, many studies proved that CHT enhance plant growth and mitigate plant abiotic stress tolerance, including rice [11-13]. The application of exogenous CHT increases drought and salt stress tolerance in plants *via* alleviation of oxidative stress [14-16].

Plants countenance different types of stresses, eg. salinity, drought, waterlogging, metal and temperature during their growth stages. Among them, salinity is one of the important abiotic stresses that hampered agricultural production worldwide. [17]. About 20% of soils over the globe are salt-affected and this percentage is estimated to be raised by 30% by the completion of 2050 [18]. Salinity stress also hampers the plants physiological and biochemical processes and induces ROS production, which damage membranes and proteins, and reduces nutrient uptake and growth. [19]. ROS accumulation in plant under salinity stress causes damage to photosynthetic pigments [20, 21]. Salt stress alter ion homeostasis and reduce transpiration, photosynthetic efficiency, and relative water contents in plants [22, 23]. Salinity creates osmotic, ionic and oxidative stress in plants [24] which finally reduced growth and yield of plant [25, 26].

Rice (*Oryza sativa* L.) is the global most important agronomical crop. As a food crop, it forms the staple food of more than three billion people, which supply about 50-80% of their daily calorie intake. [27]. Based on the high potential roles of BC and CHT under salt stress conditions, the present study aimed to investigate the role BC and CHT on comparative morpho-physiological and biochemical indices like growth parameters, photosynthetic pigments, ionic homeostasis and yield of rice.

2. Materials and Methods

2.1. Experimental Materials, Growth Condition, Design and Treatment

The experiment was carried out in Bangladesh Open University (90°38′N, 23°95′E), Gazipur, Bangladesh during the Boro season (November to May, 2022-2023). Rice variety BRRI dhan100 was used as a planting material and seeds were obtained from Bangladesh Rice Research Institute (BRRI), Gazipur, Bangladesh. Biochar was collected from the Christian Commission for the Development of Bangladesh (CCDB). The basic properties of soil were: soil pH of 6.5, organic carbon (%) of 1.686, organic matter (%) of 2.908, total N (%) of 0.17, exchangeable K 0.27 meq/100 g soil, available P, S, and Zn respectively of 12.9, 25.01, and 9.07 ppm. The basic properties of biochar were pH of 9.4, organic carbon (%) of 41.9, exchangeable Ca, Mg, and K respectively of 3.79, 2.23, and 1.84 meq/100 mL, total N (%) of 1.40, available P, Cu, Fe, Mn, and Zn respectively of 0.15, 0.05, 0.08, 0.032, and 0.012 μg/mL. Containers of 29 cm high, and 30.5 cm in diameter were used for the pot trial. Each container contained 12 kg of air-dried soil and received BRRI [28] suggested fertilizer doses (Urea, TSP, MoP, Gypsum and $ZnSO_4$ @138, 51, 63, 60 and 4 kg ha^{-1} respectively). The full dose of TSP, MoP and gypsum were applied during final soil preparation and urea was applied in three splits ($1/3^{rd}$ at 15 DAT, $1/3^{rd}$ at 30 DAT and $1/3^{rd}$ at 45 DAT). The study was carried out in a Completely Randomized Design (CRD) arrangement having three replications. The study consisted of following treatments - control, biochar @ 5%, chitosan @ 200 ppm, NaCl 80 mM, NaCl 80 mM + 5% biochar and NaCl 80 mM + 200 ppm chitosan. Before seedling transplanting, BC was weighed @ 5% by soil weight and mixed thoroughly with soil. At heading stage, plants were irrigated with NaCl solution at each 2-day interval for seven days. For CHT application, a solution of CHT (Wako, Japan) was prepared by melting in 0.01 M acetic acid. Then, during salt initiation, the plant with spraying with CHT at 9 am each 2-day interval for seven days. After seven days, morphological, physiological, and biochemical parameters were measured. Regular irrigation and other management were adopted throughout the experiment.

2.2. Measurement of Leaf Area

The leaf area was measured in top three leaves from each treatment at heading stage by using a digital leaf area meter (LICOR 3100).

2.3. Photosynthetic Pigments

A fresh leaf (100 mg) sample was extracted with 10 ml of 80% acetone for 72h. The absorbance of the extract was monitored at 645, 663, and 470 nm, respectively, for chlorophyll a, chlorophyll b, and carotenoids content. The following equations were used for calculation [29]: Chlorophyll a = 11.75 A_{663} - 2.350 A_{645}, Chlorophyll b = 18.61 A_{645} - 3.960 A_{663}, Total chlorophyll a+b = chlorophyll a + chlorophyll b and Total carotenoid = (1000A_{470} - 2.270 Chl a - 81.4 Chl b)/227.

2.4. Determination of Soil Plant Analysis Development (SPAD) Value

The concentration of leaf chlorophyll value (SPAD value) was measured by using a SPAD meter (Konica, Minolta SPAD-502 Plus, Inc., Japan). Selected expanded second leaves were used for the estimation of the SPAD values. The mean SPAD value was calculated from three readings in same leaf.

2.5. Determination of MDA Content

Lipid peroxidation was determined by measuring malondialdehyde (MDA) formation in leaf using the thiobarbituric acid (TBA) method [30]. The leaf sample (0.5 g, fresh weight) was mixed with 2 ml trichloroacetic acid (TCA, 5%) and centrifuged at 12,000 rpm for 10 min. Then, 1 ml centrifugation was mixed with 2 ml TCA (20%) containing 0.5% TBA. The test tube containing mixture was then placed in a hot water bath for 25 min. After cooling, the absorbance of the supernatant was read at 532 nm and 600 nm. The MDA content was calculated according to an extension co-efficient (155 mM^{-1} cm^{-1}).

2.6. Determination of Na^+ and K^+ Content

Straw ion (Na^+ and K^+) contents were determined by using a Na^+ ion meter (Horiba-731, Japan) and K^+ ion meter (Horiba-722, Japan). The straw was grinded by grinder and heated (95 ℃ for 30 min) with distilled water and cold at normal temperature. The sample was put to the sensor of the Horiba Na^+ and K^+ ion meter, and values were recorded.

2.7. Measurements of Plant Height, Number of Tiller and Yield Contributing Characters

After treatment application, the height of rice plants was measured by measuring scale and the number of tillers $hill^{-1}$ was counted. After plant harvest, the panicle and shoots were separated for measuring panicle $hill^{-1}$, grain $panicles^{-1}$, 1000-grain weight (g) as well as grain weight.

2.8. Statistical Analysis

One-way ANOVA (analysis of variance) with the statistics10 software was used to analyze the data. Means were compared using the least significant difference (LSD) test at $P < 0.05$ level of significance.

3. Results and Discussion

3.1. Effects of Salt Stress, BC and CHT on Growth Attributes

The results exhibited that salt stress significantly decreased the plant height of salt-susceptible BRRI dhan100 by 9.17% compared to control plants (Figure 1A). Under salt stress, BC applications improved the plant height of BRRI dhan100 by 9.25%, while the exogenous application of CHT enhanced plant height by 9.09% compared to that with salt stress alone. Figure 1B shows that salt stress significantly decreased number of tiller per hill by 29.80% in BRRI dhan100 over control plants. However, application of BC significantly increased number of tiller per hill in salt-stressed BRRI dhan100 compared to that of plants treated with salt alone. Whereas, CHT did not significant effect on number of tiller per hill in salt-stressed plant. This result in line with the recent findings [31] observed that the use of biochar has significantly boosted the plant height in salt-stressed rice. Similar result found by Khanam [32] in rice. Toan and Hanh [33] observed that chitosan played a functions of an elicitor to enhance the growth of rice plant. Hakim *et al.* [34] observed that the number of tillers $plant^{-1}$ were significantly reduced in salt susceptible rice varieties.

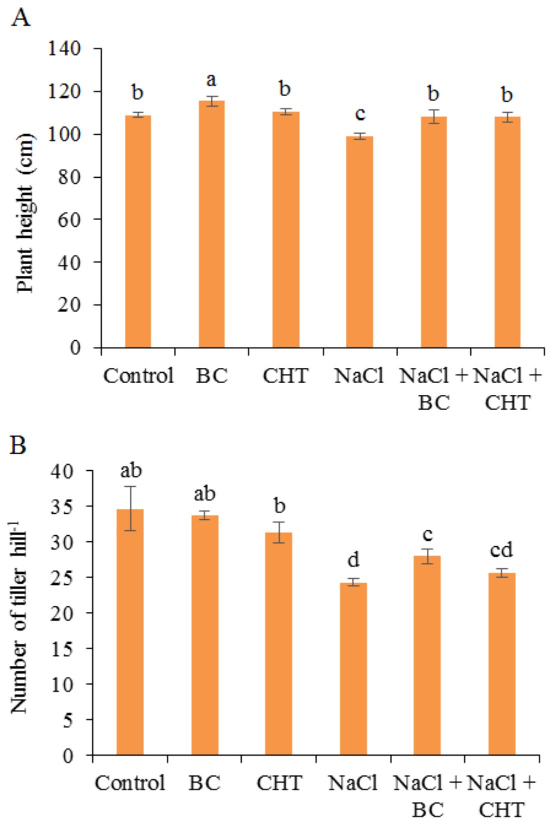

Figure 1. *Effect of biochar (BC, 5%) and chitosan (CHT, 200ppm) on plant height (cm) (A) and number of tiller* $hill^{-1}$ *(B) of rice variety BRRI dhan100 under salt stress (NaCl, 80 mM) for 7 days. Data are mean ±SD (standard deviation). Error bars represent LSD value at a 5% level of significance. Dissimilar letters indicate significant differences at* $P < 0.05$.

3.2. Effects of Salt Stress, BC and CHT on Leaf Area

Figure 2 shows salt stress decreased the leaf area of BRRI dhan100 by 19.57% compared to that of control plants. However, application of BC and/or exogenous application of CHT significantly increased leaf area to salt-stressed BRRI dhan100 by 19.50% and 25.88%, respectively. Similar observation was reported by Hakim *et al.* [34] who found that reduction of leaf area by salt stress in salt susceptible variety. These results are in agreement with the previous findings of El-Hadrami *et al.* [35], chitosan foliar application on maize and soybean plant significantly improved leaf area in comparison to the control. Like to the results of our study, Hou *et* *al.* [36] also observed that BC increased leaf area in salt-stressed cotton plant.

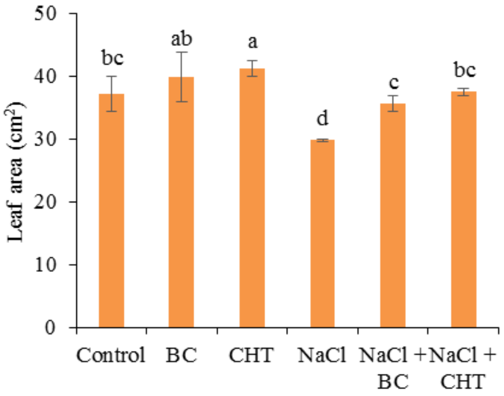

Figure 2. *Effect of biochar (BC, 5%) and chitosan (CHT, 200ppm) on leaf area (cm^2) of rice variety BRRI dhan100 under salt stress (NaCl, 80 mM) for 7 days. Data are mean ±SD (standard deviation). Error bars represent LSD value at a 5% level of significance. Dissimilar letters denote significant differences between treatments at P < 0.05.*

3.3. Effects of Salt Stress, BC and CHT on Photosynthetic Pigments and SPAD Value

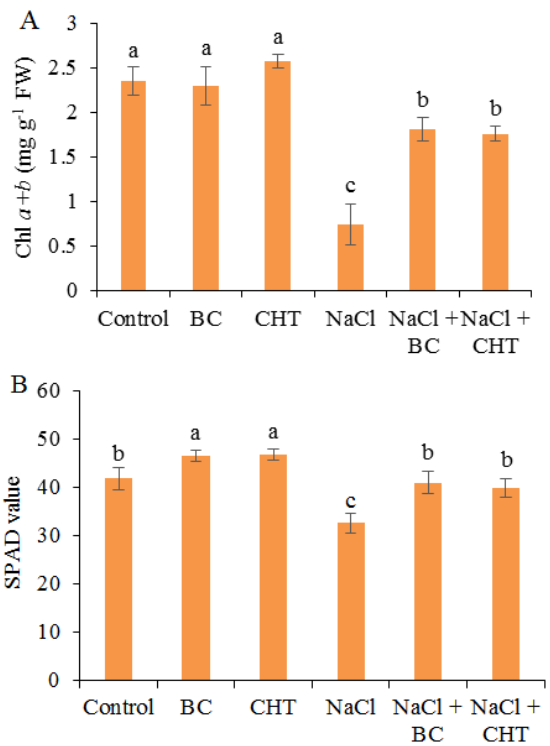

Figure 3. *Effect of biochar (BC, 5%) and chitosan (CHT, 200ppm) on Chl a+b (A) and SPAD value (B) of rice variety BRRI dhan100 under salt stress (NaCl, 80 mM) for 7 days. Data are mean ± SD (standard deviation). Error bars represent LSD value at a 5% level of significance. Dissimilar letters indicate significant differences between treatments mean at P < 0.05.*

Chlorophyll is a pigment that used to capture light and essential for photosynthesis [37]. In this study, salt stress significantly decreased the formation of total chl in BRRI dhan100, the value of which was 68.33% lower compared to that of the control (Figure 3A). The application of BC and/or foliar application of CHT significantly enhanced the total chl content in salt-stressed BRRI dhan100 which were 143.99% and 136.43% higher, respectively, compared to those of plants treated with salt alone. These findings are in agreement with that noted by Hafez *et al.* [38] who observed that soil amendment using biochar enhanced the levels of chlorophyll *a*, chlorophyll *b* in rice. In addition, Ran *et al.* [39] who reported that the chlorophyll content indicator and leaf N in rice plant were significantly affected by BC application under saline-sodic condition. On the other hand, the application of chitosan has been reported to boost Chl *a* and Chl *b* contents in rice [40], supporting that the mode of chitosan activity engage chloroplasts.

In this study, SPAD values in rice leaves were decreased with salt stress in BRRI dhan100 (Figure 3B). Salt stress significantly decreased the SPAD value of BRRI dhan100 by 22.04% compared to that of control plants. However, the application of soil amendment BC and foliar application of CHT to salt-stressed BRRI dhan100 significantly increased the SPAD value, which was 25.82%, 22.44%, respectively, compared to that of rice plants treated with salt alone. Similar observation was found by Hussain *et al.* [41] who stated that chlorophyll value (SPAD value) in rice leaves are detriment by the addition of NaCl, which might inhibit the electron transport in photosystem II (PSII). Our result is similar with Hafez *et al.* [42] where biochar application significantly increased SPAD value of rice leaves in salt-affected soil. Moreover, our finding is also supported with the result of Ullah *et al.* [43] who observed that exogenous application of CHT increased the SPAD value in salt-stressed tomato plant.

3.4. Effects of Salt Stress, BC and CHT on Lipid Peroxidation

Malondialdehyde (MDA) amount is always used to observe the level of lipid peroxidation under ROS-induced oxidative stresses [44]. Figure 4 shows that salt stress significantly increased the MDA content in BRRI dhan100 by 81.43% relative to control. Meanwhile, the application of BC and CHT significantly decreased MDA content in salt-stressed BRRI dhan100 by 28.75% and 22.64% respectively, compared with salt stress alone. The results reveal that MDA contents were considerably higher in salt-stressed rice plant than control, which implies that the stability of the cell membrane declined due to ROS generated oxidative damage. Sobahan *et al.* [45] reported that salt stress induces H_2O_2 production, which boosted the MDA content in rice. Lashari *et al.* [46] mentioned that the use of BC considerably increased the membrane stability by decreasing MDA concentration in maize plant. Similarly, Zhang *et al.* [47] observed that exogenous application of CHT decreases ROS and lipid peroxidation in lettuce under salt stress. These results indicate that BC and CHT can alleviate ROS-induced oxidative stress and restrain the stability of the cell membrane under saline conditions.

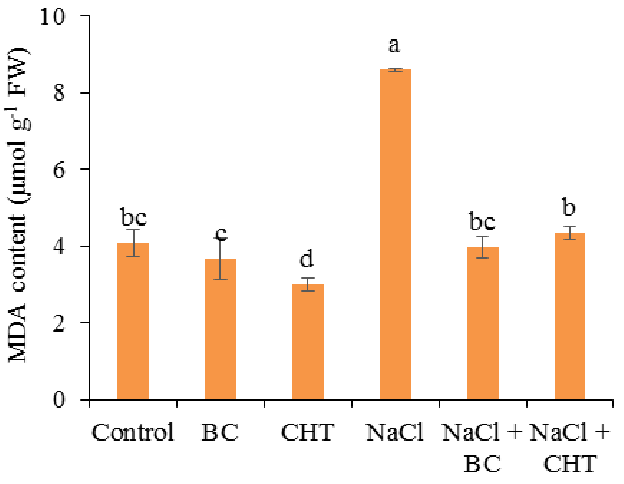

Figure 4. *Effect of biochar (BC, 5%) and chitosan (CHT, 200ppm) on MDA content of rice variety BRRI dhan100 under salt stress (NaCl, 80 mM) for 7 days. Data are mean ±SD (standard deviation). Error bars demonstrate LSD value at a 5% level of significance. Dissimilar letters imply significant differences between treatments at P < 0.05.*

3.5. Effects of Salt Stress, BC and CHT on Ion Homeostasis

The Na^+/K^+ ratio was significantly affected by salt stress in BRRI dhan100 (Figure 5). Compared to the control, the Na^+/K^+ ratio of BRRI dhan100 was increased by 5011% under salt stress. However, compared with salt stress alone, the application of BC and CHT significantly decreased Na^+/K^+ ratio by 39.16 and 50.40% respectively in salt-stressed BRRI dhan100. Therefore, BC and CHT treatments were most effectively restored the Na^+/K^+ ratio by significantly reducing straw Na^+ accumulation and enhancing K^+ uptake in BRRI dhan100. The sensitivity of rice to salinity in usual is associated with Na^+ accumulation because rice plant cannot regulate the uptake of Na^+ from saline soil, cause Na^+ transport from roots to the shoot [48]. It has been observed that the biochar application in rice reduced the shoot Na^+ and decreased the Na^+/K^+ ratio in the saline soil compared to no biochar application [31]. Moreover, Jin *et al.* [49] also found that the Na^+ ion accumulation at the stem, leaf, sheath and panicle of rice in saline-sodic soil were significantly decreased by adding biochar. Biochar addition reduced plant Na uptake by quick Na^+ binding due to its high adsorption ability and by unleashing mineral nutrients (particularly K^+, Ca^{2+}, Mg^{2+}) into the soil [50, 51]. Moreover, Hasanuzzaman *et al.* [52] mentioned that BC and CHT reducing the toxicity of Na^+ ion in jute plant by homeostasis of Na^+ and K^+ ion under salt stress conditions.

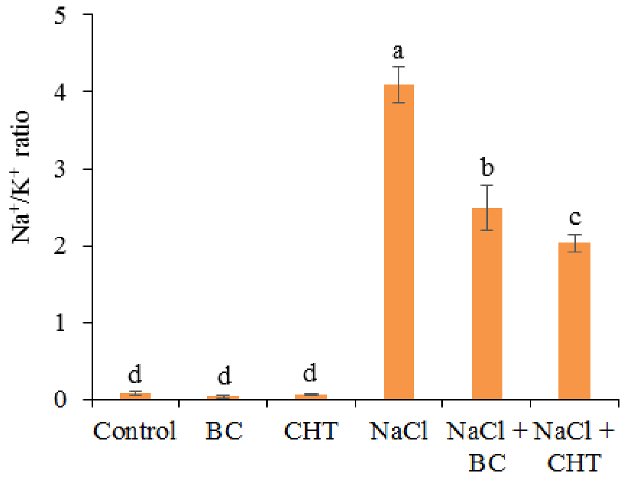

Figure 5. *Effect of biochar (BC, 5%) and chitosan (CHT, 200ppm) on Na^+/K^+ ratio of rice straw under salt stress (NaCl, 80 mM) for 7 d. Data are mean ± SD (standard deviation). Error bars demonstrate LSD value at a 5% level of significance. Dissimilar letters imply significant differences between treatments at P < 0.05.*

3.6. Effects of Salt Stress, BC and CHT on Yield and Yield Contributing Characters

Salt stress significantly decreased the grain weight hill^{-1} of BRRI dhan100 by 83.74% relative to control (Figure 6). However, the application of soil amendment BC and foliar application of CHT to salt-stressed BRRI dhan100 increased grain weight hill^{-1} by 80.78% and 79.17%, respectively, compared with salt stress alone. The data revealed that salinity stress decreased number of panicle hill^{-1} by 16.67% in BRRI dhan100 compared to that of control (Table 1). The application of BC and CHT did not improved the number of panicle hill^{-1}, compared with salt stress alone. Salt stress significantly decreased grains panicle^{-1} by 77.67% in BRRI dhan100, relative to control. The application of BC and CHT significantly increased the number of grains panicle^{-1} by 131.88% and 70.80%, respectively in salt-stressed BRRI dhan100 compared with salt stress alone. It also observed that salt stress significantly decreased 1000-grains weight by 29.41% in BRRI dhan100, compared to control. The application of BC and CHT did not changed 1000-grain weight in BRRI dhan100 (Table 1). Razzaque *et al.* [53] concluded that salt stress reduced grain weight and yield characters of rice at the reproductive stage. This result is accordance of Zhang *et al.* [54] who observed that the panicle number, grain number panicle^{-1} and 1000-grain weight of salt-sensitive variety Yangyugeng2 decreased under salt stress. Our results correlate with Sudratt and Faiyue [31] who reported that addition of biochar increased the yield and yield components of rice under salinity. Toan and Hanh [33] showed that application of

CHT solution significantly increased (~31%) yield in rice plant.

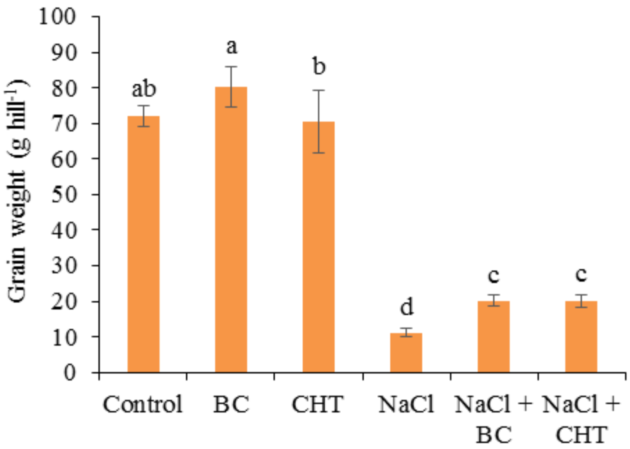

Figure 6. *Effect of biochar (BC, 5%) and chitosan (CHT, 200ppm) on grain weight of rice under salt stress (NaCl, 80 mM) for 7 d. Data are mean ±SD (standard deviation). Error bars represent LSD value at a 5% level of significance. Dissimilar letters denote significant differences between treatments at P < 0.05.*

Table 1. *Effect of biochar (5%) and chitosan (200 ppm) on panicle hill^{-1}, grains panicle^{-1} and 1000-grain weight (g) of BRRI dhan100 under salt stress (NaCl, 80 mM) condition.*

Treatments	Panicle hill^{-1}	Grains panicle^{-1}	1000-grain weight (g)
Control	34.00a	148.23c	14.31a
BC	33.00a	190.81a	14.40a
CHT	32.00a	168.92b	15.29a
NaCl	26.00b	33.09f	10.10b
NaCl+BC	26.66b	76.73d	10.37b
NaCl+CHT	25.00b	56.52e	10.76b
LSD $_{(0.05)}$	2.179	2.228	2.228
CV (%)	3.92	7.97	6.74

Note: BC= Biochar, CHT= Chitosan

4. Conclusion and Recommendations

In conclusion, current study exhibited that salt stress significantly decreased the plant growth, yield, photosynthetic pigment and increased MDA content as well as Na^+/K^+ ratio in BRRI dhan100. The application of BC (5%) or CHT (200 ppm) improved growth and yield of rice by reducing the deleterious effects of salt-stressed BRRI dhan100. Likewise, cultivation of BRRI dhan100 under saline condition should not exceed salinity 80mM. Bichar and chitosan solution are environmentally friendly and can be applied at a large scale for mass production of rice.

Abbreviations

BC	Biochar
CHT	Chitosan
NaCl	Sodium Chloride
MDA	Malondialdehyde
SPAD	Soil Plant Analysis Development
CO_2	Carbon di Oxide
ROS	Reactive Oxygen Species
BRRI	Bangladesh Rice Research Institute
CCDB	Christian Commission for the Development of Bangladesh
CRD	Completely Randomized Design
TSP	Triple Super Phosphate
MoP	Muriate of Potash
$ZnSO_4$	Zinc Sulphate
TBA	Thiobarbituric Acid
TCA	Tri-Chloroacetic Acid
ANOVA	Analysis of Variance
LSD	Least Significant Difference

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Authors Contributions

Muhammad Abdus Sobahan: Methodology, Investigation, Writing – original draft

Nasima Akter: Formal Analysis and Writing – review & editing

Md. Touhidul Islam: Conceptualization, resources

Conflicts of Interest

The authors declare no conflicts of interest.

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