# AMELIORATION OF SALT STRESS IN SOYBEAN BY *Rhizobium* BIOFERTILIZER

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*CERTIFICATE*

*This is to certify that thesis entitled, "**AMELIORATION OF SALT STRESS IN SOYBEAN BY Rhizobium BIOFERTILIZER**" submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE (MS) in AGRONOMY**, embodies the result of a piece of bona-fide research work carried out by **A.B.M. Zahurul Islam**, **Registration no. 13-05645** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

*I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.*

**Date:**
**Place: Dhaka, Bangladesh**

**Anisur Rahman, Ph.D**
Associate professor
Supervisor

**DEDICATED TO**

**MY**

**BELOVED PARENTS**

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**AMELIORATION OF SALT STRESS IN SOYBEAN BY *Rhizobium* BIOFERTILIZER**

## ABSTRACT

A pot experiment was conducted at Sher-e-Bangla Agricultural University, Dhaka during the period of September to November- 2020 to ameliorate the salt stress in soybean by application microbial biofertilizer. The two factor experiment was conducted by following CRD design with four replications. Factor A consisted of three levels of NaCl *viz;* $S_0$ = 0 ppm NaCl $pot^{-1}$ (Control), $S_1$= 1800 ppm NaCl $pot^{-1}$ and $S_2$ = 3600 ppm NaCl $pot^{-1}$ and Factor B consisted of four levels of biofertilizer *viz;* $B_0$= 0 g Rhizobium @ 50 kg seed, $B_1$= BARI RGm-907 @ 25 g/50 kg seed, $B_2$= BARI RGm-922 @ 50 g/50 kg seed and $B_3$= BARI RGm-928 @ 75 g/50 kg seed. Result revealed that salinity decreases plant height, leaf number, SPAD value, RWC, pod $plant^{-1}$, seed yield and other yield attributes. Exposure of 1800 ppm and 3600 ppm NaCl decreased seed yield by 48.34% and 56.83% in soybean compared to control by decreasing pod number. Application of different biofertilizers significantly influences growth and yield of soybean. BARI RGm-922 (50 g/50 kg seed) application recorded the highest number of pods $plant^{-1}$, seeds $pod^{-1}$ (3.11), 1000 seeds weight (88.61 g), seed yield (6.54 g $pot^{-1}$), stover yield (19.75 g $pot^{-1}$), biological yield (35.56 g $pot^{-1}$) and harvest index (24.69 %). Different types of biofertilizer application under salt stress condition recovered growth and yield loss of soybean. Among the biofertilizers BARI RGm-922 (50 g/50 kg seed) gave highest seed yield in all stresses *viz* $S_0B_2$ (9.77 g $pot^{-1}$), $S_1B_2$ (5.14 g $pot^{-1}$) and $S_2B_2$ (4.70 g $pot^{-1}$) comparable to others treatment combinations. So, it might be concluded that, the growth and yield of soybean decreased with the increasing dose of salt and application of different biofertilizers recovered growth and yield of soybean by ameliorating salt stress. However, application of BARI RGm-922 (@ 50 g/50 kg seed) as biofertiizer might be the best approach to reduce the salt induced damages in soybean.

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## ABBREVIATIONS

| Full word | Abbreviations |
|---|---|
| Agriculture | Agric. |
| Agro-Ecological Zone | AEZ |
| And others | *et al.* |
| Applied | App. |
| Bangladesh Bureau of Statistics | BBS |
| Biology | Biol. |
| Biotechnology | Biotechnol. |
| Botany | Bot. |
| Centimeter | Cm |
| Cultivar | Cv. |
| Degree Celsius | °C |
| Dry weight | DW |
| Editors | Eds. |
| Emulsifiable concentrate | EC |
| Entomology | Entomol. |
| Environments | Environ. |
| Food and Agriculture Organization | FAO |
| Fresh weight | FW |
| Gram | g |
| International | Intl. |
| Journal | J. |
| Kilogram | kg |
| Least Significant Difference | LSD |
| Liter | L |
| Triple super phosphate | TSP |
| Science | Sci. |
| Soil Resource Development Institute | SRDI |
| Technology | Technol. |
| Serial | Sl. |
| Percentage | % |

# CHAPTER I

# INTRODUCTION

Soybean (*Glycine max* L.) is the most widely cultivated legume around the world because of its versatile uses and economic importance (Liu *et al.,* 2020). Soybean is one of the most multipurpose, nutritionally and economically important legumes due to its unique seed composition (Shea *et al*., 2020). Soybean seed contains about 18 to 22% oil and 38 to 56% vegetable protein with favorable amino acid (USDA, 2018). It is a prominent source of proteins and edible oil, it has valuable uses as food, feed and oil seed crop (Liu *et al*., 2020). Globally, soybean is responsible for about 61% of total international oilseed production and occupied 6% of the world's cultivable area (SoyStat, 2019). According to USDA (2019), about 336.11 million tons of soybean produced around the world, from the cultivated area of 121.69 million hectares with an average yield of 2.76 t $ha^{-1}$. The United States, Brazil and Argentina are the leading soybean producing countries in the world and responsible for 81% of the total production. In Bangladesh, 0.986 million t of soybean produced in 59,445 ha land area, while global production was 348.7 million t in 124.9 million ha area (FAOSTAT, 2018). BBS (2018) reported that the total soybean cultivated area was 59443.46 ha and total production was 98699 t in Bangladesh. In our country, the demand for soybean as poultry feed was 0.94-1.13 million t in 2015. In Bangladesh, there are 80 oil refineries with a total production capacity of 2.9 million t. But only 48% of production capacity is utilized, so there is a huge demand for soybean in these industries (USDA, 2017).

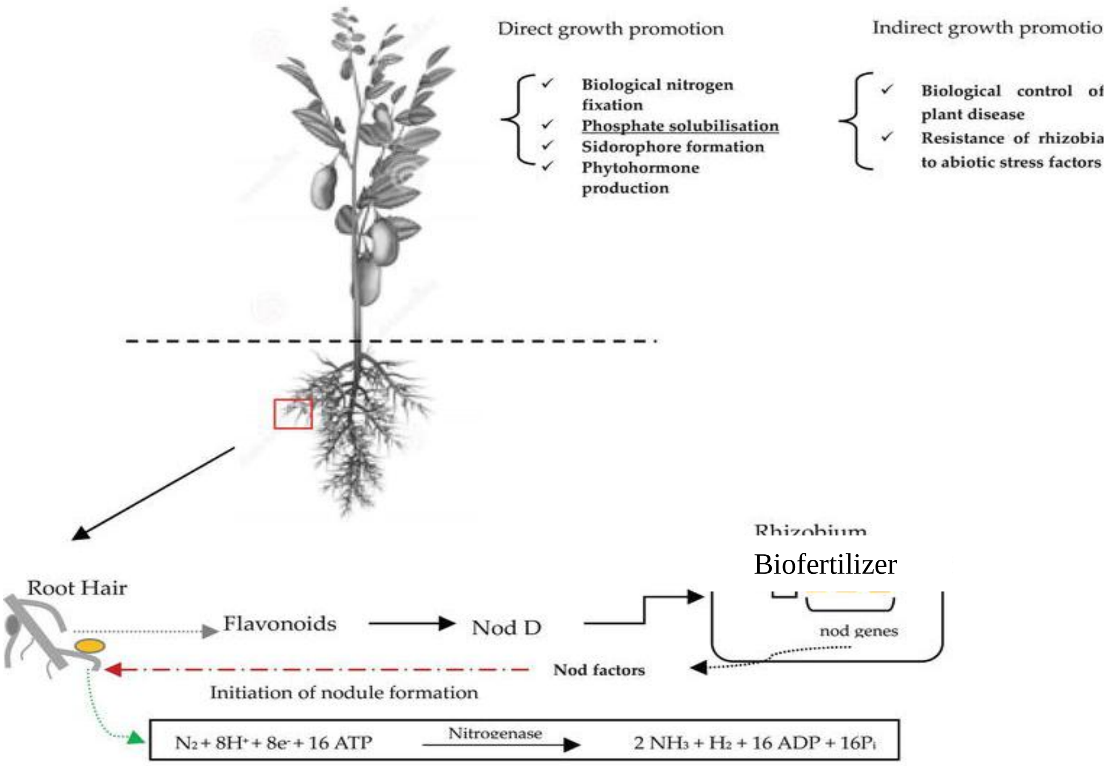
Soybean is classified as moderately salt sensitive instead of moderately salt tolerant (Katerji *et al.*, 2000). Salt tolerance of plants may be dependent on growth stage, varieties, nutrition and environment (Bischoff and Warner, 1999). Soil salinity has been reported to reduce yields, nodulation and the total nitrogen content in legume plants (Singleton and Bohlool, 1984; Lauter *et al.,* 1981). Soybean nodulation has been well known to be extremely sensitive to NaCl. A reduction in inoculation of 50 % compared to maximum nodule number and nodule dry weight in soybean occurred with 26.6 mM NaCl in solution culture (Singleton and Bohlool, 1984). Soil salinity reduced plant growth and photosynthesis due to the complex negative effects of osmotic, ionic, and nutritional interactions (Shirokova *et al.* 2000). Salinity stress increased levels of ethylene that significantly inhibited shoot and root elongation and reduces plant height and overall growth (Ma *et al.*, 1998; Klassen and Bugbee, 2002). Numerous studies have shown that soil salinity decreased rhizobial colonization and nodulation and dramatically reduced $N_2$ fixation and nitrogenase activity of nodulated legumes (Elsheikh and Wood, 1995; Zahran, 1999). Increasing salt concentration might have detrimental effects on rhizobial population (Singleton *et al.*, 1982).

Soil salinity particularly disturbed the symbiotic interaction between legumes and rhizobia (Marcar *et al.*, 1991). Because of accumulated salts in soil under salt stress condition plant wilts apparently while soil salts such as $Na^+$ and $Cl^-$ disrupt normal growth and development of plant (Khajeh-Hosseini *et al.* 2003; Farhoudi *et al*. 2007). The agronomic traits of soybean could be severely affected by high salinity, including reduction in height, leaf size, biomass, number of internodes, number of branches, number of pods, weight per plant, and weight of 100 seeds (Chang *et al.* 1994). In general, salt stress reduces the protein contents in soybean seeds (Chang *et al.* 1994; Wand *et al*. 2002). However, the effect of salt on oil content of soybean seeds is still inconclusive since experimental results varied in different field sites using different cultivars treated with different salinity levels (Chang *et al*. 1994; Wan *et al*. 2002). As more and more agricultural lands are affected by soil salinity, soybean production is being threatened.

This situation requires the adoption of techniques that leach the salts of the root environment of plants as the leaching fraction (Rhoades *et al*., 2000) and also of inputs exercising physical improvements expressed by the pore space (Benbouali *et al*., 2013), chemical by the availability of macro and micronutrients (Patil, 2010) and biological in the increase in population and diversification of soil fauna (Maiti, 2013).

Generally, the soil has insufficient nutrients for plant growth, therefore, providing fertilizer is among the efforts of farmers to increase soil fertility for soybean production. Dependence on chemical fertilizers for forthcoming farming intensification would mean more loss in soil quality, water contamination and unsustainable burden on the economic system (Mazid *et al*., 2014). Incorporated use of mineral fertilizers, organic manures, biofertilizers, etc. is the only alternate for improving soil fertility (Singh *et al*., 2014).

Biofertilizer is a fertilizer that contains a mixture of free nitrogen fixing bacteria, phosphate solvents, and nutrient solubilizing fungi with a growth-promoting formula and microbial elements needed by plants (Gupta *et al*., 2015; Kalayu, 2019).




Source: **(**Assine *et al*. 2018).

**Figure 1.** Improve plant stress condition by using biofertilizer

They improve the soil structure, restores soil nutrient, build soil organic matter, water uptake, plant growth and plant tolerance to abiotic and biotic factors (Akram *et al*., 2020). Since biofertilizers contain microorganisms that deliver nitrogen to plants and nutrients to the soil, these potential microbes would play a key role in productivity and sustainability of soil and also in protecting the environment as eco-friendly and cost-effective inputs for the farmers (Bardi and Malusà, 2012).

The use of biofertilizers to mitigate the negative impact of salinity in plant growth and development is an alternative emerging technology to improve the abiotic stress tolerance of plants (Khan *et al*., 2018). Several reports have shown that plant growth-promoting rhizobacteria (PGPR) that colonize the rhizospheres of plants, are beneficial microorganisms, capable of increasing the stress tolerance of host plants against both biotic and abiotic factors (Solanki *et al*., 2015). *Bacillus amyloliquefaciens* is a Gram-positive, non-pathogenic endospore-forming, soil-inhabiting prokaryote rhizobacterium, which colonizes the plant rhizosphere, promotes plant growth, and suppresses competing phytopathogens, such as bacterial, fungal, and fungal-like pathogens. The ability to promote plant growth is linked to the use of diverse mechanisms that include indole-3-acetic acid (IAA) synthesis (Liu *et al*., 2016), and phosphorus and potassium solubilization (Belbahri, *et al*., 2017).

It is crucial to adopt cultural techniques to ameliorate salt stress on soybean plants in an eco-friendly way. Since salinity imposes a great sequel of physiological and biochemical disorders in plants, the present study was undertaken to achieve the following objectives

i. To know the effect of salinity on soybean plant.
ii. To study the influence of biofertilizer on soybean.
iii. To understand the role of biofertilizer to combat yield reduction of soybean plant under salt stress condition.

# CHAPTER II

# REVIEW OF LITERATURE

## 2.1 Soybean

Soybean (*Glycine max* L.) is the most widely cultivated legume around the world because of its multipurpose uses and economic importance. As it is a prominent source of proteins and edible oils, it has valuable uses as food, feed and oilseedcrop (Liu *et al.,* 2020). In Bangladesh, Noakhali and Lakshmipur are the leading regions in the soybean production (Miah *et al.* 2015; Salam and Kamruzzaman, 2015). Miah *et al.* (2015) reported that due to the adaptation of improved soybean varieties living standard of soybean farmers has been improved. Improved soybean variety cultivation $ha^{-1}$ has created an opportunity for additional employment of 6.1 men $day^{-1}$. In the high growing regions of soybean, the average yield is 1813 kg $ha^{-1}$ and the net return is 25,599 Taka $ha^{-1}$. The BCR (Benefit cost ratio) of soybean is 1.43 and the DRC (Domestic Resource Cost) value is 0.55. All of these facts clarify that in Bangladesh, domestic production is more profitable than importing soybean (Salam and Kamruzzaman, 2015).

## 2.2 Importance of soybean

Soybean has a very special seed composition and because of that, it has versatile uses. It is one of the most multipurpose, nutritionally and economically important legumes due to its unique seed composition (Shea *et al.*, 2020). Globally, in 2018 soybean alone was responsible for about 61% of total international oilseed production and occupied 6% of the world's cultivable area (SoyStat, 2019).

According to the United Soybean Board (2019), the two main products of soybean are meal and oil, which have various important industrial uses. Soybean meal is responsible for 70% of soybean's value. About 97% of soybean meal is used as feed for poultry and livestock in the USA. In case of uses, soybean oil has uses as fuel, solvent, cosmetics, foam, soap and candles. In food industry, high oleic acid containing soybean oil is popular as edible oil because it provides a trans-fat free solution.

Singer *et al.* (2019) reported soybean as a major source of protein and essential amino acid for humans and livestock because of its well-balanced amino acid profile. It supplies a significant amount of protein, amino acid, oil and carbohydrate. It contains about 25% linoleic and 3% linolenic acid. Moreover, it is also enriched with antioxidant and anticarcinogenic properties (Sharma *et al.,* 2014).

Legumes are popular around the world as restorative crops, green manuring crops and cover crops and can easily be included in cropping patterns as main crop or inter-crop. Besides, these legumes show moderate tolerance against different abiotic stresses (Hasanuzzaman *et al.,* 2016). For the hay and silage, soybean can be cultivated as a pasture crop (Heuze *et al.,* 2015).

Soybean is a potential source of bioenergy, corn grain ethanol and soybean biodiesel are used as biofuels (Adie and Krisnawati, 2015). Legumes have promising positive effects on soil health, such as in soil restoration, improving soil nitrogen pool by biological nitrogen fixation (BNF) and by increasing soil organic carbon stock (Dhakal *et al.,* 2016). Soybean improves soil nitrogen (N) pool by accumulating about 53-76 kg N $ha^{-1}$ (Dabin *et al.*, 2016). Legumes improve the biological property of soil by associating with soil microbes and by fixing nitrogen to the soil without disturbing the soil natural biota (Prashar and Shah, 2016). Inter-cropping of legume increases biological diversity, the interaction between or among crop species, reduces fertilizer requirement and decreases the chance of crop failure (Meena *et al.,* 2016).

Legumes have a role in decreasing the negative effect of climate change by mitigating greenhouse gases (Hasanuzzaman *et al.,* 2019). According to Hasanuzzaman *et al.* (2019), by 2050 the world population is expected to reach 9 billion as a result, food production need to be increased by 70%. In the case of providing food security and maintaining sustainability in agriculture, legumes are considered as the jack of all trades. As a member of Fabaceae family, soybean also plays the above mentioned vital roles of the legumes.

### 2.3 Abiotic stress

Abiotic stress is not an individual one. It comprises all types of hostile environmental conditions that a plant may face in nature (Bechtold and Field, 2018).

## 2.4 Types of abiotic stress

Among different types of abiotic stresses; drought, flooding, salinity, toxic metal/metalloid stress, high temperature, low temperature, UV-radiation, pollutants etc. are the major abiotic stresses encountered by plants in nature (Hasanuzzaman *et al.,* 2016; Bechtold and Field, 2018).

## 2.5 Impact of abiotic stress on plant

Abiotic stresses or environmental stresses are the potential threats for crop productivity for upcoming decades. Plants are sessile organisms are more prone to adverse environmental conditions day by day due to continuously changing climatic conditions. Abiotic stress adversely affects plant's morphological, physiological and biochemical activity, ultimately causes a reduction in productivity (Hasanuzzaman *et al.,* 2017; Vishwakarma *et al.,* 2017). Directly or indirectly, crop productivity is related to the economy, which means abiotic stresses are potential threats to the economy (Singh *et al.,* 2015).

According to Hasanuzzaman *et al.* (2017), the productivity of crops is decreasing due to environmental stresses because of climate change. On the other hand, the world population is growing rapidly as well as the demand is also increasing for food, fiber, oil and other products and by-product yielding crops with an issue of food security. Food security is one of the major concerns of the 2030 Sustainable Development Goals (SDGs). Farming communities are on the frontline facing tremendous challenges to harvest the potential crop yield and to maintain sustainability in the agriculture sector due to climate change (FAO, 2017). In counter to the growing demand of the rapidly increased population, plant productivity is decreasing because of the negative effect of abiotic stresses. Sometimes more than 50% of crop reduction occurs due to abiotic stresses. To ensure global food security, improving plant stress tolerance is a prerequisite. For improving plant stress tolerance understanding the response of plants towards abiotic stress is a vital point (Hasanuzzaman *et al.,* 2017).

The future challenge is to parallel the demand for the food, feed, fiber, oil and biofuel, for the increasing population, which is expected to reach about 9 billion before 2050, by mitigating abiotic stresses for maximizing productivity (Noreen *et al.*, 2018).

## 2.6 Salt stress

Salt stress is considered as one of the most detrimental one among the abiotic stresses. It reduces the productivity of about 6% of the land area. About 20% of the irrigated land area and 17% of the total arable area are already salt-affected. The alarming issue is up to 50% of agricultural land loss may occur due to salinity by the next couple of decades. In soil solution, sodium chloride (NaCl) and sodium sulfate ($Na_2SO_4$) are the most commonly found soluble salts. Moreover, calcium sulfate ($CaSO_4$), magnesium sulfate ($MgSO_4$), potassium nitrate ($KNO_3$), sodium bicarbonate ($NaHCO_3$), etc. are also found in the soil solution and most of these are partially soluble in the solution. An increase in salinity in most of the cases refers to mainly an increase in $Na^+$ and $Cl^-$ ions. Both $Na^+$ and $Cl^-$ ions produce toxic conditions for the plant, but between them, $Cl^-$ is more dangerous (Hasanuzzaman *et al.*, 2013; Choudhury *et al.*, 2013).

According to Shrivastava and Kumar (2015), salt stress is a potential threat to sustainable crop production. About 20 to 50% of crop productivity diminishes due to salinity in cultivated land area. In every minute, around 3 hectares of arable land are affected by salinity resulting in 10% increase in saline affected area every year. Because of climate change saline affected area is increasing more rapidly than before (Reddy *et al.*, 2017). About 20% of the cultivated land and 33% of the irrigated area in the world are salt-affected already. About 7% of the land content high rate of salt among the salt-affected area (Kibria *et al.*, 2017). Salinity creates osmotic stress and ionic toxicity. Osmotic stress occurs due to the accumulation of a higher concentration of salt ions in the root zone outside of the root cell. Osmotic stress hinders the uptake of water and nutrient of the plants. In the later stage, a higher accumulation of salt inside the cell and tissues induces ionic toxicity. Both ionic and osmotic stresses are responsible for overproduction of ROS. Therefore, an excess concentration of ROS in plants induces deleterious oxidative stress, which causes oxidation of plant cells, along with the cell organelles and membranes. Moreover, oxidative stress hampers the plant growth, physiological activity, biochemical process, productivity and can also cause cell and plant death (Munns, 2005; Munns and Tester, 2008; Hasanuzzaman *et al.*, 2016; Choudhury *et al.*, 2017; Hasanuzzaman *et al.*, 2020b).

Seed germination, vegetative and reproductive growth and nutrient balance are adversely affected by salt stress (Hussain *et al.,* 2018). Salt concentration, duration, genotypes of the plants, phytochemical quenching capacity, age of the plant and environment are the key factors that determine the degree of deleterious effect of salt stress (Shahverdi *et al.,* 2018).

Kamran *et al.* (2020) reported salinity as one of the hazardous abiotic stresses causes significant reduction in the growth and yield of crops. Salinity induced growth and yield reduction occurs due to wide spectrum alteration physiological and biochemical activity of salt stressed plants. Salt stress affects plant physiology and biochemical activity by reducing water potential in the soil, causing ionic imbalance, ionic toxicity and overproduction of ROS, which ultimately leads to yield reduction.

**2.7 Effect of salinity on plant**

Saline soil affects plant growth, development and process of photosynthesis. It also affects protein synthesis and lipid metabolism (Parida and Das, 2005). Osmotic stress reduces photosynthetic efficiency which is resulted in partial closure of stomata (Meloni *et al.,* 2003). The nutrient imbalance and membrane destabilization are caused by soil salinity (Hasegawa *et al*., 2000). The cell growth and development are decreased in plants in responses to osmotic stress. It resulted in decreased leaf area and chlorophyll content (Shannon and Grieve, 1999).

The nutritional imbalances are also caused by decrease in the uptake of calcium ions and potassium ions in leaves and an increase in the uptake of sodium ions. In some cases, there is a requirement of low sodium ions and high potassium ions or calcium ions which are required for optimum function, but increased sodium ions resulted in metabolic disturbances. Cell swelling in plants is caused by accumulation of sodium and chloride which can affect plant enzymes. It can also result in physiological changes and reduced energy production (Larcher, 1980). The photosynthetic function is disturbed by nitrate reductase activity due to chloride ions (Xu *et al*., 2000). There are competitive interactions with nutrient ions for binding sites. It can also affect transfer of protein in root cells under excessive sodium and chloride ions in rhizosphere. It also affects processes like movement of material, deposition, and partitioning within plants (Tester and Davenport, 2003). Salts can increase in intercellular spaces resulted in cell dehydration (White. and Broadley, 2001). Oxidative stress increases due to the accumulation of reactive oxygen species which has negative impact on cell membranes, proteins, enzymes, and nucleic acids (Ruiz-Lozano *et al*., 2012). Both antioxidant enzymes and non-enzymatic antioxidants are produced by plants to protect against oxidative stress (Hasegawa *et al*., 2000).

### 2.8 Effect of salt stress on soybean

#### 2.8.1 Effect on growth

Soliman *et al.* (2020) reported that when subjected to 100 mM NaCl, shoot length and shoot DW plant$^{-1}$ decreased by 40 and 29%, respectively, in comparison to control. Intercellular $CO_2$ and stomatal conductance reduced by 26.55 and 22.13%, respectively, compared to control. Furthermore, net chlorophyll (Chl) and carotenoids (Car) content and photosynthesis decreased by 46.22, 40.23 and 42.12%, respectively, in comparison to control.

According to Kataria *et al.* (2019) investigated that leaf weight and leaf size were significantly reduced under salt stress. The leaf area reduction was 16, 28 and 38% and the reduction rate of leaf weight was 12, 15 and 31%, respectively, compared to control at 50, 75 and 100 mM NaCl stress. Moreover, 150 mM NaCl-induced salt stress remarkably reduced the root Fresh weight (FW). Fresh weight plant$^{-1}$ also decreased when subjected to salt stress (Cheng *et al.*, 2020).

Khan *et al.* (2019) carried out an experiment to investigate the different levels of salt stress, growth parameters were negatively affected. Shoot length was reduced by 9.7, 23 and 35.39% at 100, 200 and 300 mM NaCl stress, compared to control. Root length followed a similar trend. Root length reduced by 2.6, 12.46 and 24.08% when exposed to 100, 200 and 300 mM NaCl, compared to control. Moreover, shoot FW decreased by 9, 16.11 and 60.18% at 100, 200 and 300 mM NaCl, in comparison to control. Soybean plants exhibited a reduction in root FW under 100, 200 and 300 mM NaCl by 10.6, 20.28 and 35.48%, in comparison to control plants

El-Eswai *et al.* (2018) reported that salinity adversely affects plant growth, root architecture traits and biomass yield. Root architecture traits are assessed as root length, root volume, root dry weight (DW), root FW and the number of nodules formed plant$^{-1}$, negatively affected by salt stress. Moreover, at 40 and 80 mM NaCl length of the root decreased by 32.8 and 59.4%, respectively, compared to control. The volume of root also decreased by 29.2 and 54.2% at 40 and 80 mM NaCl-induced salt stress. Similarly, at 40 and 80 mM NaCl the FW of the root reduced by 33.7 and 57.4%. Moreover, DW of the root reduced by 45.5 and 72.7% at 40 and 80 mM NaCl, respectively. Salinity also reduced the shoot growth and plant biomass. Moreover, Shoot DW and shoot FW were negatively affected by salt stress. Moreover, at 40 and 80 mM NaCl length of shoot declined by 22.8 and 39.3% respectively compared to control

Egamberdieva *et al.* (2017) observed that upon exposure to 0, 50 and 75 mM NaCl shoot weight, root weight and protein content were reduced. Root length reduced by 20.28 and 35% when subjected to 50 and 75 mM NaCl, compared to control. In comparison to control, sharp reduction occurred in root surface area, by 56.74 and 78.65% under 50 and 75 mM NaCl stress, respectively. Root diameter decreased by 23.93and 31.08% under 50 mM and 75 mM NaCl stress. Plant exhibited a noticeable reduction in root volume by 58.95 and 78.95% upon exposure to 50 mM and 75 mM NaCl stress.

Hamayun *et al.* (2017) reported that shoot length, leaf number, leaf area, Chl content and transpiration rate decreased under 70 and 140 mM NaCl-induced salt stress, compared to control. Similarly, FW and DW also showed a decreasing trend under salt stress, in comparison to control.

Kataria *et al.* (2017) investigated that 0, 25, 50, 75 and 100 mM NaCl decreased the shoot length and root length in a dose-dependent manner, compared to control. Moreover, DW of seedling was also reduced when subjected to salt stress, compared to corresponding control.

Akram *et al.* (2017) reported that 150 mM NaCl-induced salt stress significantly reduced the plant height and branch number plant$^{-1}$ in different genotypes of soybean. Shu *et al.* (2017) investigated that 150 mM NaCl-induced salt stress lingered the process of germination by regulating GA (Gibberellic acid) and ABA (Abscisic acid) ratio in three soybean cultivars (Hedou-19, Nandou-12 and C-103). Moreover, the rate of germination percentage, length of radicle and germinated soybean seed FW were also reduced under salt stress, compared to control.

Baghel *et al.* (2016) reported that plant height, plant FW, plant DW, leaf area, root length, root DW, Chl a content, Chl b content, total Chl content and Chl a/b ratio were decreased at 25 and 50 mM NaCl-stressed soybean plants, in comparison to control.

Zhang *et al.* (2016) studied that shoot length, root length, shoot DW, leaf area, the total number of leaves plant$^{-1}$ and root DW of soybean decreased under salt stress. Furthermore, the number of lateral roots and the number of fibrous roots were reduced in salt stress, compared to control. Moreover, the color of root was deepened in salt-stressed plants, in comparison to control plants.

According to Klein *et al.* (2015), salinity was responsible for a noticeable reduction in root FW and DW, shoot FW and DW, total FW and DW, plant height, root length, leaf number and area of leaf in seedlings of soybean. Upon exposure to 120 mM NaCl plant height, DW plant$^{-1}$ and leaf area were reduced in two soybean cultivars (Jackson and Lee68), compared to control (Wei *et al.,* 2015).

Kumari *et al.* (2015) investigated that upon exposure to 100 mM NaCl shoot length and root length reduced by 46.67 and 50%, respectively, compared to control. In salt-stressed plants, drastic reduction (75%) was observed in the number of leaves, compared to control. Plant FW decreased by 48.65% under salt stress, compared to control. Similarly, the number of lateral roots was reduced by 33.33% when subjected to salt stress, in comparison to control. Moreover, Chl content and leaf water content were decreased by 70 and 65.51%, respectively, compared to control.

### 2.8.2 Effect on physiology

Ashraf and Harris (2013) reported that photosynthetic pigments, chl a and chl b, are greatly affected by different abiotic stresses including salinity. Accumulation of toxic Na+ reduces the content of precursor of chl biosynthesis (such as glutamate and 5-aminolevullinic acid) and thus interrupts chl biosynthesis under saline condition.

Amirjani (2010) found a dose-dependent reduction of chl content against salt stress (0, 25, 50, 100 and 200 mM NaCl). At 200 mM NaCl, chl a and chl b reduced by 44 and 27%, respectively, compared to control.

Cha-Um and Kirdmanee (2010) reported that in dark reaction, net photosynthetic rate (NPR), gs and transpiration rate (E) decreased in both varieties, Homjan (HJ) and Pathumthani 1 (PT1) varieties, under salt-stressed condition.

Romero-Aranda *et al.* (2006) reported that increase of salt in the root medium can lead to a decrease in leaf water potential and, hence, may affect many plant processes. Osmotic effects of salt on plants are the result of lowering of the soil water potential due to increase in solute concentration in the root zone. At very low soil water potentials, this condition interferes with plants ability to extract water from the soil and maintain turgor. However, at low or moderate salt concentration (higher soil water potential), plants adjust osmotically (accumulate solutes) and maintain a potential gradient for the influx of water.

Ghoulam *et al.* (2002). carried out an experiment to study the effects of salt stress on growth, inorganic ions and proline accumulation in relation to osmotic adjustment in five sugar beet cultivars and reported that salt treatment caused a significant decrease in relative water content (RWC) in sugar beet varieties.

Katerji *et al.* (1997) reported that a decrease in RWC indicates a loss of turgor that results in limited water availability for cell extension processes.

### 2.8.3 Effect on yield

Kataria *et al.* (2019) reported that total biomass accumulation, rate of photosynthesis, crop yield and harvest index decreased as the salinity level increased at 50, 75 and 100 mM NaCl. The maximum reduction of photosynthesis 54% occurred at 100 mM salt stress compared to normal conditions. The reduction in photosynthesis rate caused low carbon accumulation and led to lower yield. As the salinity increased, the number of pod $plant^{-1}$ remarkably decreased.

In an experiment, Farhangi-Abriz and Ghassemi-Golezani (2018), observed a significant reduction in biomass and seed yield under salinity stress in soybean. About 39% plant biomass reduction and about 44% yield reduction occurred under salt stress.

Akram *et al.* (2017) conducted an experiment by inducing salinity (150 mM NaCl) at the reproductive stage and used 11 genotypes of soybean to study the plant response towards the salinity stress. Experimental result revealed that BINA Soybean-1 and

BINA soybean-2 showed the maximum tolerance against the salinity stress. On the other hand, BINA soybean-4 was the one with minimum tolerance.

Baghel *et al.* (2016) reported that the number of pods plant$^{-1}$, the number of seed plant$^{-1}$ and seed weight was decreased under 0-50 mM NaCl-induced salt stress.

He *et al.* (2016) reported that, salinity decreased the yield and yield contributing parameters of 3 soybean genotypes (S111-9, S113-6 and Melrose). Salinity reduced the number of pod plant$^{-1}$ and the number of seed plant$^{-1}$ in S111-9, S113-6 and Melrose. The lowest reduction occurred in the number of pod plant$^{-1}$ (46.1%) and the number of seed plant$^{-1}$ 49.9% in S111-9 genotypes. Moreover, by 36.1and 36.7% reduction occurred in the number of pods and the number of seed plant$^{-1}$ in S113-6. The highest reduction observed in Melrose, the number of pod plant$^{-1}$ (39.7%) and the number of seed plant$^{-1}$ (51.4%).

According to Hasanuzzaman *et al.* (2016), soybean varieties encountered a significant reduction in quality traits and yield when subjected to salt stress.

El-Sabagh *et al.* (2015) noticed that salinity affects the seed yield and quality of the soybean. Due to the salinity, the yield and yield attributes of soybean in terms of branches plant$^{-1}$, number of pods plant$^{-1}$ and 1000 seed weight significantly decreased. Oil content, protein content, mineral content, soluble carbohydrate content and amino acid content decreased under salinity.

Ashraf and Foolad, (2007) reported that soybean plants exhibits yield reduction when the salinity exceeds 5 dS m$^{-1}$.

### 2.9 Bio-fertilizers

Bio-fertilizers are microbial fertilizers which contain highly efficient strains of bacteria, fungi, and algae provide plants with biogenous elements, nitrogen, phosphorus and potassium (Govedarica *et al*. 2002). Leguminous plants establish symbiotic association with specific genra of bacteria i.e. *Rhizobium*, *Bradyrhizobium*, *Azorhizobium*, *Sinorhizobium* and *Mesorhizobium* collectively known as rhizobia (Sawada *et al.* 2003). Soil or seed inoculated with these *Rhizobium* strains lead to increase the nutrient acquisition and seed yield of legumes. *Bradyrhizobium japonicum* is a strain that responsible for increase nodulation in soybean plants. PGPR are a group of bacteria which colonize the rhizosphere of the plants and improve plant growth through $N_2$ fixation, P solubilization or by converting the unavailable form of nutrients to available form. PGPR promote growth of the crop plants by releasing phytohormones such as auxins, gibberellins, indole acetic acid (IAA), secrete siderophores which provide iron to the plants, solubilize enzymes like 1-aminocyclopropane-1-carboxylate (ACC) deaminase to reduce the level of ethylene and produce antibiotics which have antagonistic effect against phytopathogens in chickpea (Kaur and Sharma 2013). PGPR with phosphate solubilizing ability improve the availability of P to the plants by lowering the soil pH through the production of organic acids and solubilization of fixed P (Singh and Singh 2014). PGPR along with *Bradyrhizobium* can improve grain yield, nutrient uptake, nodulation properties in soybean significantly than uninoculated control (Munda *et al.* 2013). Jain and Trivedi (2005) recorded significantly higher nodule number, dry weight of nodules and leghaemoglobin content with combined inoculation of PGPR and *Bradyrhizobium*. Sole use of biofertilizers are not enough to meet demand of soybean but their combined use along with inorganic fertilizers not only reduces the dose of fertilizers but also helps to eradicate many problems associated with use of excessive fertilizers.

## 2.10 Effect of biofertilizers

### 2.10.1 Effect on growth

Kamaraj and Padmavathi (2018) reported that beneficial microorganisms in biofertilizer accelerate and improve the plant growth and protect the plant from pest and disease. The plant growth characters like plant height, number of branches plant$^{-1}$, number of leaves plant$^{-1}$ and leaf area showed higher value in treatment $T_7$ (Rhizobium @ 600 gm/ha + Phosphate solubilizing bacteria @ 600 gm/ha +VAM @ 600 gm/ha) under saline condition. Because of the combined inoculation treatment, salinity condition shows only 20% reduction in plant height, 27% reduction in number of branches per plant, 27% reduction in number of leaves per plant and 31% reduction in leaf area. This may be due to the combined effect of biofertilizer produced considerable amount of plant growth promoting substances (e.g. IAA and siderophores) which stimulates growth and may also contribute to the suppression of pathogens.

Jaybhay *et al.* (2017) conducted an experiment in Pune, Maharashtra and reported that the maximum dry matter content, relative growth rate and crop growth rate was obtained by dual application of PSB and *Rhizobium*.

Chibeba *et al.* (2015) observed that co-inoculation of *Bradyrhizobium* sp. and *Azospirillum brasilense* improved dry weight of plant.

Mahanta *et al.* (2014) conducted an experiment on influence of biofertilizers and phosphorus levels on soybean and wheat rotation. They observed that best root properties observed at 50% recommended P fertilizer with PSB and VAM while highest P in rhizosphere was observed at 100% recommended dose.

Argaw (2012) observed that with dual inoculation of *Bradyrhizobium japonicum* (TAL 378) and PSB significantly increased the plant height at harvest stage and recorded the highest fresh weight of nodule volume plant$^{-1}$.

Marius *et al.* (2010) observed that rhizobacterial strain (*Baccilus pumilus* Rs3) resulted in significant increase in plant height, number of leaves and leaf area as compared to non-treated control.

Tomar *et al.* (2010) observed that with increase level of P fertilizer along with application of *Rhizobium* and PSB increased the main root length of plant.

Afzal and Bano (2009) reported that seed inoculated with PSB and *Rhizobium* along with P fertilizer resulted increase in plant height, root and shoot weight of plant.

Tahir *et al.* (2009) reported that combination of P and *Rhizobium* inoculation lead to increase the growth parameters of soybean.

Mahanta and Rai (2008) conducted an experiment on effect of PSB and arbuscular mycorrhiza (AM) on root properties in soybean-wheat cropping system to decrease dose of phosphatic fertilizers and reported that application of 50 percent rock phosphate (0.5 RP) + PSB + AM and 100 per cent superphosphate gave almost similar root density (13.71 $m^2m^{-3}$) and root cation exchange capacity. Therefore, it is concluded that dual inoculation of *Rhizobium* and PSB (Phosphate solubilizing bacteria) and combined application of phosphorus and biofertilizers improves the growth parameters such as plant height, dry matter and leaf area.

Singh *et al.* (2007) conducted an experiment to study the efficiency of *Rhizobium, Azotobacter*, phosphate solubilizing bacteria (PSB) and farmyard manure (FYM) on the performance of rainfed soybean and soil fertility. Results revealed that sole or dual inoculation of soybean with biofertilizers, application of FYM and recommended dose of fertilizer (RDF) significantly increased the plant growth parameters like plant height and dry matter accumulation of plant.

Kanase *et al.* (2006) reported that the supply of 75 % of recommended N along with application of *Rhizobium* lead to increase in plant height, leaf area index and leaf area per plant.

Malik *et al.* (2006) concluded that combined application of phosphorus (50 kg $P_2O_5$ $ha^{-1}$) along with *Rhizobium* lead to increase the growth parameters of soybean.

Lanje *et al.* (2005) also found that dual inoculation of *Rhizobium* and PSB biofertilizers gave the maximum dry matter of plant.

Menaria *et al.* (2003) reported that combined inoculation of *Rhizobium* + PSB and sole inoculation of both biofertilizers gave better plant height, dry matter accumulation, leaf area index and primary branches of soybean over uninoculated control.

Raut *et al.* (2003) found that application of 32 kg $P_2O_5$ $ha^{-1}$ along with dual inoculation with PSB and *Rhizobium* gave the maximum plant height, plant dry matter at 90 days after sowing.

Balyan *et al.* (2002) conducted an experiment in Palampur, observed that combined application of PSB and *Rhizobium* gave significantly more plant dry weight than sole inoculation of *Rhizobium* and PSB and uninoculated control.

Umale *et al.* (2002) observed from their studies that combined application of 75 kg $P_2O_5$ $ha^{-1}$ and seed inoculated with PSB gave the maximum plant height, plant dry matter and number of leaves.

### 2.10.2 Effect on physiology

Umi and Athaya (2021) carried out an experiment on the effect of biofertilizer and salinity stress on *amaranthus tricolor* L. growth and total leaf chlorophyll content and reported that biofertilizer provides more nutrients for the plant through its microbial activity, therefore, the productivity and the required nutrients for photosynthesis were provided result in increasing total leaf chlorophyll content.

Naghashzadeh (2014) reported that relative water content and cell membrane stability in inoculated plant were higher than non-inoculated plant.

Auge *et al.* (2003) that fungal hyphae were obtained water by direct uptake and transfer to the host plant, so that protection plants against stress condition.

### 2.10.3 Effect on biochemical attributes

Jaybhay *et al.* (2017) reported that dual inoculation of PSB and *Rhizobium* gave the maximum oil yield in soybean seeds.

Munda *et al.* (2013) reported that protein content was recorded at par with 50 % P as RP + PSB + VAM and 100% P as DAP.

Tahir *et al.* (2009) observed that with combined application of phosphorus fertilizer and *Rhizobium* provided 16 per cent more oil content and 3 per cent more protein content as compared to control.

Shahid *et al.* (2009) reported that application of 75 kg $P_2O_5$ $ha^{-1}$ with seed inoculation with *Rhizobium* provided the highest protein content which was, however, statistically at par with 100 kg $P_2O_5$ $ha^{-1}$ with uninoculation, whereas no significant effect on oil content trend was recorded.

Dhage and Kachave (2008 b) conducted an experiment to study the effect of dual inoculation of *Rhizobium* and PSB on quality parameters of soybean and reported that dual inoculation of *Rhizobium* and PSB gave the maximum oil and protein content but statistically at par with sole inoculation of *Rhizobium* but significantly better than sole inoculation of PSB and uninoculated seed.

Kumar *et al.* (2005) reported that in terms of oil and protein yield i.e. dual application of PSB and rhizobacteria recorded the maximum protein and oil yield i.e. 885.4 kg $ha^{-1}$ and 491.95 kg $ha^{-1}$, respectively.

Lanje *et al.* (2005) reported that dual inoculation of PSB and *Rhizobium* recorded the maximum protein content and oil content in soybean i.e. 38.2% and 19.95%, respectively over uninoculation.

**2.10.4 Effect on yield contributing characters and yield**

Pawar *et al.* (2018) conducted an experiment to study co-inoculation effect of *Bradyrhizobium japonicum* and *Pseudomonas flouroscens* on productivity and quality of soybean and observed that that inoculation of biofertilizers either sole or in combination improves the yield and yield attributes significantly.

Jaybhay *et al.* (2017) conducted a study and reported that dual inoculation of *Rhizobium* and PSB recorded the highest number of pods $plant^{-1}$ which was, however, statistically at par with sole inoculation of individual biofertilizers and significantly better than uninoculated control. However, dual inoculation of *Rhizobium* and PSB recorded significantly better seed yield than sole inoculation of individual biofertilizers and uninoculated control.

Jaga and Sharma (2015) reported that seed inoculation with VAM + PSB + *Rhizobium* gave the maximum seed yield which was, however, statistically at par with VAM + *Rhizobium* and PSB + *Rhizobium* and significantly better than sole inoculation of VAM, PSB and *Rhizobium* and uninoculated control. But in case of straw yield, treatment containing all biofertilizers was significantly better than all other treatments.

Mehta and Patel (2011) conducted an experiment and reported that higher pod length, number of pods $plant^{-1}$, number of seed $pods^{-1}$ and 100-seed weight were recorded with dual inoculation of PSB and *Rhizobium*.

Afzal *et al.* (2010) conducted an experiment on effect of co-inoculation of N fixing and P solubilizing bacteria on yield of soybean and observed that with co-inoculation of both bacteria, increased the seed yield by 38% and 12% in pots and field, respectively.

Jadhav *et al.* (2009) observed that dual inoculation of *Rhizobium* and PSB gave significantly higher number of pods plant$^{-1}$ and 100-seed weight than sole inoculation of *Rhizobium* and PSB in soybean.

Dhage and Kachave (2008a) conducted an experiment at Parbhani, Maharashtra and reported that dual inoculation of *Rhizobium* and PSB gave more straw (18.18 q ha$^{-1}$) and seed yield (13.47 q ha$^{-1}$) and significantly better than sole inoculation of both biofertilizers and uninoculated control.

Chavan *et al.* (2008) conducted an experiment in Akola, Maharashtra and reported that 100-seed weight, number of pods and other yield attributes were increased with inoculation of seed with PSB.

Reddy *et al* (2007) reported that dual inoculation of *Pseudomonas* + *Rhizobium* gave significantly higher yield attributes than without inoculation.

Sonone *et al.* (2007) observed that the highest seed yield and harvest index were obtained by application of PSB and *Rhizobium*.

Gupta *et al.* (2006) reported that seed and straw yield were influenced by inoculation of PSB along with phosphatic fertilizers.

Govindan and Thirumurugan (2005) observed that seed yield of soybean was increased by dual inoculation of PSB and *Rhizobium*.

Menaria and Singh (2004) observed that sole and dual inoculation of *Rhizobium* and PSB had a large impact on yield attributes of soybean. Maximum number of pods, number of branches and test weight was attained by dual inoculation of *Rhizobium* and PSB but statistically at par with sole inoculation of both biofertilizers and significantly better than uninoculated control.

Singh and Rai (2002) conducted an experiment at IARI, New Delhi and reported that combined application of *Pseudomonas striata* + *Aspergillus awamori* significantly improved the yield attributes i.e. number of grains and number of branches.

**2.11 Effects of biofertilizer under salt stressed condition**

Raja and Takhankar (2017) reported that seed inoculation with *Rhizobium* recorded the maximum available nitrogen in soil at the time of harvest under different stress condition.

Goswami *et al.* (2014) recorded out of all the screened isolates, *Bacillus licheniformis* strain $A_2$ showed most prominent PGP traits in vitro and it was tested in vivo for growth promotion of Groundnut (*Arachis hypogaea*) under saline soil condition. In presence of soil supplemented with 50 mM NaCl, *B. licheniformis* treated plants showed increase in fresh biomass, total length and root length by 28, 24 and 17 per cent and in absence of NaCl it was 43, 31 and 39 per cent, respectively.

Aamir *et al.* (2013) reported that under salt affected field condition where pre-isolated strains of *Rhizobium* and PGPR were used alone as well as in combination for mitigating the salinity stress on growth, nodulation and yield of mung bean and revealed that single and combined inoculation enhanced the 1000 grain weight and grain yield up to 14 and 30 per cent, respectively. Moreover, nodulation, relative water content (RWC) and total dry matter (TDM) were improved in case of inoculated plant, also improved protein content (48 %) and K/Na ratio (95 %) in grain were observed. Thus, inoculation/co-inoculation with *Rhizobium* and PGPR could be a sustainable approach to improve plant growth under salinity stress.

Sharma *et al.* (2013) studied on the salinity tolerance of naturally occurring rhizobia, isolated from the root nodules of three leguminous plants, viz., sesbania (*Sesbania sesban*), lablab (*Lablab purpureus*) and pigeonpea (*Cajanus cajan*), growing at research farm in Dubai (United Arab Emirates). The Rhizobial isolates were also found to be effective in nodulating 21-day old seedlings grown in potting soil and irrigated with saline water up to 12 $dSm^{-1}$ after inoculation. The tolerance to high levels of salinity and the survival and persistence in severe and harsh desert conditions made these Rhizobia highly valuable inoculum to improve productivity of the leguminous plants cultivated under extreme environments.

Bagheri (2011) reported the effect of *Azosprillium brasilense* significantly increased photosynthesis rates at all salinity levels. The highest plant N content was obtained from inoculated treatment in saline condition. In the inoculated barley roots, nitrogenase activity (NA) was not severely inhibited by salinity. Generally, inoculation caused plant to cope with stress effectively by increasing fructan content and $NO_3/NH_4$ ratio and decreased less the whole plant N content.

Shinde (2010) studied the efficacy of salt tolerant *Rhizobium* strains and their effect on yield of soybean and observed that SR-3 strain proved to be best strain in improving yield of soybean in saline soil.

Shewale (2009) studied the effect of salinity on rhizobium population and it's influence on growth and yield of soybean and found that the soil rhizobial population was lower in saline soil than normal soil due to increased salt concentration.

Dhage and Kachave (2008a) conducted an experiment to study the effect of phosphatic fertilizers and biofertilizers on properties of soil. The highest $NO_3^-$ nitrogen and $NH_4^+$ nitrogen was recorded with combined application of PSB and *Rhizobium* which was, however, significantly better than sole inoculation of PSB & *Rhizobium* and uninoculated control.

Gupta *et al.* (2008) studied the effect of Rhizobium strains and nitrogen fertilizer on soil pH and nitrogenase activity in nodule of salt tolerant lentil (*Lens culinaris L*.) and reported that physiochemical properties such as pH, EC (Electrical conductivity), organic carbon slightly decreased but available nitrogen of soil was increased significantly. However, CEC (Cation exchange capacity) slightly increased but available P and K decreased besides exchangeable sodium percentage was also decreased due to *Rhizobium* strain inoculation in soil salinity.

Nasef *et al.* (2004) reported that the application of N at different levels and inoculation with biofertilizer led to an increase in total porasity improves soil aggregation and possible moving salt soil under irrigation water. Also, the plants tolerated the lower salinity levels especially when combined with biofertilizer application where it counteracted the reduction of salinity on vegetative growth, pigment contents, total sugars and some minerals.

# CHAPTER III

# MATERIALS AND METHODS

This chapter presents a brief description of the crop, experimental design, treatments, and cultural operations, collection of soil and plant samples and analytical methods followed in the experiment.

## 3.1 Experimental details

### 3.1.1 Study Area

The experiment was conducted at Sher-e-Bangla Agricultural University, agronomy farm Dhaka to investigate the amelioration of salt stress in soybean by microbial biofertiloizer.

### 3.1.2 Experimental period

The experiment was conducted during the period from September to November in 2020.

## 3.2 Description of the experimental site

### 3.2.1 Geographical location

The experiment was conducted in the Agronomy field of Sher-e-Bangla Agricultural University (SAU). The experimental site is geographically situated at 23°77′ N latitude and 90°33′ E longitude at an altitude of 8.6 meter above sea level (Anon., 2004).

### 3.2.2 Agro-Ecological Zone

The experimental site belongs to the Agro-ecological zone (AEZ) of “The Modhupur Tract”, AEZ-28 (Anon., 1988 a). This was a region of complex relief and soils developed over the Modhupur clay, where floodplain sediments buried the dissected edges of the Modhupur Tract leaving small hillocks of red soils as ‘islands’ surrounded by floodplain (Anon., 1988 b). For better understanding about the experimental site has been shown in the Map of AEZ of Bangladesh in Appendix-I.

### 3.2.3 Soil

The soil for filling the pot was collected from agronomic field belongs to the general soil type, shallow red brown terrace soils under tejgaon soil series. Soil pH ranges from 5.4–5.6 (Anon., 1989). The land was above flood level and sufficient sunshine was available during the experimental period. Soil samples from 0–15 cm depths were collected from the Sher-e-Bangla Agricultural University (SAU) Farm, field. The soil analyses were done at Soil Resource and Development Institute (SRDI), Dhaka. The morphological and physicochemical properties of the soil are presented in below table.

**Table 1. The initial physical and chemical characteristics of soil use in this experiment**

| **Physical characteristics** | |
|---|---|
| Constituents | Percent |
| Sand | 26 |
| Silt | 45 |
| Clay | 29 |
| Textural class | Silty clay |
| **Chemical characteristics** | |
| Soil characteristics | Value |
| pH | 5.6 |
| Organic carbon (%) | 0.45 |
| Organic matter (%) | 0.78 |
| Total nitrogen (%) | 0.03 |
| Available P (ppm) | 20.54 |
| Exchangeable K (mg/100 g soil) | 0.10 |

**Sourse:** Soil Resources Development Institute (SRDI), Khamarbari, Farmgate, Dhaka.

### 3.2.4 Climate and weather

The climate of the experimental site was subtropical, characterized by the winter season from November to February and the pre-monsoon period or hot season from March to April and the monsoon period from May to October (Edris *et al.*, 1979). Meteorological data related to the temperature, relative humidity and rainfall during the experiment period of was collected from Bangladesh Meteorological Department (Climate division), Sher-e-Bangla Nagar, Dhaka and has been presented in Appendix-

### 3.3 Experimental materials

#### 3.3.1 Plant material

BARI Soybean-6 was used as the plant material for conducting the experiment. The important characteristics of these varieties are mentioned below:

Released: 2009

Developed: Bangladesh Agriculture Research Institute (BARI).

Primary number of branches: 2-3

Seed pod$^{-1}$: 2-3

Seed: creamy white in colour and medium in size

Protein content: 42-44%

Oil content: 20-21%

Carbohydrate content: 27%

#### 3.3.2 Earthen pot

Earthen pots of having 12 inches diameter, 12 inches height with a hole at the centre of the bottom were used.

### 3.4 Experimental treatment

There were two factors in the experiment namely different salt stress and application of different microbial biofertiloizer as mentioned below:

**Factor A**: Different salt concentration (3) *viz;*

$S_0$ = Control

$S_1$= 1800 ppm pot$^{-1}$ and

$S_2$ = 3600 ppm pot$^{-1}$

**Factor B**: Application of different microbial biofertiloizer (4) *viz;*

$B_0$ = 0 g *Rhizobium* @ 50 kg seed (Control)

$B_1$ = BARI RGm-907 @ 25 g/50 kg seed

$B_2$ = BARI RGm-922 @ 50 g/50 kg seed and

$B_3$ = BARI RGm-928 @ 75 g/50 kg seed

### 3.5 Experimental design

The experiment was laid out in Completely Random Design (CRD) with 2 factor and four replications. Total 48 unit pots will be made for the experiment with 12 treatments having 4 replication. Each pot will be of required size.

### 3.6 Detail of experimental preparation

#### 3.6.1 Seed collection

Seeds of BARI Soybean-6 were collected from Oilseed division of Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur.

#### 3.6.2 Soil preparation for pot

To prepare well pulverized and healthy soil for the experiment, soil was collected and then sun-dried and crushed. After that recommended basal dose of organic manures and fertilizers were incorporated with the prepared soil. Besides, the fertilizers and organic manures, Furadan®5 G was also mixed with the soil at the recommended dose to protect the seedlings from insect, mites and nematodes. Each pot was filled up with 10 kg well pulverized soil containing organic manures, fertilizers and insecticide.

#### 3.6.3 Fertilizer application

Fertilizer and manure dose for BARI Soybean-6 as follows:

| Fertilizers | Dose (kg $ha^{-1}$) |
|---|---|
| Cowdung | 5000 |
| Urea | 25-30 |
| Triple superphosphate | 60-70 |
| Murate of potash | 35-40 |
| Gypsum | 35-45 |

All fertilizers and manures were incorporated during final soil preparation.

### 3.7 Seed treatment

Soybean seed was coated with moist biofertilizer according with par treatment requirement and dried for one hour in shed and was used for sowing.

### 3.8 Seed sowing technique

Before seed sowing pot soil was irrigated with sufficient water to achieve the field capacity of soil for seed sowing. After that, twelve healthy seeds were sown at 5 cm depth in each pot.

### 3.9 Intercultural operations

#### i) Gap filling and thinning

Gap filling and thinning was done at 07 DAS to maintain the uniform plant density in each pot.

#### ii) Weeding, mulching and irrigation

The pots were kept weed free by regular observation and hand weeding. Mulching and irrigation applications were done when needed.

#### iii) Induce salinity treatment

The salinity treatments were applied on 15, 25 and 30 DAS. There were three salinity levels including control which was developed by adding respected amount of commercial NaCl salt to the pot as water dissolved solution. The salinity levels were $S_0$ (control), $S_1$ (1800 ppm NaCl $pot^{-1}$) and $S_2$ (3600 ppm NaCl $pot^{-1}$). When no salt added in control ($S_0$) treatment. In order to spread homogenously in each pot. The salts were dissolved in water and were added to pots for proper salinity imposition. At 15, 25 and 30 DAS 9, 4.5 and 4.5 g ( total 18 g $pot^{-1}$) NaCl was applied respectively in $S_1$ treatment, while 18, 9 and 9 g (total 36 g $pot^{-1}$) NaCl was applied respectively in $S_2$ treatment.

### 3.10 General observations of the experimental field

Regular observations were made to see the growth and visual different of the crops, due to application of different treatment were applied in the experimental pot. Incidence of Hairy caterpillar, ants were observed during vegetative growth stage and there were also some mites and nematodes were present in the experimental pot. But any bacterial and fungal disease was not observed. The flowering was not uniform.

### 3.11 Plant protection

Sumithion®57 EC was applied twice at 7 days interval (25 DAS and 33 DAS) to protect the plants from Hairy caterpillar. Furadan®5G was mixed with the soil to protect the plants from insects, mites and nematodes.

### 3.12 Harvesting

The soybean plants were uprooted carefully without disturbing the roots.

### 3.13 Collection of data

The yield and yield contributing parameters were measured at harvest. Growth, and physiological parameters were recorded during treatment duration.  Data were collected on the following parameters:

**Crop growth parameters:**

i. Plant height
ii. Number of leaves plant$^{-1}$

**Physiological parameters:**

iii. SPAD value of leaf
iv. Leaf relative water content (LRWC)

**Yield and yield contributing parameters:**

v. Number of pods plant$^{-1}$
vi. Number of seedspod$^{-1}$
vii. 1000 seed weight
viii. Seed yield plant$^{-1}$
ix. Stover yield plant$^{-1}$
x. Biological yield plant$^{-1}$
xi. Harvest index

### 3.14 Procedure of recording data

**i) Plant height (cm)**

The height of the selected plant was measured from the ground level to the tip of the plant at 15, 30, 45 and 60 DAS. Mean plant height of soybean plant were calculated and expressed in cm.

**ii) Number of leaves plant$^{-1}$**

After the completion of the treatment duration, number of leaves plant$^{-1}$ of five plants were counted and then averaged to determine the number of branches for plant$^{-1}$.

**iii) SPAD value**

After completion of the treatment duration, five leaves were randomly selected from each pot. The top, middle and bottom of each leaflet was measured with at LEAF (FT Green LLC, USA) as at LEAF value. Then, the values were then averaged, total chlorophyll content was determined by the conversion of at LEAF value into SPAD units.

**iv) Leaf relative water content (LRWC)**

Three leaflets were randomly selected from each pot and cut with scissors. Leaf relative water content (RWC) was measured according to Barrs and Weatherley (1962). Leaf relative water content was measured at 50 DAT. Leaf laminas were weighed (fresh weight, FW) and then immediately floated on distilled water in a petridish for 4 h in the dark. Turgid weights (TW) were obtained after drying excess surface water with paper towels. Dry weights (DW) were measured after drying at $80^0$C for 48 h. Then calculation was done using the following formula:

$$\text{LRWC}\ (\%) = \frac{\text{FW- DW}}{\text{TW-DW}} \times 100$$

**v) Number of pods plant$^{-1}$ (no.)**

Pods plant$^{-1}$ was counted from the 5 selected plant sample and then the average pods number was calculated.

**vi) Seeds pod$^{-1}$ (no.)**

Seeds $pod^{-1}$ was counted from splitting five $pod^{-1}$ which were sampled from sample plants and then mean value was determined.

**vii) 1000-seed weight (g)**

1000-seeds were counted which were taken from the seed stock of each pot, then weighed it in an electrical balance and data were recorded.

**viii) Seed yield (g $plant^{-1}$)**

The mean seed weight was taken by threshing the plants of each sample and then weighed it in an electrical balance and data were recorded on dry weight basis..

**ix) Stover yield (g $plant^{-1}$)**

The stover weights of soybean were calculated after threshing and separation of the seeds from the plant of sample and then weighed it in an electrical balance and data were recorded on dry weight basis.

**x) Biological yield $pot^{-1}$ (g)**

The summation of grain yield and above ground straw yield was the biological yield.
Biological yield g $pot^{-1}$ = (Grain yield + Stover yield) g $pot^{-1}$

**xi) Harvest index (%)**

Harvest index was calculated on dry weight basis with the help of following formula.

$$\text{Harvest index (HI \%)} = \frac{\text{Grain yield}}{\text{Biological yield}} \times 100$$

Here, Biological yield = Grain yield + Stover yield

**3.15 Data analysis technique**

The collected data were compiled and analyzed statistically using the analysis of variance (ANOVA) technique with the help of a computer package program name Statistix 10 Data analysis software and the mean differences were adjusted by Least Significant Difference (LSD) test at 5% level of probability (Gomez and Gomez, 1984).

# CHAPTER IV

# RESULTS AND DISCUSSION

Results obtained from the present study have been presented and discussed in this chapter with a view to amelioration of salt stress in soybean by microbial biofertilizer. The data are given in different tables and figures. The results have been discussed, and possible interpretations are given under the following headings.

## 4.1 Phenotypic appearance

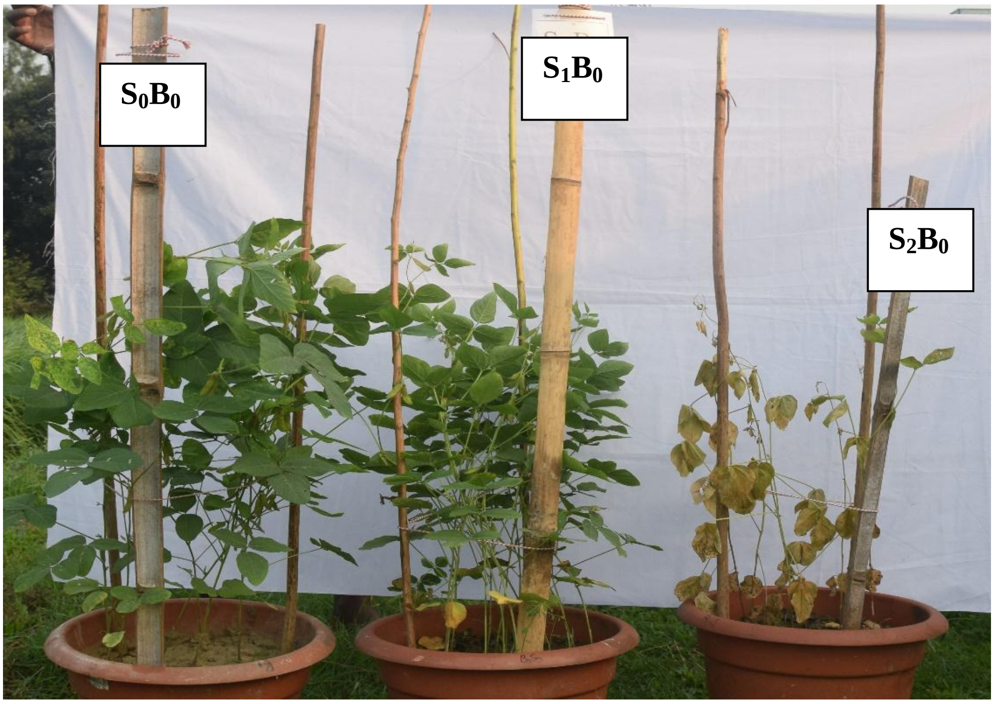



**Figure 2. Effect of salinity on plant height of soybean at 45 DAS.**

Salinity affects on soybean plant. Increasing salinity level gradually decrease plant growth and development. At moderate salinity level plant withstand some degree of salt stress but increasing stress level reduce plant height, leaf numbers, and cause yellowing and burning of the leaf due to the adverse effect of salinity.

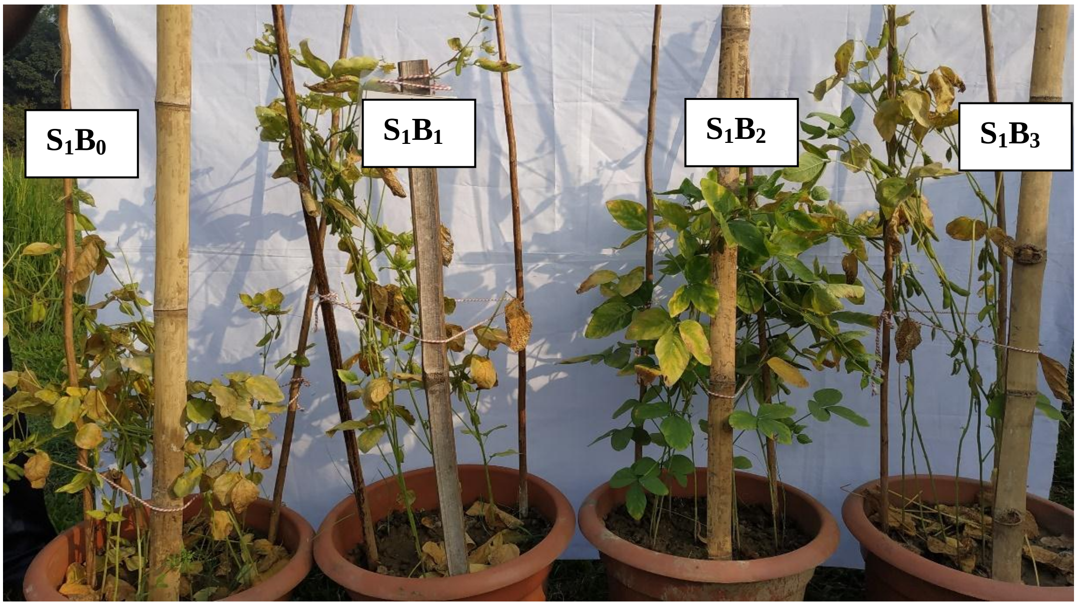



**Figure 3. Effect of biofertilizers on soybean at 60 DAS under salinity stress condition.**

Different biofertilizer influence plant growth of soybean and mitigate some degree of strees condition. Without application of biofertilizers plant growth gradually decrease due to increasing salinity levels. Among different biofertilizers application of BARI RGm-922 @ 50 g/50 kg seed performed well compared to others.

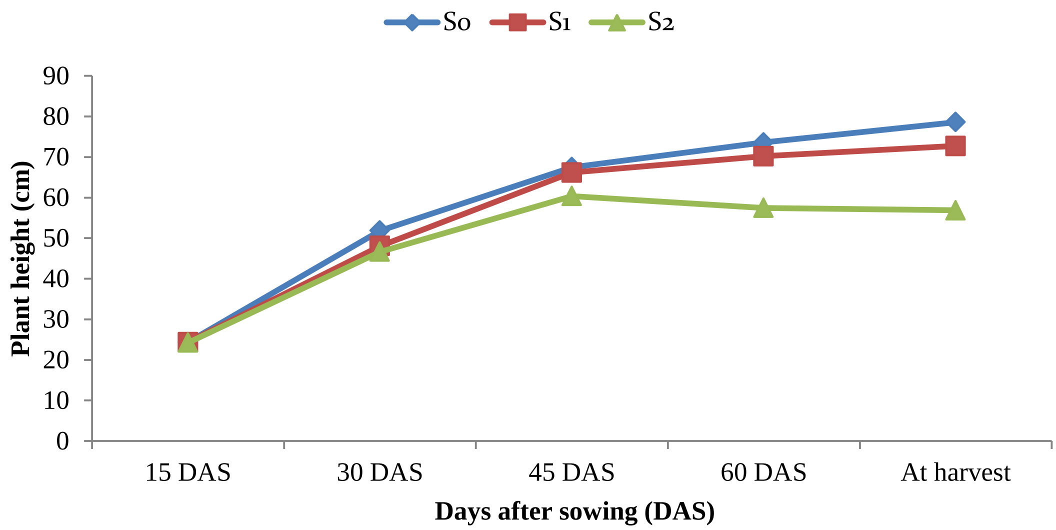
## 4.2 Crop growth parameters

### 4.2.1 Plant height (cm)

**Effect of salt stress**

Plant height is an important morphological character that acts as a potential indicator of availability of growth resources in its approach. Plant height was recorded at 15, 30, 45, 60 DAS and at harvest respectively. Different salinity level significantly effect on plant height of soybean at different days after sowing (Figure 4). From the Experiment it is found that the maximum plant height (24.39, 51.88, 67.48, 73.60 and 78.60 cm) at 15, 30, 45, 60 DAS and at harvest respectively were recorded in $S_0$ (Control) treatment. Increasing salinity level decreased plant height and the minimum plant height (24.24, 46.65, 60.38, 57.45 and 56.87 cm) at 15, 30, 45, 60 DAS and at harvest respectively were recorded in $S_2$ (140 ppm pot$^{-1}$) treatment which was (0.62

%, 10.08 %, 10.52 %, 21.94 % and 27.64 %) lower compared to control treatment. Gradual decrease in plant height might be due to the nutrient unavailability caused by increased salinity or the inhibition of cell division or cell enlargement. The result obtained from the present study was similar with the findings of El-Eswai *et al.* (2018) and they reported that salinity adversely affects plant growth.

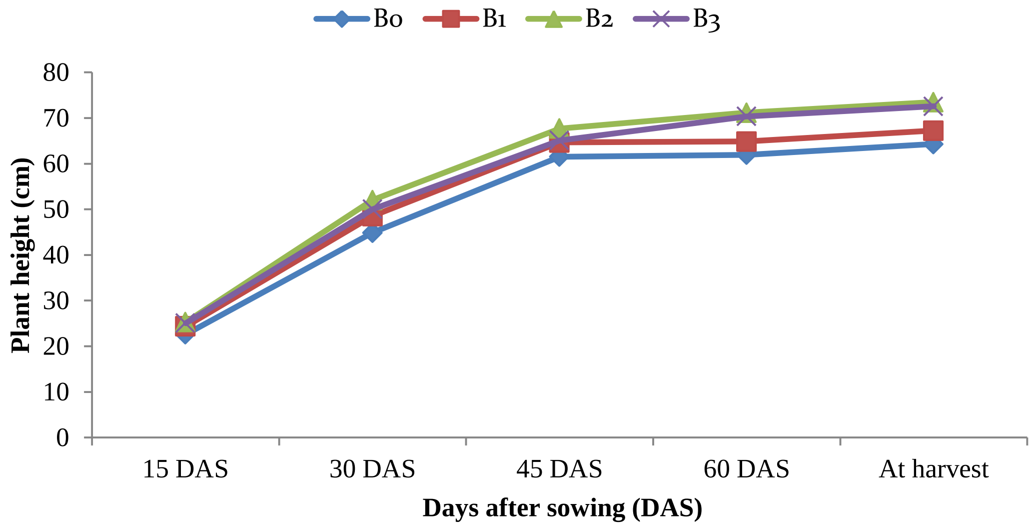



Note: $S_0$ = Control, $S_1$= 1800 ppm NaCl $pot^{-1}$ and $S_2$ = 3600 ppm NaCl $pot^{-1}$.

**Figure 4. Effect of salt stress on plant height of soybean at different DAS [$LSD_{(0.05)}$ = Ns, 0.85, 0.76, 0.50 and 0.81 at 15, 30, 45, 60 DAS and at harvest, respectively].**

### Effect of different bio-fertilizers

Application of different bio-fertilizers significantly influenced plant height of soynean at different days after sowing (Figure 5). Experimental result showed that the tallest plant (25.29, 52.02, 67.70, 71.18, and 73.48 cm) at 15, 30, 45, 60 DAS and at harvest, respectively were recorded in $B_2$ (BARI RGm-922 @50 g/50 kg seed) treatment which was 11.85 %, 15.93 %, 10.05 % 14.92 % and 12.49 % higher compared to control treatment and it was statistically similar (25.08 and 72.56 cm) with $B_3$ (BARI RGm-928 @ 75 g/50 kg seed) treatment at 15 DAS and at harvest respectively. Whereas the shortest plant (22.61, 44.87, 61.52, 61.94 and 64.30 cm) at 15, 30, 45, 60 DAS and at harvest, respectively were recorded in $B_0$ (Control) treatment. The variation of plant height was due to the various effect of different biofertilizer that produced considerable amount of plant growth promoting substances which stimulates growth and may also contribute to the suppression of stress condition. Argaw (2012) also found similar result which supported the present finding reported that with dual inoculation of *Bradyrhizobium japonicum* (TAL 378) and PSB significantly increased the plant height at harvest stage and recorded the highest fresh weight of nodule volume $plant^{-1}$. Marius *et al.* (2010) observed that rhizobacterial strain (*Baccilus pumilus* Rs3) resulted in significant increase in plant height, number of leaves and leaf area as compared to non-treated control.

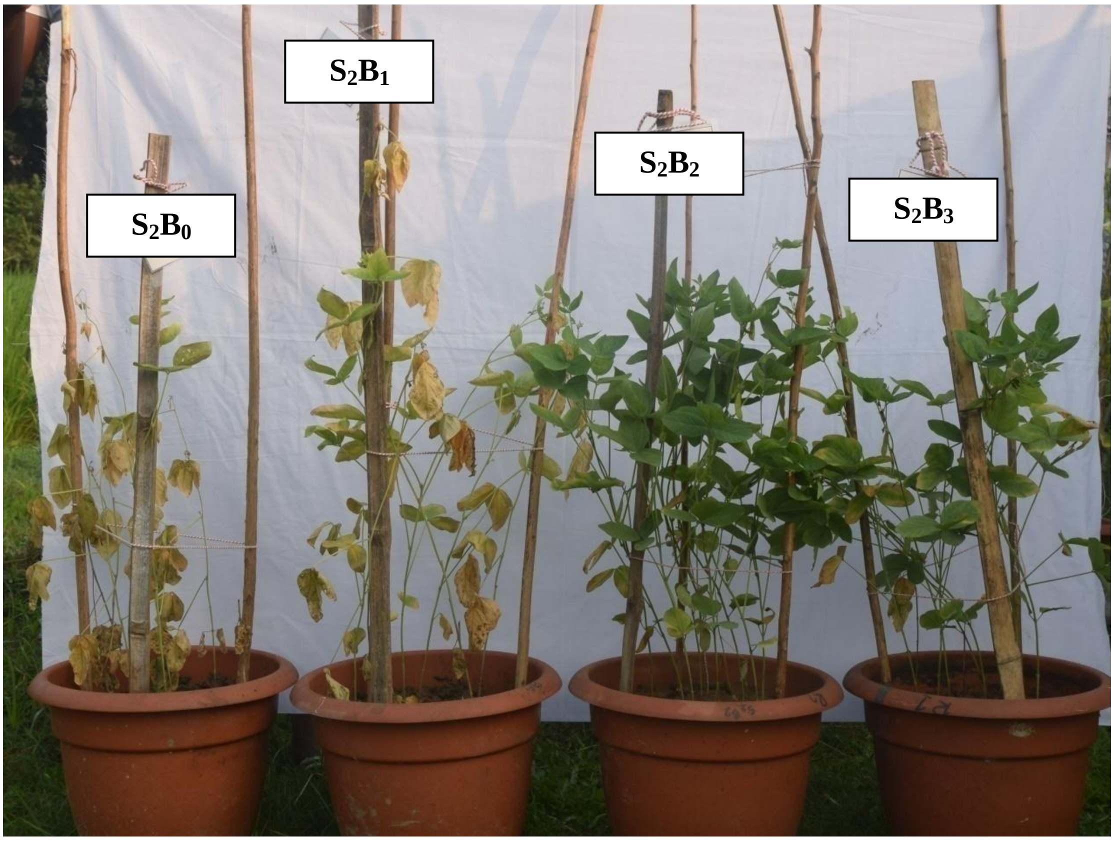



Note: $B_0$= 0 g Rhizobium @ 50 kg seed, $B_1$= BARI RGm-907 @ 25 g/50 kg seed, $B_2$= BARI RGm-922 @ 50 g/50 kg seed and $B_3$= BARI RGm-928 @ 75 g/50 kg seed.

**Figure 5. Effect of biofertilizers on plant height of soybean at different DAS [$LSD_{(0.05)}$ = 0.47, 0.98, 0.88, 0.58 and 1.01 at 15, 30, 45, 60 DAS and at harvest, respectively].**

**Combined effect of salt stress and bio-fertilizers**

Combined effect of salt stress and bio-fertilizers significantly effect on plant height of soybean at different days after sowing (Figure 6 and Table 2). It is observed that the longest plant (55.94, 71.36, 77.37 and 82.37 cm) at 30, 45, 60 and at harvest, respectively were recorded in $S_0B_2$ treatment combination which was 13.54, 10.65, 9.56 and 10.18 % higher comparable to control treatment. Whereas the minimum plant height (42.31, 57.72, 47.94 and 47.51 cm) at 30, 45, 60 DAS and at harvest, respectively were recorded in $S_2B_0$ treatment combination which was 14.13, 10.50, 32.12 and 36.45 % lower comparable to control treatment and it was statistically similar with statistically similar with $S_1B_0$ (43.03 cm) treatment combination.




Here, $S_0$ = Control, $S_1$= 1800 ppm NaCl $pot^{-1}$ and $S_2$ = 3600 ppm NaCl $pot^{-1}$.$B_0$= 0 g Rhizobium @ 50 kg seed, $B_1$= BARI RGm-907 @ 25 g/50 kg seed, $B_2$= BARI RGm-922 @ 50 g/50 kg seed and $B_3$= BARI RGm-928 @ 75 g/50 kg seed.

**Figure 6. Combined effect of salt stress and bio-fertilizers on plant height of soybean at 45 DAS.**

Goswami *et al.* (2014) reported that supplementation with *B. licheniformis* in 50 mM NaCl, treated plants showed increase in fresh biomass, total length and root length by 28, 24 and 17 % compared with control plant. Aamir *et al.* (2013) reported that inoculation/co-inoculation with *Rhizobium* and PGPR could be a sustainable approach to improve plant growth under salinity stress.

**Table 2. Combined effect of salt stress and different bio-fertilizers on plant height of soybean at different DAS**

| Treatment Combinations | Plant height (cm) | | | | |
|---|---|---|---|---|---|
| | 15 DAS | 30 DAS | 45 DAS | 60 DAS | At harvest |
| $S_0B_0$ | 22.87 | 49.27 cd | 64.49 c | 70.62 c | 74.76 b |
| $S_0B_1$ | 24.33 | 49.66 c | 66.55 b | 69.76 cd | 75.62 b |
| $S_0B_2$ | 25.30 | 55.94 a | 71.36 a | 77.37 a | 82.37 a |
| $S_0B_3$ | 25.09 | 52.63 b | 67.52 b | 76.66 a | 81.66 a |
| $S_1B_0$ | 22.52 | 43.03 f | 62.34 d | 67.27 e | 69.77 c |
| $S_1B_1$ | 24.55 | 48.99 cd | 67.40 b | 69.00 d | 71.50 c |
| $S_1B_2$ | 25.30 | 50.34 c | 67.58 b | 72.51 b | 75.01 b |
| $S_1B_3$ | 25.20 | 49.88 c | 67.44 b | 72.08 b | 74.58 b |
| $S_2B_0$ | 22.44 | 42.31 f | 57.72 f | 47.94 i | 47.51 f |
| $S_2B_1$ | 24.28 | 46.91 e | 60.02 e | 55.86 h | 55.46 e |
| $S_2B_2$ | 25.27 | 49.77 c | 63.46 cd | 63.66 f | 63.06 d |
| $S_2B_3$ | 24.95 | 47.60 de | 60.32 e | 62.33 g | 61.45 d |
| $LSD_{(0.05)}$ | Ns | 1.71 | 1.53 | 1.00 | 1.75 |
| CV(%) | 2.35 | 2.43 | 1.64 | 1.04 | 1.76 |

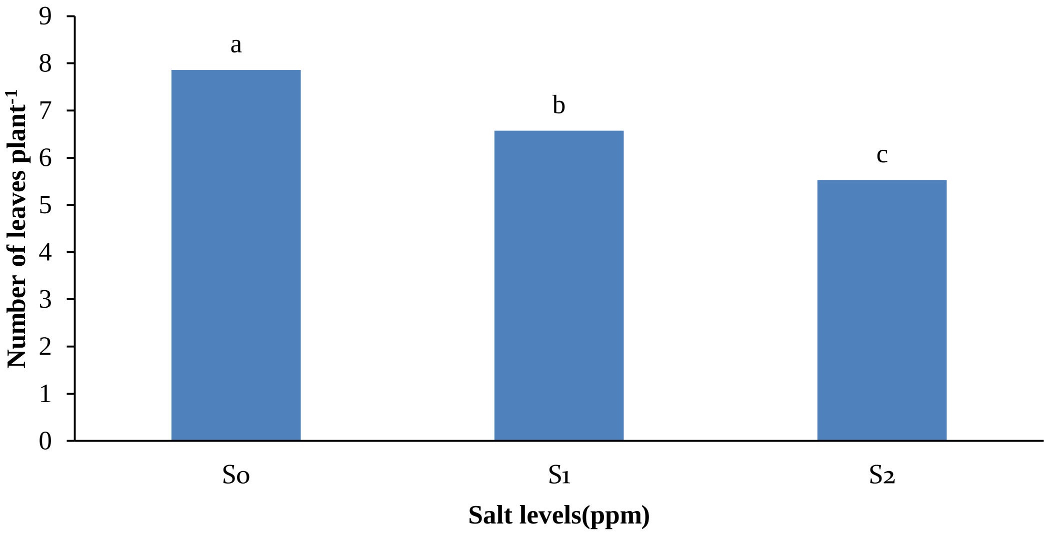
In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at 0.05 level of probability. Here, $S_0$ = Control, $S_1$= 1800 ppm pot$^{-1}$ and $S_2$ = 3600 ppm pot$^{-1}$.$B_0$= 0 g Rhizobium @ 50 kg seed, $B_1$= BARI RGm-907 @ 25 g/50 kg seed, $B_2$= BARI RGm-922 @ 50 g/50 kg seed and $B_3$= BARI RGm-928 @ 75 g/50 kg seed.

### 4.2.2 Number of leaves plant$^{-1}$

**Effect of salt stress**

A leaf is the principal lateral appendage of the vascular plant stem, usually borne above ground and specialized for photosynthesis (Tozer *et al.*, 2015). In this experiment number of leaves plant$^{-1}$ of soybean was varied with different salinity condition at 30 DAS (Figure 7). Experimental result showed that the maximum number of leaves plant$^{-1}$ of soybean (7.86) at 30 DAS was recorded in $S_0$ (Control) treatment. With the increasing salinity levels the number of leaves plant$^{-1}$ of soybean drastically reduced. So the minimum number of leaves plant$^{-1}$ of soybean (5.53) at 30

DAS was recorded in $S_2$ (140 ppm $pot^{-1}$) treatment which was 29.64 % lower comparable to control treatment. Extreme salt stress causes chlorosis, necrosis and premature senescence of adult leaves and thus limits the photosynthetic area available to support continued growth of salt-affected plants. Hamayun *et al.* (2017) reported that shoot length, leaf number, leaf area, Chl content and transpiration rate decreased under 70 and 140 mM NaCl-induced salt stress, compared to control.



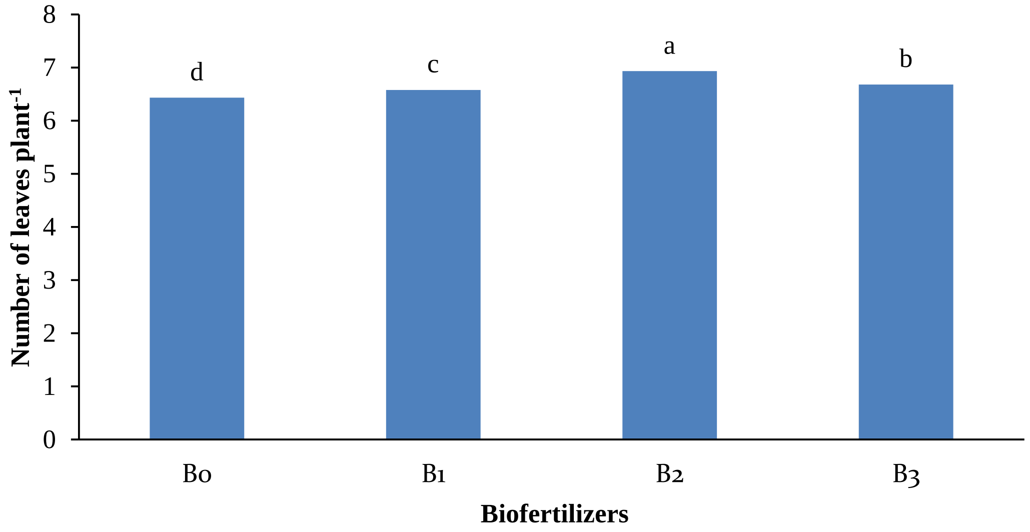

Note: $S_0$ = Control, $S_1$= 1800 ppm NaCl $pot^{-1}$ and $S_2$ = 3600 ppm NaCl $pot^{-1}$.

**Figure 7. Effect of salt stress on number of leaves $plant^{-1}$ of soybean at 30 DAS [$LSD_{(0.05)}$ = 0.05 at 30 DAS].**

### Effect of different bio-fertilizers

Bio-fertilizers application significantly influenced on number of leaves $plant^{-1}$ of soybean at 30 DAS (Figure 8). Experimental result revealed that the highest number of leaves $plant^{-1}$ of soybean (6.93) was recorded in $B_2$ (BARI RGm-922 :50 g/50 kg seed) treatment which was 7.78 % higher over control treatment. Whereas the lowest number of leaves $plant^{-1}$ of soybean (6.43) at 30 DAS was recorded in control treatment ($B_0$). Bio-fertilizer increases a group of soil bacteria which colonize the rhizosphere of the plants and improve plant growth through $N_2$ fixation, P solubilization or by converting the unavailable form of nutrients to available form. This available nutrients might have helped in enhancing number of leaves and leaf area, which impact leaf area index, thereby resulted in higher photo-assimilates and more dry matter accumulation result on improved growth of rice. Marius *et al.* (2010)

observed that rhizobacterial strain (*Baccilus pumilus* Rs3) resulted in significant increase in plant height, number of leaves and leaf area as compared to non-treated control.




Note: $B_0$= 0 g Rhizobium @ 50 kg seed, $B_1$= BARI RGm-907 @ 25 g/50 kg seed, $B_2$= BARI RGm-922 @ 50 g/50 kg seed and $B_3$= BARI RGm-928 @ 75 g/50 kg seed.

**Figure 8. Effect of biofertilizer on number of leaves plant$^{-1}$ of soybean at 30 DAS [LSD$_{(0.05)}$ = 0.06 at 30 DAS].**

**Combined effect of salt stress and bio-fertilizers**

Combined effect of salt stress and bio-fertilizers have significantly effect on n number of leaves plant$^{-1}$ of soybean at 30 DAS (Table 3). Experimental result revealed that the maximum number of leaves plant$^{-1}$ (8.10) of soybean at 30 DAS was recorded in $S_0B_2$ treatment combination which was 5.88 % higher comparable to control ($S_0B_0$). While the lowest number of leaves plant$^{-1}$ (5.25) of soybean at 30 DAS was recorded in $S_2B_0$ treatment combination which was 31.37 % lower comparable to control treatment. Application of biofertilizers keep the soil environment rich in all kinds of micro- and macro-nutrients via nitrogen fixation, phosphate and potassium solubilization or mineralization, release of plant growth regulating substances, production of antibiotics and biodegradation of organic matter in the soil thus stimulate plant growth and provide protection against stress condition. Kamaraj and

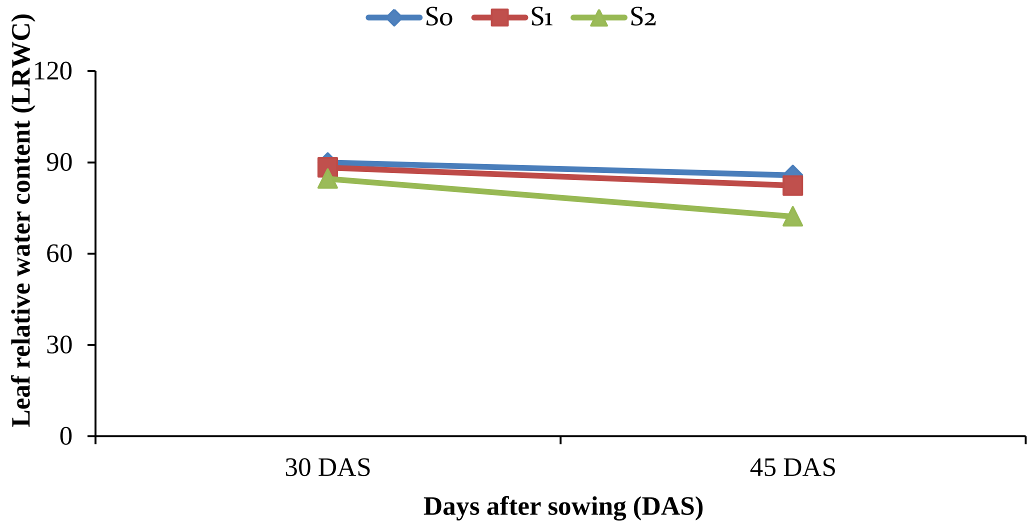
Padmavathi (2018) reported that beneficial microorganisms in biofertilizer accelerate and improve the plant growth like number of leaves $plant^{-1}$ under saline condition.

## 4.3 Physiological parameters

### 4.3.1 Leaf relative water content (LRWC)

**Effect of salt stress**

Relative water content is described as the amount of water in a leaf at the time of sampling relative to the maximal water a leaf can hold. It is an important parameter in water relation studies, e.g. it allows the calculation of the osmotic potential at full turgor (Tanentzap, 2015). In this experiment, exposure of salt significantly influenced leaf relative water content of soybean at 30 and 45 DAS (Figure 9). From the study it is found that the maximum leaf relative water content (89.93 and 85.79 %) at 30 and 45 DAS were recorded in control ($S_0$) treatment which was gradually decreasing with increasing salt concentration. The minimum leaf relative water content (84.60 and 72.18 %) were recorded in $S_2$ treatment and it was (5.92 and 15.86 %) lower comparable to control treatment. Romero-Aranda *et al.* (2006) reported that increase of salt in the root medium can lead to a decrease in leaf water potential and, hence, may affect many plant processes. Osmotic effects of salt on plants are the result of lowering of the soil water potential due to increase in solute concentration in the root zone. At very low soil water potentials, this condition interferes with plants‘ ability to extract water from the soil and maintain turgor. However, at low or moderate salt concentration (higher soil water potential), plants adjust osmotically (accumulate solutes) and maintain a potential gradient for the influx of water. Ghoulam *et al.* (2002) and Katerji *et al.* (1997) also reported that a decrease in RWC indicates a loss of turgor that results in limited water availability for cell extension processes.

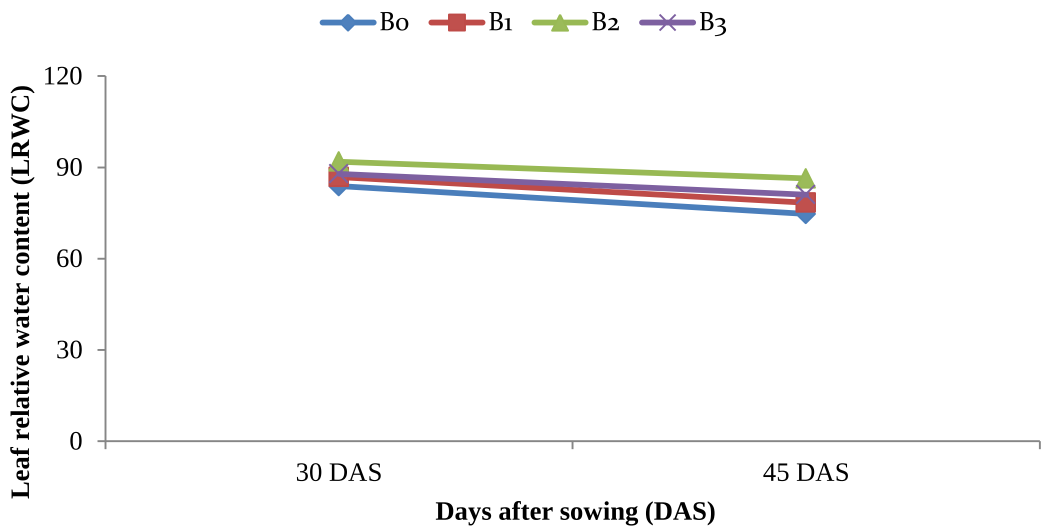



Note: $S_0$ = Control, $S_1$= 1800 ppm NaCl $pot^{-1}$ and $S_2$ = 3600 ppm NaCl $pot^{-1}$.

**Figure 9. Effect of salt stress on leaf relative water content of soybean at different DAS [$LSD_{(0.05)}$ = 0.74 and 0.59 at 30 and 45 DAS].**

**Effect of different bio-fertilizers**

Application of different bio-fertilizers significantly influenced leaf relative water content of soybean at 30 and 45 DAS (Figure 10). The present study revealed that the highest leaf relative water content (91.89 and 86.33 %) at 30 and 45 DAS were recorded in $B_2$ treatment which was (9.59 and 15.63 %) higher comparable to control treatment. Whereas the lowest leaf relative water content (83.85 and 74.66 %) were recorded in $B_0$ treatment. The present study was similar with the findings of Naghashzadeh (2014) who reported that relative water content and cell membrane stability in inoculated plant were higher than non-inoculated plant. Auge *et al.* (2003) also reported that fungal hyphae were obtained water by direct uptake and transfer to the host plant, so that protection plants against stress condition.




Note: $B_0$= 0 g Rhizobium @ 50 kg seed, $B_1$= BARI RGm-907 @ 25 g/50 kg seed, $B_2$= BARI RGm-922 @ 50 g/50 kg seed and $B_3$= BARI RGm-928 @ 75 g/50 kg seed.

**Figure 10. Effect of biofertilizers on leaf relative water content of soybean at different DAS [LSD$_{(0.05)}$ = 0.86 and 0.68 at 30 and 45 DAS].**

**Combined effect of salt stress and bio-fertilizers**

Combination of salt and bio-fertilizers have significant effect on leaf relative water content of soybean at 30 and 45 DAS (Table 3). The highest leaf relative water (94.75 and 88.84 %) at 30 and 45 DAS were recorded in $S_0B_2$ treatment combination which was (7.55 and 6.81 %) higher comparable to control treatment and statistically similar with $S_1M_2$ treatment combination recorded leaf relative water (93.63) at 30 DAS. The lowest leaf relative water content of soybean (81.27 and 63.31 %) were recorded in $S_2B_0$ treatment combination which was (7.75 and 23.53 %) lower comparable to control treatment. Aamir *et al.* (2013) reported that nodulation, relative water content (RWC) and total dry matter (TDM) were improved in case of inoculated plant, and its also improved protein content (48 %) and K/Na ratio (95 %) in grain of mung bean under salinity stress.

**Table 3. Combined effect of salt stress and different bio-fertilizers on leaf relative water content of soybean**

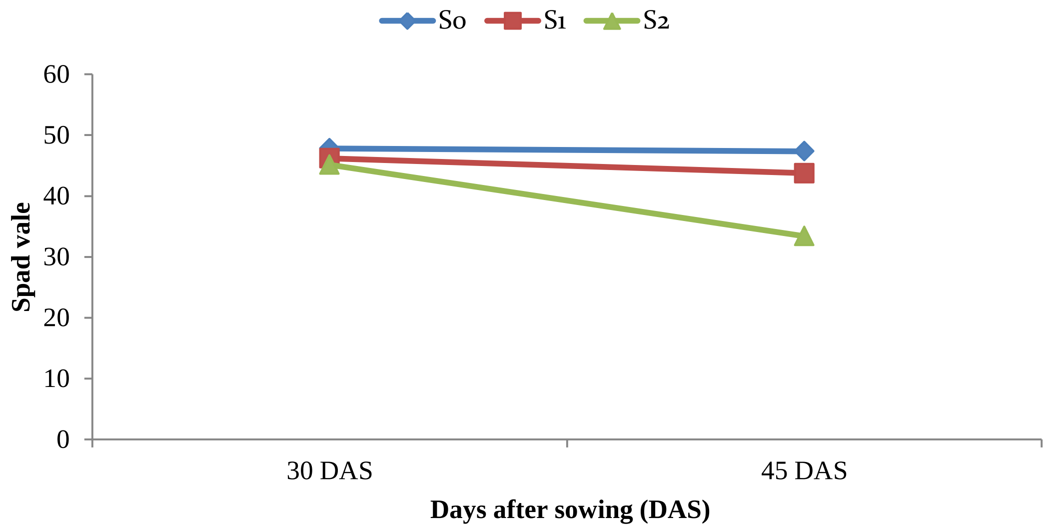
| Treatment Combinations | Leaf relative water content | |
|---|---|---|
| | **30 DAS** | **45 DAS** |
| **$S_0B_0$** | 88.10 bc | 82.79 de |
| **$S_0B_1$** | 88.17 bc | 83.85 cd |
| **$S_0B_2$** | 94.75 a | 88.84 a |
| **$S_0B_3$** | 88.71 bc | 87.69 a |
| **$S_1B_0$** | 82.18 f | 77.88 f |
| **$S_1B_1$** | 88.58 bc | 81.86 e |
| **$S_1B_2$** | 93.63 a | 85.83 b |
| **$S_1B_3$** | 88.87 b | 83.78 cd |
| **$S_2B_0$** | 81.27 f | 63.31 i |
| **$S_2B_1$** | 83.77 e | 69.52 h |
| **$S_2B_2$** | 87.31 cd | 84.33 c |
| **$S_2B_3$** | 86.07 d | 71.59 g |
| **$LSD_{(0.05)}$** | 1.49 | 1.18 |
| **CV(%)** | 1.18 | 1.02 |

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at 0.05 level of probability. Here, $S_0$ = Control, $S_1$= 1800 ppm pot$^{-1}$ and $S_2$ = 3600 ppm pot$^{-1}$.$B_0$= 0 g Rhizobium @ 50 kg seed, $B_1$= BARI RGm-907 @ 25 g/50 kg seed, $B_2$= BARI RGm-922 @ 50 g/50 kg seed and $B_3$= BARI RGm-928 @ 75 g/50 kg seed.

### 4.3.2 SPAD value

#### Effect of salt stress

SPAD value determine leaf chlorophyll concentrations. Chlorophyll is the natural compound present in green plants that gives them their color. It helps plants to absorb energy from the sun as they undergo the process of photosynthesis (Croft *et al.*, 2017). In this experiment, different salt stress condition significantly effect on spad value of soybean at 30 and 45 DAS (Figure 11). The highest spad value of soybean (47.84 and 47.35 %) at 30 and 45 DAS were recorded in control ($S_0$) treatment which was gradually decreasing with increasing salt concentration. The minimum SPAD value (45.15 and 33.41) at 30 and 45 DAS were recorded in $S_2$ treatment and which were (5.62 and 29.44 %) lower comparable to control treatment. Ashraf and Harris (2013) also found similar result which supported the present finding and reported that photosynthetic pigments, chl a and chl b, are greatly affected by different abiotic stresses including salinity. Accumulation of toxic Na+ reduces the content of precursor of chl biosynthesis (such as glutamate and 5-aminolevullinic acid) and thus interrupts chl biosynthesis under saline condition.

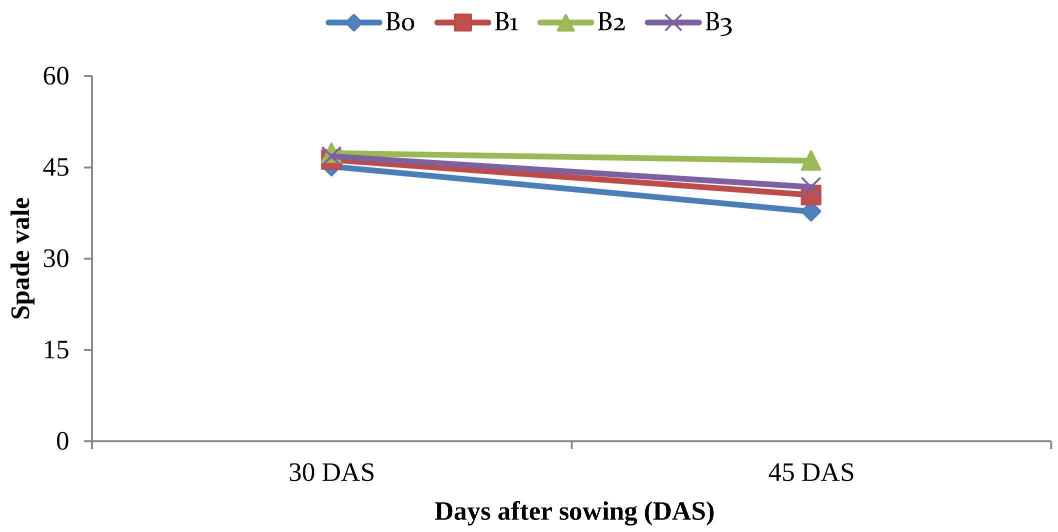



Note: $S_0$ = Control, $S_1$= 1800 ppm NaCl $pot^{-1}$ and $S_2$ = 3600 ppm NaCl $pot^{-1}$.

**Figure 11. Effect of salt stress on sapde value of soybean at different DAS [$LSD_{(0.05)}$ = 0.35 and 0.34 at 30 and 45 DAS].**

**Effect of different bio-fertilizers**

The SPAD value of soybean varied for application of different bio-fertilizers at 30 and 45 DAS (Figure 12). Our results revealed that the highest SPAD value of soybean (47.37 and 46.10 %) at 30 and 45 DAS were recorded in $B_2$ treatment which was (4.69 and 18.18 %) higher comparable to control treatment. Whereas the minimum SPAD value (45.15 and 37.72 %) at 30 and 45 DAS were recorded in $B_0$ treatment. The result obtained from the present study was similar with the findings of Umi and Athaya (2021) and reported that biofertilizer provides more nutrients for the plant through its microbial activity, therefore, the productivity and the required nutrients for photosynthesis were provided result in increasing total leaf chlorophyll content.




Note: $B_0$= 0 g Rhizobium @ 50 kg seed, $B_1$= BARI RGm-907 @ 25 g/50 kg seed, $B_2$= BARI RGm-922 @ 50 g/50 kg seed and $B_3$= BARI RGm-928 @ 75 g/50 kg seed.

**Figure 12. Effect of biofertilizer on sapd value of soybean at different DAS [$LSD_{(0.05)}$ = 0.40 and 0.39 at 30 and 45 DAS].**

**Combined effect of salt stress and bio-fertilizers**

Interaction of salt and biofertilizers significantly influence SPAD value of soybean at 30 and 45 DAS (Table 4). The highest SPAD value of soybean (48.77 and 49.24 %) at 30 and 45 DAS were recorded in $S_0B_2$ treatment combination which was (3.43 and 8.31%) higher comparable to control treatment. Whereas the lowest chlorophyll content (43.20 and 26.06 %) at 30 and 45 DAS were recorded in $S_2B_0$ treatment which was (8.38 and 42.67 %) lower comparable to control treatment.

**Table 4. Combined effect of salt stress and different bio-fertilizers on spad value of soybean at different DAS**

| Treatment Combinations | Spad vale | |
|---|---|---|
| | **30 DAS** | **45 DAS** |
| **$S_0B_0$** | 47.15 cd | 45.46 c |
| **$S_0B_1$** | 47.43 bc | 47.34 b |
| **$S_0B_2$** | 48.77 a | 49.24 a |
| **$S_0B_3$** | 48.01 b | 47.36 b |
| **$S_1B_0$** | 45.09 g | 41.64 e |
| **$S_1B_1$** | 45.83 ef | 42.25 e |
| **$S_1B_2$** | 47.31 c | 46.82 b |
| **$S_1B_3$** | 46.52 de | 44.32 d |
| **$S_2B_0$** | 43.20 h | 26.06 h |
| **$S_2B_1$** | 45.46 fg | 31.72 g |
| **$S_2B_2$** | 46.03 ef | 42.23 e |
| **$S_2B_3$** | 45.91 ef | 33.62 f |
| **$LSD_{(0.05)}$** | 0.69 | 0.68 |
| **CV(%)** | 1.04 | 1.14 |

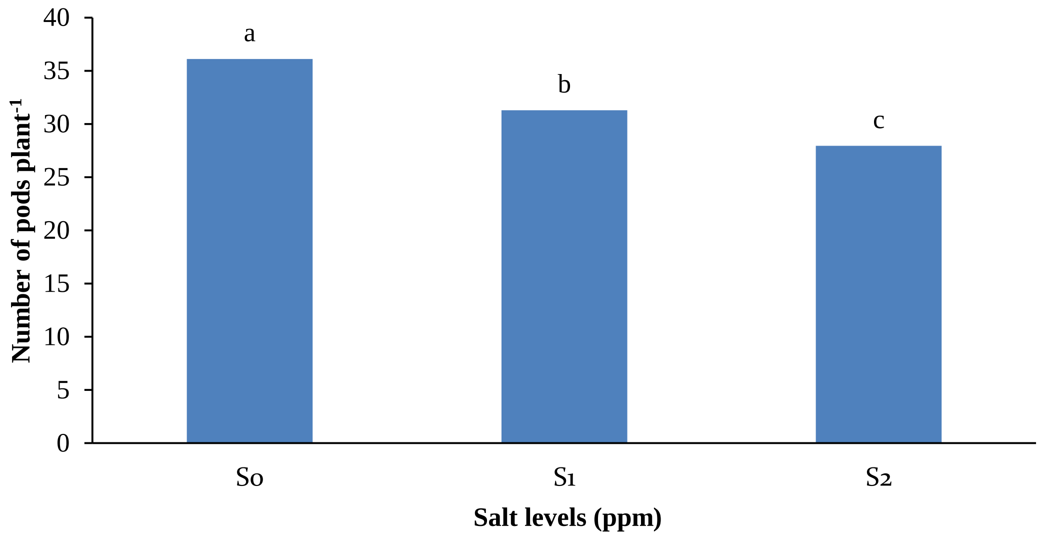
In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at 0.05 level of probability. Here, $S_0$ = Control, $S_1$= 1800 ppm NaCl pot$^{-1}$ and $S_2$ = 3600 ppm NaCl pot$^{-1}$.$B_0$= 0 g Rhizobium @ 50 kg seed, $B_1$= BARI RGm-907 @ 25 g/50 kg seed, $B_2$= BARI RGm-922 @ 50 g/50 kg seed and $B_3$= BARI RGm-928 @ 75 g/50 kg seed.

## 4.4 Yield contributing characters

### 4.4.1 Number of pods plant$^{-1}$

#### Effect of salt stress

The number of pods plant$^{-1}$ of soybean varied significantly under different salt stress condition at harvest (Figure 13). Among all the treatments, the maximum number of pods plant$^{-1}$ (36.12) was recorded in control ($S_0$) treatment and the number of pods plant$^{-1}$ decreased with increasing salinity levels in soybean. The minimum number of pods plant$^{-1}$ (27.95) was recorded in control ($S_2$) treatment which was (22.62 %) lower over control treatment. Data showed that number of pods plant$^{-1}$ significantly reduced in soybean under all salt stress levels. This difference is directly linked with chlorophyll contents (SPAD values), and photosynthesis activities in soybean leaves. The results in a reduction of the C (Carbon) assimilation and biomass production due to osmotic stress and ion imbalance high $Na^+$ and $Cl^-$ in soil and plant tissues under salt stress condition. Kataria *et al.* (2019) reported that the reduction in photosynthesis rate caused low carbon accumulation and led to lower yield. As the salinity increased, the number of pod plant$^{-1}$ remarkably decreased.

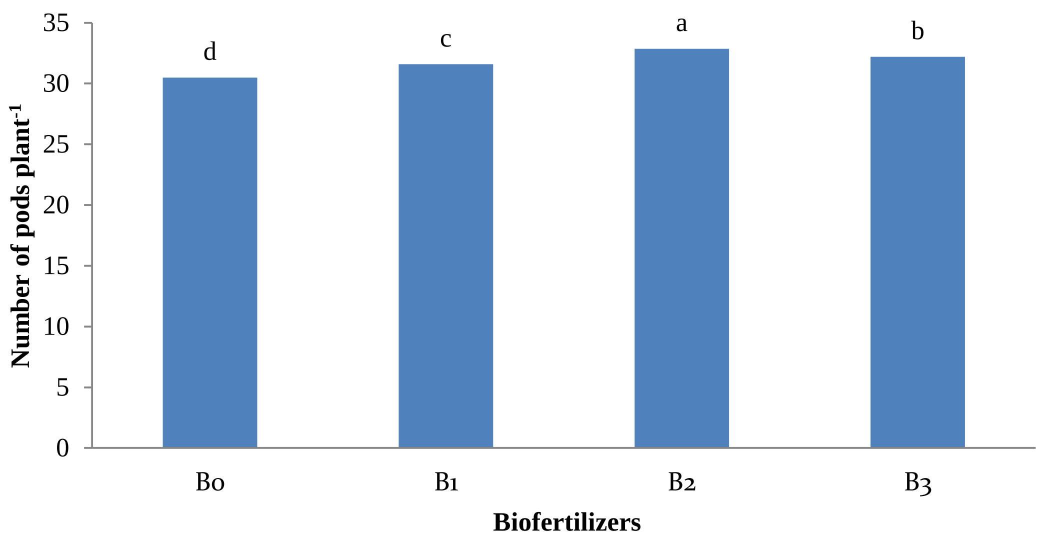



Note: $S_0$ = Control, $S_1$= 1800 ppm NaCl pot$^{-1}$ and $S_2$ = 3600 ppm NaCl pot$^{-1}$.

**Figure 13. Effect of salt stress on number of pods plant$^{-1}$of soybean at harvest [LSD$_{(0.05)}$ = 0.38].**

**Effect of different bio-fertilizers**

Different bio-fertilizers application significantly influenced the number of pods plant$^{-1}$ of soybean at harvest (Figure 14). The present study revealed that the maximum number of pods plant$^{-1}$ of soybean (32.87) was recorded in $B_2$ treatment which was (7.70 %) higher comparable to control treatment. The lowest number of pods plant$^{-1}$ of soybean (30.50) was recorded in $B_0$ treatment. The higher number of pods plant$^{-1}$ of soybean under different biofertilizer treated pot comparable to control one due to biofertilizer contains highly efficient strains of bacteria, fungi, and algae provide plants with biogenous elements, nitrogen, phosphorus and potassium (Govedarica *et* *al*. 2002). Chavan *et al.* (2008) reported that number of pods and other yield attributes were increased with inoculation of seed with PSB.




Note: $B_0$= 0 g Rhizobium @ 50 kg seed, $B_1$= BARI RGm-907 @ 25 g/50 kg seed, $B_2$= BARI RGm-922 @ 50 g/50 kg seed and $B_3$= BARI RGm-928 @ 75 g/50 kg seed.

**Figure 14. Effect of biofertilizer on number of pods plant$^{-1}$of soybean at harvest [LSD$_{(0.05)}$ = 0.43].**

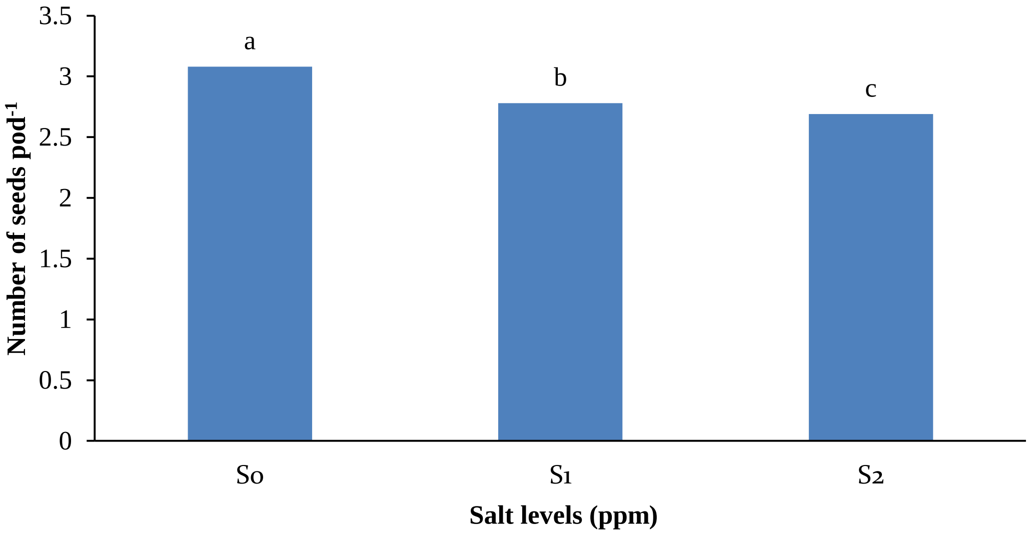
**Combined effect of salt stress and bio-fertilizers**

Combination of salt and bio-fertilizers have significant effect on number of pods plant$^{-1}$ of soybean at harvest (Table 5). The highest number of pods plant$^{-1}$ of soybean (37.20) was recorded in $S_0B_2$ treatment combination which was (5.68 %) higher comparable to control treatment ($S_0B_0$). The lowest number of pods plant$^{-1}$ (26.20) was recorded in $S_2B_0$ treatment combination which was (25.57 %) lower comparable to control treatment combination.

### 4.4.2 Number of seeds pod$^{-1}$

**Effect of salt stress**

Number of seeds pod$^{-1}$ of soybean varied significantly under different salt stress condition at harvest (Figure 15). Among all the treatments, the maximum number of seeds pod$^{-1}$ (3.08) was recorded in control ($S_0$) treatment and the number of seeds pod$^{-1}$ decreased with increasing salinity levels in soybean. The minimum number of seeds pod$^{-1}$ (2.69) was recorded in ($S_2$) treatment which was (12.66 %) lower over control treatment. Plants when exposed to excess salt condition either in soil or in solution culture, they exhibited toxicity symptoms such as: inhibition of seed germination, decrease in plant height, lower number of seeds pod$^{-1}$, seed yield and sometimes leads to death. El-Sabagh *et al.* (2015) noticed that salinity affects yield and yield attributes of soybean. Ashraf and Foolad, (2007) reported that soybean plants exhibits yield reduction when the salinity exceeds 5 dS m$^{-1}$.

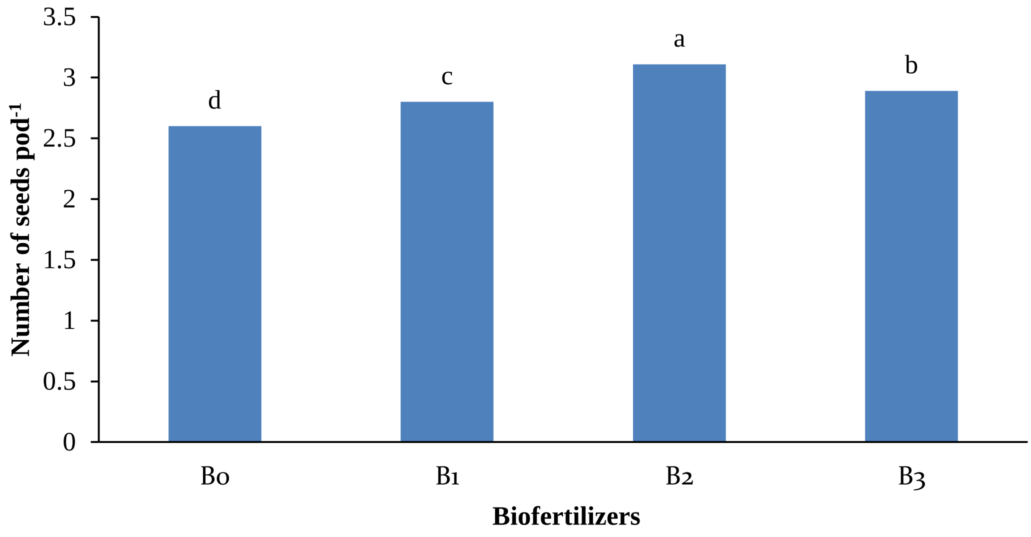



Note: $S_0$ = Control, $S_1$= 1800 ppm NaCl pot$^{-1}$ and $S_2$ = 3600 ppm NaCl pot$^{-1}$.

**Figure 15. Effect of salt stress on number of seeds pod$^{-1}$of soybean at harvest [$LSD_{(0.05)}$ = 0.05].**

**Effect of different bio-fertilizers**

Application of different bio-fertilizers have significant effect on number of seeds pod$^{-1}$ of soybean at harvest (Figure 15). The maximum number of seeds pod$^{-1}$ of soybean (3.11) was recorded in $B_2$ treatment which was (19.62 %) higher comparable to control treatment. The minimum number of pods plant$^{-1}$ of soybean (2.60) was recorded in $B_0$ treatment. Pawar *et al.* (2018) reported that that inoculation of biofertilizers either sole or in combination improves the yield and yield attributes significantly.




Note: $B_0$= 0 g Rhizobium @ 50 kg seed, $B_1$= BARI RGm-907 @ 25 g/50 kg seed, $B_2$= BARI RGm-922 @ 50 g/50 kg seed and $B_3$= BARI RGm-928 @ 75 g/50 kg seed.

**Figure 16. Effect of biofertilizer on number of seeds pod$^{-1}$of soybean at harvest [LSD$_{(0.05)}$ = 0.06].**

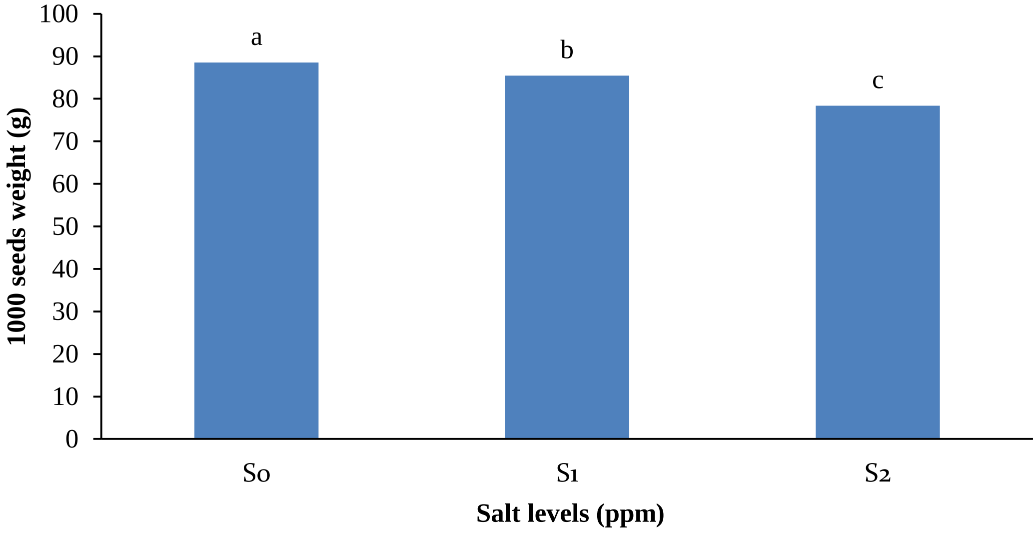
**Combined effect of salt stress and bio-fertilizers**

Combination of salt and bio-fertilizers have significant effect on number of seeds pod$^{-1}$ of soybean at harvest (Table 5). Experimental result revealed that the maximum number of seeds pod$^{-1}$ of soybean (3.33) was recorded in $S_0B_2$ treatment combination which was (11 %) higher comparable to control treatment. The minimum number of seeds pod$^{-1}$ (2.40) was recorded in $S_2B_0$ treatment combination which was (20 %) lower comparable to control treatment combination and it was statistically similar with $S_1B_0$ treatment combination.

**4.4.3 1000 seeds weight**

**Effect of salt stress**

Exposure of salinity have significant influence on 1000 seeds weight of soybean at harvest (Figure 17). The maximum 1000 seeds weight (88.56 g) was recorded in $S_0$ treatment. Whereas the minimum 1000 seeds weight (78.41 g) was recorded in $S_2$ treatment which was (11.46 %) lower over control treatment. The variation of 1000 seeds weight among different treatment due to reason that salt availability in soil can disturb normal functioning of plant metabolism, consequently leading to stunted growth and low crop productivity. According to Hasanuzzaman *et al.* (2016), soybean varieties encountered a significant reduction in quality traits and yield when subjected to salt stress. Baghel *et al.* (2016) reported that the number of pods plant$^{-1}$, the number of seed plant$^{-1}$ and seed weight was decreased under 0-50 mM NaCl-induced salt stress.

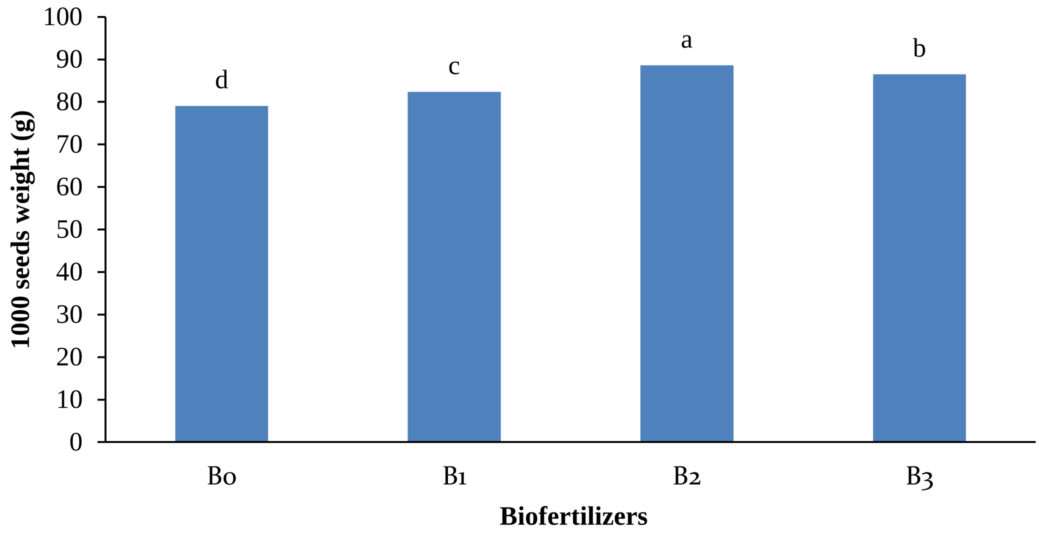



Note: $S_0$ = Control, $S_1$= 1800 ppm NaCl pot$^{-1}$ and $S_2$ = 3600 ppm NaCl pot$^{-1}$.

**Figure 17. Effect of salt stress on 1000 seeds weight of soybean at harvest [$LSD_{(0.05)}$ = 0.64].**

**Effect of different bio-fertilizers**

Application of different bio-fertilizers significantly influenced 1000 seeds weight of soybean at harvest (Figure 18). Experimental result revealed that the maximum 1000 seed weight of soybean (88.61 g) was recorded in $B_2$ treatment which was (12.07 %) higher compared to control treatment. Whereas the minimum number 1000 seed weight of soybean (79.07 g) was recorded in $B_0$ treatment. Pawar *et al.* (2018) reported that that inoculation of biofertilizers either sole or in combination improves the yield and yield attributes significantly.




Note: $B_0$= 0 g Rhizobium @ 50 kg seed, $B_1$= BARI RGm-907 @ 25 g/50 kg seed, $B_2$= BARI RGm-922 @ 50 g/50 kg seed and $B_3$= BARI RGm-928 @ 75 g/50 kg seed.

**Figure 18. Effect of biofertilizer on 1000 seeds weight of soybean at harvest [$LSD_{(0.05)}$ = 0.73].**

**Combined effect of salt stress and bio-fertilizers**

Combined effect of salt stress and bio-fertilizers significantly influenced 1000 seed weight of soybean at harvest (Table 5). Frpm the experimental result it is found that the maximum 1000 seed weight of soybean (93.50 g) was recorded in $S_0B_2$ treatment combination which was (12.38 %) higher comparable to control treatment. Whereas the minimum number of seeds pod$^{-1}$ (73.00) was recorded in $S_2B_0$ treatment combination which was (12.26 %) lower compared to control treatment combination.

**Table 5. Combined effect of salt stress and different bio-fertilizers on number of pods plant$^{-1}$, seeds pod$^{-1}$ and 1000 seeds weight (g) of soybean**

| Treatment Combinations | Pods plant (no.) | Seeds pod$^{-1}$ (no.) | 1000 seeds weight (g) |
|---|---|---|---|
| **$S_0B_0$** | 35.20 c | 3.00 b | 83.20 d |
| **$S_0B_1$** | 36.08 b | 3.00 b | 86.50 c |
| **$S_0B_2$** | 37.20 a | 3.33 a | 93.50 a |
| **$S_0B_3$** | 36.00 b | 3.00 b | 91.10 b |
| **$S_1B_0$** | 30.10 f | 2.40 d | 81.00 e |
| **$S_1B_1$** | 31.20 e | 2.70 c | 83.10 d |
| **$S_1B_2$** | 32.20 d | 3.00 b | 90.20 b |
| **$S_1B_3$** | 31.70 de | 3.00 b | 87.50 c |
| **$S_2B_0$** | 26.20 i | 2.40 d | 73.00 g |
| **$S_2B_1$** | 27.50 h | 2.70 c | 77.60 f |
| **$S_2B_2$** | 29.20 g | 3.00 b | 82.13 de |
| **$S_2B_3$** | 28.90 g | 2.68 c | 80.90 e |
| **$LSD_{(0.05)}$** | 0.76 | 0.10 | 1.27 |
| **CV(%)** | 1.66 | 2.49 | 1.05 |

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at 0.05 level of probability. Here, $S_0$ = Control, $S_1$= 1800 ppm NaCl pot$^{-1}$ and $S_2$ = 3600 ppm NaCl pot$^{-1}$.$B_0$= 0 g Rhizobium @ 50 kg seed, $B_1$= BARI RGm-907 @ 25 g/50 kg seed, $B_2$= BARI RGm-922 @ 50 g/50 kg seed and $B_3$= BARI RGm-928 @ 75 g/50 kg seed.

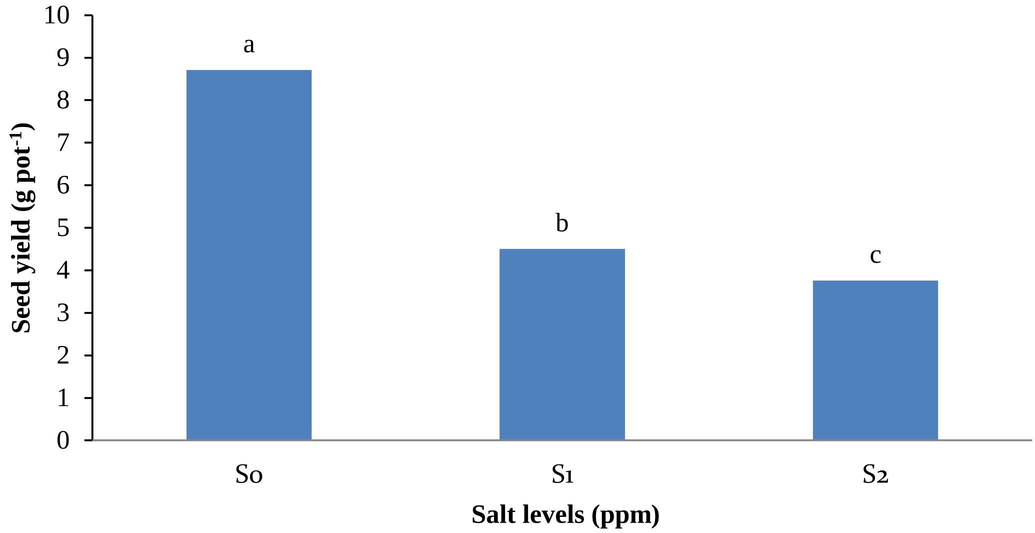
## 4.5 Yield characters

### 4.5.1 Seed yield

#### Effect of salt stress

Different level of salt stress condition significantly affect seed yield of soybean (Figure 19). The highest seed yield (8.71 g pot$^{-1}$) was recorded in $S_0$ treatment whereas the lowest seed yield (3.76 g pot$^{-1}$) was recorded in $S_2$ treatment which was (56.81 %) lower over control treatment. The reduction of seed yield with increasing salt concentration was due inhabitation of photosynthesis rate by salinity in plants which ultimately impact on plant growth, yield and yield contributing attributes.

According to Hasanuzzaman *et al.* (2016), soybean varieties encountered a significant reduction in quality traits and yield when subjected to salt stress. Ashraf and Foolad, (2007) also reported that soybean plants exhibits yield reduction when the salinity exceeds 5 dS $m^{-1}$.

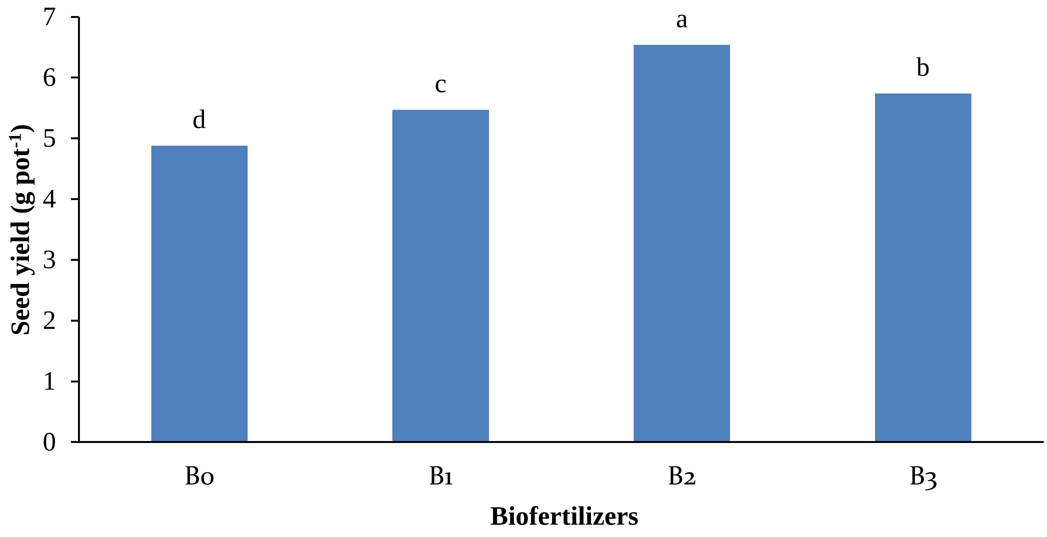



Note: $S_0$ = Control, $S_1$= 1800 ppm NaCl $pot^{-1}$ and $S_2$ = 3600 ppm NaCl $pot^{-1}$.

**Figure 19. Effect of salt stress on seed yield $pot^{-1}$ of soybean at harvest [$LSD_{(0.05)}$ = 0.05].**

**Effect of different bio-fertilizers**

Application of bio-fertilizers significantly influenced seed yield of soybean (Figure 20). Experiment result showed that the maximum seed yield (6.54 g $pot^{-1}$) was recorded in $B_2$ treatment which was (25.38 %) higher over control treatment. Whereas the minimum seed yield (4.88 g $pot^{-1}$) was recorded in $B_0$ treatment. Biofertilizers keep the soil environment rich in all kinds of macro and micro nutrients via nitrogen fixation, phosphate and potassium solubilisation or mineralization, release of plant growth regulating substances, production of antibiotics and biodegradation of organic matter in the soil (Sinha *et al*., 2014). Biofertilizers, when applied as seed or soil inoculants, multiply and participate in nutrient cycling and leads to crop productivity (Adesemoye *et al*., 2009). Chavan *et al.* (2008) also reported that yield and other yield attributes were increased with inoculation of seed with Phosphate Solubilizing Biofertilizers (PSB).




Note: $B_0$= 0 g Rhizobium @ 50 kg seed, $B_1$= BARI RGm-907 @ 25 g/50 kg seed, $B_2$= BARI RGm-922 @ 50 g/50 kg seed and $B_3$= BARI RGm-928 @ 75 g/50 kg seed.

**Figure 20. Effect of biofertilizer on seed yield pot$^{-1}$ of soybean at harvest [$LSD_{(0.05)}$ = 0.06].**

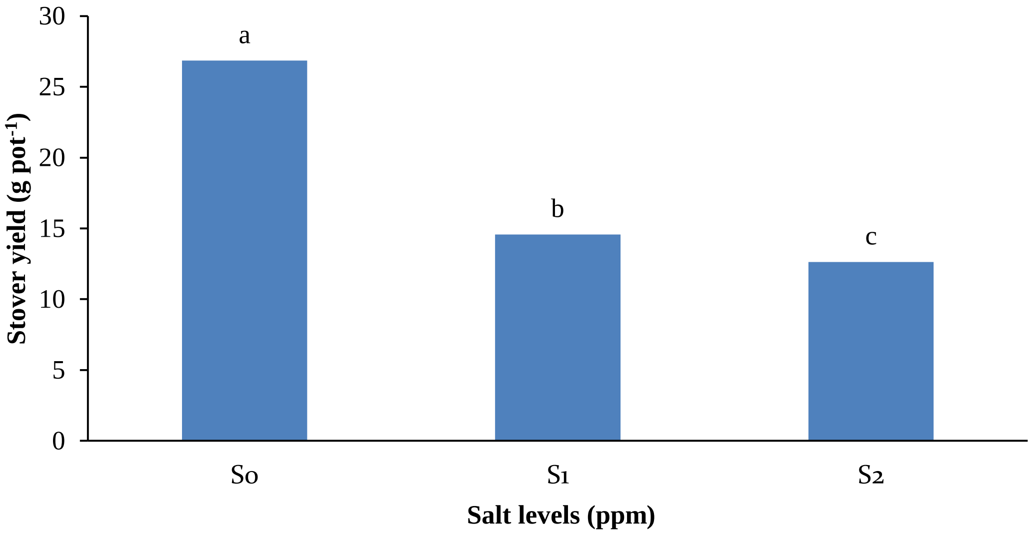
**Combined effect of salt stress and bio-fertilizers**

Interaction of salt and bio-fertilizers have significant effect on seed yield of soybean at harvest (Table 6). Experimental result revealed that the maximum seed yield of soybean (9.77 g pot$^{-1}$) was recorded in $S_0B_2$ treatment combination which was (16.48 %) higher comparable to control treatment. The lowest seed yield of soybean (2.82 g pot$^{-1}$) was recorded in $S_2B_0$ treatment combination which was (65.44 %) lower comparable to control treatment combination. Nasef *et al.* (2004) reported that the application of N at different levels and inoculation with biofertilizer led to an increase in total porasity improves soil aggregation and possible moving salt soil under irrigation water. Also, the plants tolerated the lower salinity levels especially when combined with biofertilizer application where it counteracted the reduction of salinity on vegetative growth, pigment contents, total sugars and some minerals.

### 4.5.2 Stover yield

**Effect of salt stress**

Application of different level of salt significantly influenced stover yield of soybean at harvest (Figure 21). Experimental result revealed that the highest stover yield (26.85 g pot$^{-1}$) was recorded in $S_0$ treatment whereas the lowest stover yield (12.64 g pot$^{-1}$) was recorded in $S_2$ treatment which was (52.92 %) lower over control treatment. Higher concentration of salt is toxic to plant and its can disturb normal functioning of plant metabolism, consequently leading to stunted growth and low crop productivity. Hamayun *et al.* (2017) also found similar result which supported the present finding and reported that soybean plant fresh weight and dry weight decreasing trend under salt stress, in comparison to control.

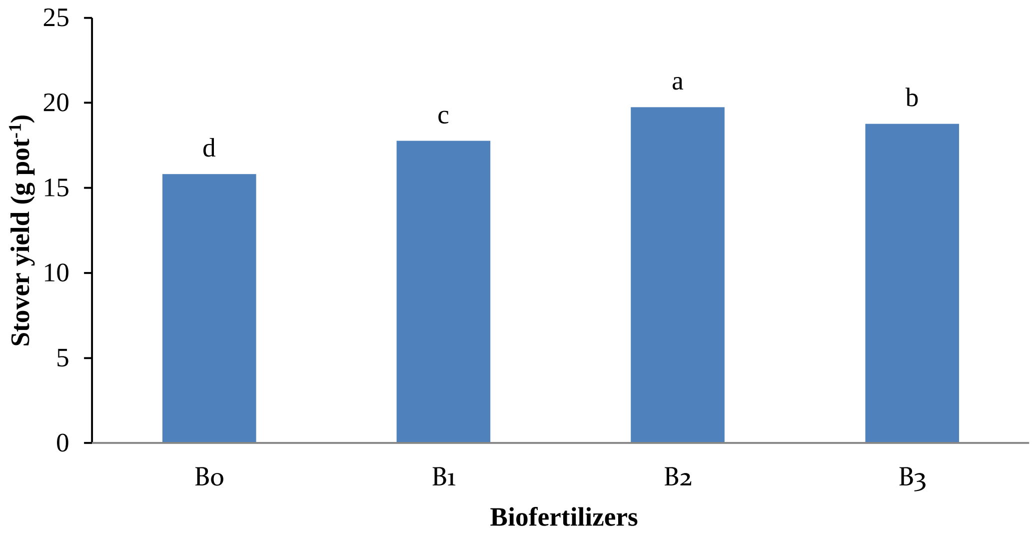



Note: $S_0$ = Control, $S_1$= 1800 ppm NaCl pot$^{-1}$ and $S_2$ = 3600 ppm NaCl pot$^{-1}$.

**Figure 21. Effect of salt stress on stover yield pot$^{-1}$ of soybean at harvest [$LSD_{(0.05)}$ = 0.33].**

**Effect of different bio-fertilizers**

Different types of bio-fertilizers application significantly influenced stover yield of soybean (Figure 22). The highest stover yield (19.75 g pot$^{-1}$) was recorded in $B_2$ treatment which was (19.94 %) higher over control treatment. Whereas the minimum stover yield (15.81 g pot$^{-1}$) was recorded in $B_0$ treatment. Gupta *et al.* (2006) reported that seed and straw yield were influenced by inoculation of PSB along with phosphatic fertilizers.




Note: $B_0$= 0 g Rhizobium @ 50 kg seed, $B_1$= BARI RGm-907 @ 25 g/50 kg seed, $B_2$= BARI RGm-922 @ 50 g/50 kg seed and $B_3$= BARI RGm-928 @ 75 g/50 kg seed.

**Figure 22. Effect of biofertilizer on stover yield pot$^{-1}$ of soybean at harvest [LSD$_{(0.05)}$ = 0.39].**

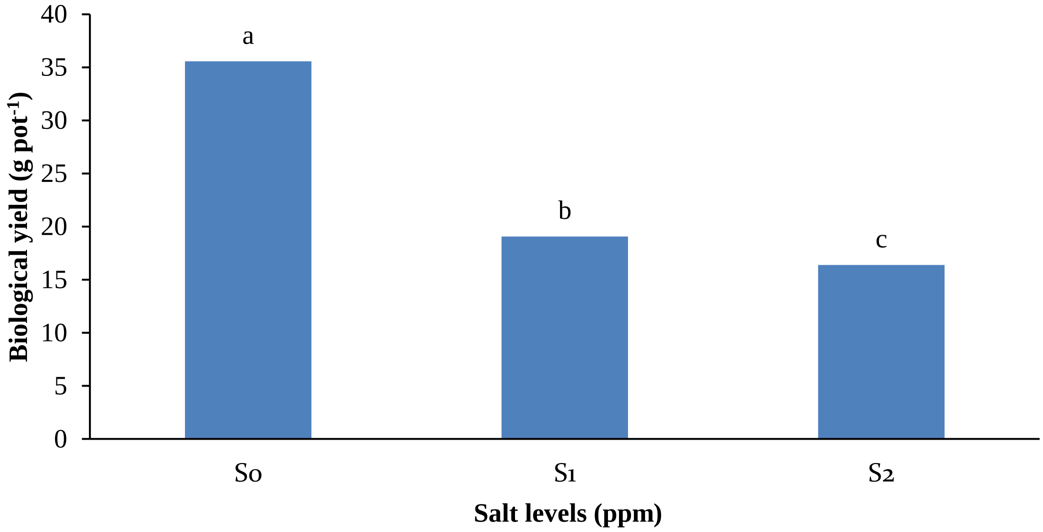
**Combined effect of salt stress and bio-fertilizers**

Different levels of salt stress and along with bio-fertilizers application significantly influenced stover yield of soybean at harvest (Table 6). Experimental results revealed that the maximum stover yield of soybean (28.63 g pot$^{-1}$) was recorded in $S_0B_2$ treatment combination which was (16.86 %) higher compared to control treatment $S_0B_0$. Whereas the minimum seed yield of soybean (10.00 g pot$^{-1}$) was recorded in $S_2B_0$ treatment combination which was (59.18 %) lower compared to control treatment combination. Aamir *et al.* (2013) reported that single and combined inoculation of *Rhizobium* and PGPR improve plant growth under salinity stress condition.

### 4.5.3 Biological yield

**Effect of salt stress**

External application of salt significantly influenced biological yield of soybean at harvest (Figure 23). The highest biological yield (35.56 g pot$^{-1}$) was recorded in $S_0$ treatment whereas the lowest biological yield (16.40 g pot$^{-1}$) was recorded in $S_2$ treatment which was (53.88 %) lower over control treatment.

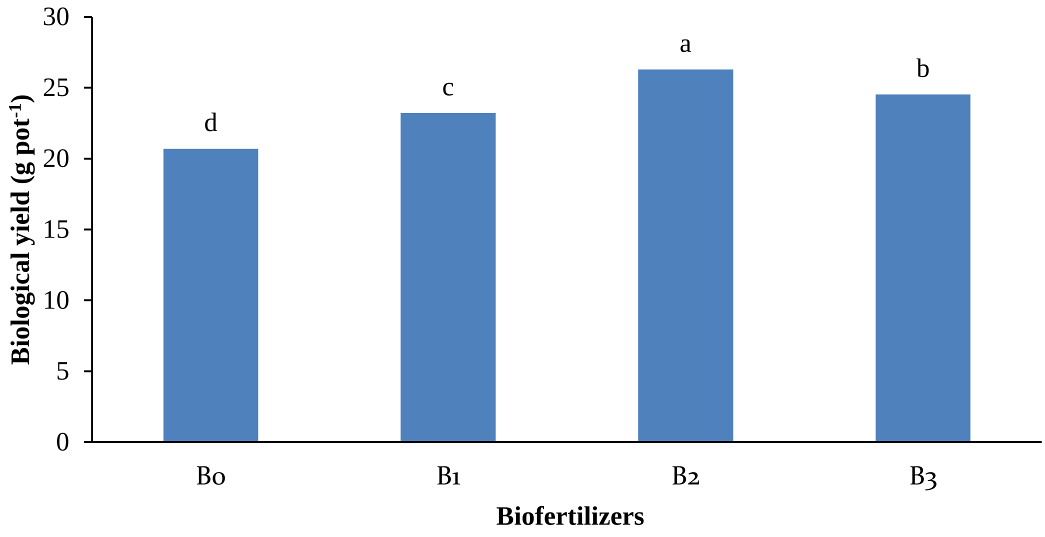



Note: $S_0$ = Control, $S_1$= 1800 ppm NaCl pot$^{-1}$ and $S_2$ = 3600 ppm NaCl pot$^{-1}$.

**Figure 23. Effect of salt stress on biological yield pot$^{-1}$ of soybean at harvest [$LSD_{(0.05)}$ = 0.43].**

**Effect of different bio-fertilizers**

Biological yield of soybean varied for the variation of biofertilizer application. (Figure 24). The highest biological yield (26.29 g pot$^{-1}$) was recorded in $B_2$ treatment which was (27.00 %) higher over control treatment. Whereas the minimum biological yield (20.70 g pot$^{-1}$) was recorded in $B_0$ treatment.




Note: $B_0$= 0 g Rhizobium @ 50 kg seed, $B_1$= BARI RGm-907 @ 25 g/50 kg seed, $B_2$= BARI RGm-922 @ 50 g/50 kg seed and $B_3$= BARI RGm-928 @ 75 g/50 kg seed.

**Figure 24. Effect of biofertilizer on biological yield pot$^{-1}$ of soybean at harvest [LSD$_{(0.05)}$ = 0.50].**

**Combined effect of salt stress and bio-fertilizers**

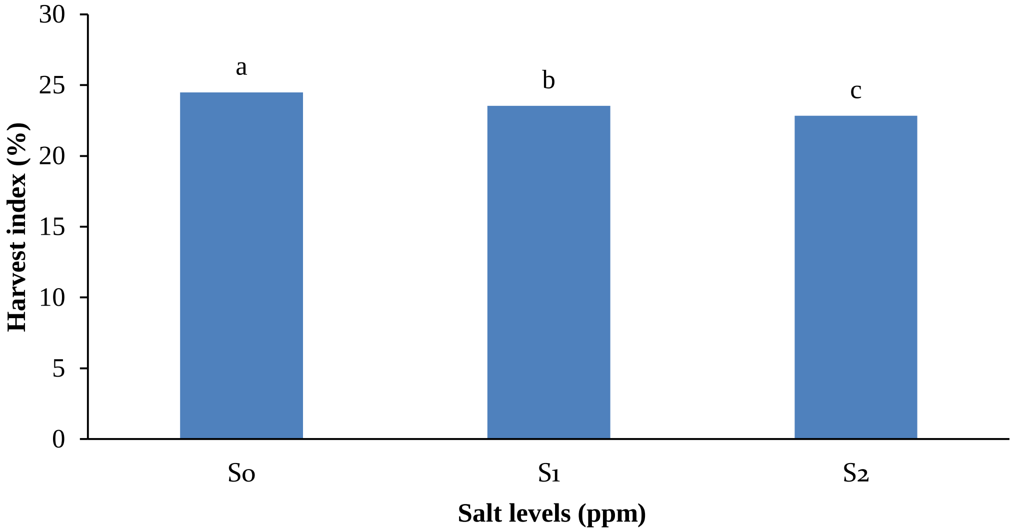
Combination of salt stress and bio-fertilizers significantly influenced biological yield of soybean (Table 6). The present study revealed that the highest biological yield of soybean (38.40 g pot$^{-1}$) was recorded in $S_0B_2$ treatment combination which was (17.58 %) higher compared to control treatment. Whereas the lowest biological yield of soybean (12.82 g pot$^{-1}$) was recorded in $S_2B_0$ treatment combination which was (60.75 %) lower compared to control treatment combination.

### 4.5.4 Harvest index

**Effect of salt stress**

Application of different level of salt significantly affect harvest index of soybean at harvest (Figure 25). The highest harvest index (24.49 %) was recorded in $S_0$ treatment whereas the minimum harvest index (22.83 %) was recorded in $S_2$ treatment which was (6.78 %) lower over control treatment. The differences of harvest index at different salt level due to reason that increasing the level of salt decreased yield contributing characters of plant which ultimately impact on harvest index. Ashraf and

Foolad, (2007) reported that soybean plants exhibits yield reduction when the salinity exceeds 5 dS $m^{-1}$.

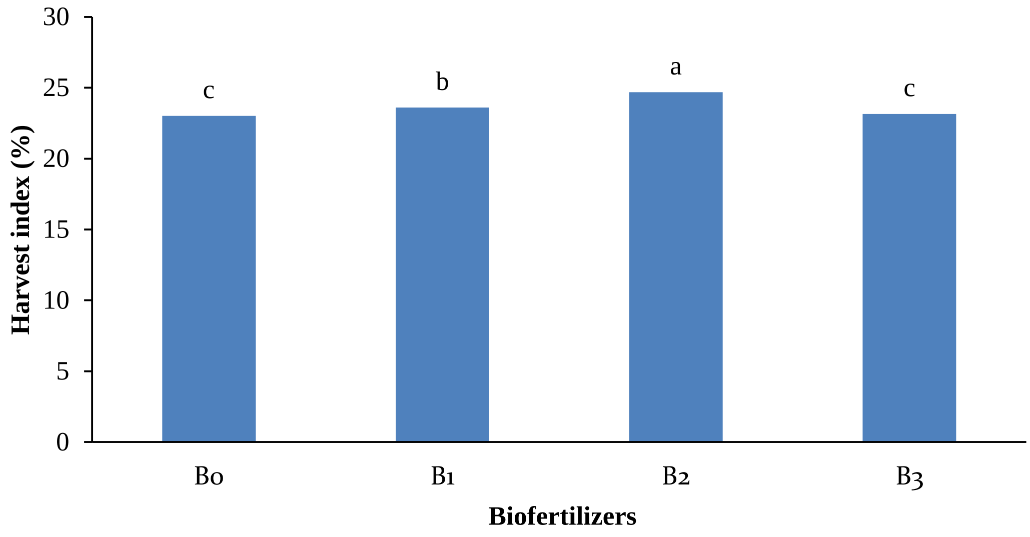



Note: $S_0$ = Control, $S_1$= 1800 ppm NaCl $pot^{-1}$ and $S_2$ = 3600 ppm NaCl $pot^{-1}$.

**Figure 25. Effect of salt stress on harvest index of soybean at harvest [$LSD_{(0.05)}$ = 0.19].**

**Effect of different bio-fertilizers**

Different type of bio-fertilizers application significantly influenced harvest index of soybean at harvest (Figure 26). Experimental result showed that the maximum harvest index (24.69 %) was recorded in $B_2$ treatment which was (7.25 %) higher over control treatment. Whereas the minimum harvest index (23.02 %) was recorded in $B_0$ treatment which was statistically similar with $B_3$ treatment. Sonone *et al.* (2007) observed that the highest harvest index of soybean was obtained by application of PSB and Rhizobium. Rhizobium fixes atmospheric nitrogen available to plant and PSB (Phosphate Solubilizing Biofertilizers) which converts insoluble phosphate into soluble forms result in proper nutrients absorption as well as enhancing the production and translocation of the dry matter content from source to sink which impact on harvest index as its depends on grain yield and biological yield of the plant.




Note: $B_0$= 0 g Rhizobium @ 50 kg seed, $B_1$= BARI RGm-907 @ 25 g/50 kg seed, $B_2$= BARI RGm-922 @ 50 g/50 kg seed and $B_3$= BARI RGm-928 @ 75 g/50 kg seed.

**Figure 26. Effect of biofertilizer on harvest index of soybean at harvest [$LSD_{(0.05)}$ = 0.22].**

**Combined effect of salt stress and bio-fertilizers**

Application of different levels of salt stress and along with bio-fertilizers significantly influenced harvest index of soybean at harvest (Table 6). Experimental result revealed that the maximum harvest index of soybean (25.45 %) was recorded in $S_0B_2$ treatment combination which was (1.88 %) higher comparable to control treatment. Whereas the minimum harvest index of soybean (21.99 %) was recorded in $S_2B_0$ treatment combination which was (11.97 %) lower comparable to control treatment combination and it was statistically similar with $S_1B_0$ (22.10 %) and $S_2B_3$ (22.13 %) treatment combination.

**Table 6. Combined effect of salt stress and different bio-fertilizers on seed yield, stover yield, biological yield (g $pot^{-1}$) and harvest index (%) of soybean at harvest**

| Treatment Combinations | Seed yield (g $pot^{-1}$) | Stover yield (g $pot^{-1}$) | Biological yield (g $pot^{-1}$) | Harvest index (%) |
|---|---|---|---|---|
| $S_0B_0$ | 8.16 c | 24.50 c | 32.66 c | 24.98 b |
| $S_0B_1$ | 8.25 c | 27.13 b | 35.38 b | 23.32 e |
| $S_0B_2$ | 9.77 a | 28.63 a | 38.40 a | 25.45 a |
| $S_0B_3$ | 8.67 b | 27.13 b | 35.80 b | 24.22 d |
| $S_1B_0$ | 3.67 h | 12.94 g | 16.61 gh | 22.10 f |
| $S_1B_1$ | 4.45 f | 13.89 f | 18.34 f | 24.27 cd |
| $S_1B_2$ | 5.14 d | 15.75 d | 20.89 d | 24.61 bc |
| $S_1B_3$ | 4.74 e | 15.75 d | 20.49 d | 23.13 e |
| $S_2B_0$ | 2.82 i | 10.00 i | 12.82 i | 21.99 f |
| $S_2B_1$ | 3.70 h | 12.25 h | 15.95 h | 23.20 e |
| $S_2B_2$ | 4.70 e | 14.88 e | 19.58 e | 24.01 d |
| $S_2B_3$ | 3.82 g | 13.44 fg | 17.26 g | 22.13 f |
| $LSD_{(0.05)}$ | 0.11 | 0.67 | 0.86 | 0.39 |
| CV(%) | 1.33 | 2.57 | 2.53 | 1.14 |

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at 0.05 level of probability. Here, $S_0$ = Control, $S_1$= 1800 ppm NaCl $pot^{-1}$ and $S_2$ = 3600 ppm NaCl $pot^{-1}$.$B_0$= 0 g Rhizobium @ 50 kg seed, $B_1$= BARI RGm-907 @ 25 g/50 kg seed, $B_2$= BARI RGm-922 @ 50 g/50 kg seed and $B_3$= BARI RGm-928 @ 75 g/50 kg seed.

# CHAPTER V

# SUMMARY AND CONCLUSION

## 5.1 Summary

A pot experiment was conducted at Sher-e-Bangla Agricultural University, Dhaka during September to November in 2020, to study the amelioration of salt stress in soybean by microbial biofertilizer. The experiment consisted of two factors, and followed Completely Random Design (CRD) with four replications. Factor A: Three levels of salt stress *viz;* $S_0$ = 0 ppm pot$^{-1}$ (Control), $S_1$= 1800 ppm NaCl pot$^{-1}$ and $S_2$ = 3600 ppm NaCl pot$^{-1}$ and Factor B: Four levels of biofertilizer application, *viz;* $B_0$= 0 g Rhizobium @ 50 kg seed, $B_1$= BARI RGm-907 @ 25 g/50 kg seed, $B_2$= BARI RGm-922 @ 50 g/50 kg seed and $B_3$= BARI RGm-928 @ 75 g/50 kg seed.

In case of different salt stress condition, plant growth deceasing with increasing salt level. The minimum plant height (24.24, 46.65, 60.38, 57.45 and 56.87 cm) at 15 DAS, 30, 45, 60 DAS and at harvest respectively were recorded in $S_2$ (3600 ppm NaCl pot$^{-1}$) treatment. In similar way, the minimum number of leaves plant$^{-1}$, leaf relative water content and spad value were recorded in $S_2$ (3600 ppm NaCl pot$^{-1}$) treatment.

Exposure of salt greatly reduced the yield and yield contributing parameters of soybean. The minimum number of number of pods plant$^{-1}$ (27.95), seeds pod$^{-1}$ (2.69), 1000 seeds weight (78.41 g), seed yield (3.76 g pot$^{-1}$), stover yield (12.64 g pot$^{-1}$), biological yield (16.40 g pot$^{-1}$) and harvest index (22.83 %) were recorded in $S_2$ treatment.

Application of biofertilizer helps to develop plant growth. maximum plant height (25.29, 52.02, 67.70, 71.18 and 73.48 cm) at 15, 30, 45, 60 DAS and at harvest, respectively were recorded in $B_2$ (BARI RGm-922 @ 50 g/50 kg seed) treatment. In similar way, the maximum number of leaves plant$^{-1}$, leaf relative water content and spad value were recorded in $B_2$ (BARI RGm-922 @ 50 g/50 kg seed) treatment.

All biofertilizers don't have same ability to contribute to yield development of soybean plant. In this experiment $B_2$ (BARI RGm-922 @ 50 g/50 kg seed) treatment played a major role for the development of yield of soybean. The maximum number of pods plant$^{-1}$, seeds pod$^{-1}$ (3.11), 1000 seeds weight (88.61 g), seed yield (6.54 g pot$^{-1}$), stover yield (19.75 g pot$^{-1}$), biological yield (35.56 g pot$^{-1}$) and harvest index (24.69 %) were recorded in $B_2$ (BARI RGm-922 @ 50 g/50 kg seed) treatment.

In case of combined effect, the highest plant growth and development was seen with the absence of salt stress condition along with the application BARI RGm-922 @ 50 g/50 kg seed as biofertilizer. The minimum plant height (22.44, 42.31, 57.72 and 47.94 cm) at 15 DAS, 30 DAS, 45 DAS and at harvest, respectively were recorded in $S_2B_0$ treatment combination. In similar way, the minimum number of leaves plant$^{-1}$, leaf relative water content and spad value were recorded in $S_2B_0$ treatment combination.

The minimum number of number of pods plant$^{-1}$ (26.20), seeds pod$^{-1}$ (2.40), 1000 seeds weight (73.00 g), seed yield (2.82 g pot$^{-1}$), stover yield (10.00 g pot$^{-1}$), biological yield (12.82 g pot$^{-1}$) and harvest index (21.99 %) were recorded in $S_2B_0$ treatment.

## 5.2 Conclusion

Salinity inhibits soybean development across the whole life-cycle, which finally leads to decreased yields. However, plants follow different and organized way to minimize the toxic effect of this stress at some certain level. So possible ways to minimize this toxic effect according to this experiment is concluded here-

- Exposure of salt decreased plant growth and yield of soybean.
- In terms of growth parameter, BARI RGm-922 (@ 50 g/50 kg seed) as biofertilizer played an excellent role to overcome and help soybean crop to tolerate the toxicity of salt stress.
- In case of combined effect, the plant growth and development was maximum with the absence of salt stress condition along with the application of BARI RGm-922 (@ 50 g/50 kg seed) as biofertilizer.

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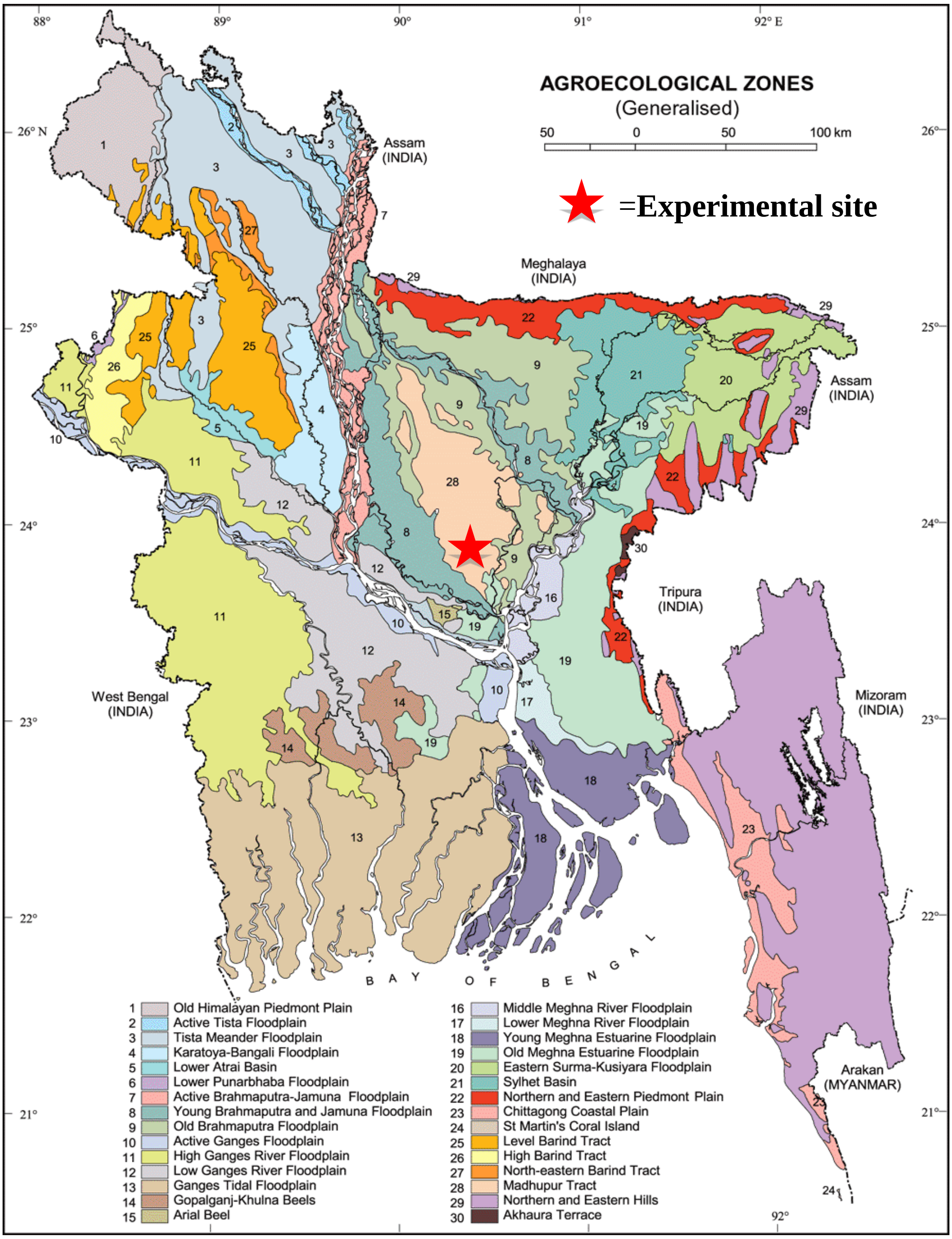
# APPENDICES

Appendix I. Map showing the experimental site under study



Appendix II. Monthly meteorological information during the period from September-2020 to November 2020.

| Year | Month | Air temperature ($^0C$) | | Relative humidity (%) | Total rainfall (mm) |
|---|---|---|---|---|---|
| | | Maximum | Minimum | | |
| 2020 | September | 23.9 | 78 | 55 | 33.2 |
| | October | 23.9 | 76 | 52 | 31.2 |
| | November | 29.6 | 19.8 | 53 | 00 |

(Source: Metrological Centre, Agargaon, Dhaka (Climate Division)

Appendix III. Analysis of variance of the data of plant height soybean at different DAS

| **Source** | **DF** | Mean square of plant height at | | | |
|---|---|---|---|---|---|
| | | 15 DAS | 30 DAS | 45 DAS | At harvest |
| Replication | 3 | 9.5008 | 52.020 | 46.215 | 10.06 |
| Salt level (S) | 2 | 0.1314$^{Ns}$ | 117.003** | 228.881** | 1161.44** |
| Biofertilizers (B) | 3 | 17.7576 | 109.539** | 71.774** | 235.16** |
| S × B | 6 | 0.0750 $^{Ns}$ | 7.233** | 5.005** | 30.02** |
| Error | 33 | 0.3278 | 1.407 | 1.130 | 0.49 |
| Total | 47 | | | | |

Ns: Non significant

**: Significant at 0.01 level of probability

*: Significant at 0.05 level of probability

Appendix IV. Analysis of variance of the data of number of leaves plant$^{-1}$,leaf relative water content of soybean at different DAS

| Source | DF | Mean square of | | |
|---|---|---|---|---|
| | | Number of leaves | Leaf relative water content | |
| | | 30 DAS | 30 DAS | 45 DAS |
| Replication | 3 | 0.0072 | 3.926 | 12.787 |
| Salt level (S) | 2 | 21.9406** | 119.395** | 800.534** |
| Biofertilizers (B) | 3 | 0.5300** | 132.675** | 288.519** |
| S × B | 6 | 0.0325** | 12.769** | 51.554** |
| Error | 33 | 0.0054 | 1.074 | 0.673 |
| Total | 47 | | | |

Ns: Non significant

**: Significant at 0.01 level of probability

*: Significant at 0.05 level of probability

Appendix V. Analysis of variance of the data of spad value of soybean at different DAS

| Source | DF | Mean square of | |
|---|---|---|---|
| | | 45 DAS | At harvest |
| Replication | 3 | 12.2207 | 8.083 |
| Salt level (S) | 2 | 29.4487** | 838.341** |
| Biofertilizers (B) | 3 | 10.8319** | 146.539** |
| S × B | 6 | 0.9117** | 32.329** |
| Error | 33 | 0.2319 | 0.224 |
| Total | 47 | | |

Ns: Non significant

**: Significant at 0.01 level of probability

*: Significant at 0.05 level of probability

Appendix VI. Analysis of variance of the data of number of pods $plant^{-1}$, seeds $pod^{-1}$ and 1000 seeds weight of soybean

| **Source** | **DF** | Mean square of | | |
|---|---|---|---|---|
| | | Pods $plant^{-1}$ | Seeds $pod^{-1}$ | 1000 seeds weight |
| Replication | 3 | 0.267 | 0.00688 | 0.056 |
| Salt level (S) | 2 | 269.790** | 0.67276** | 433.975** |
| Biofertilizers (B) | 3 | 12.123** | 0.53734** | 217.267** |
| S × B | 6 | 0.725** | 0.07109 | 1.953* |
| Error | 33 | 0.279 | 0.00506 | 0.783 |
| Total | 47 | | | |

Ns: Non significant

**: Significant at 0.01 level of probability

*: Significant at 0.05 level of probability

Appendix VII. Analysis of variance of the data of number of seed yield, stover yield, biological yield and harvest index

| **Source** | **DF** | Mean square of | | | |
|---|---|---|---|---|---|
| | | Seed yield | Stover yield | Biological yield | Harvest index |
| Replication | 3 | 5.940 | 8.723 | 4.45 | 0.9085 |
| Salt level (S) | 2 | 114.187** | 949.269** | 1721.87** | 11.1196** |
| Biofertilizers (B) | 3 | 5.664** | 34.042** | 66.48** | 6.8521** |
| S × B | 6 | 0.216** | 1.332** | 1.66** | 2.7388** |
| Error | 33 | 0.006 | 0.215 | 0.36 | 0.0728 |
| Total | 47 | | | | |

Ns: Non significant

**: Significant at 0.01 level of probability

*: Significant at 0.05 level of probability

## PLATES


Plate 1. Picture showing soybean seedling germination at different salinity level


Plate 2. Effect of different levels on salinity on soybean plant


Plate 3. Picture showing combined effect of different salinity level along with biofertilizer application on soybean plant