IMPROVING HEALTH OF SALT AFFECTED SOILS THROUGH MICROBIAL FORMULATIONS

By

DIVYA CHADHA
(J-18-M-585)

Thesis submitted to Faculty of Postgraduate Studies in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE IN AGRICULTURE

(SOIL SCIENCE AND AGRICULTURE CHEMISTRY)

Division of Soil Science and Agricultural Chemistry
Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu
Main Campus, Chatha, Jammu-180009
2020

CERTIFICATE – I

This is to certify that the thesis entitled **"IMPROVING HEALTH OF SALT AFFECTED SOILS THROUGH MICROBIAL FORMULATIONS"** submitted in partial fulfillment of the requirements for the degree of **Master of science in Agriculture (Soil Science and Agriculture Chemistry)** to the Faculty of Post-Graduate Studies, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu is a record of bonafide research carried out by **Miss Divya Chadha,** Registration No. **J-18-M-585,** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma. It is further certified that help and assistance received during the course of thesis investigation have been duly acknowledged.

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ABSTRACT

Title of the thesis	:	**IMPROVING HEALTH OF SALT AFFECTED SOILS THROUGH MICROBIAL FORMULATIONS**
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ABSTRACT

A pot experiment was conducted during Kharif season of 2019, at the Division of Soil Science and Agriculture Chemistry to study the efficacy of halophilic biofertilizers i.e. Halo-Azo, Halo-Phosphate Solubilizing Bacteria (Halo-PSB), Halo-Zinc and Halo-Mix on the soil health as well as growth and productivity of paddy (PR-113). Regular biofertilizers are ineffective under sodic soils owing to the inability of microbes to tolerate salt stress. It was hypothised that application of halophilic (salt/sodium tolerant) microbes can be beneficial for soil health and crop productivity in sodic soils. Application of these bioformulations showed significant decrease in pH, electrical conductivity and sodium content in sodic soil. Organic carbon, available nitrogen, available phosphorus, available potassium and available zinc values were increased upon application of bioformulations in sodic soil over uninoculated, respectively. Biological parameters such as, microbial biomass carbon, dehydrogenase activity, alkaline phosphatase activity and bacterial count were also increased significantly in sodic soil over corresponding control values. However, microbial biomass carbon, dehydrogenase activity and bacterial count also increased significantly in normal soil, respectively, while there was no significant difference observed in soil alkaline phosphatase activity in normal soils treated with various bioformulations. The soil fungal colonies remained significantly higher in normal soil as compared to sodic soil, even after the application of these bioformulations. These bioformulations showed significant increase in plant growth and yield in terms of plant height, dry matter accumulation, leaf area index, no. of effective panicles/m^2, length of panicle, no. of grains per panicle, grain yield, straw yield and 1000 grain weight. No impact of bioformulations was observed among N, P, K content in both grain and straw, both in sodic and non-sodic soils. N, P and K uptake in grain was significantly higher in sodic soil, as a result of inoculation with halophilic formulations, as compared to corresponding control. Nitrogen and phosphorus uptake by straw was also significantly increased in sodic soils upon inoculation with bioformulations over corresponding control, while no significant differences were observed in case of potassium. In nutshell, it was observed that the normal soils performed better on all indicators of soil health and productivity in comparison to sodic soil. Halo-Azo, Halo-PSB, Halo-Zinc and Halo-Mix had a positive impact on soil health of the sodic soils, as indicated by various physico-chemical parameters as well as significantly increased the productivity of PR-113. Exploitation of Halo-Azo, Halo-Phosphate solubilizing bacteria, Halo-Zinc and Halo-Mix as biofertilizers has enormous potential in improving the crop productivity and soil fertility under sodic soil condition owing to its cost-effectiveness.

Keywords: Halo-Azo, Halo-PSB, Halo-Zinc, Biofertilizer, Bioformulations, Soil health.

Signature of Major Advisor

Divya Chadha
Signature of student

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LIST OF ABBREVIATIONS USED

ACC deaminase	1-aminocyclopropane-1-carboxylate deaminase
Azo	*Azospirillium*
CFU	Colony-forming unit
CSSRI	Central Soil Salinity Research Institute
DHA	Dehydrogenase Activity
dS m^{-1}	desi Siemens per meter
DTPA	Diethylene Triamine Penta Acetic acid
EC	Electrical Conductivity
EC_e	Saturation extract electrical conductivity
ESP	Exchangeable Sodium Percentage
et al.	co-worker/and others
mha	million hectare
i.e.	*Id est*, that is
kg ha^{-1}	Kilogram per hectare
MBC	Microbial Biomass Carbon
meq	Milliequivalent
mg kg^{-1}	Milligram per Kilogram
$molL^{-1}$	mole per litre
NA	Nutrient Agar
NaCl	Sodium Chloride
NS	Non-Significant
PDA	Potato Dextrose Agar
PNP	cpara-Nitrophenyl phosphate
PSB	Phosphorus Solubilizer Bacteria
q ha^{-1}	Quintal per hectare
SAR	Sodium Adsorption Ratio
TPF	Tri-phenyl Formazon
TTC	Triphenyl tetrazolium chloride
VAM	Vesicular-Arbuscular Mycorrhiza
Viz.	Vide licet, namely, Such as
%	Percentage
µg g^{-1}	Microgram per gram

Chapter-I

Introduction

CHAPTER-I

INTRODUCTION

Soil health is one of the key factors for efficient agricultural production. Doran *et al.* (1996) defined soil health as "the capacity of soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality and promote plant and animal health." Healthy soils maintain community of soil organisms that help to control plant diseases, insect and weed pests, recycle essential plant nutrients, improve soil structure and ultimately improve crop production (FAO, 2008). Thus, soil health deals with integration and optimization of soil physical, chemical and biological properties for improved productivity and environmental quality. Increased demand for agriculture commodities generates incentives to convert forests and grasslands to farm fields and pastures which results in increase in soil erosion. In addition to erosion, soil health and quality is affected by other aspects of agriculture. These impacts include compaction, loss of soil structure, nutrient degradation and soil salinity. Among the soil degradation problems, salt-affected soil is one of the serious problems under arid and semi-arid lands. Soil degradation through salinity or sodicity is universal concern. Salt in soil jeopardize a crop's health first by dehydration and later by toxicity. The problem of salinity and sodicity in India is becoming more serious and is a matter of concern because of alarming increase in the area in the country under these soils.

The soil that contains excess salts which impairs its productivity is called salt affected (Chhabra, 1996). Salt affected lands are estimated at about 955 m ha^{-1} worldwide (Szabolcs, 1994), afflicting 7 percent of world's total arable land (Flowers *et al.*, 1997). In India, the area under salt affected soils is about 6.73 m ha^{-1} with the leading states being that of Gujarat (2.23 m ha^{-1}), U.P (1.3 m ha^{-1}), Maharashtra (0.61 m ha^{-1}), West Bengal (0.44 m ha^{-1}) and Rajasthan (0.38 m ha^{-1}). Salt-affected soils are classified into two main categories: saline and sodic (Abrol and Bhumbla, 1971). Saline soils are characterized by EC_e (Saturation extract electrical conductivity) values above 4 dS m^{-1} at 25^0C, ESP (Exchangeable Sodium Percentage) <15 or SAR (Sodium adsorption ratio) <13 and have pH values of less than 8.5. Salinity is a global problem that extends across all continents in more than 100 countries of the world. About 351.2

m ha^{-1} of area is saline in the world while 2.96 m ha^{-1} of land in India is saline. It has been estimated that more than 50 percent of the arable land would be salinized by the year 2050 (Jamil *et al.*, 2011). On the other hand, sodic soils have, EC_e values of less than 4 dS m^{-1} at 25^0C, high ESP (>15) or high SAR (>13) and pH values of greater than 8.5. About 581 m ha^{-1} area in the world and 3.78 m ha^{-1} area in India is under sodic soils. There is a third category termed as saline-sodic, which have both high EC and ESP.

Saline soils contain neutral soluble salts which adversely affect the growth of most crop plants. The soluble salts are chiefly sodium chloride and sodium sulphate. But, saline soils also contain appreciable quantities of chlorides and sulphates of Ca^{2+} and Mg^{2+}. These soils have good physical properties and water permeability. White salt crusts on the surface and scattered growth of crops are the indicators of salinity problem (Singh *et al.*, 2009). High salt concentrations due to ancient marine deposits, poor drainage and shallow water table conditions often tend to accentuate the salinity problem. Excessive salt accumulation in the root zone negatively affect plant growth. Salinity poses two major threats to plant growth: osmotic stress and ionic stress (Flowers and Colmer, 2008). Ion toxicity and osmotic stress cause metabolic imbalance, which in turn leads to oxidative stress (Chinnusamy *et al*., 2006). The deleterious effect of salinity affect different physiological and metabolic processes of plants. The responses to these changes are often accompanied by a variety of symptoms, such as the decrease in leaf area, increase of leaf thickness and succulence, abscission of leaves, necrosis of root and shoot, and decrease of internode lengths (Gucci and Tattini, 1997; Kozlowski and Pllardy, 1997; Parida and Das, 2005). Excessive salinity in soil water can decrease plant available water and cause plant stress. Excess salts in the root zone hinder plant roots from withdrawing water from surrounding soil. This lowers the amount of water available to the plant.

On the other hand, sodicity in soil is the presence of a high proportion of sodium ions relative to other cations. The problem of sodicity is associated with elevated soil pH and under these conditions the availability of certain plant nutrients is reduced resulting in severe loss of crop productivity. The excess sodium and high pH results in adverse soil physical properties leading to poor air-water-plant relationships (Acharya and Abrol, 1976) and crop failure on alkali soils. This condition is the result of excess sodium carbonate and bicarbonate and osmotic effect of other salts present.

Excess sodium imparts poor physical properties to soils. These soils, therefore, have extremely low permeability, are puddled easily and upon drying form compact blocks that may present a physical barrier to seed germination and root penetration. Soluble carbonates result in high soil pH (Mashhady and Rowell, 1978). This in turn brings about nutritional imbalances in the soil which prevent or limit the growth of most crops (Qadir *et al*., 2007).

The reclamation of saline-sodic is one of the main problems for humans (Szabolcs, 1994). The reclamation of these soils requires additional agricultural inputs and practices like amendments, water and infrastructure for drainage, which is costly and resource consuming. Alternative approaches need to be employed to enhance the productivity of these soils. One of the cheapest approach for reclaiming the salt affected soils is the use of microorganisms in the form of different formulations. The role of microbes in sustainable agriculture has provided new insights to agro-economy, and one of the direct benefits is the lesser reliance on chemical fertilizers and pesticides. Bioformulations are best defined as biologically active products containing one or more beneficial microbial strains in an easy to use and economical carrier materials. Usually, the term bioformulation refers to preparations of microorganisms that may be partial or complete substitute for chemical fertilization/pesticides. A good quality formulation promotes survival of bacteria maintaining available population that are sufficient to exude growth promoting effects on plants. Several bioformulations are commercially available in market by different commercial producer and different government institution. Some of the available bioformulations are *Azotobacter,* compost inoculants, phosphorus solubilizing bacteria (Bio-phospho), VAM inoculants etc. But the microbial strains available as biofertilizers for different crops do not perform effectively under salt stress and their activity decreases when used in salt affected soils due to osmolysis. Salt stress is responsible for obliteration of the microbial communities and carbon cycling in the soil.

To overcome this, attempts have been made to develop bioformulation based on halophiles, that can replace regular biofertilizer applications in salt affected soils. Halophiles are salt-loving organisms that inhabit hypersaline environments. Although salts are required for all life forms, halophiles are distinguished by their requirement of hypersaline conditions for growth. They may be classified according to their salt requirement: slight halophiles grow optimally at 0.2-0.85 mol L^{-1} (2-5%) NaCl;

moderate halophiles grow optimally at 0.85-3.4 mol L^{-1} (5-20%) NaCl; and extreme halophiles grow optimally above 3.4-5.1 mol L^{-1} (20-30%) NaCl. Halotolerant organisms can grow both in high salinity and in the absence of a high concentration of salts. Halophiles include mainly prokaryotic and eukaryotic microorganisms with the capacity to balance the osmotic pressure of the environment and resist the denaturing effects of salts. Halophilic bacteria have the ability to produce compatible solutes, especially glycine, betaines, and ectoines, which may be used as stress protectants against high salinity, thermal denaturation, desiccation and freezing as well as stabilizers of enzymes, nucleic acids, membranes and whole cells. They can produce enzymes that have optimal activity at high salinity. All halophilic microorganisms contain potent transport mechanism, generally based on Na^{+}/H^{+} antiporters, to expel sodium ions from the interior of the cells (Oren, 2002). They indirectly increase crop production (kumar *et al.*, 2014). They also enhance production of organic acids. The use of halophilic bacteria in the recovery of saline soils is based on the hypothesis that microbial activities in saline soil may favor the growth of plants under salt stress. The second hypothesis is based on the utilization of these bacteria as bio-indicators in saline wells (Arora *et al.*, 2014). The application of salt tolerant plant growth promoting bacteria (Halophiles) include overcoming of ill effects of salt affected soils by directly supporting the growth of vegetation thus enhancing crop yields. Introduction of effective halophilic bioformulation increase the availability of nutrients in such adverse soil conditions. These bioformulations play a greater role with salt tolerant varieties and further improve the crop produce and soil fertility.

In the Ravi-Tawi Command area of Jammu region near about 7500 ha of land is salt affected which is mostly of sodic nature (pH greater than 8.5, EC lesser than 4.0 dS m^{-1}, ESP greater than 15) (Sharma *et al.*, 2011). This is the region where rice is the dominant crop in kharif. Rice is an essential and most consumable cereal of Asia, feeds the majority of the world population. About more than 90 percent of the world rice is produced and consumed in the Asia and 60 percent of the world population and two- thirds of the world poor people live on this cereal (Khush and Virk, 2000). In Jammu and Kashmir, rice is grown on an area of 261.66 hectares with a production of 5456 quintals and productivity of 20.95 quintals per hectares (DES, 2012-2013). Rice is generally grown in the nitrogen deficient soils, and this nitrogen must be supplied to the field by the commercially available nitrogenous fertilizers. Grain yield of rice in salt affected soils is much lower because of its high sensitivity to salt stress (Gao *et al.*, 2007). Rice is exceptionally sensitive to salinity and sodicity at early seedling stage (Aslam *et al.,* 1988) and high yield losses have been observed because of high mortality and poor crop establishment. The increased level of salt in soil has serious effects on rice crop.

Conventional methods to remove salt from soil are expensive and may also destroy soil structure. Phytoremediation is a technique that is used to mitigate organic or inorganic contaminants in soils. The use of rhizobacteria in phytoremediation of soil contaminated with organic or inorganic metals has recently received more attention but the use of halophilic bacteria in phytoremediation is limited. A study was, therefore, proposed to assess the efficacy of halophilic bacterial strain based biofertilizers in enhancing soil health and crop yield.

This study entitled, "Improving health of Salt affected soils through microbial formulations" was proposed with the following objectives:

- To study the efficacy of microbial formulations on plant available nutrients and basic soil properties.
- To assess the effect of application of microbial formulations on soil biological properties.
- To observe the impact of microbial inoculation on uptake of nutrients and yield of crop.

Chapter-II

Review of Literature

CHAPTER-II

REVIEW OF LITERATURE

Bio-remediation is one of the cheap and eco-friendly approaches for remediation of salt affected lands as the traditional physical and chemical techniques are becoming costly. The possibility of application of halophilic bacteria in saline/sodic soil recovery and the importance of microbial diversity in soil is important in order to realistically assess their future application in the rehabilitation of degraded lands. The plant growth promoting halophilic bacteria helps in bio-remediation of salt affected soils and thereby improves the agricultural crop yields.

The work pertaining to the objectives of the study has been discussed in this chapter under the following sub- heads:

2.1 Salt-affected soils

2.2 Regular Bioformulations/Biofertilizers

2.3 Halophiles

2.4 Effect of halophiles on soil properties

2.5 Halophilic bacteria as plant growth promoter

2.1 Salt- affected soils

2.1.1 Genesis, characteristics and global distribution of salt-affected soils

Soil reaction (pH_s) and electrical conductivity (EC_e) of soil saturation extract, exchangeable sodium percentage (ESP) and sodium adsorption ratio (SAR) are the criteria used to classify the salt-affected soils. Based on these parameters, SAS are classified into two main categories: saline and sodic (Abrol *et al.*, 1980). The excess soluble salts present in saline soils- characterized by EC_e values above 4 dS m^{-1} at $25^{O}C$ render them unsuitable to grow majority of food crops. Similarly, in sodic soils excess exchangeable sodium percentage (ESP>15) adversely affects the growth and development in most crop plants (Abrol *et al.*, 1988). Besides physical and chemical weathering of rocks and primary minerals as the main processes, other factors responsible for the formation and/or accumulation of soluble salts in soils include unchecked irrigation and continuous or excessive fertilization (Tedeschi & Dell'Aquila, 2005; Quantin *et al.*, 2008). Irrigation with saline groundwater, development of saline creeps due to excessive leaching, ingress of sea water in coastal regions, congestion of natural drainage, seepage from canals, waterlogging due to faulty irrigation practices and localized redistribution of salts can lead to salinity development in perfectly normal soils. The chlorides and sulphates of Na^+, Ca^{2+} and Mg^{2+} are the dominant neutral soluble salts in saline soils. White salt crusts on the surface and scattered growth of crops are the indicators of salinity problem (Singh *et al*., 2009). High salt concentrations due to ancient marine deposits, poor drainage and shallow water table conditions often tend to accentuate the salinity problem (Horney *et al*., 2005). The factors responsible for the formation of sodic soils include alternate wet and dry seasons, groundwater containing carbonates and bicarbonates, desalinization and the reduction of sulphate ions under anaerobic conditions. In these soils, excess exchangeable sodium and high pH result in the dispersion of clay, poor physical properties and restricted water permeability (Abrol *et al*., 1988; Singh *et al*., 2009).

Sharma and Singh (2015) showed that salinity is one of the major constraints in sustainable food production in many parts of the world. The global scenario is changing day by day due to extreme climate variability, land and water degradation, biodiversity loss and trade regulations, the food and nutritional security of a burgeoning population is a cause for concern to the researchers and policy makers. So, increasing competition for productive lands and fresh water resources coupled with pervasive land use are pushing agriculture to the marginal environments, ensuring sustainable agricultural productivity and remunerative returns to the growers remain the major challenges. Globally, about 1128 m ha^{-1} area is affected by salinity and sodicity stresses. The regions with preponderance of salt affected soils are Middle East (189 m ha^{-1}) followed by Australia (169 m ha^{-1}) and North Africa (144 m ha^{-1}). South Asia, including India, has about 52 m ha^{-1} salt-affected area. In India, the area under salt-affected soils is about 6.73 m ha^{-1} with states of Gujarat (2.23 m ha^{-1}), Uttar Pradesh (1.37 m ha^{-1}), Maharashtra (0.61 m ha^{-1}), West Bengal (0.44 m ha^{-1}) and Rajasthan (0.38 m ha^{-1}) together accounting for almost 75 percent of saline and sodic soils in the country. Out of 6.72 m ha^{-1} of salt affected soils in the country, nearly 2.95 m ha^{-1} are under saline category and remaining 3.77 m ha^{-1} are sodic in nature (NRSA and Associates, 1996). Sodic soils are mostly present in the Indo-Gangetic plains of Haryana, Punjab, Uttar Pradesh and to some extent in other states including parts of Jammu and Kashmir. Contrary to this, saline soils are confined to irrigated, semi-arid and arid regions of Rajasthan, Gujarat, Karnataka, Andhra Pradesh and parts of Punjab, Himachal Pradesh and Jammu and Kashmir which includes Kandi belts of these states. Sharma *et al*. (2011) reported the impact of two important irrigation projects, which were commissioned in Jammu region utilizing the waters from Ravi and Tawi rivers on sodicity. It was observed that salt affected soils emerged at Jammu, Samba and Kathua. Areas in the command were found to have pH, sodium adsorption ratio and exchangeable sodium percentage of up to 9.9, 25.30 m. eq. L^{-1} and 78.41 m. eq. L^{-1}. Out of the total area of about 75000 ha under the canal irrigation system, 25,000 ha was observed to be either waterlogged or sodic. Saline soils in valley of Kashmir and Kandi belt of Jammu have been reported, having soluble salts in the range of 0.15 to 0.45 percent with Cl, NO_3, SO_4 and HCO_3 anions (Gupta and Arora, 2016). According to estimates, the present area under salt-affected soils (6.72 m ha^{-1}) in country would increase almost threefold to 20 million ha by 2050 (Sharma and Singh, 2014a).

As per the US Salinity Laboratory Staff, these soils have pH < 8.5, EC >4 dS m^{-1} at 25ºC and ESP <15 (Anonymous, 1954). The ratio of $[Na^+]/([Cl^-] + [SO_4^{2-}])$ in soil solution is <1.0. Bajwa (2002) stated that, the concentrations of alkali salts (CO_3^{2-} + HCO_3^-) are much lower than those of neutral salts (Cl^- and SO_4^{2-}). Many saline soils may also contain small to appreciable quantities of soluble gypsum. These soils are characterized by white encrustation of salts at the surface. Since the relative proportion of Na^+ ions with respect to Ca^{2+} and Mg^{2+} ions is low in saline soils, ESP is < 15 in these soils, the exchange complex does not have high amounts of adsorbed Na^+ ions. The dominance of Ca^{2+} and Mg^{2+} ions in soil solution and on the exchange sites and high total soluble salt results in flocculation of clay particles. The tightly adsorbed divalent (Ca^{2+} and Mg^{2+}) ions decrease the zeta potential of the soil exchange complex, which in turn results in attraction of clay particles towards each other causing flocculation. As a consequence of flocculation of clay particles, the saline soils are aggregated and highly permeable.

As per the US Salinity Laboratory Staff, alkali soils are characterized by pH >8.5, ESP >15 and EC < 4 dS m^{-1} (Anonymous, 1954). The ratio of $[Na^+]/([Cl^-] + [SO_4^{2-}])$ in soil solution is >1.0. Odell (2000) observed that sodic soils constitute a problem because their high pH and Na^+ levels cause clay swelling and dispersion, leading to a reduction in crop yields. One potential remediation method involves the stimulation of microbial populations in sodic soil to produce acid, thereby increasing the soil pH and releasing calcium, which helps to prevent soil dispersion.

2.1.2 Problems associated with salt-affected soils

Franzen (2007) observed that saline soils have salt levels high enough that either crop yields begin to suffer or cropping is impractical. Excessive salts injure plants by disrupting the uptake of water into roots and interfering with the uptake of competitive nutrients. Horneck *et al*. (2007) reported that saline soils are commonly called "white alkali" soils. They stated the harmful effect of salt on growth of crop as these salts adversely affect plant growth and productivity of most of the crop plants. High salt concentration in the soil can lead to wilting of plants even when soil moisture is adequate.

Salt-affected soils cover vast areas in arid and semi-arid regions. High evaporation in these areas results in the accumulation of large amount of salts in root zone that adversely affect the plant growth (Choudhary and Kharche, 2018). In salt-affected soils, the accumulated salts may reduce nutrient availability or cause specific ion toxicity. Nutrient uptake and use efficiency in salt affected soils is low due to salt stress and negative interactions with cations and anions present in high concentrations. Hence, a higher amount of nutrients for crops is necessary in salt affected soils. The nutritional effects of salts in sodic soils are generally caused by high soil pH. These soils usually contain less than 0.1 percent organic matter, are thus poor in native available N and hence respond to applied N. Soils with high pH values have shown positive influence on NH_3 losses (Fenn and Kissel, 1973). Organic matter decomposition and release of nutrients along with carbon, nitrogen mineralization and enzymatic activities are adversely affected by saline conditions (Ghollarata & Raiesi, 2007; Tripathi *et al.*, 2007). Higher metabolic quotients (qCO_2) and unaffected microbial biomass carbon at high EC values may indicate that salinity is a stressful factor, inducing either a shift in the microbial community with less catabolic activity or reduced efficiency of substrate utilization (Ghollarata & Raiesi, 2007). They further observed that both acid phosphatase and alkaline phosphatase activities decreased with increasing soil salinity.

Phosphate availability is reduced in saline soils not only because of ionic strength effects that reduce the activity of phosphate but also because phosphate concentrations in soil solution are highly controlled by sorption processes and by the low solubility of Ca-P minerals. Under saline-sodic or sodic conditions, high levels of external Na^+ not only interfere with K uptake but also may disrupt the integrity of root membranes and alter their selectivity (Grattan and Grieve, 1999).

Wichern *et al.* (2006) collected soil samples from two pasture sites in Heringen (Germany) and incubated them with 2 percent incorporated maize straw and at three levels of salinity (0, 15, 50 mg NaCl g^{-1} soil) at 25°C for 7 weeks. They observed that the amount of recovered maize derived particulate organic matter (POM) increased with increasing salinity indicating reduced decomposition rates. They also observed that inorganic N mainly consisted almost exclusively of NH_4^+ as the salinity level increased possibly due to decline in the nitrification process.

Salt stressed soils are known to suppress the growth of plants (Paul, 2012). Plants adversely affected by salinity grow more slowly and are, therefore, stunted. Salinity and sodicity generally affects the growth rate and it result in plants with smaller leaves, shorter stature and sometimes fewer leaves by reducing growth rate. Salt stress alters the water potential in the environment and thus, causes osmotic stress to plants. Because of high salinity, plants lose their turgor (Xiong and Zhu, 2002). Crop yield reductions results primarily from alteration of various metabolic processes in plants under salt stress. Yield reductions can be caused by reduced vegetative growth and/or by perturbation of the reproductive phases (El Falaky and Rady, 1993).

2.2 Regular Bioformulations/Biofertilizers

Biofertilizers or Bioformulations are now a popular term among agriculturists. It is being used extensively for biocontrol and plant growth promotion. Bioformulation is one of the areas of prime research importance as it affects the effectiveness of inoculants. Bioformulations generally refers to preparations of microorganism(s) that may be partial or complete substitute for chemical either fertilizers/pesticides. Microbial life at high salt concentrations is phylogenetically very diverse. Although arid soils are places with unfavourable conditions for micro-organisms growth, they are a suitable habitat for extremophilic ones, particularly, halophilic microorganisms (Garcia *et al*., 2018). Extensive and long-term application of bio-fertilizers may result in accumulation of salts, nutrients, and heavy metals that could cause adverse effects on plant growth, development of organisms of the soil, water quality and human health (Moreno *et al*., 2012). In arid and semi-arid areas affected by salts, there is low availability of water and salts are more concentrated promoting dehydration of the microorganism i.e., the microbes will lose water, causing the cells to first shrivel and subsequently fatal loss of cellular structure and function. However, the halophilic microorganisms can exchange cation and osmolytes when the salt concentration in the environment is high (Chowdhury *et al*., 2011). Halophilic enzymes are more stable than their non-halophilic counterparts owing to their polyextremophilic characteristics. These enzymes remain active in high-salt environments, thermotolerant and alkaliphilic (Moreno *et al*., 2009). Halophilic enzymes maintain solvation and solubility in low water activity (Sirisattha *et al*., 2016). A high-salt environment substantially impacts protein solubility and stability and consequently its function (Oren, 2006). In such environments, dehydration of cellular proteins becomes pronounced because of decreased water content in cells owing to water-molecule entrapment within external ionic lattices (Mevarech *et al*., 2000). The unfavorable interactions that disrupt internal microbial proteins caused by dehydration may be averted by modulating their net charge. A noticeable difference between proteins from halophiles and non-halophiles is that those of halophiles have a larger proportion of glutamate and aspartate on their surfaces (Zhang and Yi, 2013). They can promote plant growth directly by the production of phytohormones (indole acetic acids, gibberellic acid sand cytokinin), solubilization and bindings of nutrients (phosphorus, potassium, zinc and iron), biological nitrogen fixation, and ACC deaminase activity. These halotolerant organisms used as biofertilizers or bio-inoculants are finally fit into long-term for high yield and soil fertility for sustainable agriculture (Yadav and Saxena, 2018).

2.3 Halophiles

Mahadevaswamy and Nagaraju (2018) reported that salinity on soils have a profound effect on crop growth and yield. As the environment changes it makes few changes in the organism, and organism must change for their survival of the fittest. Microorganisms which live under extreme conditions have developed many strategies to overcome stress and nutrient limitation. Some of them have an impeccable potentiality to enhance the growth of crops under saline conditions by using myriad of mechanisms viz., fixing nitrogen, siderophore production, zinc solubilization, phosphorous solubilization, ACC deaminase production, commercial enzymes production etc.

Borsani *et al*. (2003) indicated that soil salinity is a major abiotic stress in plant agriculture strongly, influencing plant productivity world-wide. Therefore, an alternative strategy is to generate salt tolerant plants through genetic engineering. Due to high level of salt tolerance, halophytes are good candidates to identify salt tolerance genes. Three approaches are commonly used to identify genes important for salt tolerance. The first approach is to identify genes involved in processes known to be critical for salt tolerance (osmolyte synthesis, ion homeostasis, etc.). The second approach is to identify genes whose expression is regulated by salt stress. The third approach consists of the identification of salt tolerance determinants based on functionality.

Halophiles are the organisms that survive or even thrive in saline environments (Sarma and Sarma, 2012). These organisms are found in all the three domains of life, i.e., *Archaea, Bacteria and Eukarya*. Often, these microbes are not only challenged by one extreme, but multiple, and thus they are “polyextremophile” (Mesbah and Wiegel, 2008). These are adapted to salt concentration up to saturation hence able to grow at >300g L^{-1} NaCl concentration. Halophiles include mainly prokaryotic and eukaryotic microorganisms with the capacity to balance the osmotic pressure of the environment and resist the denaturing effects of salts.

The survival of microorganisms in hypersaline conditions requires specialized cellular and enzymatic adaptations to preserve the osmotic balance with the environment (Edbeib *et al*., 2016). Halophilic microorganisms usually adopt either of the two strategies of survival in saline environments: ‘compatible solute’ strategy and ‘salt-in’ strategy (Ventosa *et al*., 1998). Salinity tolerant soil microbes counteract osmotic stress by synthesizing osmolytes which allows them to maintain their cell turgor and metabolism (Yan *et al*., 2015).When an isosmotic balance with the medium is achieved, cell volume is maintained. Compatible solute strategy is employed by the majority of moderately halophilic and halotolerant bacteria, some yeasts, algae and fungi. In this strategy, cells maintain low concentrations of salt in their cytoplasm by balancing potential through the synthesis or uptake of organic compatible solutes and exclusion of salts from cytoplasm as much as possible. The salt-in strategy is employed by true halophiles, including halophilic archaea and extremely halophilic bacteria. These microorganisms are adapted to high salt concentrations and cannot survive when the salinity of the medium is lowered. They generally do not synthesize organic solutes to maintain the osmotic equilibrium. In this adaptation, the intercellular K^+ concentration is generally higher than that of outside, the intracellular Na^+ concentration is generally lower than that in the medium. The intracellular K^+ concentration increases with increasing external NaCl concentration in a non-linear pattern. All halophilic microorganisms contain potent transport mechanisms, generally based on Na^+/H^+ antiporters (Oren, 1999). Bioformulations are made from microbes which basically bring about the enrichment of the nutrients of the soil by enhancement of the availability of the micronutrients to different cereal crops. The application of halophilic bacteria helps in recovery of saline soils, decontamination of saline or alkaline industrial wastewater and the degradation of toxic compounds in hypersaline environments (Arora *et al*., 2014). Thus, the use of the halophiles as biofertilizers is considered as an alternative to chemical fertilizers so as to improve the fertility of the soil as well as increasing the productivity of the crops in the sustainable farming (Yadav and Saxena, 2018).

Mougith (1993) isolated halophilic fungi; *Penicillium notatum, P. chrysogenum, Aspergillus flavus* which were collected from Al- Shega province at Al-Qassim region. The growth and lipid composition of these fungi was studied at different concentrations of sodium chloride (5%, 10%, 15%, 20%). Result obtained showed that the growth was reduced as the sodium chloride concentration increased more than 5 percent. *Aspergillus flavus* tolerated up to 10 percent sodium chloride. The other two fungi tolerated up to 20 percent sodium chloride. He also founded that, with the increase in concentration of the sodium chloride, the total lipids, phospholipids, free fatty acids, free sterols, and the un saturated fatty acids in fungal mycelia also increased.

Ragab (1993) collected twenty soil samples from three different regions to obtain a distribution pattern of certain microbiological groups. Counts of total bacteria were negatively correlated with total soluble salts and positively correlated with organic carbon in surface layers. Insignificant relations were found for the effect of total soluble salts or organic carbon on nitrogen fixers (*Azotobacter* and

Clostridia). Nitrifiers were more sensitive to salts in surface layers (inversely significant correlation). On the other hand, their counts were directly correlated with the organic carbon in both soil layers. Approximately similar responses were obtained for the effect of total soluble salts and organic carbon on counts of cellulose-decomposer. The halophilic bacteria were positively correlated with total soluble salts in both soil layers and positively correlated with organic carbon only in soil surfaces.

Ravikumar *et al*. (2002) collected root and rhizosphere soil samples from twelve plant species from Pichavaram mangrove forest situated on the southeastern (Coromondel) coast of India, to study the occurrence, distribution and activity of halophilic *Azospirillum* from the mangrove environment. Total counts of halophilic *Azospirillum* from the collected samples were enumerated by serial dilution and plating. Results revealed that higher counts of *Azospirillum* (148.88 x 10^4 g^{-1} dry weight) were harboured in the roots of *Avicennia marina* and in the rhizosphere sediment sample of *Suaedamonoica* (20.0 x 10^3 g^{-1} dry weight). Muller and Oren (2003) reported that Halobacteriaceae contain molar concentrations of chloride, which is pumped into the cells by cotransport with sodium ions and/or using the light-driven primary chloride pump halorhodopsin. They contain low intracellular ion concentrations, with organic solutes providing osmotic balance.

Moradi *et al*. (2011) collected two saline soil samples from the rhizosphere of *Aeluropus littoralis* to study the salinity effect on free-living diazotroph and total bacterial populations. Soil samples with salinity of 35 and 70 dS m^{-1} were selected. The heterotrophic and free-living diazotrophic bacterial populations were counted on nutrient agar and free-nitrogen medium. Result showed that, the free-living diazotrophic and total heterotrophic bacterial populations in soil sample 1 was significantly ($P < 0.05$) more than soil sample 2. It was founded that sample 1 isolate were belong to the member of the genus *Bacillus*, whereas the other isolates belong to the member of the genera *Agrobacterium*, *Enterobacter* and *Corynebacterium*, respectively.

Reyad *et al*. (2012) isolated fourteen poly-extremophilic bacterial strains from saline and salt-affected soils in Egypt to study their morphological, physiological and biochemical characters. Cell morphology was examined using phase contrast and light microscopy. Results revealed that the number of bacterial colonies grown under the isolation conditions (10% NaCl, pH 10 and 55°C) was approximately 102 CFU per one gram of soil. The bacterial count was higher in the salt-affected inland soils than in the saline coastal habitats. The viable bacterial count was positively correlated with the total soluble salts and the organic matter content.

Caglayan *et al*. (2014) collected five soil samples from Tanneries in Turkey to study the characteristics of moderately halophilic bacteria on the salted hides. The total numbers of moderately halophilic bacteria on the salted hides were determined by spread plate technique. Results showed that the total moderately halophilic bacterial counts were found as $1.4x10^7$ to $2.1x10^2$ CFU g^{-1}.

Irshad *et al*. (2014) collected and isolated 200 halophilic bacteria from the foreshore soil of Daecheon Beach located in Chungnam and Jeonbuk Provinces in Korea. The isolation of halophilic bacteria was done on NA and R2A media supplemented with 3%, 5%, 9%, 15%, 20% and 30% NaCl. Result revealed that maximum number of halophilic bacterial colonies were observed on 3%, 5% and 9% NaCl respectively. On 15% and 20% NaCl, less growth of halophilic bacteria was obtained. There was no growth on 30% NaCl media plates.

Edbeib *et al. (*2016) observed that the unique cellular enzymatic machinery of halophilic microbes allows them to thrive in extreme saline environments. These microorganisms can prosper in hypersaline environments and has been correlated with the elevated acidic amino acid content in their proteins, which increase the negative protein surface potential. Because these microorganisms effectively use hydrocarbons as their sole carbon and energy sources, they may prove to be valuable bioremediation agents for the treatment of saline effluents and hypersaline water contaminated with toxic compounds that are resistant to degradation.

Halder *et al. (*2016) isolated halophilic bacteria from various soil samples collected from Sundarban Mangrove forest for studying the physiochemical and microbiological properties. Qualitative screenings of the isolates were done and three among them was selected having moderately good growth which was further optimized in different growth media. Halophilic bacteria were isolated on nutrient agar plates supplemented with sodium chloride. Result obtained showed that the CFU gm^{-1} of soil for samples S1, S2, S3 and S4 was 18×10^{-3}, 5×10^{-6}, 22×10^{-3} and 26×10^{-3}, respectively.

Phalke *et al*. (2017) collected soils samples from Aurangabad district (Maharashtra). They recorded that most of the soil samples were of black basalt soil and the samples were collected from rhizosphere. Few samples were of red soils, they were neutral to acidic in nature. Mostly saline and rain fed soils were recorded through-out the district. They further isolated and characterized halophilic bacteria by using morphological, biochemical and molecular analysis. On the basis of characterization confirmed strains were *P. aruginosa, P. putida, B. pumilus, B. halodenitrificans, P. rifietoensis, E. coli* and *R. meliloti.* The identified strains have salt tolerance and are highly useful against salt stressed soils and saline environments.

2.4 Effect of halophiles on soil properties

2.4.1 Effect on Plant available nutrients and basic soil properties

Zhu *et al*. (2011) isolated halophilic PSB strain *Kushneria* sp. YCWA18 from the sediment of Daqiao saltern, Jimo, Qingdao, China. They observed that the growth of halophilic PSB caused a significant increase of acidity in medium containing Ca (PO). In about 4 days, the acidity increased from pH 7.21 to 4.24. In contrast, the acidity decreased from pH 7.1 to pH 7.46 in lecithin-containing medium. Singh *et al*. (2013) planted *Jatropha curcas* L. on sodic soil to assess soil amelioration potential of the plant. Result revealed that there was significant decrease in soil pH (8.6 to 7.6), EC (1.29 to 0.98 dS m^{-1}) and ESP (20.7% to 13.8%), respectively.

Arora *et al*. (2016) conducted a field experiment in sodic soil of Indo-Gangetic plains of Uttar Pradesh, to study the effect of halophilic bacteria and their consortia in remediation of alkali soil and performance of wheat. Result obtained showed that Na content was decreased with the inoculation in surface soil layers indicative remediation potential of these strains. Inoculation of halophilic plant growth promoting isolates resulted in improved soil bio-chemical properties. It was observed that in surface soils, Na content substantially reduced and available N and P content got enhanced. Surface soil pH got reduced substantially in consortia inoculation in field experiment. Maximum reduction in Na content was noted in plots where consortia of halophilic N-fixer and phosphate solubilizers were inoculated where it was found to be 197.4 mg kg^{-1} compared to surface soil from control plot where it was 323.8 mg kg^{-1}.

Singh and Jha (2016) studied the effect of a halotolerant PGP bacterium *Serratia* sp. (SL-12) which was isolated from Sambhar saltlake, to evaluate its capability to promote growth in wheat plants (*Triticum aestivum L*) under of salt stress condition. Results revealed that with the application of isolate SL-12, there was decreased in the levels of Na^+ by (65 percent) and increase in uptake of K^+ by (39 percent), indicating a role in maintaining ionic homeostasis, and minimizing toxic ionic effects in host wheat plants.

Sahay *et al*. (2018) conducted a field experiment during the kharif season of 2017 at the village Munshikhera of Unnao, India to study the impact of two halophilic bioformulations, halophilic *azotobacter* (Halo-Azo) and halophilic phosphate solubilizing bacteria (Halo-PSB) with effective salt tolerant variety of rice (CSR 36) on crop productivity and soil fertility in sodic soil. Soil samples were collected and analyzed for pH, electrical conductivity (EC), organic carbon, available nitrogen and available phosphorus. Results showed that organic carbon, available nitrogen and available phosphorus content was increased up to 20, 9.9 and 16.8 percent respectively with the inoculation of halophilic bioformulations. Bioformulation Halo-Azo strain significantly increased the N content of post-harvest soil either alone or in combination with Halo PSB. Available phosphorus content of soil was observed significantly higher in all PSB inoculated treatments.

Shah *et al*. (2018) isolated halophilic strains from salt mines of District Karak, Pakistan, to study the diversity and enzyme production of halophiles. Isolates were then grown on different pH media for further characterization. The different pH media used were pH 3, pH 5, pH 7, pH 9 and pH 11. Using 1M HCl and 1M NaOH the pH of the media was adjusted. Result showed that all strains grow well at pH 7 with temperature 37ºC. Some strains showed growth at pH 3 while most of them grow at pH 5 keeping temperature constant. Strains also grow well at pH 9 at temperature 37ºC. They concluded that all of the strains are truly mesophilic while some isolates are psychrophilic.

Khattak *et al*. (2019) inoculated two salt-resistant halophilic *Bacillus* strains (NCCP-71 and NCCP-77) in rice crop, to study the biochemical changes in rice cultivars (NIAB-IR-9 and KSK-282) under control (0 mM) and different salt stress treatments (50, 100, and 150 mM NaCl). Results revealed that with the inoculation of halophilic strains, there was decrease in Na^+ contents and increase in K^+ contents in both varieties. Calcium ion content was increased by inoculation of NCCP-71 in both varieties. NIAB-IR-9 showed an increase in calcium ion content by NCCP-77 inoculation except at 100 mM NaCl. KSK-282 showed a decrease in calcium ion contents by the inoculation of NCCP-77. This indicated that NCCP-71 has more potential for stress alleviation.

Anees *et al*. (2020) collected soil sample from the saline fields of Shadi Khel of Karak District of Pakistan, to study the complementary potential of chitinolytic and halotolerant bacterial strains (*Pseudomonas sp*., *Thalassobacillus sp*., and *Terribacillus sp*.) in promoting the phytoremediation of saline soil. The physico-chemical analysis of the samples were conducted to measure EC, pH, Na^+, Ca^{2+}/Mg^{2+}, and K^+ at the Soil and Water Testing Laboratory (Soil Fertility) Attock, Pakistan. Result obtained showed that the EC of the saline soils significantly decreased upon treatment with the chitinolytic and halotolerant bacterial strains ($p \leq 0.05$).

2.4.2 Effect on soil biological properties

Xiang *et al*. (2011) isolated four aerobic Halos PSB from the rhizospheric soils of native plants growing on the wall of Dagong Ancient Brine well, located in Sichuan of China. These were classified to the same strain, named QW10-11, and closely related to *Bacillus megatheriumvar. phosphaticum* DSM 3228 and *B. megaterium* ATCC 14581 according to their phenotype and 16S rRNA. Morphological properties of isolates on the solid medium were examined by light microscopy and scanning electron microscopy. Results revealed that on the 10% NaCl NBRIP solid medium containing calcium phosphate, the QW10-11 colonies obtained were round configurations, smooth margin, raised elevations and slight yellow with clear halos. No pigment diffusing into the surrounding medium was found and its cell was line shaped (0.8 μm x 30 μm – 100 μm). This finding implied that QW1011 had some changes in the basic physiological processes in order to adapt to the special man-made hypersaline environment.

Akhter *et al*. (2012) collected soil samples from different salinity affected areas of Bangladesh, namely Pankhali Bazar of Dakop-Upazilla, Khulna and Dargapur Village, Assasuni-Upazilla, Satkhira. 35 colonies were isolated by pour plate method containing Ashby’s medium. Out of 35 isolates, 15 were finally selected for detailed study. Result showed that, all the strains were rod-shaped gram negative and non-spore forming. Biochemical studies showed that they were obligate aerobes, positive for catalase, oxidase and starch hydrolysis test.

Arora *et al*. (2014) proposed twenty bacterial isolates which were selected and characterized through morphological characters and biochemical tests. Three were pigmented and 17 were non-pigmented, 50% isolates exhibited amylase activity and only 15% isolates showed urease activity. Result revealed that six (30%) and two (10%) isolates showed positive results for ammonia production and phosphate solubilization activity. Salt tolerance of the endophytes was also tested. Of the 20 endophytic bacteria, seventeen (85%) isolates showed growth at 7.5% NaCl and fifteen (75%) tolerated up to 10% NaCl concentration. Overall, the growth rate of endophytes decreased with increasing concentration of NaCl in media. The endophytic bacteria were identified through 16S rRNA sequencing and mostly the isolated endophytic bacteria belong to genera *Bacillus* spp.

Arora *et al*. (2016) isolated halophilic bacteria from saline soil from coastal Gujarat and sodic soil from Indo-Gangetic plains of Uttar Pradesh and inoculated the halophilic bacteria in seeds of wheat crop. Result obtained showed that there was increase in the microbial biomass carbon up to 137 µg g^{-1} as compared to 82 µg g^{-1} in control.

Singh and Jha (2016) studied the effect of a halotolerant PGP bacterium *Serratia* sp. (SL-12) which was isolated from saltlake, to evaluate its capability to promote growth in wheat plants (*Triticum aestivum L*) under conditions of salt stress. Ability of the bacterial isolate SL-12 to colonize plants was determined by counting CFU of inoculated bacteria. Results showed that $1.4x10^3$ CFU g^{-1} bacteria was obtained from the root in inoculated plants. However, no bacterial colonies were obtained from uninoculated control plants.

2.5 Halophilic bacteria as plant growth promoter

Creus *et al*. (1997) studied the effects of salt or osmotic stress on shoot growth and water status in *Triticum aestivum* inoculated with *Azospirillum brasilense* sp. Results showed that fresh weight and dry weight were higher ($P <0.05$) in shoots from inoculated plants than in controls under 320mM NaCl, 20 percent PEG 6000, or 30 percent PEG 6000 stresses.

Sapsirisopa *et al*. (2009) conducted a pot experiment in saline soil of Thailand, to study the effect of halotolerant PSB, *B.megaterium* A12ag on growth of rice KDML 105. In their experiment, they took 7 treatments i.e. using only bacterial inoculum, bacterial inoculum and chemical fertilizer 16-16-8, bacterial inoculum, chemical fertilizer 16-0-8 and rock phosphate 0-3-0, chemical fertilizer 16-16-8, chemical fertilizer 16-0-8 and rock phosphate 0-3-0, chemical fertilizer 0-16-0, and without bacterial inoculums, chemical fertilizer and rock phosphate (control). Results revealed that the use of halotolerant PSB, *B.megaterium* A12ag increased the growth of rice and yield components. This study suggested that there are potential benefits of using salt-tolerant Bacillus as a bioinoculant for cultivation of rice KDML 105 in saline soil.

Kumar *et al*. (2010) inoculated different halophilic phospho-bacterial species of PSB in seeds of *Avicennia officinalis* which were collected from Pichavaram mangrove forest, South East coast of India. Results revealed that, *Bacillus subtilis* enhanced the average root length by 43.43% and shoot biomass by 69.39%, the *Pseudomonas aeruginosa* enhanced the root biomass by 40.0%, the *Arthrobacter ilicis* enhanced the number of primary roots by 53.57% and the *Micrococcus luteus* enhanced the number of secondary roots by 59.74% over control.

Quarshi and Sabri (2013) isolated two halophilic bacterial strains *Staphylococcus haemolyticus* (ST-9) and *Bacillus subtilis* (RH-4) from saline rhizosphere, in order to study their role in improving bacterial and plant growth alleviating salt stress. They further inoculated these halophilic bacterial strains in seeds of *Cicer arietinum* and grown up to full maturity at different salinity levels (0, 50, 100, 200 mM NaCl) in wire house under full sunlight. Results revealed that inoculated plants showed significantly increase in plant growth (germination, length, chlorophyll content, total soluble sugars and protein content).

Rajput *et al*. (2013) conducted a pot experiment on wheat crop by inoculating halophilic bacterial strain (SAL-15) which was isolated from rhizosphere soil of wheat growing at high saline and alkaline environment from Bio Saline Research Station, Pakka Anna, Faisalabad to study the use of salt-tolerant PGPR in order to improve the plants tolerance in stress environment especially salinity and promote their growth. Results revealed that with the inoculation of (SAL-15) strains in wheat crop, there was increase in plant height (63%), shoot fresh weight (59%), shoot dry weight (55.5%), root length (171%), root area (12%), and root dry weight (80%) as compared to control.

Talebi *et al*. (2013) isolated halophilic exopolysaccharide- producing bacteria from saline soil, Eshtehard, Iran. They further conducted a study by inoculating these halophilic bacteria on wheat crop, in which they took four bacterial treatments i.e. without the inoculation of bacteria (B_1), isolated bacteria TP7 (B_2), isolated bacteria TP5 (B_3), and both isolates of bacteria (B_4). Result showed that the inoculated treatments had higher dry and fresh root, shoot weights, germination rate and germination percent compared to non-inoculated treatment.

Kumar *et al*. (2014) conducted field experiment to check biofertilizer effect of halophilic phospho-bacteria on growth, yield and nutrient uptake of *Oryza sativa*. The PSB inoculated with *Oryza sativa*, increased significantly the average root length by 32.95%, average shoot length by 11.00%, numbers of roots by 38.46%, shoot biomass by 90.48% and root biomass by 3.77% as compared to control. There was increase in pigments level of total chlorophyll by 68.70%, chlorophyll-a by 53.50%, chlorophyll-b by 80.47% and carotenoids by 45.83%, as compared to control. The increase in level of carbohydrates, proteins and amino acids were 33.33%, 41.21% and 30.23%, respectively when compared to control.

Anbumalar and Kumar (2015) isolated 12 halo-bacterial strains from soil samples of various salterns in Tamilnadu, India and screened their plant growth promoting activity. They further conducted pot trials by inoculating these halo-bacterial strains in various crops like Cotton, Jatropa, Lady's finger and Sorghum in order to check the effects of these strains on plant growth. Result obtained showed that selected isolate significantly increased the plant shoot length and seed germination percentage as compared to control.

Nabti *et al*. (2015) reported that halotolerant PGPB stimulate plant growth under high salinity by using similar mechanisms like halo-sensitive PGPB, such as synthesis of indole acetic acid (IAA), gibberellins (GA), cytokinins (CK), abscisic acid (ABA), solubilization of insoluble rhizosphere or non-rhizosphere soil and benefit plant growth by numerous processes.

Orhan (2016) isolated 18 halotolerant and halophilic bacteria from salt-affected soils of the East Anatolian region, in order to study their plant growth promoting abilities *in vitro* and in a hydroponic culture. Out of the tested bacteria, eight were inoculated with *Triticum aestivum* in a hydroponic culture. Results showed that, under salt stress (200 mM NaCl), the selected bacterial strains significantly increased the root and shoot length and total fresh weight of the plants. The growth rate of the plants inoculated with bacterial strains ranged from 62.2% and 78.1%.

Shah *et al*. (2016) isolated halotolerant species i.e. *Thalassobacillusdenorans* (NCCP-58) and *Oceanobacilluskapialis* (NCCP-76), from salt mines near Karak, Pakistan which were used to evaluate its effect on rice variety (Basmati-385) in soil contaminated with different concentrations (control, 50, 100 and 150 mM) of NaCl. Result obtained showed that there was significant increase in germination percentage and germination rate occurred in seeds primed with bacterial strains as compared to non-primed seeds. Root and shoot lengths were more in plants raised from primed seeds than non-treated seeds. Plants raised from inoculated seeds showed a significant increase in fresh and dry weight of seedling after 15 days, increase in photosynthetic pigment and carotenoid contents which occurred in 28 days old plants inoculated with bacterial strains under different saline conditions. Bacterial strains *O. Kapialis* (NCCP-76) was more responsive in term of physiological and biochemical parameters than *T. denorans* sp. (NCCP-58).

Singh and Jha (2016) studied the effect of a halotolerant PGP bacterium Serratia sp. (SL-12) which was isolated from saltlake, to evaluate its capability to promote growth in wheat plants (*Triticum aestivum* L) under conditions of salt stress. Results revealed that with the inoculation of SL-12 into wheat plants growing under salt stress (150-200 mM). There was significant increase in shoot length, root length, fresh weight and dry weight i.e., 35%, 27%, 31% and 34%, respectively.

Sharma *et al*. (2016) isolated five diazotrophic halo tolerant bacteria (MBE01, MBE02, MBE03, MBE04, and MBE05) from the roots of a halophyte, *Arthrocnemum indicum*, to study their ability to promote growth and tolerate salinity of peanut. They further conducted pot experiment in which peanut seeds were pre-inoculated with bacterial inoculum (MBE02, MBE03 and positive strain). Result obtained showed that seedlings inoculated with MBE01, MBE02, and MBE05 had significantly lower shoot Na^+/K^+ ratios in comparison to non- inoculated seedlings (SNK test $P < 0.05$). Rest of the isolates failed to exhibit similar effect. The shoot Ca^+ content was, also, significantly higher in seedlings inoculated with all isolates but not with the MBE01. Treatment of seedlings with MBE01 significantly lowered the root Na^+/K^+ ratio as compared to non-treated samples. However, other bacterial isolates failed to exhibit similar effect.

Arora *et al.* (2016) observed that the plant growth promoting halophilic bacteria helps in bio-remediation of salt affected soils and thereby improves the agricultural crop yields. Bio-inoculation of seeds of wheat with plant growth promoting halophilic bacteria resulted in increase of 18.1 and 24.2 percent in grain and straw yield, respectively under sodic conditions.

Borhan *et al.* (2017) isolated *Enterobacter cloacae* (B_1), a halotolerant phosphate solubilizing bacterial strain from the rhizosphere of healthy rice plant in Kafr Elsheikh Governorate, Egypt to study the impact of these halotolerant bacteria on growth and yield of wheat crop. Results revealed that, *Enterobacter cloacae* (B_1) effectively increased growth characteristics including plant height (cm) (9.96%), fresh weight (g plant) (86.78%), dry weight (g plant) (58.36%) and flag leaf area (cm^2) (53.68%) and physiological characteristics including chlorophyll pigments contents, chl. a, b and total ($\mu g/cm^2$) (31.91%, 35.81% and 33.33%), respectively. There was increase in yield characteristics such as spike length (cm) (13.14%), spikelets number (13.58%), grains number/spike (23.16%), 1000 kernels weight (g) (29.37%), biological weight ($g\ m^{-2}$) (55.92%) and grains weight ($g\ m^{-2}$) (41.18%) as compare to control.

Sahay *et al.* (2018) conducted a field experiment in village Munshikhera, district Unnao to study the efficacy of halophilic bio fertilizers, Halo *Azotobacter* (Halo-Azo) and Halo Phosphate Solubilizing Bacteria (Halo-PSB) on the growth and productivity of salt tolerant variety of paddy (CSR-36) and residual fertility of post-harvest soil. Results showed that inoculation of bioformulations improves the germination per cent ranged from 10 to 16 percent as compare to control. Germination per cent, plant establishment per cent, number of effective tillers per hill, number of grains per panicles, test weight and grain yield were recorded 1.4, 1.5, 1.75, 2.21, 1.1 and 3.6 percent respectively higher with inoculation of Halo-PSB than Halo-Azo. Co-inoculation treatment of Halo-Azo and Halo-PSB significantly improved the all growth parameter recorded as compare to control. Co-inoculation treatment of both bioformulation significantly increased the germination per cent, plant establishment per cent and grain yield as compare to alone inoculation of Halo-Azo and Halo-PSB.

Yadav and Saxena (2018) isolated the halotolerant microbiomes from diverse saline habitat including *Actinobacteria, Bacteroidetes, Euryarchaeota, Firmicutes, Proteobacteria, and Spirochaetes.* They further isolated the halotolerant plant growth promoting (PGP) microbes such as *Ammoniphilus, Arthrobacter, Azospirillum, Bacillus, Brevibacillus, Brevibacterium,Haloarcula, Halobacillus, Halococcus, Haloferax, Halolamina, Halomonas, Halorubrum, Haloterrigena, Lysinibacillus, Marinobacter, Marinospirillum, Oceanobacillus, Paenibacillus, Penicillium, Pontibacillus, Pseudomonas, Sediminibacillus, Sporosarcina, Streptomyces, Thalassobacillus,* and *Thermonema*. They observed that the halophilic microbes have ability to produce phytohormones (indole acetic acids, gibberellic acids, and cytokinin), solubilize and bind nutrients (phosphorus, potassium, zinc, and siderophores), besides eliciting plant defence reactions against pathogens and also helps in plant growth under harsh saline environments. The halophilic PGP microbes increased the plant growth, yields, and nutrient uptake under the saline condition. It was observed that the salt-tolerant microbes with multifarious PGP attributes could be applied for plant growth and ameliorations of salt stress.

Zhang *et al*. (2018) isolated 305 halophilic bacteria strains from paddy soil in Taoyuan, China. Among these, 162 strains were tested for salt-tolerance 67.3%, 28.4% and 9.3%, of the strains could grow in media with NaCl concentrations of 50, 100, and 150 g L^{-1}, respectively. Further, among 162 strains, 30 halotolerant strains were screened for their plant-growth-promoting activities under axenic conditions at 3, 6, 9 and 12 g L^{-1} NaCl. Results revealed that 43% - 97% of the strains improved rice germination energy or germination capacity, while 63% - 87% increased shoot and root lengths.

Chapter-III

Material and Methods

CHAPTER-III

MATERIAL AND METHODS

The present investigation entitled "Improving Health of Salt Affected Soils through Microbial Formulations" was conducted during 2019-2020 at Division of Soil Science and Agricultural Chemistry, Sher-e-Kashmir University of Agricultural Science and Technology, Chatha, Jammu (J&K). The details of material used and methods adopted during the investigation have been presented in this chapter.

3.1 General Description

3.1.1 Experimental Location

The experiment was conducted at the Division of Soil Science and Agriculture Chemistry, Sher-e-Kashmir University of Agricultural Science and Technology, Chatha, Jammu (J&K). The experiment was laid out during summer season in the sub-tropical zone at latitude of 32.43^{O} North and longitude of 74.54 ^{O} East. Seedlings were planted 30 cm apart in pots.

3.1.2 Climate

The study area Chatha (Jammu) falls in sub-tropical zone of Jammu and Kashmir. The area receives an annual precipitation of about 1200 mm, most of which is received as monsoonal rains during July to September (about 70 percent). The mean annual maximum and minimum temperature are 29.6^{o}C and 16.7^{o}C, respectively. The highest temperature during the year does not ordinarily exceed 48^{o}C in summers and the minimum does not go below 4^{o}C in winter months.

3.1.3 Soil Types

Generally, the soil in the study area are Inceptisols, with texture varying from clay loam to sandy clay loams. The area has scattered patches of sodicity especially in the low-lying areas where water stagnation is a problem. The pH of these sodic soils reach over 10.0, with a high ESP (Sharma *et al.*, 2011).

3.2 Pot Experiment

3.2.1 Soil collection for pots

To conduct the pot experiment, bulk soil was collected from the Chatha farm of Sher-e-Kashmir University of Agricultural Science and Technology, Jammu.

After collecting the soil, it was ground and passed through 5.0-mm sieve and 12 kg of soil was filled in each pot. 30 pots were taken, among which 15 pots were kept as normal soil and sodicity was induced in remaining 15 pots. Sodicity was artificially induced by preparing a solution of sodium bicarbonate with water. A mixture of 24 g of sodium bicarbonate dissolved in 2 l of water was poured in each of the 15 pots. This treatment was replicated thrice till the pH obtained was greater than 9. They were analysed over a few days till a constant pH of 9.46±(0.10) was obtained. The properties of both soil types are presented in Table 3.1.

Table 3.1: Initial soil properties of the potted soils.

Soil Type	**Normal**	**Sodic**
Parameters		
pH	7.90	9.72
EC (dS m^{-1})	0.36	0.84
Organic Carbon (%)	0.98	0.44
N (kg ha^{-1})	207.4	88.0
P (kg ha^{-1})	9.35	10.16
K (kg ha^{-1})	211.9	156.8
Available Zn (mg kg^{-1})	0.82	0.63
Available Na (mg kg^{-1})	36.7	397.5
MBC (µg g^{-1})	102.5	64.3
Dehydrogenase activity (µg TPF g^{-1} hr^{-1})	19.0	14.7
Alkaline Phosphatase activity (PNP h^{-1} g^{-1})	26.4	12.3
Bacteria (CFU g^{-1})	56.4	22.6
Fungi (CFU g^{-1})	21.4	8.5

3.2.2 Experimental treatments

A pot-culture study was set up with Factorial Completely Randomized Design with three replications to test influence on soil properties and plant growth promoting activity of selected four halophilic bacterial bioformulations. Two factors were taken viz., Soil types and biofertilizers (halophilic bioformulations). Under soils, two types were taken i.e. normal rice growing soils and sodic soils. Five levels of bioformulations were also taken. These levels were control (B_0), Inoculation with Halo-Azo (B_1), Inoculation with Halo-PSB (B_2), Halo-Zinc inoculation (B_3) and Inoculation with consortium of bioformulations (Halo-Azo+Halo-PSB+Halo-Zinc) (B_4). There were a total of ten treatment combinations with 3 replications taking the number of pots to 30. A description of the treatments is given below in table 3.2.

Table 3.2: Details of treatment combinations

FACTOR 1: Soil type	
S_0 -	Normal soil
S_1 -	Sodic soil
FACTOR 2: Biofertilizers (B)	
B_0	Un-inoculated
B_1	Halo-Azo inoculation
B_2	Halo-PSB inoculation
B_3	Halo-Zinc inoculation
B_4	Halo-Mix (Halo-Azo + Halo-PSB + Halo- Zinc inoculation)

3.2.3 Plant material

The experiment consists of 150 seedlings of rice variety PR 113. Twenty-one days old seedling were selected. In each pot 5 plants are transplanted. Proper cultural practices were given to the plants during the entire course of study.

3.2.4 Bioformulations and their application

Biofertilizers i.e. (Halo-Azo, Halo-PSB, Halo-Zinc and Halo-Mix) were obtained from CSSRI, Lucknow. Seedling root dip was done in 100 times diluted formulations for one hour. After one hour, these seedlings were transplanted in pots. (Plate no. 3.1).

3.3 Soil sampling and analysis

3.3.1 Collection and preparation of soil samples

To study the impact of treatments on soil properties, soil samples from each experimental pot were collected at harvesting stage. Composite soil samples from 15 cm depth were collected from four points in the pot with the help of screw type auger. The soil samples, thus collected, were dried in shade, grounded, sieved through 2 mm plastic sieve and stored in polythene bags for analysis of basic soil properties and nutrient status. However, fresh soil samples were used for estimation of soil biological properties. Moisture correction was carried out for fresh soil samples, by keeping a replicate in the hot air oven and ascertaining the soil moisture content, which was then deducted from the weight taken for the analysis of biological properties.

3.3.2 Soil Chemical Properties

3.3.2.1 Soil pH

Soil pH was determined in 1: 2.5 soil water suspensions using digital pH meter following procedure as described by Jackson (1973).

3.3.2.2 Electrical conductivity (dS m^{-1})

The electrical conductivity of the supernatant in the soil water extract was read with the help of digital EC meter (Jackson, 1967).

3.3.2.3 Organic carbon (%)

It was determined according to wet digestion method of Walkley and Black (1934).

3.3.3 Soil nutrient status

3.3.3.1 Nitrogen (kg ha^{-1})

The available nitrogen (N) was estimated by alkaline potassium permanganate method as given by Subbiah and Asija (1956) and expressed in kg ha^{-1}.

3.3.3.2 Phosphorus (kg ha^{-1})

Available phosphorus in soil was determined by Stannous Chloride reduced ammonium molybdate method using Olsen's extractant (Olsen *et al.*, 1954) and determined on ultra violet visible spectrophotometer at 660 nm wave length and the results were expressed in kg ha^{-1}.

3.3.3.3 Potassium (kg ha^{-1})

Potassium was extracted with neutral normal ammonium acetate as per the procedure given by Jackson (1973). Potassium was estimated on the Flame Photometer and expressed in kg ha^{-1}.

3.3.3.4 Available Zinc (mg kg^{-1})

Available Zn in soil samples was determined by the method of Lindsay and Norvell (1978). In this method, 10 g of soil was extracted with 20 ml DTPA extracting solution by shaking for 2 hrs on a mechanical shaker. The suspension was

PLATE NO 3.1 Inoculation of rice seedlings with different bioformulations.

then filtered and available zinc was determined by atomic absorption spectrophotometer (Thermo 303) using respective cathode lamp.

3.3.3.5 Available Sodium (mg kg^{-1})

Available sodium in soil was extracted with 1N neutral ammonium acetate and determined on the flame photometer.

3.3.4 Soil biological properties

3.3.4.1 Microbial biomass carbon

Microbial biomass carbon (MBC) was determined using the chloroform fumigation extraction methods of Vance *et al*. (1987). To estimate MBC with the chloroform fumigation extraction method (CFE-MBC), a paired set of 20 g fresh soil samples maintained at $4^{O}C$ were taken, one part was fumigated with ethanol-free chloroform in a desiccator and another was kept under identical conditions without fumigation. Fumigated and non-fumigated samples were then treated with 0.5 M K_2SO_4. The C in the fumigated and non-fumigated extracts was measured and the difference obtained was used to calculate MBC.

3.3.4.2 Dehydrogenase enzyme activity

Dehydrogenase activity was determined by monitoring the rate of production of triphenyl formazan (TPF) from tri-phenyl tetrazolium chloride (TTC), using the method of Casida *et al*. (1964).

3.3.4.3 Phosphatase activity (PA)

The method of determination of phosphatase activity in soils was suggested by Tabatabai and Bremner (1969). It involves colorimetric estimation of the *p*-nitrophenol released by phosphomonoesterases activity. The soil is incubated with buffer (at 11 for alkaline phosphomonoesterases activities) sodium *p*-nitrophenyl phosphate solution and toluene. Alkaline phenol has a yellow colour which is measured in colorimeter. The $CaCl_2$-NaOH treatment is used for extraction of *p*-nitrophenol.

3.3.4.4 Microbial Count

The serial dilution and plating techniques suggested by Rao (1999), was employed for isolation and identification of viable bacteria count. Media were prepared for desired micro flora. The autoclaved and cooled medium was poured into sterile plates and allowed to solidify. One gram of sieved (2 mm) soil was added to 9 ml sterile water blank and shaken for 15-20 minutes. Serial dilutions of 10^{-2} ,10^{-3}, 10^{-4},10^{-5}, 10^{-6} and 10^{-7} were prepared and 0.1 ml of aliquots of various dilutions were added, over cooled and solidified medium in petriplates. Pour plate method was employed. The plates were rotated for uniform distribution of bacterial cells in the aliquot under the media and allowed to solidify. After the media solidified, the plates were inverted and incubated at 280°C for 3-4 days. The appearance of colonies on the surface of medium in the plates was observed. Viable count of microbes was noted using dilution plate technique by employing nutrient agar (NA), potato dextrose agar medium (PDA) and ken knights media, respectively. The population was expressed as colony forming units (CFU g^{-1} soil).

3.4 Collection of plant samples

Plant samples from each experimental pot were collected at harvesting stage. Plant sampling was done during the last week of November. The data on plant i.e. growth parameters and yield parameters were recorded and the procedures followed are described below:

3.4.1 Plant growth parameters

3.4.1.1 Height of plants

The height of plants (cm) was measured from the surface of soil to the tip of plant with the help of meter scale at harvesting stages.

3.4.1.2 Dry matter accumulation

After harvesting, plant samples were kept in paper bags and dried in hot air oven at 60± 2^{O}C till the samples attained constant weight.

3.4.1.3 Leaf area index

Three plants were selected from each pot and were cut close to the ground. All the leaves were removed from these plants, counted and categorized into three groups of large, medium and small size. A representative leaf from each category was selected and its leaf area was measured.

$$\text{Leaf area index} = \frac{\text{Leaf area (m}^2\text{)}}{\text{Ground area (m}^2\text{)}}$$

3.4.1.4 Root : Shoot ratio

The root:shoot ratio is one measure to assess the overall health of plant. To measure the root:shoot ratio, remove the plants from soil and wash off any loose soil. Blot the plants removing any free surface moisture. Dry the plants in an oven set to low heat (100^0F) overnight. Then, allow the plants to cool in a dry environment. Once the plants have cooled, weigh them on a scale. Separate the root from the top. Separately weigh and record the root and top for each plant. (Dry weight for roots/dry weight for top of plant = root/shoot ratio).

3.4.2 Yield attributes

3.4.2.1 Number of effective panicles per meter square

At harvest, only the grain filled panicle bearing shoots of all the plants counted for recording number of plants/m^2 were taken in to account for expressing number of effective panicles/m^2.

3.4.2.2 Panicle length

Panicle length (cm) was found out by counting between the lowest internode and terminal of the panicle as a mean of ten values at maturity stage.

3.4.2.3 Number of grains per panicle

Ten effective panicles/ears were randomly selected from the net area of each pot, threshed and the grains so obtained were counted and averaged to find out number of grains per panicle.

3.4.2.4 Yield

Grain and straw yield per pot was recorded after harvesting of crop.

3.4.2.5 Harvest index

The ratio of economic yield to the biological yield was computed to find out the harvest index by using the formula as given by Nichiporovich (1967):

$$\text{Harvest index (percent)} = \frac{\text{Economic yield (Grain)}}{\text{Biological yield (Grain+Straw)}} \times 100$$

3.4.2.6 1000- Grain weight

A random sample from bulk produce of each treatmental pot was taken out of which 1000-grains were counted and weighed. The weight so recorded was expressed in grams as test weight.

3.4.3 Plant analysis

The plant samples collected at harvest were washed with distilled water and dried in an oven at 65^{o}C. These dried samples were powdered and analysed for the nutrients.

3.4.3.1 Nitrogen

Nitrogen content in samples was determined by digesting the sample using concentrated H_2SO_4 and digestion mixture consisting of K_2SO_4, $CuSO_4$ and Se and then distilled in an alkaline medium as per the standard procedure described by Jackson (1973).

3.4.3.2 Diacid digestion of plant samples for P and K

One gram of powdered plant samples were pre-digested with 10 ml of concentrated nitric acid and kept overnight. The samples were then digested with 10ml of diacid mixture (HNO_3:$HClO_4$ in 10:4 ratio) until a white residue was left and the solution was clear. The residue was cooled, made up to a known volume with distilled water and used for further analysis.

3.4.3.2.1 Phosphorus

Phosphorus was estimated by Vanado-molybdate phosphoric acid yellow colour method. The intensity of yellow colour was read using a spectrophotometer at 430 nm wave length (Jackson, 1973).

3.4.3.2.2 Potassium

Potassium content in the digested sample was determined using flame photometer (Jackson, 1973).

3.4.3.3 Nutrient uptake

Nutrient uptake was calculated using nutrient concentration in grain and straw samples and multiplying it with the grain and straw yields for an area of one hectare.

3.5 Statistical Analysis

The data recorded during the investigation was statistically analysed as per Factorial Completely Randomized Design using Microsoft 365 excel data tool.

Chapter-IV

Results

CHAPTER-IV

RESULTS

The results pertaining to the present investigation on influence of halophilic bioformulations on soil properties and physiological parameters of coarse rice are presented in this chapter.

4.1 Chemical Parameters

The effect of different bioformulations on the basic soil properties and available nutrients is presented in this section under different sub-heads.

4.1.1 pH

The data pertaining to soil pH as influenced by application of halophilic microbial formulations to rice are presented in table 4.1. The mean value of soil pH for S_0 and S_1 were 7.97 and 8.94, respectively, whereas the mean obtained by application of different bioformulation treatments were 8.66, 8.32, 8.42, 8.55 and 8.33 for B_0, B_1, B_2, B_3 and B_4, respectively. The soil pH remained significantly higher in S_1 as compared to S_0, even after application of bioformulation. However, it was lower than the initial soil pH of sodic soil. Bioformulation application in S_1 revealed that application of B_1, B_2, B_3 and B_4 formulations decreased soil pH significantly over control with values of 8.68, 8.96, 8.99 and 8.61, respectively. Treatment S_1B_4 (8.61) among the S_1 factors had the lowest pH which was at par with S_1B_1 (8.68), but was significantly lower than S_1B_2 (8.96) and S_1B_3 (8.99). A significant increase in pH was also observed in S_0B_3 (8.12), while all other treatments were at par, within the normal soil.

4.1.2 Electrical Conductivity (EC)

The mean value of soil EC for S_0 and S_1 were 0.25 dS m^{-1} and 0.46 dS m^{-1}, respectively, whereas the mean obtained by application of different treatments inoculants were 0.46, 0.33, 0.32, 0.31 and 0.36 dS m^{-1} for B_0, B_1, B_2, B_3 and B_4, respectively (table 4.2). The soil EC was significantly higher in S_1 as compared to S_0. However, it was lower than the initial EC of sodic soil. Bioformulation inoculation in sodic soil revealed that application of B_1, B_2, B_3 and B_4 decreased soil EC significantly over control (0.66 dS m^{-1}) with values of 0.42, 0.38, 0.37 and 0.46 dS m^{-1}, respectively. Treatment S_1B_3 (0.37 dS m^{-1}) of among the S_1 factors had the lowest EC which was at par with S_1B_2 (0.38 dS m^{-1}), but was significantly lower than S_1B_1 (0.42

dS m^{-1}) and S_1B_4 (0.46 dS m^{-1}). Overall, the EC indicated that the soils were not saline.

Table 4.1 Effect of halophilic bio-fertilizer formulations on soil pH.

Treatments	Normal (S_0)	Sodic (S_1)	Mean
Control (B_0)	7.86	9.46	**8.66**
Halo-Azo (B_1)	7.95	8.68	**8.32**
Halo- PSB (B_2)	7.88	8.96	**8.42**
Halo-Zinc (B_3)	8.12	8.99	**8.55**
Halo-Mix (B_4)	8.04	8.61	**8.33**
Mean	**7.97**	**8.94**	

CD

B 0.17

S 0.11

BxS 0.24

Table 4.2 Effect of halophilic bio-fertilizer formulations on Electrical Conductivity (dS m^{-1}) of soil.

Treatments	Normal (S_0)	Sodic (S_1)	Mean
Control (B_0)	0.26	0.66	**0.46**
Halo-Azo (B_1)	0.24	0.42	**0.33**
Halo- PSB (B_2)	0.26	0.38	**0.32**
Halo-Zinc (B_3)	0.24	0.37	**0.31**
Halo-Mix (B_4)	0.26	0.46	**0.36**
Mean	**0.25**	**0.46**	

CD

B 0.04

S 0.02

BxS 0.05

4.1.3 Organic Carbon (OC)

A perusal of data in table 4.3 revealed that the mean value of soil organic carbon for S_0 and S_1 were 0.89 percent and 0.57 percent, respectively. The OC was significant higher in normal soils as compared to sodic soils. The mean obtained by application of bioformulations were 0.59, 0.84, 0.69, 0.73 and 0.81% for B_0, B_1, B_2, B_3 and B_4, respectively. Application of bioformulations in sodic soils, significantly increased organic carbon over control (0.25%). Treatment S_1B_4 (0.70%) among the S_1 factors had the highest organic carbon which was at par with treatment S_1B_1 (0.68%), but was significantly higher than the treatment S_1B_2 (0.60%) and S_1B_3 (0.59%). A significant increase in soil organic carbon was also observed in S_0B_1 (0.99%) over S_0B_0 (0.93%).

Table 4.3 Effect of halophilic bio-fertilizer formulations on soil Organic Carbon (%).

Treatments	Normal (S_0)	Sodic (S_1)	Mean
Control (B_0)	0.93	0.25	**0.59**
Halo-Azo (B_1)	0.99	0.68	**0.84**
Halo- PSB (B_2)	0.77	0.60	**0.69**
Halo-Zinc (B_3)	0.86	0.59	**0.73**
Halo-Mix (B_4)	0.91	0.70	**0.81**
Mean	**0.89**	**0.57**	

CD	
B	0.10
S	0.07
BxS	0.15

4.1.4 Available Nitrogen

A perusal of data in table 4.4 revealed that the available nitrogen in soil varied from 140.3 kg ha^{-1} to 185.2 kg ha^{-1} among the bioformulation treatments. The mean value of available nitrogen for S_0 and S_1 were 199.3 kg ha^{-1} and 140.4 kg ha^{-1} respectively. The mean values obtained by application of bioformulations were 140.3, 185.2, 174.3, 167.2, 182.2 kg ha^{-1} for B_0, B_1, B_2, B_3 and B_4, respectively. The available nitrogen in soil was significantly higher in S_0 as compared to S_1. Application of bioformulations in sodic soils, significantly increased available nitrogen over control (86.6 kg ha^{-1}). Treatment S_1B_1 (168.2 kg ha^{-1}) among the sodic soil had the highest soil available nitrogen which was at par with treatment S_1B_4 (159.9 kg ha^{-1}), but was significantly higher than the treatment S_1B_2 (148.8 kg ha^{-1}) and S_1B_3 (138.5 kg ha^{-1}). There were no significant differences in soil available nitrogen in normal soils due to application of various bioformulations.

4.1.5 Available Phosphorus

The data pertaining to available phosphorus as influenced by application of halophilic microbial formulations to rice are presented in table 4.5. The mean value of soil available phosphorus for S_0 and S_1 were 11.7 kg ha^{-1} and 13.3 kg ha^{-1}, respectively, whereas the mean obtained by application of different bioformulations were 10.8, 11.9, 13.7, 12.4 and 13.6 kg ha^{-1} for B_0, B_1, B_2, B_3 and B_4, respectively. The available phosphorus in soil was significantly higher in S_1 as compared to S_0. Application of bioformulations in sodic soils, significantly increased soil available phosphorus over control (11.1 kg ha^{-1}) except in case of B_3 (Halo-Zinc). Treatment S_1B_4 (14.8 kg ha^{-1}) among the S_1 factor had the highest soil available phosphorus which was at par with treatment S_1B_2 (14.7 kg ha^{-1}), but was significantly higher than the treatment S_1B_3 (12.5 kg ha^{-1}). A significant increase in soil available phosphorus was also observed in S_0B_2 (12.8 kg ha^{-1}), S_0B_3 (12.4 kg ha^{-1}) and S_0B_4 (12.3 kg ha^{-1}) over S_0B_0 (10.4 kg ha^{-1}).

Table 4.4 Effect of halophilic bio-fertilizer formulations on available nitrogen (kg ha^{-1}) in soil.

Treatments	Normal (S_0)	Sodic (S_1)	Mean
Control (B_0)	194.1	86.6	**140.3**
Halo-Azo (B_1)	202.2	168.2	**185.2**
Halo- PSB (B_2)	199.9	148.8	**174.3**
Halo-Zinc (B_3)	196.0	138.5	**167.2**
Halo-Mix (B_4)	204.4	159.9	**182.2**
Mean	**199.3**	**140.4**	

CD

B 10.70

S 6.77

BxS 15.13

Table 4.5 Effect of halophilic bio-fertilizer formulations on available phosphorus (kg ha^{-1}) in soil.

Treatments	Normal (S_0)	Sodic (S_1)	Mean
Control (B_0)	10.4	11.1	**10.8**
Halo-Azo (B_1)	10.5	13.3	**11.9**
Halo- PSB (B_2)	12.8	14.7	**13.7**
Halo-Zinc (B_3)	12.4	12.5	**12.4**
Halo-Mix (B_4)	12.3	14.8	**13.6**
Mean	**11.7**	**13.3**	

CD

B 1.13

S 0.71

BxS 1.59

4.1.6 Available Potassium

A perusal of data in table 4.6 revealed that the mean value of available potassium for S_0 and S_1 were 214.4 kg ha^{-1} and 173.8 kg ha^{-1}, respectively, whereas the mean obtained by application of different bioformulations were 180.9, 200.4, 194.7, 192.4 and 202.2 kg ha^{-1} for B_0, B_1, B_2, B_3 and B_4, respectively. The available potassium in soil remained significantly higher in S_0 as compared to S_1. Application of bioformulations in sodic soils, significantly increased soil available potassium over control (152.7 kg ha^{-1}). Treatment S_1B_4 (187.2 kg ha^{-1}) among the S_1 factors had the highest soil potassium which was at par with treatment S_1B_1 (183.8 kg ha^{-1}), but was significantly higher than the treatment S_1B_2 (173.9 kg ha^{-1}) and S_1B_3 (171.3 kg ha^{-1}). Within the normal soils, the application of bioinoculants did not result in any significant changes.

Table 4.6 Effect of halophilic bio-fertilizer formulations on available potassium (kg ha^{-1}) in soil.

Treatments	**Normal (S_0)**	**Sodic (S_1)**	**Mean**
Control (B_0)	209.2	152.7	**180.9**
Halo-Azo (B_1)	216.9	183.8	**200.4**
Halo- PSB (B_2)	215.4	173.9	**194.7**
Halo-Zinc (B_3)	213.4	171.3	**192.4**
Halo-Mix (B_4)	217.1	187.2	**202.2**
Mean	**214.4**	**173.8**	

CD

B	5.87
S	3.71
BxS	8.30

4.1.7 Available Zinc (DTPA extractable)

A perusal of data in table 4.7 revealed that the available zinc in the soil varied from 0.66 mg kg^{-1} to 0.86 mg kg^{-1} among all treatment combinations. The mean value of soil available zinc for S_0 and S_1 were 0.79 mg kg^{-1} and 0.74 mg kg^{-1}, respectively, whereas the mean obtained by application of different bioformulations were between 0.76 mg kg^{-1} and 0.79 mg kg^{-1}. The available zinc in soil was significantly higher in S_0

as compared to S_1. Application of bioformulations in sodic soils, significantly increased soil zinc over control (0.66 mg kg^{-1}). Treatment S_1B_3 (0.79 mg kg^{-1}) among the S_1 factors had the highest soil available zinc which was at par with treatment S_1B_2 (0.78 mg kg^{-1}), S_1B_1 (0.75 mg kg^{-1}) and S_1B_4 (0.73 mg kg^{-1}). A significant higher in soil available zinc was also observed in S_0B_0 (0.86 mg kg^{-1}), while all other treatments were at par among themselves within normal soils.

Table 4.7 Effect of halophilic bio-fertilizer formulations on available zinc (mg kg^{-1}) in soil.

Treatments	Normal (S_0)	Sodic (S_1)	Mean
Control (B_0)	0.86	0.66	**0.76**
Halo-Azo (B_1)	0.78	0.75	**0.77**
Halo- PSB (B_2)	0.80	0.78	**0.79**
Halo-Zinc (B_3)	0.73	0.79	**0.76**
Halo-Mix (B_4)	0.79	0.73	**0.76**
Mean	**0.79**	**0.74**	

CD

B 0.06

S 0.04

BxS 0.09

4.1.8 Available Sodium

The data pertaining to sodium content in soil as influenced by application of microbial formulations to rice are presented in table 4.8. Perusal of data revealed that the sodium content in the soil was decreased from 154.0 mg kg^{-1} to 110.3 mg kg^{-1} upon application of bioformulations. The mean value of sodium for S_0 and S_1 were 33.3 mg kg^{-1} and 209.6 mg kg^{-1}, respectively. The means obtained by application of different bioformulations were 154.0, 112.0, 114.6, 116.4 and 110.3 mg kg^{-1} for B_0, B_1, B_2, B_3 and B_4, respectively. The available sodium was significantly higher in S_1 as compared to S_0, even after application of bioformulation. However, it was lower than the initial sodium content of sodic soil. Bioformulation application within sodic soil decreased sodium in soil significantly over control (274.7 mg kg^{-1}) with values of 190.4, 195.11, 200.3 and 187.5 mg kg^{-1}, respectively. Treatment S_1B_4 (187.5 mg kg^{-1}) of among the S_1 factor had the lowest sodium content which was at par with S_1B_1 (190.4 mg kg^{-1}), but was significantly lower than S_1B_2 (195.1 mg kg^{-1}) and S_1B_3 (200.3 mg kg^{-1}). No variations were observed among the treatments within the normal soils, with respect to soil sodium content.

Table 4.8 Effect of halophilic bio-fertilizer formulations on available sodium (mg kg^{-1}) in soil.

Treatments	Normal (S_0)	Sodic (S_1)	Mean
Control (B_0)	33.4	274.7	**154.0**
Halo-Azo (B_1)	33.5	190.4	**112.0**
Halo- PSB (B_2)	34.2	195.1	**114.6**
Halo-Zinc (B_3)	32.6	200.3	**116.4**
Halo-Mix (B_4)	33.1	187.5	**110.3**
Mean	**33.3**	**209.6**	

CD

B 3.64

S 2.30

BxS 5.15

4.2 Biological Parameters

The effect of different bioformulations on the soil biological properties is reported in various sub-sections below:

4.2.1 Microbial Biomass Carbon (MBC)

A perusal of data in table 4.9 revealed that the microbial biomass carbon in the soil varied from 69.1 µg g^{-1} to 120.1 µg g^{-1} among all treatment combinations. The mean value of soil microbial biomass carbon for S_0 and S_1 were 113.8 µg g^{-1} and 84.8 µg g^{-1}, respectively, whereas the mean obtained by application of different bioformulations were 88.6, 102.8, 99.3, 100.4 and 105.5 µg g^{-1} for B_0, B_1, B_2, B_3 and B_4, respectively. The microbial biomass carbon in soil was significantly higher in S_0 as compared to S_1. Application of bioformulations in sodic soils, significantly increased soil microbial biomass carbon over control (69.1 µg g^{-1}). Treatment S_1B_4 (90.8 µg g^{-1}) among the S_1 factors had the highest soil microbial biomass carbon which was at par with treatment S_1B_1 (90.7 µg g^{-1}), S_1B_2 (86.0 µg g^{-1}) and S_1B_3 (87.4 µg g^{-1}). A significant increase in soil microbial biomass carbon was also observed in S_0B_4 (120.1 µg g^{-1}) over S_0B_0 (108.0 µg g^{-1}), while all other treatments were at par within the normal soils.

Table 4.9 Effect of halophilic biofertilizer formulations on microbial biomass carbon (µg g^{-1}) in soil.

Treatments	Normal (S_0)	Sodic (S_1)	Mean
Control (B_0)	108.0	69.1	**88.6**
Halo-Azo (B_1)	114.8	90.7	**102.8**
Halo- PSB (B_2)	112.5	86.0	**99.3**
Halo-Zinc (B_3)	113.5	87.4	**100.4**
Halo-Mix (B_4)	120.1	90.8	**105.5**
Mean	**113.8**	**84.8**	

CD

B	6.60
S	4.18
BxS	9.34

4.2.2 Dehydrogenase activity (DHA)

A perusal of data in table 4.10 revealed that the mean value of dehydrogenase activity for S_0 and S_1 were 24.0 µg TPF g^{-1} hr^{-1} and 15.6 µg TPF g^{-1} hr^{-1}, respectively, whereas the mean obtained by application of different bioformulations were 14.20, 21.9, 21.6, 20.0 and 21.3 µg TPF g^{-1} hr^{-1} for B_0, B_1, B_2, B_3 and B_4, respectively**.** The dehydrogenase activity in soil was significantly higher in S_0 as compared to S_1. Application of bioformulations in sodic soils, significantly increased soil dehydrogenase activity over control (10.1 µg TPF g^{-1} hr^{-1}). Treatment S_1B_2 (18.0 µg TPF g^{-1} hr^{-1}) among the S_1 factors had the highest soil dehydrogenase activity which was at par with treatments S_1B_1 (17.1 µg TPF g^{-1} hr^{-1}), S_1B_3 (16.2 µg TPF g^{-1} hr^{-1}) and S_1B_4 (16.7 µg TPF g^{-1} hr^{-1}). A significant increase in soil dehydrogenase activity was also observed in S_0B_1 (26.8 µg TPF g^{-1} hr^{-1}) over S_0B_0 (18.3 µg TPF g^{-1} hr^{-1}), while all other treatments were at par within normal soils.

4.2.3 Alkaline Phosphatase activity (PA)

A perusal of data in table 4.11 revealed that the alkaline phosphatase activity in the soil varied from 11.2 µg PNP h^{-1} g^{-1} to 35.4 µg PNP h^{-1} g^{-1} among the various treatment combinations. The mean value of alkaline phosphatase activity for S_0 and S_1 were 32.2 µg PNP h^{-1} g^{-1} to 22.0 µg PNP h^{-1} g^{-1}, respectively, whereas the mean obtained by application of different bioformulations were 20.1, 29.2, 31.8, 25.7 and 28.6 µg PNP h^{-1} g^{-1} for B_0, B_1, B_2, B_3 and B_4, respectively**.** The alkaline phosphatase activity in soil was significantly higher in S_0 as compared to S_1. Application of bioformulations in sodic soils, significantly increased soil alkaline phosphatase activity over control (11.2 µg PNP h^{-1} g^{-1}). Treatment S_1B_2 (28.2 µg PNP h^{-1} g^{-1}) among the S_1 factors had the highest soil alkaline phosphatase activity which was at par with treatment S_1B_4 (26.0 µg PNP h^{-1} g^{-1}), S_1B_1 (23.1 µg PNP h^{-1} g^{-1}) and S_1B_3 (21.3 µg PNP h^{-1} g^{-1}). No significant difference in soil alkaline phosphatase activity was observed in normal soils treated with various bioformulations.

Table 4.10 Effect of halophilic biofertilizer formulations on dehydrogenase activity (µg TPF g^{-1} hr^{-1}) in soil.

Treatments	Normal (S_0)	Sodic (S_1)	Mean
Control (B_0)	18.30	10.10	**14.20**
Halo-Azo (B_1)	26.82	17.14	**21.98**
Halo- PSB (B_2)	25.25	18.07	**21.66**
Halo-Zinc (B_3)	23.81	16.23	**20.02**
Halo-Mix (B_4)	25.91	16.71	**21.31**
Mean	**24.02**	**15.65**	

CD

B	2.93
S	1.85
BxS	4.14

Table 4.11 Effect of halophilic biofertilizer formulation on alkaline phosphatase activity (µg PNP h^{-1} g^{-1}) in soil.

Treatments	Normal (S_0)	Sodic (S_1)	Mean
Control (B_0)	29.0	11.2	**20.1**
Halo-Azo (B_1)	35.3	23.1	**29.2**
Halo- PSB (B_2)	35.4	28.2	**31.8**
Halo-Zinc (B_3)	30.1	21.3	**25.7**
Halo-Mix (B_4)	31.2	26.0	**28.6**
Mean	**32.2**	**22.0**	

CD

B	6.70
S	4.24
BxS	9.48

4.2.4 Microbial count

4.2.4.1 Soil Bacteria

A perusal of data in table 4.12 revealed that the bacterial colonies in the soil varied from 26.3 CFU g^{-1} to 67.0 CFU g^{-1}. The mean value of bacterial colonies for S_0 and S_1 were 64.9 CFU g^{-1} and 43.0 CFU g^{-1}, respectively, whereas the mean obtained by application of different bioformulations were 46.2, 58.2, 54.7, 51.4 and 59.3 CFU g^{-1} for B_0, B_1, B_2, B_3 and B_4, respectively**.** Thebacterial colonies in soil were significantly higher in S_0 as compared to S_1. Application of bioformulations in sodic soils, significantly increased soil bacterial colonies over control (26.3 CFU g^{-1}). Treatment S_1B_4 (54.3 CFU g^{-1}) among the S_1 factors had the highest soil bacterial colonies which was significantly higher than S_1B_1 (49.3 CFU g^{-1}), S_1B_2 (44.0 CFU g^{-1}) and S_1B_3 (41.3 CFU g^{-1}). Within normal soils, application of Halo-Azo (B_1) resulted in highest bacterial count compared to all other treatments. (Plate no. 4.1).

Table 4.12 Effect of halophilic biofertilizer formulations on microbial count (Bacteria) (CFU g^{-1}) in soil.

Treatments	**Normal (S_0)**	**Sodic (S_1)**	**Mean**
Control (B_0)	66.1	26.3	**46.2**
Halo-Azo (B_1)	67.0	49.3	**58.2**
Halo- PSB (B_2)	65.5	44.0	**54.7**
Halo-Zinc (B_3)	61.6	41.3	**51.4**
Halo-Mix (B_4)	64.3	54.3	**59.3**
Mean	**64.9**	**43.0**	

CD

B	3.45
S	2.18
BxS	4.88

4.2.4.1 Soil Fungi

A perusal of data in table 4.13 revealed that the fungal colonies in the soil varied from 6.6 to 24.4 CFU g^{-1}. The mean value of fungal colonies for S_0 and S_1 were 22.9 CFU g^{-1} and 7.8 CFU g^{-1}, respectively, whereas the mean obtained by application of different bioformulations were 17.0, 15.4, 13.7, 15.1 and 15.5 CFU g^{-1} for B_0, B_1, B_2, B_3 and B_4, respectively. The soil fungal colonies were significantly higher in S_0 as compared to S_1, even after application of bioformulation. However, no significant differences were observed among treatments within individual soils, both normal and sodic.

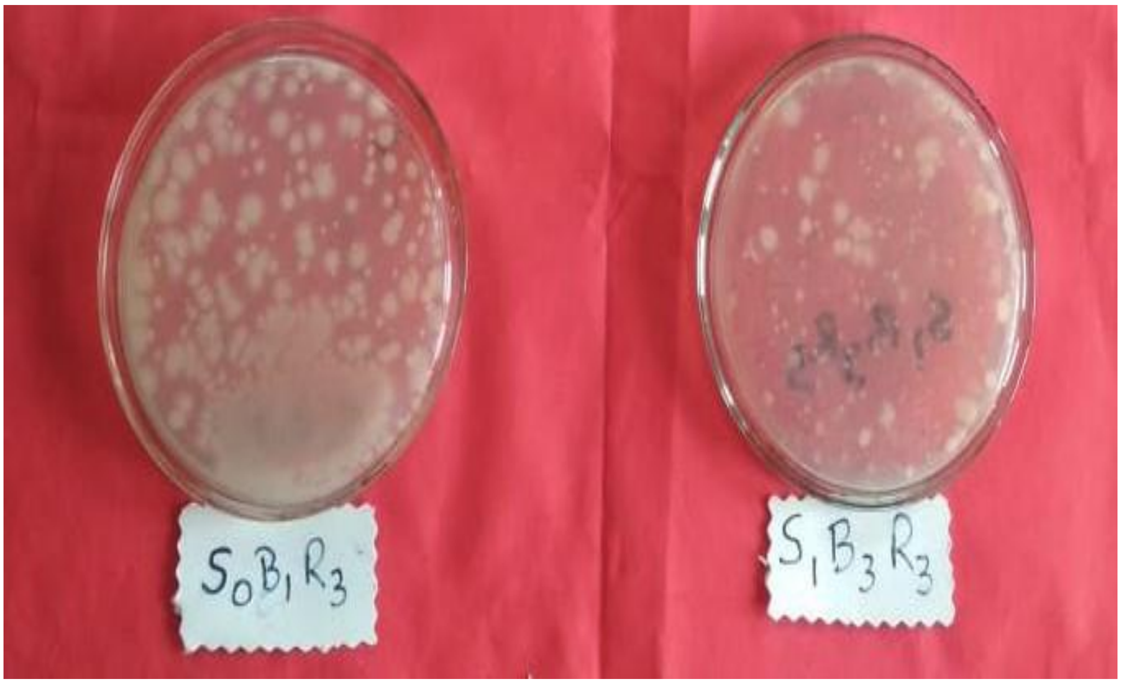

PLATE 4.1 Comparison of bacterial counts between normal and sodic soils.

Table 4.13 Effect of halophilic biofertilizer formulations on microbial count (Soil Fungi) (CFU g^{-1}).

Treatments	Normal (S_0)	Sodic (S_1)	Mean
Control (B_0)	24.4	9.6	**17.0**
Halo-Azo (B_1)	22.2	8.6	**15.4**
Halo- PSB (B_2)	20.7	6.8	**13.7**
Halo-Zinc (B_3)	23.6	6.6	**15.1**
Halo-Mix (B_4)	23.6	7.3	**15.5**
Mean	**22.9**	**7.8**	

CD

B	2.64
S	1.67
BxS	3.73

4.3 Physiological Parameters

4.3.1 Plant growth and Biomass

4.3.1.1 Plant Height

The data pertaining to the plant height at harvest is presented in table 4.14. The mean value of plant height for S_0 and S_1 were 103.6 cm and 75.8 cm, respectively, whereas the mean obtained by application of different bioformulations were 85.7, 91.5, 90.6, 90.6 and 90.1 cm for B_0, B_1, B_2, B_3 and B_4, respectively. The plant height was significantly higher in S_0 as compared to S_1. Application of bioformulations in sodic soils, significantly increased plant height over control (66.5 cm). Treatment S_1B_1 (79.6 cm) among the S_1 factors had the highest plant height which was at par with treatment S_1B_3 (78.6 cm), S_1B_2 (77.3 cm) and S_1B_4 (77.2 cm). No significant differences in plant height was observed for different treatments under normal soils (Plate no. 4.2).

4.3.1.2 Dry matter accumulation

The data pertaining to the dry matter accumulation at harvest stage is presented in table 4.15. The mean value of dry matter accumulation for S_0 and S_1 were 1619.1 g/m^2 and 890.5 g/m^2 respectively, whereas the mean obtained by application of different bioformulations were 1094.7, 1282.8, 1258.5, 1286.8 and 1351.2 g/m^2 for B_0, B_1, B_2, B_3 and B_4, respectively. The dry matter accumulation was significantly higher in S_0 as compared to S_1. Application of bioformulations in sodic soils, significantly increased dry matter accumulation over control (620.0 g/m^2). Treatment S_1B_1 (1040.7g/m^2) among the S_1 factor had the highest dry matter accumulation. A significant increase in dry matter accumulation was also observed in S_0B_4 (1737.3 g/m^2) over S_0B_0 (1569.3 g/m^2).

Table 4.14 Effect of halophilic biofertilizer formulations on Plant Height (cm) of rice crop.

Treatments	Normal (S_0)	Sodic (S_1)	Mean
Control (B_0)	104.9	66.5	**85.7**
Halo-Azo (B_1)	103.4	79.6	**91.5**
Halo- PSB (B_2)	104.0	77.3	**90.6**
Halo-Zinc (B_3)	102.6	78.6	**90.6**
Halo-Mix (B_4)	103.1	77.2	**90.1**
Mean	**103.6**	**75.8**	
CD			
B 3.62			
S 2.29			
BxS 5.12			

Table 4.15 Effect of halophilic biofertilizer formulations on dry matter accumulation (g/m^2) of rice crop.

Treatments	Normal (S_0)	Sodic (S_1)	Mean
Control (B_0)	1569.3	620.0	**1094.7**
Halo-Azo (B_1)	1525.0	1040.7	**1282.8**
Halo- PSB (B_2)	1592.0	925.0	**1258.5**
Halo-Zinc (B_3)	1671.7	902.0	**1286.8**
Halo-Mix (B_4)	1737.3	965.0	**1351.2**
Mean	**1619.1**	**890.5**	
CD			
B 160.8			
S 101.7			
BxS 227.4			

4.3.1.3 Leaf area index

The data pertaining to the leaf area index of rice affected by different microbial formulations have been presented in table 4.16. The mean value of leaf area index for S_0 and S_1 were 0.167 and 0.060, respectively, whereas the mean obtained by application of different bioformulations were 0.102, 0.116, 0.109, 0.121 and 0.121 for B_0, B_1, B_2, B_3 and B_4, respectively. The leaf area index was significantly higher in S_0 as compared to S_1. Application of bioformulations in sodic soils, significantly

PLATE 4.2 Comparison of growth under different treatments.

increased leaf area index over control (0.041). Treatment S_1B_3 (0.070) among the S_1 factors had the highest leaf area index which was at par with treatment S_1B_1 (0.068) and S_1B_4 (0.067). There was no significant differences in leaf area index among various bioformulation treatments within normal soils.

Table 4.16 Effect of halophilic biofertilizer formulations on leaf area index of rice crop.

Treatments	Normal (S_0)	Sodic (S_1)	Mean
Control (B_0)	0.164	0.041	**0.102**
Halo-Azo (B_1)	0.163	0.068	**0.116**
Halo- PSB (B_2)	0.166	0.052	**0.109**
Halo-Zinc (B_3)	0.171	0.070	**0.121**
Halo-Mix (B_4)	0.175	0.067	**0.121**
Mean	**0.167**	**0.060**	

CD

B 0.009

S 0.006

BxS 0.013

4.3.1.4 Root: Shoot ratio

The data pertaining to the root shoot ratio of rice affected by different microbial formulations have been presented in table 4.17. The mean value of root shoot ratio for S_0 and S_1 were 1.55 to 3.62 respectively, whereas the mean obtained by application of

Table 4.17 Effect of halophilic biofertilizer formulations on root shoot ratio of rice crop.

Treatments	Normal (S_0)	Sodic (S_1)	Mean
Control (B_0)	1.40	3.43	**2.41**
Halo-Azo (B_1)	1.31	3.49	**2.40**
Halo- PSB (B_2)	1.54	3.57	**2.56**
Halo-Zinc (B_3)	1.79	3.99	**2.89**
Halo-Mix (B_4)	1.72	3.61	**2.67**
Mean	**1.55**	**3.62**	

CD

B 0.56

S 0.36

BxS 0.80

different bioformulations were 2.41, 2.40, 2.56, 2.89 and 2.67 for B_0, B_1, B_2, B_3 and B_4, respectively. The root shoot ratio was significantly higher in S_1 as compared to S_0.

Bioformulations did not have significant impact on root shoot ratio of rice both in sodic and normal soils.

4.3.2 Yield attributes and Yield

4.3.2.1 Number of effective panicles/m^2

The data pertaining to the number of effective panicle/m^2 of rice affected by different microbial formulations have been presented in table 4.18. The mean value of effective panicle/m^2 for S_0 and S_1 were 226.3 and 139.6 respectively, whereas the mean obtained by application of different bioformulations were 178.3, 176.7, 182.5, 196.2 and 191.2 for B_0, B_1, B_2, B_3 and B_4, respectively. The effective panicle/m^2 was significantly higher in S_0 as compared to S_1. Application of Halo-Zinc in sodic soils, significantly increased effective panicle/m^2 over control (126.7) while all other treatments were at par with Halo-Zinc. There were no significant differences among the bioformulations treatments within normal soils.

Table 4.18 Effect of halophilic biofertilizer formulations on number of effective panicles/m^2 of rice crop.

Treatments	Normal (S_0)	Sodic (S_1)	Mean
Control (B_0)	230.0	126.7	**178.3**
Halo-Azo (B_1)	211.7	141.7	**176.7**
Halo- PSB (B_2)	230.0	135.0	**182.5**
Halo-Zinc (B_3)	235.0	157.3	**196.2**
Halo-Mix (B_4)	245.0	137.3	**191.2**
Mean	**226.3**	**139.6**	

CD

B 17.77

S 11.24

BxS 25.14

4.3.2.2 Length of Panicle

The data pertaining to the length of panicle of rice affected by different microbial formulations is presented in table 4.19. The mean value of length of panicle for S_0 and S_1 were 21.9 cm to 17.0 cm respectively, whereas the mean obtained by application of different bioformulations were 18.4, 19.2, 18.7, 21.5 and 19.3 cm for B_0, B_1, B_2, B_3 and B_4, respectively. The length of panicle was significantly higher in S_0 as compared to S_1. Application of Halo-Zinc significantly increased length of panicle over control (15.0 cm) in sodic soils, while there was no significant differences within normal soil as a result of bioformulations.

Table 4.19 Effect of halophilic biofertilizer formulations on length of panicle (cm) of rice crop.

Treatments	Normal (S_0)	Sodic (S_1)	Mean
Control (B_0)	21.8	15.0	**18.4**
Halo-Azo (B_1)	20.8	17.6	**19.2**
Halo- PSB (B_2)	21.5	15.9	**18.7**
Halo-Zinc (B_3)	22.6	20.5	**21.5**
Halo-Mix (B_4)	22.7	16.0	**19.3**
Mean	**21.9**	**17.0**	

CD

B 1.87

S 1.19

BxS 2.65

4.3.2.3 Number of grains/panicle

The data pertaining to the number of grains/panicle of rice affected by different microbial formulations is presented in table 4.20. The mean value of no. of grains/panicle for S_0 and S_1 were 112.4 to 76.3 respectively, whereas the mean obtained by application of different bioformulations were 84.0, 92.8, 94.3, 100.2 and 100.3 for B_0, B_1, B_2, B_3 and B_4, respectively. The number of grains/panicle was significantly higher in S_0 as compared to S_1. Application of bioformulations in sodic soils, significantly increased number of grains/panicle over control (59.7). Treatment S_1B_3 (84.7) among the S_1 factors had the highest number of grains/paniclewhich was at par with treatment S_1B_1 (80.0). A significant increase in number of grains/paniclewas also observed in S_0B_4 (120.7) over S_0B_0 (108.3).

4.3.2.4 Grain Yield (q ha ^{1})

The data pertaining to the grain yield of rice affected by different microbial formulations is presented in table 4.21. The mean value of grain yield for S_0 and S_1 were 56.6 q ha^{-1} and 30.6 q ha^{-1} respectively, whereas the mean obtained by application of different bioformulations were 36.8, 42.3, 45.0, 47.2 and 46.7 q ha^{-1} for B_0, B_1, B_2, B_3 and B_4, respectively. The grain yield was significantly higher in S_0 as compared to S_1. Application of bioformulations in sodic soils, significantly increased grain yield over control (18.6 q ha^{-1}). Treatment S_1B_3 (34.6 q ha^{-1}) among the S_1 factors had the highest grain yield which was at par with treatment S_1B_1 (34.4 q ha^{-1}),

S_1B_2 (32.1 q ha^{-1}) and S_1B_4 (33.1 q ha^{-1}). No significant increase in grain yield was observed in normal soil as a result of bioformulation applications.

Table 4.20 Effect of halophilic biofertilizer formulations on number of grains/panicle of rice crop.

Treatments	Normal (S_0)	Sodic (S_1)	Mean
Control (B_0)	108.3	59.7	**84.0**
Halo-Azo (B_1)	105.7	80.0	**92.8**
Halo- PSB (B_2)	111.7	77.0	**94.3**
Halo-Zinc (B_3)	115.7	84.7	**100.2**
Halo-Mix (B_4)	120.7	80.0	**100.3**
Mean	**112.4**	**76.3**	
CD			
B 11.2			
S 7.1			
BxS 15.9			

Table 4.21 Grain yield (q ha^{-1}) of rice as influenced by different halophilic treatments.

Treatments	Normal (S_0)	Sodic (S_1)	Mean
Control (B_0)	55.1	18.6	**36.8**
Halo-Azo (B_1)	50.3	34.4	**42.3**
Halo- PSB (B_2)	57.8	32.1	**45.0**
Halo-Zinc (B_3)	59.8	34.6	**47.2**
Halo-Mix (B_4)	60.3	33.1	**46.7**
Mean	**56.6**	**30.6**	
CD			
B 7.19			
S 4.54			
BxS 10.16			

4.3.2.5 Straw Yield (q ha^{-1})

The data pertaining to the straw yield of rice affected by different microbial formulations is presented in table 4.22. The mean value of straw yield for S_0 and S_1 were 105.2 q ha^{-1} and 58.4 q ha^{-1} respectively, whereas the mean obtained by application of different bioformulations were 72.5, 85.9, 80.8, 81.4 and 88.3 q ha^{-1} for B_0, B_1, B_2, B_3 and B_4, respectively. The straw yield was significantly higher in S_0 as compared to S_1. Application of bioformulations in sodic soils, significantly increased straw yield over control (43.3 q ha^{-1}). Treatment S_1B_1 (69.6 q ha^{-1}) among the S_1 factors had the highest straw yield which was at par with treatment S_1B_4 (63.3 q ha^{-1}), S_1B_2 (60.3 q ha^{-1}) and S_1B_3 (55.5 q ha^{-1}). No significant increase in straw yield was observed in normal soil due to application of bioformulations.

Table 4.22 Straw yield (q ha ¹) of rice as influenced by different halophilic treatments.

Treatments	Normal (S_0)	Sodic (S_1)	Mean
Control (B_0)	101.8	43.3	**72.5**
Halo-Azo (B_1)	102.1	69.6	**85.9**
Halo- PSB (B_2)	101.3	60.3	**80.8**
Halo-Zinc (B_3)	107.3	55.5	**81.4**
Halo-Mix (B_4)	113.4	63.3	**88.3**
Mean	**105.2**	**58.4**	

CD

B	10.6
S	6.7
BxS	15.0

4.3.2.6 Harvest Index

The data pertaining to the harvest index of rice affected by different microbial formulations is presented in table 4.23. The mean value of harvest index for S_0 and S_1 were 54.4 percent and 53.2 percent respectively, whereas the mean obtained by application of different bioformulations were 50.2, 48.8, 56.6, 60.0 and 53.4 percent for B_0, B_1, B_2, B_3 and B_4, respectively. No significant differences in harvest index were observed under various treatment combinations.

4.3.2.7 1000 Grain weight

A perusal of data in table 4.24 revealed that the mean value of 1000 grain weight for S_0 and S_1 were 23.4 g and 16.5 g respectively, whereas the mean obtained by application of different bioformulations were 19.0, 20.0, 19.7, 20.6 and 20.4 g for B_0, B_1, B_2, B_3 and B_4, respectively. The 1000 grain weight remained significantly higher in S_0 as compared to S_1. Application of bioformulations in sodic soils, significantly increased 1000 grain weight over control (15.1 g). Treatment S_1B_3 (17.5 g) among the S_1 factors had the highest 1000 grain weight which was at par with treatment S_1B_1 (17.3 g), S_1B_2 (16.2 g) and S_1B_4 (16.4 g). A significant increase in

1000 grain weight was also observed in S_0B_4 (24.3 g) over S_0B_0 (22.9 g), while all other treatments were at par within normal soils.

Table 4.23 Effect of halophilic biofertilizer formulations on Harvest index (%) of rice crop.

Treatments	Normal (S_0)	Sodic (S_1)	Mean
Control (B_0)	52.6	47.9	**50.2**
Halo-Azo (B_1)	49.8	47.7	**48.8**
Halo- PSB (B_2)	57.5	55.6	**56.6**
Halo-Zinc (B_3)	56.6	63.3	**60.0**
Halo-Mix (B_4)	55.3	51.5	**53.4**
Mean	**54.4**	**53.2**	

CD

B 11.47

S 7.26

BxS 16.23

Table 4.24 Effect of halophilic biofertilizer formulations on 1000 grains weight (g) of rice crop.

Treatments	Normal (S_0)	Sodic (S_1)	Mean
Control (B_0)	22.9	15.1	**19.0**
Halo-Azo (B_1)	22.6	17.3	**20.0**
Halo- PSB (B_2)	23.2	16.2	**19.7**
Halo-Zinc (B_3)	23.8	17.5	**20.6**
Halo-Mix (B_4)	24.3	16.4	**20.4**
Mean	**23.4**	**16.5**	

CD

B 1.23

S 0.78

BxS 1.74

4.4 Plant analysis

4.4.1 Nutrient concentration

4.4.1.1 Nitrogen concentration in grains

A perusal of data in table 4.25 revealed that the mean value of nitrogen content in grains for S_0 and S_1 were 1.19 percent and 1.14 percent respectively, whereas the mean obtained by application of different bioformulations were 1.15, 1.20, 1.15, 1.13 and 1.18 percent for B_0, B_1, B_2, B_3 and B_4, respectively. No significant differences in nitrogen content in grains were observed under various treatment combinations.

4.4.1.2 Nitrogen concentration in straw

A perusal of data in table 4.26 revealed that the mean value of nitrogen content in straw for S_0 and S_1 were 0.73 percent and 0.70 percent respectively, whereas mean obtained by application of different bioformulations were 0.70, 0.74, 0.71, 0.68 and 0.75 percent for B_0, B_1, B_2, B_3 and B_4, respectively. No significant differences in nitrogen content in straw were observed under various treatment combinations.

Table 4.25 Nitrogen concentration in grains (%) of rice as influenced by different treatments.

Treatments	Normal (S_0)	Sodic (S_1)	Mean
Control (B_0)	1.19	1.10	**1.15**
Halo-Azo (B_1)	1.23	1.18	**1.20**
Halo- PSB (B_2)	1.15	1.15	**1.15**
Halo-Zinc (B_3)	1.17	1.10	**1.13**
Halo-Mix (B_4)	1.19	1.16	**1.18**
Mean	**1.19**	**1.14**	

CD

B NS

S NS

BxS NS

Table 4.26 Nitrogen concentration in straw (%) of rice as influenced by different treatments.

Treatments	Normal (S_0)	Sodic (S_1)	Mean
Control (B_0)	0.72	0.67	**0.70**
Halo-Azo (B_1)	0.73	0.75	**0.74**
Halo- PSB (B_2)	0.76	0.67	**0.71**
Halo-Zinc (B_3)	0.69	0.67	**0.68**
Halo-Mix (B_4)	0.77	0.72	**0.75**
Mean	**0.73**	**0.70**	

CD

B NS

S NS

BxS NS

4.4.1.3 Phosphorus concentration in grains

A perusal of data in table 4.27 revealed that the mean value of phosphorus content in grains for S_0 and S_1 were 0.21 percent and 0.23 percent respectively, whereas the mean obtained by application of different bioformulations were 0.22, 0.21, 0.24, 0.20 and 0.24 percent for B_0, B_1, B_2, B_3 and B_4, respectively. No significant differences in phosphorus content in grains were observed under various treatment combinations.

Table 4.27 Phosphorus concentration in grains (%) of rice as influenced by different treatments.

Treatments	Normal (S_0)	Sodic (S_1)	Mean
Control (B_0)	0.22	0.22	**0.22**
Halo-Azo (B_1)	0.19	0.23	**0.21**
Halo- PSB (B_2)	0.21	0.26	**0.24**
Halo-Zinc (B_3)	0.20	0.19	**0.20**
Halo-Mix (B_4)	0.23	0.24	**0.24**
Mean	**0.21**	**0.23**	

CD

B	NS
S	NS
BxS	NS

4.4.1.4 Phosphorus concentration in straw

A perusal of data in table 4.28 revealed that there were no significant differences in phosphorus content in straw under various treatment combinations. The mean value of phosphorus content in straw for S_0 and S_1 were 0.13 percent and 0.14 percent respectively, whereas the mean obtained by application of different bioformulations were 0.13, 0.13, 0.14, 0.14 and 0.15 percent for B_0, B_1, B_2, B_3 and B_4, respectively.

4.4.1.5 Potassium concentration in grains

A perusal of data in table 4.29 revealed that the mean value of potassium content in grains for S_0 and S_1 were 0.29 percent to 0.18 percent respectively. The mean values for potassium concentration obtained by application of different bioformulations were 0.22, 0.24, 0.24, 0.24 and 0.23 percent for B_0, B_1, B_2, B_3 and B_4, respectively. Potassium concentration was higher in rice grains grown under normal soils as compared to sodic soils. No impact of bioformulations was however, observed on the potassium concentration in grains.

Table 4.28 Phosphorus concentration in straw (%) of rice as influenced by different treatments.

Treatments	Normal (S_0)	Sodic (S_1)	Mean
Control (B_0)	0.13	0.13	**0.13**
Halo-Azo (B_1)	0.13	0.13	**0.13**
Halo- PSB (B_2)	0.14	0.14	**0.14**
Halo-Zinc (B_3)	0.13	0.14	**0.14**
Halo-Mix (B_4)	0.14	0.16	**0.15**
Mean	**0.13**	**0.14**	

CD	
B	NS
S	NS
BxS	NS

Table 4.29 Potassium concentration in grains (%) of rice as influenced by different treatments.

Treatments	Normal (S_0)	Sodic (S_1)	Mean
Control (B_0)	0.27	0.17	**0.22**
Halo-Azo (B_1)	0.29	0.18	**0.24**
Halo- PSB (B_2)	0.28	0.20	**0.24**
Halo-Zinc (B_3)	0.30	0.17	**0.24**
Halo-Mix (B_4)	0.28	0.18	**0.23**
Mean	**0.29**	**0.18**	

CD	
B	0.03
S	0.02
BxS	0.04

4.4.1.6 Potassium concentration in straw

A perusal of data in table 4.30 revealed that the mean value of potassium content in straw for S_0 and S_1 were 1.44 percent and 0.52 percent respectively, whereas the mean obtained by application of different bioformulations were 0.91, 1.02, 1.04, 0.91 and 0.94 the mean for B_0, B_1, B_2, B_3 and B_4, respectively. Potassium concentration was significantly higher in rice straw grown under normal soils as compared to sodic soils. No impact of bioformulations were however, observed on the potassium concentration in straw.

Table 4.30 Potassium concentration in straw (%) of rice as influenced by different treatments.

Treatments	Normal (S_0)	Sodic (S_1)	Mean
Control (B_0)	1.52	0.44	**0.98**
Halo-Azo (B_1)	1.51	0.53	**1.02**
Halo- PSB (B_2)	1.45	0.62	**1.04**
Halo-Zinc (B_3)	1.34	0.47	**0.91**
Halo-Mix (B_4)	1.35	0.53	**0.94**
Mean	**1.44**	**0.52**	

CD

B 0.23

S 0.15

BxS 0.33

4.4.2 Nutrient uptake

4.4.2.1 Nitrogen uptake in grain

A perusal of data in table 4.31 revealed that the mean value of nitrogen uptake in grain for S_0 and S_1 were 67.35 kg ha^{-1} and 34.85 kg ha^{-1} respectively, whereas the mean obtained by application of different bioformulations were 43.13, 51.04, 51.81, 54.13 and 55.39 kg ha^{-1} for B_0, B_1, B_2, B_3 and B_4, respectively. The nitrogen uptake was significantly higher in S_0 as compared to S_1. Application of bioformulations in sodic soils, significantly increased nitrogen uptake over control (20.55 kg ha^{-1}). Treatment S_1B_1 (40.30 kg ha^{-1}) among the S_1 factors had the highest nitrogen uptake which was at par with treatment S_1B_4 (38.42 kg ha^{-1}), S_1B_3 (38.14 kg ha^{-1}) and S_1B_2 (36.85 kg ha^{-1}). No significant increase in nitrogen uptake was observed in normal soil as a result of bioformulation applications.

4.4.2.2 Nitrogen uptake in straw

A perusal of data in table 4.32 revealed that the mean value of nitrogen uptake in straw for S_0 and S_1 were 76.89 kg ha^{-1} to 40.87 kg ha^{-1} respectively, whereas the mean obtained by application of different bioformulations were 50.60, 63.19, 58.52, 55.63 and 66.47 kg ha^{-1} for B_0, B_1, B_2, B_3 and B_4, respectively. The nitrogen uptake was significantly higher in S_0 as compared to S_1. Application of *Azospirillium* based bioformulations in sodic soils, significantly increased nitrogen uptake over control (28.62 kg ha^{-1}). Treatment S_1B_1 (52.36 kg ha^{-1}) among the S_1 factors had the highest nitrogen uptake which was at par with treatment S_1B_4 (45.80 kg ha^{-1}) and S_1B_2 (40.16

kg ha^{-1}). A significant increase in nitrogen uptake in straw was also observed in S_0B_4 (87.14 kg ha^{-1}) over S_0B_0 (72.58 kg ha^{-1}).

Table 4.31 Nitrogen uptake in grain (kg ha^{-1}) of rice as influenced by different treatments.

Treatments	Normal (S_0)	Sodic (S_1)	Mean
Control (B_0)	65.71	20.55	**43.13**
Halo-Azo (B_1)	61.79	40.30	**51.04**
Halo- PSB (B_2)	66.77	36.85	**51.81**
Halo-Zinc (B_3)	70.13	38.14	**54.13**
Halo-Mix (B_4)	72.37	38.42	**55.39**
Mean	**67.35**	**34.85**	

CD

B 10.75

S 6.80

BxS 15.20

Table 4.32 Nitrogen uptake in straw (kg ha^{-1}) of rice as influenced by different treatments.

Treatments	Normal (S_0)	Sodic (S_1)	Mean
Control (B_0)	72.58	28.62	**50.60**
Halo-Azo (B_1)	74.03	52.36	**63.19**
Halo- PSB (B_2)	76.87	40.16	**58.52**
Halo-Zinc (B_3)	73.84	37.42	**55.63**
Halo-Mix (B_4)	87.14	45.80	**66.47**
Mean	**76.89**	**40.87**	

CD

B 9.81

S 6.20

BxS 13.87

4.4.2.3 Phosphorus uptake in grain

A perusal of data in table 4.33 revealed that the mean value of phosphorus uptake in grain for S_0 and S_1 were 12.14 kg ha^{-1} and 6.99 kg ha^{-1} respectively, whereas the mean obtained by application of different bioformulations were 8.17, 8.88, 10.37, 9.40 and 10.99 kg ha^{-1} for B_0, B_1, B_2, B_3 and B_4, respectively. The phosphorus uptake was significantly higher in S_0 as compared to S_1. Application of bioformulations in sodic soils, significantly increased phosphorus uptake over control (3.99 kg ha^{-1}).

Treatment S_1B_2 (8.27 kg ha^{-1}) among the S_1 factors had the highest phosphorus uptake which was at par with treatment S_1B_4 (7.98 kg ha^{-1}), S_1B_1 (7.97 kg ha^{-1}) and S_1B_3 (6.73 kg ha^{-1}). Although, S_1B_3 was not significantly higher over control (S_1B_0). No significant increase in phosphorus uptake in grain was observed in normal soil as a result of bioformulation applications.

Table 4.33 Phosphorus uptake in grain (kg ha^{-1}) of rice as influenced by different treatments.

Treatments	Normal (S_0)	Sodic (S_1)	Mean
Control (B_0)	12.34	3.99	**8.17**
Halo-Azo (B_1)	9.80	7.97	**8.88**
Halo- PSB (B_2)	12.487	8.27	**10.37**
Halo-Zinc (B_3)	12.06	6.73	**9.40**
Halo-Mix (B_4)	14.00	7.98	**10.99**
Mean	**12.14**	**6.99**	

CD

B	2.62
S	1.66
BxS	3.71

4.4.2.4 Phosphorus uptake in straw

A perusal of data in table 4.34 revealed that the mean value of phosphorus uptake in straw for S_0 and S_1 were 14.02 kg ha^{-1} to 8.23 kg ha^{-1} respectively, whereas the mean obtained by application of different bioformulations were 9.30, 11.37, 11.18, 11.09 and 12.67 kg ha^{-1} for B_0, B_1, B_2, B_3 and B_4, respectively. The phosphorus uptake was significantly higher in S_0 as compared to S_1. Application of bioformulations in sodic soils, significantly increased phosphorus uptake over control (5.76 kg ha^{-1}). Treatment S_1B_4 (9.88 kg ha^{-1}) among the S_1 factors had the highest phosphorus uptake which was at par with treatment S_1B_1 (9.14 kg ha^{-1}), S_1B_2 (8.46 kg ha^{-1}) and S_1B_3 (7.88 kg ha^{-1}). A significant increase in phosphorus uptake in straw was also observed in S_0B_4 (15.47 kg ha^{-1}) over S_0B_0 (12.84 kg ha^{-1}).

4.2.5 Potassium uptake in grain

A perusal of data in table 4.35 revealed that the mean value of potassium uptake in grain for S_0 and S_1 were 16.12 kg ha^{-1} and 5.58 kg ha^{-1} respectively, whereas the mean obtained by application of different bioformulations were 8.98, 10.58, 11.26, 12.04 and 11.37 kg ha^{-1} for B_0, B_1, B_2, B_3 and B_4, respectively. The potassium uptake was significantly higher in S_0 as compared to S_1. Application of bioformulations in sodic soils, significantly increased potassium uptake over control (3.20 kg ha^{-1}). Treatment S_1B_2 (6.41 kg ha^{-1}) among the S_1 factors had the highest potassium uptake which was at par with treatment S_1B_1 (6.31 kg ha^{-1}), S_1B_4 (6.05 kg ha^{-1}) and S_1B_3 (5.91 kg ha^{-1}). A significant increase in potassium uptake in grain was also observed in S_0B_3 (18.18 kg ha^{-1}) and S_0B_4 (16.69 kg ha^{-1}) over S_0B_0 (14.77 kg ha^{-1}).

Table 4.34 Phosphorus uptake in straw (kg ha^{-1}) of rice as influenced by different treatments.

Treatments	Normal (S_0)	Sodic (S_1)	Mean
Control (B_0)	12.84	5.76	**9.30**
Halo-Azo (B_1)	13.60	9.14	**11.37**
Halo- PSB (B_2)	13.90	8.46	**11.18**
Halo-Zinc (B_3)	14.29	7.88	**11.09**
Halo-Mix (B_4)	15.47	9.88	**12.67**
Mean	**14.02**	**8.23**	

CD

B	1.70
S	1.08
BxS	2.41

Table 4.35 Potassium uptake in grain (kg ha^{-1}) of rice as influenced by different treatments.

Treatments	Normal (S_0)	Sodic (S_1)	Mean
Control (B_0)	14.77	3.20	**8.98**
Halo-Azo (B_1)	14.86	6.31	**10.58**
Halo- PSB (B_2)	16.11	6.41	**11.26**
Halo-Zinc (B_3)	18.18	5.91	**12.04**
Halo-Mix (B_4)	16.69	6.05	**11.37**
Mean	**16.12**	**5.58**	

CD

B	1.85
S	1.17
BxS	2.61

4.4.2.6 Potassium uptake in straw

A perusal of data in table 4.36 revealed that the mean value of potassium uptake in straw for S_0 and S_1 were 148.1 kg ha^{-1} and 30.7 kg ha^{-1} respectively, whereas the mean obtained by application of different bioformulations were 87.9, 95.9, 91.3, 85.3 and 92.6 kg ha^{-1} for B_0, B_1, B_2, B_3 and B_4, respectively. Potassium uptake by straw was significantly higher in normal soils as compared to sodic soils. No significant differences in potassium uptake in straw, was however observed with respect to bioformulations.

Table 4.36 Potassium uptake in straw (kg ha^{-1}) of rice as influenced by different treatments.

Treatments	**Normal (S_0)**	**Sodic (S_1)**	**Mean**
Control (B_0)	156.1	19.6	**87.9**
Halo-Azo (B_1)	154.9	36.9	**95.9**
Halo- PSB (B_2)	145.7	36.9	**91.3**
Halo-Zinc (B_3)	144.2	26.4	**85.3**
Halo-Mix (B_4)	151.6	33.6	**92.6**
Mean	**148.1**	**30.7**	

CD

B	25.00
S	15.81
BxS	35.36

Chapter-V

Discussion

CHAPTER-V

DISCUSSION

The results of present investigation entitled "Improving health of salt-affected soils through microbial formulations" presented in the previous chapter are discussed here in the light of study duly supported by available relevant literature and shreds of evidence to establish cause and effect relationship. The entire discussion has been partitioned into following major parts:

5.1 Chemical Parameters

5.2 Biological Parameters

5.3 Physiological Parameters

5.4 Plant nutrient concentration and uptake

5.1 Chemical Parameters

The soil's chemical condition affects soil-plant relationships by determining the capacity of soil to hold, supply, and cycle nutrients and influence the movement and availability of water. The normal soils and sodic soils used for the transplantation of rice crop were analyzed for their chemical properties before and after transplantation of rice crop.

5.1.1 Soil pH, EC and available sodium

Soil pH is the single soil characteristics, which elucidates an overall picture of the medium for plant growth including nutrient supply trend, fate of added nutrients, salinity/sodicity status, soil aeration, soil minerology and ultimate weather conditions of the region (Zhao, 2011). High pH of the sodic soils is related with the increase in sodium content of these soils in combination with carbonates and bicarbonates. It essentially means that if the sodium can be removed from the soil, it will bring down the soil pH as well.

In case of S_1 (Sodic soils), the pH of the soil decreased upon application of salt tolerant bioformulations in comparison to control, with maximum decrease in pH measured under treatment Halo-mix (8.61). The differences between bioformulation application and control (S_1B_0) were significant. However, the mean soil pH remained significantly higher in sodic soil (8.94) as compared to normal soil (7.97), even after application of bioformulation. Similar results have been reported by Sahay *et al.* (2018).

The higher pH of sodic soils may be due to presence of soluble and exchangeable Na^+ along with CO_3^{2-}, HCO_3^- ions. The high pH of sodic soils has no direct adverse effect on plant growth, but it frequently results in lowering the availability of some essential plant nutrients. The halotolerant bio-fertilizers formulations showed potential effect on the soil pH and minimized the negative effect of sodic soil on the crop and improved the quality of the soil. However, these microorganisms produce organic acid, thereby decreasing the soil pH and releasing calcium, which helps to prevent soil dispersion. Arora *et al.* (2016) also reported that soil pH was reduced by applying Halo-Mix in sodic soils. Soil respiration (CO_2 emission), organic matter decomposition and root exudates may be responsible for reduction in pH of sodic soils (Singh *et al.*, 2013). Root exudates are made up of organic acids, sugars, amino acids, harmones and mucilage (Jamaluddin and Shukla, 2012) that also play important role to reduce soil pH. The increase in plant biomass due to application of halo-tolerant bioformulations could have certain extent affected this process.

The decrease in pH can be directly linked to the reduction in soil sodium content at harvest following the same trend as that of pH. There was significant decrease in available sodium content of the soil upon application of bio-formulations in sodic soils, while no significant differences were observed in normal soils. Halo-mix application recorded highest decrease in sodium (187.5 mg kg^{-1}) in sodic soils over control. The sodium remained significantly higher in sodic soils (209.6 mg kg^{-1}) as compared to normal soil (33.3 mg kg^{-1}), even after application of bioformulation. The high content of sodium indicates the high salinity in soil and are associated with high OH^- content, where it produces a negative effect on soil stability generating salt crusts (Garcia *et al.*, 2018). Halophilic bacteria improved sodium stress by increasing the relative humidity in plants and ion homeostasis (Soleimani *et al.*, 2016). These organisms can produce bacterial exopolysaccharides (EPS) to bind cations as well as increase the population density of EPS-producing bacteria in the root zone which would reduce the content of Na^+ available for plant uptake, thus help in alleviating salt stress in plants growing in saline environments. Han and Lee, (2005). Arora *et al.* (2016) founded that Na content decreased with inoculation in surface soil layers indicative remediation potential of these strains. They observed that with the co-inoculation of Halophilic consortia and FYM, maximum decrease in sodium content (323.8 to 197.4 mg kg^{-1}) was observed. It has been reported that halophilic microorganisms can use different ions such as Na^+ or Cl^- which are important for their survival. Na^+ can be used for halophilic bacteria as amino acid transport dependent (Na^+ symport). On the other hand, anions such as Cl^- participates in the volume increase during cell division and can act as co-transporter with sodium ions using the light-driven primary chloride pump halorhodopsin (Muller and Oren, 2003).

The beneficial effect of *Azospirillum* and halotolerant N-fixers inoculation was also evident (Creus *et al*., 1997; Arora *et al*., 2015). *Azospirillum* inoculated wheat seedlings subjected to osmotic stress developed significant better water status than non-inoculates seedlings (Creus *et al*., 1998). It was observed that in surface soils, Na content substantially reduced and microbial biomass, enzyme activities as well as available N and P content got enhanced. Surface soil pH got reduced substantially in inoculated treatments. Maximum reduction in pH was noted in treatments where consortia of halotolerant N-fixer, phosphate solubilizer and halo-zinc were inoculated.

The result trends for electrical conductivity were similar to those of pH and available sodium. EC is a soil parameter that indicates the total concentration of soluble salts and is a direct measurement of salinity. Application of bioformulations in sodic soil significantly decreased the EC over where no bioformulation was applied. However, no such change was observed in normal soils. The soil EC remained significantly higher in sodic soils (0.46 dS m^{-1}) as compared to normal soils (0.25 dS m^{-1}), even after application of bioformulation. The decrease in pH of the soil might has also enhanced the discharge of inorganic salts thereby decreasing EC of the soil (Liu *et al*., 2007 and Bai, 2010). Gandhi and Muralidharan, (2016) reported that the rice crop treated with selected zinc solubilizer bacteria inoculants significantly decreased EC of the soil as compared to control. A decrease in EC to 3.25 dS m^{-1} from 4.28 dS m^{-1} with the inoculation of PSB in sweet basil (*O.basilicum*) was reported by Sahay and Patra, (2014).

5.1.2 Organic Carbon (OC)

Application of halophilic bioformulations significantly increased soil organic carbon at harvest in sodic soils. Similar results have been reported by Sahay *et al.*

(2018). They found that with the co-inoculation treatment of Halo-Mix (Halo-Azo and Halo- PSB) in sodic soil, organic carbon was increased up to 20 percent. Singh and Najar, (2007) recorded that the organic carbon (1.08 percent) was highest with the application of combined inoculation of *Rhizobium* + *Azotobacter* + PSB + FYM. Organic carbon is an indicator of organic fraction in the soil formed from microbial decomposition of organic residues. It acts as major regulating factor of organic forms of nitrogen, phosphorus, sulphur and trace elements in the soils (Stevenson, 1982).

The organic carbon was significantly higher in normal soil (0.89 percent) as compared to sodic soil (0.57 percent). With the onset of salinity and sodicity levels, native soil organic matter is rapidly lost as its solubility, decomposability and accessibility increases. Concurrently, C inputs into the soil are decreased. The low status of organic carbon in sodic soil could be due to strongly alkaline condition which might have dissolved the humic substances of soil and further loss from soil. Soil organisms use organic matter as a source of energy and food. Thus, the organic matter added to the soil is converted by oxidative decomposition to simpler substances which are made available in stages for plant growth and the residue is transformed into humus. The roots of the plants release substances or exudates, into the soil. These exudates include sugar, amino acids, vitamins, long chain carbohydrates, enzymes and lysates which are released when roots cell break. These exudates are decomposed primarily by bacteria. These bacteria will respire the carbon compounds through the TCA cycle; however, fermentation is also present (Khatoon *et al.*, 2017).

5.1.3 Available nutrients

The effect of biofertilizer formulations on available nitrogen in soil is recorded highest in case of normal soil, under the treatment Halo-mix (204.4 kg ha^{-1}) and the least nitrogen was recorded under the control (194.1 kg ha^{-1}), whereas in case of S_1, highest nitrogen was observed with the inoculation of Halo-Azo treatment (168.2 kg ha^{-1}) and the least nitrogen was observed in control (86.6 kg ha^{-1}). Application of Halo-bioformulations significantly increased the available N content in soil in sodic soils. Similar results have been showed by Sahay *et al*. (2018) in which they observed that with the inoculation treatment of Halo-Mix (Halo-Azo and Halo- PSB) in sodic soil, available nitrogen was increased up to 9.9 percent, respectively. The application of halophilic bacteria increases the available nitrogen by converting the organic nitrogen into the inorganic form. Nitrogen (N) is one of the most important nutrients for plant growth and its availability often limits plant productivity, i.e. plant biomass can only increase if more N is made available to them. During microbial decomposition of organic material N and other nutrients bound in dead organic matter are released as ammonium (NH_4^+) and made available for uptake by both microorganisms and plants. By recycling nutrients essential for plant growth, soil microorganisms are vital for the maintenance of soil fertility. The beneficial effect of *Azospirillum* and halotolerant N-fixers inoculation was also evident (Creus *et al*., 1997; Arora *et al*., 2015). *Azospirillum* inoculated wheat seedlings subjected to osmotic stress developed significant better water status than non-inoculated seedlings (Creus *et al*., 1998). Further, the available nitrogen in soil remained significantly higher in normal soil (199.3 kg ha^{-1}) as compared to sodic soil (140.4 kg ha^{-1}). In saline ecosystems the conditions created by the density of the soil and the water regime are limiting for nitrogen fixation (Rice and Paul, 1971). In sodic soil, the high pH declines the organic carbon status of sodic which indicates low status of available nitrogen in these soils.

The effect of biofertilizer formulations on available phosphorus in soil is recorded highest in case of S_0, under the treatment Halo-PSB (12.8 kg ha^{-1}) and the lowest phosphorus was recorded under the control (10.4 kg ha^{-1}), whereas in case of S_1, highest phosphorus was observed with the inoculation of Halo-mix treatment (14.8 kg ha^{-1}) and the least phosphorus was observed in control (11.1 kg ha^{-1}). The trend was similar to that of available nitrogen in the sense that application of bioformulations resulted in increased available phosphorus content in the soil. Similar results have been observed by Sahay *et al*. (2018) in which they observed that with the inoculation treatment of Halo-Mix (Halo-Azo and Halo-PSB) in sodic soil, available phosphorus was increased by up to 16.8 percent, respectively. The application of bio-phosphate increased the availability of N, K and especially P in the soil. The availability of phosphorus increased by the application of bio-phosphate which increased the population of phosphate dissolving microorganisms surrounding the roots, that the high density increased the P solubility until it became available to the plants (Rao, 1982; Harley and Smith, 1983). Arora *et al*. (2016) reported that inoculation of halophilic bacteria and FYM significantly increased the phosphorus (9.9 to 15.6 kg ha^{-1}) availability in sodic soil. Use of phosphate solubilizing bacteria has been reported to be promising in reducing phosphate fixation and increasing the phosphorous availability from soluble and insoluble phosphatic fertilizers (Mahadevaswamy and Nagaraju, 2018). The most common mechanism used by these microorganisms for solubilizing tri-calcium phosphate seems to be acidification of the medium via biosynthesis and release of a wide variety of organic acids (Rodriguez and Fraga, 1999). Strains of *Bacillus licheniformis* and *B. amyloliquefaciens* were found to produce mixtures of lactic, isovaleric, isobutyric, and acetic acids. Other organic acids, such as glycolic acid, oxalic acid, malonic acid, succinic acid, citric acid and propionic acid, have also been identified among phosphate solubilizers (Illmer and Schinner, 1992; Banik and Dey 1982; Chen *et al.*, 2006). Alternative possibilities other than organic acids include the release of H^+ to the outer surface in exchange for cation uptake or ATPase which can constitute alternative ways, with the help of H^+ translocation, for solubilization of mineral phosphates (Rodriguez and Fraga, 1999).

The effect of biofertilizer formulations on available potassium in soil is recorded highest in case of S_0, under the treatment Halo-mix (217.1 kg ha^{-1}) and the least potassium was observed under control (209.2 kg ha^{-1}), whereas in case of S_1, highest potassium was observed under treatment Halo-mix (187.2 kg ha^{-1}) and the least potassium was observed under control (152.7 kg ha^{-1}). Halophilic bacteria produces IAA and ACC which reduces ethylene production and increase potassium concentration. Singh and Najar, (2007) recorded that the combined inoculation of *Rhizobium* + *Azotobacter* + PSB + FYM significantly increased available potassium (222.3 kg ha^{-1}). It has been observed that halophilic organisms are able to influence soil fertility through soil processes viz. decomposition, mineralization, and storage/ release of nutrients (Parmar and Sindhu, 2013). These organisms solubilize the insoluble K to soluble forms of K by various mechanisms including production of inorganic and organic acids, acidolysis, polysaccharides complexolysis, chelation, and exchange reactions (Etesami *et al.*, 2017). During acidolysis, these organisms in soil decrease the local pH by producing succinic, citric, gluconic, alpha-keto gluconic and oxalic acids. Production of protons (H^+) and organic acids anions not only enhances the chelation of cations which are bound to K but also helps for acidolysis of surrounding environment of microbes (Uroz *et al.*, 2009; Parmar and Sindhu, 2013). During the process of acidolysis, rhizospheric microbes can chelate Al and Si cations associated with K minerals and by doing so they also enhance the exchangeable K in soil solution (Romheld and Kirkby, 2010).

The highest available zinc is recorded in case of S_0, under the control (0.86 mg kg^{-1}) and the least zinc was observed under treatment Halo-Zinc (0.73 mg kg^{-1}), whereas in case of S_1, highest zinc was observed under treatment Halo-Zinc (0.79 mg kg^{-1}) and the least zinc was observed under control (0.66 mg kg^{-1}). Ramesh *et al*. (2014) reported that strains MDSR7 and MDSR14 (zinc solubilizer bacteria) produced substantially higher soluble zinc content with significant decline in pH. Many Indian soil exhibit Zn deficiency. The conditions that make unavailability of zinc to plants are high pH, low organic matter content, high usage of P fertilizer, less textured soil and utilization of synthetic fertilizer to correct Zn deficiency which results in availability of zinc after seven days of application. An alternative eco-friendly approach to overcome Zn deficiency constraint in plants and soil is by the application of microbial inoculants as a biofertilizer. Rhizospheric microorganisms play a vital role in the conversion of unavailable form of metal to available form through solubilization mechanism (Kumar *et al*., 2014). Gandhi and Muralidharan, (2016) reported that with the inoculation of zinc solubilizer bacteria (*Acinetobacter* sp.) in rice crop, there was significant increase in exchangeable zinc, crystalline and amorphous bound zinc and a decrease organically complexed and carbonate bound zinc as compared to control. Dinesh *et al*. (2018) also reported that with the inoculation of zinc solubilizer bacteria, significantly increased available zinc in soil up to (2.96 mg kg^{-1}) as compared to control.

Overall, it was observed that all the available nutrients studied were in significantly higher concentration in normal soils as compared to sodic soils, except for available phosphorus which followed a reverse trend. High pH, the main characteristic of sodic soils, tends to decrease the availability of nitrogen, potassium and zinc. However, in case of available phosphorus the decline is only up to around a pH of 8.5. Increase sodium content competes with calcium, which decreases the precipitation of phosphorus to tricalcium phosphate. Furthermore, it was noted that no significant impact of bioformulations was observed within the normal soils on their available nutrient content. The bioformulations used were based on halophilic microorganisms who proliferate in saline/sodic environments. Their activity is mainly confined to sodium rich soils.

5.2 Biological Parameters

5.2.1 Microbial Biomass Carbon (MBC)

In sodic soils, highest microbial biomass carbon was observed with the inoculation of Halo-mix treatment (90.8 µg g^{-1}) and the least microbial biomass carbon (MBC) was observed in control, (69.1 µg g^{-1}) where no bioformulation was inoculated. The mean microbial biomass carbon was significantly higher in normal soil (113.8 µg g^{-1}) as compared to sodic soil (84.8 µg g^{-1}). The highest soil microbial biomass carbon values in normal soil may be due to the highest counts of soil microbes and their activities, also high availability of organic carbon, nutrients, optimum moisture and aeration (Doran *et al*., 2006). Salinity and sodicity exert deterimental effects on both microbial biomass and thereby on metabolic quotient. Therefore, the metabolic quotient may indicate the response of microbial population under stress (Wong *et al*., 2008). Arora *et al*. (2016) reported that with the co-inoculation treatment of consortia + FYM, MBC was significantly increased (82 to 129 µg g^{-1}). Wong *et al*. (2004) reported that the levels of MBC was very low in the saline-sodic soil compared to the normal degraded soil. The low levels of MBC and respiration rates were the result of the low levels of soil organic carbon. In our study also we have obtained very low organic carbon under sodic soils, which improved upon application of bioinoculants.

5.2.2 Enzymatic Activity

Both dehydrogenase activity and phosphatase activity was affected by the type of soil and application of halophilic formulations. The effect of biofertilizer formulations on enzymatic activity were observed in both type of soils unlike most other parameters. In sodic soils the quantum of increase in enzymatic activity was, however, higher and significant, upon application of bioformulations. In normal soils only the dehydrogenase activity was significantly affected by application of bioformulations, while a visual rise was observed from phosphatase data which was not statistical higher than control.

Arora *et al*. (2016) observed that with the inoculation of halophilic consortia, dehydrogenase activity was increased (16.8 µg TPF g^{-1} hr^{-1}) as compared to control (12.4 µg TPF g^{-1} hr^{-1}). Soil dehydrogenases are the major representatives of the oxidoreductase enzymes class (Gu *et al*., 2009). Dehydrogenases play a significant role in the biological oxidation of soil organic matter (OM) by transferring hydrogen from organic substrates to inorganic acceptors (Zhang *et al*., 2010). The observation on activity of DHA in sodic soils is close conformity with results reported by Zahir *et al*. (2001), they reported positive correlation between dehydrogenase and organic carbon content in soil and concluded that dehydrogenase activity decreases in alkali soils as compared with normal and saline soils due to low organic carbon content in alkali soil. At high salt concentrations, proteins are destabilized due to enhanced hydrophobic interactions. The activity and stability of the enzymes depend not only on the concentrations but also on the nature of the salt. Halophilic enzymes have highly negative surface charge with hydrated carboxyl groups shifted by high salt content, which protects against unfolding of the proteins (Joo and Kim, 2005; Asolkar 2009). Brzezinska *et al*. (2001) found that active dehydrogenases can utilize both oxygen and other compounds as terminal electron acceptors, although anaerobic microorganisms produce most dehydrogenases.

Behera *et al*. (2017) reported that with the inoculation of PSB-37, the maximum phosphatase activity in soil was (85.6 μml^{-1}). Plants and microbes release phosphatase enzyme to mineralize organic P compounds. These enzymes are key agents in organic P mineralization and play important roles in plant response to limited P availability or increasing P demand (Dakora and Phillips, 2002). Phosphatases are the enzymes catalyzing the hydrolysis of both esters and anhydrides of phosphoric acid (Schmidt and Laskowski, 1961). Generally, alkaline phosphatase predominates in soils with neutral or slightly alkaline pH. It has been observed that activity of soil enzymes depends on microbial growth and availability of resources and nutrients (Sinsabaugh and Moorhead, 1994). The activity of alkaline phosphatase might be dependent on availability of labile phosphorus in the soils (Singh *et al*., 2012). When soil microorganisms are phosphorus limited, they produce acid or alkaline phosphatase that release inorganic phosphatase from organic matter (Allison *et al*., 2011).

5.2.4 Microbial count (Bacteria and Fungi)

Microbial count for both bacteria and fungi was significantly higher in normal soils as compared to sodic soils, as evident from overall mean values. The reduced and/or altered microbial activities in saline and sodic soils are likely either due to direct toxic effects of salts on microbial communities (Rietz and Haynes, 2003) or the salinity and sodicity may exert toxic effects on vegetation (crop, grass and tree) (Wong *et al*., 2010), which in turn, lead to decreased organic matter inputs (crop residues, litter and fine roots) in the soil and, consequently, significant decrease in microbial activities (Singh *et al*., 2015). At the same time application of bioformulations in sodic soils resulted in different trends for bacterial and fungal count. Bacterial count was significantly higher in inoculated sodic soils over un-inoculated sodic soil. In sodic soil, there is low availability of water and the salts are concentrated promoting a dehydration of the microorganisms. However, the halophilic microorganisms can exchange cations and osmolytes when the salt concentrations in the environment are elevated (Chowdhury *et al*., 2011). They accumulate osmolytes in their cells which enhances salt tolerance (Asghar *et al*., 2012) to combat with low osmotic and metric potentials in the soil solution. Hence, their capacity to tolerate high salt concentrations is associated with the endospore formation, which allows them to colonize different sites such as saline soils, playing a key role in processes like carbon, nitrogen, sulfur and phosphorus cycles (Mandic-Mulec *et al*., 2015). Singh and Jha, (2016) reported that with the inoculation of halotolerant PGP bacterium *Serratia* sp. (SL-12) in wheat plants (*Triticum aestivum L*) significantly increased the bacterial colonies (1.4×10^3 CFU g^{-1}) as compared to control. Ravikumar *et al*. (2002) also observed the higher counts of *Azospirillum* (148.88×10^4 g^{-1} dry weight) in the roots of *Avicennia marina* and in the rhizosphere sediment sample of *Suaeda monoica* (20.0×10^3 g^{-1} dry weight). Arora *et al*. (2016) reported that the highest CFU per gram soil (1.38×10^8) was found in the rhizospheric soil of *Salvadora persica* followed by 4×10^7 CFU g^{-1} and 2.86×10^6 CFU g^{-1} in *Sphaeranthus indicus* and *Suadea maritima* species, respectively.

In fungi, a low osmotic potential decreases spore germination and the growth of hyphae and changes the morphology (Jupiner and Abbott, 2006) and gene expression (Liang *et al*., 2007), resulting in the formation of spores with thick walls. Fungi have been reported to be more sensitive to osmotic stress than bacteria. There is a significant reduction in the total fungal count in soils salinized with different concentrations of sodium chloride. The reduction rate in fungal population due to sodicity is appeared to be higher than that of bacterial population. This indicates that fungi are more susceptible to sodicity stress as compared with bacteria.

5.3 Physiological Parameters

5.3.1 Plant growth and Biomass

All the plant growth and biomass related parameters, such as plant height, leaf area index (LAI), root:shoot ratio and dry matter accumulation (DMA) were affected by sodicity. Plant height, LAI and DMA were significantly higher in normal soils as compared to sodic soils, while root:shoot ratio followed a reverse trend. Application of bioformulations in sodic soils significantly increased plant height, LAI and DMA in sodic soils, however, no such impact was observed in normal soils.

In case of sodic soils, highest plant height was observed under treatment Halo-Azo (79.6 cm) which was at par with other bioformulation treatments but significantly higher than corresponding control. Inoculation of halo biofertilizers in rice crop significantly increased plant height up to 116.3 cm as compared to control (Sahay *et al*., 2018). Borham *et al*. (2017) who reported that the *Enterobacter cloacae* effectively increased growth characteristics of plant height up to 9.96 percent. Shamma and Shahwany, (2014) reported that agronomic traits and yields have been increased when plants were inoculated with combined bio-fertilizers, and that is because of the application of bio-fertilizers which may be attributed to their role by enhancing plant growth due to the availability of different nutrients including N,P and K in addition to several micronutrients. Nghia *et al*. (2017) reported that there was significant increase in plant height (23.4 cm) in rice crop with the inoculation of the halophilic bacterial strains. Thus, they concluded that IAA concentration production from these strains can promote root and biomass of rice seedlings, thus it could help plant to take up more nutrients in soil and consequently it helps to improve biomass and yield of rice plant. There was significant increase in plant height (47.5 cm) in basil (*Ocimum basilicum*) with the inoculation of PSB, respectively (Sahay and Patra, 2014).

Vaid *et al*. (2014) reported that there was increase in dry matter accumulation (12.9 percent) in rice crop with the inoculation of Zn solubilizer bacteria (*Burkholderia* and *Acineobacter*). Sahay and Patra, (2014) found that with the inoculation of halophilic PSB in *O. basilicum*, dry matter content was significantly increased (17.81 to 26.43 percent) over control. The NaCl concentration caused a considerable decrease in lengths and fresh and dry weights of shoot and root while bio-priming alleviated the adverse effect. Plant growth promotion by bacteria have the ability to solubilize inorganic and/or organic P from their soil after their inoculation in soil or plant seeds (Rodriguez and Fraga, 1999; Singh *et al*., 2010). According to Mishra *et al*. (2010) inoculation of bacterial strains caused a considerable increase in root and shoot length and dry matter production of shoot and root of *Cicer arietinum* seedlings. A greater amount of DMA in halophilic inoculated plants is attributable to more efficient absorption, utilization, and assimilation of soil moisture, regulating stomatal openings, and thus enhanced dry-matter production (Bethlenfalvay *et al*., 1988).

Malik *et al*. (2015) reported that the zinc solubilizer bacteria significantly improved the leaf area in mungbean (*Vigna radiata*). Ravikumar *et al*. (2010) also reported that with the inoculation of halophilic PSB (*Bacillus subtilis*) on *Avicennia officinalis*, leaf area was increased up to 70.55 percent. *Psuedomonas putida* and *Novosphingobium* sp. are reported to reduce salt-stress induced damage in citrus plants by lowering the level of abscisic acid (ABA) and salicylic acid (SA), reducing the efficiency of photosystem II (Fv/Fm), increasing accumulation of IAA in the leaf and inhibiting accumulation of root chloride and proline during salt stress (Vives-Peris *et al*., 2018). Chlorophyll and carotene contents of plant leaves were significantly reduced with the increase in salt stress but plant inoculated with bacteria had more leaf chlorophyll contents. Yildirim *et al*. (2011) also reported that these bacterial bio-fertilizer increase the chlorophyll and carotenoid content in NaCl conditions which in turn affects the leaf area. Halophiles or PGPR produces lumichrome and riboflavin which can act as a microbe-to-plant signal compounds that able to stimulate plant growth. Lumichrome can accelerate appearance of leaves (more rapid development) and leaf expansion (enhanced growth). In addition, it can increase plant height and overall leaf area, resulting in improved production of biomass (Dakora *et al*., 2015). These organisms also enhanced activity of cytokinin (growth promoter), of which P is a major component and which promotes leaf growth through enhanced cell division and cell expansion (Li *et al*., 1994).

Tariq *et al*. (2007) reported that the activity of plant growth promoting rhizobacteria to mobilize indigenous soil zinc in rice (*Oryza sativa* L.). They observed that the inoculation showed positive impact on root length (54%), root weight (74%), root volume (62%), root area (75%) as compared to control. The longer the length of the plant roots, the more the absorption of nutrients from the soil (Yafizham and Abubakar, 2009). The halophilic organisms produced Indole Acetic Acid (IAA), which helps in production of longer roots with extensive root hair which indirectly helps in nutrient uptake (Hassan and Bano, 2015; Khalid *et al*., 2013; Kang *et al*., 2014). Vasudevan *et al*. (2002) reported that the increased length of root with comparison to the control plants on CV. IR24 variety with the four biological preparations *Bacillus* spp. Maximum fresh weight, dry weight, shoot length and root length of plant were found in the case of "Consortium 5% NaCl" (CSSRYI and CSSRO2) treated pot, 194.5 percent increase in fresh weight, 98.97 percent increase in dry weight, 15.37 cm increase in shoot length and 7.4 cm increase in root length as compared to the uninoculated control maize crop (Arora *et al.,* 2016). These organisms also produce a vital enzyme, 1- aminocyclopropane-1-carboxylate (ACC) deaminase to reduce the level of ethylene in the root of developing plants thereby increasing the root length and growth (Penrose and Glick, 2001).

However, apart from increase in root length the inoculation also positively impacts shoot growth, which balances out he root:shoot ratio. Sharma *et al*. (2015) reported that with the inoculation of PGPR isolates in peanut plant significantly increased the shoot (14-70 percent) and root length (12.9 to 36 percent), respectively. Improvement in shoot and root biomass ranged between 21-44 percent and 36-64 percent over the non-inoculated control, respectively. The synthesis of phytohormone IAA is a frequently used mechanism of PGPR to enhance plant growth (Dimkpa *et al*., 2009; Glick, 2012). IAA regulates several aspects of growth and development by controlling critical biological process, such as lateral root initiation, cell enlargement, cell division and increase root surface area (Zhao, 2011). Kumar *et al*. (2010) reported that with the inoculation of halophilic PSB (*Bacillus subtilis* and *Pseudomonas aeruginosa*), average root length (43.4 percent), shoot length (40.0 percent), root biomass (26.3 percent) and shoot biomass (69.3 percent) was enhanced.

5.3.2 Yield attributes and Yield

5.3.2.1 Yield attributes

As expected, the rice plant transplanted in sodic soil had lower number of effective panicles in comparison those that were grown in normal soils. Application of bioformulations increased the effective panicles in sodic soils, with application of Halo-zinc recording the maximum, which was significantly higher that the control for sodic soils. Similar trend was also observed in length of panicle of the rice crop. Vaid *et al*. (2014) also reported that zinc solubilizer bacterial inoculations significantly increased the number of effective tillers up to 13.3 percent in rice crop. Horan *et al*. (2002) and Rabie and Almahdini, (2005) concluded that negative effects of salinity and sodicity that can cause a decrease in plant growth, reduction in biomass, plant stands are shorter due to the effect of osmotic stress and nutrient deficiencies. Inoculation of bioformulations improves the germination percent (1.4 percent), plant establishment (1.5 percent) and number of effective panicles/m^2 (1.75 percent) (Sahay *et al.,* 2018).

As with most of the parameters, the number of grains per panicle were highest in normal soils in comparison to sodic soils. Within sodic soils, application of bioformulations resulted in significant increase in the number of grains per panicle. In case of sodic soilhighest number of grains per panicle were observed in treatment Halo-Zinc (84.7) which was statistically at par with treatment Halo-Azo, Halo-mix and Halo-PSB but significantly higher than control for sodic soil. Vaid *et al*. (2014) observed similar results. They reported that with the inoculation of Zn solubilizer bacteria in rice crop, significant increase in the number of grains/panicle up to 12.8 percent was observed, as compared to control. Sahay *et al*. (2018) also reported that with the inoculation of Halo-Azo and Halo-PSB significantly increased grains per panicle. Sapsirisopa *et al*. (2009) reported that with the inoculation of salt-tolerant *Bacillus megaterium* on rice seedling significant increase in grains per panicle (34.00 to 76.67) was observed.

5.3.2.2 Yield

Grain yield has traditionally been a direct parameter used in assessing soil productivity in different kinds and rates of fertilizer applications. Yield is a function of complex inter-relationships of its components, which are determined from the growth rhythms in vegetative phase and its subsequent reflection in reproduction phase of the plant. Vigorously growth plants are able to absorb larger quantity of mineral nutrients through well-developed root system.

The mean grain yield was higher in normal soils as compared to sodic soils. The grain yield ranged from 55.6 to 30.6 q ha^{-1} among the different treatments. In case of normal soils (S_0), highest grain yield (60.3 q ha^{-1}) was recorded in treatment (Halo-Mix) which was statistically at par with all other treatments. In case of sodic soils (S_1), highest value recorded in treatment Halo-zinc (34.6 q ha^{-1}) which was statistically at par with treatment Halo-Azo, Halo-PSB and Halo-mix but was significantly higher than control for sodic soils. The results can be corroborated with the finding of Kumar *et al*. (2004) who reported that application of zinc enriched organic manures and Zn solubilizers on the yield of the turmeric crop. The increased growth and yield in comparison to control were due to the improvement of the soil which increased the nutrient uptake by the crop and minimized the negative effect of sodic soil by halotolerant bio-fertilizer formulations. Higher yield due to inoculation of halophilic organisms may be attributed to the greater potential of conversion of tryptophan to IAA and the increased area of leaf for photo assimilation (Ullah and Bano, 2019).

Productivity of rice in salt/alkalinity affected areas is very low, but it can be reasonably raised by application of microbial inoculants (Ponnamperuma, 1994). These reports are also in agreements with (Subramani and Solaimalai, 2000) and (Ramanathan *et al*., 2004). It is well known that the availability of nitrogen in the soils is one of the plant-growth limiting factor (Singla *et al*., 2014; Singla and Inubushi, 2015). A foliar application of *Azospirillum* significantly increased the grain yield and straw yield of rice (Kanniyan *et al*., 1980). Chavan *et al*. (2010) also reported that biofertilizers that possess a high capacity for N_2 fixation (*Azotobacter tropicalis*) and consist of phosphate solubilizing bacteria (*Burkhoderiaunamae*) have a high potential for growth and yield enhancement of corns and vegetables. These biofertilizer bioformulations improved soil quality, yield components i.e., tillers number, number of panicles and number of grains ultimately increased grain yield due to the better nutrient supply throughout the crop growth period. Arora *et al*. (2016) reported that grain yield of wheat in field increased from 3497 kg ha^{-1} to 4129 kg ha^{-1} when consortia of halophilic N-fixers and P-solubilizers where inoculated in seed.

Wagar *et al*. (2004) studied the influence of bacteria containing ACC deaminase enzyme inoculation on growth and yield of wheat. They found that bacteria containing the enzyme significantly increased grain and straw yield, root length and weight, number of tillers and the amount of nitrogen, phosphorus and potassium in grain and straw compared to control. They recognized that all these effects were due to the reduction of ACC ethylene level in plant.

Similar trend was also observed in the yield of straw as in case of grain yield. Within sodic soil (S_1), highest straw yield was recorded in treatment Halo-Azo (69.6 q ha^{-1}) which was statistically at par with treatment Halo-mix (63.3 q ha^{-1}) and Halo-PSB (60.3 q ha^{-1}) while statistically lower straw yield value was recorded under the control (43.3 q ha^{-1}). Some strains of halophilic organisms or PGPR can promote relatively large amounts of gibberellins, leading to enhanced plant shoot growth (Jha and Saraf, 2015). These organisms also produce secondary metabolites and volatile organic compounds (VOC) which stimulate plant growth, resulting in increased shoot biomass in the rhizosphere (Bailly and Weisskopf, 2012).The inoculation with *Azospirillum* can cause increase in the dry weight and in the accumulation of total nitrogen of the plant, both in the straw and grain yield, in the germination rate of the seeds and in changes in the duration of the plants growth stages (Boddey and Dobereiner, 1988; Sumner, 1990; Fages, 1994; Pandey *et al.*, 1998). The results can be correlated with the finding of some authors (Creus *et al.*, 1998) who reported that *Azospirillum* inoculated wheat seedlings subjected to osmotic stress developed significant higher coleoptiles, with higher fresh weight and better water status than non-inoculated seedlings. Sahay *et al.* (2018) reported that with the co-inoculation of Halo-Azo and Halo-PSB, grain yield and straw yield was improved as compared to control. Arora *et al.* (2016) reported that straw yield also increased from 5.03 to 6.24 t ha^{-1} with inoculation of these halophilic isolates.

No significant difference among treatments were observed as far as Harvest index was concerned. This indicates that the impact of sodicity as well as application of bioinoculants is observed both on shoots and grains. As far as the test weight is concerned, the results showed that highestand significant 1000 grain weight (g) in normal soils was recorded in inoculation treatment Halo-mix (24.3 g) and lowest value was recorded under treatment Halo-Azo (22.6 g). Both were at par with each other. In case of sodic soil, highest 1000 grain weight was observed under Halo-Zinc (17.5 g) and least value was recorded under control (15.1 g). Shivran *et al.* (2015) reported that with the inoculation of zinc solubilizer in wheat crop, test weight was increased. Yafizham and Abubakar, (2009) reported that bio-phosphate applications at the rate 10 and 20g/hill significantly affected the weight of 1000 grains of lowland rice by 2.1 percent and 11.4 percent, respectively. Microorganisms with phosphate solubilizing activity increased the availability of the soluble phosphate and enhanced the plant growth by improving biological nitrogen fixation (Mohammadi, 2011). Inoculation of halo biofertilizers in rice crop significantly increased test weight up to 1.1 percent (Sahay *et al.*, 2018). Gandhi and Muralidharan, (2016) reported that with the inoculation of *Acinetobacter* sp. (zinc solubilizer), the 1000 grain weight of rice plant significantly increased (17.6 g) as compared to control.

5.4 Plant nutrient concentration and uptake

5.4.1 Nutrient concentration

The results showed that there were no significant differences observed in nitrogen content in grains under various treatment combinations in normal as well as sodic soils, as far as total nitrogen and phosphorus were concerned. However, in case of potassium the mean values for sodic and non-sodic indicated that a significantly lower concentrations in both grain and straw of paddy plant grown under sodic soils. It has been generally observed that plants exposed to saline environment (NaCl) take up high amounts of Na^+, whereas the uptake of K^+, is significantly reduced (Marschner, 1995).

5.4.2 Nutrient uptake

Overall the nutrient uptake (N, P and K) was significantly higher in normal soils as compared to sodic soils. This was largely because of higher dry matter yield in case of normal soils especially in case of N and P. Further, the application of bioformulations in sodic soils significantly increased N, P and K uptake in grains over the non-inoculated sodic soil treatment. Similar trend of increase in nutrient uptake was also observed for straw, however it was not significant for all of the bioinoculants. Increased N uptake is implicated to gibberellin produced by *Azospirrillum* sp. The improved grain and straw yield may be attributed to the increased grain filling due to enhanced nutrient uptake and increased photosynthesis (Gulnaz *et al*., 2017). The results are in accordance with the findings of Singh *et al*. (2016) for nitrogen uptake of wheat. Many PSB inoculation studies have shown increased phosphorus uptake both in pot experiments and under field conditions (Elizabeth *et al*., 2017). Ghaderi *et al.* (2008) demonstrated that the rate of P released by *Pseudomonas putida*, *Pseudomonas fluorescens* CHAO and Tabriz *Pseudomonas fluorescens* was 51, 29 and 62 percent, respectively. Similarly, improved phosphorus uptake and increased grain yield of wheat were reported following inoculation of phosphate solubilizing *Pseudomonas* and *Bacillus* species (Walpola and Yoon, 2012). Vyas and Gulati, (2009) also found growth promotion and higher nutrient uptakes in response of PSB inoculation in the cereals. The role of phosphate solubilizing bacteria increases in the availability of phosphorus in soil through secretion of phosphatase enzyme which leads to transfer organic phosphorus to their available forms (El-yazeid *et al*., 2007; Eid *et al*., 2006). Consequently, it enhances phosphorus absorption and accumulation in plant tissues. Solubilization of soil fixed P through microbes making it available for increased plants absorption and its greater expression in leaf concentration (Rosa *et al.,* 2020). Inoculation of phosphate solubilizing bacteria directly increases phosphate availability to the plants by 15 times in the soil (Seneviratne and Jayasinghearachchi, 2005).

Singh *et al*. (1986) reported that concentration of potassium increased significantly due to application of Zn in wheat grain and straw. K is considered a macronutrient for plants and is the most abundant cation within plant cells. The K has a number of important functions within plants, including balancing the charges of cellular anions, enzyme activation, control of stomatal opening/closing and serving osmoticum for cellular growth. The increment of potassium might be due to effect of different strain groups and nutrient mobilizing microorganism which help in availability of metals and their forms in the composted material and increased levels of extracted minerals (Yada *et al*., 2013). Singh *et al*. (1987) observed that the uptake of potassium increased with application of zinc up to 10 ppm in wheat.

Mean values indicated that nutrient uptake was significantly higher in non-sodic soils. Differences in nutrients accumulation between plants growing on sodic and normal soils may be due to the differences in the availability of nutrients in soil (Pandey *et al*., 2008). The N, P, K content decreases with increase in soil sodium (Singh and Abrol, 1985).

Chapter-VI

Summary and Conclusion

CHAPTER-VI

SUMMARY AND CONCLUSIONS

A study was carried to assess the impact of microbial formulations on health of salt affected soils under rice crop. Four halotolerant formulations i.e. Halo-Azo, Halo-PSB, Halo-Zinc and Halo-Mix were used to inoculate the seedlings. The major objectives of the experiment were to compare normal and sodic soils after the inoculation of these halotolerant bioformulations, and impact of these bioformulations on soil as well as on rice crop. The salient findings of the investigation are summarized and concluded below:

6.1 Chemical parameters

The normal and sodic soil used for the transplantation of rice crop was analyzed for their chemical properties. The results showed that different soil treatments affected the properties of the soil.

In case of sodic soils, soil pH, EC and available sodium significantly decreased with application of all of the bioformulations. Further, the soil pH remained significantly higher in sodic soil (8.94) as compared to normal soil (7.97), even after application of bioformulation. The decrease in pH can be directly linked to the reduction in soil sodium content at harvest following the same trend as that of pH. There was significant decrease in available sodium content of the soil upon application of bio-formulations in sodic soils, while no significant differences were observed in normal soils. The sodium also remained significantly higher in sodic soils (209.6 mg kg^{-1}) as compared to normal soil (33.3 mg kg^{-1}), in spite of application of bioformulations. Application of halophilic bioformulations significantly increased soil organic carbon at harvest in sodic soils. In sodic soil, highest soil organic carbon was observed with the co-inoculation of Halo-Mix formulations. Overall, the organic carbon was significantly higher in normal soil (0.89 percent) as compared to sodic soil (0.57 percent). In case of sodic soils, highest nitrogen was observed with the inoculation of Halo-Azo treatment (168.2 kg ha^{-1}) and the least nitrogen was observed in control (86.6 kg ha^{-1}). Application of Halo-bioformulations significantly increased the available N, P, K and zinc content of the sodic soil. Overall, it was observed that all the available nutrients studied were in significantly higher concentration in normal soils as compared to sodic soils, except for available phosphorus which followed a reverse trend. High pH, the main characteristic of sodic soils, tends to decrease the availability of nitrogen, potassium and zinc. However, in case of available phosphorus the decline is only up to around a pH of 8.5. Increase sodium content competes with calcium, which decreases the precipitation of phosphorus to tricalcium phosphate.

6.2 Biological Parameters

Soil biological properties can be assessed by examining the process level biological indices. Soil enzyme assays are process level indicators and are a means of determining the potential of a soil to degrade or transform substrates. It is a useful indicator of a soil's capacity to carryout important steps in nutrient cycling, nitrification, oxidation and other process. The effect of different halophilic bio-fertilizer formulations on biological properties of soil like, microbial biomass carbon, dehydrogenase activity, phosphatase activity and microbial count were studied. In sodic soils, highest microbial biomass carbon was observed with the inoculation of Halo-mix treatment (90.8 $\mu g\ g^{-1}$) and the least microbial biomass carbon (MBC) was observed in control, (69.1 $\mu g\ g^{-1}$), where no bioformulation was inoculated. The microbial biomass carbon was significantly higher in normal soil (113.8 $\mu g\ g^{-1}$) as compared to sodic soil (84.8 $\mu g\ g^{-1}$). The effect of biofertilizer formulations on enzymatic activity were observed in both type of soils unlike most other parameters. In sodic soils, the quantum of increase in enzymatic activity was, however, higher and significant, upon application of bioformulations. In normal soils, only the dehydrogenase activity was significantly affected application of bioformulations, while a visual rise was observed from phosphatase data which was not statistical higher than control. Microbial count for both bacteria and fungi was significantly higher in normal soils as compared to sodic soils, as evident from overall mean values. At the same time application of bioformulations in sodic soils resulted in different trends for bacterial and fungal count. Bacterial count was significantly higher in inoculated sodic soils over un-inoculated sodic soil. In sodic soil, there is low availability of water and the salts are concentrated promoting a dehydration of the microorganisms.

6.3 Growth and yield Parameters:

The effect of different halophilic bio-fertilizer formulations on rice growth and yield components, transplanted in normal and sodic soil on growth and yield parameters like, plant height (cm), dry matter accumulation (g/m^2), leaf area index, root shoot ratio, number of effective panicles/m^2 , length of panicle (cm) , number of grains per panicle, grain and straw yield, harvest index and 1000 grain weight were studied. All the plant growth and biomass related parameters, such as plant height, leaf area index (LAI), root:shoot ratio and dry matter accumulation (DMA) were affected by sodicity. Plant height, LAI and DMA were significantly higher in normal soils as compared to sodic soils, while root:shoot ratio followed a reverse trend. Application of bioformulations in sodic soils significantly increased plant height, LAI and DMA in sodic soils, however, no such impact was observed in normal soils. The rice plant transplanted in sodic soil had lower number of effective panicles in comparison those that were grown in normal soils. Application of bioformulations increased the effective panicles in sodic soils, with application of Halo-zinc recording the maximum, which was significantly higher that the control for sodic soils. Similar trend was also observed in length of panicle of the rice crop. The number of grains per panicle were highest in normal soils in comparison to sodic soils. Within sodic soils, application of bioformulations resulted in significant increase in the number of grains per panicle. In case of sodic soil, highest number of grains per panicle were observed in treatment Halo-Zinc (84.7) which was statistically at par with treatment Halo-Azo, Halo-mix and Halo-PSB but significantly higher than control for sodic soil. The mean grain yield was higher in normal soils as compared to sodic soils. The mean grain yield ranged from 55.6 to 30.62 q ha^{-1} among the different treatments. In case of normal soils (S_0), highest grain yield (60.3 q ha^{-1}) was recorded in treatment (Halo-Mix) which was statistically at par with all other treatments. In case of sodic soils (S_1), highest value recorded in treatment Halo-zinc (34.6 q ha^{-1}) which was statistically at par with treatment Halo-Azo, Halo-PSB and Halo-mix but was significantly higher than control for sodic soils. Straw yield followed similar trend as the grain yield. As far as the test weight is concerned, it was significantly higher in normal soils.

6.4 Nutrient Concentration and uptake

The mean value of nitrogen content in grains for S_0 and S_1 were 1.19 percent and 1.14 percent, whereas the mean value of nitrogen content in straw for S_0 and S_1 were 0.73 percent and 0.70 percent, respectively. The mean value of phosphorus content in grains for S_0 and S_1 were 0.21 percent and 0.23 percent, whereas the mean value of phosphorus content in straw for S_0 and S_1 were 0.13 percent and 0.14 percent, respectively. However, there were no significant differences in nitrogen and phosphorus content in grains and straw under various treatment combinations in normal as well as sodic soil. The mean value of potassium content in grains for S_0 and S_1 were 0.29 percent and 0.18 percent, whereas the mean value of potassium content in straw was S_0 and S_1 were 1.44 percent to 0.52 percent, respectively. However, there was no impact of bioformulations on K concentration in grains and straw. Overall, the nutrient uptake (N, P and K) was significantly higher in normal soils as compared to sodic soils. This was largely because of higher dry matter yield in case of normal soils especially in case of N and P. Further, the application of bioformulations in sodic soils significantly increased N, P and K uptake in grains over the non-inoculated sodic soil treatment. Similar trend of increase in nutrient uptake was also observed for straw, however it was not significant for all of the bioinoculants.

Conclusion

Halotolerant strains of bio-fertilizers viz. Halo-*azospirillium*, Halo-phosphorus solubilizing bacteria, Halo-zinc and Halo-mix (combination of all three) were found to be effective in sodic soils. Application of halotolerant bio-formulations had a significant effect in promoting soil health and fertility of sodic soil and improved physico-chemical properties of soil. Application of halotolerant bio-formulations enhanced growth and yield of rice cultivated in sodic soils in comparison to sodic soil where no bioformulation was applied. Inoculation with halotolerant bio-formulations could be helpful in ameliorating the ill-effects of salt affected soils cost-effectively, as cost of gypsum is prohibitive and availability is limited. Although, normal soils still performed better, continuous application over a number of years of these bioformulations may effectively bridge the yield and soil health gap.

The formulations can be an effective remedial measure for the farmers and other stake holders. For further refinement in the future, the pot studied need to be replicated in the field for practical purposes. The cost-effective management options for sodic soils can further be refined through studies on new salt tolerant and thermotolerant strains. Application of multi-strain bacterial growth consortia could be an effective approach and need to be further explored.

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CERTIFICATE-IV

Certified that all the necessary corrections as suggested by the external examiner and the Advisory committee have been duly incorporated in the thesis entitled **"Improving Health of Salt Affected Soils Through Microbial Formulations"** submitted by **Ms. Divya Chadha**, Registration No. **J-18-M-585.**

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