

**“ROLE OF MICROBIAL CONSORTIA IN RECLAMATION OF
SODIC SOIL”**

by

Mr. Patole Amit Sarjerao

(Reg.No. 018/ 246)

A thesis submitted to the

**MAHATMA PHULE KRISHI VIDYAPEETH,
RAHURI - 413 722, DIST. AHMEDNAGAR,
MAHARASHTRA, INDIA**

In partial fulfilment of the requirements for the degree
of

MASTER OF SCIENCE (AGRICULTURE)

In

AGRICULTURAL MICROBIOLOGY



**DEPARTMENT OF PLANT PATHOLOGY AND
AGRICULTURAL MICROBIOLOGY**

**POST GRADUATE INSTITUTE
MAHATMA PHULE KRISHI VIDYAPEETH,
RAHURI - 413 722, DIST. AHMEDNAGAR,
MAHARASHTRA, INDIA**

2021

**“ROLE OF MICROBIAL CONSORTIA IN RECLAMATION OF
SODIC SOIL”**

by

Mr. Patole Amit Sarjerao

(Reg.No. 018/ 246)

A thesis submitted to the
**MAHATMA PHULE KRISHI VIDYAPEETH,
RAHURI - 413 722, DIST. AHMEDNAGAR,
MAHARASHTRA, INDIA**

In partial fulfilment of the requirements for the degree
of

MASTER OF SCIENCE (AGRICULTURE)
in
AGRICULTURAL MICROBIOLOGY

APPROVED BY

Dr. R.B. Sonawane
(Chairman and Research Guide)

Dr. R.T. Gaikwad
(Committee Member)

Dr. A.G. Durgude
(Committee Member)

Dr. V.S. Wani
(Committee Member)

**DEPARTMENT OF PLANT PATHOLOGY AND
AGRICULTURAL MICROBIOLOGY
POST GRADUATE INSTITUTE
MAHATMA PHULE KRISHI VIDYAPEETH,
RAHURI - 413 722, DIST. AHMEDNAGAR,
MAHARASHTRA, INDIA**

2021

CANDIDATE'S DECLARATION

I hereby declare that this thesis or part
there of has not been submitted
by me or other person to any
other University or Institute
for a Degree or
Diploma

Place: M.P.K.V., Rahuri

Date:

(A.S. Patole)

Dr. R.B. Sonawane

Assistant Professor of Plant Pathology,
Onion and Grape Research Station,
Pimpalgaon Baswant,
Tal. Niphad, Dist. Nashik 422 209

CERTIFICATE

This is to certify that the thesis entitled **“ROLE OF MICROBIAL CONSORTIA IN RECLAMATION OF SODIC SOIL”** submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar (Maharashtra) in partial fulfilment of the requirement for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **AGRICULTURAL MICROBIOLOGY**, embodies the result of a piece of bonafide research work carried out by **Mr. PATOLE AMIT SARJERAO** under my guidance and supervision and that no part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been duly acknowledged.

Place : M.P.K.V., Rahuri

(R.B. Sonawane)

Date :

Dr. T.K. Narute
Head,
Department, Plant Pathology and
Agricultural Microbiology,
Post Graduate Institute,
Mahatma Phule Krishi Vidyapeeth,
Rahuri-413 722, Dist. Ahmednagar (M.S)

CERTIFICATE

This is to certify that the thesis entitled **“ROLE OF MICROBIAL CONSORTIA IN RECLAMATION OF SODIC SOIL”** submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar (Maharashtra) in partial fulfilment of the requirement for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **AGRICULTURAL MICROBIOLOGY**, embodies the result of a piece of bonafide research work carried out by **Mr. PATOLE AMIT SARJERAO** under guidance and supervision of **Dr. R.B. SONAWANE**, Assistant Professor of Plant Pathology, Onion and Grape Research Station, Pimpalgaon Baswant, Tal. Niphad, Dist. Nashik and that no part of the thesis has been submitted for any other degree or diploma.

Place : M.P.K.V., Rahuri

(T.K. Narute)

Date : / /2021

Dr. P. N. Rasal

Associate Dean,
Post Graduate Institute,
Mahatma Phule Krishi Vidyapeeth,
Rahuri - 413 722, Dist. Ahmednagar,
Maharashtra (INDIA)

CERTIFICATE

This is to certify that the thesis entitled **“ROLE OF MICROBIAL CONSORTIA IN RECLAMATION OF SODIC SOIL”** submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar (Maharashtra) in partial fulfilment of the requirement for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **AGRICULTURAL MICROBIOLOGY**, embodies the result of a piece of bonafide research work carried out by **Mr. PATOLE AMIT SARJERAO** under guidance and supervision of **Dr. R.B. SONAWANE**, Assistant Professor of Plant Pathology, Onion and Grape Research Station, Pimpalgaon Baswant, Tal. Niphad, Dist. Nashik and that no part of the thesis has been submitted to any other University for Degree or Diploma.

Place : M.P.K.V., Rahuri.

(P.N. Rasal)

Date : / / 2021

ACKNOWLEDGEMENT

“Education plays fundamental role in personal and social development and teacher plays a fundamental role in imparting education. Teachers have crucial role in preparing young people not only to face the future with confident but also build up it with purpose and responsibility. There is no substitute for teacher pupil relationship”. I start in the name of GOD Hanuman-who has bestowed upon me all the physical and mental attributes that I possess and skills to cut through and heal a fellow human

With a sense of high resolve and reverence. I express my sincere and deep sense of gratitude to adorable Dr. R. B. Sonawane, Assistant Professor of Plant Pathology, Onion and Grape Research Station, Pimpalgaon Baswant, who is chairman of my advisory committee. I have no word to express heartfelt thanks to him for his blessings, invaluable inspiring guidance, unfailing encouragement, suggestions, research in site, unique supervision, constructive criticism, scholarly advice, sympathetic attitude and keen interest, throughout the investigation and preparation of this manuscript.

My sincere thanks to Dr. A. S. Dhawan, Hon. Vice-Chancellor, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar and also thanks to Dr. K. P. Vishwanatha, former Hon. Vice-Chancellor, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar, Dr. A.L. Pharande, Dean, Faculty of Agriculture, M.P.K.V., Rahuri and Dr. P. N. Rasal, Associate Dean (PGI), MPKV, Rahuri and Dr. T.K. Narute, Head, Department of Plant Pathology and Agricultural Microbiology, MPKV., Rahuri for providing me the necessary facilities during my study.

I have immense pleasure in expressing my whole hearted sense of gratitude and appreciation for Dr. C.D. Deokar, Associate Dean, College of Agriculture, Dhule for his blessings, inspiring suggestions, enthusiastic interest and encouragement which provided me solace during the tenure of investigation and preparation of this manuscript.

I would be ever grateful to the members of my Advisory Committee, Dr. R. T. Gaikwad, Associate Professor, Department of Plant Pathology and Agricultural Microbiology, Dr. A.G. Durgude, Analytical Chemist Micronutrient Research Project, Department of Soil Science and Agricultural Chemistry, Dr. V.S. Wani, Associate Professor, Department of Statistics for providing me inspiring suggestions and encouragement during the tenure of investigation.

I will take this opportunity to record my deep sense of gratitude to thank Dr. A.M. Navale, Associate Professor and Dr. S. V. Kolase, Associate Professor, Department of Plant Pathology and Agril. Microbiology for providing necessary facilities and keen support during the course of this research work. I convey my thanks to all the staff members of my department Mr. P.S. Kachole, Mrs. M.N. Nangre Madam, Mrs. Jagdale Madam, Mrs. Lambe Madam, Mrs. Kavita Madam, Mr. Abhijit sir, Mr. Laxman, Miss. Rupali, Miss. Kamini, Sonawane mama and field labour Mrs. Khalekar mavshi for their timely help and co-operation during the conduct of this experimental work. I am highly obliged to all dignitaries of Mahatma Phule Krishi Vidyapeeth, Rahuri as well as very thankful to all respected authorities of Post Graduate Institute,

MPKV, Rahuri for providing enthusiastic academic environment during my studies.

I express my eternal gratitude towards my friends Anantkumar, Dattatray, Namdev, Rajesh, Somnath, Sachin, Shubham, Aditya, Ramu, Dipak, Niranjana as well as juniors Viraj, Pradip, Akash, Harihar, Shubham.

I am grateful to all my seniors such as Tushar Sir, Akash Sir, Nilesh Sir, Nagesh Sir, Joel Sir, Dhananjay Sir, Maitri Sir, Appasaheb Sir, Rahul Sir, Pandurang sir who encouraged me at each and every step.

A word of thanks should not come in between us but still from the depth of my heart I convey my devoted thanks to Rohini who always motivated me and to all my brothers Sumit Dada, Akash Dada, Manoj, Mayur, Vikas, Mahesh, Shubham, Ajay and my friend as well as sisters Snehal, Sushma, Divya, Shweta, Rutuja madam, Komal madam, Pranita, Vaishnavi, Megha, Arohi.

I am sincerely thankful to the authors, past and present whose literature has been cited.

I would like to convey my cordial thanks to “Krishi Mitra Ekata Manch” family, specially to karyakarini 2019 and 2020.

*I regard my sincere gratitude to the **Mahatma Phule Krishi Vidyapeeth**, Rahuri, Dist. Ahmednagar for providing me an opportunity to undertake my post graduate studies in this “**Institute of Excellence**”.*

It will be my preposterousness to express my deep sense of exaltation for my father Mr. Sarjerao Shripati Patole and my mother Mrs. Ratna Sarjerao Patole for their supreme sacrifice, solicitous contrivance and blissful for which I have reached to this stage of my career. I wish to express my unbounded love to my grandfather late Shripati Patole, my Aajji, Kaka, Kaki and Cousins.

Besides this, several people have knowingly and unknowingly helped me in the successful completion of this project. I owe heart full thanks to all.

Place : M.P.K.V., Rahuri.

(A.S. Patole)

Date : / /2021

CONTENTS

Chapter No.	Title	Page No.
	CANDIDATE'S DECLARATION	iii
	CERTIFICATE OF RESEARCH GUIDE	iv
	CERTIFICATE OF HEAD OF THE DEPARTMENT	v
	CERTIFICATE OF ASSOCIATE DEAN	vi
	ACKNOWLEDGEMENT	vii
	CONTENTS	ix
	LIST OF TABLES	xiii
	LIST OF FIGURES	xiv
	LIST OF PLATES	xiv
	LIST OF ABBREVIATIONS AND SYMBOLS	xv
	ABSTRACT	xvii
1.	INTRODUCTION	1
2.	REVIEW OF LITERATURE	4
	2.1 Salt-affected soils	4
	2.1.1 Sodic soils : Defination, salt present and their concentration, effect on plant growth and distribution	4
	2.2 Definition and classification of salt tolerant and salt loving microorganisms	6
	2.3 Isolation of salt tolerant bacteria	6
	2.4 Characterization of salt tolerant bacteria	9
	2.5 Effect of halotolarant bacteria on pH and electrical conductivity and its application for reclamation of sodic soil	11
	2.6 Salt tolerant bacteria as bioremediation agent of problematic soils	12
	2.7 Sodic soil and Gypsum	13
	2.7.1 Effect of gypsum on the microflora of sodic soils	13
	2.8 Organic matter and biological reclamation of sodic soil	14
	2.8.1 Farmyard manure mixture	15
	2.8.2 Effect of green manure on the microflora of sodic soils	15
	2.8.3 Leaf Litter Compost	16

	2.8.4	Urban compost	16
	2.9	Effect of halophilic (salt tolerant) bacteria on chemical properties of acidic/saline soil	16
3.		MATERIALS AND METHODS	17
	3.1	Materials	17
	3.1.1	Glasswares	17
	3.1.2	Equipments	17
	3.1.3	Miscellaneous Material	17
	3.1.4	Culture Media	17
	3.1.5	Chemicals and reagents for microbiological analysis	18
	3.2	Methods	18
	3.2.1	Aseptic precautions	18
	3.2.2	Sterilization	18
	3.2.3	Description of sampling site of sodic soils	18
	3.2.4	Sample collection	18
	3.2.5	Determination of pH _s , EC _e , Exchangeable sodium percentage and Organic Carbon of sodic soil	19
	3.2.5.1	pH of Saturation paste extract (pH _s)	19
	3.2.5.2	Electrical conductivity of saturation paste extract (EC _e)	19
	3.2.5.3	Exchangeable Sodium Percentage (ESP)	19
	3.2.5.4	Organic Carbon (OC)	20
	3.2.6	Isolation of bacteria from collected sodic soil samples	20
	3.2.6.1	Isolation of salt tolerant bacteria from sodic soil sample	20
	3.2.7	Characterization of bacterial isolates	21
	3.2.7.1	Physiological characterization bacterial isolates	21
	3.2.7.1.1	Testing of isolates to different concentrations of NaCl	21
	3.2.7.2	Morphological characterization	21
	3.2.7.2.1	Gram reaction	21
	3.2.7.2.2	Phenotypic characterization	22
	3.2.7.3	Biochemical characterization	22
	3.2.7.3.1	Methyl red test	22

		3.2.7.3.2	Starch hydrolysis	23
		3.2.7.3.3	Gelatin hydrolysis	23
		3.2.7.3.4	Hydrogen sulfide production	24
		3.2.7.3.5	Catalase test	24
		3.2.7.3.6	Oxidase test	24
		3.2.7.3.7	Denitrification	25
		3.2.8	Experimental Detail	25
		3.2.8.1	Experimental Location	25
		3.2.8.2	Soil	25
		3.2.8.3	Climate	25
		3.2.8.4	Design and Layout	25
		3.2.8.5	Experiment Details	26
		3.2.8.6	Treatment Details	26
		3.2.8.7	Replications	27
		3.2.9	Pot Culture Experiment	27
		3.2.9.1	Pot filling with sodic soil	28
		3.2.9.2	Application of Organic components (Factor A)	28
		3.2.9.3	Application of inoculums of Salt tolerant bacteria (Factor B)	28
		3.2.9.4	Sowing	28
		3.2.9.5	Fertilizer Application	28
		3.2.9.6	Crop Management	28
		3.2.9.7	Irrigation	28
		3.2.9.8	Harvesting	28
		3.2.10	Observations to be recorded	29
		3.2.10.1	Plant Growth Parameters (quantitative characters)	29
		3.2.10.1.1	Height of Plant (cm)	29
		3.2.10.1.2	Number of Leaves per Plant	29
		3.2.10.1.3	Fresh weight of plant (g)	29
		3.2.10.1.4	Dry weight of plant (g)	29
		3.2.10.1.5	Yield	29
		3.2.10.2	Soil quality parameter test	29
		3.2.11	Statistical Analysis	29

4.	RESULTS AND DISCUSSION	30
	Laboratory Studies	30
	4.1 Isolation of salt tolerant bacteria from sodic soil	30
	4.2 Morphological, physiological and biochemical characterization of isolates	30
	4.2.1 Morphological characters	30
	4.2.2 Physiological characterization	31
	4.2.2.1 Testing of Isolates to different concentration of NaCl	31
	4.2.2.2 Testing of Isolates at different temperatures	32
	4.2.3 Biochemical characters	32
	4.3 Identification of Salt tolerant bacteria	33
	4.4 Preparation of Consortia	34
	Pot Culture Experiment	34
	4.5 Pot Culture Experiment	34
	4.5.1 Plant Height (cm)	34
	4.5.2 Number of Leaves	36
	4.5.3 Fresh weight of leaves per plant (g)	37
	4.5.4 Dry Weight of leaves per plant (g)	39
	4.5.5 pH _s	41
	4.5.6 EC _e (dS m ⁻¹)	42
	4.5.7 Exchangeable Sodium Percentage (%)	44
	4.5.8 Organic carbon (%)	45
5.	SUMMARY AND CONCLUSION	47
6.	LITERATURE CITED	50
7.	APPENDIX	61
8.	VITAE	62

LIST OF TABLES

Table No.	Description	Page No.
3.1	Treatment details	26
3.2	Treatment combinations	27
4.1	Isolation of salt tolerant bacterial isolates from sodic soils	31
4.2	Growth of Isolates at Different NaCl Concentrations	31
4.3	Growth of isolates at different temperatures	32
4.4	Biochemical characterization of salt tolerant bacterial isolates	32
4.5	Effect of application of salt tolerant bacteria and organic components on height (cm) of Spinach	35
4.6	Effect of application of salt tolerant bacteria and organic components on number of leaves of Spinach	36
4.7	Effect of application of salt tolerant bacteria and organic components on fresh weight (g) of Spinach	38
4.8	Effect of application of salt tolerant bacteria and organic components on dry weight (g) of leaves per plant Spinach	40
4.9	Effect of application of salt tolerant bacteria and organic components on pH _s at harvesting of Spinach	42
4.10	Effect of application of salt tolerant bacteria and organic components on EC _e (dS m ⁻¹) at harvesting of Spinach	43
4.11	Effect of application of salt tolerant bacteria and organic components on ESP (%) at harvesting of Spinach	44
4.12	Effect of application of salt tolerant bacteria and organic components on organic carbon (%) at harvesting of Spinach	46

LIST OF FIGURES

Figure No.	Description	Between pages
3.1	Layout plan of pot culture experiment	26-27

LIST OF PLATES

Plate No.	Description	Between page
1.	General View of Pot Culture Experiment of Spinach – “Role of Microbial Consortia in Reclamation of Sodic Soil”	26-27
2.	Pure Culture Plates of salt tolerant bacterial isolates	31-32
3.	Gram Staining of Salt tolerant Bacterial isolates	31-32
4.	Growth of <i>Pseudomonas spp.</i> at different NaCl concentrations	31-32
5.	Growth of <i>Azotobacter spp.</i> at different NaCl concentrations	31-32
6.	Growth of <i>Bacillus spp.</i> at different NaCl concentrations	31-32
7.	Growth of <i>Pseudomonas spp.</i> at different temperature levels	31-32
8.	Growth of <i>Azotobacter spp.</i> at different temperature levels	31-32
9.	Growth of <i>Bacillus spp.</i> at different temperature levels	31-32
10.	Biochemical characterization of Salt tolerant <i>Pseudomonas spp.</i>	31-32
11.	Biochemical characterization of Salt tolerant <i>Azotobacter spp.</i>	31-32
12.	Biochemical characterization of Salt tolerant <i>Bacillus spp.</i>	31-32
13.	Broth Cultures of Salt tolerant bacterial isolates	33-34

LIST OF ABBREVIATIONS AND SYMBOLS

%	:	Per cent
(-)	:	Absent
(+)	:	Present
/	:	Per
@	:	At the rate of
C.D.	:	Critical difference
C.V.	:	Coefficient of Variation
cfu	:	Colony forming unit
cm	:	Centimeter
CO ₂	:	Carbon dioxide
Cu	:	Copper
cv.	:	Cultivar
DAS	:	Date after sowing
DAT	:	Date after transplanting
DMSO	:	Dimethyl sulfoxide
EC _e	:	Electrical Conductivity of saturation paste extract
ESP	:	Exchangeable Sodium Percentage
<i>et al.</i>	:	Co-workers
Etc	:	Et cetera (and so forth)
FCRD	:	Factorial Completely Randomized design
Fe	:	Iron
Fig.	:	Figure (s)
FYM	:	Farm Yard Manure
g	:	gram (s)
GA	:	gibberellic acid
ha	:	Hectare
STB	:	Salt Tolerant Bacteria
hr.	:	Hour (s)
i.e.	:	That is
IAA	:	Indole acetic acid
<i>in vitro</i>	:	In Laboratory
INM	:	Integrated Nutrient Management
K	:	Potassium
K ₂ O	:	Potassium oxide
kg	:	Kilogram (s)

M	:	Mill
M.S.	:	Maharashtra State
mg	:	Milligram
ml	:	Milliliter (s)
mm	:	Millimeter (s)
Mn	:	Manganese
MPKV	:	Mahatma Phule Krishi Vidyapeeth
MPN	:	Most probable number
MR	:	Methyl red test
MT	:	Metric Tonne (s)
N	:	Nitrogen
No.	:	Number
OC	:	Organic Carbon
°C	:	Degree Celsius
P	:	Phosphorus
PGPR	:	Plant growth promoting rhizobacteria
pH	:	Potential of Hydrogen
pp.	:	Page (s)
PSB	:	Phosphate solubilizing bacteria
Q	:	Quintal
R	:	Replication
RBD	:	Randomized block design
RDF	:	Recommended dose of fertilizers
RDN	:	Recommended dose of nitrogen
S.E.	:	Standard error
S.E. $m \pm$	:	Standard error mean
<i>spp.</i>	:	Species
t	:	Ton (s)
T	:	Treatment
Tab	:	Table
TSS	:	Total soluble solids
<i>viz.</i>	:	Videlicet (namely)
VP	:	Vogues-Proskauer test
X	:	Multiplied by

ABSTRACT

“ROLE OF MICROBIAL CONSORTIA IN RECLAMATION OF SODIC SOIL”

by

Mr. Patole Amit Sarjerao

A candidate for the degree

of

MASTER OF SCIENCE (AGRICULTURE)

in

AGRICULTURAL MICROBIOLOGY

POST GRADUATE INSTITUTE,
MAHATMA PHULE KRISHI VIDYAPEETH,

RAHURI-413 722

2021

Research Guide	: Dr. R. B. Sonawane
Department	: Plant Pathology and Agril. Microbiology

The studies on “Role of Microbial Consortia in Reclamation of Sodic Soil” was conducted during 2019-20 at Department of Plant Pathology and Agricultural Microbiology, MPKV., Rahuri. In this study, the isolation of 03 salt tolerant bacteria were carried out from salt affected soils of D block farm, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri. The collected samples of the university farm were subjected for initial chemical analysis and isolation of Salt tolerant bacteria. The initial chemical analysis values of Sodic soil were pH_s 8.61, EC_e 3.12 dSm⁻¹, organic carbon 0.28% and ESP 15.99%.

The three bacterial isolates collected from Sodic soil of D block farm, Post Graduate Institute, MPKV., Rahuri were characterized and studied for their morphological, physiological and biochemical characteristics. The cultures were maintained at Department of Plant Pathology and Agricultural Microbiology, MPKV., Rahuri.

The isolates *viz.*, STB-1, STB-2 and STB-3 were examined for colony colour, Cell shape and Gram reaction. All the 3 isolates were white in colony colour and colonies were round, circular in shape. The bacteria were rod shaped and singular or paired habitat. The isolates STB-1 and STB-2 were Gram negative in reaction and isolate STB-3 was Gram positive in reaction

All the STB-1, STB-2 and STB-3 bacterial isolates were tested for biochemical characterization viz., starch hydrolysis, H₂S production, catalase test, oxidase test, denitrification, methyl red test and gelatin liquefaction. Out of these 3 bacterial isolates, all were positive in oxidase and catalase in reaction, 2 isolates were positive in starch hydrolysis reaction and 1 was negative in starch hydrolysis, H₂S production, denitrification, gelatin liquefaction.

All the 03 bacterial isolates showed growth at ambient temperature. However, isolate STB-1 was positive growth at 4⁰C and negative at 37⁰C whereas STB-2 and STB-3 showed negative growth at 4⁰C.

A pot culture experiment was conducted to study the response of application of organic amendments and salt tolerant bacteria on growth and yield of Spinach crop.

The results of pot culture experiment indicated that application of organic amendments to sodic soil viz., FYM @ 5 t/ha + Gypsum @ 5 t/ha along with inoculation of consortium of salt tolerant bacteria (*Pseudomonas* sp + *Azotobacter* sp + *Bacillus* sp.) seems to be promising in enhancing the growth parameters of Spinach crop.

Similarly, the effects of these isolates were studied on the chemical properties viz., pH_s, EC_e, ESP and OC of the Sodic soil. All the salt tolerant isolates reduced the pH_s, EC_e and ESP values of the Sodic soil as compared to the initial values. The application of FYM @ 5t/ha + Gypsum @ 5 t/ha along with consortium of salt tolerant bacterial isolates (*Pseudomonas* sp. + *Bacillus* sp. + *Azotobacter* sp) was found more efficient in reducing the pH_s, EC_e and ESP ranging from 8.11 to 8.40 over initial 8.61, 2.22 to 2.79 dS m⁻¹ over initial 3.12 dS m⁻¹, 11.43 to 13.80% over initial 15.99 % respectively . On the contrary the organic carbon percentage of sodic soil was found significantly increased from 0.28 to 0.38% over 0.28% initial due to application of FYM @ 5t/ha + Gypsum @ 5 t/ha along with consortium of salt tolerant bacterial isolates (*Pseudomonas* sp. + *Bacillus* sp. + *Azotobacter* sp.).

The study thus showed that the selected isolates and organic amendment could be used for plant growth promotion under salinity stress condition of the soil and for the reclamation of sodic soil.

1. INTRODUCTION

Healthy soils are required to support the growth of crop plant. The term healthy soil is used for such soils which promote the vigorous growth and yields of the crop plants. Such soils have pH in the range of 5.5 to 7.0, exchangeable sodium percentage less than 15%, electrical conductivity of 1.0 dS m^{-1} besides soil organic matter and soil biota. In the process of crop cultivations due to non-judicious soil management and climatic conditions large areas of soils became sick and now turned as acidic soils or salt affected soils. The salt affected soils include saline soils, saline-sodic and sodic soils where crop plant development is restricted and yield of crop plants are considerably reduced.

Saline and sodic soils are distributed widely around the world with a total of 932 million of ha; either natural or human-induced found three predominant environmental distributions such as arid-semi arid regions, coastal areas and humid regions (Shukla *et al.*, 2011). Salt induced land degradation adversely affects the productivity of agricultural land worldwide. Salt-affected soils occupy 6.73 million of ha area (2.1 % of geographical area) of India out of which about 56 per cent are sodic and the remainder 44 per cent saline. Sodic soils in large parts of Indo-Gangetic plains have naturally formed due to rapid alternate wetting and drying conducive to alkali hydrolysis, sodium saturation and high pH development. Sodic soils are characterized by variable electrical conductivity of soil saturation paste (EC_e , mostly $< 4 \text{ dSm}^{-1}$), high pH_s (> 8.5) and high ESP (> 15). Contrary to the saline soils having excessive levels of chlorides and sulphates of Na^+ , Ca^{2+} and Mg^{2+} , sodic soils contain high amounts of CO_3^{2-} and HCO_3^- salts.

The major soil agricultural problems are soil sodicity, by which plant growth is either depressed or entirely prevented due to excessive built up of high sodium concentration and/or other cations in the soil, respectively. The sodic lands contain high concentration of sodium in their soil. Sodic alkali land all over the country is the major problem in sustainable agriculture.

High exchangeable sodium, high pH and low calcium and magnesium combine to cause the soil to disperse, meaning that individual soil particles act

independently. The dispersion of soil particles destroys soil structure and prevents water movement into and through the soil by clogging pore spaces. Sodic soils are characterized by a poor soil structure, they have low infiltration rate, they are poorly aerated and difficult to cultivate. Thus, sodic soils adversely affect the plant growth (Anonymous, 1998).

The composition developed herein employs the use of microbial consortia and farm yard manure/organic manure/agricultural waste and sugar/molasses and or jiggery. Manures will increase the organic matter content of the sodic soil while at the same time sugar will provide nutrient to microbes for their multiplication and enrichment in the same soil.

In India, there is a widespread salinity problem in Maharashtra, Andhra Pradesh, Gujrat, Rajasthan, Orissa, Uttar Pradesh, Haryana, Punjab, Karnataka, Madhya Pradesh and Tamil Nadu. In Maharashtra about 1.06 m ha (3.4 % of 30.76 m ha) soils are degraded by the problems of salinity and sodicity (Challa *et al.*, 1995).

These extreme microbes contribute directly or indirectly in soil health and crop productivity. The soil's biochemical and biological properties are also interlinked with microbial activity. Considering research conducted primarily in India has demonstrated beneficial effect of biological reclamation of sodic soil in particular to alkaline and highly sodic soil (More, 1994).

The applications of halophilic bacteria include recovery of salt affected soil by directly supporting the growth of vegetation thus indirectly increasing crop yields in salt stress. The biotic approach 'plant microbe interaction' to overcome salinity/sodicity problems has recently received a considerable attention from many workers throughout the world. Halophilic bacteria provide a high potential for biotechnological applications for at least two reasons: (1) their activities in natural environments with regard to their participation in biogeochemical processes of C, N, S, and P, the formation and dissolution of carbonates, the immobilization of phosphate and the production of growth factors and nutrients (Rodriguez-Valera, 1988); and (2) their nutritional requirements are simple.

The use of halophilic bacteria in the recovery of saline soils is covered by the following hypotheses. The first hypothesis is that microbial activities in saline soil

may favour the growth of plants resistant to soil salinity. The second hypothesis is based on the utilization of these bacteria as bio-indicators in saline wells. Indicator microorganisms can be selected by their abilities to grow at different salt concentrations. Halophilic microbes are also found to remove salt from saline soils (Arora *et al.*, 2013; Bhuva *et al.*, 2013). There are reports that potential salt tolerant bacteria isolated from soil or plant tissues and having plant growth promotion trait, helps to alleviate salt stress by promoting seedling growth and increased biomass of crop plants grown under salinity stress (Arora *et al.*, 2014 a, b). Both physical and chemical methods of their reclamation are not cost-effective and also the availability of mineral gypsum or other chemical amendments is a problem. The applications of halophilic bacteria include recovery of salt affected soils by directly supporting the growth of vegetation thus indirectly increasing crop yields in salt affected soils.

The Outline of Research work is proposed with the following objectives.

1. Isolation of Salt tolerant bacteria from Sodic soil.
2. Characterization of Salt tolerant bacteria for morphological, physiological and biochemical characters.
3. To prepare consortia of effective Salt tolerant bacteria for sodic soil.
4. To test effective Salt tolerant bacteria under pot culture condition for Spinach crop in sodic soil.

2. REVIEW OF LITERATURE

The literature available on various aspects of salt tolerant bacteria and halophilic bacteria isolated from sodic soil and their use in reclamation of salt affected soil is reviewed under following sub-headings:

2.1 Salt-affected soils

The salt -affected soils need to be classified into various groups for developing special systems of management for specific types of problem and constraints in the production of crops. The US Salinity Laboratory Staff grouped salt-affected soils into three distinct classes, *viz.*, (i) saline soils, (ii) alkali soils, and (iii) saline-alkali soils in 1954. But keeping in view the experiences about the effects of two common kinds of salts (neutral salts and alkali salts) on the properties of these soils and plant growth in India and other countries, salt-affected soils have been grouped into two categories only *viz.*, (i) saline soils, (ii) alkali soils, in the soil map of the world (Szabolcs, 1974). On the basis of nature, characteristics and plant growth relationship in salt affected soils, three main groups of these soils have been distinguished as i) Saline soil, ii) Sodic soil, iii) Saline-sodic soil.

2.1.1 Sodic soils : Defination, salt present and their concentration, effect on plant growth and distribution

The alkali soils are characterized by pH more than 8.5, ESP more than 15% and EC lower than 4 dSm⁻¹ at 25⁰C as per the US Salinity Laboratory Staff, (Anonymous, 1954). However, experiences sodic soils or Indian alkali soils show that the EC is limitless if originating from high concentration of carbonates and bicarbonates of sodium. Otherwise, EC is less than 4 dSm⁻¹ at 25⁰C.

Bajwa (2002) studied that Sodic soils have sufficient sodium saturation and alkalinity to adversely affect plant growth and crop productivity. Sodic soils often have a black color due to dispersion of organic matter and a greasy or oily-looking surface with little or no vegetative growth. Carbonates ($\text{CO}_3^{2-} + \text{HCO}_3^-$) of sodium are the dominant salts. The concentration of neutral salts (Cl^- and SO_4^{2-}) is much lower. Sparingly soluble gypsum, which may be present in many saline soils, is nearly absent in alkali soils.

Abrol *et al.* (1988) studied that the highly sodic soils, dissolved organic matter (OM) often accumulates on the surface imparting a dark colour which are commonly referred to as black sodic soils. The upper surface of sodic soils is often very hard and virtually impenetrable to water while the sub-surface layers remain saturated with water. After drying, deep and 1-2 cm wide cracks develop on the surface of these soils.

Singh *et al.* (2010) reported that about 6.73 M ha area is salt-affected; sodic and saline soils constitute ~56 per cent (3.77 M ha) and 44 per cent (2.96 M ha) of the total salt-affected area in India, respectively. Sodicty is a severe obstacle to sustainable crop production in 11 states. Uttar Pradesh has the largest area (1.35 M ha) under sodic soils constituting nearly 36 per cent of the total. Besides Uttar Pradesh, Gujarat (14.36 %), Maharashtra (11.21 %), Tamil Nadu (9.41 %), Haryana (4.86 %) and Punjab (4.02 %) have high sodicty problem and together represent about 80 per cent of the total sodic lands in India. In Maharashtra, highest sodicty affected area is in Ahmednagar district (265000 ha) followed by Nashik (40000 ha), Aurangabad (31000 ha), Pune (26000 ha) and Solapur (20000 ha).

Wicke *et al.* (2011) prepared map the global extent of salt-affectedness by using Harmonized World Soil Database in a Geographic Information System (ESRI ArcGIS 9.3.1) and estimated that salt stress diminishes the productivity of over 1100 M ha area globally of which saline, sodic and saline sodic soils constitute roughly 60, 26 and 14 per cent, respectively.

Damodaran *et al.* (2013) studied that a range of microorganisms have been tested for overcoming salt-stress in a cost-effective and environmental friendly way. Although effective in alleviating salt stress in crops, use of microbial inoculants is limited due to higher costs and lack of technical know-how. To circumvent these constraints, a low-cost microbial bioformulation ‘CSRBIO’, based on a consortium of *Bacillus pumilus*, *Bacillus thuringensis* and *Trichoderma harzianum* on dynamic media, has been developed. It acts as a soil conditioner and nutrient mobilize and significantly increases the productivity of rice, banana, vegetables and gladiolus in sodic soils.

Sharma *et al.* (2016) studied that Salt induced land degradation adversely affects the productivity of ~1000 million hectares (M ha) of agricultural land worldwide.

Salt-affected soils occupy 6.73 M ha area in India of which ~56 per cent are sodic and the remainder 44 per cent saline. Sodic soils in large parts of Indo-Gangetic plains have naturally formed due to rapid alternate wetting and drying conducive to alkali hydrolysis, sodium saturation and high pH development. Sodic soils are characterized by variable electrical conductivity of soil saturation paste (E_{ce} , mostly $< 4 \text{ dSm}^{-1}$), high pHs (> 8.2) and high ESP (> 15). Contrary to the saline soils having excessive levels of chlorides and sulphates of Na^+ , Ca^{2+} and Mg^{2+} , sodic soils contain high amounts of CO_3^{2-} and HCO_3^- salts.

2.2 Definition and classification of salt tolerant and salt loving microorganisms

Salt tolerant bacteria do not require NaCl for growth, although they grown in high salinity and in environments devoid of high concentration of salt. Salt loving can be classified differently by different scientists on the basis of their response or requirement of NaCl for their growth and development as under.

Larsen (1962) proposed four groups of microorganisms based on NaCl salt requirement as (i) non- halophilic grows best at less than 2 per cent salt, (ii) slight halophilic grows best at 2-5 per cent salt, (iii) moderate halophilic grows best a 5-20 per cent salt and (iv) extreme halophilic grows best at 20 – 30 per cent salt.

2.3 Isolation of Salt tolerant bacteria

Halophiles are extremophilic organisms that thrive in environments with very high concentration of salt. The name comes from the Greek for “Salt loving” while the term is perhaps most often applied to some halophiles. Halophiles are categorized slight, moderate or extreme by the extent of their halotolerance.

Quesada *et al.* (1982) studied that the soil samples collected from rhizosphere soils obtained from a series of hypersaline soils near Alicante in Spain. The microbial counts from rhizosphere soil were always significantly higher than those from root-free soils. They isolated spore forming *Bacillus* from hypersaline habitat. These bacterial isolates appeared to be typical moderate halophiles and Gram positive in reaction but about half of them grew on normal media with only 0.9 % NaCl, a notable difference from moderately halophilic aquatic bacteria.

Imhoff (1993) classified that the four groups of halophilic bacteria according to the salt requirement for optimum growth as (i) non-halophilic (grow below 0.2 M NaCl), (ii) slightly Halophilic (grow at 0.2 to 1.2 M NaCl), (iii) moderately halophilic (grow at 1.2 to 2.5 M NaCl) and (iv) extreme halophilic (grow at more than 2.5 M NaCl).

Arias *et al.* (2007) studied abundance, morphological and physiological characters of six groups of bacteria *viz.* aerobic, proteolytic, ammonifiers, nitrifiers, denitrifiers and N-free medium in six toposequences of salt-affected soils in the semi-arid region of Argentina. Abundance of most groups of bacteria was positively related with soil organic matter content and inversely related with pH, exchangeable sodium and soil depth. All the groups examined were present in the salt crusts. All bacterial groups were affected by NaCl than by Na₂SO₄ in laboratory test.

Khan *et al.* (2007) isolated the halotolerant bacterial isolates from sodic soil on modified nutrient agar media, Ashby's agar media and Pikovskaya's agar media using soil dilution pour plate method.

Nabti *et al.* (2007) studied that the salt affected soil samples collected and isolated bacterial isolate from that salt- affected soil and was identified as *Azospirillum brasilense* using phenotypic analysis and 16S RNA based phylogeny. The inoculation of this osmotolerant rhizosphere strain was tested for its effect on growth of wheat under saline stress conditions. Its effect of promoting plant growth of wheat was significantly superior to that of *A. brasilense* sp.7, the non- halotolerant strain under saline conditions.

Zahran *et al.* (2010) isolated bacteria from salt marshes and saline soils of Egypt and successfully grown on mannitol N-free medium and studied nitrogen fixing ability. The nitrogen fixing activity was then demonstrated for these isolates in modified Staniers medium using acetylene reduction assay. These bacterial isolates possessed appreciable nitrogenase activity under non-saline conditions; however at 5 per cent NaCl only 60 per cent of the isolates exhibited a high rate of this activity and 25 per cent were completely negative under these conditions.

Nabti *et al.* (2013) collected rhizosphere soil samples of potato from University of Bejaia-Algeria and isolated two RBA-E 23 and RBA-E32 new halophilic

Bacillus strains from rhizosphere soil. They screened these two osmotolerant isolates with different NaCl concentrations ranging from 1.72-1000 mM/lit. NaCl.

Trivedi and Arora (2013) isolated salt tolerant *Rhizobium* species from coastal saline soils, Organisms tolerated a higher salt concentration upto 5%, also grow at pH 4.0 and 4.5 level in the laboratory conditions..

Kochar and Gera (2015) reported that the bacterial counts of sodic soils on Jensen's, malate, Pikovskaya's, King's B and soil extract agar media ranges from 3.44 – 6.23, 4.17 – 6.44, 3.77 – 5.45, 3.26 – 6.06 and 5.85 – 8.00 log cfu g⁻¹ soil respectively.

Vivekanand *et al.* (2015) reported that application of saline –tolerant rhizobacteria helps in improvement of crop production in saline soils.

Hafeez *et al.* (2015) studied that population of bacteria gradually decreases with storage time and maximum population was observed at zero day (41) of formulation. The population of *Azotobacter* declined upto 02 log at 108 cfu/g after six months of storage which is within the permissible limit. Thus it is concluded that the stress tolerant strains of biofertilizer formulations in fly ash could be a better option for the growth and yield of the crop in sodic type of soil.

Arora *et al.* (2016) isolated two halophilic bacteria from sodic soils from Indo-Gangetic plains of Uttar Pradesh. The bacterial isolates were characterized, screened for salt tolerance and plant promoting traits and tested for plant growth promotion in sodic soil in field (pH 9.4) experiment. Salt removal efficiency of the strains were tested in liquid media.

Hafeez *et al.* (2018) isolated 55 halotolerant bacterial strains *viz.*, *Azotobacter* (40) and phosphate solubilizing bacteria (15) from soil samples collected from different 10 villages out of which 8 samples were sodic and 2 were saline sodic reaction in Allahabad district. It was also observed that sodicity or salinity levels of the soil samples affected the bacterial population. The maximum cfu-count were found in soil of Panwari Village, which was 7.4 cfu x 10⁵/g soil in Nutrient agar medium, 8.6 cfu x 10⁴/g soil in Ashby's agar medium and 6.3 cfu x 10⁵/g soil in Pikovaskaya agar medium. Several research workers also have isolated salt tolerant *Pseudomonas* from various sources like seawater (Rosenberg, 1983; Claudia Fendrich, 1988), saline infested zone of

wheat rhizosphere (Upadhyay *et al.*, 2009; Zahir Ahmad Zahir *et al.*, 2009), saline soils (Anukool Vaishnav, 2016) in world wide.

2.4 Characterization of salt tolerant bacteria

Hasnain and Taskeen (1989) isolated apparently 20 salt-tolerant bacteria from rhizosphere of *Leptochloa fusca* (Lf) and *Atriplex rhogodoidales* (Ar) from saline patches of Lahore (Pakistan) at 0.5 M NaCl plates initially and at 1M NaCl finally. Out of 20 bacterial isolates 4 from Lf and 3 from Ar were able to grow at 1M salt concentration. Slight variation was observed in colonial morphology of these strains, except Lf-5b. All strains were Gram- negative rods, Lf-5b and Ar-5b were non-motile, while the rest of the isolates were motile. Catalase and oxidase enzymes were present in Lf-2a, Lf-4a and Ar-5b while Lf-4b was positive for oxidase only. Slight variation in the 20 biochemical characters of these isolates was observed. According to Bergey's classification these isolates fall in four groups. Lf-2b and Ar-5b belongs to Gram- negative aerobic rods; Lf-4a and Ar-5a to Gram-negative facultative anaerobic rods. Ar-3b to Gram-negative anaerobic rods; Lf-5b to Gram-positive anaerobic bacteria.

Sanchez-Porro *et al.* (2003) isolated 122 moderately halophilic bacteria from water and sediment of different hypersaline environments in South Spain. Several phenotypic features were determined, the most of the isolates were identified as members of the genera : *Salinivibrio* (55 isolates), *Bacillus- Salibacillus* (29 isolates), *Halomonas* (25 isolates), *Chromohalobacter* (2 isolates) and only eight isolates were not identified.

Ivanova *et al.* (2004) characterized seven slightly halophilic and neutrophilic *Pseudoalteromonas* strain by phylogenetic, genetic and physiological properties and placed them within a novel species, *Pseudoalteromonas aliena* sp. nov. which grew mainly between 4 and 30⁰C and at 3-6 per cent NaCl concentration. They were haemolytic and cytotoxic and were able to degrade starch, gelatin and Tween 80.

Prado *et al.* (2006) isolated 52 strains of moderately halophilic bacteria from hypersaline sediment of Lake Tebenquiche, Chile. The morphological, physiological, biochemical and nutritional characteristics of the strains matched to those described for the genus *Chromohalobacter*. Cells were Gram –negative, non-spore-forming, rod shaped and motile, produced black pigmentation.

Perk *et al.* (2006) isolated a marine bacterial strain, designated FR1311T from sea-water sample from Ganghwa Island, South Korea. Cells were Gram –negative, facultatively anaerobic, catalase and oxidase positive, motile, oval or rod shaped and halophilic (optimum sea-salt concentration for growth of 5-6 %). Phylogenetic analysis of its 16S rRNA gene sequence revealed that it represented a distinct line within the genus *Photobacterium* for which *Photobacterium ganghwense* sp. nov. was proposed.

Green *et al.* (2006) isolated moderately halophilic marine bacteria of shellfish which was Gram-negative, aerobic and hydrocarbon degrading. On the basis of phylogenetic and phenotypic analysis it related to the genus *Marinobacter*, grew optimally in media with 3-6 per cent NaCl and at 25-30⁰C and named *Marinobacter algicola* sp. nov.

Tiquia *et al.* (2007) characterized 125 halophilic and hotolerant bacterial isolates of distinct morphologies and subjected to physiological test, using API strips, identified by 16S rDNA sequence analysis and compared with sequences from Genbank. Results indicated that the SSU rRNA sequences of the bacterial isolates were similar to six major genera, *Bacillus*, *Staphylococcus*, *Halobacillus*, *Paenabacillus*, *Halomonas* and *Clostridium*. Half of the isolates sequenced were of *Bacillus* spp. The API assay showed that the majority of the isolates were positive for the enzymes tryptophane deaminase, gelatinase and β -galactosidase. Indole production, acetoin production and citrate utilization were not observed for any isolates. Fermentation of carbohydrates was observed for very few isolates. They observed six different types of cellular morphologies (long rods, short rods, V-shaped rods, streptobacilli, cocci and spirilli), six different types of colony morphologies (filamentous, circular, lobate, rhizoid, filiform and undulate) on streak plate. The majority of the isolates were from Gram-positive taxa and were non-motile rods, spirilli or cocci.

Tanasupawat *et al.* (2008) isolated halophilic bacteria from fish sauce (Nam-pla) in Thailand. A total fifteen isolates obtained were characterized for morphological, cultural, physiological and biochemical characteristics. They grouped them into three groups, Group A- the moderately rod-shaped halophilic bacteria (4 isolates), Group B- the extremely rod-shaped halophilic bacteria (10 isolates) and Group C- sphere-shaped bacterium (1 isolate).

Essghaier *et al.* (2009) isolated 148 halotolerant to halophilic bacteria from four shallow salt lake of Tunisia and selected 30 effective and active biocontrol isolates by *in vitro* screening. They were phenotypically characterized on the basis of morphological, physiological and chemical analyses. The colony and cell morphology, motility, spore production, Gram staining, pigmentation and growth at optimal pH, temperature and salinity as well as 16S rRNA gene sequence was determined. Identification revealed that 33.4 per cent of the biocontrol isolates belonged to the genus *Bacillus* and 40 per cent to *Staphylococcus*.

Ramya *et al.* (2012) isolated moderately halophilic bacteria from saline water collected from Asian sea bass identified it as *Bacillus laterosporus*, a Gram-positive, bacilli, non-motile, catalase positive and aerobic. Optimum growth was observed at 35°C and pH 8.

Nabti *et al.* (2013) isolated two RBA-E23 and RBA-E32 new halophilic bacterial strains from the rhizosphere soil samples of potato, from sea front at University of Bejaia-Algeria. They identified these strains based on Gram staining, mobility and other biochemical characters by API system 50CHB/E (bio-Merieux, Marcy l'Etoile, France) and 16S rDNA analysis. The strains were identified as *Bacillus licheniformis* and *Bacillus* sp.

2.5 Effect of salt tolerant bacteria on pH and electrical conductivity and its application for reclamation of sodic soil

Krishnamurti and Kate (1951) reported that solution containing glucose and peptone when inoculated with inoculums of *Bacillus subtilis* increased the conductivity of the media appreciably. This results attributed to the initial activity of bacteria through their multi-enzymes systems in splitting up the peptone in the medium into the amino acids which was absorbed by bacteria as nutrient for their growth. When they estimated amino acids formed in the medium at various stages of bacterial growth they found close parallelism between their concentration and the electrical conductivity. Seelig and Richardson (1991) reported that sodic soils are usually the most expensive type of soil to reclaim. Sodic soils can be reclaimed by adding chemical amendments *viz.* gypsum, lime, elemental sulfur and sulphuric acid, adding organic matter or deep tillage.

Reclaiming sodic soils is slow because poor soil structure. Any reclamation of sodic soils is long-term endeavor. Complete reclamation may never be achieved.

Wang *et al.* (2010) reported that three halophilic bacteria i.e. *Pseudomonas mendocina*, *Burkholderia glumae* and *Acinetobacter johnsonii* might have potential for remediation of the degraded wetland in Yellow River Delta of China.

Hafeez *et al.* (2015) reported about halo tolerant *Azotobacter* sp. and its bio-formulations. The efficiency of N₂ fixing ability of many *Azotobacter chroococcum* isolates was generally better in non saline and slightly saline strains in comparison to saline strains.

Arora *et al.* (2016) reported that bio-inoculation of seeds of wheat with plant growth promoting halobacteria resulted in increase of 18.1 and 24.2 per cent in grain and straw yield, respectively under sodic conditions. The inoculation with these bacterial isolates can promote the growth of plants in salt affected soil due to production of hormone auxin and thus enhanced root growth. The soil pH of sodic soil reduced from 9.4 to 8.6 when consortia of halophilic bacteria was applied.

2.6 Salt tolerant bacteria as bioremediation agent of problematic soils

Kargi and Dincer (2000) treated synthetic wastewater composed of diluted molasses, urea, phosphate and different amounts of salt (0 to 5 % sodium chloride) with *Halobacter halobium* in an aeration tank operated in fed-batch mode. The results were compared with those obtained with the activated- sludge culture alone. *Halobacter* addition produced significantly greater COD removal rates and efficiencies at salt conc. greater than 2 % salt. Results indicated that saline waste water containing more than 2 % salt can be treated effectively by *Halobacter*- supplemented, activated-sludge cultures, with COD removal efficiencies greater than 85 per cent with in 9 hr of fed-batch operation.

Abed *et al.* (2004) studied bioremediation of saline soils at a laboratory scale. The saline soils collected from six different locations were treated with product SLR-80, which was a blend of TRX-80 catalyst and aerobic bacteria and found that the bacteria were very efficient in reducing the E_{Ce} values in the first week of application by almost 50 per cent for all of the soil samples, further they noticed that after 104 days the reduction of E_{Ce} was gradual and reached to almost 99.9 per cent for some samples.

Wang *et al.* (2010) reported that three halophilic bacteria i.e. *Pseudomonas mendocina*, *Burkholderia glumae* and *Acinetobacter johnsonni* might have potential for remediation of the degraded wetland in Yellow River Delta of China.

Shivanand and Mugeraya (2011) reported that halophilic bacteria are able to survive in a wide range of salinities and opinioned detailed study is needed to exploit the potential applications of these microorganisms for their commercial viability, including protein and/or enzyme stabilizers, cosmetic actives, therapeutic agents, bioremediation and also in improving the salinity tolerance of crop plants by gene transfer.

2.7 Sodic soil and Gypsum

Gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) has been shown to be beneficial to sodic soils, as when it is combined with soil, Ca^{2+} ions are introduced, which can then exchange with Na^+ ions adsorbed to the surface of clay particles. Release of calcium leads to a decrease in 1) the percentage of exchangeable Na^+ ions present in the soil (ESP), 2) soil pH (through the precipitation of HCO_3^- and CO_3^{2-} complexes) (Gupta and Abrol, 1990), and 3) soil slaking (Ca^{2+} ions are not as readily hydrated as Na^+ ions) (Lehrsch *et al.*, 1993).

2.7.1 Effect of gypsum on the microflora of sodic soils

Gypsum has been demonstrated as having a mostly deleterious effect on the microflora of sodic soils. For example Tyurin *et al.*, (1960), observed that the application of gypsum to sodic soils can cause a temporary decrease in N mineralization rate, as well as microbial activity.

Carter (1986) found that the long-term gypsum treatment (11.2 t/ha) of four acid sodic soils (ranging in pH from 4.5 to 6.5) reduced the overall level of microbial N by 10 to 43 per cent, increased the C:N ratio of the biomass, and reduced mineralisable N by 10 to 54 per cent.

Gasoni *et al.* (1986), demonstrated that treatment of an alkaline sodic soil (pH 8.4) with 4 t/ha gypsum, followed by sulphuric acid treatment, decreased the number of bacteria and fungi isolated from the soil by 72 and 40 per cent, respectively. However, as the number of microorganisms in the soil could be increased to approximately that of the untreated soil by adding ground *Vicia villosa* and *Medicago polymorpha* leaves, it is thought that this decline in microbial numbers was more due to the destructive effects of

sulphuric acid, than from the gypsum treatment. Converse to these studies, Bajpai *et al.*, (1976), found that gypsum application increased the total microbial biomass, microbial activity and numbers of bacteria within sodic soils.

2.8 Organic matter and biological reclamation of sodic soil

Considerable research, conducted primarily in India, has demonstrated the beneficial effect of adding organic matter to sodic soils, in particular to alkaline and highly sodic soils (Swarup, 1986; Varma and Mathur, 1990; Gill *et al.*, 1991; Swarup, 1991; Swarup, 1992; Dubey and Mondal, 1993; More, 1994).

Organic amendments are known to improve the structure of sodic soils, as well as increase the amounts of Fe, Mn, and Zn available to crops (Swarup, 1985; 1992). The ameliorative effect of organic matter can be attributed to 1) the biotransformation of organic matter into organic acids and CO₂, and 2) an increase in microbial biomass and soil enzyme activities (Schnurer and Rosswall, 1982; Dick *et al.*, 1988). More details of these mechanisms are given below:

1. The biotransformation of organic matter into organic acids and CO₂ by microorganisms in alkaline sodic soil containing CaCO₃ causes a reduction in soil pH, and eventually a reduction in the ESP. Organic acids, and aqueous, dissolved CO₂ (carbonic acid) react with CaCO₃ within the soil, freeing Ca²⁺ ions. These Ca²⁺ ions may then exchange with Na⁺ ions adsorbed to clay particles, allowing Na⁺ ions to be leached down further into the soil profile, thus decreasing the ESP (Lehrsch *et al.*, 1993).

To increase production of organic acids, in particular Volatile Fatty Acids, and to maintain acid levels necessary for successful soil amelioration, regular addition of fresh, readily degradable organic matter is necessary because organic acids will be mineralised by soil microorganisms (Tsutsuki and Ponnampereuma, 1987).

2. An increase in microbial biomass and soil enzyme activities leads to increased quantities of fungal hyphae and bacterial polysaccharides, which can bind micro- and macro-aggregates, preventing slaking and dispersion of soil (Tisdall and Oades, 1982; Lynch and Elliot, 1983).

2.8.1 Farmyard manure mixture

Farmyard manure, like crop waste, is one of the most readily obtainable sources of organic matter on farms. Each pig produces up to 3.2 kg of waste per day, and each cow produces between 24.5 kg and 46.6 kg of waste per day (Haug, 1993). With such an output it would be advantageous to utilize this resource for the reclamation of sodic soils, especially considering manures contain both readily available nutrients, which encourage rapid microbial growth (hence acid, CO₂, hyphae, and polysaccharide production) and more stable, bulking organic matter (which can reduce effects of mechanical disturbance and rainfall).

In experiments by Gaffar *et al.* (1992), farmyard manure, in particular, chicken manure, was found to decrease the sodium adsorption ratio (SAR) in a sodic soil, to a depth of 60 cm. The decrease in SAR thus, improved the growth of sorghum planted in this soil. Gaffar and co-workers went further to show that soil treated with farmyard and chicken manure experienced a reduction in pH.

The reclaiming effects of manure on alkaline sodic soils can be further increased by pre-incubating manure before application to the soil. For example, Swarup (1986), showed that farmyard manure submerged for 30 days and then mixed with a sodic soil improved rice yield.

In a separate experiment by Tomar *et al.* (1987) the application of farmyard manure to a wheat crop sown in a sodic soil, this time incubated aerobically for 15 weeks with rock phosphate and pyrites, led to an improvement in yield.

Swarup, 1986; Tomar *et al.*, (1987) studied that, whilst a large portion of the yield improvements reported in these studies are likely to be due to a nutrient response, some of the improvements may also be attributable to VFAs and CO₂, formed during the anaerobic incubation of the manure, reacting with carbonates in the soil and decreasing the soil pH and ESP

2.2 Effect of green manure on the microflora of sodic soils

Green manuring has been shown to have a beneficial effect on the microflora of alkaline sodic soils. For instance, in work by Rao and Pathak (1996), addition of the green manure *Sesbania cannabina* to several alkaline sodic soils ranging

in pH from 8.4 to 10, and in ESP from 3 to 73, was found to increase soil C and N levels, as well as decrease soil pH and ESP levels, stimulating the biological activity of the soils.

2.7.3 Leaf Litter Compost

Mahdy (2011) observed that Compost (animal wastes + plant residues) , Decreasing EC and SAR. Organic amendments co-applied with chemical amendments seemed to have a high value for reducing soil pH, soil salinity and sodicity.

2.7.4 Urban compost

Mojiri (2011) reported that application of Municipal wastewater, Decreasing soil pH and bulk density, while increasing EC and OM content of soil.

Sarfraz *et al.* (2017) studied that integrated and site specific use of MSWC slightly reduced the pHs, ECe and SAR compared to the initial status of soil while organic matter, available P, extractable K and Zn was enhanced over the initial values. A slight increase in heavy metals such as Co, Cu and Pb was also observed with the application of MSWC in soil.

2.9 Effect of halophilic(salt tolerant) bacteria on chemical properties of acidic/saline soil

Abed *et al.* (2004) studied bioremediation of saline soils at a laboratory scale and reported that bacteria were very efficient in reducing the EC values in the first week of application by almost 50 per cent for all the soil samples. After that the reduction of EC was gradual and reached to almost 99.9 per cent after 104 days for some samples.

3. MATERIAL AND METHODS

The present research work entitled, “Role of Microbial Consortia in Reclamation of Sodic Soil” was carried out in Glass house of Department of Plant Pathology and Agricultural Microbiology, Mahatma Phule Krishi Vidyapeeth, Rahuri - 413 722. Details of materials used and methods followed during the period of investigation are described briefly in the succeeding paragraph.

3.1 Materials

3.1.1 Glasswares

Standard Borosil brand glasswares viz., petriplates, conical flask, measuring cylinder, spirit jar, volumetric flask, beaker, glass rod, test tubes, pipettes of 0.1 ml and 1 ml, microscopic glass slides, coverslips etc. were used.

3.1.2 Equipments

The common laboratory equipments such as autoclave, oven, BOD incubator, refrigerator, deep- freeze, weighing balance, microscope, laminar air flow cabinet, spectronic-20, pH meter, electrical conductivity meter, rotary shaker etc. were used.

3.1.3 Miscellaneous material

Earthen pots, labels, polythene paper bags, cotton wool, blotting paper, permanent marker, sterile water, test tube stand, rubber band, sterilized plastic bottles, inoculating needles, spirit lamp etc. were used as and when required during present investigation.

3.1.4 Culture media

The culture media used during the investigation are given as below with their composition and uses.

Nutrient Agar (NA)

Beef extract	: 3.0 g
Peptone	: 5.0 g
Agar	: 15.0 g
Distilled water	: 1000 ml

Nutrient Agar (NA) was supplemented with 3, 5 and 7 % NaCl for isolation of halophilic bacteria i.e. slight (tolerance to 3 % and 5 % NaCl) and moderate halophilic (tolerance to 7% NaCl) respectively as well as for their maintenance.

3.1.3 Chemicals and reagents for microbiological analysis

The chemicals and reagents used for microbiological media preparations, identification of bacterial isolates based on conventional method were Gram staining reagents, 3 per cent hydrogen peroxide, Methyl red test reagent (Beishier, 1991 and Smibert and Krieg, 1994).

3.2 Methods

3.2.1 Aseptic precautions

The normal aseptic technology for bacteriological work was used throughout the study.

3.2.2 Sterilization

Media, cotton wool and organic amendments etc. were sterilized in autoclave at 15 lbs pressure (121.5°C) for 15 min. The problematic soils used for plant growth promotion activity and reclamation studies were sterilized in horizontal autoclave at 15 lbs pressure for 30 min. for three conjugative days by following standard procedure. The earthen pots were surface sterilized with 5% aqueous solution of copper sulphate.

3.2.3 Description of sampling site of sodic soils :

D-Block of Post Graduate Institute Farm, MPKV, Rahuri was selected for isolation of salt tolerant bacteria from Sodic soil.

3.2.4 Sample collection

The soil sample was collected randomly from salt affected soils of D Block of Post Graduate Institute Farm, Mahatma Phule Krishi Vidyapeeth., Rahuri of Ahmednagar District of Western Maharashtra region. The soil sample was taken at the depth of 15-20 cm using a hand auger having diameter of 5 cm and length of 12 cm from 4-5 places in the same field/area. Samples of the same area were mixed together to obtain the composite sample for each areas. The composite soil samples were collected in sterilized polythene bag and stored at 4°C for further examination.

3.2.5 Determination of pH_s , EC_e , Exchangeable sodium percentage and Organic Carbon of sodic soil

The problematic soils used in present investigation were analyzed for pH_s and EC_e , ESP, OC (Singh *et al.*, 2005).

3.2.5.1 pH of Saturation paste extract (pH_s)

Weighed 250g of sodic soil sample and added in a container. Added distilled water to the soil while stirring it with a spatula. At saturation the soil paste will glisten as it reflects light, flowed slightly when the container is tipped and the paste slides freely and cleanly off the spatula for all soils except clay. After mixing allowed the sample to stand for at least one hour and then rechecked for saturation. (Free water should not collect on soil surface. If the paste stiffens or looses its glisten add more water and remix). Transferred the saturated paste to the Buchner funnel fitted with filter paper and applied vacuum. Determined pH_s of the extract using pH meter (Richards, 1968).

3.2.5.2 Electrical conductivity of saturation paste extract (EC_e)

Weighed 250 g of sodic soil sample and added in a container. Added distilled water to the soil while stirring it with a spatula. At saturation the soil paste will glisten as it reflects light, flowed slightly when the container is tipped and the paste slides freely and cleanly off the spatula for all soils except clay. After mixing allowed the sample to stand for at least one hour and then rechecked for saturation. (Free water should not collect on soil surface. If the paste stiffens or looses its glisten add more water and remix). Transferred the saturated paste to the Buchner funnel fitted with filter paper and applied vacuum. The extract is collected. Determined EC_e of the extract using EC meter (Richards, 1968).

3.2.5.3 Exchangeable Sodium Percentage (ESP)

Taken 5 g of soil in a centrifuge tube and added 33 ml of reagent ammonium acetate to the tube. Stopper, and shaken for 5 min. Removed the stopper and centrifuged at $RCF = 1000$ until the supernatant liquid is clear. This usually requires 5min. Decanted the supernatant liquid as completely as possible into 100 ml volumetric flask. Extracted the soil with total of 3 times by this procedure, decanting into the same flask. Dilute to volume, mixed and determined the amount of extracted sodium by flame photometer. Flame photometric analysis is directly made upon aliquots of the extract.

Determined saturation percentage Obtained saturation extract and determine soluble sodium concentration by flame photometer. The determination of exchangeable sodium in ammonium acetate and soluble sodium in saturation extract with help of flame photometer determined (Richards, 1968).

$$\text{ESP} = \frac{\text{Exchangeable sodium (Cmol(p}^+)/\text{kg)}}{\text{Cation exchange capacity (Cmol(p}^+)/\text{kg)}}$$

3.2.5.4 Organic Carbon (OC)

Transferred 1 g of soil sample finely ground and passed through 0.5 mm sieve into 500 ml Erlynmeyer flask, added by means of pipette 10 ml of 1N potassium dichromate solution and swirled the flask gently. Added 20 ml of concentrated H₂SO₄ by measuring cylinder. Swirled the flask by hand for a two minute and set aside on an asbestos pad for exactly half an hour. At the end of half hour, added 200 ml distilled water, 10ml H₂PO₄, 0.2 g of NaF and 1 ml diphenylamine indicator and shake the content of flask. Titrated contents of the flask against 0.5N Ferrous sulphate / ferrous ammonium sulphate solution till the violet blue colour changes to green. Noted the burette reading at this point (Nelson and Sommer, 1982).

3.2.6 Isolation of bacteria from sodic soil samples

Salt tolerant bacteria *viz.* slightly and moderately salt tolerant were isolated from sodic soil samples on nutrient agar medium containing 3, 5 and 7 per cent NaCl, respectively according to their salinity requirements (Kushner, 1993) by serial dilution plate technique.

3.2.6.1 Isolation of salt tolerant bacteria from sodic soil samples

The labeled 9 ml sterile water blanks as well as sterile petriplates for plating 10⁻² to 10⁻⁷ dilutions of bacteria were prepared in duplicates. 1gm soil sample was added into 9 ml water blank to make stock solution. This was vigorously shaken to obtain uniform suspension and 1ml of suspension was transferred to previously prepared water blank with sterile pipette under aseptic conditions to make 10⁻¹ dilution and thus further serial dilutions were carried out upto 10⁻⁷. Then 1ml aliquot of each dilutions (10⁻² to 10⁻⁷) were transferred to separate sterile petriplates in duplicate having NA medium containing 7 per cent NaCl to isolate moderately Salt tolerant bacteria and NA medium containing 3 per cent NaCl and 5 per cent to isolate slightly salt tolerant bacteria. Thus

each selected dilution of each soil sample as well as sediment sample was analyzed in duplicates. The suspension was mixed by gentle rotation of the petriplate. Upon solidification of the media, the plates were kept in polythene bags to avoid desiccation and incubated in inverted position at 37⁰C for 3-7 days as halophilic bacteria prefer this temperature (Asan and Ozcan, 2008).

3.2.7 Characterization of bacterial isolates

3.2.7.1 Physiological characterization bacterial isolates

3.2.7.1.1 Testing of isolates to different concentrations of NaCl

To confirm the salt tolerant nature of bacterial isolates, they were tested against NaCl salt of three concentrations. For this, NA medium supplemented with 3, 5 and 7 per cent NaCl were used to differentiate isolated bacteria into slight (tolerance to 3 %, 5 % salt) and moderate (tolerance to 7 % salt) salt tolerant categories, respectively, according to their salinity requirements (Kushner, 1993). This was the additional procedure to screen the true salt tolerant bacteria. The slants of NA with above NaCl concentrations were prepared on which each salt tolerant bacterial isolate was inoculated by streaking and incubated at 37⁰C for 2-7 days. After completion of incubation period, growth of salt tolerant bacterial isolates was observed and rated as +++ = Full growth, ++ = Moderate growth, + = Poor growth, - = No Growth.

3.2.7.2 Morphological characterization

All the selected isolates were examined for the colony morphology, cell shape, pigmentation and Gram reaction, as per the standard procedures given by Anonymous (1957) and Barthalomew and Mittewer (1950).

3.2.7.2.1 Gram reaction

The bacterial isolates were characterized for their Gram reaction. A thin bacterial smear of one day old culture was made on a clean slide, fixed by gentle heating and Gram stained was performed for all the bacterial isolates by standard procedures given by Cappuccino and Sherman (1999). The isolates which retained dark blue or violet colour of primary stain (crystal violet) were Gram- positive, whereas those that lose the crystal violet and counter stained by safranin (appeared red) were referred as Gram-negative.

3.2.7.2.2 Phenotypic characterization

These halophilic bacterial isolates were examined for their colony morphology as per standard procedures given by Cappuccino and Sherman (1999) by using nutrient agar media at appropriate NaCl concentration according to the salt requirement of each of bacterial isolates to be characterized. Similarly, all the isolates were examined for their cell morphology (cell shape) using the trinocular microscope.

3.2.7.3 Biochemical characterization

The important biochemical test to differentiate salt tolerant bacteria were carried out as outlines in Bergey's Manual of Systematic Bacteriology (9th edition). The biochemical tests to differentiate selected salt tolerant bacteria were as follows:

3.2.7.3.1 Methyl red test

MRVP medium (Methyl Red Voges-Proskauer medium) : Composition per liter.

Peptone	: 5.0 g
Glucose	: 5.0 g
Dipotassium phosphate	: 5.0 g
pH 7.5 ± 0.2 at 25°C	

30 g and 50 g NaCl were dissolved in 200ml water separately and then all above ingredients were added to it and finally volume was made up to 1.0 lit. (to obtain 3 and 5 % NaCl concentrations). This was mixed thoroughly and gently heated until all the ingredients dissolved and autoclave sterilized. This broth was used for the differentiation of bacteria based on acid production (Methyl Red test) and acetone production (Voges-Proskauer reaction).

0.1 ml bacterial suspension (approximate 10⁸ CFU/ml) was inoculated to the MRVP medium and incubated at 37°C for 48 hours to 1 week or until bacterial growth was observed. For result testing, 5 drops of the methyl red solution (methyl red 0.1 g dissolved in 300 ml of 95 per cent ethanol and made up volume to 500 ml with distilled water) was added directly to the broth. The development of a stable red color in the medium indicated a positive result.

3.2.7.3.2 Starch hydrolysis

Starch hydrolysis agar	: Composition per liter
Soluble starch	: 20.0 g
Beef extract	: 3.0 g
Tryptose	: 10.0 g
Agar	: 15.0 g

pH 7.4 ± 0.2 at 25°C .

30 g and 50 g NaCl were dissolved in 200ml water separately and then all above ingredients were added to it and finally volume was made up to 1.0 lit (to obtain 3 % and 5 % NaCl concentrations). This was mixed thoroughly and gently heated until all the ingredients dissolved and autoclave sterilized. This medium was used for the differentiation of bacteria based on amylase production.

A starch hydrolysis agar plate was inoculated with each bacterium to be tested and incubated at 37°C for 48 hours to 1 week or until bacterial growth was observed. The plate was then flooded with iodine solution (iodine 2 g and potassium iodide 4 g were dissolved in distilled water and made volume to 600 ml, solution was kept in the dark place), and the result was observed. Blue color of the agar around the colony of the test organism indicated negative result (no starch hydrolysis), while a clear zone around the colony of the test organism indicated positive result (positive starch hydrolysis).

3.2.7.3.3 Gelatin hydrolysis

Nutrient gelatin	: Composition per liter
Gelatin	: 120.0 g
Beef extract	: 3.0 g
Peptone	: 5.0 g

pH 6.4 ± 0.2 at 25°C .

30 g and 50 g NaCl were dissolved in 200ml water separately and then all above ingredients were added to it and finally volume was made up to 1.0 lit. (to obtain 3 % and 5 % NaCl concentrations). This was mixed thoroughly and gently heated until all the ingredients dissolved and autoclave sterilized. This medium was used for the differentiation of bacteria based on their ability to liquefy gelatin.

Each bacterium to be tested was stabbed deep into the gelatin medium. The inoculated gelatin medium was incubated at 37°C for 48 hours to 1 week or until bacterial growth was observed. Gelatin hydrolysis was indicated by the liquification of the medium after the tube was kept at 4°C for 20-30 minutes.

3.2.7.3.4 Hydrogen sulfide production

Lead acetate agar : Composition per liter

Proteose peptone : 5.0 g

Peptone : 15.0 g

Glucose : 1.0 g

Lead acetate : 0.2

Sodium thiosulfate : 0.08 g

Agar : 15.0 g

pH 6.6 ± 0.2 at 25°C

30 g and 50 g NaCl were dissolved in 200ml water separately and then all above ingredients were added to it and finally volume was made up to 1.0 lit. (to obtain 3 % and 5 % NaCl concentrations). This was mixed thoroughly and gently heated until all the ingredients dissolved and autoclave sterilized. This medium was used for the observation of hydrogen sulfide production.

Each bacterium to be tested was stabbed deep into the lead acetate agar slant and also streaked on the surface of the slant, then incubated at 37°C for 48 hours to 1 week or until bacterial growth was observed. Brownish color formed on the surface and along the line of the stab indicated the positive result.

3.2.7.3.5 Catalase test

A loopful of bacterial culture was transferred on the surface of a glass slide. 1 or 2 drops of 3 per cent hydrogen peroxide (40 % H₂O₂ 7.5 ml was diluted with distilled water to 100 ml) was added over the culture. Rapid appearance of gas bubbles indicated a positive result.

3.2.7.3.6 Oxidase test

The test isolates were inoculated and cultures were incubated at $28 \pm 2^\circ\text{C}$ for 24 hours. After incubation, the filter paper is soaked into tetramethyl-phenylenediamine dihydrochloride and dried the filter paper. Then the overnight growth

of each test organism was inoculated on the surface of dried filter paper which is soaked into tetramethylenediamine dihydrochloride. The isolates showing colour change to maroon was reported as oxidase positive.

3.2.7.3.7 Denitrification

Nitrate broth

Peptone	5.00 g
Beef extract	3.00 g
KNO ₃	1.00 g
Distilled water	1000 ml

Nitrate broth tubes with inverted Durham's tube without air bubbles inside were inoculated with overnight growth culture of the test organism and were incubated at 25⁰C for 15 days. After incubation, the inverted Durham's tubes were observed for the accumulation of gas were recorded as positive.

3.2.8 Experimental Detail

3.2.8.1 Experimental Location

The pot culture experiment was conducted at Glass house of Department of Plant Pathology and Agricultural Microbiology, Post Graduate Institute, MPKV, Rahuri.

3.2.8.2 Soil

The pot culture experiment consists of Sodic soil collected from the D-block of PGI Farm, MPKV, Rahuri.

3.2.8.3 Climate

Geographically the Instructional Research Farm of Central Campus, Post Graduate Institute M.P.K.V., Rahuri located at eastern part of Ahmednagar district. This area comes under arid to semi-arid zone (scaracity tract) with an annual rainfall of 500-750 mm in two peak values, one in the month of July and another in the month of September. The average rainfall is 520 mm. The rainfall was erratic and distributed unevenly in rainy days.

3.2.8.4 Design And Layout

The pot culture experiment was laid out in Factorial Completely Randomized Block Design with 3 replications and 16 treatments. The plan of layout is illustrated in Fig. 3.1.

3.2.8.5 Experiment Details

1. Crop : Spinach
2. Season : *Rabi* (Nov-Jan)
3. Location : Instructional P.G. Farm, Department of Plant Pathology and Agricultural Microbiology, M.P.K.V., Rahuri
4. Date of sowing : 15-11-2019
5. Experimental design : Factorial Completely Randomized Design (FCRD.)
6. Treatments : 16 + 1
7. Replications : 3
8. Total Experimental pots : 49 (48 + 1)
9. Soil : Sodic
10. Sodic soil (Kg/pot) : 5 kg
11. Seed rate : 30-40 kg ha⁻¹
12. Fertilizer dose : 80:30:0 kg NPK ha⁻¹
13. Site of sodic soil : D Block of Post Graduate Institute farm, MPKV, collection Rahuri.

3.2.8.6 Treatment Details

Table 3.1 Treatment details

Sr. No.	Factor A	Factor B
1.	FYM @5t/ha+Gypsum @5t/ha (A ₁)	STB-1
2.	Urban compost @ 5t/ha+Gypsum @5t/ha (A ₂)	STB-2
3.	Leaf litter compost @5t/ha+Gypsum @5t/ha (A ₃)	STB-3
4.	Green leaf manuring (Glyricidia) @ 5t/h + Gypsum @ 5 t/ha (A ₄)	Consortia (STB-1+STB-2 +STB-3)

(STB-1: Salt Tolerant Bacteria-1, STB-2: Salt Tolerant Bacteria-2,
STB-3: Salt Tolerant Bacteria-3)

Table 3.2 Treatment combinations

Treatment No.	Treatment combinations
T ₁	FYM @ 5 t/ha + Gypsum @ 5 t/ha + STB-1
T ₂	FYM @ 5 t/ha + Gypsum @ 5 t/ha + STB-2
T ₃	FYM @ 5 t/ha + Gypsum @ 5 t/ha + STB-3
T ₄	FYM @ 5 t/ha + Gypsum @ 5 t/ha + Consortia
T ₅	Urban compost @ 5 t/ha + Gypsum @ 5 t/ha + STB-1
T ₆	Urban compost @ 5 t/ha + Gypsum @ 5 t/ha + STB-2
T ₇	Urban compost @ 5 t/ha + Gypsum @ 5 t/ha + STB-3
T ₈	Urban compost @ 5 t/ha + Gypsum @ 5 t/ha + Consortia
T ₉	Leaf litter component @ 5 t/ha + Gypsum @ 5 t/ha + STB-1
T ₁₀	Leaf litter component @ 5 t/ha + Gypsum @ 5 t/ha + STB-2
T ₁₁	Leaf litter component @ 5 t/ha + Gypsum @ 5 t/ha + STB-3
T ₁₂	Leaf litter component @ 5 t/ha + Gypsum @ 5 t/ha + Consortia
T ₁₃	Green leaf manuring (Glyricidia) 5 t/ha + Gypsum @ 5 t/ha + STB-1
T ₁₄	Green leaf manuring (Glyricidia) 5 t/ha + Gypsum @ 5 t/ha + STB-2
T ₁₅	Green leaf manuring (Glyricidia) 5 t/ha + Gypsum @ 5 t/ha + STB-3
T ₁₆	Green leaf manuring (Glyricidia) 5t/ha + Gypsum @ 5 t/ha + Consortia
Control	-

3.2.8.7 Replications

All treatments were replicated three times.

3.2.9 Pot Culture Experiment

A pot-culture study was conducted with factorial completely randomized design(Plate 1)(Fig. 1) with two factors

A. Factor A viz., A₁ = FYM @ 5 t/ha + Gypsum @ 5 t/ha,

A₂ = Urban Compost@5t/ha + Gypsum @ 5 t/ha,

A₃ = Leaf litter compost @ 5 t/ha + Gypsum @ 5 t/ha,

A₄ = Green leaf manuring (Glyricidia)@5t/ha + Gypsum @ 5 t/ha,

and

B. Factor B viz., 1. STB-1 (Isolate-1),

2. STB-2 (Isolate-2),

3. STB-3 (Isolate-3) and

4. Consortia of STB-1+STB-2+STB-3 with total 16 treatments in three replications to test plant growth promoting activity of bacterial isolates singly and in combination.

3.2.9.1 Pot filling with sodic soil

The surface sterilized earthen pots of 5 kg capacity were filled with sterilized sodic soils. A control pot was kept.

3.2.9.2 Application of Organic components (Factor A)

The organic components (FYM, Urban compost, Leaf litter compost and Green leaf manure) was applied as per the treatments in sodic soil. The pots containing sodic soil was kept for incubation for 30 days prior to sowing of Spinach crop.

Incubation period was required for exchange of Na from clay complex by chemical amendments like gypsum and organic amendments for its biological degradation which helped for easily replacement of Na from clay complex.

3.2.9.3 Application of inoculums of Salt tolerant bacteria (Factor B)

The 4 nutrient broth containing 1. STB-1, 2. STB-2, 3. STB-3, and 4. Consortia was prepared of volume 500 ml each. Prepared broth was applied to the sodic soil 20 ml per pot according to treatments and allowed to incubate for 30 days.

3.2.9.4 Sowing

Spinach seeds was sown in the pots containing sodic soil @ 10-15 seeds per pot.

3.2.9.5 Fertilizer Application

The recommended dose of fertilizer was applied in each replication in the form of urea and single super phosphate. It is applied as basal dose.

3.2.9.6 Crop Management

To check weed growth, intercultural operations were carried out two times at 15 and 30 days interval. The thinning operation was done at 15 and 25 DAS to maintain uniform plant population in all pots *i.e.* 5 plants per pot.

3.2.9.7 Irrigation

After planting one irrigation was given and subsequent irrigations were given at an interval of 7-8 days.

3.2.9.8 Harvesting

40 DAS, the Spinach plants were uprooted carefully (plate 1).

3.2.10 Observations to be recorded

3.2.10.1 Plant Growth Parameters (quantitative characters)

The crop was harvested at appropriate maturity stage. The plant growth observations *viz.*, height of plant (cm), number of leaves per plant, fresh weight and dry weight were recorded at harvesting.

3.2.10.1.1 Height of Plant (cm)

The plant height was measured from base of the plant (first nodal mark) of base to the tip of the fully opened leaf and expressed in centimeter. Plant height was recorded at time of harvesting.

3.2.10.1.2 Number of Leaves per Plant

Number of leaves per plant were counted manually at randomly selected three plants from each plot. Then average number of leaves of plants were taken as number of leaves per pot.

3.2.10.1.3 Fresh weight of plant (g)

Average fresh weight per plant were recorded using weighing balance from each pot.

3.2.10.1.4 Dry weight of plant (g)

Average dry weight per plant were recorded using weighing balance from each pot.

3.2.10.1.5 Yield

Fresh weight of the leaves of Spinach plant at harvesting was treated as yield of Spinach.

3.2.10.2 Soil quality parameter test

The soil sample taken from the each pot of the experiment for the testing of pH_s, EC_e, Exchangeable Sodium Percentage and organic carbon.

3.2.11 Statistical Analysis

The observations recorded during course of investigation were subjected to statistical analysis by adoption appropriate Model “Analysis of variance”. According to the procedure described by (Panse and Sukhatme, 1967). Critical difference (CD) with in the treatment was calculated in order to compare the treatment at 1 and 5 per cent level of significance only.

4. RESULTS AND DISCUSSION

The results obtained during the present investigation on use of salt tolerant bacteria in agriculture are described below in subsequent sub-heads.

4.1 Isolation of salt tolerant bacteria from sodic soil

The isolation of salt tolerant bacteria were carried out from salt affected soils *i.e.* from sodic soil of D block of Mahatma Phule Krishi Agricultural University, Rahuri (Plate 2).

The sodic soils of D block of instructional farm, MPKV., Rahuri were used for the isolation of salt tolerant bacteria. Three soil samples from salt affected areas was collected and brought to laboratory. The collected samples of the university farm was subjected for initial chemical analysis and isolation of the halophilic bacteria. The initial chemical analysis values of sodic soil are pH_s 8.61, EC_e 3.12 dSm⁻¹, Organic Carbon 0.28% and ESP 15.99%. The initial chemical analysis of soil was carried out at Department of Soil Science and Agricultural Chemistry, Mahatma Phule Krishi Vidyapeeth, Rahuri during the year 2018-19 to 2019-20. The isolation resulted in at least three bacterial isolates of distinct colony types from sodic soil were isolated and designated as STB-1, STB-2 and STB-3 (Table 4.1).

4.2 Morphological, physiological and biochemical characterization of isolates

Bacterial isolates from sodic soil collected from D block farm, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri were characterized and studied for their morphological, biochemical and physiological characteristics. The cultures were maintained in refrigerator at 4⁰ C at Department of Plant Pathology and Agricultural Microbiology, MPKV., Rahuri for further studies.

4.2.1 Morphological characters

The isolates of salt tolerant bacteria STB-1, STB-2 and STB-3 were examined for colony colour, Cell shape and Gram reaction. Out of these, all 3 isolates were white in colony colour and colonies were round, circular in shape. The bacteria were rod shaped and singular or pair habitat. The isolates STB-1 and STB-2 were Gram negative in reaction (Holt *et al.*, 1994) (Mary and Rita, 1985) and isolate STB-3 was Gram positive in reaction (Plate 3)(Table 4.1) (Hensyl *et al.*, 1994).

Table 4.1 Isolation of salt tolerant bacterial isolates from sodic soils.

Location	Isolate No.	Colony colour	Shape of colony/ Forms	Gram Reaction
D Block, MPKV,Rahuri Tal. Rahuri Dist. Ahmednagar	Isolate-1 (STB-1)	White	Round colonies, Rod shape bacteria	–ve
	Isolate-2 (STB-2)	White	Round,colonies rod shape bacteria	–ve
	Isolate-3 (STB-3)	White	Circular shape bacteria	+ ve

4.2.2 Physiological characterization:

All isolates were tested physiologically on the basis of NaCl concentration and temperature.

4.2.2.1 Testing of Isolates to different concentration of NaCl

All 3 isolates were streaked on Nutrient Agar medium plates containing different NaCl concentrations showed isolate-1(STB-1), isolate-2(STB-2), isolate-3 (STB-3) grew prolific at 3 and 5 per cent *i.e.* slightly salt tolerant and moderate at 7 per cent salt concentration *i.e.* moderately salt tolerant (Plate 4, 5, 6)(Table 4.2).

Table 4.2. Growth of Isolates at Different NaCl Concentrations

Salt Concentration(W/V)	Isolate-1 (STB-1)	Isolate-2 (STB-2)	Isolate-3 (STB-3)
3 %	+++	+++	+++
5 %	+++	+++	+++
7 %	++	++	++

+++ = Full growth ++ = Moderate growth + = Poor growth - = No Growth

Several research workers confirmed the halophilic nature on various media containing different concentration of NaCl salt. The slightly halophilic nature was determined in presence of NaCl concentration 2-3 per cent (Yoon *et al.*, 2005; Ivanova *et al.*, 2004), moderately halophilic nature in presence of NaCl concentration 0-10 per cent (Sanchez-Porro *et al.*, 2003; He *et al.*, 2005; Prado *et al.*, 2006; Asan and Ozcan, 2008; Ramya *et al.*, 2012) and extreme halophilic bacteria in presence of NaCl concentration 20 per cent (Rohban *et al.*, 2009).

4.2.2.2 Testing of Isolates at different temperatures

When two isolates exposed to 4⁰C, 41⁰C and 37⁰C, it was observed that isolate 1 survived at 41⁰C Show full growth whereas isolate 2 shows moderate at same. At 37⁰C all isolates showed full growth (Plate 7, 8, 9)(Table 4.3).

Table 4.3 Growth of isolates at different temperatures

Temperature	Isolate-1 (STB-1)	Isolate-2 (STB-2)	Isolate-3 (STB-3)
4 ⁰ C	++	-	-
37 ⁰ C (Ambient temperature)	+++	+++	+++
41 ⁰ C	-	++	++

+++ = Full growth ++ = Moderate growth + = Poor growth - = No Growth

4.2.3 Biochemical characters

All the STB-1 to STB-3 bacterial isolated were tested for biochemical characterization *viz.*, starch hydrolysis, H₂S production, Catalase test, oxidase test, denitrification, Methyl Red test and gelatin liquefaction.

Result indicated that all were positive in oxidase and catalase in reaction. Out of 3 bacterial isolates, 2 isolates were positive in starch hydrolysis reaction and 1 was negative in starch hydrolysis, H₂S production, Denitrification, Gelatin liquefaction (Plate 10, 11, 12)(Table 4.4).

Table 4.4 Biochemical characterization of salt tolerant bacterial isolates

Isolate Test	Isolate-1 (STB-1)	Isolate -2 (STB-2)	Isolate-3 (STB-3)
Starch hydrolysis	-	+	+
H ₂ S Production	+	+	-
Catalase	+	+	+
Oxidase	+	+	+
Denitrification	-	+	+
Gelatin liquefaction	+	-	+
Methyl Red	-	+	-

4.3 Identification of Salt tolerant bacteria

The salt tolerant bacteria were tentatively identified on the basis of morphological, physiological and biochemical characterization. The isolate-1 was found to be *Pseudomonas* spp. (STB-1), isolate-2 was *Azotobacter* spp. (STB-2) and isolate-3 was *Bacillus* spp. (STB-3)(Plate 2).

When *Azotobacter* is grown on media with different NaCl concentrations, it was observed that at all 3, 5, And 7 per cent concentrations *Azotobacter* can grow. The result co-relates the findings of Islam *et al.*, 2008. According to Bergey's manual of Systematic Bacteriology (Holt *et al.*, 1994) at more than 1 % NaCl concentration only *A. chroococcum*, *A. vinelandii* and *A. armeniacus* can survive. Zahran (1999) found that the nitrogen-fixing bacteria was able to grow in media containing 300 mM NaCl. Similarly, Ravikumar *et al.*, (2004) reported that some types of *Azotobacter* isolated from mangrove sediments viz., *A. chroococcum*, *A. berijerinkii*, *A. vinelandii* can survive upto 3 % NaCl concentrations

Saribay (2003) and Jadhav *et al.*, (2010) reported that the highest density of *Azotobacter* spp. occurs around 28⁰C whereas another study found that the optimum temperature for growth of *Azotobacter* spp. was around 30⁰C, which is found similar to present investigation. These result also showed that increasing and decreasing of temperature affect the growth of *Azotobacter* spp. Jadhav *et al.*, (2010) observed that *Azotobacter* spp. was well grown and could efficiently fixed nitrogen at temperature between 25⁰C to 40⁰C.

Several research workers also have isolated salt tolerant *Pseudomonas* from various sources like seawater (Rosenberg, 1983; Claudia Fendrich, 1988), saline infested zone of wheat rhizosphere (Upadhyay *et al.*, 2009; Zahir Ahmad Zahir *et al.*, 2009), saline soils (Anukool Vaishnav *et al.*, 2016) in world wide.

Kumar *et al.*, (2016) isolated *Pseudomonas aeruginosa* from oil-contaminated soil and showed that the isolate shown salinity tolerance upto 6 per cent.

Ahmed Gaber *et al.*, (2016) screened one bacterial isolate as indole acetic acid (IAA) producer on the basis of IAA colorimetric assay under 2 per cent of NaCl in the medium. Based on morphological, physiological and biochemical characteristics

presented in Bergey's Manual of Systematic Bacteriology and on 16SrRNA homology, this isolate identified as *Pseudomonas fluorescens*.

Truper *et al.* (1991) isolated whole range of halophilic bacteria of Gram positive rods and cocci, e.g. *Bacillus*, *Micrococcus* and *Salinicoccus*.

Zahran *et al.* (1992) isolated halophilic bacteria from saline soils of Egypt. They were Gram positive bacteria of the genera *Bacillus* and *Micrococcus* and were dominant among other Gram-positive bacteria in saline soils.

Ramya *et al.* (2012) isolated moderately halophilic bacteria from saline water collected from Asian sea bass which was identified as *Bacillus laterosporus*, a Gram-positive, bacilli, non- motile, catalase positive and aerobic.

4.4 Preparation of Consortia

Nutrient broth of three bacteria viz; *Pseudomonas sp.*, *Azotobacter sp.* and *Bacillus sp.* were prepared separately in three conical flasks by addition of 2-3 loopful of colonies of 48 hr old culture in flask. These flasks were kept on rotary shaker at 120 rpm for 5 days. In fourth flask, nutrient broth prepared and 2-3 loopfuls of colonies of each bacteria of 48 hr old culture was added and kept on rotary shaker at 120 rpm for 5 days. The fourth nutrient broth flask containing STB-1, STB-2 and STB-3 was treated as consortia (Plate 13).

4.5 Pot Culture Experiment

A pot culture experiment was conducted to study the response of application of organic components and salt tolerant bacteria on growth and yield of Spinach crop in Sodic soil.

The result in respect of plant height, number of leaves, fresh and dry weight as well as soil samples analysis for pH_s, EC_e, organic carbon and ESP after harvesting of Spinach were recorded were presented in Table 4.5 to Table 4.12.

Gaikwad (2015) reported that all the halophilic bacterial isolates increased the germination percentage, shoot length and root length of the tomato cv. Dhanshree under blotter paper test.

4.5.1 Plant height (cm)

The results (Table 4.5) of plant height were significant in respect of application of organic components, salt tolerant bacteria and their interactions.

As regards the application of organic components in sodic soil, the treatment A₁= FYM @ 5 t/ha + Gypsum @ 5 t/ha recorded maximum height (31.58 cm) of plants which was at par with the treatment A₂= Urban Compost @ 5 t/ha + Gypsum @ 5 t/ha (31.17 cm) while the minimum height was recorded in the treatment A₄= Green leaf manuring @ 5 t/ha + Gypsum @ 5 t/ha (28.00 cm) after 40 days of sowing.

The results of inoculation of salt tolerant bacteria in Sodic soil revealed that treatment Consortia (STB-1+STB-2+STB-3) recorded maximum plant height (30.92 cm) which was followed by treatment STB-1 = *Pseudomonas* sp. (30.83 cm) and STB-3 = *Bacillus* sp. (29.92 cm) and minimum was recorded in STB-2 = *Azotobacter* sp. (29.25 cm).

The interaction *viz.*, application of FYM @ 5 t/ha + Gypsum @ 5 t/ha + inoculation of consortia (*Pseudomonas* sp. + *Azotobacter* sp. + *Bacillus* sp.) recorded maximum plant height (33.67 cm) which is followed by application of urban compost @ 5 t/ha + Gypsum @ 5 t/ha and STB-3.

Bashan *et al.* (1998) reported that application of mixture of halotolerant bacteria significantly increased plant height, fresh weight and dry weight of mangrove seedling under salt stressed condition.

Table 4.5 Effect of application of salt tolerant bacteria and Organic components on height (cm) of Spinach

Treatments	FYM @ 5 t/ha + Gypsum @ 5 t/ha (A ₁)	Urban Compost @ 5t/ha + Gypsum @ 5 t/ha (A ₂)	Leaf litter compost @ 5 t/ha + Gypsum @ 5 t/ha (A ₃)	Green leaf manuring (Glyricidia) @ 5t/ha+ Gypsum @ 5t/ha (A ₄)	Mean
<i>Pseudomonas</i> sp. (STB-1)	31.67	30.00	33.67	28.00	30.83
<i>Azotobacter</i> sp. (STB-2)	30.00	31.00	30.00	26.00	29.25
<i>Bacillus</i> sp. (STB-3)	31.00	33.67	28.00	27.00	29.92
Consortia (STB-1+ STB-2+ STB-3)	33.67	30.00	29.00	31.00	30.92
Mean	31.58	31.17	30.17	28.00	30.23
	SE ±	CD at 5%		Control	25
Factor A	0.08	0.22			
Factor B	0.08	0.22			
Interaction A x B	0.20	0.50			

Similar elongation in root and shoot length of maize under 6 dSm^{-1} NaCl stress in the presence of PGPR was observed by Nadeem *et al.*, (2006).

As increase in plant height was due to application of organic amendments and inoculation of halobacteria reported in sodic soil as similar to Sanjay Arora *et al.*, (2016). The present results are, therefore, in close agreement with these reports.

4.5.2 Number of Leaves

The results (Table 4.6) of number of leaves were non significant in respect of inoculation of salt tolerant bacteria, application of organic amendments and their interactions.

As regards the application of organic components in Sodic soil, the treatment A_1 = FYM @ 5 t/ha + Gypsum @ 5 t/ha recorded maximum number of leaves (6.75) of plants as compared to control (5.00) were non significant with all treatments and it was followed by the treatment A_2 = Urban Compost @ 5 t/ha + Gypsum @ 5 t/ha (6.58), A_4 = Green leaf manuring @ 5 t/ha + Gypsum @ 5 t/ha (6.33) and A_3 = Leaf litter compost @ 5 t/ha + Gypsum 5 t/ha + Gypsum @ 5 t/ha (6.25) after 40 days of sowing.

Table 4.6 Effect of application of salt tolerant bacteria and Organic components on number of leaves of Spinach

Treatments	FYM @ 5 t/ha + Gypsum @ 5 t/ha (A_1)	Urban Compost@ 5 t/ha + Gypsum @ 5 t/ha (A_2)	Leaf litter compost @ 5 t/ha + Gypsum @ 5 t/ha (A_3)	Green leaf manuring (Glyricidia) @ 5 t/ha+ Gypsum @ 5 t/ha (A_4)	Mean
<i>Pseudomonas</i> sp. (STB-1)	7.33	6.00	7.67	5.33	6.58
<i>Azotobacter</i> sp. (STB-2)	6.00	6.33	6.33	6.00	6.17
<i>Bacillus</i> sp. (STB-3)	5.67	7.67	5.33	6.33	6.25
Consortia (STB-1+ STB-2+ STB-3)	8.00	6.33	5.67	7.67	6.92
Mean	6.75	6.58	6.25	6.33	6.48
	SE $\pm$	CD at 5%		Control	5.00
Factor A	0.30	NS			
Factor B	0.30	NS			
Interaction A x B	0.60	NS			

The results of inoculation of salt tolerant bacteria in Sodic soil revealed that, number of leaves were non-significant. It was observed that treatment Consortia (STB-1 +STB-2+STB-3) recorded maximum number of leaves (6.92) as compared to control (5.00) and it was followed by the treatment STB-1 = *Pseudomonas* sp. (6.58), treatment STB-3 = *Bacillus* sp. (6.25) and STB-2 = *Azotobacter* sp. (6.17).

The interaction viz., application of FYM @ 5 t/ha + Gypsum @ 5 t/ha + inoculation of consortia (*Pseudomonas* sp. + *Azotobacter* sp. + *Bacillus* sp.) recorded maximum number of leaves (8.00) as compared to control (5.00) which is followed by application of Urban compost @ 5 t/ha + Gypsum @ 5 t/ha and STB-3.

An increase (45 %) in root length along with shoot length, fresh weight and the number of leaves per plant of pea under salt stress was also reported by Zahir *et al.* (2008) when inoculated with *P. fluorescens*.

The number of leaves were non significant reported by application of organic components and inoculation of halobacteria reported in sodic soil. Similar results reported by Arora *et al.* (2016). The present results are, therefore, in close agreement with these reports.

4.5.3 Fresh Weight of leaves per plant (g)

The results (Table 4.7) of fresh weight of leaves per plant were significant in respect of application of organic components, inoculation of salt tolerant bacteria and their interactions.

As regards the application of organic components in Sodic soil, the treatment A₁= FYM @ 5 t/ha + Gypsum @ 5 t/ha recorded significantly superior maximum fresh weight (35.15 g/plant) than rest of treatments and which was at par with treatment A₂= Urban Compost @ 5 t/ha + Gypsum @ 5 t/ha (33.67 g/plant), followed by treatment A₄= Green leaf manuring @ 5 t/ha + Gypsum @ 5 t/ha (32.86 g/plant) while the minimum fresh weight was recorded in the treatment A₃= Leaf litter compost @ 5 t/ha + Gypsum @ 5 t/ha (31.68 g/plant) after 40 of sowing.

The data in relation to the inoculation of salt tolerant bacteria in Sodic soil revealed that, treatment Consortia (STA-1+ STA-2+ STA-3) recorded significantly superior maximum fresh weight (35.71 g/plant) which was at par with treatment STB-1 =

Pseudomonas sp. (34.17 g/plant), followed by STB-3 = *Bacillus* sp. (32.18 g/plant) and STB-2 = *Azotobacter* sp. (31.27 g/plant).

The interaction viz; A₁ = FYM @ 5 t/ha + Gypsum @ 5 t/ha + Consortia (38.03 g/plant), A₂ = Urban Compost + Gypsum @ 5 t/ha + STB-1 i.e. *Pseudomonas* sp. (38.03 g per plant) And A₃ = Leaf litter compost @ 5 t/ha + Gypsum @ 5 t/ha + Consortia (38.03 g/plant) recorded maximum fresh weight among the other interactions and at par with A₄ = Green leaf manuring (Glyricidia) @ 5 t/ha + Gypsum @ 5 t/ha + STB-1. It was observed that the fresh weight was ranged from 28.73 to 38.03 g per plant.

Bashan *et al.* (1998) reported that application of mixture of halotolerant bacteria significantly increased plant height, fresh weight and dry weight of mangrove seedling under salt stressed condition.

Table 4.7 Effect of application of salt tolerant bacteria and Organic components on fresh weight (g) of Spinach

Treatments	FYM @ 5 t/ha + Gypsum @ 5 t/ha (A ₁)	Urban Compost @ 5 t/ha + Gypsum @ 5 t/ha (A ₂)	Leaf litter compost @ 5 t/ha + Gypsum @ 5 t/ha (A ₃)	Green leaf manuring (Glyricidia) @ 5 t/ha + Gypsum @ 5 t/ha (A ₄)	Mean
<i>Pseudomonas</i> sp. (STB-1)	34.67	38.03	28.73	35.28	34.17
<i>Azotobacter</i> sp. (STB-2)	33.43	28.73	30.67	32.27	31.27
<i>Bacillus</i> sp. (STB-3)	34.47	34.47	29.27	30.53	32.18
Consortia (STB-1+ STB-2+ STB-3)	38.03	33.43	38.03	33.37	35.71
Mean	35.15	33.67	31.68	32.86	33.34
	SE $\pm$	CD at 5%		Control	24.80
Factor A	0.26	0.76			
Factor B	0.26	0.76			
Interaction A x B	0.51	1.51			

Mayak *et al.* (2004) reported that the plant growth- promoting bacteria *Achromobacter piechaudii* isolated from soil samples of Arava region of southern Israel, significantly increased the fresh and dry weight of tomato seedlings in presence of NaCl salt up to 172 mM and conferred resistance in tomato plant to salt stress.

An increase (45 %) in root length along with shoot length, fresh weight and the number of leaves per plant of pea under salt stress was also reported by Zahir *et al.* (2008) when inoculated with *P. fluorescens*.

Yildirim *et al.* (2008) isolated *Bacillus subtilis* EY2, *Bacillus atrophaeus* EY6 and *Bacillus spharicus* GC subgroup BEY30 from highly salty soils in Upper Coruh Valley in Turkey and used for seed inoculation of Radish. These bacteria positively affected growth parameters such as fresh and dry weight of shoot and root, chlorophyll content and leaf relative water content in saline conditions and thus can ameliorate deleterious effects of salt stress on radish plants.

As increase in fresh weight per plant was due to application of organic components and inoculation of halobacteria reported in Sodic soil similar results as Arora *et al.* (2016). The present results are, therefore, in close agreement with these reports.

4.5.4 Dry Weight of leaves per plant (g)

The results (Table 4.8) of dry weight of leaves per plant were significant in respect of application organic components, inoculation of salt tolerant bacteria and their interactions.

As regards the application of organic components in Sodic soil, the treatment A₁= FYM @ 5 t/ha + Gypsum @ 5 t/ha recorded significantly superior maximum dry weight (3.54 g/plant) than rest of treatments and A₁ was at par with A₂= Urban Compost @ 5 t/ha + Gypsum @ 5 t/ha (3.39 g/plant) and which was followed by the treatment A₄= Green leaf manuring@5t/ha + Gypsum @ 5 t/ha (3.29 g/plant) while the minimum dry weight was recorded in the treatment A₃= Leaf litter compost @ 5 t/ha + Gypsum 5t/ha + Gypsum @ 5 t/ha (3.18 g/plant) after 40 days of sowing.

The results of inoculation of salt tolerant bacteria in Sodic soil revealed that, treatment Consortia (STA-1+STA-2+STA-3) recorded maximum dry weight (3.42 g/plant) which was at par with the treatments STA-3 =*Bacillus* sp. (3.36 g/plant) and STA-1 = *Pseudomonas* sp. (3.35 g/plant) and the minimum dry weight per plant was recorded by the treatment STA-2 = *Azotobacter* sp.(3.27 g/plant).

The interaction viz; application of FYM @ 5 t/ha + Gypsum @ 5 t/ha + Consortia (3.82 g per plant), 2) A₂ = Urban Compost @ 5 t/ha + Gypsum @ 5 t/ha + STA-3 = *Bacillus* sp. (3.82 g per plant) And 3) A₃= Leaf litter compost @ 5 t/ha +

Gypsum @ 5 t/ha + STA-1 = *Pseudomonas* sp. (3.82 g per plant) show maximum dry weight among the interactions. It was observed that the dry weight was ranged from 2.88 to 3.82 g per plant among the interaction combinations.

Bashan *et al.* (1998) reported that application of mixture of halotolerant bacteria significantly increased plant height, fresh weight and dry weight of mangrove seedling under salt stressed condition.

Mayak *et al.* (2004) reported that the plant growth- promoting bacteria *Achromobacter piechaudii* isolated from soil samples of Arava region of southern Israel, significantly increased the fresh and dry weight of tomato seedlings in presence of NaCl salt up to 172 mM and conferred resistance in tomato plant to salt stress.

Table 4.8 Effect of application of salt tolerant bacteria and Organic components on dry weight (g) of leaves per plant Spinach

Treatments	FYM @ 5 t/ha + Gypsum @ 5 t/ha (A ₁)	Urban Compost@ 5 t/ha + Gypsum @ 5 t/ha (A ₂)	Leaf litter compost @ 5 t/ha + Gypsum @ 5 t/ha (A ₃)	Green leaf manuring (Glyricidia) @ 5 t/ha+ Gypsum @ 5 t/ha (A ₄)	Mean
<i>Pseudomonas</i> sp. (STB-1)	3.48	3.37	3.82	3.23	3.36
<i>Azotobacter</i> sp. (STB-2)	3.37	3.48	2.88	3.34	3.27
<i>Bacillus</i> sp. (STB-3)	3.48	3.82	3.08	3.06	3.35
Consortia (STB-1+STB-2 +STB-3)	3.82	2.88	2.93	3.54	3.42
Mean	3.54	3.39	3.18	3.29	3.35
	SE ±	CD at 5%		Control	2.57
Factor A	0.03	0.08			
Factor B	0.03	0.08			
Interaction A x B	0.05	0.15			

Yildirim *et al.* (2008) isolated *Bacillus subtilis* EY2, *Bacillus atrophaeus* EY6 and *Bacillus spharicus* GC subgroup BEY30 from highly salty soils in Upper Coruh Valley in Turkey and used for seed inoculation of Radish. These bacteria positively affected growth parameters such as fresh and dry weight of shoot and root,

chlorophyll content and leaf relative water content in saline conditions and thus can ameliorate deleterious effects of salt stress on radish plants.

As increase in dry weight per plant was due to application of organic amendments and inoculation of halobacteria reported in sodic soil similar to Arora *et al.* (2016). The present results are, therefore, in close agreement with these reports.

4.5.5 pH_s

The results (Table 4.9) of pH_s of Sodic soil were significant in respect of inoculation of salt tolerant bacteria, application of organic components and their interactions.

As regards the application of organic components in Sodic soil, the treatment A₁ = FYM @ 5 t/ha + Gypsum @ 5 t/ha significantly recorded maximum pH_s reduction (8.20) than rest of treatments as compared to initial pH_s (8.61) and minimum pH_s reduction was recorded in the A₄ = Green Leaf litter manuring + Gypsum 5t/ha (8.33) after 40 days of sowing.

The results of inoculation of salt tolerant bacteria in Sodic soil revealed that treatment Consortia (STB-1+STB-2+STB-3) shows maximum reduction in pH_s (8.21) significantly superior than rest of treatments and followed by the treatment STB-1 = *Pseudomonas* sp. recorded maximum reduction pH (8.23), STB-3 = *Bacillus* sp. (8.26) and minimum pH reduction was recorded by the treatment STB-2 = *Azotobacter* sp. (8.29).

The results of interaction of application of organic components and inoculation of halobacteria, the treatment combination i.e. A₁ = FYM @ 5 t/ha + Gypsum @ t/ha + Consortia (8.11), 2) A₂ = Urban Compost @ 5 t/ha + Gypsum @ 5 t/ha + STB-3 = *Bacillus* sp. (8.11) And 3) A₃ = Leaf litter compost @ 5 t/ha + Gypsum @ 5 t/ha + STB-1 = *Pseudomonas* sp. (8.11) recorded maximum pH_s reduction among the interactions. It was observed that the pH_s reduction was ranged from 8.11 to 8.40 among the interaction combinations as compared with initial pH_s (8.61).

Similar results were reported by Esther Hoberg *et al.*, (2005) the bacterium *Pseudomonas fluorescens* Pf-5 grown in liquid media (pH 6.5) with phosphate adsorbed to the Fe- oxide/hydroxide goethite (Goe-P) and with soluble phosphate (0.1 mM or 1.0 mM P as KH₂PO₄). The pH of the was decreased by *P. fluorescens* by 1.4–2.4

units., *P. fluorescens* exuded mainly citrate and only small amounts of two to three other organic acids irrespective of P source or time

The reduction in pH_s due to application of organic amendments along with salt tolerant bacteria might be due to secretion of different organic acids by salt tolerant bacteria might lowered the pH_s of Sodic soil. Similar results were recorded by Arora *et al.* (2016).

Table 4.9 Effect of application of salt tolerant bacteria and Organic components on pH_s at harvesting of Spinach

Treatments	FYM @ 5 t/ha + Gypsum @ 5 t/ha (A ₁)	Urban Compost@ 5 t/ha + Gypsum @ 5 t/ha (A ₂)	Leaf litter compost @ 5 t/ha + Gypsum @ 5 t/ha (A ₃)	Green leaf manuring (Glyricidia) @ 5 t/ha+ Gypsum @ 5 t/ha (A ₄)	Mean
<i>Pseudomonas</i> sp. (STB-1)	8.21	8.25	8.11	8.37	8.23
<i>Azotobacter</i> sp. (STB-2)	8.25	8.24	8.27	8.40	8.29
<i>Bacillus</i> sp. (STB-3)	8.24	8.11	8.30	8.38	8.26
Consortia (STB-1+STB-2+STB-3)	8.11	8.27	8.28	8.18	8.21
Mean	8.20	8.22	8.24	8.33	8.25
	SE ±	CD at 5%		Control	8.61
Factor A	0.004	0.01			
Factor B	0.004	0.01			
Interaction A x B	0.007	0.02			

4.5.6 EC_e (dS m⁻¹)

The results (Table 4.10) of EC_e of Sodic soil were significant in respect of inoculation of salt tolerant bacteria, application organic components and their interactions.

As regards the application of organic components in Sodic soil, the treatment A₁= FYM @ 5 t/ha + Gypsum @ 5 t/ha recorded significantly superior maximum EC_e reduction (2.42 dS m⁻¹) as compared to initial value 3.12 dS m⁻¹ and than the treatments A₂= Urban Compost @ 5 t/ha + Gypsum @ 5 t/ha (2.44 dS m⁻¹), A₃= Leaf

litter compost @ 5 t/ha + Gypsum @ 5t/ha + Gypsum @ 5 t/ha (2.49 dS m⁻¹) and A₄= Green leaf manuring @ 5 t/ha + Gypsum @ 5 t/ha (2.66 dS m⁻¹) after 40 days of sowing.

The results of inoculation of salt tolerant bacteria in Sodic soil, it was observed that treatment Consortia (STB-1+STB-2+STB-3) recorded maximum reduction in EC_e (2.44 dS m⁻¹) which was significantly superior than STB-1 = *Pseudomonas* sp. (2.49 dS m⁻¹), STB-3 = *Bacillus* sp. (2.52 dS m⁻¹) and STB-2 = *Azotobacter* sp. (2.56 dS m⁻¹).

The results of interaction of application of organic components and inoculation of salt tolerant bacteria, the treatment combination i.e. A₁ = FYM @ 5 t/ha + Gypsum @ 5 t/ha + Consortia (2.22 dS m⁻¹), 2) A₂ = Urban Compost @ 5 t /ha + Gypsum @ 5 t/ha + STB-3 i.e. *Bacillus* sp. (2.22 dS m⁻¹) And 3) A₃= Leaf litter compost @ 5 t/ha + Gypsum @ 5 t/ha + STB-1 i.e. *Pseudomonas* sp. (2.22 dS m⁻¹) recorded maximum EC_e reduction among the interactions. It was observed that the EC_e was ranged from 2.22 to 2.79 dS m⁻¹ among the interaction combinations as compared to initial value (3.12 dS m⁻¹).

Table 4.10 Effect of application of salt tolerant bacteria and Organic components on EC_e (dS m⁻¹) at harvesting of Spinach

Treatments	FYM @ 5 t/ha + Gypsum @ 5 t/ha (A ₁)	Urban Compost@ 5 t/ha + Gypsum @ 5 t/ha (A ₂)	Leaf litter compost @ 5 t/ha + Gypsum @ 5 t/ha (A ₃)	Green leaf manuring (Glyricidia) @ 5 t/ha+ Gypsum @ 5 t/ha (A ₄)	Mean
<i>Pseudomonas</i> sp. (STB-1)	2.46	2.51	2.22	2.79	2.49
<i>Azotobacter</i> sp. (STB-2)	2.51	2.48	2.55	2.72	2.56
<i>Bacillus</i> sp. (STB-3)	2.48	2.22	2.61	2.75	2.52
Consortia (STB-1+STB-2+STB-3)	2.22	2.55	2.57	2.40	2.44
Mean	2.42	2.44	2.49	2.66	2.50
	SE ±	CD at 5%		Control	3.12
Factor A	0.004	0.01			
Factor B	0.004	0.01			
Interaction A x B	0.008	0.02			

Similar results were reported by Abed *et al.* (2004) bioremediation of saline soils at a laboratory scale and reported that bacteria were very efficient in reducing the EC values in the first week of application by almost 50 % for all the soil samples.

As increase in EC_e reduction was due to application of organic components and inoculation of halobacteria reported in sodic soil as compared to initial EC_e . Similar results were recorded by Arora *et al.*, (2016). The present results are, therefore, in close agreement with these reports.

4.5.7 Exchangeable Sodium Percentage (%)

The results (Table 4.11) of ESP of Sodic soil were significantly superior in respect of inoculation of salt tolerant bacteria, application organic components and their interactions.

Table 4.11 Effect of application of salt tolerant bacteria and Organic components on ESP (%) at harvesting of Spinach

Treatments	FYM @ 5 t/ha + Gypsum @ 5 t/ha (A ₁)	Urban Compost@ 5 t/ha + Gypsum @ 5 t/ha (A ₂)	Leaf litter compost @ 5 t/ha + Gypsum @ 5 t/ha (A ₃)	Green leaf manuring (Glyricidia) @ 5 t/ha+ Gypsum @ 5 t/ha (A ₄)	Mean
<i>Pseudomonas</i> sp. (STB-1)	12.09	12.41	11.43	13.72	12.42
<i>Azotobacter</i> sp. (STB-2)	12.41	12.22	12.73	13.80	12.79
<i>Bacillus</i> sp. (STB-3)	12.22	11.43	12.69	13.64	12.50
Consortia (STB-1+STB-2 +STB-3)	11.43	12.73	13.01	11.61	12.20
Mean	12.04	12.20	12.47	13.19	12.48
	SE $\pm$	CD at 5%		Control	15.99
Factor A	0.05	0.15			
Factor B	0.05	0.15			
Interaction A x B	0.10	0.28			

As regards the application of organic components in Sodic soil, the treatment A₁= FYM @ 5 t/ha + Gypsum @ 5 t/ha recorded maximum ESP reduction (12.04%) than rest of treatments as compared to initial ESP (15.99%) and which was significantly superior over the treatment A₂= Urban Compost @ 5 t/ha + Gypsum @ 5

t/ha (12.20%), A₃= Leaf litter compost @ 5 t/ha + Gypsum @ 5 t/ha + Gypsum @ 5 t/ha (12.47%) and A₄= Green leaf manuring @ 5 t/ha + Gypsum @ 5 t/ha (13.19%) after 40 days of sowing.

The results of inoculation of salt tolerant bacteria in Sodic soil, it was observed that treatment Consortia (STB-1+STB-2+STB-3) recorded maximum reduction ESP (12.20%) which significantly superior over the treatments STB-1 = *Pseudomonas* sp. (12.42%), STB-3 = *Bacillus* sp. (12.50%) and minimum ESP reduction was recorded by the treatment STB-2 = *Azotobacter* sp. (12.79%).

The results of interaction of application of organic components and inoculation of salt tolerant bacteria, the treatment combination i.e. A₁ = FYM @ 5 t/ha + Gypsum @ 5 t/ha + Consortia (11.43%), 2) A₂ = Urban Compost + Gypsum @ 5 t/ha + STB-3 i.e. *Bacillus* sp. (11.43%) And 3) A₃= Leaf litter compost @ 5 t/ha + Gypsum @ 5 t/ha + STB-1 i.e. *Pseudomonas* sp. (11.43%) recorded maximum ESP reduction among the interactions. It was observed that the ESP reduction was ranged from 11.43 to 13.80% among the interaction combinations as compared with initial ESP (15.99%).

The reduction in ESP was due to application of organic components and inoculation of salt tolerant bacteria reported in Sodic soil as compared to initial ESP. Similar results were recorded by Arora *et al.* (2016). The present results are, therefore, in close agreement with these reports.

4.5.8 Organic carbon (%)

The results (Table 4.12) of organic carbon of sodic soil were significant in respect of application organic components, inoculation of salt tolerant bacteria and their interactions.

As regards the application of organic components in Sodic soil, the treatment A₁= FYM @ 5 t/ha + Gypsum @ 5 t/ha recorded maximum organic carbon (0.36%) as compared to initial value 0.28% and which was at par with all the treatment A₂= Urban Compost @ 5 t/ha + Gypsum @ 5 t/ha (0.34%) and followed by the treatments A₃= Leaf litter compost @ 5 t/ha + Gypsum 5 t/ha + Gypsum @ 5 t/ha (0.32%) and A₄= Green leaf manuring @ 5 t/ha + Gypsum @ 5 t/ha (0.31%).

The results of inoculation of salt tolerant bacteria in Sodic soil, it was observed that treatment Consortia (STB-1+STB-2+STB-3) and STB-1 = *Pseudomonas*

sp. recorded maximum organic carbon (0.34%) which was at par with the treatments STB-3 = *Bacillus* sp. (0.33%) and minimum organic carbon was recorded by the treatment STB-2 = *Azotobacter* sp. (0.31%).

The results of interaction of application of organic amendments and inoculation of salt tolerant bacteria revealed that, the treatment combination i.e. A₁ = FYM @ 5 t/ha + Gypsum @ 5 t/ha + Consortia (0.38%), 2) A₂ = Urban Compost @ 5 t/ha + Gypsum @ 5 t/ha + STB-3 i.e. *Bacillus* sp. (0.38%) And 3) A₃ = Leaf litter compost @ 5 t/ha + Gypsum @ 5 t/ha + STB-1 i.e. *Pseudomonas* sp. (0.38) recorded maximum organic carbon among the interactions. It was observed that the organic carbon was ranged from 0.28 to 0.38% among the interaction combinations.

The slightly increase in organic carbon was due to application of organic components and inoculation of salt tolerant bacteria reported in sodic soil as compared to initial organic carbon.

Table 4.12 Effect of application of salt tolerant bacteria and organic components on Organic carbon (%) at harvesting of Spinach

Treatments	FYM @ 5 t/ha + Gypsum @ 5 t/ha (A ₁)	Urban Compost@ 5 t/ha + Gypsum @ 5 t/ha (A ₂)	Leaf litter compost @ 5 t/ha + Gypsum @ 5 t/ha (A ₃)	Green leaf manuring (Glyricidia) @ 5 t/ha+ Gypsum @ 5 t/ha (A ₄)	Mean
<i>Pseudomonas</i> sp. (STB-1)	0.36	0.33	0.38	0.30	0.34
<i>Azotobacter</i> sp. (STB-2)	0.33	0.35	0.29	0.28	0.31
<i>Bacillus</i> sp. (STB-3)	0.35	0.38	0.28	0.30	0.33
Consortia (STB-1+STB-2 +STB-3)	0.38	0.29	0.33	0.35	0.34
Mean	0.36	0.34	0.32	0.31	0.33
	SE $\pm$	CD at 5%		Control	0.28
Factor A	0.005	0.02			
Factor B	0.005	0.02			
Interaction A x B	0.01	0.03			

5. SUMMARY AND CONCLUSION

The studies on “Role of Microbial Consortia in reclamation of Sodic Soil” was conducted during 2018-19 to 2019-20 at Department of Plant Pathology and Agricultural Microbiology, MPKV., Rahuri. In this studies, the isolation of 03 salt tolerant bacteria were carried out from salt affected soils collected from D block farm, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri from Ahmednagar district of Western Maharashtra region. The collected sample of the university farm was subjected for initial chemical analysis and isolation of the salt tolerant bacteria. The initial chemical analysis values of Sodic soil were pH_s 8.61, EC_e 3.12 dS m⁻¹, organic carbon 0.28% and ESP = 15.99%.

Three bacterial isolates isolated from Sodic soil of D block farm, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri were characterized and studied for their morphological, physiological, biochemical characteristics. The cultures were maintained at Department of Plant Pathology and Agricultural Microbiology, MPKV., Rahuri.

The isolates STB-1, STB-2 and STB-3 were examined for colony colour, Cell shape and Gram reaction. All 3 isolates were white in colony colour and colonies are round, circular in shape. The bacteria were rod shape and singular or pair habitat. The isolates STB-1 and STB-2 were Gram negative in reaction and isolate STB-3 was Gram positive in reaction.

All the STB-1, STB-2 and STB-3 bacterial isolated were tested for biochemical characterization *viz.*, starch hydrolysis, H₂S production, Catalase test, oxidase test, denitrification, Methyl Red test and gelatin liquefaction. Out of these 3 bacterial isolates, all were positive in oxidase and catalase in reaction. Out of these 3 bacterial isolates, 2 isolates were positive in reaction and 1 was negative in starch hydrolysis, H₂S production, Denitrification, Gelatin liquefaction.

All the 03 bacterial isolates showed growth at ambient temperature i.e. room temperature. However, isolate-1 (STB-1) was positive growth at 4⁰C and negative

at 41⁰C whereas STB-2 and STB-3 were negative at 4⁰C. The growth of three isolates were observed white in colour on nutrient agar medium.

A pot culture experiment was conducted to study the response of application of organic amendments and salt tolerant bacteria on growth and yield of Spinach.

The results of application of organic amendments to sodic soil viz; FYM @ 5 t/ha + Gypsum @ 5 t/ha along of inoculation of consortia of salt tolerant bacteria (*Pseudomonas spp.* + *Azotobacter spp.* + *Bacillus spp.*) incubated 30 days prior to sowing of Spinach crop helped to increase all growth parameters like height, number of leaves, fresh weight, dry weight of plant followed by application of green leaf manuring @ 5 t/ha + Gypsum @ 5 t/ha + inoculation of consortia of salt tolerant bacteria incubated 30 days prior to sowing.

As regards the results of pH_s, EC_e, and ESP, it was observed that the application of organic amendments viz., FYM @ 5 t/ha + Gypsum @ 5 t/ha along of inoculation of consortia of salt tolerant bacteria (*Pseudomonas spp.* + *Azotobacter spp.* + *Bacillus spp.*) helped to reduce pH_s ranging from 8.11 to 8.40 as compared to initial pH_s 8.61; EC_e 2.22 to 2.79 dSm⁻¹ as compared to initial value 3.12 dSm⁻¹ and ESP 11.43 to 13.80% as compared to initial value 15.99%.

The results of application of organic amendments viz., FYM @ 5 t/ha + Gypsum @ 5 t/ha along of inoculation of consortia of salt tolerant bacteria (*Pseudomonas spp.* + *Azotobacter spp.* + *Bacillus spp.*) was statistically significant and helped to increase organic carbon ranging from 0.28 to 0.38% over 0.28% initial value.

Thus from results of present research work it can be concluded that:

1. Isolation of salt tolerant bacteria from Sodic soil.
2. On the basis of morphological, physiological and biochemical characteristics of salt tolerant bacteria, they were identified as *Pseudomonas sp.*, *Azotobacter sp.*, *Bacillus sp.*
3. Effective consortia of salt tolerant bacteria *Azotobacter sp.*, *Bacillus sp.*, *Pseudomonas sp.* were prepared and used for reclamation of Sodic soil.
4. Organic amendments FYM @ 5 t/ ha + Gypsum @ 5 t/ha was found more effective than other organic amendments .

5. The treatment of FYM @ 5 t/ ha + Gypsum @ 5 t/ha along with consortia of salt tolerant bacteria (*Azotobactor sp.*+ *Bacillus sp.* + *Pseudomonas sp.*) was found more efficient in recording growth parameters viz., plant height, number of leaves, fresh weight, dry weight and yield over other organic amendments and salt tolerant bacteria.
7. The treatment of FYM @ 5 t/ ha + Gypsum @ 5 t/ha along with consortia of salt tolerant bacteria (*Azotobactor sp.*+ *Bacillus sp.* + *Pseudomonas sp.*) helped to reduce pH_s , EC_e, ESP of sodic soil and helps to increase organic carbon of sodic soil.
8. Therefore sodic soil was said to be reclaimed with added advantage of increased growth parameters and yield of Spinach.

6. LITERATURE CITED

- Abed, N.A., Amayreh, J., Afifi, A.A. and Hiyari, G.A. 2004. Bioremediation of Jordanian saline soil : A laboratory study. *Commun. Soil Sci. Plant Ana.* **35**(9-10) : 1457- 1467.
- Abrol, I.P., Yadav, J.S.P. and Massoud, F.I. 1988. Salt-affected soils and their management. FAO Soils Bulletin. 39, Food and Agriculture Organization of the United Nations, Rome.
- Ahmed Gaber, Mohamed el-awady, Nagwa i. Elarabi, Salah A. Moustafa, 2016. Overexpression of Synechocystis glutaredoxin-2 improves the growth of *Pseudomonas fluorescens* under salt stress. *Romanian Biotechnological Letters*, Vol. 22, No. 6.
- Anonymous, 1954. U.S. Salinity Laboratory Staff. Diagnosis and improvement of saline and alkali soils. U.S. Dept. Agric. Handbook No. **60**(5) : 69-82.
- Anonymous, 1998. FAO Corporate Document Repository. Saltysoils. <http://www.fao.org/docrep/R4082E/r4082e08.htm>.
- Anukool Vaishnav, Sarita Kumari, Shekhar Jain, Devendra Kumar Choudhary, Kanti Prakash Sharma. 2016. Molecular characterization of potential salt tolerant bacteria for soybean growth promotion. *International Journal of Bioassays*. **5**(2) : 18-22..
- Arias, R.S., Galizzi, A.F., Sagardoy, M.A., Peinemann, N. and Ares, A. 2007. Bacteria related to the nitrogen cycle in salt-affected soils of Argentina. *J. Basic Microbiol.* **35** (4) : 269-275.
- Arora, Sanjay, Patel, P., Vanza, M. and Rao, G.G. 2014a. Isolation and Characterization of endophytic bacteria colonizing halophyte and other salt tolerant plant species from Coastal Gujarat. *Afr. J. Microbiol.Res.* **8**(17): 1779-1788.
- Arora, Sanjay, Trivedi, R. and Rao, G.G. 2013. Bioremediation of costal and inland salt affected soils using halophytes plants and halophilic soil microbes. CSSRI Annual Report 2012-13, CSSRI, Karnal, India, pp.94-100.
- Arora, Sanjay, Vanza, M., Mehta, R., Bhuva, C. and Patel, P. 2014b. Halophilic microbes for bio-remediation of salt affected soils. *Afr. J. Microbiol. Res.* **8**(33): 3070- 3078.

- Arora, Sanjay, Y.P. Singh, Meghna Vanza and Divya Sahni. 2016. Bio-remediation of saline and sodic soils through halophilic bacteria to enhance agricultural production. *J. Soil and Water Conservation*. **15**(4) : 302-305.
- Asan, M. and Ozcan, N. 2008. Comparision of enzyme production by halophilic bacteria in sterilized and non-sterilized condition. *J. Appl. Biol. Sci.* **2**(3) :15-19.
- Bajpai, P.D., Gupta, B.R., and Singh, C. 1976. Some microbiological studies in salt-affected soils II. Effect of gypsum and mulch on selected soil microflora and evolution of carbon dioxide. *J. Indian Soc. Soil Sci.* **24** : 48-52.
- Bajwa, M.S. 2002. Soil salinity and alkalinity. *In* : Fundamentals of soil science. Ind. Soc. of Soil Sci. Narayanasamy, G. (Eds). pp. 281-307.
- Barthalomew, J.W. and Mittewar, J. 1950. A Simplified bacterial strain, *Strain Tech.* **25** : 153.
- Bashan, Y., Puente, M.E., Myrold, D.D. and Toledo, G. 1998. *In vitro* transfer of fixed nitrogen fro diazotrophic filamentous Cyanobacteria to black mangrove seedlings. *FEMS Microbiol. Ecol.* **26** : 165-170.
- Beishier, L. 1991. Microbiology in practice (5th eds.), New York : Harper Collins. Bergey's manual of determinative bacteriology, (9th eds.), Baltimore: Williams and Wilkins, Marylan, USA.
- Bhuva, C., Arora, Sanjay and Rao, G.G. 2013. Efficacy of halophilic microbes for salt removal from coastal saline soils. National Seminar with the theme "Microbes and Human Welfare", Bharathidasan University, Tiruchirappalli, India.
- Bio-Gro's Complete Fertility System-Alkaline, Saline and Sodic Soils, www.biogro.com, pp: 1-13.
- Cappuccino, J.G. and Sherman, N. 1999. Microbiology : A laboratory manual. California : Benjamin/Cummings Sci.
- Carter, M.R. 1986. Microbial biomass and mineralizable nitrogen in solonetzic soils: influence of gypsum and lime amendments. *Soil Biol. Biochem.* **18** : 531-537.
- Challa, O., Vadivelu, S. and Sehgal, J. 1995. Soils of Maharashtra for optimizing land use, NBSS and LUP in co-operation with Department of Agriculture, Govt. of Maharashtra. NBSS Publ. No. 54.

- Claudia Fendrich. 1988. *Halovibrio variabilis* gen. nov. sp. nov., *Pseudomonas halophila* sp. nov. and a New Halophilic Aerobic Coccoid Eubacterium from Great Salt Lake, Utah, USA. *Systematic and Applied Microbiology*. **11** : 36-43.
- Damodaran, T., Rai, R.B., Jha, S.K., Sharma, D.K., Mishra, V.K., Dhama, K., Singh, A.K. and Sah, V. 2013. Impact of social factors in adoption of “CSRBIO” A cost effective, ecifriendly biogrowth enhancer for sustainable crop production. *South Asian J. Experiment Biol.* **3**:158-165.
- Dick, R.P., Myrold, D.D., and Kerle, E.A. 1988. Microbial biomass and soil enzyme activities in compacted and rehabilitated skid trail soils. *Soil Sci. Soc. Am. J.* **52** : 512-516.
- Dubey, S.K., and Mondal, R.C. 1993. Sodic soil reclamation with saline water in conjunction with organic and inorganic sediments. *Arid Soil Res. and Rehab.* **7** : 219-231.
- Essghaier, B., Fardeau, M.L., Cayol, J.L., Hajlaoui, M.R., Boudabous, A., Jijakli, H. and Sadfi-Zouaoui, N. 2009. Biological control of grey mould in strawberry fruits by halophilic bacteria. *J. Applied Microbiol.* **106** : 833-846.
- Gaffar, M.O., Ibratrim, Y.M. and Wahab, D.A.A. 1992. Effect of farmyard manure and sand on the performance of sorghum and sodicity of soils. *J. Indian Soc. Soil Sci.* **40** : 540- 543.
- Gaikwad, R.T. 2015. Use of Halophilic (salt loving) bacteria in Agriculture. Ph.D. Thesis submitted to Mahatma Phule Krishi Vidyapeeth., Rahuri. page 120.
- Gasoni, L., Rimolo, M. and Giambiagi, N. 1986. Efecto de diversas enmiendas sobre la flora bacteriana y fungica de un suelo alcalino - sodico de la Pama Deprimida. *Revue d' Ecologie et de Biologie du Sol.* **23** : 351-365.
- Gill, H.S., Rao, D.L.N. and Meelu, O.P. 1991. Effect of anaerobic and aerobic incubation on nitrogen mineralisation from urea and farmyard manure in a sodic soil. *J. Indian Soc. Soil Sci.* **39** : 63-67.
- Green, D.H., Bowman, J.P., Smith, E. A., Gutierrez, T. and Bolch, C.J.S. 2006. *Marinobacter algicola* sp.nov., isolated from laboratory cultures of paralytic shellfish toxin-producing dinoflagellates. *Int. J. Systematic Evolutionary Microbiol.* **56**(3) : 523 527.

- Gupta, R.K., and Abrol, I.P. 1990. Salt affected soils: Their reclamation and management for crop production. *Adv. Soil Sci.* **11** : 224-288.
- Hafeez, M., Lawrence, R., Ramteke, P.W., Suresh, B.G., Bharose, R. and Masih, H. 2018. Halotolerant bacterial diversity isolated from sodic soil samples of Allahabad, Uttar Pradesh. *J of Pharmacology and Phytochemistry.* **7**(5) : 527-531.
- Hafeez, M., Masih, H., Lawrence, R. and Ramteke, P.W. 2015. Halotolerant *Azotobacter* sp. and its formulations to screen, different carrier materials and shelf life study. *New Agriculturist.* **26**(2) : 351-356.
- Haug, R.T. 1993. The practical handbook of compost engineering. Lewis Publishers, 159.
- He, J., Wang, T., Sun, J.Q., Gu, L.F. and Li, S.P. 2005. Isolation and characterization of moderately halophilic bacterium accumulated ectoine as main compatible solute. *Acta Microbiologica Sinica.* **45**(6) : 900-904.
- Hensyl, W.R. 1994. *Bergey's Manual of Systematic Bacteriology 9th edition*. John. G. Holt and Stanley, T. Williams (Eds.) Williams and Wilkins, Baltimore, Philadeipha, Hong kong, London, Munich, Sydney, Tokyo.
- Hasnain, S. and Taskeen, N. 1989. Characterization of salt tolerant bacteria isolated from the rhizosphere of *Leptochloa fusca* and *Atriplex rhogodoidales*. *Pak. J. Pharm. Sci.* **2**(2): 47-53.
- Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T and Williams, S.T. 1994. *Bergey's Manual of Determinative Bacteriology*, 9th ed, Willams and Wilkins Co. Baltimore.
- Imhoff, J.F. 1993. Osmotic adaption in halophilic and halotolerant microorganisms. In : R.H. Vreeland and L.I. Hochstein (eds.). *The Biology of Halophilic Bacteria* : Boca Raton, CRC press. pp. 211-212.
- Islam, M.Z., Sharif, D.I. and Hossain, M.A. 2008. A comparative study of *Azotobacterspp.* From different soil samples. *J. Soil. Nature*, **2**(3) : 16-19.
- Ivanova, E.P., Gorshkova, N.M., Zhukova, N.V., Lysenko, A.M., Zelepuga, E.A., Prokofeva, N.G., Mikhailov, V.V., Nicolau, D.V. and Christen, R. 2004. Characterization of *Pseudoalteromonas* distinct-like sea-water isolates and description of *Pseudoalteromonas aliena* sp. nov. *Int. J. Syst. Evolu. Microbiol.* **54**(5) : 1431-1437.

- Jadhav, G.G., Salunkhe, D.S., Nerkar, D.P. and Bhadekar, R.K. 2010. Isolation and characterization of salt-tolerant nitrogen-fixing micro-organism from food. *J. Eur. Asia. Bio. Sci.*, **4** : 33-40.
- Kargi, F. and Dincer, A.R. 2000. Use of halophilic bacteria in biological treatment of saline wastewater by fed-batch operation. *Water Environ. Res.* **72**(2) : 170-174..
- Khan, M.S., Zaidi, A. and P.A. Wani. 2007. Role of phosphate-solubilizing microorganisms in sustainable agriculture- A review, *Agron. Sustain. Dev.* 27:29-43.
- Kochar, H.K. and Gera, R. 2015. Isolation of Salt tolerant Rhizobacteria and their Plant Growth Promoting activities from Sodic Soils of Harayana. *Intl. J. of Res.* **2**(11) : 215-227.
- Krishnamurti, K. and Kate, S.R. 1951. Changes in electrical conductivity during bacterial growth. *Nature*. 168 : 170. www.nature.com.
- Kumar, K., Amaresan, N., Madhuri, K. and Kannamma Usharani, G. 2016. Isolation and Characterization of Salt Tolerant Plant Growth Promoting Rhizobacteria from Plants Grown in Tsunami Affected Regions of Andaman and Nicobar Islands. *Geomicrobiological Journal*. **33** : 942-947.
- Kushner, D.J. 1993. Growth and nutrition of halophilic bacteria. In: R. H. Vreeland and L. I. Hochstein (eds.). *The Biology of Halophilic Bacteria*. Boca Raton, CRC press. pp. 87- 89.
- Larsen, H. 1962. Halophilism. In : R. H. Vreeland and L. I. Hochstein (eds.). *The Biology of Halophilic Bacteria* : 87, Boca Raton, CRC press.
- Lehrsch, G.A., Robbins, C.W. and Hansen, C.L. 1993. Cottage cheese (acid) whey effects on sodic soil aggregate stability. *Arid soil Res. Rehab.* **8** : 19-31.
- Lynch, J.M. and Elliot, L.F. 1983. Minimising the potential phytotoxicity of wheat straw by microbial degradation. *Soil Biol. Biochem'* **15** : 221-222.
- Mahdy, A.M., 2011. Comparative effects of different soil amendments on amelioration of saline-sodic soils. *Soil Water Res.* **6** : 205–216.
- Mary, L.G. and Rita, R.V. 1985. Enumeration, isolation and characterization of N₂ fixing bacteria from sea water. Department of Microbiology, University of Maryland. **50**(2) : 18-19.

- Mayak, S.T., Tipora, T. and Glick, B.R. 2004. Plant growth promoting bacteria confer resistance in tomato plants to salt stress. *Plant Physiol.* **42** : 565-572.
- More, S.D. 1994. Effect of farm wastes and organic manures on soil properties, nutrient availability, and yield of rice-wheat grown on sodic vertisol. *J. Indian Soc. Soil Sci.* **42** : 253-256.
- Mojiri, A. 2011. Effects of municipal wastewater on physical and chemical properties of saline soil. *J. Biol. Environ. Sci.* **5** : 71-76.
- Nabti, E.H., Sahnoune, M., Adjrad, S., Dommelen, A.V., Ghoul, M., Schmid, M. and Hartmann, A. 2007. A halophilic and osmotolerant *Azospirillum brasiliense*. *Engineering in Life Sci.* **7**(4) : 354-360.
- Nabti, E.H., Mokrane, N., Ghoul, M., Manyani, H., Dary, M. and Megias, M.G. 2013. Isolation and characterization of two halophilic *Bacillus* (*B. licheniformis* and *Bacillus* sp.) with antifungal activity. *J. Eco. Heal. Env.* **1**(1) : 13-17.
- Nadeem, S.M., Hussain, I., Naveed, M., Asghar, H.N., Zahir, Z.A. and Arshad, M. 2006. Performance of plant growth promoting rhizobacteria containing ACC-deaminase activity for improving growth of maize under salt stressed conditions. *Pak. J. Agric. Sci.* **43**(3-4) : 114-121.
- Nelson, D.W and Sommers, L.E. 1982. Total carbon and organic matter in method of soil analysis, part 2 page, A.L (Ed) American Society of Agronomy Inc. Madison, USA., pp. 539-577.
- Panse, V.G. and Sukhatme, P.V. 1967. Statistical methods for agricultural workers. ICAR. New Delhi. 4th Edition. pp. 158-162.
- Perk, Y.D., Baik, K.S., Seong, C.N., Bae, K.S., Kim, S. and Chun, J. 2006. *Photobacterium ganghwense* sp. Nov., a halophilic bacterium isolated from sea-water. *Int. J. of Syst. Evol. Microbiol.* **56**(4) : 745-749.
- Prado, B., Lizama, C., Aguilera, M., Ramos-Cormenzama, A., Fuentes, S., Campos, V. and Monteoliva-Sanchez, M. 2006. *Chromobacter nigrandesensis* sp. nov. a moderately halophilic, Gram-negative bacterium isolated from lake Tebenquiche on the Atacama Salten, Chile. *Int. J. Syst. Evol. Microbiol.* **56**(3) : 647-651.

- Quesada, E., Ventosa, A., Rodriguez-Valera, F., Ramos- Cormenzana, A. 1982. Types and properties of some bacteria isolated from hyper saline soils. *J. Appl. Bacteriol.* **53** : 155-161.
- Ramya, S.R., Asha, K.R.T. and Baskaran, N. 2012. Isolation and characterization of bacterial strains from sea water of Mandaicad in Tamil Nadu, India. *Int. J. Pharma. Biol. Arch.* **3**(6) : 1527-1532.
- Rao, D.L.N. and Pathak, H. 1996. Ameliorative influence of organic matter on biological activity of salt-affected soils. *Arid Soil Res. Rehab.* **10** : 311-319..
- Ravikumar, S., Kathiresan, K., Ignatiammal, T.M., Baba, M.S. and Shanthi, S. 2004. Nitrogen fixing *Azotobacters* from mangrove habitat and their utility as marine biofertilizers. *Journal of Experimental Marine Biology and Ecology*, **31**(2): 5-17.
- Richards, L.A 1968. Diagnosis and improvement of saline and alkaline soils. Agriculture Handbook No. 60, United Soil Salinity Laboratory Staff, Oxford and IBH Publ., Co Culcutta, pp. 1-156.
- Rodriguez-Valera, F. 1988. Characteristics and microbial ecology of hypersaline environments. In: Rodriguez-Valera F (ed) *Halophilic Bacteria*, Vol. 1: CRC Press, Boca Raton, Fla, pp 3-30.
- Rohban, R., Amoozegar, M.A. and Ventosa, A. 2009. Screening and isolation of halophilic bacteria producing extracellular hydrolyses from Howz Soltan lake, Iran. *J. Ind. Microbiol. Biotechnol.* **36** : 333-340.
- Rosenberg, T. 1983. *Pseudomonas halodurans* sp. nov., a halotolerant bacterium. *Archives of Microbiology.* **136** : 117-123.
- Sanchez-Porro, S., Martin, S., Mellado, E. and Ventosa, A. 2003. Diversity of moderately halophilic bacteria producing extracellular hydrolytic enzymes. *J. Applied Microbiol.* **94** : 295-300.
- Sarfraz, M., Qureshi, M.A., Mehdi, S.M., Zaka, M.A., Asifa Naz, A., Ehsan, M., Nawaz, M.Q., Rizwan, M., 2017. Integrated use of MSWC and phosphorus for improving productivity of rice-wheat rotation in salt affected soils. *Intern. J. Biosci.* **10**(3) : 54-62.
- Saribay, G.F. 2003. Growth and nitrogen fixation dynamics of *Azotobacterchroococcum* nitrogen-free and OMW containing medium. M.Sc. thesis in Graduate

School of Natural and Applied Sciences, Middle East Technical University, Turkey.

- Seelig, B.D. and Richardson, J.L. 1991. Salinity and sodicity in North Dakota soils. North Dakota State Uni. Extn. Service EB 57.
- Sharma, D.K., Anshuman Singh, Sharma, P.C., Dagar, J.C. and Chaudhari, S.K. 2016. Sustainable Management of Sodic Soils for Crop Production: Opportunities and Challenges. *Journal of Soil Salinity and Water Quality*. **8**(2) : 109-130.
- Shivanand, P. and Mugeraya, G. 2011. Halophilic bacteria and their compatible solutes – osmoregulation and potential applications. *Current Sci.* **100**(10) : 1516-1521.
- Shukla, S.K., Singh, K., Singh, B. and Gautam, N.N. 2011. Biomass productivity and nutrient availability of *Cynodon dactylon* (L.) Pers. growing on soils of different sodicity stress. *Biomass Bioenergy*. **35**(8) : 3440-3447.
- Singh G., Bundela DS, Sethi M, Lal K and Kamra SK. 2010. Remote sensing and geographic information system for appraisal of salt affected soils in India. *J. Environ. Qual.* **39** : 5-15.
- Singh, D., Chhonkar, P.K. and Dwivedi, B.S. 2005. Manual on soil, plant and water analysis. Westville Publishing House, New Delhi. pp. 23-27.
- Smibert, R.M. and Krieg, N.R. 1994. Phenotypic characterization. In : Gerhardt, P., Murray, R.G.E., Wood, W.A. and Krieg, N.R. (eds.). Manual of methods for general biotechnology. Washington, D.C.: Ameri. Soci. For Microbiol.
- Swarup, A. 1985. Yield and nutrition of rice as influenced by pre submergence and amendments in a highly sodic soil. *J. Indian Soil Sci. Soc.* **33**(2) : 352-354.
- Swarup, A. 1986. Effect of gypsum, pyrites, farmyard manure, and rice husk on the availability of zinc and phosphorus to rice in submerged sodic soil. *J. Indian Soc. Soil Sci.* **34** : 844-848.
- Swarup, A. 1991. Effect of gypsum, green manure, farmyard manure and zinc fertilization on the zinc, iron, and manganese nutrition of wetland rice on a sodic soil. *J. Indian Soc. Soil Sci.* **39** : 530-536.
- Swarup, A. 1992. Effect of organic amendments on the nutrition and yield of wetland rice and sodic soil reclamation. *J. Indian Soc. Soil Sci.* **40** : 816-822.

- Szabolcs, I. 1974. Salt-affected soils in Europe, Martinus Nijhoff, The Hague; Res. Inst. of Soil Sci. and Agril. Chem., Acad. of Sci., Budapest.
- Tanasupawat, S., Namwong, S., Kudo, T. and Itoh, T. 2008. Identification of halophilic bacteria from fish sauce (Nam-pla) in Thailand. *J. Culture Collections*. 6 : 69-75.
- Tiquia, S. M., Davis, D., Hadid, H., Kasparian, S., Ismail, M., Sahly, R., Shim, J., Singh, S. and Murray, K.S. 2007. Halophilic and halotolerant bacteria from river water and shallow groundwater along the Rouge river of South Eastern Michigan. *Expt. Tech.* **28** : 297-307.
- Tisdall, J.M., and Oades, J.M. 1982. Organic matter and water-stable aggregates in soils. *J. Soil Science*. **33** : 141-163.
- Tomar, N.K., Sharma, A.K., and Gupta, A.P. 1987. Effect of pre-incubated phosphates, manure and pyrites on the phosphorus transformation in a sodic soil. *J. Indian Soc. Soil Sci.* **35** : 432-440.
- Trivedi, R. and Arora, S. 2013. Characterization of acid and salt tolerant *Rhizobium* sp. isolated from saline soils of Gujarat. *Int. Res. J. Chem.* **3**(3) : 8-13.
- Truper, H.G., Severin, J., Wohlfarth, A., Muller, E. and Galinski, E.A. 1991. Halophily, taxonomy, phylogeny and nomenclature. In : Rodriguez-Valera F. (Ed.). General and applied aspects of halophilic microorganisms. Plenum Press, New York. pp. 3-7.
- Tsutsuki, K. and Ponnamperna, F.N. 1987. Behaviour of anaerobic decomposition products in submerged soils. Effects of organic material amendment, soil properties, and temperature. *Soil Sci. Plant Nutrit.* **33** : 13-33.
- Tyurin, I.V., Antipov-Karatev, I.N. and Chuztrervskii, M.G. 1960. Reclamation of solonchic soils in the U.S.S.R. Acad. Sci. U.S.S.R. (translated from Russian by Israel Scientific Translations, Jerusalem, Israel, 1967, **I** : 48-151.
- Upadhyay, S.K., Singh, D.P. and Ratul Saikia. 2009. Genetic Diversity of Plant Growth Promoting Rhizobacteria Isolated from Rhizospheric Soil of Wheat Under Saline Condition. *Current Microbiology*. **59** : 489-496
- Varma, S. and Mathur, R.S. 1990. The effects of microbial inoculation on the yield of wheat when grown in straw amended soil. *Biolog. Wastes*. **33** : 9-16.

- Vivekanandan, M., Karthik, R. and Leela, A. 2015. Improvement of crop productivity in saline soils through application of saline-tolerant rhizosphere bacteria- Current Perspective. *Int. J. Adv. Res.* **3**(7) : 18-22.
- Wang, Z.Y., Xin, Y.Z., Gao, D.M., Li, F.M., Morgan, J. and Xing, B.S. 2010. Microbial community characteristics in a degraded wetland of the Yellow River Delta. *Pedosphere*, **20**(4) : 466-478.
- Wicke, B., E. Smeets, V. Dornburg, B. Vashev, T. Gaiser, W. Turkenburg and A. Faaij. 2011. The global technical and economic potential of bioenergy from salt-affected soils. *Energy Environ. Sci.* **4**:2669-2681.
- Yildirim, E., Donmez, M.S. and Turan, M. 2008. Use of bioinoculants in ameliorative effects on radish plants under salinity stress. *J. Plant Nutri.* **31**(12) : 127-129.
- Yoon, J.H., Kang, S.J. and Oh, T.K. 2005. *Marinomonas dokdonensis* sp. nov. isolated from sea-water. *Int. J. Syst. Evol. Microbiol.* **55**(6) : 2303-2307.
- Zahir Ahmad Zahir, Usman Ghani, Muhammad Naveed, Sajid Mahmood Nadeem, Hafiz Naeem Asghar, 2009. Comparative effectiveness of *Pseudomonas* and *Serratia* sp. containing ACC-deaminase for improving growth and yield of wheat (*Triticum aestivum* L.) under salt-stressed conditions. *Archives of Microbiology*. **191** : 415-424.
- Zahir Ahmad Zahir, Usman Ghani, Muhammad Naveed, Sajid Mahmood Nadeem, Hafiz Naeem Asghar. 2009. Comparative effectiveness of *Pseudomonas* and *Serratia* sp. containing ACC-deaminase for improving growth and yield of wheat (*Triticum aestivum* L.) under salt-stressed conditions. *Archives of Microbiology*. **191** : 415-424.
- Zahran, H.H., Moharram, S.M. and Mohammad, H.A. 1992. Some ecological and physiological studies on bacteria isolated from salt affected soils of Egypt. *J. Basic Microbiol.* **32** : 405-413.
- Zahir, Z.A., Munir, A., Asghar, H.N., Shaharoon, B. and Arshad, M. 2008. Effectiveness of Rhizobacteria containing ACC deaminase for growth promotion of peas (*Pisum sativum*) under drought conditions. *J. Microbiol Biotechnol.* **18**(5) : 958-963.

- Zahran, H.H. 1999. Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in acid climate. *Microbiol. Mol. Biol. Rev.*, **63** : 968-989.
- Zahran, H.H., Ahmed, M.S. and Afkar, E.A. 2010. Isolation and characterization of nitrogen fixing moderately halophilic bacteria from saline soil of Egypt. *J. Basic Microbiol.* **50**(3) : 285-289.
- Zahran, H.H., Moharram, S.M. and Mohammad, H.A. 1992. Some ecological and physiological studies on bacteria isolated from salt affected soils of Egypt. *J. Basic Microbiol.* 32 : 405-413.

7. APPENDIX

Composition of different culture media used

1. Nutrient Agar medium

Ingredient	Quantity
Agar	15 gm
Beef extract	5 gm
Peptone	10 gm
NaCl	5 gm
Distilled Water	1000 ml
pH	7.0

2. Nutrient Broth medium

Ingredient	Quantity
Beef extract	5 gm
Peptone	10 gm
NaCl	5 gm
Distilled Water	1000 ml
pH	7.0

8. VITAE

MR. PATOLE AMIT SARJERAO
MASTER OF SCIENCE (AGRICULTURE)
IN
AGRICULTURAL MICROBIOLOGY
2021

Title of thesis		:	“Role of Microbial Consortia in Reclamation of Sodic soil”
Major field		:	Agricultural Microbiology
Biographical information		:	
Personal	Date of Birth	:	06/11/1996
	Place of Birth	:	At Post - Asad, Tal - Kadegaon, Dist – Sangli, State - Maharashtra.
	Father’s Name	:	Mr. Sarjerao Shripati Patole
	Mother’s Name	:	Mrs. Ratna Sarjerao Patole
Educational	Bachelor Degree Obtained	:	Loknete Mohanrao Kadam College of Agriculture, Kadegaon, Tal. Kadegaon, Dist. Sangli
	Class	:	First class
	Name of University	:	Mahatma Phule Krishi Vidyapeeth, Rahuri.
Address		:	At Post - Asad, Tal - Kadegaon, Dist - Sangli, Pin code -415 303
Email-id		:	amitpatole07@gmail.com
Contact Number		:	9527517512