AMELIORATION OF SALT STRESS IN RICE PLANT BY THE UTILIZATION OF BIOCHAR

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AMELIORATION OF SALT STRESS IN RICE PLANT BY THE UTILIZATION OF BIOCHAR

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CERTIFICATE

This is to certify that the thesis entitled **'AMELIORATION OF SALT STRESS IN RICE PLANT BY THE UTILIZATION OF BIOCHAR'** submitted to the faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE** in **AGRONOMY**, embodies the results of a piece of bona fide research work carried out by **MST. MUNNI KHANAM**, Registration No. **17-08314** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that any help or source of information, received during the course of this investigation has been duly acknowledged.

Dated: December, 2018
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DEDICATED

TO

MY BELOVED PARENTS

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All praises and compliments are due to the Supreme Regulator and Ruler of the Universe for the blessing upon the successful accomplishment of education, to complete the research work and thesis leading of Master of Science (MS) in Agronomy.

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The Author

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ABSTRACT

A Pot experiment was conducted in the experimental shed of Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka during Aman season (2018 July-2018 December) to ameliorate the salinity stress in rice as influenced by different level of biochar. The experiment was laid out in two factor CRD design with four replications. There were 12 treatments (3 salinity level × 4 level of biochar). Mixture of water with commercial NaCl was used as salinity treatments. Salinity level were S_0 = 0 ppm NaCl, S_1 = 1600 ppm NaCl, S_2 = 2800 ppm NaCl. Biochar level were B_0 = 0 t ha^{-1}, B_1 = 2 t ha^{-1}, B_2 = 4 t ha^{-1}, B_3 = 6 t ha^{-1}. The salinity treatments were applied on 20-40 DAT. Salt stress significantly reduced growth and yield including plant height, number of tillers $hill^{-1}$, number of effective tillers $hill^{-1}$, panicle length, number of filled grains $panicle^{-1}$, 1000 grain weight, grain yield , straw yield. With the rise of salinity level the adverse effect of salinity was more clearly visible. Application of 2800 ppm NaCl caused death of rice plant after 60 days of transplanting. Intrusion of 1600 ppm NaCl caused 28% yield reduction 2800 ppm NaCl caused 100% yield loses. However, application of different doses of Biochar ameliorates salt-induced damage in rice plant to a certain extent. Application of Biochar at 4 t ha^{-1} showed better result than other under 1600 ppm NaCl stress regarding growth and yield.

LIST OF CONTENTS

CONTENT (Cont'd)

CONTENT (Cont'd)

CONTENT (Cont'd)

CONTENT (Cont'd)

LIST OF TABLES

LIST OF FIGURES

Content (cont'd)

LIST OF APPENDICES

LIST OF ACRONYMS

Acronyms	Full word
AEZ	Agro ecological zone
BRRI	Bangladesh Rice Research Institute
BBS	Bangladesh Bureau of Statistics
panicle^{-1}	Per panicle
Cm	Centimeter
CV	Coefficient of Variation
DAT	Days After Transplanting
et al.	And others (et alibi)
FAO	Food and Agriculture Organization
Gm	Gram
Ha	Hectare
HI	Harvest Index
Kg	Kilogram
Hill^{-1}	Per hill
LSD	Least Significance Difference
m^2	Square Meter
MS	Master of Science
no.	Number
NS	Non-Significant
%	Percent
P^H	Hydrogen ion concentration
ROS	Reactive oxygen species
SAU	Sher-e- Bangla Agricultural University
SRDI	Soil Resources and Development Institute
t ha^{-1}	Ton per hectare

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CHAPTER 1

INTRODUCTION

Rice (Oryza sativa L.) is the staple food crop of more than half of the world's population (Anonymous, 2009). Rice is the second most widely grown cereal and primary source of food for more than half of the world population, and about 90% of the world rice is grown in Asia which is carrying about 60% of the world population (Haque et al., 2015). But the production of rice is decreasing day by day due to different environmental factors such as salinity stress. Among various environmental stresses, salinity is one of the most brutal environmental factors limiting the productivity of crop plant. A considerable amount of land in the world is affected by salinity which is increasing day by day. More than 45 million hectares (M ha) of irrigated land which account to 20% of total land have been damaged by salt worldwide and 1.5 M ha are taken out of production each year due to high salinity levels in the soil (Pitman and Läuchli, 2002; Munns and Tester, 2008). On the other hand, increased salinity of agricultural land is expected to have destructive global effects, resulting in up to 50% loss of cultivable lands by the middle of the twenty-first century (Mahajan and Tuteja, 2005).

Most of Bangladesh's coastal region lies on the southwest coastal region of the country. The majority of the saline land (0.65 million ha) exists in the districts of Satkhira, Khulna, Bagerhat, Barguna, Patuakhali, Pirojpur and Bhola on the western coast and a smaller portion (0.18 million ha) in the districts of Chittagong, Cox's Bazar, Noakhali, Lakshmipur, Feni and Chandpur. Farmers mostly cultivate low yielding, traditional rice varieties. Most of the land kept fallow in the summer or pre-monsoon hot season (March-early June) and autumn or post-monsoon season (October- February) because of soil salinity, lack of good quality irrigation water and late draining condition. In the recent past, with the changing degree of salinity of southwest coastal region of Bangladesh, crop production becomes very risky and crop yields, cropping intensity, production levels of crop and people's quality of livelihood are much lower than that in the other parts of the country. Cropping intensity in saline area of Bangladesh is relatively low, mostly 170% ranging from 62% in Chittagong coastal region to 114% in Patuakhali coastal region (FAO, 2007). The salinity of the soils is

either derived from tidal flooding with saline water at high spring tides or from sporadic inundation with salt water during cyclonic storm surge. Salinity is a major adverse environmental constraint for plant productivity, limiting the utilization of about 800 million ha of agricultural land globally (Yang *et al.*, 2011). Salinization of agricultural soils is a worldwide concern, especially in irrigated land. Salt induces osmotic stress by limiting absorption of water from soil, and ionic stress results from high concentrations of potentially toxic salt ions within plant cells. Salinity largely reduces the yield of rice. It causes membrane damage, nutrient imbalance, enzymatic inhibition, metabolic dysfunction, photosynthesis inhibition, and hampers other major physiological and biochemical processes. Salinity causes damage in plant start from germination and exist till death of plant in rice. Yield components related to final grain yield are also severely affected by root-zone salinity. Primary branches per panicle, panicle length, spikelet per panicle, number of filled spikelet, and seed weight per panicle are significantly reduced by salinity.

The increase in rice production under salt stress condition can be achieved by use of organic matter. Biochar is an organic matter. It is environment-friendly fertilizer. It is a fine-grained charcoal that is rich in organic carbon, produced by pyrolysis or by heating biomass in a low oxygen environment and it increase soil fertility. Biochar is favorable to optimize root morphology and physiological characteristics in rice and increases rice yield significantly. It could significantly change the approach to agriculture in Bangladesh as food poisoning from fertilizer is one of the biggest concerns of daily consume in salt-stressed croplands. Biochar with highly porous structure increase water holding capacity of sandy soil (Downie *et al*., 2009). Biochar is often regarded as a soil conditioner because its application rapidly increases the soil fertility and plant growth by supplying and retaining nutrients. The application of biochar to soil is considered to have the potential for long-term soil carbon sequestration, as well as for improving plant growth and suppressing soil pathogens. It could be applied as soil amendments for alleviating salt stress and enhancing crop productivity in salt-stressed rice field. Biochar is like organic matter that can effectively reduce the absorption of crops to Na^{+}, reduce the salinity stress to crops, supply minerals nutrients, increase soil organic carbon (Chaganti and Crohn, 2015).

With conceiving the above thinking in mind, the present research work has been undertaken in order to fulfil the following objectives:

- To know the effect of salinity on rice plant
- To know the effect of biochar on rice plant
- To study the role of biochar on rice plant in ameliorating salt stress.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Rice

Rice (*Oryza sativa L.*) is centre of lives of about half of world's population and it is possibly the oldest domesticated grain. It is mainly used as a source of energy due to quick digestion of proteins than others cereals. Rice can be used in different food processing industries such as snack food, beverages, bran oil, syrup and it is believed that rice has medicinal value (Ali *et al.*, 2014). In Asia both indica and japonica cultivated rice derived from *Oryza rufipogon* which was domesticated in China about 8,200-13,500 years ago (Wei *et al.*, 2012). After that rice cultivation spread all over the world very rapidly.. In Bangladesh, about 76% peoples live in rural areas and around 47.5% manpower involved in agricultural activities. In Bangladesh agriculture contributes about 19.3% of GDP in which 11.6% comes from rice (Bangladesh Finance Bureau, 2014). Due to the tropical climatic condition Bangladesh is suitable for rice cultivation and cultivated all over the country except southern hilly area. Rice is grown all year round in Bangladesh with three distinct seasons (*Aus*, *Aman* and *Boro*) and grown in four ecosystems namely irrigated (*Boro*), rainfed (transplanted *Aus* and *Aman*), rainfed upland (direct-seeded *Aus*) and deepwater (broadcast *Aman*) (Hussain, 2012).

2.2 Abiotic Stress

Abiotic stress is defined as the negative impact of non-living factors on the living organisms in a specific environment. The non-living variable must influence the environment beyond its normal range of variation to adversely affect the population performance or individual physiology of the organism in a significant way. Not even a single environmental condition is totally free from plant stress. Every plant has to face some extent of stress. Plants are subjected to various abiotic stresses such as low temperature, salt, drought, floods, heat, oxidative stress and heavy metal toxicity during their life cycle that hampers plant growth and development. These stresses may create toxic chemical components such as Reactive oxygen species (ROS) that contain4hydrogen per oxide (H_2O_2), superoxide (O^{2-}), hydroxyl (OH^-) and many more (Dasgupta *et al.*, 2014). Abiotic stress is a major factor in maintaining food security and world economy. Mostly under abiotic stress plant faces increase amount of

ROS accumulation in plant which is a threat to plant both in cellular and organ level (Keunen *et al.*, 2013). Abiotic stress hinders the production in many areas. Due to global climate change and unpredictable environmental conditions natural hazards are more common which is worsening the production condition (Mittler, 2002). Plants are facing more abiotic stresses as higher amount of toxic and antagonistic materials are released from industrial areas which pollutes both soil and irrigation channel. Abiotic stress hampers plant metabolism that negatively affect plant growth and development. If stresses continues rising and stays for a longer period of time it may result in plant death.

2.3 Salinity

Salinity is one of the major abiotic stresses in arid and semi-arid regions but salt-affected soils have been recorded in practically all the climatic regions and more than 800 million hectares of land or over 6% of the world surfaces are salt affected. Sodium chloride is the most soluble, pervasive, and superabundant salt in the world (FAO, 2008; Munns and Tester, 2008). Rapid population growth and subsequent food shortage especially in Asia and Africa and advancing salinity in arable land due to climate change have increased the importance of finding salt tolerant genotypes (Blumwald *et al.*, 2004). In the arid and semiarid regions, high rate of evapotranspiration and lack of inorganic salts leaching from the soil surface layers have given rise to increase salinity and sodicity. Salinity is the saltiness or amount of salt dissolved in a body of water. Saline soil is characterized by the presence of toxic levels of sodium and its chlorides and sulphates (Rajaravindran and Natarajan, 2012).). About one million ha of land of coastal and offshore areas are affected by varying degrees of salinity. In Bangladesh there are approximately 2.85 million ha of coastal soil. According to the report of Soil Resource Development Institute (SRDI, 2010) of Bangladesh, about 0.203 million ha of land is very slightly (2-4 dSm^{-1}), 0.492 million ha is slightly (4-8 dSm^{-1}), 0.461 million ha is moderately (8-12dSm^{-1}) and 0.49 million ha is strongly (>12 dSm^{-1}) salt affected soils in southwestern part of the coastal area of Bangladesh which occur in the southern parts of the Ganges tidal floodplain, in the young Meghna estuarine floodplain and in tidal areas of the Chittagong coastal plain and offshore islands. These coastal saline soils are distributed unevenly in 64 thanas of 13 coastal districts coverings agroecological zones (AEZ) of the country. The majority of the saline land (0.65 million ha) exists in the districts of Satkhira, Khulna, Bagerhat,

Barguna, Patuakhali, Pirojpur and Bhola on the western coast and a smaller portion (0.18 million ha) in the districts of Chittagong, Cox's Bazar, Noakhali, Lakshmipur, Feni and Chandpur. Large fluctuations in salinity levels over time are also observed at almost all sites in these regions. The common trend is an increase in salinity with time, from November- December to March-April until the onset of the monsoon rains. The factors causing salinization are numerous, including salt composition, climate, topography of lands and human activities (Blumwald *et al*., 2004). In terms of salt composition, various cations and anions are involved in salinization but the most important ion precipitate are Na^+ and Cl^- where Na^+ particularly causes the soil dispersion while Cl^- causes high toxicity and nutrient imbalances in plants. Excess salt in the soil influences plant activities including physiological, biochemical and molecular processes and crop production is suppressed by salinity in terms of quality and quantity. Intensity of salinity depends on the amount of salt in irrigation water, texture and structure of soils, type of plants, plant growth stages and irrigation schedules. At low intensity of salinity the damages are due to osmotic stress, nutritional imbalances and ion toxicity. At low salt concentration, shoot dehydration is the primary response of plants to osmotic stress (Carvajal *et al.*, 1999) and at moderate up to high salt concentration, nutritional imbalances due to interferences of saline ions and their toxicity caused by accumulating the ions especially Na^+ and Cl^- are the main effects of salinity on physiological and biochemical activities in plants (De-Pascale *et al.*, 2003a; De-Pascale *et al.*, 2003b).

2.4 Effect of salinity on plant

2.4.1. Germination Stage

Salinity severely affects plants especially at germination stage (Sosa *et al.*, 2005). Seed germination is very important stage for the successful establishment of healthy seedlings which are very sensitive to salinity as compared to other vegetative stages. Salinity accumulates the toxic ion in plants causing a mineral imbalance. The essential ions are reduced and do not meet the demand resulting in hindrance in normal physiological activities of plant. High salt stress retards seed germination process while low salt stress causes seed dormancy (Khan and Weber, 2008). To cope with such nutritional limitation, seeds develop a mechanism of maintaining low water potential (Allen, 1994) or other specific tolerance mechanism to prevent the damage due to salt stress. Salinity disturbs germination in a

number of ways. From reducing the osmotic potential of soil which declines in water inhibitions by seed (Khan and Weber, 2008) to the creation of ionic toxicity which alters enzymes action involved in nucleic acid metabolism. Other impacts of salt stress on seed germination include change in metabolism of protein. Seeds are more susceptible to salt stress due to close association to surface of the soil. With sodium chloride accumulation to a toxic level in soil, ionic stress decreases the rate of germination. Water absorption by the seed is reduced because of lower water potential caused by salt stress thus posing toxic effects to the developing embryo, resulting in delay in germination process (Khan and Ungar, 1984). The average time of seed germination is dependent on salinity stress, strength and genotypes. With increasing trend of salinity stress there is always decreasing rate of germination. Salinity has negative effect on the vigor index by raising salt concentration in the growing medium. Rice is extremely sensitive to salinity during germination, young seedling and early developmental stages for most commonly used rice varieties. However, in contrast, observed that rice is relatively salt tolerant at germination and in some cases is not affected significantly up to 16.3 dsm^{-1} of salinity. High levels of soil salinity can significantly inhibit seed germination and seedling growth, due to the combined effects of high osmotic potential and specific ion toxicity.

2.4.2. Growth stage

Once the seed has germinated, next goal for plant growth is crop establishment. Salinity causes reduction in crop establishment by reducing shoot growth, blocking leaf development and expansion, reducing growth of internodes and promoting abscission of leaf (Ziska *et al*.,1990). Salinity accelerates a number of factors in plants like osmotic stress, ion toxicity and nutrient imbalance; these are identified as most prominent causes of reduction in crop growth which finally lead to crop failure. However, different stages like germination, vegetative growth, flowering, seed establishment and grain filling of crops behave differently with salinity. The main harmful effects of salinity are reduced germination and emergence, stand and establishment of seedlings and enhanced chlorosis and senescence of leaves. To cope with osmotic stress, plants reduce the leaf area and increase the rooting density (Guo *et al.*, 2002). Increased amounts of salt in the soil pose a serious threat to different processes of plants which results in reduction of crop productivity. Epstein *et al* (1980) reported reduction in the uptake of essential ions in the plants due to salinity causes alteration in metabolic rates

and leading to reduction in growth rate. Excessive salt concentration in root zone of plant causes change in plant water relations. To deal with the increased amounts of salinity, the osmotic potential decreases (Kaymakanova and Stoeva, 2008). Salinity causes reduction in turgidity in plant cells due to reduction in water uptake by the plant. Low water uptake reduces cell division and regulation of stomata aperture which ultimately lead to low photosynthesis and finally death of plant tissues (Munns *et al.*, 2002). Reduction in turgor pressure results in stomata closure which causes reduction in gaseous exchange through transpiration (Munns and Tester, 2008). Other physiological activities under the influence of salinity include changes in membrane permeability leading to destabilization of membrane proteins and reduction in the process of photosynthesis (Ashraf and Shahbaz, 2003). Lowering of photosynthesis rate happens due to reduction in enzymes and pigments carrying out photosynthesis (Ashraf and Harris, 2013). It was found that the additional increase of leaf Na^+ and Cl^- also causes the production of ROS followed by reduced photosynthetic capacity leading to low plant growth (Nazar *et al.*, 2011). Many processes which are related with plant physiology and biochemistry are affected by salinity like photosynthesis (Hayat *et al.*, 2010), water conductance through stomata (Perez-Perez *et al.*, 2009), various biomolecules and plant-water relations. All these adversely affected biological processes ultimately reduces crop yield. Plants adopt various strategies in response of salinity that allow them to deal with the problem. Plants with growth in high salt concentration, have more thickness of leaves (Waisel, 1991), epidermis, cell walls and cuticles. The high salt concentration, increases mesophyll cell layers and cell size (Zekri and Parsons, 1990), due to more extension in cell wall at high turgor pressure (Munns and Termaat, 1986). Plants grown in salt stress conditions have large in number but narrow xylem vessels as compared to plant grown in salt free media (Walker *et al.*, 1985). Salinity increases the density of stomata of lower side of leaves and leaf thickness (Raafat *et al.*, 1991) with palisade tissues (Hussein *et al.*, 2012); however, it reduces number of cells per leaf. Salinity reduces the number of stomata on the surface of epidermis (Cavisoglu *et al.*, 2007), the total leaf area, (Awang *et al.*, 1993), leaf plastochron index (Bray and Reid, 2002). Vascular bundle length, xylem rows, number of vessels have also been reported to decline due to salinity (Hussein *et al.*, 2012). In rice, stem diameter was reported to be reduced (Pimmongkol *et al.*, 2002), while trichome and stomata density increased. Salt stress reduced cell size while trichome and

stomata density increased. Salt stress reduced cell size, epidermal thickness of leaves, apical meristem, diameter of cortex and central cylinder (Reinhardt and Rost, 1995). Salinity caused thickening of endodermis as well as exodermises and increased development of sclerenchymatous tissues (Javed *et al.*, 2001).

2.4.3. Yield

Salinity causes about 50% downfall in crop growth, productivity and yield throughout the globe. Nahar and Hasanuzzaman, (2009) came with a result that that salt stress decreased different components of yield in *V. radiata* and rice. Kafi and Goldam, (2000) determined the response of plants against salinity stress and concluded that salinity poses a serious problem in vegetative and reproductive stage in the plants. Differences in yield response of rice to soil salinity can be related to climatic variations. In particular, a low relative humidity of the air during the growing season can enhance the yield losses per unit increase of salt concentration because the potential yield is higher in the dry season, as a consequence of longer and more intense solar radiation in the dry season than in the wet season. It has been well documented that the effect of salinity on seedling growth, seedling establishment, grain yield components such as tiller number has successively lead to a reduction in grain yield (Khatun *et al.*, 1995). Salinity also resulted in a decrease of the 1000 grain weight and increased sterility, regardless of the season and development stage (Khatun *et al.*, 1995)

Mechanism of salt stress on plant

2.5.1 Osmotic effect

Plants are stressed under high salt concentrations either by increased osmotic potential or by toxic effects of high ionic concentrations (Brady and Weil, 2002). In osmotic or H_2O deficit environments, soluble salts reduce the water potential and make water not freely available to plants for uptake which is the major reason for stunted growth under salinity. It is very difficult to distinguish between either water deficiency is due to salinity or drought (Nawaz *et al.*, 2010). The water potential of soil controls new leaf formation. Rapidly growing cells have the capacity to store higher levels of salts in their expanding vacuoles, so the growth of the new leaves is not restricted due to gathering of salts in the cytoplasm. Root and shoot growth is more disturbed because of water stress than salt specific effect during the early

days of stress (Munns, 2002). At moderate osmotic stress, root growth is not much affected whereas the reduction of shoot growth is maximum (Hsiao and Xu, 2000). Damage due to osmotic effect is governed by plant species, time period of stress, types of cells and tissues and the method of stress application.

2.5.2 Specific ion effect

Ion specific toxicity, generally, is because of certain ions like sodium, chloride and sulphate which are taken up in larger quantities than routine. It affects the crop right from emergence to physiological maturity. Crops fail especially when specific ions affect at lateral growth stages. Regarding tolerance against salt stress different crops have different levels of responses. Most of the higher plants especially agricultural crops are highly susceptible to this stress. Under saline or sodic environments, high concentrations of sodium and chloride ions coupled with low concentration of potassium ions was observed in leaves of wheat varieties (Maas *et al.*, 1986). Mostly the salts are accumulated in the older leaves of plants. With higher concentration of salt accumulation there may be death of leaves; this happens when the salt concentration is too high, hence cannot be retained inside the vacuoles. In such cases the excessive salts go to the cytoplasm where they affect the normal mechanisms of enzyme action. On the other hand, excessive salts cause cell dehydration by being accumulated in the cell walls. In defense against this effect, plants either try to restrict the salt entry in their bodies or reduce the amount of salts in their cytoplasm. Concentration of sodium in the cytoplasm of the root cells is from 10-30 mM (Tester and Davenport, 2003). Due to high concentrations of sodium and chloride ions inside leaf sap, root and shoot fresh weight reduces up to 50% .

2.5.3 Nutritional imbalance

Ions discrepancy is caused by higher accumulation of sodium and chloride and consequently less absorption of the other minerals such as calcium, manganese and potassium. Elevated Na+: K+ ratio causes enzyme inactivation and affects normal metabolic functions of the plants (Booth and Beardall, 1991). Building up of salt deposition disturbs water relations of the plants; this results in limited uptake and utilization of important nutrients. As a result, metabolic activities of the cell and functioning of the enzymes is disturbed (Lacerda et al., 2003). Nutrients and salt interaction cause deficiencies and imbalances of the major

nutrients. More uptake of Na+ causes reduction in the uptake of potassium and symptoms like potassium deficiency are observed. The regulation of calcium within the plant under saline condition is a crucial parameter of plant salt tolerance. Potassium is main component for protein formation, osmoregulation, photosynthesis and maintenance of cell turgor pressure. Decrease in potassium ion uptake in due to salinity stress was observed (Marcar et al., 1991). K+ along with Ca^{2+} are necessary for maintaining the integrity and proper working of the cell membranes (Wenxue *et al.*, 2003). Sufficient amount of K+ in plant cell under salinity depends on the uptake on selection basis of the potassium ions and discriminatory compartmentalization of K^+ and Na^+ ions in the shoots.

2.5.4. Oxidative stress

A major effect of salinity is elevation in production of ROS e.g. H_2O_2, O^{2-}, and OH^-(Mittler, 2002; Munns, 2002). Proteins, lipids and nucleic acids are damaged oxidatively by ROS and hence negatively affect the normal cellular metabolism. Reduction of oxygen causes formation of these ROS that disturb plant metabolic routes ROS production occurs at minute level during the normal body and cell growth but increased production occurs during stressed conditions (Laloi *et al.*, 2004). Osmotic effect inhibits the stomata opening and decreases the CO_2 supply for photosynthesis which stimulates the deposition of super oxides in chloroplast. This deposition of super oxides promotes the photoinhibation and photo oxidation in plant cells (Ashraf, 2009). Plants have unique appliances to salvage these ROS such as stimulation of the enzymes of antioxidative pathway (Smirnoff, 2005)

2.6 Biochar

2.6.1 Biochar for crop improvement

The term ‘biochar’ was coined by Read to describe charcoal used for soil improvement. Like most charcoal, biochar is produced by pyrolysis of biomass in low or anaerobic conditions and has the potential to mitigate climate change, via carbon sequestration. It increases pH of acidic soils, agricultural productivity, and provides protection against some foliar and soil-borne diseases and reduces pressure on forests (Ndameu, 2011). It is a stable solid, rich in carbon, and can endure in soil for thousands of years. Historically**,** Pre-Columbian Amazonians were believed to have used biochar to enhance soil productivity. They produced

it by smoldering of agricultural wastes. Biochar production and application has been proposed as one of the options that mitigates climate change (Lehmann, 2007) and improving soil fertility and crop productivity (Major *et al*., 2010). Its porosity is very beneficial for improving soil structure and water holding capacity (Karhu *et al*., 2011 and Vaccari *et al*., 2011) hence mitigating the increasing drought stress in dryland agriculture due to climate change. Biochar is a technology that normally provides conditions suitable for crop improvement by providing the necessary nutrients for growth, development as well as the yield. For instance (Alburquerque *et al*., 2013, Khan *et al*., 2013, Rajkovich *et al.,* 2012, Schulz and Glaser, 2012 and Asai *et al*., 2009) found from their studies using different methods in biochar study such as greenhouse/glasshouse, field laboratory and microcosm on the effect of biochar on plant and recorded a significant positive change in plant growth and development. But the nature of the plant growth and development is subjected to many factors. In the case of (Rajkovich *et al*., 2012) different types of biochar were applied including corn stover, dairy manure paper sludge, and food. Food for instance included in the Rajkovich *et al*. (2012) study was not specifically stated as to whether it is corn, millet, sorghum (cereal) or root and tubers based food but only stated its source implying it cannot be pinpointed which food biochar is for a specific crop. Schulz and Glaser (2012) and Asai *et al*. (2009) recorded an increase in the yield of oat crop by the use of babecue charcoal. Considering the cost involved in the pyrolysis. Different biomass could have been used to bring out different results in order to make meaningful comparison.

From the review of literatures it may be concluded that salinity and different level of biochar application had significant influence on rice and other crops to produce increased plant growth and yield character.

CHAPTER 3

MATERIALS AND METHODS

A brief description about experimental site, climatic condition, planting materials, treatments, experimental design and layout, crop growing procedure, intercultural operations, data collection and statistical analysis were described in the chapter. The details of experimental materials and methods are described below:

3.1 Location

The experiment was conducted at the Experimental shed of the Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka during the period from July to December, 2018. The location of the experimental site has been shown in Appendix I.

3.2 Climate

The geographical location of the experimental site was under the subtropical climate and its climatic conditions is characterized by three distinct seasons, namely winter season from the month of November to February, the premonsoon period or hot season from the month of March to April and monsoon period from the month of May to October Details of the meteorological data of the experimental period were presented in Appendix II.

3.3 Soil

The soil of the experimental area belonged to the Modhupur tract (AEZ No. 28). It was a medium high land with non-calcarious dark grey soil. The pH value of the soil was 5.6. The physical and chemical properties of the experimental soil have been shown in Appendix III.

3.4 Materials

3.4.1. Plant material

BRRI dhan62 was used as plant material in the experiment. The features of the varietiey is presented below: BRRI dhan62, a high yielding variety of rice, was used as a test crop. The variety was developed by the Bangladesh Rice Research Institute (BRRI), Joydebpur, Gazipur as a short duration T. Aman rice. BRRI dhan62 is a zinc enriched rice variety with

19 mgkg^{-1} Zinc and 9% protein. The life duration of this rice variety is 100 days and yields about 3.5-4.5 t ha^{-1}.

3.4.2 Plastic pot

Empty plastic pots with 18 inch depth were used for the experiment. Ten kilogram sun-dried soils were put in each pot. After that, pots were prepared for seed sowing.

3.5 Design and layout

The experiment was laid out in Completely Randomized Design (CRD) with two factor and four replications using 48 plastic pots..

Conduction of the experiment

3.6 Seed collection

Seeds of BRRI dhan62 collected from Bangladesh Rice Research Institute (BRRI), Joydebpur, Gazipur, Dhaka.

3.7 Pot Preparation

The collected soil was sun dried, crushed sand sieved. The soil, fertilizers and organic matter were mixed well before placing the soils in the pots. Soils of the pots were poured in polythene bag. Each pot was filled up with 10 kg soil. Pots were placed at the net house of Sher-e-Bangla Agricultural University. The pots were pre-labeled for each biochar and treatment. The pot treated with biochar was adjusted with soil to make the weight 10 kg in each pot. Finally, water was added to bring soil water level to field capacity.

3.8 Fertilizer Application

Urea, Triple super phosphate, Muriate of potash, Gypsum and Cowdung were applied in the experimental pots @ 250 kg ha^{-1}, 110 kg ha^{-1}, 140 kg ha^{-1}, 50 kg ha^{-1}, 300 gpot^{-1}respectively. One-third of urea and the whole amount of other fertilizers were incorporated with soil at final pot preparation before transplanting. Rest of the Urea were applied in two equal splits one at 30 days after transplanting (DAT) and second at 45 days after transplanting pot.

3.9 Preparation and application of biochar

Biochar collected from FAB lab, SAU and then biochar was grinded followed by sieving for using in the pot. Then recommended biochar was added to the soil of each pot along with fertilizers at the time of final pot preparation.

3.10 Sowing of seeds in seedbed

Seeds were washed several times with fresh water and soaked in a dark place for about 48 hours. The uniformly germinated seeds were then transferred to the field and were took about 20-25 days to produce seedling.

3.11 Uprooting and transplanting of seedlings

The seedlings were carefully uprooted from the field. Seedling of 25 days old were transplanted into respective pots on 9^{th} August 2018. There were three hills per pot and one seedling was used per hill.

3.12 Intercultural operations

After establishment of seedlings, various intercultural operations were accomplished for better growth and development of the rice seedlings.

3.12.1 Gap filling and thinning

After sowing seeds continuous observation was kept. It was observed that no single seed failed to germinate. So, there was no need of gap filling. Keen observation was made for thinning to maintain 1 seedling per pot.

3.12.2 Weeding and irrigation

Sometimes there were some small aquatic weeds observed in pots that were uprooted by hand pulling. About 3-4 cm depth of water was maintained in the pot until the crop attained maturity although a 2-3 days drying period was provided after tillering stage to suppress the weeds.

3.12.3 Insect and diseases

There was no infection of diseases in the field but leaf roller (Chaphalocrosis medinalis) was found in the field and used Malathion @ 1.12 L ha^{-1} at 30 DAT with using a hand sprayer.

At the end, rats attack occurred. Pesticide mixed with dried fish was applied at the corner of the pots. From heading onwards, the pots were netted to protect the rice grain from the attack of birds.

3.14 Salinity treatment

The salinity treatments were applied on 20-40 DAT. There were three salinity levels including control where developed by adding respected amount of commercial NaCl salt to the soil/pot as water dissolved solution. The salinity levels were S_0 (control), S_1 (1600 ppm NaCl), S_2 (2800 ppm NaCl). When no salt added in control (S_0) while 18 g salts in S_1, 28 g salts in S_2 in each pot.

3.15 Treatments

The experiment consisted of two factors as mentioned below:

Factor A: 3 salinity level (s)

1. S_0 - Control (no salt application)
2. S_1 - application of 1600 ppm NaCl
3. S_2 - application of 2800 ppm NaCl

Factor B: 4 biochar level (B)

1. B_0= Control (no biochar application)
2. B_1=application of 2 t ha^{-1} biochar
3. B_2= application of 4 t ha^{-1} biochar
4. B_3= application of 6 t ha^{-1} biochar

Treatment combination: Twelve treatment combinations were as follows

i. $S_0 \times B_0$

ii. $S_0 \times B_1$

iii. $S_0 \times B_2$

iv. $S_0 \times B_3$

v. $S_1 \times B0$

vi. $S_1 \times B_1$

vii. $S_1 \times B_2$

viii. $S_1 \times B_3$

ix. $S_2 \times B_1$

x. $S_2 \times B_2$

xi. $S_2 \times B_3$

xii. $S_2 \times B_4$

3.16 General observation of the experimental pots

Observations were made regularly and the plants looked normal green. No lodging was observed at any stage. The maximum tillering, panicle initiation, and flowering stages were not uniform.

3.17 Harvesting and Processing

The experimental crop was harvested at maturity when 80% of the inflorescence turned reddish yellow in colour. Harvesting was done in the morning to avoid shattering. The crop was sun dried properly by spreading them over floor and seeds were separated from the inflorescence by beating the bundles with the help of bamboo sticks. The seeds thus collected were dried in the sun for reducing the moisture in the seed to about 9% level. The husk and straws were also dried in the sun and weight was recorded.

3.18 Data collection

Data were collected on the following parameter:

3.18.1. Crop growth parameters

a) Plant height hill^{-1}

b) Number of tiller hill^{-1}

c) No. of leaves hill^{-1}

d) Leaves area meter (cm^2)

3.18.2. Yield contributing parameters

a) Number of effective tillers hill^{-1}

b) Panicle length (cm)

c) Number of filled grains panicle^{-1}

d) Total grains hill^{-1}

e) Thousand grain weight (g)

3.18.3. Yield parameters

a) Grain yield pot^{-1} (g)
b) Straw yield pot^{-1} (g)

3.19 Procedure of sampling growth contributing parameter

3.19.1 Plant height

The height of the rice plants was recorded from 20 days after transplanting (DAT) at 20 days interval up to harvest, beginning from the ground level up to tip of the leaf was counted as height of the plant. Mean plant height was calculated and expressed in cm.

3.19.2 Number of tillers hill^{-1}

Total tillers which had at least one leaf visible were counted. It includes both productive and unproductive tillers. Data on tiller number hill^{-1} were counted from 3 selected hills and average value was recorded.

3.19.3 Number of leaves hill^{-1}

No. of leaves were counted at 20, 40, 60 days**.** Total number of leaves were counted from three hills and then averaged to leaves per hill.

3.19.4 Leaf area index

Leaf area index was measured manually at the time of 40, 60 and at harvest.

3.20 Procedure of sampling yield contributing parameter

3.20.1 No. of effective tiller hill^{-1}

Effective tiller number hill^{-1} was counted at harvesting. There were three hills in each pot.

The effective tiller number hill^{-1} was counted from the pot.

3.20.2 Panicle length

Panicle length was recorded at harvesting. Panicle length was recorded from the basal nodes of the rachis to apex of each panicle

3.20.3 Number of filled grains panicle^{-1}

The number of filled grains was calculated by counting the number of filled grain from all panicles hill^{-1}. No. of filled grain was counted at harvesting.

3.20.4. Total grain hill^{-1}

Grains from all panicles were counted and average number of grains for each panicle was determined. Filled and unfilled grains are also determined from the panicle.

3.20.5. 1000-grain weight

All grains collected from each pot was oven dried. Thoushands grain were separated and weighed.

3.21. Procedure of sampling yield parameter

3.21.1 Grain yield pot^{-1}

Grain yield was recorded at harvest. Threshing was done and grains were separated then sun dried and weighed.

3.21.2. Straw yield per pot^{-1}

It was counted at harvesting. The straw were separated by threshing found from each pot and weighed after sun drying.

3.22. Statistical analysis

The data obtained for different parameters were statistically analyzed following computer based software statistix 10 and mean differences among treatments were tested with LSD Test at 5% level of probability.

CHAPTER 4

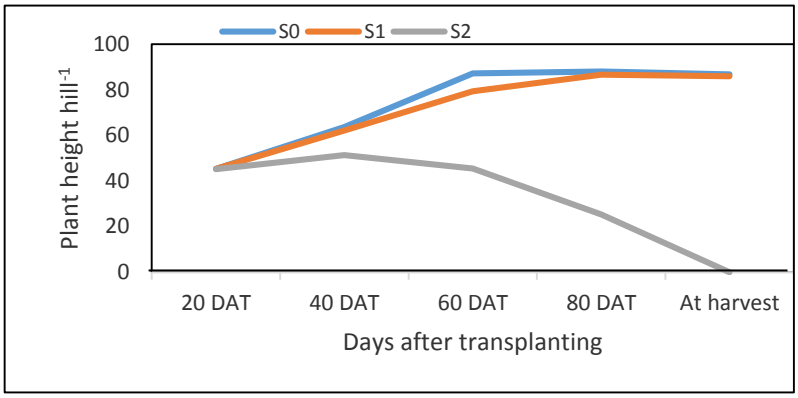
RESULTS AND DISCUSSION

4.1 Growth Parameters

4.1.1 Plant height

4.1.1.1 Effect of salinity

Significant variation was observed on plant height at different level of salinity (Figure 1 and appendix IV). From 20 DAT to harvest plant height decreased with increasing the level of salinity. The highest plant height was observed at control (S_0) at 80 DAT (87.97 cm) which was statistically similar with S_1 treatment. On the other hand the highest salinity level (S_2) gave the lowest plant height (51.36, 45.45, 25.34 cm) at 40 and 60 DAT and final harvest, respectively. The magnitude of decrease in plant height increased with time and salinity. At harvest, the plant died at treatment S_2. Alam *et al.* (2001) stated that stunted plant growth is as the most common symptom of salinity at the vegetative stage

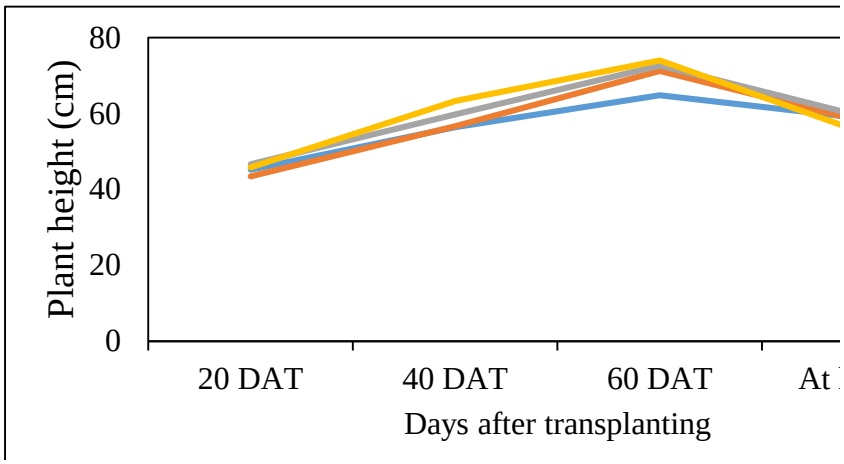


S_0 = Control (no salinity), S_1 = 1600 ppm NaCl, S_2=2800 ppm NaCl

Figure 1. Effect of salinity on plant height hill^{-1} at different days after transplanting (LSD $_{(0.05)}$) = 3.63, 14.68, 12.49, 4.73 at 40, 60, 80 DAT and harvest, respectively]

4.1.1.2 Effect of biochar

The plant height was significantly influenced by different level of biochar application from 20 to 40 DAT of rice (Figure 2 and appendix IV). The highest plant height was recorded at 20 DAT with the application of 4 t ha^{-1} Biochar. Van Zwieten *et al.* (2010) reported a nearly 30-40 per cent increase in wheat height when biochar produced from paper mill sludge was applied at a rate of 10 t ha^{-1} to an acidic soil. Gonzaga *et al*. (2019) also reported that biochar increased the plant growth of mustard by approximately 30 to 224%



B_0= Control (no biochar application), B_1= application of biochar 2 t ha^{-1}, B_2= application of biochar 4 t ha^{-1}, B_3= application of 6 t ha^{-1} biochar

Figure 2. Effect of biochar on plant height hill^{-1} of rice at different days after transplanting [LSD ($_{0.05}$) = 2.66, 4.19 at 20 and 40 DAT, respectively]

4.1.1.3 Combined effect of salinity and biochar

Plant height (cm) was varied significantly due to interaction of salinity and biochar at all the growth stages and at harvest which was shown in Table 1 and appendix IV. Combination of S1B3 showed the tallest plant (69.05 cm) at 40 DAT which was statistically similar with S0B1, S0B2, S1B2. At 60 DAT, the tallest plant (88.09 cm) was obtained from S0B2 which was statistically similar to S0B0, S0B1, S0B3, S1B0, S1B1, S1B2, S1B3. On the other hand, the shortest plant (30.31 cm) was observed from S2B0 at 60 DAT which was statistically similar to S2B1, S2B2, S2B3. At harvest, the highest plant height was obtained from S0B2 treatment which was statistically similar to S0B0, S0B1, S0B3,

S_1B_0, S_1B_1, S_1B_2, S_1B_3. The plant died at S_2B_0, S_2B_1, S_2B_2, S_2B_3 treatment at harvest period.

Table 1. Combined effect of salinity and different level of biochar on plant height hill^{-1} at different growth period of T. Aman rice

Treatments	Plant height (cm) at			
	20 DAT	40 DAT	60 DAT	At harvest
S_0B_0	44.27	61.25	87.21	86.74
S_0B_1	45.85	63.43	85.52	87.26
S_0B_2	48.28	68.11	88.09	89.67
S_0B_3	42.49	61.78	87.86	83.53
S_1B_0	45.53	58.85	76.85	89.33
S_1B_1	43.74	56.96	80.90	86.03
S_1B_2	44.74	63.38	78.98	87.69
S_1B_3	47.44	69.05	80.66	80.95
S_2B_0	45.93	49.17	30.31	0
S_2B_1	40.83	49.54	47.29	0
S_2B_2	46.78	47.72	50.88	0
S_2B_3	47.58	59.03	53.33	0
LSD$_{(0.5)}$	4.61	7.25	29.31	9.47
CV (%)	7.07	8.54	28.88	11.43

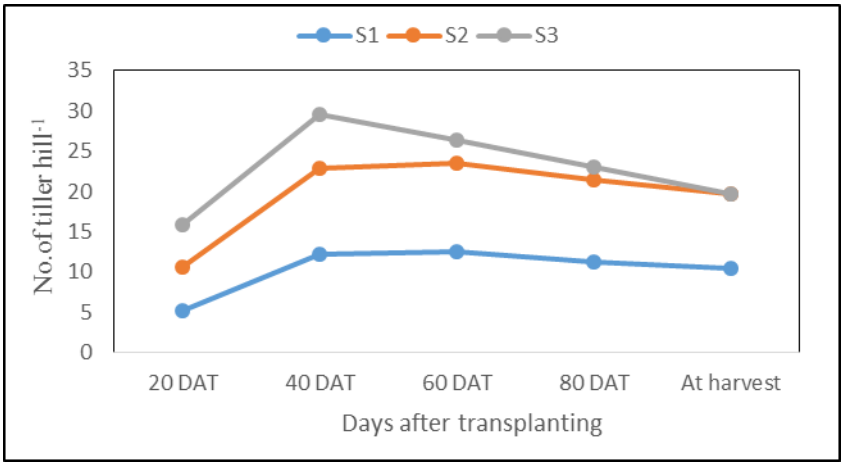
S_0 = Control (no salinity), S_1 = 1600 ppm NaCl, S_2 = 2800 ppm NaCl**,** B_0 = Control (no biochar application), B_1= application of biochar 2 t ha^{-1}, B_2= application of biochar 4 t ha^{-1}, B_3 = application of biochar 6 t ha^{-1}

Values with different letters are significantly different at 5% level of probability

4.1.2 No. of tillers hill^{-1}

4.1.2.1 Effects of salinity

Salinity intrusion has affected tillers production throughout the entire growth period of rice**.** Tiller no. recorded at 40, 60 DAT and at harvest of rice plants have been presented in Figure 3 and appendix V. At 40 DAT, the highest no of tiller hill^{-1} (12.49) was recorded from S_0 treatment, which was statistically similar with S_1 treatment. The number of tiller decreased with increasing the salinity level. The majority of tiller increased with increasing the salinity and growth duration. There were no alive tiller at S_2 at harvest. It was reported that number of tiller decreased progressively with increase in salinity level (Javed and Khan, 1975).



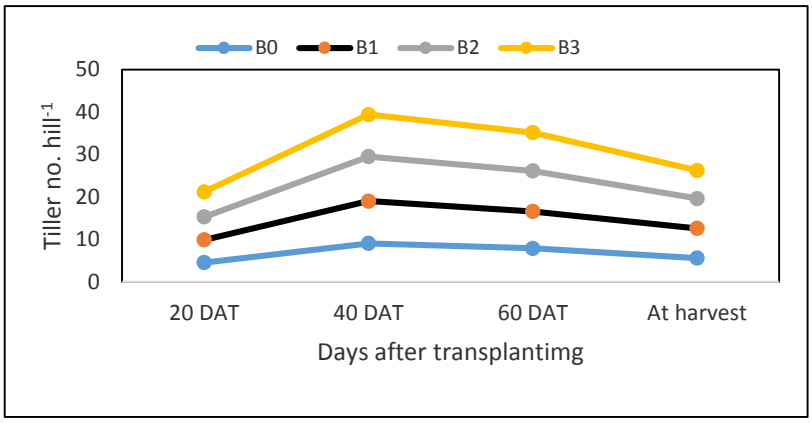
S_0 = Control (no salinity), S_1 = 1600 ppm NaCl, S_2=2800 ppm NaCl

Figure 3. Effect of salinity on the number of tillers hill^{-1} of rice at different days after transplanting (LSD (0.05) = 1, 1.57, 1.15, 0.7 at 40, 60 DAT and at harvest, respectively]

4.1.2.2 Effect of biochar

Tiller hill^{-1} was significantly influenced by different levels of biochar application at early growth stages of rice (Figure 4 and appendix V). At 20 DAT tiller hill^{-1} increased with increasing level of biochar application. The highest number of tiller hill^{-1} (5.90) were observed from 6 t ha^{-1} of biochar (B_3) at 20 DAT which was statistically similar with B_1, B_2 treatment. Whereas the lowest tiller number hill^{-1} (4.57) were observed from 0 t ha^{-1} of biochar application (B_0). At 40 and 60 DAT, tiller number was not significantly influenced

by biochar application. At harvest, the highest tiller hill^{-1} (7.05) were counted from 4 t ha^{-1} of biochar application (B_2) that statistically similar to B_1 and B_3 treatments



B_0= Control (no biochar application), B_1= application of biochar 2 t ha^{-1}, B_2= application of biochar 4 t ha^{-1} , B_3= application of biochar 6 t ha^{-1}

Figure 4. Effect of biochar on number of tillers hill^{-1} of rice at different days after transplanting [LSD $_{(0.05)}$ = 0.67 and 0.8 at 20 DAT and harvest, respectively]

4.1.2.3 Combined effect of salinity and biochar

Combination between salinity and different levels of biochar exerted significance effect on tiller no hill^{-1} at 60 DAT and at harvest (Table 2 and appendix V). At 60 DAT, the highest tiller (13.15) were observed from (S_0B_2) that was statistically similar to S_0B_0, S_0B_1, S_0B_3, S_2B_1, S_1B_2, S_1B_3. At 60 DAT, lowest number of tiller (1.38) recorded from S_2B_0 with 280 ppm NaCl with no biochar. At harvest, there was no alive plant at S_2B_0, S_2B_1, S_2B_2. .

Table 2. Combined effect of salinity and different level of biochar on number of tiller hill^{-1} at different growth period of T. Aman rice

Treatments	Tillers hill^{-1} (No.) at			
	20 DAT	40 DAT	60 DAT	At harvest
S_0B_0	4.51	12.25	12.97	9.65
S_0B_1	5.19	12	12.28	10.72
S_0B_2	5.22	13	13.15	10.70
S_0B_3	5.85	11.25	11.58	10.56
S_1B_0	4.70	9.63	9.40	7.25
S_1B_1	5.70	11.47	11.32	10.2
S_1B_2	5.38	11.40	12.05	10.45
S_1B_3	5.77	10.83	10.98	9.25
S_2B_0	4.50	5.38	1.38	0
S_2B_1	5.15	6.40	2.50	0
S_2B_2	5.65	7	3.40	0
S_2B_3	6.08	7.59	4.50	0.0000

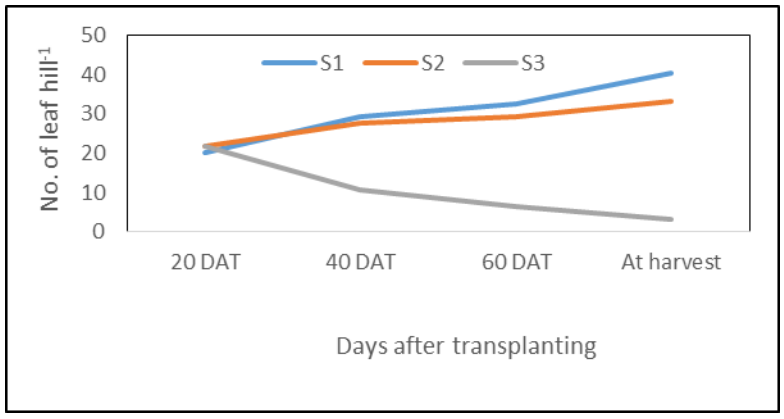
Values with different letters are significantly different at 5% level of probability

S_0 = Control (no salinity), S_1 = 1600 ppm NaCl, S_2=2800 ppm NaCl, B_0= Control (No biochar application), B_1= application of biochar 2 t ha^{-1}, B_2= application of biochar 4 t ha^{-1}, B_3= application of biochar 6 t ha^{-1}

4.1.3 No. of leaves hill^{-1}

4.1.3.1 Effect of salinity

A critical analysis of mean data (Figure 5 and appendix VI) revealed that different salinity level had significant influence at 40, 60 and 80 DAT. No. of leaf decreased with increasing level of salinity at all sampling data. At 40 DAT, the highest leaf number (29.43) were recorded at control (S0) that statistically similar with S1 (27.55) with application of 160 ppm NaCl and and lowest leaf hill-1 (10.80) were counted from S2 with 280 ppm NaCl application. The highest leaf (32.58, 40.46) were counted at S0 and the lowest leaves hill-1 was (6.26, 3) were counted from S2 with 280 ppm NaCl at 60 DAT. This result showed that number of leaves hill-1 decreased gradually with the increasing salinity levels. Similar results was reported Dabnath (2003)



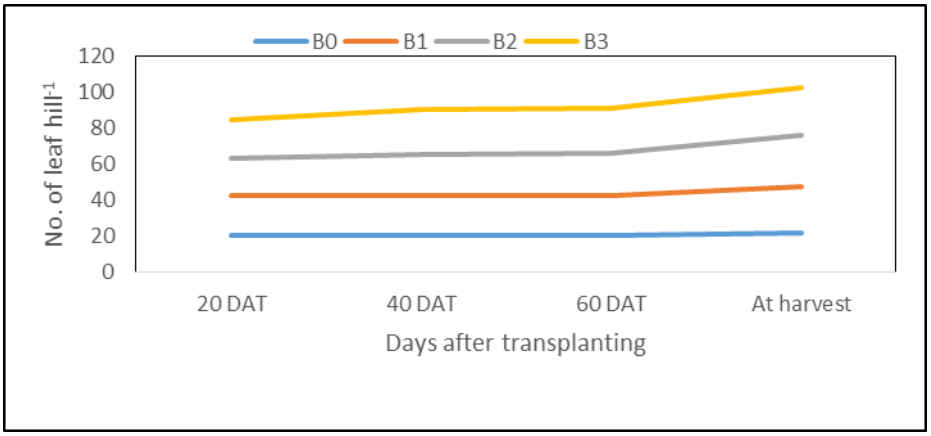
S_0 = Control (no salinity), S_1 = 1600 ppm NaCl, S_2=2800 ppm NaCl

Figure 5. Effect of salinity on number of leaf hill^{-1} of rice at different days after transplanting [LSD (0.05) = 1.99, 2.79, 3.08 at 40, 60 and at harvest, respectively]

4.1.3.2 Effect of biochar

A critical analysis of mean data (Figure 6 and appendix VI) revealed that different biochar levels had non-significant influence on leaf hill^{-1} at 20 DAT but had significant influence at 40, 60 and 80 DAT. At 40 DAT, the highest leaf (24.68) were counted fromB_3 with application of 6 t ha^{-1} biochar that were statistically similar with (23.06) followed by B_2 and

lowes leaf (20.54) were counted from B_0 by B_1. At 60 DAT and at harvest, the highest number of leaf (24.48, 23.8) were counted from B_3 and B_2 with application of 6 and 4 t ha^{-1} biochar, respectively which were statistically similar to B_1 (22.46) and the lowest number of leaf (20.18 and 21.74) were counted from B_0 with no biochar application which were statistically similar with B_1 (22.46) with application of 2 t ha^{-1} biochar.



B_0= Control (No biochar application), B_1= application of biochar 2 t ha^{-1}, B_2= application of biochar 4 t ha^{-1}, B_3= application of biochar 6 t ha^{-1}

Figure 6. Effect of biochar on the number of leaf $hill^{-1}$ of rice at different days after transplanting (LSD (0.05) = 2.30, 3.21, 3.55 at 40 and 60 DAT and at harvest respectively]

Table 3. Combined effect of salinity and different level of biochar on number of leaf hill^{-1} at different growth period of T. Aman rice

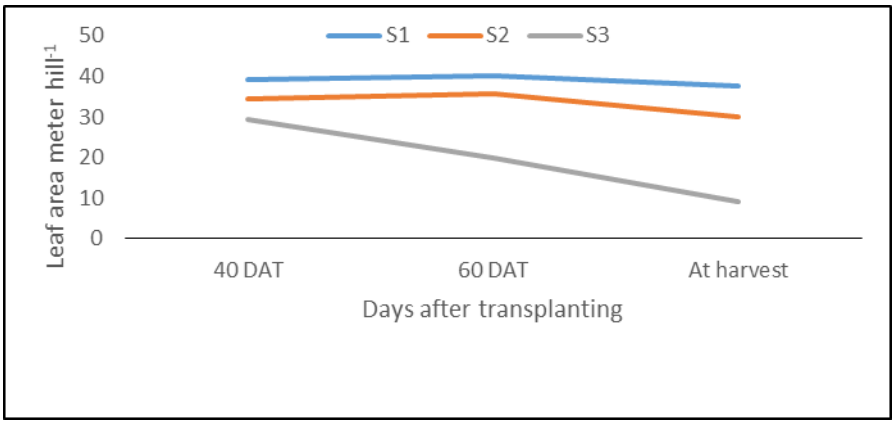
Treatments	Leaves hill^{-1} (No.) at			
	20 DAT	40 DAT	60 DAT	At harvest
S_0B_0	19.585	29.24	32.98	41.33
S_0B_1	21.750	29	32.33	41.16
S_0B_2	20.150	29.59	32.58	39.73
S_0B_3	19.418	29.89	32.45	39.63
S_1B_0	20.085	24.08	24.30	23.88
S_1B_1	22.828	28.15	29.43	33.36
S_1B_2	21.200	29.05	31.45	39.66
S_1B_3	22.750	28.92	32.23	36.79
S_2B_0	21.750	8.31	3.25	0
S_2B_1	21.650	9.13	5.63	3.5
S_2B_2	22.000	10.55	7.38	6.75
S_2B_3	21.825	15.23	8.78	1.75
LSD$_{(0.50)}$	NS	NS	NS	6.15
CV (%)	15.21	12.28	17.05	16.69

Values with different letters are significantly different at 5% level of probability Note: S_0=Control (no salinity), S_1 = 1600 ppm NaCl, S_2=2800 ppm NaCl**,** B_0= Control (no biochar application), B_1= application of biochar 2 t ha^{-1}, B_2= application of biochar 4 t ha^{-1} , B_3= application of biochar 6 t ha^{-1}

4.1.4 Leaf area meter

4.1.4.1 Effect of salinity

Leaf area meter was significantly influenced by different level of salinity application at all growth stages of rice shown in (Figure 7 and appendix VII). Leaf area meter decreased with increasing level of salinity. At 40 DAT, the highest leaf area meter (39.29) observed with no salinity application (S_0) and lowest leaf area meter (29.49) were recorded at 280 ppm NaCl (S_2). The highest leaf area meter (40.11, 37.57) were recorded with no NaCl application (S_0) which was statistically similar to (35.6, 37.57) with application of 160 ppm NaCl application (S_1) at 60 and at harvest.

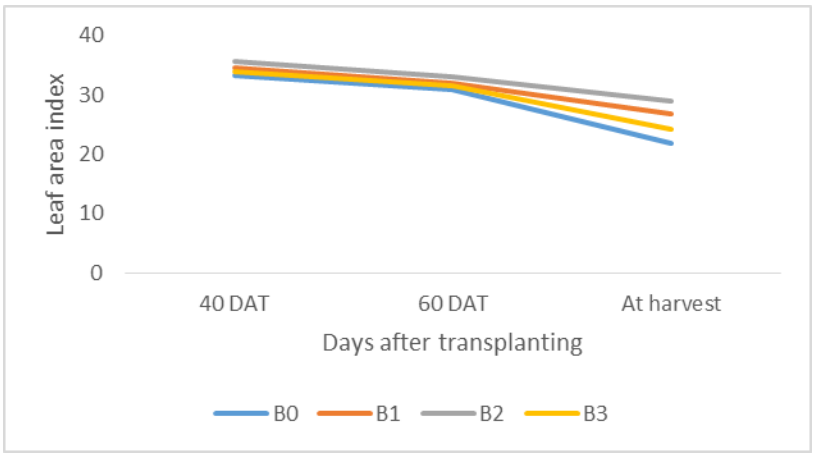


S_0 = Control (no salinity), S_1 = 1600 ppm NaCl, S_2=2800 ppm NaCl

Figure 7. Effect of salinity on leaf area meter of rice at different days after transplanting (LSD $_{(0.05)}$ = 3.09, 7.22, 4.62 at 40, 60 DAT and at harvest, respectively]

4.1.4.2 Effect of biochar

Different level of biochar had non-significant influence on leaf area meter (figure 8 and appendix XII). Leaf area meter increased with increasing of time. At 40, 60 and 80 DAT, the highest leaf area meter (35.74, 33.07, 29.01) were obtained with 4 t ha^{-1} biochar application (B_2) and the lowest leaf area meter 33.20, 30.97, 21.92 were recorded with no biochar application (B_0)



B_0= Control (no biochar application), B_1= application of biochar 2 t ha^{-1}, B_2= application of biochar 4 t ha^{-1} , B_3= application of biochar 6 t ha^{-1}

Figure 8. Effect of biochar on leaf area meter of rice

4.1.4.3 Combined effect of salinity and biochar

Leaf area meter non-significantly influenced by interaction of different level of NaCl and biochar application (Table 4 and appendix VII).

Table 4 . Combined effect of salinity and different level of biochar on leaf area meter at different growth period of T. Aman rice.

Treatments	Leaf area index (cm^2)		
	40 DAT	60 DAT	At harvest
S_0B_0	37.58	39.99	38.30
S_0B_1	37.78	39.29	37.21
S_0B_2	41.87	40.71	37.71
S_0B_3	39.91	40.44	37.04
S_1B_0	36.77	38.94	27.44
S_1B_1	35.38	35.54	31.07
S_1B_2	33.79	34.81	32.27
S_1B_3	31.39	33.47	29.27
S_2B_0	25.26	14	0.000

Treatments	Leaf area index (cm^2)		
	40 DAT	60 DAT	At harvest
S_2B_1	30.39	21.48	12.35
S_2B_2	31.57	23.68	17.04
S_2B_3	30.76	21.01	6.64
LSD(0.5)	NS	NS	NS
CV (%)	12.48	31.42	25.20

S_0 = Control (no salinity), S_1 = 1600 ppm NaCl, S_2 =2800 ppm NaCl B_0 = Control (No biochar application), B_1 = application of biochar 2 t ha^{-1}, B_2 = application of biochar 4 t ha^{-1} , B_3 = application of biochar 6 t ha^{-1},

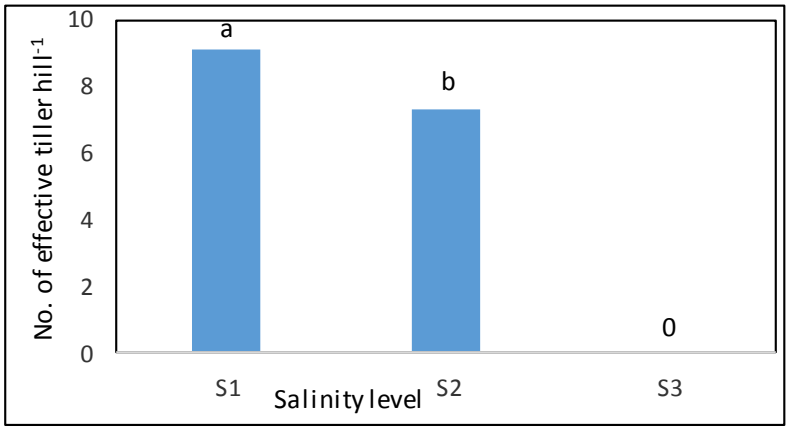
Values with different letters are significantly different at 5% level of probability.

4.2 Yield contributing parameter

4.2.1 Effective tillers $hill^{-1}$

4.2.1.1 Effect of salinity

Salinity stress reduced the number of effective tillers $hill^{-1}$ to a great extent (Table 5 and appendix VIII). Increase in salinity strength proportionally decreased the number of effective tillers $hill^{-1}$. Number of effective tillers $hill^{-1}$ was 9.09, 7.34 and 0 in S_0, S_1, S_2. Khatun *et al.* (1995) found that salinity delayed flowering, reduced the number of productive tillers, the number of fertile florets per panicle. Salt tolerance indexes in terms of seed yield, seed weight $panicle^{-1}$, spikelet number $panicle^{-1}$ and tiller number $plant^{-1}$ were reduced with increasing salinity (Zeng *et al.*, 2002)

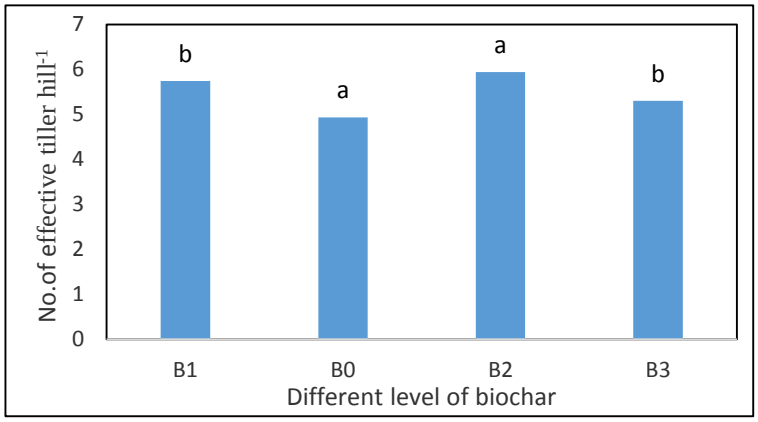


S_0= control (no salinity), S_1 = 1600 ppm, NaCl, S_2=2800 ppm NaCl

Figure 9. Effect of salinity on the number of effective tillers hill^{-1} of rice (LSD $_{(0.05)}$ 0.35 at harvest.

4.2.1.2 Effect of biochar

Biochar application showed significant difference on number of effective tiller hill-1 (Table 5 and appendix VIII . Result found from this experiment revealed that the highest number of effective tillers hill-1 (5.94) was recorded in B2 (application of biochar 4 t ha-1) which was statistically similar with (5.74) with application of 2 t ha-1 (B1) and the lowest number of effective tiller hill-1 (4.93) was achieved by B0 (no biochar application) which was statistically similar with B3 (6 t ha-1) biochar application .



B_0 = Control (no biochar application), B_1 = application of biochar 2 t ha^{-1}, B_2 = application of biochar 4 t ha^{-1} , B_3 = application of biochar 6 t ha^{-1}

Figure 10. Effect of biochar on number of effective tillers hill^{-1} of rice (LSD (0.05) = 0.40).

4.2.1.3 Combined effect of salinity and biochar on effective tillers hill^{-1}

Combined effect between salinity and different levels of biochar showed significant effect on number of effective tillesr hill^{-1} (Table 5 and appendix VIII). The highest number of effective tillers hill^{-1} (9.33) was observed by interaction of 0 ppm NaCl with application of 4 t ha^{-1} biochar (S_0B_2). Application of 280 ppm NaCl does not produce effective tiller in combined with biochar. No effective tiller was found as all the plant died with S_2 treatment during harvesting period.

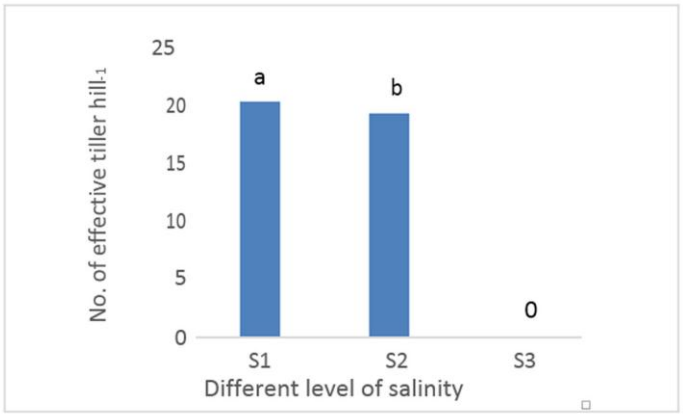
4.2.2 Panicle length

4.2.2.1 Effect of salinity

Panicle length was significantly affected by salinity level (Table 5 and appendix VIII). Panicle length reduced with rise in salinity level. It was found that the longest panicle (20.34 cm) was found from the salinity level S_0 (0 ppm NaCl) which was sinificantly different from all othe treatments followed by S_1 (160 ppm NaCl) and S_2 (280 ppm NaCl). The plant died at S_2 treatment and no panicle length was found. Alam *et al.* (2001) reported that salinity severely reduces the panicle length and panicle weight, thereby reducing the grain yield.

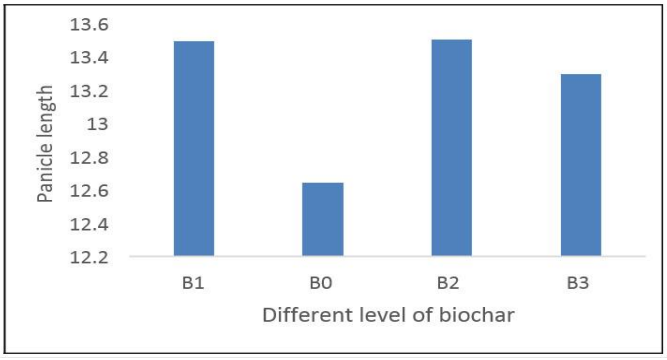
4.2.2.2 Effect of biochar

Panicle length was non-significantly influenced by different levels of biochar application



S_0 = Control (no salinity), S_1 = 1600 ppm NaCl, S_2=2800 ppm NaCl

Figure 11. Effect of salinity on panicle length of rice (LSD $_{(0.05)}$) = 0.97 at harvest



B_0= Control (no biochar application), B_1= application of biochar 2 t ha^{-1}, B_2= application of biochar 4 t ha^{-1} , B_3= application of biochar 6 t ha^{-1}

Figure 12: Effect of biochar on panicle length of rice .

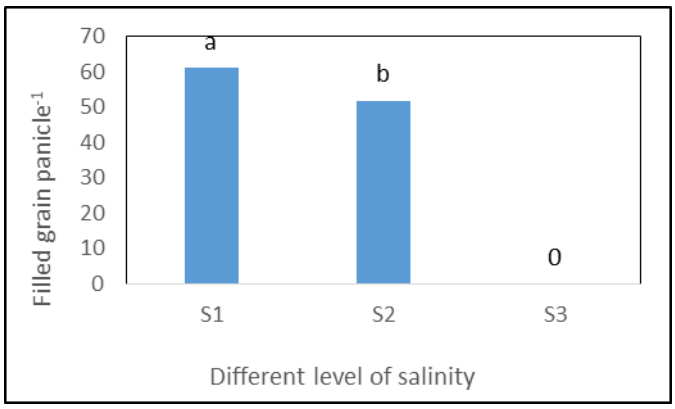
4.2.2.3 Combined effect of salinity and biochar on panicle length

Combination of salinity and different levels of biochar showed significant effect on panicle length. (Table 6 and appendix VIII). The highest panicle length (21 cm) was observed with combination of 0 ppm NaCl and 4 t ha^{-1} biochar (S0B2) which was statistically similar with S0B0, S0B1, S0B3, S1B1, S1B2, S1B3. No plant was alive at combination of S2B0, S2B1, S2B2, S2B3 treatments that's why no panicle was found in that treatment.

4.2.3 Filled grain $panicle^{-1}$

4.2.3.1 Effect of salinity

Salinity intrusion affected number of filled grain panicle-1 throughout the growing period (Table 5 and appendix VIII). Increasing salinity level decreased the number of filled grain panicle decrease in S1 and S2 compared to control. Highest filled grain panicle-1 (61.07) was found at control (S0) whereas at highest salinity level the plant was not alive and no filled grain was observed. This result was in line with Nahar and Hasanuzzaman (2009). They reported that increase in salinity decreased the numbers of filled grains panicle-1.

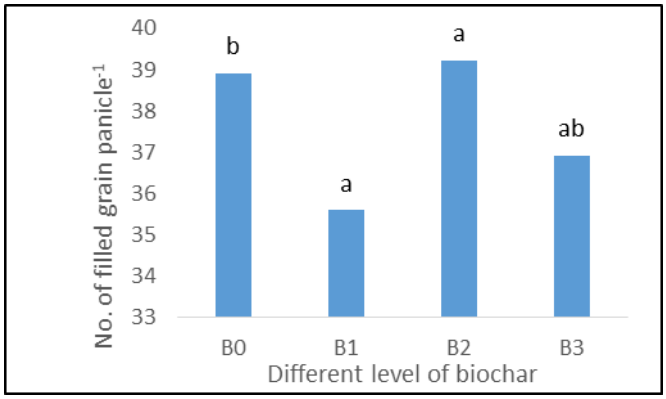


S_0 = Control (no salinity), S_1 = 1600 ppm NaCl, S_2=2800 ppm NaCl

Figure 13. Effect of salinity on filled grains $panicle^{-1}$ of rice at harvesting (LSD $_{(0.05)}$ = 2.41)

4.2.3.2 Effect of biochar

No. of filled grains varied significantly due to different levels of biochar application (Table 5 and appendix VIII). The highest filled grains (39.22) was observed from B_2 (application of Biochar 4 t ha^{-1}) which was similar to B_1 (38.91) and B_3 (36.91)



B_0= Control (No biochar application), B_1= 2 t ha^{-1}application of biochar, B_2= application of biochar 4 t ha^{-1} , B_3= application of biochar 6 t ha^{-1}

Figure 14. Effect of biochar on filled grains panicle^{-1}of rice (LSD $_{(0.05)}$ = 2.75 4.3.3.3

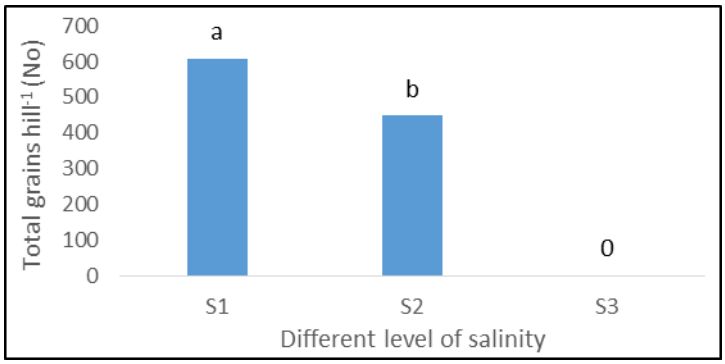
Combined effect of salinity and biochar on filled grains panicle^{-1}

Filled grain was significantly affected by the combination of salinity and biochar which was shown at Table 5 and appendix VIII. Highest filled grain (62.82) was obtained at the combination of 0 ppm NaCl and 2 t ha-1 biochar (S0B1) which was statistically similar with S0B0, S0B2. On the other hand there was no plant found at the combination of S2B0, S2B1, S2B2, S2B3 treatment as no plant was alive.

4.2.4 Total grains hill^{-1}

4.2.4.1 Effect of salinity

Total grain were different at different strength of salinity level (Fig 18 and appendix 8). The maximum total grains hill-1 (608.67) was noticed at S0 where no NaCl was application. Total grain number was highest in this treatment as effective tiller was highest. The plant died at S2 treatment where 2800 ppm NaCl was applied.

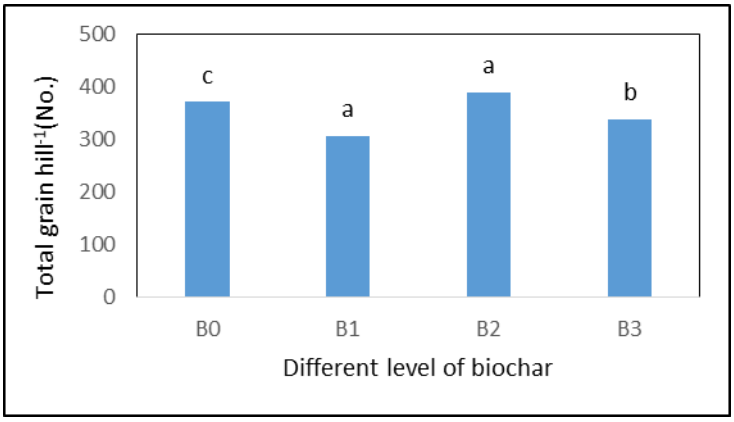


S_0 = Control (no salinity), S_1 = 1600 ppm NaCl, S_2=2800 ppm NaCl

Figure 15. Effect of salinity on total grain hill^{-1} of rice (LSD $_{(0.05)}$ = 26.41

4.2.4.2 Effect of biochar

Significant difference was found on total grain due to the effect of varied biochar application (Table 12 and appendix 8). Higher grain (390.57) was found at B_2 treatment where 4 t ha^{-1} biochar were applied. On the other hand lower grain (307.99) was observed from B_0 where no biochar was added.



B_0= Control (No biochar application), B_1= application of biochar 2 t ha^{-1}, B_2= application of biochar 4 t ha^{-1} , B_3= application of biochar 6 t ha^{-1}

Figure 16. Effect of biochar on total grain hill^{-1} of rice [LSD $_{(0.05)}$ = 26.41

4.2.4.3 Combined effect of salinity and biochar on total grain hill^{-1}

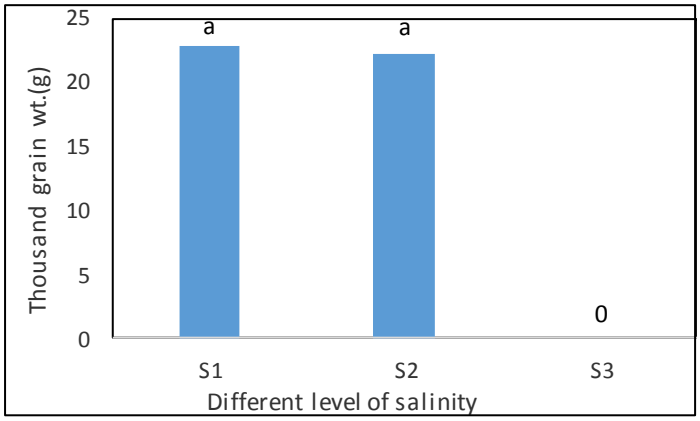
Interaction effect between salinity and different levels of biochar showed significanteffect on total grain hill^{-1} (Table 13 and appendix VIII). The highest grain (635.95) was observed with combination of 0 ppm NaCl and 4 t ha^{-1} biochar (S_0B_2) which was statistically similar with

S_0B_0 and S_0B_1. No plant alive at S_2 treatment with combination of all doses of biochar and no grain was observed.

4.2.5 1000 grain wt.

4.2.5.1 Effect of salinity

Increase in salinity strength reduced 1000 grain weight (Table 4 and appendix VIII). The highest weight (22.90 g) was found at control (S_0) which was statistically similar with S_1. There was no plant found in salinity treatment S_3. Khatun and Flowers (1995) reported that 1000 grain weight decreases with increase in salinity.

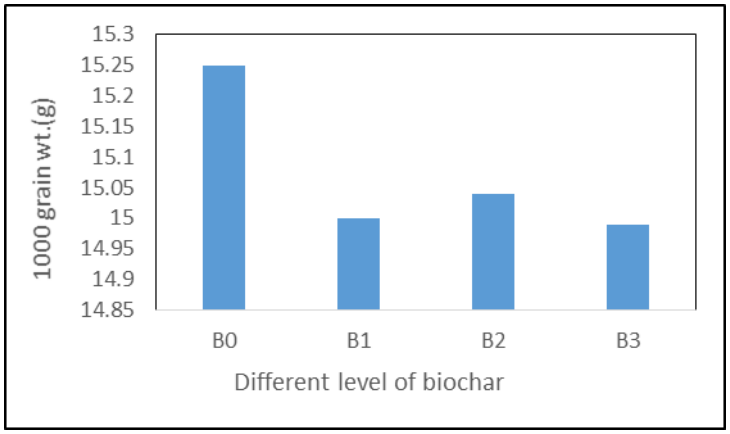


S_0 = Control (no salinity), S_1 = 1600 ppm NaCl, S_2=2800 ppm NaCl

Figure 17 . Effect of salinity on thousand grain hill^{-1} of rice (LSD $_{(0.05)}$ = 0.67

4.2.5.2 Effect of biochar

Different levels of biochar application had non-significant influence on1000 grain wt. (Table 6 and appendix VIII). The highest 1000 grain wt. (15.25 g) was obtained from B_1 (application of Biochar 2 t ha^{-1}) while the lowest 1000 grain wt. (14.99 g) from B_3 where 6 t ha^{-1} biochar were added.



B_0 = Control (no biochar application), B_1 = application of biochar 2 t ha^{-1}, B_2 = application of biochar 4 t ha^{-1} , B_3 = application of biochar 6 t ha^{-1}

Figure 18. Effect of different level of biochar on thousand grain wt. of rice.

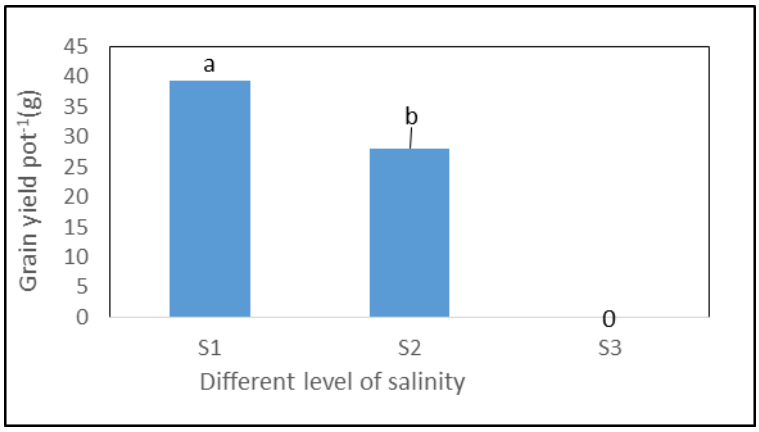
4.2.5.3 Combined effect of salinity and biochar on 1000 grain wt. pot^{-1}

Combination of salinity and different levels of biochar showed significant effect on 1000 grain wt. (Table 13 and appendix VIII). The highest 1000 grain wt (23.25 g) was observed with combination of 0 ppm NaCl and 0 t $hill^{-1}$ biochar (S_0B_0)) which was statistically similar with S_0B_1, S_0B_2, S_0B_3, S_1B_1, S_1B_2, S_1B_3. No plant alive from combination of S_2B_0, S_2B_1, S_2B_2, S_2B_3.

4.2.6 Grain yield pot^{-1}

4.2.6.1 Effect of salinity level

Significant variation was observed in grain yield pot^{-1} due to different salinity treatments (Fig 22 and appendix IX). Grain yield decrease with increasing salinity as effective tiller, grain number and 1000 grain wt. decreased. The maximum grain yield pot^{-1} (39.21 g) was recorded in control condition. After increase in saline, the plant died and no grain yield were found. Linghe and Shannon, (2001) stated that salt stress is responsible for grain yield reduction



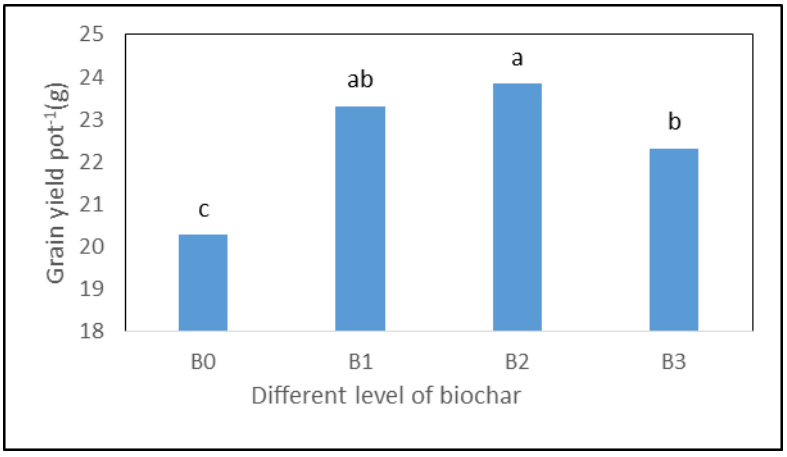
S_0 = Control (no salinity), S_1 = 1600 ppm NaCl, S_2=2800 ppm NaCl

Figure 19 . Effect of salinity on grain yield pot^{-1} of rice (LSD $_{(0.05)}$= 1.24 at harvest

4.2.6.2 Effect of biochar

Grain yield varied significantly for different level of biochar application (Figure 21 and appendix IX). The highest grain yield pot^{-1} (23.85 g) was achieved by B_2 where 4 t ha^{-1} was added which was statistically similar with B_1. The lowest grain yield pot^{-1} (20.2 obtained from B_0. Grain is higher in B_2 than B_3 because biochar perform better upto a

certain level. After crossing that level , it cause negative impact. That's why grain yield is low in B_3



B0= Control (no biochar application), B1= application of biochar 2 t ha-1, B2= application of biochar 4 t ha-1 , B3= application of biochar 6 t ha-

Figure 20. Effect of biochar on grain yield pot-1 of rice (LSD (0.05) = 1.43 at harvest] 42

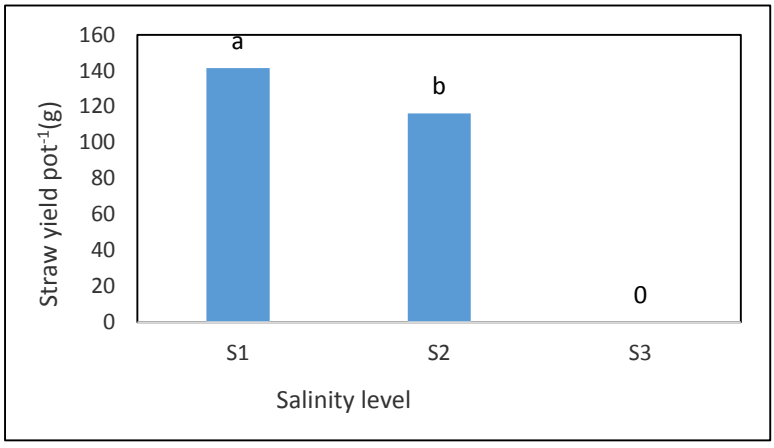
4.2.6.3 Combined effect of salinity and biochar on grain yield pot^{-1}

Grain yield was significantly affected by the combination of salinity and biochar which was shown at Table 5 and appendix IX. The highest grain yield (40.19 g) was obtained at the combination of 0 ppm NaCl and 4 t ha^{-1} biochar (S_0B_2) which was statistically similar with S_0B_0, S_0B_1, S_0B_3. On the other hand there was no plant found at the combination of S_2B_0, S_2B_1, S_2B_2, S_2B_3

4.2.7 Straw yield pot^{-1}

4.2.7.1 Effect of salinity level

Sharp decreases in straw yield pot-1 were observed due to salinity increase (Fig 25 and appendix IX). The maximum straw yield pot-1 (141.46 g) was recorded in control condition. However, when the salt stress increased straw yield pot-1 was decreased. The plant died at S2 treatment. Siddique et al. (2015) mentioned that reduced straw yield under salinity condition might be due to inhibited photosynthesis under salinity stress that caused less amount of nutrient uptake by the plant.



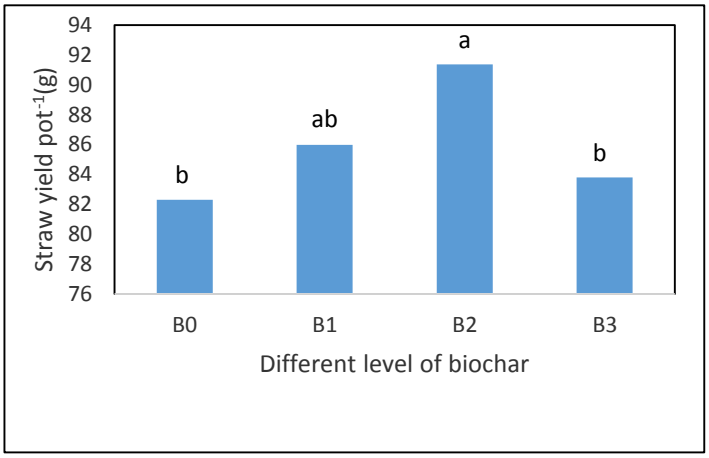
S_0 = Control (no salinity), S_1 = 1600 ppm NaCl, S_2=2800 ppm NaCl

Figure 21. Effect of salinity on straw yield pot^{-1}of rice (LSD $_{(0.05)}$=

5.13 at harvest

4.2.7.2 Effect of biochar

Straw yield varied significantly for different level of biochar application (Figure 21 and appendix IX). The highest straw yield pot^{-1} (91.37 g) was achieved by B_2 where 4 t ha^{-1} was added which statistically similar with B_1. The lowest straw yield pot^{-1} (82.31g) obtained from B_0. Application of 6 t ha^{-1} biochar reduced the staw yield because biochar perform better upto a certain level (4 t ha^{-1}). After crossing that limit, biochar cause negative effect on straw yield . That is why straw yield is minimum in B_3 than B_2



B_0= Control (no biochar application), B_1= application of biochar 2 t ha^{-1}, B_2= application of biochar 4 t ha^{-1}, B_3= application of biochar 6 t ha^{-1}

Figure 22. Effect of biochar on straw yield pot^{-1} of rice [LSD $_{(0.05)}$= 5.93]

4.2.7.3 Combined effect of salinity and biochar on straw yield pot^{-1}

Straw yield was significantly influenced by the interaction of salinity and biochar which was shown at Table 5 and appendix IX. The highest straw yield (45.74 g) was obtained at the combination of 0 ppm NaCl and 4 t ha^{-1} biochar (S_0B_2) which was statistically similar with S_0B_0, S_0B_1, S_0B_2, S_0B_3. On the other hand there was no plant observed by the interaction of S_2B_0, S_2B_1, S_2B_2, S_2B_3.

Table 5. Combined effect of salinity and different level of biochar on yield contributing parameter of rice

Treatments	Effective tillers $hill^{-1}$ (No.)	Panicle length (cm)	Filled grains $panicle^{-1}$ (No.)	Total grains $hill^{-1}$ (No.)	1000-grain wt. (g)
S_0B_0	9.03 ab	20.58 a	62.65 a	597.78 ab	23.250 a
S_0B_1	9.09 ab	19.86 a	62.82 a	619.16 ab	22.750 ab
S_0B_2	9.33 a	21 a	61.17 ab	635.95 a	22.625 ab
S_0B_3	8.95 ab	19.9 a	57.67 bc	581.79 b	22.975 ab
S_1B_0	5.75 e	17.35 b	44.08 d	326.20 e	21.750 b
S_1B_1	8.12 c	20.60 a	53.92 c	496.14 c	23.000 ab
S_1B_2	8.5 bc	19.51 a	56.50 bc	535.76 c	22.500 ab
S_1B_3	7 d	19.98 a	53.07 c	432.70 d	22.020 ab
S_2B_0	0 f	0 c	0 e	0 f	0 c
S_2B_1	0 f	0 c	0 e	0 f	0 c
S_2B_2	0 f	0 c	0 e	0 f	0 c
S_2B_3	0 f	0 c	0 e	0 f	0 c
$LSD_{(0.5)}$	0.70	1.94 (NS)	4.83	45.73	1.35
CV (%)	8.87	10.2	8.90	9.03	6.21

Table 6. Combined effect of salinity and different level of biochar on yield contributing parameter of rice

Treatments	Grain yield pot^{-1} (g)	Straw yield pot^{-1} (g)
S_0B_0	38.66 a	141.24 a
S_0B_1	39.58 a	141.99 a
S_0B_2	40.19 a	145.74 a
S_0B_3	38.39 a	136.87 a
S_1B_0	22.13 d	105.70 d
S_1B_1	30.35 bc	116.00 c
S_1B_2	31.34 b	128.37 b
S_1B_3	28.54 c	114.56 c
S_2B_0	0 e	0 e
S_2B_1	0 e	0 e
S_2B_2	0 e	0 e
S_2B_3	0 e	0 e
$LSD_{(0.5)}$	2.48	10.27
CV (%)	7.7	8.31

CHAPTER 5

SUMMARY AND CONCLUSION

A Pot experiment was carried out at the Agronomy net house of Sher-e-Bangla Agricultural University, Dhaka during the period from July 2018 to December 2018 to evaluate the amelioration of salinity stress by utilization of biochar in rice plant (*Oryza sativa*). The experiment consisting of 12 treatments combinations that was laid out in CRD design with four replications. Fourty eight plastic pot with 18 inch depth were used to conduct the experiment. There were twelve treatment combinations. The treatments were S_0 (control), S_1 (1600 ppm NaCl), S_2 (2800 ppm NaCl) and four biochar level- B_0 (control), B_2 (2 t ha^{-1}), B_2 (4 t ha^{-1}), B_3 (6 t ha^{-1}).

Results showed that, with increasing salinity growth contributor decreased viz. plant height, tiller no. leaf no and leaf area index. Highest plant height (63.64, 87.17 cm) was at control (S0) and the lowest plant height (51.36, 45.45 cm) was found at S2 treatment at 40 and 60 DAT. Number of tillers hill-1 also showed similar result. At 40, 60 DAT and at harvest, the highest plant height (12.12, 12.49, 10.41 cm) was found at control (S0) compared to S1 and S2. However,intrusion of 280 ppm NaCl caused death of plant after 60 days of transplanting. Significant effect of salinity was seen on yield contributing characters viz. number of effective tiller, panicle length, filled grains panicle-1, 1000 grain weight, grain yield and straw yield. Highest number of effective tillers (9.09), filled grains panicle-1 (61.07) and 1000 grain wt. (22.90 g) was found at control (S0) compared to S1 and S2. Induction of 1600 ppm NaCl during 20-40 DAT caused 28% yield lose. On the other hand, intrusion of 2800 ppm NaCl during 20-40 DAT caused 100% yield lose. Biochar had significant influence on growth parameter. With increasing the level of biochar, the growth parameter such as plant height, tiller no. leaf no. increased. Application of biochar under salt stress also ameliorate salt induced damage to a certain extent. Addition of biochar at 4 t ha−1 under 1600 ppm salt stress reduced 42% yield lose. The results in this study revealed that salt stress caused growth and yield reduction even death of plant. Application of different doses of biochar can ameliorate salt-induced damage to a certain extent. However application of 4 t ha^{-1} biochar performed best compared to other doses under salt stress condition.

RECOMMENDATIONS

Based on the results of the present study, the following recommendations may be drawn

1. Such study is needed in different agro-ecological zones (AEZ) of Bangladesh for regional compliance and other performance.
2. Another experiment may be carried out with different doses of NaCl and biochar for specific effect.

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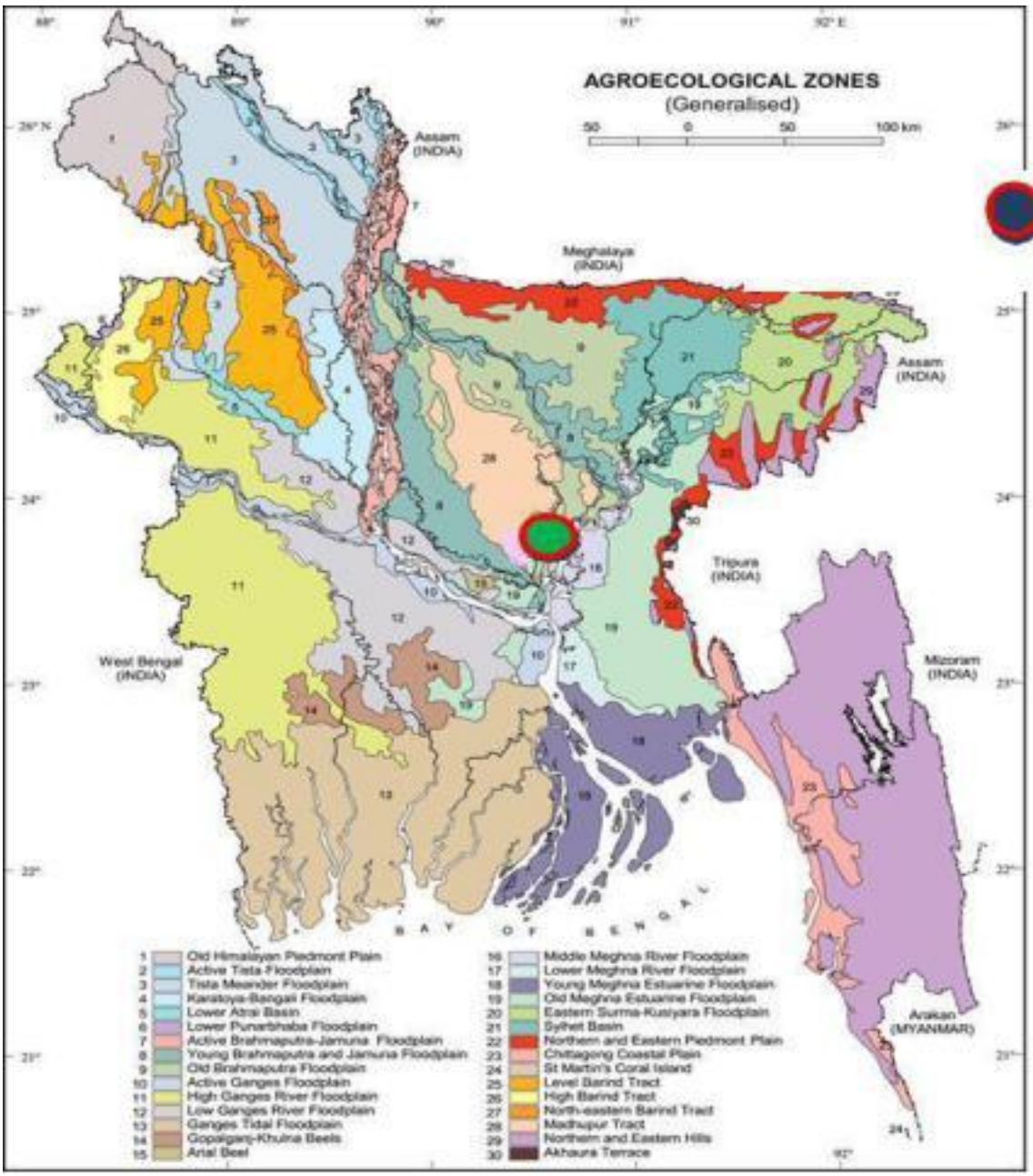
APPENDICES

Appendix Ⅰ. Map showing the experimental site under the study



Appendix Ⅱ. Monthly average temperature, average relative humidity and total rainfall and average sunshine of the experimental site during the period from July- December, 2018

Month	Average Temperature (°C)		Average Relative Humidity (%)	Rainfall (mm)	Average sunshine (hr)
	Minimum	Maximum			
June, 2018	23.2	35.5	78	312	5.4
July, 2018	24.5	36.0	83	563	5.1
August, 2018	23.5	36.0	81	319	5.0
September, 2018	24.4	34.5	81	279	4.4
October, 2018	25	32	79	175	6
November, 2018	21	30	65	35	8
December, 2018	15	29	74	15	9

Source: Bangladesh Meteorological Department (Climate & Weather Division), Agargoan, Dhaka-1212.

Appendix III. Physico-chemical properties of soil in the study area

Characteristics	**Value/concentration**
Particle size analysis.	
% Sand	26
% Silt	45
% Clay	29
Textural class	silty-clay
Ph	6.3
Organic matter (%)	1.8
Total N (%)	.09
Phosphorus microgram/g soil	13.1
Potassium (ml equivalent/100 g soil	0.19

Appendix IV. Mean sum square values of the data for plant height at different days after transplanting

Source of Variation	DF	Mean Sum square values of plant height			
		20 DAT	40 DAT	60 DAT	At harvest
Replication	3	20.08	11.717	238.48	28.9
Salinity	2	0.08 NS	713.466**	7867.52**	39814**
Biochar	3	21.27*	124.424**	198.28 NS	44.9 NS
Salinity*Biochar	6	24.88*	68.626*	126.60 *	16.6*
Error	33	10.25	25.393	416.24	43.3
Total	47				

Appendix V. Mean sum square values of the data for tiller number at different days after transplanting

Source of Variation	DF	Mean Sum square values of tiller number			
		20 DAT	40 DAT	60 DAT	At harvest
Replication	3	1.85715	1.242	6.059	0.234
Salinity	2	0.16744NS	133.803 **	419.85**	522.09**
Biochar	3	3.62459*	3.975 NS	5.524 NS	5.08**
Salinity*Biochar	6	0.18436	2.296 NS	4.29 *	2.2*
Error	33	0.64479	1.942	4.767	0.938
Total	47				

Appendix VI. Mean sum square values of the data for leaf number at different days after transplanting

Source of Variation	DF	Mean Sum square values of leaf number			
		20 DAT	40 DAT	60 DAT	At harvest
Replication	3	2.97	1.75	29.66	11.57
Salinity	2	12.60NS	1682.83 NS	3298.24**	6342.44**
Biochar	3	5.24NS	36.12 NS	43.25*	100.01**
Salinity*Biochar	6	3.17NS	12.39 NS	15.35 NS	62.52**
Error	33	10.44	7.69	15.03	18.30
Total	47				

Appendix VII. Mean sum square values of the data for leaf area meter at different days after transplanting

Source of Variation	DF	Mean Sum square values of leaf number		
		40 DAT	60 DAT	At harvest
Replication	3	6.781	8.05	122.46
Salinity	2	383.44**	1778.31**	3503.34**
Biochar	3	13.57 NS	9.27 NS	113.74 NS
Salinity*Biochar	6	28.45 NS	42.15 NS	60.99 NS
Error	33	18.41	100.76	41.40
Total	47			

Appendix VIII. Mean sum square values of the data for yield contributing parameter

Source of Variation	DF	Mean Sum square values of tiller number				
		Effective tiller	**Panicle length**	Filled grain panicle^{-1} (No.)	Total grain hill^{-1} (No.)	**1000-grain weight**
Replication	3	0.424	2.32	30.0	718	0.53
Salinity	2	372.74 **	2104.61 **	17352.2 **	1591537 **	2727.52 **
Biochar	3	2.46 **	1.95 NS	35.6*	16026 **	0.17 NS
Salinity*Biochar	6	1.89 **	3.62 NS	52.1**	9844 **	0.67*
Error	33	0.236	1.82	11.2	1011	0.88
Total	47					

Appendix IX. Mean sum square values of the data for grain and straw yield contributing parameter

Source of Variation	DF	Mean Sum square values of leaf number	
		Grain yield	**Straw yield**
Replication	3	0.83	16.5
Salinity	2	6532.76**	91048.7**
Biochar	3	29.90**	188.7**
Salinity*Biochar	6	20.68**	106.2*
Error	33	2.98	51.0
Total	47		