Project ID: 559

Competitive Research Grant

Sub-Project Completion Report

on

Symbiotic and Molecular Characterization of Potential Saline Tolerant Rhizobial Strains and Biofertilizer Production for Soybean and Groundnut

Project Duration

July 2017 to September 2018

Soil Science Division
Bangladesh Agricultural Research Institute (BARI)
Gazipur

Submitted to
Project Implementation Unit-BARC, NATP 2
Bangladesh Agricultural Research Council
Farmgate, Dhaka-1215

September 2018

Competitive Research Grant (CRG)

Sub-Project Completion Report

on

Symbiotic and Molecular Characterization of Potential Saline Tolerant Rhizobial Strains and Biofertilizer Production for Soybean and Groundnut

Project Duration

July 2017 to September 2018

Soil Science Division
Bangladesh Agricultural Research Institute (BARI)
Gazipur

Submitted to
Project Implementation Unit-BARC, NATP 2
Bangladesh Agricultural Research Council
Farmgate, Dhaka-1215

September 2018

Citation

F. Alam, M. A. H. Bhuiyan and M. A. Hossain 2018. Symbiotic and Molecular Characterization of Potential Saline Tolerant Rhizobial Strains and Biofertilizer Production for Soybean and Groundnut. A report of Competitive Research Grant Sub-Project under National Agricultural Technology Program-Phase II Project (NATP-2), Bangladesh Agricultural Research Council (BARC), Farmgate, Dhaka, Bangladesh.

Project Implementation Unit
National Agricultural Technology Program-Phase II Project (NATP-2)
Bangladesh Agricultural Research Council (BARC)
New Airport Road, Farmgate, Dhaka - 1215
Bangladesh.

Edited and Published by:

Project Implementation Unit
National Agricultural Technology Program-Phase II Project (NATP-2)
Bangladesh Agricultural Research Council (BARC)
New Airport Road, Farmgate, Dhaka - 1215
Bangladesh.

Acknowledgement

The execution of CRG sub-project has successfully been completed by Bangladesh Agricultural Research Institute (BARI) using the research grant of USAID Trust Fund and GoB through Ministry of Agriculture. We would like to thank to the World Bank for arranging the grant fund and supervising the CRGs by BARC. It is worthwhile to mention the cooperation and quick responses of PIU-BARC, NATP 2, in respect of field implementation of the sub-project in multiple sites. Preparing the project completion report required to contact a number of persons for collection of information and processing of research data. Without the help of those persons, the preparation of this document could not be made possible. All of them, who made it possible, deserve thanks. Our thanks are due to the Director PIU-BARC, NATP 2 and his team who given their whole hearted support to prepare this document. We hope this publication would be helpful to the agricultural scientists of the country for designing their future research projects in order to generate technology as well as increasing production and productivity for sustainable food and nutrition security in Bangladesh. It would also assist the policy makers of the agricultural sub-sectors for setting their future research directions.

Published in: September 2018

Printed by: [Name of press with full address]

Acronyms

AEZ	Agro-Ecological Zone
ARS	Agricultural Research Station
B	Boron
BARC	Bangladesh Agricultural Research Council
BARI	Bangladesh Agricultural Research Institute
Ca	Calcium
Co-PI	Co- Principal Investigator
Cu	Cupper
CRD	Complete Randomized Design
CRG	Competitive Research Grant
CV	Co-efficient of Variation
DAS	Days After Sowing
DNA	Deoxyribo Nucleic Acid
Fe	Iron
FRG	Fertilizer Recommendation Guide
GR	Groundnut *Rhizobium*
GPS	Global Positioning System
K	Potassium
Mg	Magnesium
MoP	Muriate of Potash
NATP	National Agricultural Technology Program
OM	Organic Matter
P	Phosphorus
PCR	Polymerase Chain Reaction
PCR	Project Completion Report
PI	Principal Investigator
PIU	Project Implementation Unit
RARS	Regional Agricultural Research Station
S	Sulphur
SE	Standard Error
SR	Soybean *Rhizobium*
ST	Strip Tillage
YEM	Yeast Extract Mannitol
YEMA	Yeast Extract Mannitol Agar
Zn	Zinc

Table of Contents

Executive Summary

A project was implemented by Soil Science Division, Bangladesh Agricultural Research Institute (BARI) during 2017-2018 with the goal to isolate of salt-tolerant rhizobia species from coastal saline area of Bangladesh, characterize and produce biofertilizers by those effective salt tolerant bacteria and apply it for soybean and groundnut production. For isolating of salt tolerant *Rhizobium* bacteria, 273nodule samples were collected from soybean and groundnut root from different fields of saline stress soils (Noakhali, Lakhmipur, Cox's Bazar, Satkhira, Bhola, Borguna, Patuakhali) of Bangladesh using global positioning system (GPS) record along with crop history. Nodules were collected aseptically, purified strains and preserved at 4^0C for short time preservation and kept in -20^0C, -80^0C for long time preservation. The salt tolerance study was carried out on YEM agar plate with each modified by NaCl at 0, 2, 4, 8, 16, 32 and 48 dSm^{-1}. All isolated bacteria were tested by growing on plate up to >48 dS m^{-1} level. Finally selected bacterial strains have been used for peat based biofertilizer production. Seedlings were grown in glass tube to check the infection ability of *Rhizobium* (strain SR1 to SR20 for soybean and strain GR1 to GR20 for groundnut) and found nodule formation in seedling roots within 30-50 days after inoculation. Two sets of pot experiment were conducted at net house of Soil Science Division, BARI during 2017-2018 to know the performance of salt tolerance*Rhizobium* bacteria in soybean (strain SR1 to strain SR20) and groundnut (strain GR1 to strain GR20)at 0, 4, 8, 12, 16 dSm^{-1} salinity level.Field experiments were carried out for soybean whereas biofertilizer strain SR1 to strain SR10 were experimented at Cox's Bazar, Noakhali and biofertilizer strain SR11 to strain SR20 were tested at Patuakhali and Satkhira, respectively.Similarly, another field experimentwas carried out for groundnut whereas biofertilizer strain GR1 to strain GR10 were experimented at Cox's Bazar, Noakhali and biofertilizer strain GR11 to strain GR20 were tested at Patuakhali and Satkhira, respectively. Application of salt tolerance *Rhizobium* biofertilizer on soybean and groundnut, the inoculated plants exhibited better performance in nodule numbers, nodule weights, aboveground biomass, root biomass, and plant height than non-inoculated plants, indicating that all *Rhizobium* sp. effectively enhanced nodulation and growth parameters on soybean and groundnut plant than non-inoculatedplants in all salinity levels under pot and field native saline condition.In pot trials, plants inoculated with *Rhizobium* sp. showed higher pod yield, stover yield and seed yield than non-inoculated plants, revealing the positive impact of *Rhizobium* sp. on yield parameters of soybean and groundnut. Soybean plants inoculated with Rhizobium sp.SR15 produced highest seed yield than other strains inoculated and non-inoculated plantsat different salinity levels. Similarly, in pot trails, groundnut plants inoculated with *Rhizobium* sp.GR13 showed highest nut yield of groundnut than other strains inoculated and non-inoculated plantsat different salinity levels.Soybean plants inoculated with *Rhizobium* sp.SR7 tested in field recorded best result at Cox's Bazar and Noakhali,and*Rhizobium* sp.SR15 tested in field showed best result at Patuakhali andSatkhira, respectively.In the same way, groundnut plants inoculated with *Rhizobium* sp.GR9recorded best result at Cox's Bazar, Noakhali and *Rhizobium* sp.GR13showed best result at Patuakhali, Satkhira, respectively. Therefore, all strains *Rhizobium*have capable on salt toleranceand established an effective symbiotic association with soybean and groundnut and that why, they were responsible for increased growth and biomass, and improved yield characteristics of soybean and groundnut in pot and field trialsat all the locations. DNA was isolated from *Rhizobium* strains, amplified by PCR, and purified DNA were sequenced. The better strains sequence result showed that these strains were belonging to bacteria kingdom, proteo bacteria phylum, alpha proteo bacteria class, rhizobiales order, rhizobiaceae family, and genus *Rhizobium*.

CRG Sub-Project Completion Report (PCR)

A. Sub-project Description

1. Title of the CRG sub-project: Symbiotic and Molecular Characterization of Potential Saline Tolerant Rhizobial Strains and Biofertilizer Production for Soybean and Groundnut.
2. Implementing organization: Bangladesh Agricultural Research Institute (BARI)
3. Name and full address with phone, cell and E-mail of PI/Co-PI (s):

Dr. Faridul Alam
PI &Senior Scientific Officer
Soil Science Division, BARI, Gazipur-1701
Phone: 02-49270075, Mobile: 01819338131
E-mail : farid443@yahoo.com
&
Dr. Md. Asadul Haque Bhuiyan
Co-PI & Principal Scientific Officer
Research wing, BARI, Gazipur
Phone: 02-49270075, Mobile: 01720051657
E-mail: mahbhuiyan63@yahoo.com

4. Sub-project budget (Tk):
   4.1 Total : 3000000/-
   4.2 Revised (if any) : 3000000/-
5. Duration of the sub-project:
   5.1 Start date (based on LoA signed):12July 2017
   5.2 End date :30 September 2018
6. Justification of undertaking the sub-project:

Nitrogen is one of the most abundant elements on earth, but its availability often limits plant growth and crop production (Alam *et al.,* 2015). It is converted to plant available forms by chemical fixation through industrial production and/or biological nitrogen fixation (BNF) involving micro-organisms. Salinity in the arid and semi-arid region of the world is a serious threat to agriculture. Saline soils cover about 380-995 million hectares of the earth's land surface. The ground water in salt affected areas is also saline and thus unfit for crop production. Therefore, the agriculture sectors face a big challenge to meet the requirements of food and raw materials for ever increasing population of the world. In case of Bangladesh, the coastal area covers about 20% of the total area and 30% of the net cultivable area (Ahmed *et al.,* 2013). Due to the climate change and decreased flow rate freshwater from the north, about 1 (one) million hectare of land in the southern region is adversely affected by salinity (Ahmed *et al.,* 2013). Millions of people living in this salinity affected regions are severely affected by the depletion of soil fertility, scarcity of freshwater and environmental pollution leading to reduction of agricultural and forest productivity and metabolic diseases. These vast saline areas provide grazing, fodder, fuel, feed and raw material to some extent that could be increased significantly under better management. Through the poverty alleviation is the prime issue of the country but changes of world climate appeared to be foremost challenge against any national issue including poverty alleviation. No processes of development and eradication of poverty can be conceived of without putting caring for environment and sustainable development at the center-stage. Day by day the condition of the people living in these vast salinities affected areas becoming more vulnerable in respect of all sorts of things needed to get rid of poverty.

As such, technological development particular biotechnological development is a necessity to increase soil fertility and crop production, tackle diseases of humans, plants and fishes through the development of effective remedies, develop indigenous resources, conserve genetic diversity and adopt adequate measures toward controlling various pollutants including chemical fertilizers, pesticides and arsenic. Biotechnological method, which is used to improve soil as well as saline soil characteristics, is the inoculation of soil with microorganisms as called biofertilizers that are able to process available components of plant nutrients. *Rhizobia* are used to increase of nitrogen absorption in soil. Though, people in the salinity affected regions in Bangladesh live in vulnerable conditions in respect of food security. No systemic study is available in the literature providing information regarding existing indigenous salt-tolerant *Rhizobium* bacteria for soybean and groundnut, which could be effectively used in agricultural practices. Moreover, the prices of chemical fertilizers are rapidly increasing day by day and are reaching beyond the farmers' purchase level. Investigations of the diversity, dynamics, and characteristics of rhizobia residing in saline stress soils of different AEZs have practical importance in both ecology and agriculture. Such studies not only facilitate the discovery of unknown rhizobia but also selecting effective combinations of *Rhizobium*-legume genotypes to increase nitrogen fixation. Some strains of *Rhizobium* must have been adapted in saline stress soil. Most of generic biofertilizers are suitable for normal soil, however, it does not show good result in coastal saline soil due to high salt concentration which restrict to microbial living. In natural condition, many microorganisms have been adapted to grow in saline environment. Therefore, some strains of *Rhizobium* must have been adapted in saline soil in Bangladesh also. Hence, it is utmost important to screen-out salt-tolerant *Rhizobium* strains and their application in coastal saline soil for boosting up soil fertility as well as soybean and groundnut production.

7. Sub-project goal: Discovery of salt-tolerant Rhizobia species and biofertilizers production for promoting soil fertility, and soybean and groundnut production in saline affected areas of Bangladesh.

8. Sub-project objective (s):
   i. To isolate, identify and preserve effective rhizobial strains collected from soybean and groundnut root nodules from saline stress areas of Bangladesh.
   ii. To study symbiotic, biochemical and molecular characteristics of effective rhizobial strains and identify their taxonomy (family, genus and species).
   iii. To use these effective strains for biofertilizer production for soybean and groundnut cultivation.

9. Implementing location (s): Soil Microbiology Laboratory; Net house, Soil Science Division; Kuruskul, Cox's Bazar; Subornochor, Noakhali; Kuakata, Patuakhali and ARS, Satkhira,

10. Methodology in brief:

i) Collection and Isolation of *Rhizobium* from nodules:

Nodules from soybean and groundnut root (273 samples) were collected from different fields of saline soils (Noakhali, Lakhmipur, Cox's Bazar, Satkhira, Bhola, Borguna, Patuakhali) of Bangladesh using global positioning system (GPS) record along with crop history (Table 1, Table 2). Nodules were collected aseptically and crushed, streaked onto yeast extract mannitol agar (YEMA) plate and incubated at 30^0C for 2-3 days. A final selection of the bacterial strains was done by comparing morphological (colony) characteristics and transferred to agar slants prepared with the corresponding plating media.

Table 1. Rhizobia bacterial strains were collected from soybean field

Sl no.	Name	Origin	EC (dSm^{-1})	GPS position	AEZ	Cropping patern
01	*Rhizobium* SR1	Subornochor, Noakhali	3.2	Latitude: 22.94^{o}N, Longitude: 90.82^{o}E	18	Soybean - T. aus -T.aman
02	*Rhizobium* SR2	Kalia, Doulatkhan, Bhola	3.8	Latitude: 22.35^{o}N Longitude: 90.45^o E	18	Soybean - Fallow-T.aman
03	*Rhizobium* SR3	Bangla Bazar, Doulatkhan, Bhola	4.8	Latitude: 22.35^{o}N Longitude: 90.40^o E	18	Soybean - T. aus -T.aman
04	*Rhizobium* SR4	Hazir Hat, Komol Nagar, Lakhmipur	3.8	Latitude: 22.94^{o}N Longitude: 90.82^{o}E	18	Soybean - Fallow-T.aman
05	*Rhizobium* SR5	Hazir Hat, Komol Nagar, Lakhmipur	3.6	Latitude: 22.94^{o}N, Longitude: 90.82^{o}E	18	Soybean - Fallow-T.aman
06	*Rhizobium* SR6	Hazir Hat, Komol Nagar, Lakhmipur	4.6	Latitude: 22.94^{o}N Longitude: 90.82^{o}E	18	Soybean - T. aus -T.aman
07	*Rhizobium* SR7	Hazir Hat, Komol Nagar, Lakhmipur	3.9	Latitude: 22.94^{o}N, Longitude: 90.82^{o}E	18	Soybean - Fallow-T.aman
08	*Rhizobium* SR8	Hazir Hat, Komol Nagar, Lakhmipur	3.4	Latitude: 22.94^{o}N, Longitude: 90.82^{o}E	18	Soybean - Fallow-T.aman
09	*Rhizobium* SR9	Hazir Hat, Komol Nagar, Lakhmipur	3.5	Latitude: 22.94o N, Longitude: 90.82^{o}E	18	Soybean - Fallow-T.aman
10	*Rhizobium*SR10	Hazir Hat, Komol Nagar, Lakhmipur	3.6	Latitude: 22.94^{o}N, Longitude: 90.82^{o}E	18	Soybean - T. aus -T.aman
11	*Rhizobium* SR11	Hazir Hat, Komol Nagar, Lakhmipur	3.5	Latitude: 22.94^{o}N, Longitude: 90.82^{o}E	18	Soybean - Fallow-T.aman
12	*Rhizobium* SR12	Hazir Hat, Komol Nagar, Lakhmipur	3.6	Latitude: 22.94^{o}N, Longitude: 90.82^{o}E	18	Soybean - Fallow-T.aman
13	*Rhizobium* SR13	Hazir Hat, Komol Nagar, Lakhmipur	3.6	Latitude: 22.94^{o}N, Longitude: 90.82^{o}E	18	Soybean - Fallow-T.aman
14	*Rhizobium* SR14	Hazir Hat, Komol Nagar, Lakhmipur	3.6	Latitude: 22.94^{o}N, Longitude: 90.82^{o}E	18	Soybean - Fallow-T.aman

15	*Rhizobium* SR15	Hazir Hat, Komol Nagar, Lakhmipur	5.0	Latitude: 22.94°N, Longitude: 90.82°E	18	Soybean - T. aus -T.aman
16	*Rhizobium* SR16	Subornochor, Noakhali	3.4	Latitude: 22.94°N, Longitude: 90.82°E	18	Soybean - T. aus -T.aman
17	*Rhizobium* SR17	Challatoli, Doulatkhan, Bhola	3.9	Latitude: 22.35°N, Longitude: 90.45° E	18	Soybean - Fallow-T.aman
18	*Rhizobium* SR18	Hazir Hat, Komol Nagar, Lakhmipur	3.6	Latitude: 22.94°N, Longitude: 90.82°E	18	Soybean - Fallow-T.aman
19	*Rhizobium* SR19	Hazir Hat, Komol Nagar, Lakhmipur	3.5	Latitude: 22.94°N, Longitude: 90.82°E	18	Soybean - Fallow-T.aman
20	*Rhizobium* SR20	Hazir Hat, Komol Nagar, Lakhmipur	3.6	Latitude: 22.94o N, Longitude: 90.82o E	18	Soybean - Fallow-T.aman

Table 2. Rhizobia bacterial strains were collected from groundnut field

Sl no.	Name	Origin	EC (dSm^{-1})	GPS position	AEZ	Cropping patern
01	*Rhizobium* GR1	Nayapara, Bangla Bazar, Cox's Bazar	3.1	Latitude: 21.26°N, Longitude: 92.02° E Elevation:7.2 m	23	Groundnut- T. aus-T.aman
02	*Rhizobium* GR2	Vomoriaghona, Eidgah, Cox's Bazar	3.4	Latitude: 21.34°N, Longitude: 92.05° E Elevation:7.4 m	23	Groundnut-Fallow-T.aman
03	*Rhizobium* GR3	Pokkhali, Eidgah, Cox's Bazar	4.7	Latitude: 21.32°N, Longitude: 92.01° E Elevation:7.3 m	23	Groundnut-Fallow-T.aman
04	*Rhizobium* GR4	Dakkin para, Moheskhali, Cox's Bazar	3.5	Latitude: 21.30°N, Longitude: 91.56° E Elevation:7.2 m	23	Groundnut-Fallow-T.aman
05	*Rhizobium* GR5	Possimpara, Moheskhali, Cox's Bazar	3.6	Latitude: 21.30°N, Longitude: 91.56° E Elevation:7.2 m	23	Groundnut-Fallow-T.aman
06	*Rhizobium* GR6	Tulatali, Kuakata, Patuakhali	3.4	Latitude: 21.50°N, Longitude: 90.07° E Elevation:7.5 m	18	Groundnut-Fallow-T.aman
07	*Rhizobium* GR7	Kuakata, Kolapara, Patuakhali	2.8	Latitude: 21.30°N, Longitude: 91.56° E Elevation:7.2 m	18	Groundnut-Fallow-T.aman
08	*Rhizobium* GR8	Aslampur, Chorphason, Vhola	3.7	Latitude: 22.11°N, Longitude: 90.48° E Elevation:7.8m	18	Groundnut-Fallow-T.aman
09	*Rhizobium* GR9	Zinnogor, Chorphason, Bhola	4.5	Latitude: 22.11°N, Longitude: 90.47° E Elevation:7.8 m	18	Groundnut-Fallow-T.aman
10	*Rhizobium* GR10	Ilisiapara, Joarianala, Ramu, Cox's bazar	3.8	Latitude: 21.27°N, Longitude: 91.05° E Elevation: 8.2 m	23	Groundnut-Fallow-T.aman

Sl no.	Name	Origin	EC (dSm^{-1})	GPS position	AEZ	Cropping patern
11	*Rhizobium* GR11	Dailpara, Khuruskul Road, Cox's Bazar	3.5	Latitude: 21.29°N, Longitude: 91.59° E Elevation:7.2 m	23	Groundnut-Fallow-T.aman
12	*Rhizobium* GR12	Pokkhali, Eidgah, Cox's Bazar	3.2	Latitude: 21.32°N, Longitude: 92.01° E Elevation:7.3 m	23	Groundnut-Fallow-T.aman
13	*Rhizobium* GR13	Dakkin para, Moheskhali, Cox's Bazar	5.8	Latitude: 21.30°N, Longitude: 91.56° E Elevation:7.2 m	23	Groundnut-Fallow-T.aman
14	*Rhizobium* GR14	Ilisiapara, Joarianala, Ramu, Cox's bazar	3.4	Latitude: 21.27°N, Longitude: 92.05° E Elevation: 8.2 m	23	Groundnut-Fallow-T.aman
15	*Rhizobium* GR15	Tulatali, Kuakata, Patuakhali	4.8	Latitude: 21.50°N, Longitude: 90.07° E Elevation:7.5 m	18	Groundnut-Fallow-T.aman
16	*Rhizobium* GR16	Nayapara, Kuakata, Patuakhali	3.6	Latitude: 21.50°N, Longitude: 90.07° E Elevation:7.3 m	18	Groundnut-Fallow-T.aman
17	*Rhizobium* GR17	Aslampur, Chorphason, Bhola	3.5	Latitude: 22.11°N, Longitude: 90.48° E Elevation:7.8m	18	Groundnut-Fallow-T.aman
18	*Rhizobium* GR18	DakkinRatanpur, Bhola sadar, Bhola	3.6	Latitude: 22.38°N, Longitude: 90.39° E Elevation:7.8 m	18	Groundnut-Fallow-T.aman
19	*Rhizobium* GR19	Dakkin para, Moheskhali, Cox's Bazar	3.7	Latitude: 21.30°N, Longitude: 91.56° E Elevation:7.2 m	23	Groundnut-Fallow-T.aman
20	*Rhizobium* GR20	Kuakata, Kolapara, Patuakhali	3.8	Latitude: 21.30°N, Longitude: 91.56° E Elevation:7.2 m	18	Groundnut-Fallow-T.aman

ii) Purification and preservation of the isolated strains:

The isolated strains were purified by streak plate method. The purified strains were transferred to agar slants and preserved as stock culture in a refrigerator at 4^0C. In addition, the culture was mixed with 50% (v/v) glycerol andkept in -20^0C and -80^0C for long time preservation.

iii) Colony morphology:

The colony morphology of isolates was examined on YEMagar plates. After an incubation of 2-3 days at 30°C, individual colonieswere characterized based on their colour, shape, appearance, motility, transparency, Congo red and Gram stain reaction(Aneja 2003).

iv) Salt tolerance assays:

The bacterial strains were re-cultured in YEM broth at 30°C for 24-48 hours. The cell suspension was tenfold serial diluted to 10^{-4} to 10^{-8} before being used as bacterial samples.

The salt tolerance study was carried out by spreading 0.1 ml of each sample on YEM agar plate with each modified by NaCl at 0, 2, 4, 8, 16,32 and 48 dSm^{-1}. The plates were incubated at ambient temperature (28±2°C) for 3 days. The salt tolerant bacteria were selected and preserved on salt containing medium.

v) Inoculum preparation and biofertilizer production:

Processing of inoculant materials

Peat was used as carrier material for inoculant. In Bangladesh, there is a great reserve of peat soil in greater Faridpur, Khulna and Sylhet districts. After collection of the peat, soil materials were first air-dried thoroughly. The air-dried materials were ground to fine powder using a laboratory grinding mill so as to pass through an 80-mesh sieve. Powdered peats were neutralized with 5% $CaCO_3$ to raise the pH to 6.8. The inoculant materials were weighed and then taken in cotton plugged Erlenmeyer flasks and sterilized in an autoclave at 121°C and 15 psi of steam pressure for 3 hours. The sterilized materials were then transferred into the polyethylene bags and sealed carefully to avoid any contamination.

Preparation of broth cultures

To prepare the broth cultures, the *Rhizobium* strains were first sub-cultured in plates from the test tube stock. Yeast mannitol broth medium were taken in Erlenmeyer flasks and sterilized for 20 minutes at 121°C and a steam pressure of 15 psi. After cooling the medium, a small portion of *Rhizobium* culture was aseptically transferred from the plates to the liquid medium in the flasks with the help of an inoculating needle. The flasks were then placed on an electric shaker and shaken at a slow speed to enhance rhizobial growth. The broth culture was ready after 4-9 days depending on the rate of growth (Fast or slow growing) or when the medium in the flasks showed dense growth. It is called "mother culture". After checking the culture for purity and proper growth, the culture was transferred from tubes to large flasks containing sterile liquid medium for 4-9 days. Rhizobial broth was grown on YEM medium in large flasks on a horizontal shaker. After proper growth, the broth was checked for free from contamination. The broths were used having viable cells higher than 10^9/ml cells.

Inoculation, incubation and storage

A high-count broth cultures were injected into peat containing polyethylene bags aseptically (40% moisture holding capacity materials) with the help of a sterilized syringe in inoculation chamber and the puncture were sealed by adhesive tape. The contents were mixed by rolling in hand, incubated at 28°C for two weeks and then stored at 4°C. Finally, these biofertilizer packets were used for soybean and groundnut production.

vi) Seedling infectivity test:

In order to check the infection ability of *Rhizobium* on soybean and groundnut seedlings roots, *Rhizobium* was grown in yeast extract mannitol (YEM) broth culture at 30°C for 48h and 0.1 ml of this broth culture was added to the YEM agar slants tube (60 ml glass test tube, diameter 3 cm) having 1-2 seedlings and kept for 3-5 weeks at room temperature under aseptic condition and monitored nodulation infectivity. The nitrogen-free nutrient solution was supplied as a nutrient source to YEM agar slant tubes twice in a week.

vii) Molecular characterization of *Rhizobium* strains:

Cultivation of bacteria and DNA extraction

Selected effective salt tolerantbacteria were cultured in test tubes containing 3 ml of YEM liquid broth by shaking in a rotary shaker at 180 rpm, 30°C for 48 hours, and the cultures were centrifuged at 18,000 rpm for 5 minutes at 4°C. Genomic DNA was extracted from the pellet using DNA isolation kit (Promega, USA) and DNA yield was quantified using a spectrophotometer. The extracted bacterial DNA were used as a template for PCR amplification.

Olegonucleotide primers:

One pair of primers, which were specific for housekeeping gene (16S rRNA) of every morphologically and biochemically identified bacteria, were used to amply a fragment of 16S rRNA gene by PCR. The specific primers weredesigned from 16S rRNA gene sequences.

PCR amplification:

About 50 ng of template DNA was used to amply fragments of the 16S rRNA gene by PCR. PCR was carried out in a final volume of 25 µL containing 1 µL template DNA, 12.5 µL master mix (including polymerase, buffer, dNPT, Mg^{2+}, promega company), 1 µl forward primer, 1 µl reverse primer, 9.5 µL sterile water. PCR amplification was performed staring with 5 min denaturing step at 95°C, followed by 30 cycle of denaturation at 95°C for 30 sec, annealing at 48°C for 30 sec, extension at 72°C for 1.5 min, and final extension 72°C for 5 min. The PCR products were assessed by electrophoresis on 1% agarose gel. DNA bands were visualized by UV illumination and photographed with gel documentation system. PCR products were purified using gel purification Kit and were kept for sequence.

Sequencing and phylogenetic analysis

DNA sequencing was performed using an Applied Biosystems 3730 automated sequencer with the M13 primer to obtain nearly full-length bacterial 16S rDNA sequences. The bidirectional gene sequences were compiled using DNAMAN software (DNAMAN version 4.11, LynnonBiosoft, San, Ramon, CA, USA), and the sequences were analyzed using MEGA 5.2 software. The consensus sequences were used in a BLAST search of the NCBI Gene Bank database. Phylogenetic analysis was conducted using MEGA version 5.2, and a neighbor-joining tree were constructed using Kimura 2-parameter distances with 1000 replicates to estimate bootstrap support. The compiled sequence of the *Rhizobium* strains were deposited in the Gene Bank database and assigned accession number.

Statistical analysis

All data were statistically analyzed using an RCB design. Treatment effects of measured variables were tested by analysis of variance (ANOVA), and comparisons among treatment means were made using the least significant difference (LSD) multiple range test calculated at 5% level of probability ($P \leq 0.05$). Statistical procedures were carried out with the software program Statistix 10™.

11. Results and Discussion:

i) Morphology and biochemical traits of Rhizobium isolates

The characters of 40 bacterial isolates (20 isolates from soybean and 20 isolates from groundnut) were studied followingthe standard microbiological and biochemical methods. As standardmicrobiological methods, the morphology and Gram staining wasinvestigated. The physiological and biochemical characters such as Congo red,Gram staining, salt tolerancewas investigated. The cells of Rhizobium isolates were examined underlight microscope and found that the cells were rod shape and motile(Table 3, Table 4). The isolates absorbed counterstain as they were Gramnegative bacteria. Vincent (1970) stated that Rhizobium was gram negative, rod shaped and generally motile which was in line with the present study. The isolates were observed to lack the ability to absorb Congo red from YEMA medium containing this dye where colonies were colorless white or very faintly pink colonies which is inagreement with the findings of Wang et al. (1999). Congo red isthought to formcolored colloidal complex with ions on the cell surface,the colonies absorbed little dye and remain colorless or became slightlypink after 2 days of incubation that proved all colonies were belongs to Rhizobium.Bacteria were grown on YEM agar plate containing NaCl at 0, 8, 16, 24, 32 and 48 dSm^{-1}. Final selection of bacteria was confirmed who showed salt tolerance >48dS m^{-1} salinity level(Fig. 1) and preservedsalt containing media for further pot experimentation.

Table 3. Morphological and bio-chemical characterization of isolated Rhizobia from soybean

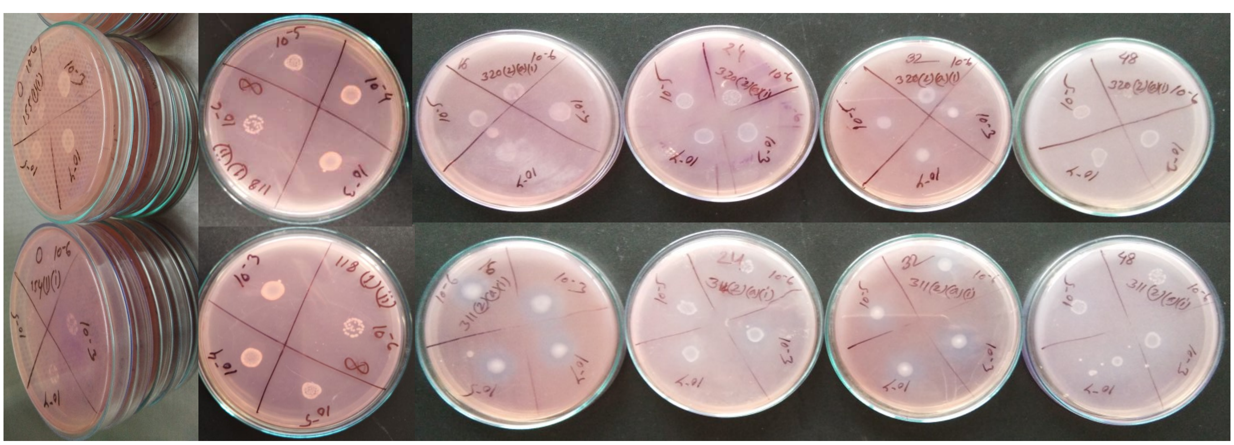
Sl no.	Name of isolate	Cell shape	Motility	Gram Reaction	Congo red test	Culture growth 28±2°C, 48h	
						Plate	Broth
01	*Rhizobium* SR1	Rod	Motile	(-) ve	(-) ve	Quick	Quick, Mucilage
02	*Rhizobium* SR2	Rod	Motile	(-) ve	(-) ve	Quick	Quick, Mucilage
03	*Rhizobium* SR3	Rod	Motile	(-) ve	(-) ve	Slow	Slow, Mucilage
04	*Rhizobium* SR4	Rod	Motile	(-) ve	(-) ve	Slow	Slow, Mucilage
05	*Rhizobium* SR5	Rod	Motile	(-) ve	(-) ve	Medium	Medium, Mucilage
06	*Rhizobium* SR6	Rod	Motile	(-) ve	(-) ve	Quick	Quick, Mucilage
07	*Rhizobium* SR7	Rod	Motile	(-) ve	(-) ve	Quick	Quick, Mucilage
08	*Rhizobium* SR8	Rod	Motile	(-) ve	(-) ve	Slow	Slow, Mucilage
09	*Rhizobium* SR9	Rod	Motile	(-) ve	(-) ve	Slow	Slow, Mucilage
10	*Rhizobium*SR10	Rod	Motile	(-) ve	(-) ve	Medium	Medium, Mucilage
11	*Rhizobium* SR11	Rod	Motile	(-) ve	(-) ve	Quick	Quick, Mucilage
12	*Rhizobium* SR12	Rod	Motile	(-) ve	(-) ve	Quick	Quick, Mucilage
13	*Rhizobium* SR13	Rod	Motile	(-) ve	(-) ve	Slow	Slow, Mucilage
14	*Rhizobium* SR14	Rod	Motile	(-) ve	(-) ve	Slow	Slow, Mucilage
15	*Rhizobium* SR15	Rod	Motile	(-) ve	(-) ve	Medium	Medium, Mucilage
16	*Rhizobium* SR16	Rod	Motile	(-) ve	(-) ve	Quick	Quick, Mucilage
17	*Rhizobium* SR17	Rod	Motile	(-) ve	(-) ve	Quick	Quick, Mucilage
18	*Rhizobium* SR18	Rod	Motile	(-) ve	(-) ve	Slow	Slow, Mucilage
19	*Rhizobium* SR19	Rod	Motile	(-) ve	(-) ve	Slow	Slow, Mucilage
20	*Rhizobium* SR20	Rod	Motile	(-) ve	(-) ve	Medium	Medium, Mucilage

Table 4. Morphological and bio-chemical characterization of isolated Rhizobium from groundnut

Sl no.	Name of isolate	Cell shape	Motility	Gram Reaction	Congo red test	Culture growth 28±2°C, 48h	
						Plate	Broth
01	T_1: *Rhizobium* GR1	Rod	Motile	(-) ve	(-) ve	Quick	Quick, Mucilage
02	T_2: *Rhizobium* GR2	Rod	Motile	(-) ve	(-) ve	Quick	Quick, Mucilage
03	T_3: *Rhizobium* GR3	Rod	Motile	(-) ve	(-) ve	Slow	Slow, Mucilage
04	T_4: *Rhizobium* GR4	Rod	Motile	(-) ve	(-) ve	Slow	Slow, Mucilage
05	T_5: *Rhizobium* GR5	Rod	Motile	(-) ve	(-) ve	Medium	Medium, Mucilage
06	T_6: *Rhizobium* GR6	Rod	Motile	(-) ve	(-) ve	Quick	Quick, Mucilage
07	T_7: *Rhizobium* GR7	Rod	Motile	(-) ve	(-) ve	Quick	Quick, Mucilage
08	T_8: *Rhizobium* GR8	Rod	Motile	(-) ve	(-) ve	Slow	Slow, Mucilage
09	T_9: *Rhizobium* GR9	Rod	Motile	(-) ve	(-) ve	Slow	Slow, Mucilage
10	T_{10}: *Rhizobium* GR10	Rod	Motile	(-) ve	(-) ve	Medium	Medium, Mucilage
11	T_1: *Rhizobium* GR11	Rod	Motile	(-) ve	(-) ve	Quick	Quick, Mucilage
12	T_2: *Rhizobium* GR12	Rod	Motile	(-) ve	(-) ve	Quick	Quick, Mucilage
13	T_3: *Rhizobium* GR13	Rod	Motile	(-) ve	(-) ve	Slow	Slow, Mucilage
14	T_4: *Rhizobium* GR14	Rod	Motile	(-) ve	(-) ve	Slow	Slow, Mucilage
15	T_5: *Rhizobium* GR15	Rod	Motile	(-) ve	(-) ve	Medium	Medium, Mucilage
16	T_6: *Rhizobium* GR16	Rod	Motile	(-) ve	(-) ve	Quick	Quick, Mucilage
17	T_7: *Rhizobium* GR17	Rod	Motile	(-) ve	(-) ve	Quick	Quick, Mucilage
18	T_8: *Rhizobium* GR18	Rod	Motile	(-) ve	(-) ve	Slow	Slow, Mucilage
19	T_9: *Rhizobium* GR19	Rod	Motile	(-) ve	(-) ve	Slow	Slow, Mucilage
20	T_{10}: *Rhizobium* GR20	Rod	Motile	(-) ve	(-) ve	Medium	Medium, Mucilage

Salinity: dSm^{-1}

0 8 16 24 32 48



Photograph 1: *Rhizobium* bacterial culture and characterization salt tolerance assay

ii) Seedling infectivity test:

Infection ability were checked in test tube and pot culture of soybean and groundnut seedlings.Forty isolates of effective *Rhizobium* (strain SR1 to strain SR20 for soybean and strain GR1 to strain GR20 for groundnut) were used for infection ability test and all bacteria were successfully produced nodules in soybean and groundnut roots both test tube and pot culture (Table 5, Fig. 2).

Table 5: Nodulation infectivity tests of characterized *Rhizobium* in soybean and groundnut

Sl no.	Name of isolate from Soybean	Infectivity Test		Name of isolate from Groundnut	Infectivity Test	
		Glass tube	Pot expt.		Glass tube	Pot expt.
01	*Rhizobium* SR1	+	+	*Rhizobium* GR1	+	+
02	*Rhizobium* SR2	+	+	*Rhizobium* GR2	+	+
03	*Rhizobium* SR3	+	+	*Rhizobium* GR3	+	+
04	*Rhizobium* SR4	+	+	*Rhizobium* GR4	+	+
05	*Rhizobium* SR5	+	+	*Rhizobium* GR5	+	+
06	*Rhizobium* SR6	+	+	*Rhizobium* GR6	+	+
07	*Rhizobium* SR7	+	+	*Rhizobium* GR7	+	+
08	*Rhizobium* SR8	+	+	*Rhizobium* GR8	+	+
09	*Rhizobium* SR9	+	+	*Rhizobium* GR9	+	+
10	*Rhizobium*SR10	+	+	*Rhizobium* GR10	+	+
11	*Rhizobium* SR11	+	+	*Rhizobium* GR11	+	+
12	*Rhizobium* SR12	+	+	*Rhizobium* GR12	+	+
13	*Rhizobium* SR13	+	+	*Rhizobium* GR13	+	+
14	*Rhizobium* SR14	+	+	*Rhizobium* GR14	+	+
15	*Rhizobium* SR15	+	+	*Rhizobium* GR15	+	+
16	*Rhizobium* SR16	+	+	*Rhizobium* GR16	+	+
17	*Rhizobium* SR17	+	+	*Rhizobium* GR17	+	+
18	*Rhizobium* SR18	+	+	*Rhizobium* GR18	+	+
19	*Rhizobium* SR19	+	+	*Rhizobium* GR19	+	+
20	*Rhizobium* SR20	+	+	*Rhizobium* GR20	+	+

Photograph. 2. Nodulation infectivity tests of characterized *Rhizobium* and detection of most potential species

iii) Inoculum preparation and biofertilizer production:

A final selection of the bacterial strains was used for carrier (peat) based inoculums preparation and biofertilizer production for soybean and groundnut.A total 40 strains of effective salt tolerant *Rhizobium* (strain SR1 to strain SR20 for soybean and strain GR1 to strain GR20 for groundnut) were used for biofertilizer production for soybean and groundnut (Fig. 3).

Photograph. 3. Biofertilizer production for soybean and groundnut

viii) Pot experiment trials:

In order to check the infection ability and performance of *Rhizobium* on soybean and groundnut roots in saline stress condition, two pot experiments were performed in net house condition with 0, 4, 8, 12 and 16 dSm^{-1} level of NaCl in pot soil. A total 40 strains of effective salt tolerant *Rhizobium* (strain SR1 to strain SR20 for soybean and strain GR1 to strain GR20 for groundnut) were used in pot trails for soybean and groundnut.

Pot Expt. 01:

Performanceof salt tolerant rhizobial strains on soybean production atdifferent salinity level under pot culture

Objectives:

i) To study the nodulation capacity of salt tolerant rhizobial strains on soybean production.
ii) To study the performance of salt tolerant rhizobial strains on growth and yield of soybean at different salinity level.

Materials and methods:

A pot experiment was carried out at the net house of Soil Science Division, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur (24.00^o N latitude, 90.25^o E longitude and 8.4 m elevation) during rabi season of 2017. Seeds were shown on 05 December 2017. The pot soil belongs to the Chiata series of Grey Terrace Soil. The experiment was laid out in Complete Randomized Design (CRD) with three replications. There were 21 treatments viz. T_1: *Rhizobium* SR1, T_2: *Rhizobium* SR2, T_3: *Rhizobium* SR3, T_4: *Rhizobium* SR4, T_5: *Rhizobium* SR5, T_6: *Rhizobium* SR6, T_7: *Rhizobium* SR7, T_8: *Rhizobium* SR8, T_9: *Rhizobium* SR9, T_{10}: *Rhizobium* SR10, T_{11}: *Rhizobium* SR11, T_{12}: *Rhizobium* SR12, T_{13}: *Rhizobium* SR13, T_{14}: *Rhizobium* SR14, T_{15}: *Rhizobium* SR15, T_{16}: *Rhizobium* SR16, T_{17}: *Rhizobium* SR17, T_{18}: *Rhizobium* SR18, T_{19}: *Rhizobium* SR19, T_{20}: *Rhizobium* SR20 and T_{21}: Control. The pot soils were modified with 0, 4, 8, 12, 16 dS m^{-1}salinity level by adding NaCl on to the pot. The tested crop was soybean (cv. BARI Soybean-6). Peat based rhizobial inoculum containing 10^8 cells g^{-1} inoculumwas used at the rate of 1.5 kg ha^{-1}. Seeds were mixed thoroughly with inoculum (20:1 ratio) before sowing. Ten seeds were sown in each pot 1 cm soil depth. Seeds were used at the rate of 75 kg ha^{-1}. Phosphorus, potassium, sulphur, zinc and boron @ $P_{42}K_{40}S_{40}Zn_5B_1$ kg ha^{-1} were used in the form of TSP, MoP, gypsum, zinc sulphate and boric acid, respectively. All P, K, S, Zn, B were applied at the time of pot preparation. All the intercultural operations such as irrigation, weeding, insect control etc. were done as and when necessary. Nodules were collected carefully by uprooting and five sample plants were selected randomly from each unit plot at 50 percent flowering stage. Nodules were separated from the roots, counted and then oven-dried and weighed. Data on yield and yield components were recorded at maturity. The crop was harvested on 02 March 2018. The initial soil samples at a depth of 0-15 cm from the experimental fields were collected and analyzed following standard methods (Table 6).

Table 6. Initial fertility status of the soil samples of the pot soil

Soil Properties	pH	OM %	Ca	Mg	K	Total N %	P	S	B	Cu	Fe	Zn
			meq $100g^{-1}$				µg g^{-1}					
Pot soil	6.7	0.92	4.6	2.5	0.07	0.08	11	4	0.54	1.5	42	0.35
Critical level	-	-	2.0	0.5	0.12	-	7	10	0.20	0.20	4	0.6

Methods of chemical analysis:

Soil pH was measured by a combined glass calomel electrode (Jackson, 1958). Organic carbon was determined by wet oxidation method (Walkley and Black). Total N was determined by modified Kjeldahl method. Calcium, K and Mg were determined by NH_4OAc extraction method. Copper, Fe, Mn and Zn were determined by DTPAextraction followed by AAS reading. Boron was determined by $CaCl_2$ extraction method. Phosphorus was determined by Bray and Kurtz method (Acid soils). Sulphur was determined by $CaH_4(PO_4)_2.H_2O$ extraction followed by turbidimetric turbidity method with $BaCl_2$.

Statistical analysis

All data were statistically analyzed using a RCBD design. Treatment effects on measured variables were tested by analysis of variance (ANOVA), and comparisons among treatment means were made using the least significant difference (LSD) multiple range test calculated at 5% level of probability ($P \leq 0.05$). Statistical procedures were carried out with the software program Statistix 10™.

Results and Discussion

At 50% flowering stage of soybean, the numbers of noduleswere recorded higher in inoculated *Rhizobium* bacteria compared to non-inoculated control. The highest number of nodule (28.7 plant^{-1}) were recorded with *Rhizobium* SR15 treatment at 0 salinity level which was identical to all treatments except T_1, T_2, T_3, T_4, T_6, T_8, T_9, T_{10} and T_{21} (Table 7). However, the nodule numbers were recorded the highest(29.2 plant^{-1}) with *Rhizobium* SR15 treatment at 4 dS m^{-1} salinity level which was differed with all treatments except T_7, T_{10}, T_{12},T_{16}, $T_{17}T_{18}$. On the other hand, the salinity level at 8 dS m^{-1}, the highest number of nodules (24.0 plant^{-1}) were recorded with *Rhizobium* SR16and *Rhizobium* SR17 treatment which was identical with all the treatments except T_1, T_4, T_5, T_6, T_8, T_9, T_{10}, $T_{14}$and T_{21}. Moreover, the salinity level at 12 dS m^{-1}, the highest number of nodules (20.9 plant^{-1}) were recorded with *Rhizobium* SR17 treatment which was identical with all the treatments except T_1, T_3, T_5, T_{11}, T_{13} and T_{21}. Furthermore, the salinity level at 16 dS m^{-1}, the highest number of nodules (18.3 plant^{-1}) were recorded with *Rhizobium* SR7 treatment which was identical with all other treatments except T_1, T_3, T_5, T_{11}, T_{13} and T_{21}.Similarly, the nodule weight was recorded higher in inoculated *Rhizobium* bacteria compared to non-inoculated control. The highest nodule weight (0.31 g plant^{-1}) was recorded with *Rhizobium* SR15 treatment at 0 salinity level which was differed with all treatments except T_6, T_7, T_{11}, T_{12}, T_{14}, T_{16}, T_{17}, T_{18} T_{19}, and T_{20} (Table 7). However, the nodule weight was recorded the highest(0.28 g plant^{-1}) with *Rhizobium* SR12, *Rhizobium* SR15, *Rhizobium* SR17 treatments at 4 dS m^{-1} salinity level which was differed with all treatments except T_7, T_{11}, T_{13}, T_{16}, T_{18}, T_{19} and T_{20}. On the other hand, the salinity level at 8 dS m^{-1}, the highest nodule weight (0.22 g plant^{-1}) were recorded with *Rhizobium* SR15 treatment which was identical with all the treatments except T_1, T_5, T_{13} and T_{21}. Moreover, the salinity level at 12 dS m^{-1}, the highest nodule weight (0.21 g plant^{-1}) were recorded with *Rhizobium* SR15 treatment which was identical with all the treatments except T_3 and T_{21}. Furthermore, the salinity level at 16 dS m^{-1}, the highest nodule weight (0.16 g plant^{-1}) were recorded with *Rhizobium* SR3, *Rhizobium* SR8 *Rhizobium* SR13 and *Rhizobium* SR15 treatments which was identical with T_6, T_7, T_{12}, T_{16}, T_{17}, T_{18} and T_{20} treatments but differed with all other treatments. Alam et al (2015) found that both numbers and weights of nodules were higher in

soybean plants inoculated with *Rhizobium* sp. BARIRGm901 than in non-inoculated plants.

Table 7. Nodulation production of soybean inoculated by *Rhizobium* at different salinity level

Treatment	Nodule number plant^{-1}					Nodule weight (g plant^{-1})				
Salinity level (dSm^{-1})	0	4	8	12	16	0	4	8	12	16
T_1: *Rhizobium* SR1	22.5b-e	21.5b-h	12.5f	11.5f	10.7e	0.22b-f	0.21c-h	0.15cd	0.16ab	0.09g
T_2: *Rhizobium* SR2	20.0c-f	18.5d-i	19.7a-d	20.0a	13.5a	0.16f	0.21b-g	0.18abc	0.17ab	0.12d-f
T_3: *Rhizobium* SR3	19.3def	23.3b-g	19.5a-d	13.3de	12.7de	0.17ef	0.18fgh	0.18abc	0.12b	0.16a
T_4: *Rhizobium* SR4	19.7c-f	15.3i	18.8b-e	19.2ab	12.8ab	0.19c-f	0.19e-h	0.19abc	0.18ab	0.13cde
T_5: *Rhizobium* SR5	23.0a-e	17.2hi	14.5ef	14.2cde	11.7cde	0.23b-f	0.17fgh	0.12d	0.18ab	0.09fg
T_6: *Rhizobium* SR6	20.0c-f	21.7b-h	16.8c-f	19.5a	18.2a	0.24a-f	0.22b-f	0.16a-d	0.17ab	0.13a-e
T_7: *Rhizobium* SR7	25.2a-d	26.2ab	21.2abc	20.5a	18.3a	0.28ab	0.24a-e	0.21ab	0.20a	0.14a-d
T_8: *Rhizobium* SR8	19.0ef	17.8ghi	16.8c-f	19.2ab	16.2ab	0.19c-f	0.20d-h	0.16a-d	0.19ab	0.16a
T_9: *Rhizobium* SR9	17.8ef	18.3f-i	18.3b-e	19.0ab	11.5ab	0.18def	0.17gh	0.16bcd	0.19ab	0.11d-f
T_{10}:*Rhizobium*SR10	15.2f	24.2a-e	16.3def	19.2ab	13.7ab	0.17ef	0.16h	0.18a-d	0.17ab	0.13cde
T_{11}: *Rhizobium* SR11	23.0a-e	20.8b-i	20.3a-d	11.2e	10.6e	0.24a-e	0.27ab	0.19abc	0.19ab	0.10efg
T_{12}: *Rhizobium* SR12	23.7a-e	24.2a-d	19.8a-d	19.6a	13.0a	0.26abc	0.28a	0.20abc	0.19ab	0.14a-d
T_{13}: *Rhizobium* SR13	27.6ab	21.1b-h	21.4abc	15.0b-e	14.4b-e	0.22b-f	0.26abc	0.16cd	0.17ab	0.16a
T_{14}: *Rhizobium* SR14	27.8ab	20.3c-i	18.3b-e	17.7a-d	13.9a-d	0.25a-e	0.26abc	0.18abc	0.20a	0.12def
T_{15}: *Rhizobium* SR15	28.7a	29.2a	22.1ab	17.4a-d	15.2a-d	0.31a	0.28a	0.22a	0.21a	0.16a
T_{16}: *Rhizobium* SR16	26.8ab	24.0a-f	24.0a	20.1a	14.4a	0.29ab	0.25abc	0.21abc	0.17ab	0.14a-d
T_{17}: *Rhizobium* SR17	26.6ab	25.9abc	24.0a	20.9a	14.8a	0.26abc	0.28a	0.20abc	0.19ab	0.14a-d
T_{18}: *Rhizobium* SR18	28.2ab	24.2a-d	20.2a-d	18.9ab	13.6ab	0.25a-d	0.27ab	0.19abc	0.20a	0.14a-d
T_{19}: *Rhizobium* SR19	26.7ab	19.8d-i	21.0abc	18.2abc	14.6abc	0.24a-f	0.25a-d	0.19abc	0.20a	0.13b-e
T_{20}: *Rhizobium* SR20	25.6abc	20.9b-i	23.4a	16.7a-d	14.6a-d	0.24a-f	0.26abc	0.17a-d	0.19ab	0.14a-d
T_{21}: Control	0.0g	0.0j	0.0g	0.0f	0.0f	0.00g	0.00i	0.00e	0.00c	0.00h
SE (±)	3.05	2.81	2.30	2.17	2.07	0.04	0.03	0.03	0.04	0.02
Level sig.	***	***	***	***	***	***	***	***	**	***
CV (%)	16	16.7	15.2	16	19.1	22.2	15.2	19.9	20	16.2

Means followed by common letter are not significantly different at 5% level by DMRT

In the same way, the shoot weight was recorded higher in inoculated *Rhizobium* bacteria compared to non-inoculated control. The highest shoot weight (6.00 g plant^{-1}) was recorded with *Rhizobium* SR15 treatment at 0 salinity level which was differed with all treatments except T_4,T_6, T_7, T_{14}, T_{16}, T_{17} and T_{18}(Table 8). However, the shoot weight was recorded the highest(5.92 g plant^{-1}) with *Rhizobium* SR15 treatment at 4 dS m^{-1} salinity level which was identical with all treatments except T_1, T_3, T_4, T_5, T_9, $T_{10}T_{11}$, T_{12}, T_{19}, T_{20} and T_{21}. On the other hand, the salinity level at 8 dS m^{-1}, the highest shoot weight (4.97 g plant^{-1}) was recorded with *Rhizobium* SR15 treatment which was differed with all the treatments except T_7, T_{12}, T_{13}, T_{14}, T_{16}, T_{17} and T_{18}.Moreover, the salinity level at 12 dS m^{-1}, the highest shoot weight (4.55 g $plant^{-1}$) was recorded with *Rhizobium* SR15 treatment which was identical with T_5 T_7, T_{10}, T_{12}, T_{14}, T_{16}, T_{17}, T_{18} and T_{20} but differed with all treatments.Furthermore, the salinity level at 16 dS m^{-1}, the highest shoot weight (2.96 g $plant^{-1}$) was recorded with *Rhizobium* SR17 treatment which was identical with T_7,T_9, $T_{14}$and T_{17}treatments but differed with all other treatments. Alam et al (2015) found that the number and weight of nodules increasedininoculatedsoybeangenotypes,asanalmost twofoldincrease in nodule number was observedininoculatedplantsrelativetonon-inoculatedplants of the same genotype.

Table 8. Dry matter production of soybean inoculated by *Rhizobium* at different salinity level

Treatment	Shoot weight (g $plant^{-1}$)					Root weight (g $plant^{-1}$)				
Salinity level (dSm^{-1})	0	4	8	12	16	0	4	8	12	16
T_1: *Rhizobium* SR1	4.79b-i	4.13c-g	3.79bcd	3.04cde	1.42g	0.34def	0.21g	0.28bcd	0.26bc	0.25bc
T_2: *Rhizobium* SR2	3.31jk	4.90a-e	3.79bcd	2.42ef	1.96b-f	0.26fg	0.22fg	0.29bcd	0.29abc	0.24bc
T_3: *Rhizobium* SR3	4.25g-j	4.62b-e	2.64fgh	2.39ef	2.05b-e	0.34def	0.25fg	0.28bcd	0.25bcd	0.26abc
T_4: *Rhizobium* SR4	5.35a-g	4.69b-e	3.68cd	2.45ef	2.33bcd	0.33def	0.27efg	0.25de	0.27bc	0.25bc
T_5: *Rhizobium* SR5	4.17hij	3.34fgh	2.44fg	3.83abc	1.74efg	0.35def	0.20g	0.27cde	0.27bc	0.26abc
T_6: *Rhizobium* SR6	5.57a-e	4.74a-e	3.66cde	2.37ef	1.95cdf	0.36cde	0.28d-g	0.26cde	0.24cd	0.23cd
T_7: *Rhizobium* SR7	5.73abc	5.12a-d	4.91a	4.06ab	2.94a	0.37cde	0.31c-f	0.31a-d	0.30abc	0.29abc
T_8: *Rhizobium* SR8	3.87ijk	4.93a-e	3.42def	2.68def	2.27bcd	0.33def	0.21g	0.30a-d	0.27bc	0.27abc
T_9: *Rhizobium* SR9	4.41f-j	3.75efg	3.47de	3.07cde	2.45abc	0.30ef	0.21g	0.25de	0.23cd	0.26abc
T_{10}:*Rhizobium*SR10	4.39f-j	3.08gh	3.10d-g	3.62a-d	2.16b-e	0.35cde	0.23fg	0.28bcd	0.26bc	0.24bc
T_{11}: *Rhizobium* SR11	4.53e-i	4.26b-g	3.80bcd	3.20b-e	1.50fg	0.35de	0.38a-d	0.30a-d	0.28bc	0.28abc
T_{12}: *Rhizobium* SR12	4.48e-i	4.51b-f	4.01a-d	3.68abc	1.98b-f	0.36cde	0.38abc	0.33abc	0.31ab	0.28abc
T_{13}: *Rhizobium* SR13	4.68c-i	4.74a-e	4.12a-d	3.10cde	1.93d-g	0.32def	0.40abc	0.31a-d	0.29abc	0.30ab
T_{14}: *Rhizobium* SR14	5.69a-d	5.35ab	4.01a-d	4.27a	2.46ab	0.35de	0.44ab	0.28bcd	0.28bc	0.31ab
T_{15}: *Rhizobium* SR15	6.00a	5.92a	4.97a	4.55a	2.96a	0.48a	0.46a	0.37a	0.36a	0.32a
T_{16}: *Rhizobium* SR16	5.47a-f	5.19abc	4.07a-d	4.15a	2.15b-e	0.46ab	0.41abc	0.32abc	0.30abc	0.27abc
T_{17}: *Rhizobium* SR17	5.01a-h	4.76a-e	4.57abc	4.05ab	2.95a	0.39bcd	0.40abc	0.34ab	0.29abc	0.30ab
T_{18}: *Rhizobium* SR18	5.86ab	5.45ab	4.76ab	4.41a	2.39bcd	0.44abc	0.39abc	0.33ab	0.28bc	0.30ab
T_{19}: *Rhizobium* SR19	4.62d-i	4.48b-f	3.34def	3.19b-e	2.33bcd	0.37cde	0.34b-e	0.28bcd	0.26bc	0.26abc
T_{20}: *Rhizobium* SR20	4.17hij	3.93d-g	3.17d-g	3.91abc	2.06b-e	0.39bcd	0.43ab	0.31a-d	0.24bcd	0.28abc
T_{21}: Control	2.78k	2.45h	2.25g	1.91f	1.71efg	0.19g	0.20g	0.21e	0.18d	0.17d
SE (±)	0.55	0.59	0.51	0.46	0.25	0.04	0.05	0.03	0.04	0.03
Level sig.	***	**	***	***	***	***	***	*	*	*
CV (%)	14.2	16.3	16.8	17.2	14.3	14.5	18.6	14.1	16.2	14.8

Means followed by common letter are not significantly different at 5% level by DMRT

Likewise, the root weight was recorded higher in inoculated *Rhizobium* bacteria compared to non-inoculated control. The highest root weight (0.48g plant^{-1}) was recorded with *Rhizobium* SR15 treatment at 0 salinity level which was differed with all treatments except T_{16} and T_{18} (Table 8). However, the root weight was recorded the highest(0.46g plant^{-1}) with *Rhizobium* SR15 treatment at 4 dS m^{-1} salinity level which was differed with all treatments except T_{11}, T_{12}, T_{13}, T_{16}, T_{17}, T_{18} and T_{20}. On the other hand, the salinity level at 8 dS m^{-1}, the root weight was recorded the highest(0.37g plant^{-1}) with *Rhizobium* SR15 treatment which was differed with all treatments except T_7, T_8, T_{11}, T_{12}, T_{13}, T_{16}, T_{17}, T_{18} and T_{20}.Moreover, the salinity level at 12 dS m^{-1}, the highest root weight (0.36g plant^{-1}) was recorded with *Rhizobium* SR15 treatment which was differed with all treatments except T_2, T_7, T_{12}, T_{13}, T_{14}, T_{16} and T_{17}.Furthermore, the salinity level at 16 dS m^{-1}, the highest root weight (0.32g plant^{-1}) was recorded with *Rhizobium* SR3, *Rhizobium* SR13, *Rhizobium* SR15 treatment which was identical with all treatments but differed with T_1, T_2, T_4, T_6, T_{10}, and T_{21}.Thesignificantincreasesin above-ground and rootbiomass of inoculated soybean genotypes might result from a high associationwith symbiotic *Rhizobium* sp. that promoted plant growth. Jones (1998) showed that highernodulation and biomass yields of inoculated plantscould be attributed to high nitrogen fixation incorporated into nitrogen biosynthesis.

Table 9. Plant height and collar diameter of soybean inoculated by *Rhizobium* at different salinity level

Treatment	Plant height (cm)					Collar diameter (mm)				
Salinity level (dSm^{-1})	0	4	8	12	16	0	4	8	12	16
T_1: *Rhizobium* SR1	49.0	46.8def	42.1	40.2c-f	36.2a	1.58cde	1.60def	1.18gh	0.78ij	0.76de
T_2: *Rhizobium* SR2	54.6	48.2cde	44.8	44.6cde	38.6a	1.94abc	1.97a-e	1.18gh	0.86hij	1.01a-d
T_3: *Rhizobium* SR3	53.1	49.2cde	40.8	41.8def	36.3a	2.05abc	1.96a-e	1.13gh	0.65j	0.92b-e
T_4: *Rhizobium* SR4	49.8	46.3def	47.8	44.4cde	33.6a	1.59cde	2.04a-d	1.48c-h	1.18c-i	0.95b-e
T_5: *Rhizobium* SR5	51.5	48.0cde	44.4	45.2b-e	36.3a	2.17ab	1.61def	1.77a-f	0.87g-j	0.90cde
T_6: *Rhizobium* SR6	48.3	50.5b-e	49.8	45.8a-e	36.6a	2.10abc	1.72cde	1.93a-d	1.07d-j	0.91cde
T_7: *Rhizobium* SR7	55.6	54.8a-d	51.2	46.8a-d	38.6a	2.29ab	2.22abc	2.00abc	1.85a	1.22ab
T_8: *Rhizobium* SR8	48.2	47.3def	46.2	45.1b-e	37.1a	2.07abc	1.96a-e	1.64a-g	0.95f-j	0.67e
T_9: *Rhizobium* SR9	48.6	42.3ef	50.3	46.3a-e	36.2a	1.29abc	2.04a-d	1.35e-h	1.39a-g	0.88cde
T_{10}:*Rhizobium*SR10	44.6	43.8ef	40.2	43.4cde	33.7a	2.01abc	1.46ef	1.55b-h	1.57a-d	0.79de
T_{11}: *Rhizobium* SR11	52.6	50.5b-e	45.8	43.1cde	37.4a	1.62cde	1.80b-e	1.21gh	1.00e-j	0.91cde
T_{12}: *Rhizobium* SR12	56.0	56.6abc	49.4	46.6a-d	39.2a	1.89abc	2.20abc	1.24fgh	0.91g-j	1.06a-d
T_{13}: *Rhizobium* SR13	52.0	59.4ab	48.0	45.2b-e	37.0a	1.91abc	2.00a-e	1.26fgh	1.07d-j	0.98bcd
T_{14}: *Rhizobium* SR14	53.3	54.2a-d	51.7	49.5abc	37.1a	1.78bcd	2.19abc	1.63a-g	1.37a-h	0.92b-e
T_{15}: *Rhizobium* SR15	57.0	60.8a	53.0	51.9a	40.1a	2.34a	2.42a	2.11a	1.74ab	1.30a
T_{16}: *Rhizobium* SR16	54.6	58.5ab	55.0	48.9abc	39.5a	2.32a	2.35a	2.08ab	1.21c-i	0.97bcd
T_{17}: *Rhizobium* SR17	54.0	50.9b-e	51.1	49.2abc	38.6a	2.32a	1.96a-e	1.98abc	1.64abc	1.17abc
T_{18}: *Rhizobium* SR18	52.8	55.0a-d	52.6	48.1a-d	39.2a	2.27ab	1.99a-e	1.81a-f	1.28b-i	0.83de
T_{19}: *Rhizobium* SR19	54.0	55.4a-d	53.1	51.3ab	39.3a	1.97abc	2.32ab	1.42d-h	1.46a-e	0.95b-e
T_{20}: *Rhizobium* SR20	53.0	53.8a-d	48.6	43.2cde	40.7a	2.19ab	1.98a-e	1.38e-h	1.49a-f	1.04a-d
T_{21}: Control	40.7	38.2f	36.8	33.5f	0.0b	1.13e	1.12f	1.07h	1.00e-j	0.81de
SE (±)	4.9	4.53	5.19	3.17	4.12	0.26	0.27	0.27	0.26	0.15
Level sig.	NS	**	NS	**	***	**	**	**	**	*
CV (%)	11.7	10.9	13.3	8.54	14.1	16.7	16.8	21.2	26.2	19.4

Means followed by common letter are not significantly different at 5% level by DMRT

The collar diameter was recorded higher in inoculated *Rhizobium* bacteria compared to non-inoculated control. The highest collar diameter (2.34 mm) was recorded with *Rhizobium* SR15 treatment at 0 salinity level which was identical with all treatments except T_1, T_4, T_{11}, T_{14} and T_{21} (Table 9). However, the collar diameter was recorded the highest(2.42 mm) with *Rhizobium* SR15 treatment at 4 dS m^{-1} salinity level which was identical with all treatments except T_1, T_5, T_6, T_{10} T_{11} and T_{21}. On the other hand, the salinity level at 8 dS m^{-1}, the highest collar diameter (2.11 mm) was recorded with *Rhizobium* SR15 treatment which was differed with all the treatments except T_5, T_6, T_7, T_{14}, T_{16}, T_{17} and T_{18}. Moreover, the salinity level at 12 dS m^{-1}, the highest collar diameter (1.85 mm) was recorded with *Rhizobium* SR7 treatment which was differed with all the treatments except T_9, T_{14}, T_{15}, T_{17}, T_{20}. Furthermore, the salinity level at 16 dS m^{-1}, the highest collar diameter (1.30 mm) was recorded with *Rhizobium* SR15 treatment which was identical with T_1, T_7, T_{12}, T_{17}, T_{20} treatment but differed with all other treatments.

Table 10. Pod length and pod numbers of soybean inoculated by *Rhizobium* at different salinity level

Treatment	Pod length plant^{-1}					No. of pod plant^{-1}				
Salinity level (dSm^{-1})	0	4	8	12	16	0	4	8	12	16
T_1: *Rhizobium* SR1	3.83bcd	3.37cde	3.30c-f	3.13cd	0	22.0ef	25.3abc	20.3a-d	14.0	0
T_2: *Rhizobium* SR2	3.73de	3.93abc	3.37b-f	3.17bcd	0	23.8a-f	25.2abc	21.8a-d	14.3	0
T_3: *Rhizobium* SR3	3.87bcd	3.20de	3.77a-e	2.97de	0	23.5a-f	22.8abc	20.8a-d	15.3	0
T_4: *Rhizobium* SR4	3.93bcd	3.37cde	3.43b-f	3.13cd	0	22.3def	24.3abc	21.0a-d	16.0	0
T_5: *Rhizobium* SR5	3.83bcd	3.73a-d	3.87a-d	3.50a-d	0	24.7a-f	23.3abc	20.0b-e	16.3	0
T_6: *Rhizobium* SR6	4.17a-d	4.00abc	3.77a-e	3.63abc	0	25.3a-e	24.3abc	21.0a-d	15.7	0
T_7: *Rhizobium* SR7	4.43ab	4.33a	4.10ab	3.67abc	0	26.8a-d	25.8ab	23.2abc	17.0	0
T_8: *Rhizobium* SR8	3.63de	3.13de	3.60a-e	3.57a-d	0	22.2ef	20.8cd	19.8b-e	15.7	0
T_9: *Rhizobium* SR9	3.97bcd	3.63bcd	3.33c-f	3.33a-d	0	23.0b=f	22.3bc	19.0cde	15.7	0
T_{10}:*Rhizobium*SR10	3.87bcd	3.50b-e	3.27c-f	3.10cd	0	21.7ef	21.0cd	18.3de	15.0	0
T_{11}: *Rhizobium* SR11	3.98bcd	3.76a-d	3.47b-f	3.21bcd	0	20.2fg	24.7abc	21.3a-d	14.7	0
T_{12}: *Rhizobium* SR12	3.81cd	4.04ab	3.12ef	3.52a-d	0	23.3b-f	25.1abc	22.3a-d	14.8	0
T_{13}: *Rhizobium* SR13	4.36abc	4.00abc	3.69a-e	3.76ab	0	23.1b-f	22.2bc	21.6a-d	17.1	0
T_{14}: *Rhizobium* SR14	3.94bcd	3.62bcd	3.21def	3.60abc	0	22.2def	24.9abc	23.8ab	15.3	0
T_{15}: *Rhizobium* SR15	4.68a	4.38a	4.22a	3.77ab	0	28.0a	27.2a	24.4a	19.8	0
T_{16}: *Rhizobium* SR16	4.12a-d	3.67bcd	3.96abc	3.41a-d	0	27.4ab	25.4abc	21.0a-d	14.9	0
T_{17}: *Rhizobium* SR17	4.08a-d	4.34a	3.93a-d	3.26a-d	0	27.1abc	27.1a	23.6ab	18.3	0
T_{18}: *Rhizobium* SR18	3.74d	4.01abc	3.77a-e	3.86a	0	22.9b-f	21.1cd	19.8b-e	16.9	0
T_{19}: *Rhizobium* SR19	4.06bcd	3.88abc	3.74a-e	3.61abc	0	22.1ef	21.2bcd	20.1b-e	17.6	0
T_{20}: *Rhizobium* SR20	3.59de	3.97abc	3.42b-f	3.43a-d	0	22.7c-f	21.8bc	18.2de	14.7	0
T_{21}: Control	3.13e	2.90e	2.73f	2.40e	0	16.3g	17.0d	16.0e	12.7	0
SE (±)	0.30	0.32	0.36	0.30	-	2.30	2.28	2.10		-
Level sig.	*	**	*	**	-	**	*	*	NS	-
CV (%)	9.37	10.5	12.5	10.9	-	12.1	11.9	12.3	14.6	-

Means followed by common letter are not significantly different at 5% level by DMRT

Pod length was recorded higher in inoculated *Rhizobium* bacteria compared to non-inoculated control. The highest pod length (4.68 cm plant^{-1}) was recorded with *Rhizobium* SR15 treatment at 0 salinity level which was differed with all treatments except T_6, T_7, T_{13}, T_{16} and T_{17} (Table 10). However, the pod length was recorded the highest(4.38 cm plant^{-1}) with *Rhizobium* SR7 *Rhizobium* SR15 *Rhizobium* SR17 treatment at 4 dS m^{-1} salinity level which was differed with all treatments except T_2, T_5, T_{11}, T_{13}, T_{19} and T_{20}. Moreover, the highest pod length (4.68 cm plant^{-1}) was recorded with *Rhizobium* SR15 treatment at 8 dS m^{-1} salinity level which was identical with all treatments except T_1, T_2, T_4, T_9, T_{10}, T_{11}, T_{12}, T_{14} and T_{21} .On the other hand, the salinity level at 12 dS m^{-1}, the length of pod was recorded the highest (3.86 cm plant^{-1}) with *Rhizobium* SR18 which was identical with all treatments except T_1, T_2, T_3, T_4, T_{10}, T_{11} and T_{21}.The salinity levels at 16 dS m^{-1}, there was no pod formation found due to strong salinity.

The pod number was recorded higher in inoculated *Rhizobium* bacteria compared to non-inoculated control. The pod number was recorded the highest(28.0 pod plant^{-1}) with *Rhizobium* SR15 treatment at 0dS m^{-1} salinity level which was differed with all treatments except T_2, T_3, T_5, T_6, T_7, T_{16} and T_{17} (Table 10). However, the salinity levels at 4dS m^{-1}, the pods number was recorded the highest(27.2 pod plant^{-1}) with *Rhizobium* SR15 treatment which was identical with all treatments except T_8, T_9, T_{10}, T_{13}, T_{18}, T_{19}, T_{20} and T_{21}. Moreover, the pod number were recorded the highest(24.4 pod plant^{-1}) with *Rhizobium* SR15 treatment at 8 dS m^{-1} salinity level which was identical with all treatments except T_5, T_8, T_9, T_{10}, T_{18}, T_{19}, T_{20} and T_{21}. On the other hand, the salinity levels at 12 dS m^{-1}, the pod number was recorded non-significant in all treatments. The salinity levels at 16 dS m^{-1}, there was no pod formation found due to strong salinity.

The number of seeds per pod was recorded higher in inoculated *Rhizobium* bacteria compared to non-inoculated control. The highest number of seeds per pod (2.64 pod plant^{-1}) was recorded with *Rhizobium* SR15 treatment at 0dS m^{-1} salinity level which was identical with all treatments except T_1, T_3, T_5, T_{10}, T_{13}, T_{14}, T_{16}, T_{19}, $T_{20}$and T_{21} (Table 11). However, the number of seeds per pod was recorded the highest(2.45 plant^{-1}) with *Rhizobium* SR15 treatment at 4dS m^{-1} salinity level which was differed with all treatments except T_4, T_6, T_{13}, T_{14}, T_{16} and T_{17}. But, the salinity levels at 8 dS m^{-1}, the number of seeds per pod was recorded the highest(2.40 plant^{-1}) with *Rhizobium* SR17 treatment which was identical with all treatments except T_3, T_{10}, T_{11}, T_{13}, T_{20} and T_{21}. Moreover, the highest number of seeds per pod (2.26 pod plant^{-1}) was recorded with *Rhizobium* SR15 treatment at 12 dS m^{-1} salinity level which was differed with all treatments except T_2, T_5, T_6, T_7, T_{13}, T_{16} and T_{17}. The salinity levels at 16 dS m^{-1}, there no pod formation found due to strong salinity.

Thousand seed weight was recorded higher in inoculated *Rhizobium* bacteria compared to non-inoculated control. The highest thousand seed weight (138.2g) was recorded with *Rhizobium* SR16 treatment at 4 dS m^{-1} salinity level which was identical with all treatments except T_2, T_3, T_8, T_{12}, T_{13}, T_{18} and T_{21} (Table 11). However, the thousand seed weight was recorded the highest(72.5g) with *Rhizobium* SR7 treatment at 12 dS m^{-1} salinity level which was identical with all treatments except T_1, T_2, T_4, T_5, T_6, T_{11}, T_{12}, T_{14} and T_{21}. Thousand seed weight in all salinity levels was recorded non-significant with 0 and 4 dS m^{-1} salinity level, respectively. The salinity levels at 16 dS m^{-1}, there no seed formation found due to strong salinity.

Table 11. Seed number and 1000 seed weight of soybean inoculated by *Rhizobium* at different salinity level

Treatment	No. of seed pod^{-1}					1000 seed weight (g)				
Salinity level (dSm^{-1})	0	4	8	12	16	0	4	8	12	16
T_1: *Rhizobium* SR1	1.95b-g	1.86b-f	1.96a-f	1.58fg	0	122.8	127.7a-d	167.5	45.8i	0
T_2: *Rhizobium* SR2	2.12a-e	1.58d-g	2.10a-d	1.92a-f	0	125.3	113.6b-g	105.8	52.2d-i	0
T_3: *Rhizobium* SR3	1.94b-g	1.93b-f	1.67d-g	1.88b-f	0	125.5	110.6d-g	105.4	57.6a-i	0
T_4: *Rhizobium* SR4	2.06a-f	2.09abc	2.35a	1.86b-f	0	109.8	123.6a-f	113.7	49.9f-i	0
T_5: *Rhizobium* SR5	1.90c-g	1.43fg	2.06a-d	2.04a-e	0	132.9	121.0a-g	147.0	54.3b-i	0
T_6: *Rhizobium* SR6	2.04a-f	2.10abc	2.18abc	2.34a	0	125.8	132.5ab	102.4	50.6e-i	0
T_7: *Rhizobium* SR7	2.54ab	2.31ab	2.27a	2.19abc	0	137.2	136.4a	121.3	72.5a	0
T_8: *Rhizobium* SR8	2.42a-d	1.89b-f	2.03a-e	1.71d-g	0	129.3	106.7fg	112.0	64.5a-f	0
T_9: *Rhizobium* SR9	2.13a-e	1.81b-f	2.09a-d	1.64efg	0	127.8	128.9a-d	112.9	61.4a-h	0
T_{10}:*Rhizobium*SR10	1.52efg	1.78c-f	1.75c-f	1.58fg	0	124.5	136.1a	104.6	69.1abc	0
T_{11}: *Rhizobium* SR11	2.02a-f	1.75c-f	1.78b-f	1.55fg	0	116.8	126.0a-f	157.0	49.0ghi	0
T_{12}: *Rhizobium* SR12	2.24a-d	1.51efg	2.24ab	1.84b-f	0	124.9	113.2b-g	101.0	54.1c-i	0
T_{13}: *Rhizobium* SR13	1.94b-g	2.02a-d	1.56fg	1.90a-f	0	126.6	107.7efg	109.4	65.2a-e	0
T_{14}: *Rhizobium* SR14	1.86d-g	1.96a-e	2.16abc	1.77c-f	0	112.5	124.9a-f	114.8	55.9b-i	0
T_{15}: *Rhizobium* SR15	2.64a	2.45a	2.36a	2.26ab	0	132.2	121.5a-g	122.7	60.1a-i	0
T_{16}: *Rhizobium* SR16	1.87d-g	2.22abc	2.30a	2.23ab	0	130.3	138.2a	127.8	71.7a	0
T_{17}: *Rhizobium* SR17	2.49abc	2.24abc	2.40a	2.09a-d	0	139.4	133.0a	123.5	67.2a-d	0
T_{18}: *Rhizobium* SR18	2.40a-d	2.00a-e	2.18abc	1.72d-g	0	128.3	102.9g	111.0	63.1a-g	0
T_{19}: *Rhizobium* SR19	1.99b-f	1.89b-f	2.12a-d	1.57fg	0	127.1	131.1abc	113.8	60.5a-i	0
T_{20}: *Rhizobium* SR20	1.44fg	1.58d-g	1.58efg	1.69d-g	0	124.1	126.3a-e	109.4	69.3ab	0
T_{21}: Control	1.34g	1.24g	1.22g	1.29g	0	116.2	112.9c-g	102.5	47.6hi	0
SE **(±)**	0.31	0.25	0.23	0.21	-	10.8	9.57	18.9	7.48	-
Level sig.	**	**	**	**	-	NS	**	NS	**	-
CV (%)	18.4	16.2	14.0	14.6	-	10.6	9.56	19.6	15.5	-

Means followed by common letter are not significantly different at 5% level by DMRT

The stover yield was recorded higher in inoculated *Rhizobium* bacteria compared to non-inoculated control. The highest stover yield (9.40 g plant^{-1}) was recorded with *Rhizobium* SR17 treatment at 0 salinity level which was differed with all treatments except T_2, T_4, T_6, T_7, T_9, T_{12}, T_{14}, T_{18} and T_{19} (Fig. 4). However, the stover yield was recorded the highest(9.64 g plant^{-1}) with *Rhizobium* SR15 treatment at 4 dS m^{-1} salinity level which was differed with all treatments except T_2, T_4, T_7, T_9, T_{12}, T_{17}, T_{18} and T_{19}. On the other hand, the salinity level at 8 dS m^{-1}, stover yield was recorded non-significant with all the treatments.

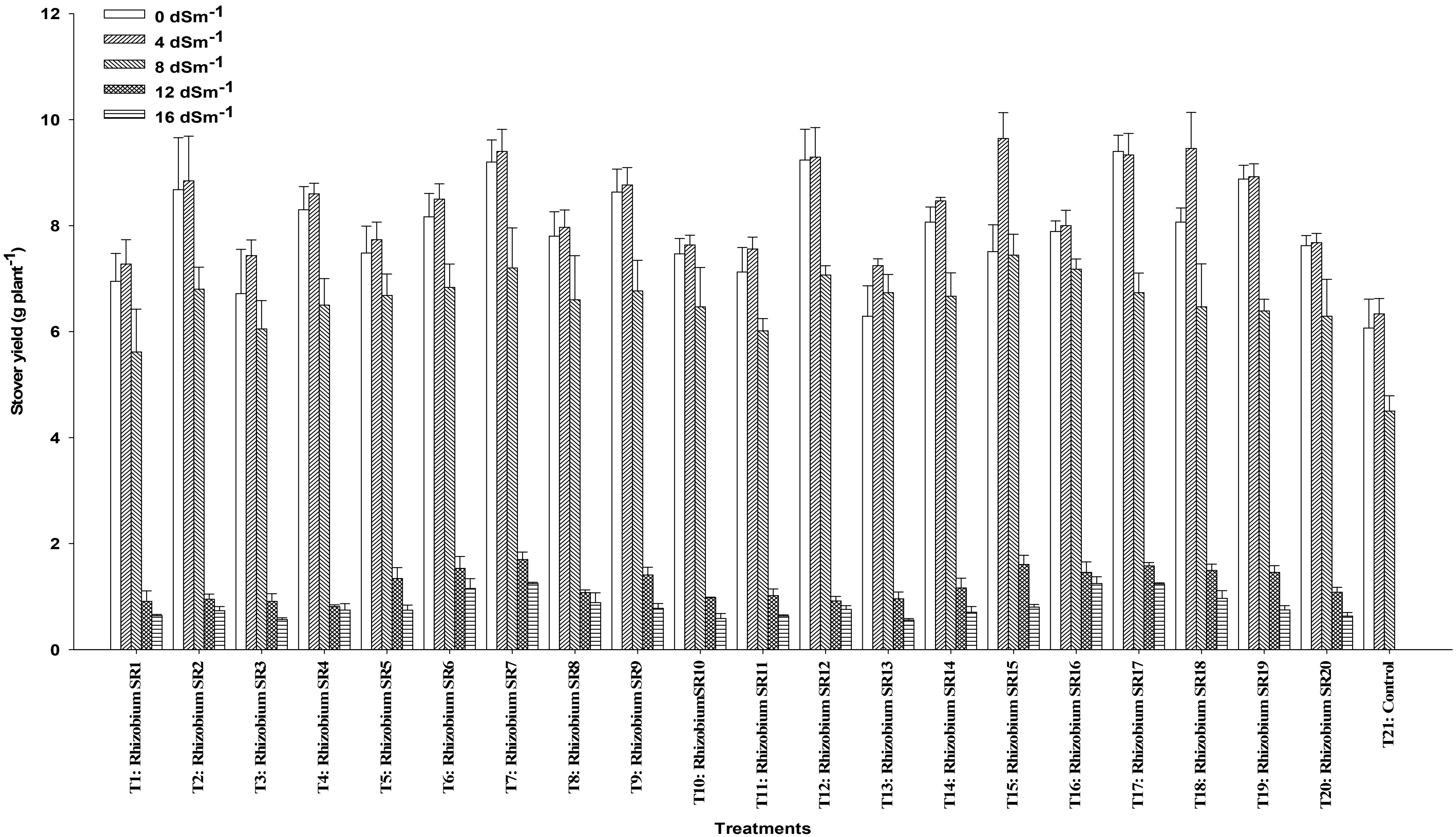
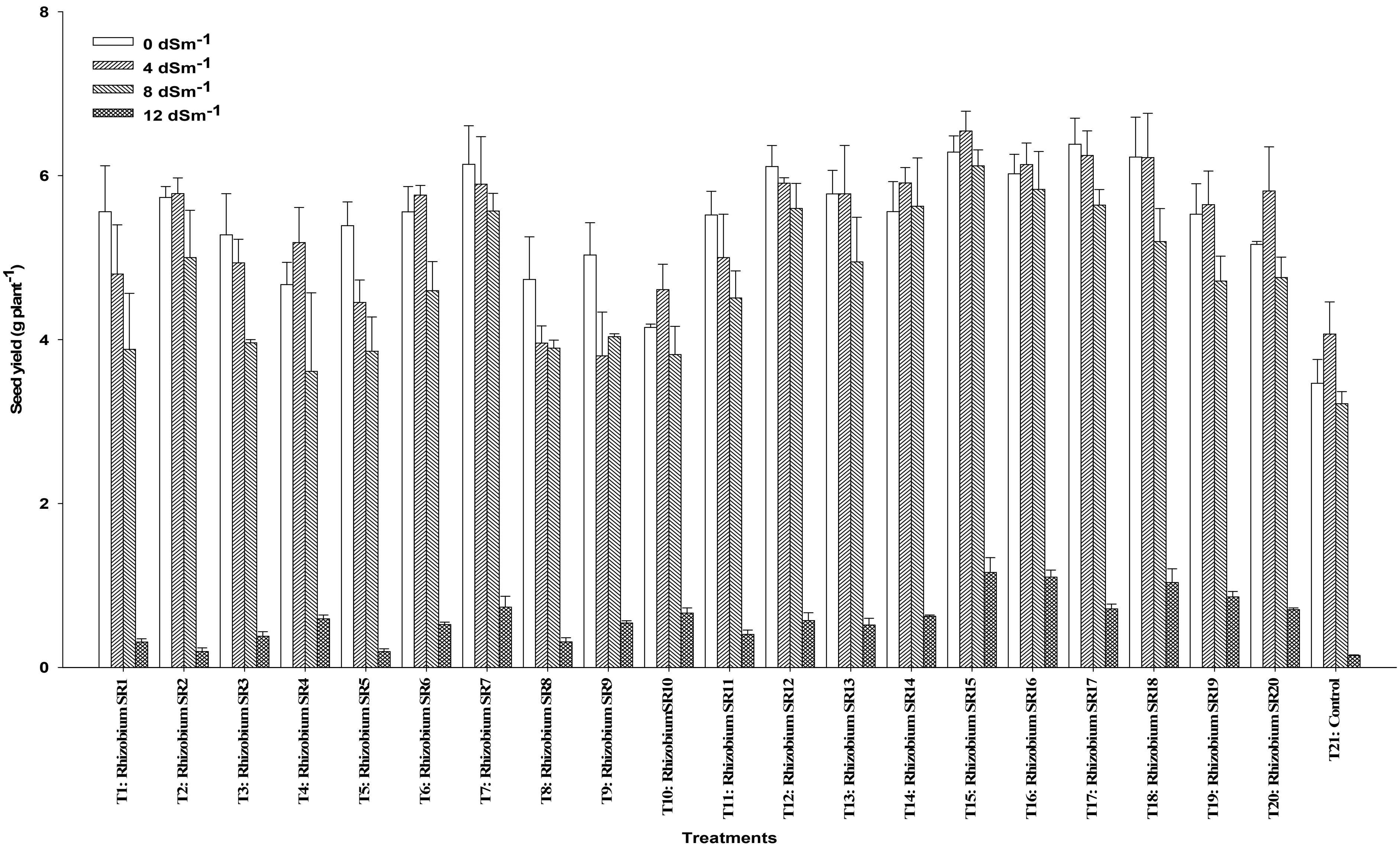


Fig. 4.Stover yield of soybean inoculated by *Rhizobium*sp at different salinity level



Fig, 5. Seed yield of soybean inoculated by *Rhizobium*sp at different salinity level

Moreover, the salinity level at 12 dS m^{-1}, the highest stover yield (1.70 g $plant^{-1}$) was recorded with *Rhizobium* SR7 treatment which was differed with all the treatments except T_5, T_6, T_9, T_{15}, T_{16}, T_{17}, T_{18} and T_{19}. Furthermore, the salinity level at 16 dS m^{-1}, the highest stover yield (1.25 g $plant^{-1}$) was recorded with *Rhizobium* SR7 *Rhizobium* SR16 treatment which was differed with T_6 and T_{17}. The seed yield was recorded higher in inoculated *Rhizobium* bacteria compared to non-inoculated control. The highest seed yield (6.38 g $plant^{-1}$) was recorded with *Rhizobium* SR17 treatment at 0 salinity level which was identical with all treatments except T_3, T_4, T_8, T_9, T_{10}, T_{20} and T_{21} (Fig. 5). However, the seed yield was recorded the highest(6.54 g $plant^{-1}$) with *Rhizobium* SR15 treatment at 4 dS m^{-1} salinity level which was differed with all treatments except T_2, T_6, T_7, T_{11}, T_{12}, T_{13}, T_{16}, T_{17}, T_{18}, T_{19} and T_{20}. On the other hand, the salinity level at 8 dS m^{-1}, the seed yield was recorded the highest(6.12 g $plant^{-1}$) with *Rhizobium* SR15 treatment which was differed with all treatments except T_2, T_7, T_{12}, T_{13}, T_{14}, T_{16}, T_{17} and T_{18}. Moreover, the salinity level at 12 dS m^{-1}, the highest seed yield (1.16 g $plant^{-1}$) was recorded with *Rhizobium* SR15 treatment which was differed with all the treatments except T_{16}, T_{18}. Furthermore, the salinity level at 16 dS m^{-1}, there was no seed yield found due to strong salinity.

Conclusion:

Application of *Rhizobium*biofertilizer on soybean, the plants exhibited better performance in nodule numbers, nodule weights, aboveground biomass, root biomass, and plant height than non-inoculated plants, indicating that all *Rhizobium* sp. effectively enhanced nodulation and growth parameters on soybean plant than non-inoculated soybean in all salinity levels. Soybean plants inoculated with *Rhizobium* sp. showed higher pod yield, stover yield, and seed yield than non-inoculated plants, revealing the positive impact of *Rhizobium* sp. on yield parameters of soybean. Soybean plants inoculated with *Rhizobium* sp. showed better performance comparatively *Rhizobium* sp.SR15> *Rhizobium* sp.SR16>*Rhizobium* sp.SR17>*Rhizobium* sp.SR18>*Rhizobium* sp.SR7>*Rhizobium* sp.SR12>*Rhizobium* sp.SR14>*Rhizobium* sp.SR13>*Rhizobium* sp.SR19>*Rhizobium* sp.SR2>*Rhizobium* sp.SR20>*Rhizobium* sp.SR6>*Rhizobium* sp.SR11>*Rhizobium* sp.SR3>*Rhizobium* sp.SR1>*Rhizobium* sp.SR4>*Rhizobium* sp.SR5>*Rhizobium* sp.SR9>*Rhizobium* sp.SR10> *Rhizobium* sp.SR8> considering average seed yield of soybean at different salinity levels than non-inoculated plants. Therefore, *Rhizobium* sp. established an effective symbiotic association with soybean and was responsible for increase growth and biomass, and improve yield characteristics of soybean at different salinity levels.

Photograph 4. *Rhizobium* strains R1-R20 tested on Soybean with 0, 4, 8, 12 and 16 dS/m salinity

Pot Expt. 02: Performance of salt tolerant rhizobial strains on groundnut production at different salinity level under pot culture

Objectives:

i. To study the nodulation capacity of salt tolerant rhizobial strains on groundnut production.
ii. To study the performance of salt tolerant rhizobial strains on growth and yield of groundnut at different salinity level.

Materials and methods:

The experiment was conducted at the net house of Soil Science Division, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur (24.00^o N latitude, 90.25^o E longitude and 8.4 m elevation) duringrabi season of 2017. The experiment was laid out in Complete Randomized Design (RCD) with three replications. There were 21 treatments viz. T_1: *Rhizobium* GR1, T_2: *Rhizobium* GR2, T_3: *Rhizobium* GR3, T_4: *Rhizobium* GR4, T_5: *Rhizobium* GR5, T_6: *Rhizobium* GR6, T_7: *Rhizobium* GR7, T_8: *Rhizobium* GR8, T_9: *Rhizobium* GR9, T_{10}: *Rhizobium* GR10, T_{11}: *Rhizobium* GR11, T_{12}: *Rhizobium* GR12, T_{13}: *Rhizobium* GR13, T_{14}: *Rhizobium* GR14, T_{15}: *Rhizobium* GR15, T_{16}: *Rhizobium* GR16, T_{17}: *Rhizobium* GR17, T_{18}: *Rhizobium* GR18, T_{19}: *Rhizobium* GR19, T_{20}: *Rhizobium* GR20 and T_{21}: Control. Strainswere testedat 0, 4, 8, 12, 16 dS m^{-1} salinity level. The tested crop was groundnut (cv. BARI Chinabadam-8). Seeds were sown on 05 December 2017.The pot soil belongs to the Chiata series of Grey Terrace Soil.Peat based rhizobial inoculum containing 10^8 cells g^{-1} inoculumwas used at the rate of 1.5 kg ha^{-1}. Seeds were mixed thoroughly with inoculum (20:1 ratio) before sowing. Ten seeds were sown in each pot in 1 cm soil depth. Seeds were used at the rate of 75 kg ha^{-1}. Phosphorus, potassium, sulphur, zinc and boron @ $P_{42}K_{40}S_{40}Zn_5B_1$ kg ha^{-1} were used in the form of TSP, MoP, gypsum, zinc sulphate and boric acid, respectively. All P, K, S, Zn, B were applied at the time of pot preparation. All the intercultural operations such as irrigation, weeding, insect control etc. were done as and when necessary. Nodules were collected by uprooting carefully andfive sample plants were selected randomly from each unit plot at the 50 percent flowering stage. Nodules were separated from the roots, counted and then oven-dried and weighed. Data on yield and yield components were recorded at maturity. The crop was harvested on 02 April 2018. The initial soil samples at a depth of 0-15 cm from the experimental fields were collected and analyzed following standard methods (Table 6).

Methods of chemical analysis:

Organic carbon was determined by wet oxidation method (Walkley and Black). Total N was determined by modified Kjeldahl method. Soil pH was measured by a combined glass calomel electrode (Jackson, 1958). Calcium, K and Mg were determined by NH_4OAc extraction method. Copper, Fe, Mn and Zn were determined by DTPAextraction followed by AAS reading. Boron was determined by $CaCl_2$ extraction method. Sulphur was determined by $CaH_4(PO_4)_2.H_2O$ extraction followed by turbidimetric turbidity method with $BaCl_2$. Phosphorus was determined by Bray and Kurtz method (Acid soils) and Modified Olsen method (Neutral + Calcareous soils).

Statistical analysis

All data were statistically analyzed using two factor analysis. Treatment effects on measured variables were tested by analysis of variance (ANOVA), and comparisons among treatment means were made using the least significant difference (LSD) multiple range test calculated at

5% level of probability ($P \leq 0.05$). Statistical procedures were carried out with the software program Statistix 10™.

Results and Discussion

Groundnut growing at 50% flowering stage, the number of nodules was noted higher in inoculated *Rhizobium* bacteria compared to non-inoculated control. The number of nodules was recorded the highest (53.6 plant^{-1}) with *Rhizobium* GR13 treatment at 0 salinity level which was identical with all treatments except T_{12}, T_{14}, T_{15}, $T_{15}$and T_{19}(Table 12). However,the nodule numbers were documented the highest(57.2 plant^{-1}) with *Rhizobium* GR13 treatment at 4 dS m^{-1} salinity level which was differed with all treatments except T_{11}, T_{12},T_{14}, T_{15}, T_{16}, T_{17},T_{18},T_{19} and T_{20}. On the other hand, the salinity level at 8 dS m^{-1}, the highest number of nodules (50.9 plant^{-1}) was recorded with *Rhizobium* GR13 treatment which was differed with all the treatments except T_{11}, T_{14}, T_{15}, $T_{18}$and T_{19}. Moreover, the salinity level at 12 dS m^{-1}, the highest number of nodules (37.8 plant^{-1}) was recorded with *Rhizobium* GR13 treatment which was differedwith all treatments except with T_{11}, T_{12}, T_{14}, T_{15}, and T_{18}.Furthermore, the salinity level at 16 dS m^{-1}, the highest number of nodules (22.5 plant^{-1}) were recorded with *Rhizobium* GR9 treatment which was differed with all other treatments except T_6, T_7, T_{13}, T_{15} and T_{16}.

The nodule weight of groundnut was recorded similarly higher in inoculated *Rhizobium* bacteria compared to non-inoculated control. The highest nodule weight (0.74 g plant^{-1}) was documented with *Rhizobium* GR13 treatment at 0 salinity level which was identical with all treatments except T_1, T_{11}, T_{12}, T_{18}, T_{19}, T_{20} and T_{21} (Table 12). However, the nodule weight was recorded the highest(0.72 g plant^{-1}) with *Rhizobium* GR13 treatment at 4 dS m^{-1} salinity level which was differed with all treatments except T_{15} and T_{18}. On the other hand, the salinity level at 8 dS m^{-1}, the highest nodule weight (0.49 g plant^{-1}) was noted with *Rhizobium* GR13 treatment which was varied with all the treatments except T_9, T_{14} and T_{19}. Moreover, the salinity level at 12 dS m^{-1}, the highest nodule weight (0.37 g plant^{-1}) was recorded with *Rhizobium* GR13 treatment which was differed with all the treatments except T_9, T_{11} and T_{12}. Furthermore, the salinity level at 16 dS m^{-1}, the highest nodule weight (0.29 g plant^{-1}) was noted with *Rhizobium* GR13, *Rhizobium* GR14 treatment which was identical with T_{11}, T_{15}, T_{17}, T_{18}, and T_{20} but differed with rest of the treatments.

Table 12. Nodulation production of groundnut inoculated by *Rhizobium* at different salinity level

Treatment	Nodule number plant^{-1}					Nodule weight (g plant^{-1})				
Salinity level (dSm^{-1})	0	4	8	12	16	0	4	8	12	16
T_1: *Rhizobium* GR1	35.8def	24.8e	23.8f	12.8h	10.7f	0.57bcd	0.36d	0.29d	0.16ef	0.09h
T_2: *Rhizobium* GR2	37.3def	26.2e	25.3ef	21.7def	13.5c-f	0.65abc	0.39cd	0.25d	0.17ef	0.10h
T_3: *Rhizobium* GR3	39.0def	26.7e	26.2ef	13.3h	12.7def	0.64abc	0.41cd	0.27d	0.12f	0.17b-g
T_4: *Rhizobium* GR4	38.0def	27.7e	25.5ef	21.7def	12.8def	0.63a-d	0.42cd	0.26d	0.18def	0.13fgh
T_5: *Rhizobium* GR5	38.7def	30.5de	27.8ef	14.2gh	11.7ef	0.60a-d	0.37cd	0.32cd	0.18def	0.09h
T_6: *Rhizobium* GR6	33.3f	30.3de	25.2f	19.5e-h	18.2abc	0.59a-d	0.42cd	0.26d	0.17ef	0.13e-h
T_7: *Rhizobium* GR7	35.2ef	35.2cde	24.5f	20.5efg	18.3abc	0.64abc	0.36d	0.35bcd	0.26b-e	0.14e-h
T_8: *Rhizobium* GR8	39.0def	36.5b-e	30.2def	19.2fgh	16.2b-e	0.68abc	0.47cd	0.30d	0.25cde	0.19cde
T_9: *Rhizobium* GR9	41.2cdef	37.0b-e	33.3de	25.7c-f	22.5a	0.71ab	0.50bc	0.42abc	0.28a-d	0.21bcd
T_{10}:*Rhizobium*GR10	35.2ef	26.5e	26.3ef	22.5def	13.7b-f	0.61a-d	0.39cd	0.34bcd	0.23cde	0.13gh
T_{11}: *Rhizobium* GR11	38.2def	38.8a-e	45.2abc	35.3ab	16.9bcd	0.52cd	0.41cd	0.28d	0.35ab	0.25abc
T_{12}: *Rhizobium* GR12	45.2abcd	41.4a-e	37.3cd	35.9a	13.8b-f	0.56bcd	0.47cd	0.32bcd	0.35ab	0.22bcd
T_{13}: *Rhizobium* GR13	53.6a	57.2a	50.9a	37.8a	18.6ab	0.74a	0.72a	0.49a	0.37a	0.29a
T_{14}: *Rhizobium* GR14	49.9abc	48.7a-d	46.3ab	37.2a	16.3b-e	0.62a-d	0.47cd	0.43ab	0.22c-f	0.29a
T_{15}: *Rhizobium* GR15	52.2ab	52.9abc	49.4ab	30.7abc	18.6ab	0.65abc	0.62ab	0.31d	0.17ef	0.26ab
T_{16}: *Rhizobium* GR16	42.9b-f	55.3ab	41.7bc	28.7bcd	18.2abc	0.60a-d	0.45cd	0.28d	0.19c-f	0.19c-f
T_{17}: *Rhizobium* GR17	36.7def	56.2ab	31.6def	22.8def	15.1b-f	0.61a-d	0.41cd	0.30d	0.23c-f	0.25abc
T_{18}: *Rhizobium* GR18	49.0abc	54.6abc	44.5abc	30.7abc	16.4b-e	0.56bcd	0.63ab	0.34bcd	0.29abc	0.26ab
T_{19}: *Rhizobium* GR19	44.8a-e	51.3abc	43.4abc	28.2bcd	17.1bcd	0.52cd	0.47cd	0.42abc	0.22c-f	0.17d-g
T_{20}: *Rhizobium* GR20	38.4def	41.7a-e	28.8ef	26.5cde	14.2b-f	0.46d	0.46cd	0.29d	0.25b-e	0.25abc
T_{21}: Control	0.0g	0.0f	0.0g	0.0i	0.0g	0.00e	0.00e	0.00e	0.00g	0.00i
SE **(±)**	4.78	9.81	4.02	3.50	2.48	0.09	0.07	0.06	0.05	0.03
Level sig.	***	**	***	***	***	***	***	***	***	***
CV (%)	14.9	31.5	15.1	17.8	20.2	18.1	18.5	22.2	28.6	21.7

Means followed by common letter are not significantly different at 5% level by DMRT

The groundnut shoot weight was recorded higher in inoculated *Rhizobium* bacteria compared to non-inoculated control. The shoot weight was noted the highest(6.41 g plant^{-1}) with *Rhizobium* GR9 treatment at 0 dS m^{-1} salinity level which was differed with all treatments except T_6, T_{13} and T_{14}(Table 13). However, the shoot weight was recorded the highest(5.75 g plant^{-1}) with *Rhizobium* GR9 treatment at 4 dS m^{-1} salinity level which was varied with all treatments except T_3, T_4, T_6, T_8, T_{12}, T_{13}, T_{14}, T_{18} and T_{19}. On the other hand, the salinity level at 8 dS m^{-1}, the highest shoot weight (4.15 g plant^{-1}) was documented with *Rhizobium* GR14 treatment which was identical with all the treatments except T_1, T_3, T_5, T_8, T_{10}, T_{11}, T_{15}, T_{20} and T_{21}. Moreover, the salinity level at 12 dS m^{-1}, the highest shoot weight (3.41 g plant^{-1}) was recorded with *Rhizobium* GR9 treatment which was differed with all treatments except T_1, T_7, T_9, T_{10}, T_{11}, T_{13}, T_{19} and

T_{20}.Furthermore, the salinity level at 16 dS m^{-1}, the highest shoot weight (2.94 g $plant^{-1}$) were recorded with *Rhizobium* GR7 treatment which was differed with all other treatments.

Table 13. Dry matter production of groundnut inoculated by *Rhizobium* at different salinity level

Treatment	Shoot weight (g $plant^{-1}$)					Root weight (g $plant^{-1}$)				
Salinity level (dSm^{-1})	0	4	8	12	16	0	4	8	12	16
T_1: *Rhizobium* GR1	5.12b-e	4.13c-f	3.45b-e	3.04a-d	1.65fg	0.37b-e	0.27fgh	0.28	0.34a-d	0.24c-g
T_2: *Rhizobium* GR2	4.65cde	4.23c-f	3.69a-d	2.42ef	1.96c-f	0.26e	0.26gh	0.29	0.30a-e	0.25c-g
T_3: *Rhizobium* GR3	4.25ef	4.62a-e	2.97efg	2.39ef	2.05b-f	0.34de	0.28e-h	0.35	0.28c-g	0.27b-f
T_4: *Rhizobium* GR4	5.35bcd	4.69a-d	4.02ab	2.45ef	2.33bc	0.33de	0.28e-h	0.35	0.35a-d	0.30abc
T_5: *Rhizobium* GR5	4.17ef	3.34f	2.77fg	2.83c-f	1.74efg	0.35cde	0.26gh	0.27	0.37abc	0.23d-g
T_6: *Rhizobium* GR6	5.57abc	4.74a-d	3.66a-d	2.37f	1.95c-f	0.40bcd	0.32d-h	0.35	0.31a-e	0.22e-g
T_7: *Rhizobium* GR7	4.40de	4.32c-f	3.91ab	3.06a-d	2.94a	0.34de	0.29e-h	0.29	0.38ab	0.34a
T_8: *Rhizobium* GR8	5.20b-e	4.93abc	3.42b-e	2.68def	2.27bcd	0.33de	0.36b-f	0.30	0.31a-e	0.30a-d
T_9: *Rhizobium* GR9	6.41a	5.75a	3.81ab	3.41ab	2.45b	0.46abc	0.41abc	0.37	0.38a	0.33ab
T_{10}:*Rhizobium*GR10	4.39de	3.42ef	3.10d-g	3.29abc	2.16b-e	0.35cde	0.34c-g	0.35	0.35a-d	0.23efg
T_{11}: *Rhizobium* GR11	4.81cde	4.53b-f	3.40b-f	3.04a-d	1.58fg	0.40bcd	0.38a-d	0.31	0.24efg	0.22efg
T_{12}: *Rhizobium* GR12	5.10b-e	4.93abc	3.65a-d	2.42ef	1.89c-f	0.37b-e	0.37a-d	0.33	0.23efg	0.24c-g
T_{13}: *Rhizobium* GR13	6.10ab	5.68ab	3.97ab	3.06a-d	1.95c-f	0.57a	0.46a	0.37	0.31a-e	0.28a-e
T_{14}: *Rhizobium* GR14	5.51abc	5.02abc	4.15a	3.45a	2.14b-e	0.48ab	0.41abc	0.33	0.30a-e	0.21fg
T_{15}: *Rhizobium* GR15	4.86cde	4.23c-f	2.77fg	2.80c-f	1.74efg	0.54a	0.42abc	0.32	0.31a-e	0.24c-g
T_{16}: *Rhizobium* GR16	4.96cde	4.47b-f	3.66a-d	2.31f	1.61fg	0.54a	0.36b-e	0.37	0.28d-g	0.22efg
T_{17}: *Rhizobium* GR17	4.55cde	4.28c-f	3.71a-d	2.86b-f	1.94c-f	0.47abc	0.41abc	0.32	0.29d-f	0.21efg
T_{18}: *Rhizobium* GR18	5.19b-e	5.19abc	3.76abc	2.58bef	1.60fg	0.58a	0.39a-d	0.33	0.20g	0.28a-e
T_{19}: *Rhizobium* GR19	4.92cde	5.28abc	3.81ab	3.07a-d	1.79def	0.58a	0.42abc	0.33	0.23efg	0.27b-f
T_{20}: *Rhizobium* GR20	4.42de	4.17c-f	3.16c-f	2.96a-e	1.63fg	0.56a	0.43ab	0.35	0.21fg	0.24c-g
T_{21}: Control	3.24f	3.58def	2.53g	2.29f	1.29g	0.27e	0.24h	0.24	0.24efg	0.18g
SE (±)	0.52	0.60	0.31	0.28	0.24	0.05	0.04	0.04	0.04	0.03
Level sig.	**	**	**	**	***	***	***	NS	**	**
CV (%)	12.9	16.1	10.9	12.5	15.2	17.3	15.1	16.1	18.4	15.8

Means followed by common letter are not significantly different at 5% level by DMRT

Rhizobium inoculated root of groundnutrecorded higher root weight compared to non-inoculated control. The highest root weight (0.58 g $plant^{-1}$) was recorded with *Rhizobium* GR18, *Rhizobium* GR19 treatments at 0 salinity level which was differed with all treatments except T_9, T_{13}, T_{14}, T_{15}, T_{16}, $T_{17}$and T_{20}. (Table 13). However, the root weight was noted the highest(0.46 g $plant^{-1}$) with *Rhizobium* GR13 treatment at 4 dS m^{-1} salinity level which was differed with all treatments except T_9, T_{11}, T_{12}, T_{14}, T_{15}, T_{17}, T_{18}, T_{19} and T_{20}. On the other hand, the salinity level at 8 dS m^{-1}, the root weight was documented non-significant with all treatments.Moreover,the root weight was recorded the highest(0.38 g plant^{-1}) with *Rhizobium* GR9 treatment at 12 dS m^{-1} salinity level which was differed with all treatments except T_1, T_2, T_4, T_5, T_6, T_7, T_8, T_{10}, T_{13}, T_{14} and T_{15}. Moreover, the salinity level at 16 dS m^{-1}, the highest root weight (0.34 g plant^{-1}) was recorded with *Rhizobium* GR7 treatment which was differed with all treatments except T_4, T_8, T_9, T_{13} and T_{18}.

Table 14. Plant height and nut per plant of groundnut inoculated by *Rhizobium* at different salinity level

Treatment	Plant height (cm)					Nut plant^{-1}				
Salinity level (dSm^{-1})	0	4	8	12	16	0	4	8	12	16
T_1: *Rhizobium* GR1	32.3gh	31.6g	32.9c-g	24.6g-j	21.4efg	17.0cde	16.0e-h	14.0efg	5.0cd	1.0f
T_2: *Rhizobium* GR2	33.8fgh	35.7c-g	32.8d-g	26.6e-i	20.0gh	19.2 a-d	18.7c-g	13.2fgh	6.3abc	1.0f
T_3: *Rhizobium* GR3	37.8c-g	33.0efg	31.1efg	23.6hij	22.2d-g	20.8 a-d	17.8c-g	13.3e-h	5.5bc	2.7a-d
T_4: *Rhizobium* GR4	41.6a-d	34.2d-g	32.6d-g	27.2d-h	22.2d-g	20.2 a-d	17.5d-g	14.3d-g	7.7a	1.5ef
T_5: *Rhizobium* GR5	36.6d-h	32.4fg	29.8fg	27.3c-h	25.8a-d	16.5de	17.0d-f	12.0fgh	5.2bc	2.7a-d
T_6: *Rhizobium* GR6	36.1d-h	30.7g	29.9fg	23.1 hij	25.2a-d	17.0cde	15.3fgh	10.8gh	5.3bc	2.0de
T_7: *Rhizobium* GR7	35.7e-h	31.8g	31.1efg	22.1 ij	20.7fgh	16.3de	15.5fgh	11.2fgh	5.2bc	2.0de
T_8: *Rhizobium* GR8	41.7a-d	36.9b-g	34.3b-f	27.9 b-h	25.2a-d	21.2 a-d	19.7b-f	16.0c-f	6.6ab	2.7a-d
T_9: *Rhizobium* GR9	44.1ab	43.2a	36.0a-e	32.0 a-d	28.2a	22.2ab	20.5b-e	15.8c-f	7.8a	3.3a
T_{10}:*Rhizobium*GR10	31.8hi	39.0a-e	28.0gh	27.0 e-i	23.7b-f	17.7b-e	14.7gh	13.0fgh	5.0cd	3.2ab
T_{11}: *Rhizobium* GR11	35.6fgh	36.6b-g	34.4b-f	24.9 f-j	23.5b-g	18.1 a-d	21.3a-d	21.0ab	2.7e	2.6a-d
T_{12}: *Rhizobium* GR12	36.2d-h	40.8abc	36.1a-e	29.8 b-f	22.4d-g	19.7 a-d	21.4a-d	22.6ab	3.1e	2.9abc
T_{13}: *Rhizobium* GR13	46.6a	44.1a	41.3a	34.9a	24.3b-e	22.7a	25.8a	23.6a	3.5e	3.2ab
T_{14}: *Rhizobium* GR14	42.6abc	42.1ab	39.8ab	26.9 e-i	26.7ab	20.3 a-d	20.5b-e	19.2a-d	3.4e	2.5bcd
T_{15}: *Rhizobium* GR15	44.1ab	40.2a-d	38.6abc	30.8 a-e	25.2a-d	21.8abc	22.7abc	20.7abc	3.5de	3.1ab
T_{16}: *Rhizobium* GR16	41.3a-e	42.1ab	38.2a-d	31.4 a-e	25.4a-d	19.0 a-d	22.6abc	20.7abc	3.5de	2.2cde
T_{17}: *Rhizobium* GR17	42.7abc	40.6abc	37.5a-d	32.3 abc	26.2abc	20.0 a-d	21.6a-d	21.3ab	3.3e	2.1de
T_{18}: *Rhizobium* GR18	43.0abc	42.6ab	38.3a-d	32.6 ab	25.3a-d	22.3ab	25.7a	23.1ab	2.9e	2.1de
T_{19}: *Rhizobium* GR19	42.8abc	41.2abc	38.2a-d	31.3 a-e	25.0a-e	19.5 a-d	24.2ab	19.3abc	3.3e	2.1de
T_{20}: *Rhizobium* GR20	38.7b-f	38.6a-f	36.1a-e	29.2 b-g	22.8c-g	19.9a-d	20.9a-e	18.2b-e	3.2e	2.0de
T_{21}: Control	26.1i	24.1h	22.4h	20.1 j	17.8h	13.1e	12.4h	8.50h	0.6f	0.2g
SE (±)	2.81	3.08	2.84	2.44	1.78	2.44	2.46	2.45	0.76	0.39
Level sig.	***	***	***	***	**	*	***	***	***	***
CV (%)	8.94	10.2	10.1	10.8	9.18	15.5	15.4	17.9	21.1	21.1

Means followed by common letter are not significantly different at 5% level by DMRT

The plant height was recorded higher in inoculated plantcompared to non-inoculated control. The highest plant height (46.6 cm) was noted with *Rhizobium* GR13 treatment at 0 salinity level which was differed with all treatments except T_4, T_8, T_9, T_{14}, T_{15}, T_{16}, T_{18} and T_{19}(Table 14). However, the plant height was recorded the highest(43.2 cm) with *Rhizobium* GR9 treatment at 4 dS m^{-1} salinity level which was differed with all treatments except T_{10}, T_{12}, T_{13}, T_{14}, T_{15}, T_{16}, T_{18} and T_{19}. On the other hand, the salinity level at 8 dS m^{-1}, the highest plant height (41.3 cm) were recorded with *Rhizobium* GR13 treatment which was identical with all the treatments except T_1, T_2, T_3, T_4, T_5, T_6, T_8,T_{10}, T_{12} and T_{21}. Moreover, the salinity level at 12 dS m^{-1}, the highest plant height (34.9 cm) was documented with *Rhizobium* GR13 treatment which was differed with all the treatments except T_9, T_{15}, T_{16}, T_{17}, T_{18} and T_{19}. Furthermore, the salinity level at 16 dS m^{-1}, the highest plant height (28.2 cm) was recorded with *Rhizobium* GR9 treatment which was identical with alltreatments except T_1, T_2, T_3, T_4, T_7,T_{10}, T_{11}, T_{12}, T_{13}, T_{20} and T_{21}.

The highest nut plant^{-1} (22.2 nut plant^{-1}) was documented with *Rhizobium* GR9 treatment at 0 salinity level which was identical with all treatments except T_1, T_5, T_6, T_7, T_{10} and T_{21} (Table 14). However, the nut plant^{-1} was recorded the highest(25.8 nut plant^{-1}) with *Rhizobium* GR13 treatment at 4 dS m^{-1} salinity level which was varied with all treatments except T_{11}, T_{12}, T_{15}, T_{16}, T_{17}, T_{18}, T_{19} and T_{20}. On the other hand, the salinity level at 8 dS m^{-1}, the nut plant^{-1} was recorded the highest(23.6 nut plant^{-1}) with *Rhizobium* GR13 treatment which was differed with all treatments except T_{11}, T_{12}, T_{14}, T_{15}, T_{16}, T_{17}, T_{18} and T_{19}. Moreover, the salinity level at 12 dS m^{-1}, the highest nut plant^{-1} (7.85 nut plant^{-1}) was documented with *Rhizobium* GR9 treatment which was differed with all the treatments except T_2, T_4 and T_8. Furthermore, the salinity level at 16 dS m^{-1}, the highest nut plant^{-1} (3.3 nut plant^{-1}) was recorded with *Rhizobium* GR9 treatment which was differed with all treatments except T_3, T_5, T_8 T_{10}, T_{11}, T_{12}, T_{13} and T_{15}.

The highest kernel (2.0 kernel nut^{-1}) was recorded with *Rhizobium* GR9 treatment at 0 salinity level which was differed with all treatments except T_1, T_2, T_{10}, T_{11}, T_{13}, T_{12}, T_{13}, T_{18}, and T_{19} (Table 15). However, the kernel was recorded the highest(1.90 kernel nut^{-1}) with T_6, T_9 and T_{16} treatment at 4 dS m^{-1} salinity level which was identical with all treatments except T_1, T_3, T_5, T_{11} and T_{21}. On the other hand, the salinity level at 8 dS m^{-1}, the highest kernel (2.0 kernel nut^{-1}) was recorded with *Rhizobium* GR8 treatment which was identical with all the treatments except T_3, T_6, T_7, T_{16}, T_{17}, T_{18}, T_{19}, T_{20} and T_{21}. Moreover, the salinity level at 12 dS m^{-1}, the highest kernel (1.75 kernel nut^{-1}) was recorded with *Rhizobium* GR9 treatment which was identical with all the treatments except T_6, T_{11}, T_{14}, T_{17}, T_{20} and T_{21}. Furthermore, the salinity level at 16 dS m^{-1}, the highest kernel (1.53 kernel nut^{-1}) was recorded with *Rhizobium* GR13 treatment which was differed with all the treatments except T_3, T_9 and T_{15}.The 100 nut weight was recorded the highest(64.6 g) with *Rhizobium* GR13 treatment at 0 dS m^{-1} salinity level which was differed with all treatments except T_3, T_8, T_9 T_{12}, T_6, T_{17}, T_{18} and T_{19} (Table 15). However, the 100 nut weight was recorded the highest(56 g) with T_{13} treatment at 4 dS m^{-1} salinity level which was identical with all treatments except T_4, T_7, T_{17}, T_{20} and T_{21}. On the other hand, the salinity level at 8 dS m^{-1}, the highest 100 nut weight (52.0 g) was recorded with *Rhizobium* GR9 treatment which was identical with all the treatments except T_2, T_{10}, T_{11}, T_{12}, T_{20}, and T_{21}. Moreover, the salinity level at 12 dS m^{-1}, the highest 100 nut weight (61.5 g) was recorded with *Rhizobium* GR9 treatment which was differed with all the treatments except T_8. Furthermore, the salinity level at 16 dS m^{-1}, the highest 100 nut weight (54.8 g) was recorded with *Rhizobium* GR9 treatment which was differed with all the treatments except T_5, and T_8.

Table 15. Karnel and 100 nut weight of groundnut inoculated by *Rhizobium* at different salinity level

Treatment	Kernel nut^{-1}					100 nut weight (g)				
Salinity level (dSm^{-1})	0	4	8	12	16	0	4	8	12	16
T_1: *Rhizobium* GR1	1.85a-d	0.55f	1.76a-e	1.60a-d	1.00efg	49.4fgh	49.8a-d	47.8a-e	44.6ef	41.3def
T_2: *Rhizobium* GR2	1.90ab	1.80ab	1.80a-d	1.70ab	1.25b-e	57.8a-e	53.2ab	43.3de	49.9b-e	43.3c-f
T_3: *Rhizobium* GR3	1.60b-f	1.30cd	1.50cde	1.40 a-e	1.35a-d	61.3abc	50.6 a-d	47.3 a-e	49.8cde	43.2c-f
T_4: *Rhizobium* GR4	1.50c-g	1.55abc	1.95ab	1.35 a-e	0.90fg	53.7c-f	44.0c-f	48.5a-d	48.8cde	42.1c-f
T_5: *Rhizobium* GR5	1.50c-g	1.45bc	1.70a-e	1.65abc	0.95fg	48.8fgh	50.4 a-d	51.5a	53.3bc	49.9abc
T_6: *Rhizobium* GR6	1.20g	1.90a	1.55b-e	1.30b-e	0.95fg	41.4hi	49.3 a-d	47.8 a-e	48.4cde	45.0b-e
T_7: *Rhizobium* GR7	1.50c-g	1.55abc	1.40de	1.55 a-e	0.90fg	56.0b-f	40.5ef	48.3a-d	49.3cde	45.9bcd
T_8: *Rhizobium* GR8	1.40efg	1.85a	2.00a	1.50 a-e	0.80gh	57.8a-e	48.9 a-d	48.0 a-e	56.0ab	52.7ab
T_9: *Rhizobium* GR9	2.00a	1.90a	1.75a-e	1.75a	1.45ab	62.2ab	55.9a	52.0a	61.5a	54.8a
T_{10}:*Rhizobium*GR10	1.65a-e	1.58abc	1.85abc	1.45 a-e	1.10def	55.1b-f	48.6a-e	44.7cde	52.8bcd	46.1bcd
T_{11}: *Rhizobium* GR11	1.69 a-e	1.03df	1.59a-e	1.27cde	1.00efg	52.8d-g	51.4 a-d	45.1b-e	42.3f	36.4fgh
T_{12}: *Rhizobium* GR12	1.60b-f	1.80 ab	1.70a-e	1.37 a-e	1.25b-e	61.1abc	52.2abc	45.3b-e	45.6ef	40.3def
T_{13}: *Rhizobium* GR13	1.87abc	1.83ab	1.72a-e	1.63a-d	1.53a	64.6a	56.0a	50.6ab	48.5cde	40.6def
T_{14}: *Rhizobium* GR14	1.53b-g	1.55abc	1.65a-e	1.22de	1.17c-f	50.4efg	51.8abc	47.5 a-e	45.4ef	39.8def
T_{15}: *Rhizobium* GR15	1.67 a-e	1.82ab	1.70a-e	1.45 a-e	1.42abc	55.4b-f	54.9a	50.5ab	47.9c-f	38.5d-g
T_{16}: *Rhizobium* GR16	1.23fg	1.90a	1.55b-e	1.17e	1.03efg	44.8gh	50.1 a-d	47.1 a-e	46.7ef	38.0d-g
T_{17}: *Rhizobium* GR17	1.50c-g	1.55abc	1.33e	1.45 a-e	1.07efg	59.3a-d	43.6def	47.3 a-e	46.9def	37.3efg
T_{18}: *Rhizobium* GR18	1.70 a-e	1.72ab	1.53b-e	1.50 a-e	1.03efg	58.8a-d	48.9 a-d	49.0abc	47.7c-f	37.3efg
T_{19}: *Rhizobium* GR19	1.64 a-e	1.70ab	1.75a-e	1.42a-e	1.25b-e	58.9a-d	54.2ab	48.3a-d	44.2ef	28.7hi
T_{20}: *Rhizobium* GR20	1.48d-g	1.58abc	1.52cde	1.25cde	1.17c-f	50.1efg	46.3b-e	42.5e	43.9ef	30.3ghi
T_{21}: Control	0.70h	0.74ef	0.68f	0.70f	0.55h	36.4i	36.4f	31.4f	29.4g	27.8i
SE **(±)**	0.18	0.19	0.21	0.20	0.13	4.04	4.09	2.77	3.00	4.12
Level sig.	***	***	**	**	***	***	**	***	***	***
CV (%)	14.3	15.2	16.1	17.9	15.4	9.15	10.2	7.25	7.70	12.4

Means followed by common letter are not significantly different at 5% level by DMRT

The kernel weight of 100 nut was noted higher in inoculated *Rhizobium* bacteria compared to non-inoculated control. The highest kernel weight of 100 nut (44.7 g) was documented with *Rhizobium* GR8 treatment at 0 dS m^{-1} salinity level which was varied with all treatments except $T_{7,}$ $T_{9,}$ $T_{13,}$ T_{18} and T_{19} (Table 16). However, the kernel weight of 100 nut was recorded the highest (44.0 g) with *Rhizobium* GR9 treatment at 4 dS m^{-1} salinity level which was identical with all treatments except T_1, T_3, T_5, T_7, T_{10}, T_{11}, T_{20} and $T_{21.}$ On the other hand, the salinity level at 8 dS m-1, the highest kernel weight of 100 nut (43.0 g) was recorded with *Rhizobium* GR9 treatment which was differed with all the treatments except $T_8$and$T_{13.}$ Moreover, the salinity level at 12 dS m^{-1}, the highest kernel weight of 100 nut (38.4 g) was recorded with *Rhizobium* GR8 treatment which was differed with all the treatments except T_3, T_5, T_6, T_7, T_9,and T_{10}. Furthermore, the salinity level at 16 dS m^{-1}, the highest kernel weight of 100 nut (35.9 g) was noted with *Rhizobium* GR9 treatment which was differed with all the treatments except T_3, T_4, T_5, T_6, T_7, T_8, T_{10} and T_{13}.But shelling percent was non-significant in all treatments at all salinity level.

Table 16. Kernel weight and shelling percent of groundnut inoculated by *Rhizobium* at different salinity level

Treatment	Kernel weight of 100 nut (g)					Shelling %				
Salinity level (dSm^{-1})	0	4	8	12	16	0	4	8	12	16
T_1: *Rhizobium* GR1	27.5ij	36.8c-f	32.7c-g	30.8cd	29.5b-e	55.5d	74.0	68.5	69.4	71.7
T_2: *Rhizobium* GR2	37.2d-g	40.4a-e	32.9c-g	30.9cd	27.5e	64.6bcd	75.9	76.1	62.4	64.8
T_3: *Rhizobium* GR3	39.1b-f	36.7c-f	30.5efg	33.8a-d	34.9abc	63.9bcd	72.8	64.6	68.2	82.4
T_4: *Rhizobium* GR4	35.6e-h	39.5a-e	34.6cde	35.4abc	32.8a-e	67.5a-d	89.7	71.8	73.0	82.6
T_5: *Rhizobium* GR5	31.3hi	37.9b-f	31.7d-g	35.9ab	32.0a-e	64.1bcd	77.8	61.2	67.3	64.2
T_6: *Rhizobium* GR6	33.2gh	40.0a-e	35.9cde	33.8a-d	33.2a-e	82.7a	81.5	75.2	69.7	74.2
T_7: *Rhizobium* GR7	41.0a-d	36.5def	36.3b-e	34.1a-d	32.2a-e	75.3abc	91.5	76.4	69.4	71.4
T_8: *Rhizobium* GR8	44.7a	40.7a-e	42.0ab	38.4a	34.8a-e	77.7ab	84.4	88.9	68.6	67.2
T_9: *Rhizobium* GR9	42.6abc	44.0a	43.0a	38.3a	35.9a	68.9a-d	79.2	82.8	62.5	68.1
T_{10}:*Rhizobium*GR10	35.2fgh	33.0f	36.4b-e	33.8a-d	34.1a-e	65.2bcd	68.0	81.4	64.4	74.6
T_{11}: *Rhizobium* GR11	30.8hi	34.9ef	33.9c-f	29.9d	27.5e	59.2cd	69.6	75.5	70.7	66.4
T_{12}: *Rhizobium* GR12	35.9e-h	42.1a-d	34.6cde	29.6d	27.4e	58.9cd	71.3	76.4	65.2	66.6
T_{13}: *Rhizobium* GR13	44.1ab	43.2ab	38.3abc	32.8bcd	31.6a-e	68.7a-d	77.1	75.9	68.3	69.2
T_{14}: *Rhizobium* GR14	36.9d-g	42.5abc	36.9bcd	30.5cd	29.4b-e	67.2a-d	74.8	70.6	67.6	58.7
T_{15}: *Rhizobium* GR15	35.5e-h	41.6a-d	34.2cde	32.4bcd	29.8b-e	71.9a-d	75.9	74.6	71.7	69.8
T_{16}: *Rhizobium* GR16	34.6fgh	38.3a-f	32.6c-g	31.6bcd	28.1de	70.7a-d	73.1	69.5	67.6	68.5
T_{17}: *Rhizobium* GR17	39.4b-f	38.2a-f	33.4c-f	31.7bcd	27.8e	66.8a-d	73.8	72.0	67.6	65.7
T_{18}: *Rhizobium* GR18	42.7abc	40.2a-e	36.7bcd	30.3d	28.3de	65.8bcd	72.6	75.0	64.2	68.9
T_{19}: *Rhizobium* GR19	40.6a-e	40.0a-e	27.0g	29.7d	28.8cde	69.0a-d	74.3	56.0	67.6	68.0
T_{20}: *Rhizobium* GR20	37.8c-g	35.7ef	28.0fg	31.4bcd	29.4b-e	71.1a-d	71.0	65.9	71.6	62.7
T_{21}: Control	22.5j	21.5g	19.5h	21.45e	19.5f	61.9bcd	59.1	62.5	72.8	70.1
SE (±)	2.54	2.90	2.96	2.48	2.96	8.13	8.56	8.37	6.00	10.3
Level sig.	***	***	***	**	**	NS	NS	NS	NS	NS
CV (%)	8.53	9.29	10.7	9.45	12.0	14.7	13.8	14.2	10.8	18.3

Means followed by common letter are not significantly different at 5% level by DMRT

The non-inoculated control plant recorded lowerstover yield compared to inoculated plant. The highest stover yield (9.38 g plant^{-1}) was recorded with *Rhizobium* GR9 treatment at 4 salinity level which was identical with all treatments except T_6, T_7, T_{14},T_{16}, $T_{17}$and T_{21} (Fig.7). However, the salinity level at 0, 8, 12 and 16 dS m^{-1}, stover yield was recorded non-significant with all the treatments, respectively.The inoculated plantdocumented higher nut yield compared to non-inoculated control. The highest nut yield (30.5 g plant^{-1}) was recorded with *Rhizobium* GR13 treatment at 0 salinity level which was differed with all treatments except T_2, T_4, T_8, T_9, T_{15}, T_{17}, T_{18} and T_{20} (Fig.8). However, the nut yield was recorded the highest(32.5 g plant^{-1}) with *Rhizobium* GR13 treatment at 4 dS m^{-1} salinity level which was differed with all treatments except T_9, T_{15}, T_{16}, T_{18} and T_{19}. Moreover, the nut yield was noted the highest(25.1 g plant^{-1}) with *Rhizobium* GR18 treatment at 8 dS m^{-1} salinity level which was varied with all treatments except T_9, T_{11}, T_{12}, T_{13}, T_{14}, T_{15}, T_{16} and T_{17}. While, the salinity level at 12 dS m^{-1}, the highest nut yield (21.8 g plant^{-1}) was recorded with *Rhizobium* GR13 treatment which was diverged with all the treatments except T_{12}, T_{14}, T_{15}, T_{16}, T_{17}, T_{18}, T_{19} and T_{20}. On the other hand, the salinity level at 16 dS m^{-1}, the nut yield was recorded the highest (6.05 g plant^{-1}) was recorded with *Rhizobium* GR13 treatment which was identical with all the treatments except T_1, T_2, T_{11}, T_{12}, T_{20} and T_{21}.

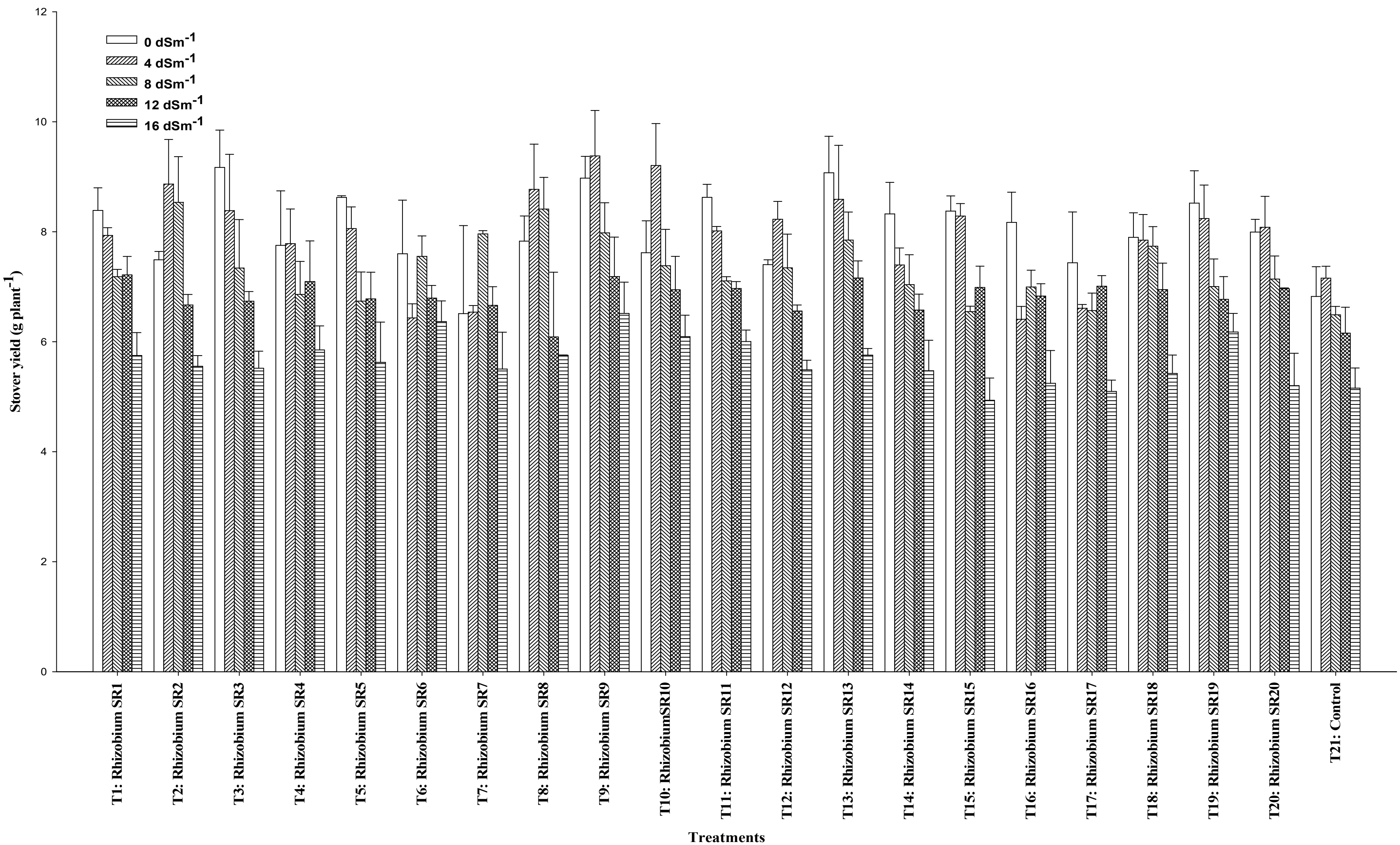


Fig.7. Stover yield of groundnut inoculated by *Rhizobium* at different salinity level

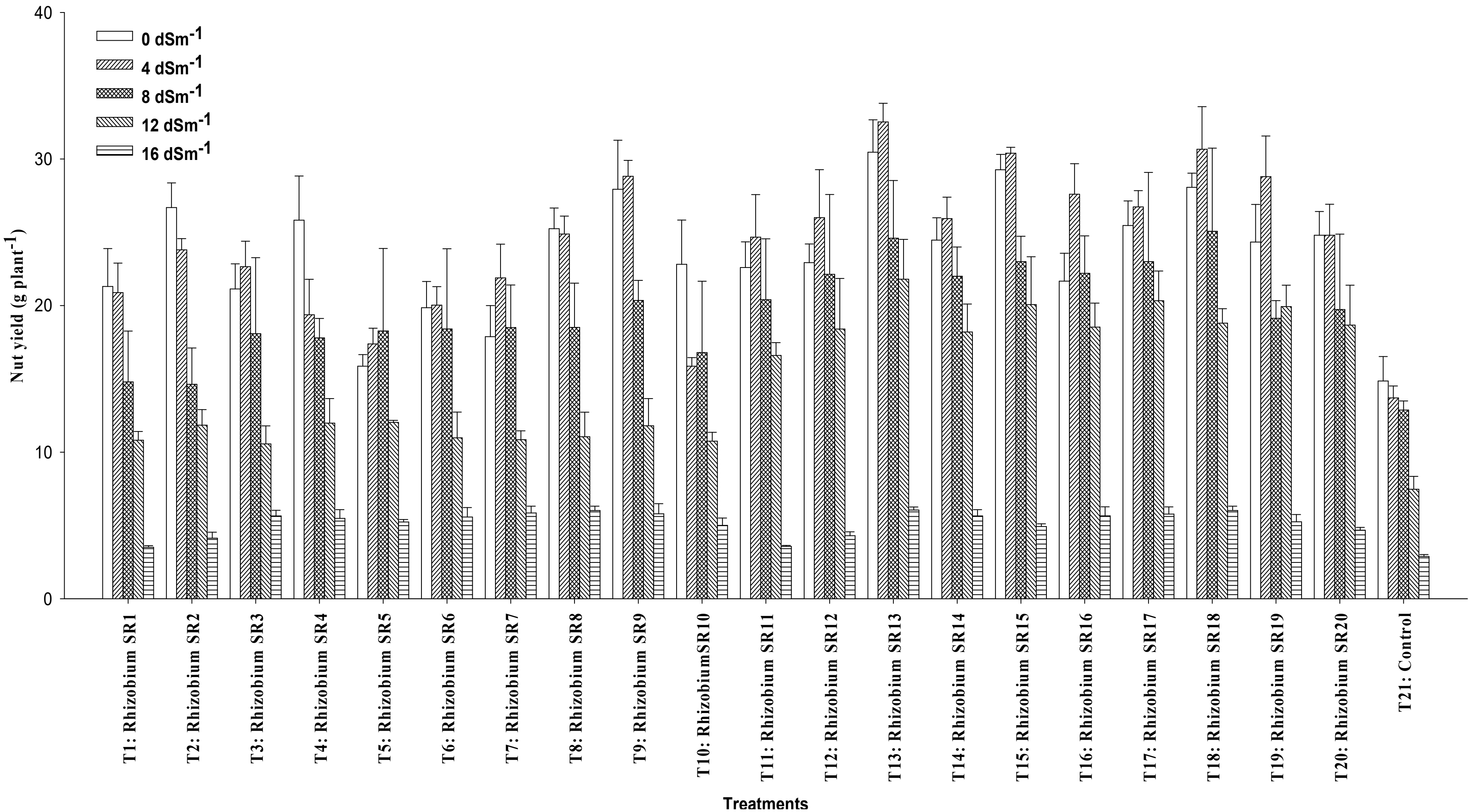


Fig.8. Nut yield of groundnut inoculated by *Rhizobium* at different salinity level

Conclusion:

Rhizobium biofertilizer applied plants exhibited better performance in nodule numbers, nodule weights, aboveground biomass, root biomass, and plant height than non-inoculated plants, representing that all *Rhizobium* sp. effectively enhanced nodulation and growth parameters on groundnut plant than non-inoculatedgroundnut in all salinity level. *Rhizobium*inoculated Groundnut plants showed higher pod yield, stover yield, and seed yield than non-inoculated plants, revealing the positive impact of *Rhizobium* sp. on yield parameters of groundnut. Groundnut plants inoculated with *Rhizobium* sp. showed better performance comparatively with *Rhizobium* sp.GR13> *Rhizobium* sp.GR18>*Rhizobium* sp.GR15>*Rhizobium* sp.GR18>*Rhizobium* sp.GR17>*Rhizobium* sp.GR19>*Rhizobium* sp.GR14>*Rhizobium* sp.GR16>*Rhizobium* sp.GR9>*Rhizobium* sp.GR20>*Rhizobium* sp.GR11>*Rhizobium* sp.GR8>*Rhizobium* sp.GR2>*Rhizobium* sp.GR4>*Rhizobium* sp.GR3>*Rhizobium* sp.GR7>*Rhizobium* sp.GR6>*Rhizobium* sp.GR10>*Rhizobium* sp.GR12> *Rhizobium* sp.GR5> considering average nut yield of groundnut at different salinity levels than non-inoculated plants. Therefore, *Rhizobium* sp. established an effective symbiotic association with groundnut and was responsible for increased growth and biomass, and improved yield characteristics of groundnut at different salinity levels.

Photograph 5. *Rhizobium* strains R1-R20 tested on groundnut with 0, 4, 8, 12 and 16 dS/m salinity

xv) Field experiment trials:

The ultimate test for an effective strain of *Rhizobium* is to find out how well it is able to establish and perform under field conditions. For the field experiments, four locations (Patuakhali, Satkhira, Lakhmipur and Cox's Bazar) were selected. Carrier-based salt tolerant *Rhizobium* inoculum was used in field experiments to know the potential *Rhizobium* biofertilizer(s) performance in soybean and groundnut production in saline soil.

Expt. 03: Adaptive field trials of salt tolerant rhizobial strains on soybean production

Objectives

i. To study on nodulation capacity of salt tolerant rhizobial strains on soybean under field condition.
ii. To study on performance of salt tolerant rhizobial strains on growth and yield of soybean under field condition.

Materials and Methods

Two set of field experiment was carried out during rabi season whereas first set of experiment wereconductedon 10 January 2018 at Subornochor, Noakhali and another location wasKhuruskul, Cox's Bazar on 20 January 2018, respectively.The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. The treatments were eleven *Rhizobium* isolates which were replicated into three blocks. There were 11 treatments viz. T_1: *Rhizobium* SR1, T_2: *Rhizobium* SR2, T_3: *Rhizobium* SR3, T_4: *Rhizobium* SR4, T_5: *Rhizobium* SR5, T_6: *Rhizobium* SR6, T_7: *Rhizobium* SR7, T_8: *Rhizobium* SR8, T_9: *Rhizobium* SR9, T_{10}: *Rhizobium* SR10, T_{11}:control. The second set of experiment was design on 10 February 2018 at Benerpota, Satkhira and another location on 20 February 2018 at Kuakata, Patuakhali, respectively. Similarly, the experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. The treatments were eleven *Rhizobium* isolates which were replicated into three blocks. There were 11 treatments viz. T_1: *Rhizobium* SR11, T_2: *Rhizobium* SR12, T_3: *Rhizobium* SR13, T_4: *Rhizobium* SR14, T_5: *Rhizobium* SR15, T_6: *Rhizobium* SR16, T_7: *Rhizobium* SR17, T_8: *Rhizobium* SR18, T_9: *Rhizobium* SR19, T_{10}: *Rhizobium* SR20, T_{11}:control. The carrier-based salt tolerant *Rhizobium* inoculum was used in field experiments to know the performance of effective *Rhizobium* inoculum in soybean production in saline soil. The tested crop was soybean (cv. BARI Soybean-6). Peat based rhizobial inoculum containing 10^8 cells g^{-1} inoculumwas used at the rate of 1.5 kg ha^{-1}. Seed were mixed thoroughly with inoculum (20:1 ratio) before sowing. Seeds were used at the rate of 75 kg ha^{-1}. Phosphorus, potassium, sulphur, zinc and boron ($P_{42}K_{40}S_{40}Zn_5B_1$ kg ha^{-1}) were used in the form of TSP, MoP, gypsum, zinc sulphate and boric acid, respectively. All P, K, S, Zn, B were applied at the time of land preparation. All the intercultural operations such as irrigation, weeding, insect control etc. were done as and when necessary. Nodules were collected by carefully uprooting five sample plants selected randomly from each unit plot at the 50 percent flowering stage. Nodules were separated from the roots, counted and then oven-dried and weighed. Data on yield and yield components were recorded at maturity. The soybean was harvested on 12 May 2018 at Cox's Bazar and 18 May 2018 at Noakhali. On the other hand, the soybean was harvested at Patuakhali on 10 May 2018 and at Satkhira. on 5 May 2018. The initial soil samples at a depth of 0-15 cm from the experimental fields were collected and analyzed following standard methods (Table17).

Table 17. Initial fertility status of the soil samples of the pot soil

Soil Properties	pH	OM %	Ca	Mg	K	Total N %	P	S	B	Cu	Fe	Zn
			meq 100g^{-1}				µg g^{-1}					
Cox's Bazar soil	5.9	1.3	5.1	1.7	0.13	0.07	3.0	26	0.22	2.0	78	1.86
Noakhali soil	5.4	1.4	5.4	1.9	0.26	0.74	3.0	51	0.22	2.0	63	0.76
Critical level	-	-	2.0	0.5	0.12	-	7	10	0.20	0.20	4	0.6

Statistical analysis

All data were statistically analyzed using a RCBD design. Treatment effects on measured variables were tested by analysis of variance (ANOVA), and comparisons among treatment means were made using the least significant difference (LSD) multiple range test calculated at 5% level of probability ($P \leq 0.05$). Statistical procedures were carried out with the software program Statistix 10™.

Results and Discussion

Weobservedpoornodulation in the controlsamples; however, biofertilizer application increased nodule formation in the roots of groundnut. Biofertilizer application to the plant significantly(P < 0.05) increased both the number and weight of nodules in groundnut roots.The highest numbers of nodule (24.5 plant^{-1} at Cox' Bazar and 26.2 plant^{-1} at Noakhali) were recorded with *Rhizobium* SR7 treatment which was identical with all treatments except T_1, T_5, T_{10}, T_{11} at Cox's Bazarand T_1, T_5,T_{11} at Noakhali, respectively(Table 18). The highest numbers of nodule (25.4 plant^{-1} at Kuakata and 27.4 plant^{-1} at Satkhira) were recorded with *Rhizobium* SR15 treatment which was identical with all treatments except T_1, T_2, T_4, T_8 at Kuakata and T_4, T_{11} at Satkhira, respectively(Table 19).However, the nodule weight was recorded the highest(0.25 g plant^{-1} at Cox' Bazar and 0.26 g plant^{-1} at Noakhali) with *Rhizobium* SR7 treatment which was differed with all treatments except T_3, T_4, T_{10} at Cox' Bazar and identical with all treatments except T_{11}at Noakhali, respectively. However, the nodule weight was recorded the highest(0.22 g plant^{-1} at Kuakata and 0.35 g plant^{-1} at Satkhira) with *Rhizobium* SR15 treatment which was identical with all treatments except T_{11} at Kuakata and differed with all other treatments at Noakhali, respectively.

Table 18. Nodulation and dry matter production of soybean inoculated by *Rhizobium*strains

Treatment	Nodule number plant^{-1}		Nodule weight (g plant^{-1})		Shoot weight (g plant^{-1})		Root weight (g plant^{-1})	
Locations	Cox's Bazar	Noakhali	Cox's Bazar	Noakhali	Cox's Bazar	Noakhali	Cox's Bazar	Noakhali
T_1: *Rhizobium* SR1	15.8d	17.5c	0.19b	0.20a	4.74abcd	4.12abc	0.40	0.31ab
T_2: *Rhizobium* SR2	23.0ab	24.7ab	0.21ab	0.23a	4.26bcd	3.80bc	0.38	0.30ab
T_3: *Rhizobium* SR3	22.8ab	24.5ab	0.21ab	0.21a	4.51bcd	4.01abc	0.38	0.33ab
T_4: *Rhizobium* SR4	22.1abc	23.8ab	0.22ab	0.22a	5.35ab	4.01abc	0.44	0.28b
T_5: *Rhizobium* SR5	17.8cd	19.5bc	0.16b	0.20a	5.92a	4.97a	0.46	0.37a
T_6: *Rhizobium* SR6	20.1abcd	21.8abc	0.16b	0.21a	5.19abc	4.07abc	0.41	0.32ab
T_7: *Rhizobium* SR7	24.5a	26.2a	0.25a	0.26a	4.76abcd	4.57ab	0.40	0.34ab
T_8: *Rhizobium* SR8	20.1abcd	22.2abc	0.16b	0.21a	5.45ab	4.76ab	0.39	0.33ab
T_9: *Rhizobium* SR9	21.6abc	23.3ab	0.18b	0.19a	4.48bcd	3.34c	0.34	0.28b
T_{10}: *Rhizobium* SR10	19.6bcd	21.3abc	0.19ab	0.21a	3.93cd	3.17c	0.43	0.31ab
T_{11}: Control	0.00e	0.0d	0.00c	0.00b	3.43d	3.00c	0.32	0.22c
SE (±)	2.19	2.51	0.03	0.03	0.66	0.56	0.06	0.03
Level sig.	***	***	***	**	*	*	NS	**
CV (%)	14.2	15.1	20.0	20.7	17.12	17.10	17.39	11.9

Means followed by common letter are not significantly different at 5% level by DMRT

Table 19. Nodulation and dry matter production of soybean inoculated by *Rhizobium* strains

Treatment	Nodule number plant^{-1}		Nodule weight (g plant^{-1})		Shoot weight (g plant^{-1})		Root weight (g plant^{-1})	
Locations	Kuakata	Satkhira	Kuakata	Satkhira	Kuakata	Satkhira	Kuakata	Satkhira
T_1: *Rhizobium* SR11	22.0bcd	23.7ab	0.19a	0.21b	4.62ab	2.64cd	0.25abc	0.28
T_2: *Rhizobium* SR12	21.4cd	23.1ab	0.20a	0.22b	4.13abc	3.79b	0.21cd	0.28
T_3: *Rhizobium* SR13	23.1abcd	24.8ab	0.16a	0.19b	4.90a	3.79b	0.22bcd	0.29
T_4: *Rhizobium* SR14	20.0d	21.7b	0.18a	0.19b	4.69ab	3.68b	0.27abcd	0.25
T_5: *Rhizobium* SR15	25.4a	27.4a	0.22a	0.35a	3.34dce	2.44d	0.20d	0.27
T_6: *Rhizobium* SR16	24.3abc	26.0ab	0.21a	0.22b	4.74ab	3.66b	0.28ab	0.26
T_7: *Rhizobium* SR17	24.7abc	26.3ab	0.20a	0.23b	5.12a	4.91a	0.31a	0.31
T_8: *Rhizobium* SR18	21.9bcd	23.6ab	0.19a	0.20b	4.93a	3.42bc	0.21cd	0.30
T_9: *Rhizobium* SR19	22.7abcd	24.3ab	0.19a	0.22b	3.75bcd	3.47bc	0.21cd	0.25
T_{10}: *Rhizobium* SR20	25.1ab	26.8ab	0.17a	0.16b	3.08de	3.10bcd	0.23bcd	0.28
T_{11}: Control	0.0e	0.0c	0.00b	0.00c	2.46e	2.26d	0.21d	0.20
SE (±)	1.62	2.73	0.02	0.05	0.49	0.42	0.03	0.03
Level sig.	***	***	***	*	***	***	*	NS
CV (%)	14.8	20.0	18.3	17.8	14.7	15.1	16.7	13.6

Means followed by common letter are not significantly different at 5% level by DMRT

On the other hand, the highest shoot weight (5.92 g plant^{-1} at Cox' Bazar and 4.97 g plant^{-1} at Noakhali) were recorded with *Rhizobium* SR5 treatment which was identical with all the treatments except T_2, T_3, T_9, T_{10}, T_{11} at Cox' Bazar and T_2, T_9, T_{10}, T_{11} at Noakhali, respectively.On the other hand, the highest shoot weight (5.12 g plant^{-1} at Kuakata and 4.91 g plant^{-1} at Satkhira) were recorded with *Rhizobium* SR17 treatment which was identical with all the treatments except T_{15}, T_{19}, T_{20}, T_{21} at Kuakata and differed with all other treatments at Satkhira, respectively.Moreover, the highest root weight (0.37 g plant^{-1} at Noakhali) was recorded with *Rhizobium* SR5 treatment which was identical with all the treatments except T_9 and T_{11}.Furthermore, the root weight was non-significant in all treatments at Cox's Bazar. Moreover, the highest root weight (0.31 g plant^{-1} at Kuakata) was recorded with *Rhizobium* SR17 treatment which was differed with all the treatments except T_1 T_4 T_6.Furthermore, the root weight was non-significant in all treatments at Satkhira.

The collar diameter was recorded higher in inoculated *Rhizobium* bacteria compared to non-inoculated control. The highest collar diameter (2.42 mm at Cox' Bazar and 2.11 at Noakhali) was recorded with *Rhizobium* SR5 treatment which was identical with all treatments except T_2, T_{11}at Cox' Bazaranddiffered with all the treatments except T_6, T_7 at Noakhali, respectively(Table 20).The highest collar diameter (2.22 mm at Kuakata and 2.00 at Satkhira) was recorded with *Rhizobium* SR5 treatment which was identical with all treatments except T_2, T_5, T_{10}, T_{11} at Kuakata anddiffered with all the treatments except T_5, T_6, T_{10} at Satkhira, respectively (Table 21).The plant height was recorded non-significant in all treatments both Cox's Bazar and Noakhali, respectively. The plant height was recorded the highest (54.8 cm at Kuakata and 51.2 cm at Satkhira) was recorded with *Rhizobium* SR17 treatment which was differed with all treatments except T_1, T_6 at Kuakata andidentical with all the treatments except T_1, T_2, T_{10}, T_{11} at Satkhira, respectively.

The pod length was recorded non-significant in all treatments at Cox' Bazar but it was found the highest (4.22 cm) with *Rhizobium* SR5 treatment which was identical with all treatments except T_2, T_3, T_4, $T_{10}$and T_{11} at Noakhali. The pod length was recorded the highest (4.33 cm) with *Rhizobium* SR17 treatment which was differed with all treatments except T_3, T_6 at Kuakata but it was found non-significant in all treatments at Satkhira.

Table 20. Collar diameter, plant height and pod formation of soybean inoculated by *Rhizobium* strains

Treatment	Collar diameter (mm)		Plant height (cm)		Pod length (cm)	
Locations	Cox's Bazar	Noakhali	Cox's Bazar	Noakhali	Cox's Bazar	Noakhali
T_1: *Rhizobium* SR1	2.00ab	1.26e	59.4	48.0	4.00	3.69abcd
T_2: *Rhizobium* SR2	1.80bc	1.21e	50.5	45.8	3.76	3.47bcd
T_3: *Rhizobium* SR3	2.20ab	1.24e	56.6	49.4	4.04	3.12d
T_4: *Rhizobium* SR4	2.19ab	1.63cd	54.2	51.7	3.62	3.21cd
T_5: *Rhizobium* SR5	2.42a	2.11a	60.8	53.0	4.38	4.22a
T_6: *Rhizobium* SR6	2.35a	2.08a	58.5	55.0	3.67	3.96ab
T_7: *Rhizobium* SR7	1.96ab	1.98ab	50.9	51.1	4.34	3.93ab
T_8: *Rhizobium* SR8	1.99ab	1.81bc	55.0	52.6	4.01	3.77abc
T_9: *Rhizobium* SR9	2.32ab	1.42de	55.4	53.1	3.88	3.74abcd
T_{10}: *Rhizobium* SR10	1.98ab	1.38de	53.8	48.6	3.97	3.42bcd
T_{11}: Control	1.31c	1.24e	44.2	40.0	3.34	3.27cd
SE (±)	0.26	0.11	4.87	4.07	0.35	0.31
Level sig.	*	***	NS	NS	NS	*
CV (%)	15.6	9.43	10.8	10.2	10.9	10.4

Means followed by common letter are not significantly different at 5% level by DMRT

Table 21. Collar diameter, plant height and pod formation of soybean inoculated by *Rhizobium* strains

Treatment	Collar diameter (mm)		Plant height (cm)		Pod length (cm)	
Locations	Kuakata	Satkhira	Kuakata	Satkhira	Kuakata	Satkhira
T_1: *Rhizobium* SR11	1.96abc	1.13cef	49.2abc	40.8bcd	3.20de	3.77
T_2: *Rhizobium* SR12	1.60bcd	1.18ef	46.8bcd	42.1bcd	3.37cde	3.30
T_3: *Rhizobium* SR13	1.97abc	1.18def	48.2bcd	44.8abc	3.93ab	3.37
T_4: *Rhizobium* SR14	2.04ab	1.48bcdef	46.3bcd	47.8ab	3.37cde	3.43
T_5: *Rhizobium* SR15	1.61bcd	1.77abc	48.0bcd	44.4abc	3.73bc	3.87
T_6: *Rhizobium* SR16	1.72abc	1.93ab	50.5ab	49.8a	4.00ab	3.77
T_7: *Rhizobium* SR17	2.22a	2.00a	54.8a	51.2a	4.33a	4.10
T_8: *Rhizobium* SR18	1.96abc	1.64abcd	47.3bcd	46.2abc	3.13de	3.60
T_9: *Rhizobium* SR19	2.04ab	1.35cdef	42.3de	50.3a	3.63bcd	3.33
T_{10}: *Rhizobium* SR20	1.46cd	1.55abcde	43.8cde	40.2cd	3.50bcd	3.27
T_{11}: Control	1.13d	1.08f	38.3e	36.9d	2.91e	2.74
SE (±)	0.26	0.22	2.8	3.47	0.24	0.41
Level sig.	*	**	**	**	***	NS
CV (%)	17.7	18.1	7.53	9.48	8.35	14.4

Means followed by common letter are not significantly different at 5% level by DMRT

The pod per plant was recorded the highest (27.2 pods plant^{-1} at Cox' Bazar and 24.4 pods plant^{-1} at Noakhali) which was identical with all treatments except T_1, T_8 T_9, T_{10}, T_{11} at Cox' Bazarandexcept T_8, T_9, T_{10}, T_{11} at Noakhali, respectively. However, the pod per plant was recorded the highest (25.8 pods plant^{-1} at Kuakata) which was identical with all treatments except T_8, T_{10}, T_{11} at Kuakata but it was found non-significant in all treatments at Satkhira.

Table 22. Yield and yield attributes of soybean inoculated by *Rhizobium* strains

Treatment	Pods plant^{-1}		1000-seed weight (g)		Stover yield (t ha^{-1})	
Locations	Cox's Bazar	Noakhali	Cox's Bazar	Noakhali	Cox's Bazar	Noakhali
T_1: *Rhizobium* SR1	22.2bcd	21.6abc	107.7de	109.4	3.83	3.61ab
T_2: *Rhizobium* SR2	24.7abcd	21.3abc	126.0abc	157.0	3.71	3.33b
T_3: *Rhizobium* SR3	25.1ab	22.3abc	113.2cde	101.0	3.66	3.65ab
T_4: *Rhizobium* SR4	24.9abc	23.8ab	124.9abc	114.8	3.63	3.33b
T_5: *Rhizobium* SR5	27.2a	24.4a	121.5bcd	122.7	3.61	3.84ab
T_6: *Rhizobium* SR6	25.4ab	21.0abc	138.2a	127.8	3.55	3.73ab
T_7: *Rhizobium* SR7	27.1a	23.6ab	133.0ab	123.5	3.43	4.16a
T_8: *Rhizobium* SR8	21.1d	19.8bcd	102.9e	111.0	3.33	3.26b
T_9: *Rhizobium* SR9	21.2cde	20.1bcd	131.1ab	113.8	3.26	3.43ab
T_{10}: *Rhizobium* SR10	21.8bcd	18.2cd	126.3abc	109.4	3.13	3.46ab
T_{11}: Control	17.8e	16.4d	110.7cde	107.5	2.42	2.34c
SE (±)	1.68	1.97	7.96	17.1	0.43	0.39
Level sig.	***	*	**	NS	NS	*
CV (%)	9.30	11.46	8.03	17.7	15.5	13.8

Means followed by common letter are not significantly different at 5% level by DMRT

Table 23. Yield and yield attributes of soybean inoculated by *Rhizobium* strains

Treatment	Pods plant^{-1}		1000-seed weight (g)		Stover yield (t ha^{-1})	
Locations	Kuakata	Satkhira	Kuakata	Satkhira	Kuakata	Satkhira
T_1: *Rhizobium* SR11	22.8ab	20.8	110.6	105.4	3.67a	3.01c
T_2: *Rhizobium* SR12	25.3ab	20.3	127.7	167.5	3.26a	3.06bc
T_3: *Rhizobium* SR13	25.2ab	21.8	113.6	105.8	3.50a	3.26abc
T_4: *Rhizobium* SR14	24.3ab	21.0	123.6	113.7	3.16a	3.28abc
T_5: *Rhizobium* SR15	23.3ab	20.0	121.0	147.0	3.90a	3.77a
T_6: *Rhizobium* SR16	24.3ab	21.0	132.5	102.4	3.67a	3.17bc
T_7: *Rhizobium* SR17	25.8a	23.2	136.4	121.3	3.73a	3.03c
T_8: *Rhizobium* SR18	20.8bc	19.8	106.7	112.0	3.46a	3.63ab
T_9: *Rhizobium* SR19	22.3ab	19.0	128.9	112.9	3.55a	2.92c
T_{10}: *Rhizobium* SR20	21.0bc	18.3	136.1	104.6	3.48a	3.12bc
T_{11}: Control	17.1c	16.1	112.9	102.5	2.21b	2.205d
SE (±)	2.16	1.96	9.95	20.6	0.36	0.28
Level sig.	*	NS	NS	NS	*	**
CV (%)	11.5	11.9	9.92	12.5	13.0	10.8

Means followed by common letter are not significantly different at 5% level by DMRT

Similarly, thousand seed weight was recorded the highest (138.2 g) with *Rhizobium* SR6 treatment at Cox's Bazar which was differed with all treatments except T_4, T_7,T_9, T_{10} and it was found non-significant with all treatments at Noakhali (Table 22). Similarly, the thousand seed weight was recorded non-significant in all treatments in both locations.The stover yield was recorded non-significant in all treatments at Cox's Bazar but at Noakhali, it was recorded the highest (4.16 t ha^{-1}) with *Rhizobium* SR7 treatment which was differed with all treatments except T_1, T_3, T_5, T_6T_9 and T_{10}. The highest stover yield (3.90 t ha^{-1} at Kuakata and 3.77 t ha^{-1} at Satkhira) was recorded with *Rhizobium* SR15 treatment which was identical with all treatments except T_{11} at Kuakata andT_1, T_2, T_6, T_9, T_{10}, T_{11} at Satkhira, respectively (Table 23).The seed yield was recorded the highest (1.96 t ha^{-1} at Cox's Bazar and 2.10 t ha^{-1} at Noakhali) with *Rhizobium* SR7 treatment which was identical with all treatments except T_{10}, T_{11} at Cox's Bazarand T_1, T_5 T_9, T_{11} at Noakhali, respectively (Fig. 10). The seed yield was recorded the highest (1.95 t ha^{-1} at Kuakata and 1.98 t ha^{-1} at Satkhira) was recorded with *Rhizobium* SR15 treatment which was identical with all treatments except T_7, T_9, T_{10}, T_{11} at Kuakata andT_2, T_4, T_9, T_{10}, T_{11} at Satkhira, respectively (Fig. 11). Stover yield and seed yield were higher might be due to higher nodulation in soybean root. Alam et al (2015) reported that nodule numbers on all soybean genotypes correlated positively with stover yield and seed yield of soybean.

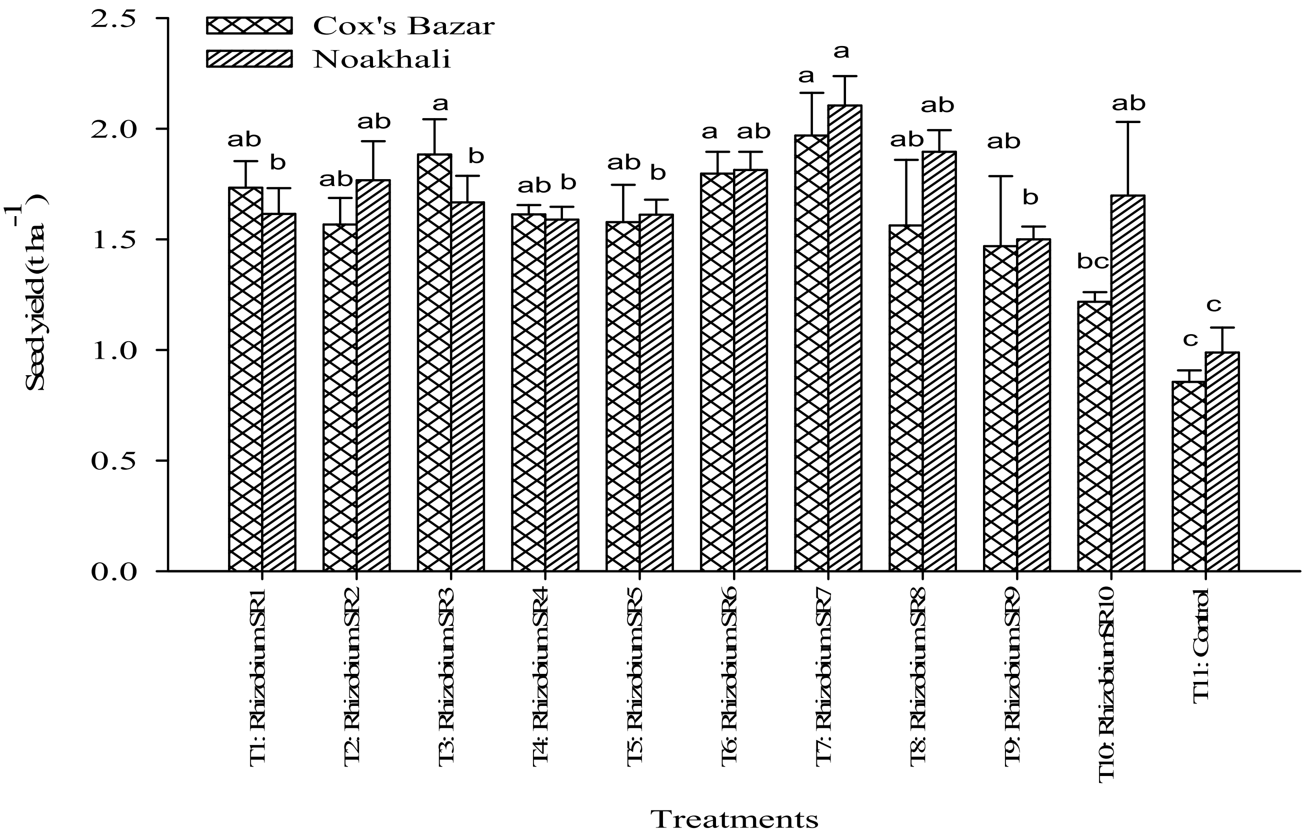


Fig.10. Seed yield of soybean inoculated by *Rhizobium* strains at Cox's Bazar and Noakhali

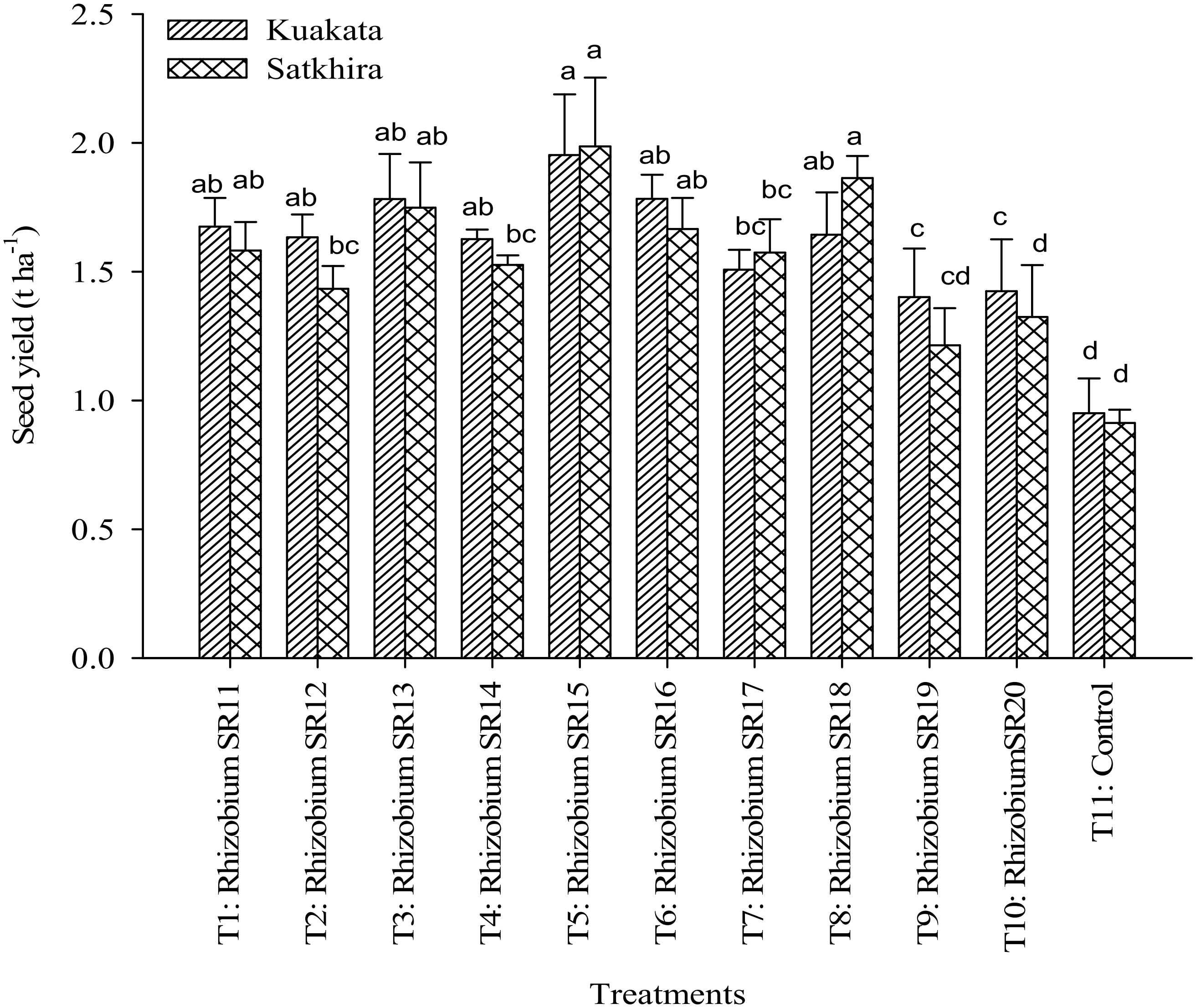


Fig.11. Seed yield of soybean inoculated by *Rhizobium* strains at Kuakata and Satkhira

Conclusion:

Application of *Rhizobium* biofertilizer on soybean, the plants exhibited better performance in nodule numbers, nodule weights, aboveground biomass, root biomass, and plant height than non-inoculated plants, indicating that all *Rhizobium* sp. effectively enhanced nodulation and growth parameters on soybean plant than non-inoculated soybean in field native saline soil. Soybean plants inoculated with *Rhizobium* sp. showed higher pod yield, stover yield, and seed yield than non-inoculated plants, revealing the positive impact of *Rhizobium* sp. on yield parameters of soybean. Soybean plants inoculated with *Rhizobium* sp.SR1 to *Rhizobium* sp.SR10 strains at

Cox's Bazar and Noakhali showed comparatively better performance with *Rhizobium* sp.SR7> *Rhizobium* sp.SR6>*Rhizobium* sp.SR3>*Rhizobium* sp.SR8>*Rhizobium* sp.SR1>*Rhizobium*sp.SR2>*Rhizobium* sp.SR4>*Rhizobium* sp.SR5>*Rhizobium* sp.SR9>*Rhizobium* sp.SR10 considering average seed yield of soybean in both location than non-inoculated plants. Likewise, Soybean plants inoculated with *Rhizobium* sp.SR11 to *Rhizobium* sp.SR20 strains at Kuakata and Satkhira showed comparatively better performance with *Rhizobium* sp.SR15> *Rhizobium* sp.SR18>*Rhizobium* sp.SR13>*Rhizobium* sp.SR16>*Rhizobium* sp.SR11>*Rhizobium* sp.SR12>*Rhizobium* sp.SR14>*Rhizobium* sp.SR17>*Rhizobium* sp.SR19>*Rhizobium* sp.SR20 considering average seed yield of soybean in both location than non-inoculated plants. Therefore, *Rhizobium* sp. established an effective symbiotic association with soybean and was responsible for increased growth and biomass, and improved yield characteristics of soybean at all location in field levels.

Photograph 6. Experiment field of Soybean

Expt. 04: Adaptive field trials of salt tolerant rhizobial strains on groundnut production

Objectives

i. To study on nodulation capacity of salt tolerant rhizobial strains on groundnut under field condition.
ii. To study on performance of salt tolerant rhizobial strains on growth and yield of groundnut under field condition.

Materials and methods

The field experiment was conducted during rabi season of 2018. First set of experiment were design at two location like Subornochor, Noakhali on 10 January 2018 andat Khuruskul, Cox's Bazaron 20 January 2018,respectively. The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. The treatments were eleven *Rhizobium* isolates which were replicated into three blocks. There were 11 treatments of first experiment viz. T_1: *Rhizobium* GR1, T_2: *Rhizobium* GR2, T_3: *Rhizobium* GR3, T_4: *Rhizobium* GR4, T_5: *Rhizobium* GR5, T_6: *Rhizobium* GR6, T_7: *Rhizobium* GR7, T_8: *Rhizobium* GR8, T_9: *Rhizobium* GR9, T_{10}: *Rhizobium* GR10 and T_{11}:control. The second set of experiment were design as same as two sites at Benerpota, Satkhira on 10 February 2018 and at Kuakata, Patuakhalion 20 February 2018, respectively. Similarly, the experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. The treatments were eleven *Rhizobium* isolates which were replicated into three blocks. There were 11 treatments viz. T_1: *Rhizobium* GR11, T_2: *Rhizobium* GR12, T_3: *Rhizobium* GR13, T_4: *Rhizobium* GR14, T_5: *Rhizobium* GR15, T_6: *Rhizobium* GR16, T_7: *Rhizobium* GR17, T_8: *Rhizobium* GR18, T_9: *Rhizobium* GR19, T_{10}: *Rhizobium* GR20 andT_{11}:control. The carrier-based salt tolerant *Rhizobium* inoculum was used in field experiments. The tested crop was groundnut (cv. BARI Groundnut-6). Peat based rhizobial inoculum containing 10^8 cells g^{-1} inoculumwas used at the rate of 1.5 kg ha^{-1}. Seeds were mixed thoroughly with inoculum (20:1 ratio) before sowing. Seeds were used at the rate of 75 kg ha^{-1}. Phosphorus, potassium, sulphur, zinc and boron @ $P_{42}K_{40}S_{40}Zn_5B_1$ kg ha^{-1} were used in the form of TSP, MoP, gypsum, zinc sulphate and boric acid, respectively. All P, K, S, Zn, B were applied at the time of land preparation. All the intercultural operations such as irrigation, weeding, insect control etc. were done as and when necessary. At the 50 percent flowering stage,nodules were collected by carefully uprooting five sample plants selected randomly from each unit plot. Nodules were separated from the roots, counted and then oven-dried and weighed. Data on yield and yield components were recorded at maturity. The groundnut was harvested on 12 May 2018 at Cox's Bazar, 18 May 2018 at Noakhali,10 May 2018 at Patuakhali and 5 May 2018at Satkhira.The initial soil samples at a depth of 0-15 cm from the experimental fields were collected and analyzed following standard methods (Table 24).

Table 24. Initial fertility status of the soil samples of the pot soil

Soil Properties	pH	OM %	Ca	Mg	K	Total N %	P	S	B	Cu	Fe	Zn
			meq 100g^{-1}				µg g^{-1}					
Kuakata soil	5.0	1.42	5.2	1.7	0.39	0.07	2.0	75	0.10	2.6	84	2.1
Satkhira soil	5.9	2.49	6.0	2.1	0.53	0.13	2.0	131	0.42	2.4	88	2.16
Critical level	-	-	2.0	0.5	0.12	-	7	10	0.20	0.20	4	0.6

Statistical analysis

All data were statistically analyzed using a RCBD design. Treatment effects on measured variables were tested by analysis of variance (ANOVA), and comparisons among treatment means were made using the least significant difference (LSD) multiple range test calculated at 5% level of probability ($P \leq 0.05$). Statistical procedures were carried out with the software program Statistix 10™.

Results and Discussion

At 50% flowering stage of soybean, the numbers of nodules were recorded higher in inoculated *Rhizobium* bacteria compared to non-inoculated control. The highest numbers of nodule (56.8 plant^{-1} at Cox' Bazar and 64.1 plant^{-1} at Noakhali) were recorded with *Rhizobium*GR9 treatment which was differed with all treatments except T_1, T_3, T_4, T_5, T_8at Cox's Bazarand T_3, T_5 at Noakhali, respectively(Table 25). The highest numbers of nodule (59.0 plant^{-1} at Kuakata and 61.6 plant^{-1} at Satkhira) were recorded with *Rhizobium* GR13 treatment which was differed with all treatments at Kuakata and differed with all treatments except T_9 at Satkhira, respectively(Table 26).However, the nodule weight was recorded the highest(0.57 g plant^{-1} at Cox' Bazar and 0.50 g plant^{-1} at Noakhali) with *Rhizobium*GR9 treatment which was identical with all treatments except T_1, T_{10}, T_{11} at Cox' Bazar and T_5, T_7, T_{11}at Noakhali, respectively.However, the nodule weight was recorded the highest(0.60 g plant^{-1} at Kuakata and 0.46 g plant^{-1} at Satkhira) with *Rhizobium* GR13 treatment which was differed with all treatments at Kuakata and differed with all other treatments except T_1, T_2, T_4, T_9 at Noakhali, respectively.On the other hand, the highest shoot weight (5.70 g plant^{-1} at Cox' Bazar and 3.97 g plant^{-1} at Noakhali) were recorded with *Rhizobium*GR3 treatment which was identical with all the treatments except T_5, T_7, T_{10}, T_{11} at Cox' Bazar and T_5, T_8, T_{10}, T_{11} at Noakhali, respectively.

Table 25. Nodulation and dry matter production of groundnut inoculated by *Rhizobium* strains

Treatment	Nodule number plant^{-1}		Nodule weight (g plant^{-1})		Shoot weight (g plant^{-1})		Root weight (g plant^{-1})	
Locations	Cox's Bazar	Noakhali	Cox's Bazar	Noakhali	Cox's Bazar	Noakhali	Cox's Bazar	Noakhali
T_1: *Rhizobium* GR1	48.5abc	46.8bcd	0.35b	0.37abc	4.5ab	3.40abc	0.38	0.31
T_2: *Rhizobium* GR2	40.7cde	44.0cde	0.39ab	0.36abc	4.9ab	3.65ab	0.37	0.33
T_3: *Rhizobium* GR3	54.3ab	55.6ab	0.51ab	0.44ab	5.7a	3.97a	0.46	0.37
T_4: *Rhizobium* GR4	49.6abc	51.3bc	0.50ab	0.44ab	5.0ab	4.15a	0.41	0.33
T_5: *Rhizobium* GR5	52.7ab	55.1ab	0.40ab	0.32bc	4.2bc	2.77cd	0.42	0.32
T_6: *Rhizobium* GR6	45.1bcd	41.7de	0.42ab	0.38abc	4.5abc	3.66ab	0.36	0.37
T_7: *Rhizobium* GR7	34.9de	37.3e	0.40ab	0.28c	4.3bc	3.71ab	0.41	0.32
T_8: *Rhizobium* GR8	47.8abc	49.5bcd	0.45ab	0.39abc	5.2ab	3.76cd	0.39	0.33
T_9: *Rhizobium* GR9	56.8a	64.1a	0.57a	0.50a	5.3ab	3.81ab	0.42	0.33
T_{10}: *Rhizobium* GR10	32.1e	35.4e	0.36b	0.36abc	4.2bc	3.16bcd	0.43	0.35
T_{11}: Control	0f	46.8f	0.00c	0.00d	3.4c	2.44d	0.32	0.23
SE (±)	5.18	4.44	0.09	0.07	0.59	0.36	0.05	0.04
Level sig.	***	***	**	**	*	**	NS	NS
CV (%)	15.1	12.5	21.9	20.1	15.6	12.7	16.4	15.4

Means followed by common letter are not significantly different at 5% level by DMRT

Table 26. Nodulation and dry matter production of groundnut inoculated by *Rhizobium* strains

Treatment	Nodule number plant^{-1}		Nodule weight (g plant^{-1})		Shoot weight (g plant^{-1})		Root weight (g plant^{-1})	
Locations	Kuakata	Satkhira	Kuakata	Satkhira	Kuakata	Satkhira	Kuakata	Satkhira
T_1: *Rhizobium* GR11	41.7d	50.2b	0.30b	0.33ab	4.13bcde	3.45bc	0.27cd	0.28
T_2: *Rhizobium* GR12	43.3cd	47.0b	0.36b	0.34ab	4.23bcde	3.69ab	0.26d	0.29
T_3: *Rhizobium* GR13	59.0a	61.6a	0.60a	0.46a	4.62abcd	2.97cde	0.28bcd	0.35
T_4: *Rhizobium* GR14	46.7bcd	45.6b	0.43b	0.35ab	4.69abc	4.02a	0.28bcd	0.35
T_5: *Rhizobium* GR15	49.7bc	46.4b	0.37b	0.24b	3.34e	2.77de	0.26cd	0.27
T_6: *Rhizobium* GR16	44.0bcd	41.7b	0.34b	0.28b	4.74abc	3.66ab	0.32bcd	0.35
T_7: *Rhizobium* GR17	45.7bcd	42.9b	0.35b	0.25b	4.32bcde	3.91ab	0.29bcd	0.29
T_8: *Rhizobium* GR18	46.0bcd	46.2b	0.40b	0.32ab	4.93ab	3.42bc	0.36ab	0.30
T_9: *Rhizobium* GR19	51.0b	51.4ab	0.44b	0.31ab	5.75a	3.81ab	0.41a	0.37
T_{10}: *Rhizobium* GR20	40.0d	42.1b	0.38b	0.28b	3.42de	3.10cd	0.34abc	0.35
T_{11}: Control	0.0e	0.0c	0.30c	0.33c	3.57cde	2.54e	0.23d	0.23
SE (±)	3.53	4.90	0.07	0.08	0.58	0.25	0.04	0.04
Level sig.	***	***	**	**	*	**	**	NS
CV (%)	10.2	13.9	20.63	20.1	16.4	8.93	15.3	15.8

Means followed by common letter are not significantly different at 5% level by DMRT

On the other hand, the highest shoot weight (5.75 g plant^{-1} at Kuakata and 4.02 g plant^{-1} at Satkhira) was recorded with *Rhizobium* GR19 and *Rhizobium* GR14 treatment which was identical with all the treatments except T_1, T_1, T_5, T_{10}, T_{11} at Kuakata and differed with all treatments except T_2, T_6, T_7, T_9 at Satkhira, respectively.Moreover, the highest root weight (0.41 g plant^{-1} at Kuakata) was recorded with *Rhizobium* GR19 treatment which was differed with all the treatments except T_8, T_{10}.Furthermore, the root weight was non-significant in all treatments at Cox' Bazar, Noakhaliand Satkhira.

Table 27. Plant height and pod formation of groundnut inoculated by *Rhizobium* strains

Treatment	Plant height (cm)		Nut plant^{-1}		Karnel nut^{-1}	
Locations	Cox's Bazar	Noakhali	Cox's Bazar	Noakhali	Cox's Bazar	Noakhali
T_1: *Rhizobium* GR1	40.8ab	36.1ab	21.6abc	18.2b	1.55a	1.52
T_2: *Rhizobium* GR2	36.6b	36.1ab	21.4c	22.6ab	1.80a	1.70
T_3: *Rhizobium* GR3	44.1a	41.3a	25.8a	23.6a	1.83a	1.72
T_4: *Rhizobium* GR4	42.1ab	39.8ab	20.5abc	19.2ab	1.03b	1.65
T_5: *Rhizobium* GR5	40.2ab	38.6ab	22.7abc	20.7ab	1.82a	1.70
T_6: *Rhizobium* GR6	42.1ab	38.2ab	22.6abc	20.7ab	1.90a	1.55
T_7: *Rhizobium* GR7	40.6ab	37.5ab	21.3bc	21.3ab	1.55a	1.33
T_8: *Rhizobium* GR8	42.6a	38.3ab	25.7ab	23.1ab	1.72a	1.53
T_9: *Rhizobium* GR9	41.2ab	38.2ab	24.2abc	19.3ab	1.70a	1.75
T_{10}: *Rhizobium* GR10	38.6ab	34.4b	20.9c	21.0ab	1.58a	1.59
T_{11}: Control	26.4c	24.4c	14.3d	12.8c	1.11b	0.954
SE (±)	2.78	2.81	2.13	2.36	0.19	0.24
Level sig.	***	**	*	*	**	NS
CV (%)	8.62	9.40	11.9	14.3	14.6	19.1

Means followed by common letter are not significantly different at 5% level by DMRT

Table 28. Plant height and pod formation of groundnut inoculated by *Rhizobium* strains

Treatment	Plant height (cm)		Nut plant^{-1}		Karnel nut^{-1}	
Locations	Kuakata	Satkhira	Kuakata	Satkhira	Kuakata	Satkhira
T_1: *Rhizobium* GR11	31.6c	32.9abc	16.0bcd	14.0	0.55d	1.76abc
T_2: *Rhizobium* GR12	35.7bc	32.8abc	18.7abc	13.2	1.80ab	1.80ab
T_3: *Rhizobium* GR13	33.0bc	31.1abc	17.8 abc	13.3	1.30c	1.50bc
T_4: *Rhizobium* GR14	34.2bc	32.6abc	17.5 abc	14.3	1.55abc	1.95a
T_5: *Rhizobium* GR15	32.4c	29.8bc	17.0 abc	12.0	1.45bc	1.70abc
T_6: *Rhizobium* GR16	30.7c	29.9bc	15.3bcd	10.8	1.90a	1.55bc
T_7: *Rhizobium* GR17	31.8c	31.1abc	15.5bcd	11.2	1.55abc	1.40c
T_8: *Rhizobium* GR18	36.9abc	34.3ab	19.7ab	16.0	1.85ab	2.00a
T_9: *Rhizobium* GR19	43.2a	36.0a	20.5a	15.8	1.90a	1.75abc
T_{10}: *Rhizobium* GR20	39.0ab	28.0cd	14.7cd	13.0	1.58abc	1.85ab
T_{11}: Control	24.2d	22.3d	12.5d	8.50	0.73d	0.67d
SE (±)	3.04	2.83	2.15	2.47	0.20	0.18
Level sig.	***	*	*	NS	***	***
CV (%)	11.0	11.2	15.7	19.3	16.6	1.63

Means followed by common letter are not significantly different at 5% level by DMRT

The plant height was observed higher in inoculated *Rhizobium* bacteria compared to non-inoculated control. Inoculated plant recorded the highest plant height (42.6cm) with *Rhizobium*GR8at Cox's Bazar which was identical with all treatments except T_2, T_{11}. Similarly, the plant height was recorded the highest (41.3 cm) at Noakhali with *Rhizobium*GR8 treatment which was identical with all the treatments except T_{10}, T_{11} (Table 27). The highest plant height (43.2 cm at Kuakata and 36.0 cm at Satkhira) was recorded with *Rhizobium* GR19 treatment which was differed with all treatments except T_{18}, T_{20} at Kuakata and identical with all the treatments except T_5, T_6, T_{10} at Satkhira, respectively (Table 28).The nut per plant was recorded highest (25.8 nut plant^{-1} at Cox's Bazar and23.6 nut plant^{-1} at Noakhali) with *Rhizobium*GR3 treatment which was identical with all treatments except T_2, T_7, T_9, T_{10} atCox's Bazar and T_{11} at Noakhali, respectively.The nut per plant was recorded the highest (20.5 nut plant^{-1} at Kuakata) was recorded with *Rhizobium* GR19 treatment which was differed with all treatments except T_2, T_3, T_4, T_5, T_8 at Kuakata but it was recorded non-significant with all the treatments at Satkhira.The kernel per plant was recorded highest (1.90kernel nut plant^{-1}) with *Rhizobium*GR6 treatment which was identical with all treatments except T_{11}atCox's Bazar but same treatment recorded non-significant in all treatments at Cox' Bazarat Noakhali. The kernel per nut was recorded the highest (1.9 kernel nut^{-1} at Kuakata and 2.0 kernel nut^{-1} at Satkhira) with *Rhizobium* GR19 and *Rhizobium* GR19 treatment which was identical with all treatments except T_1, T_3, T_5, T_{11} at Kuakata and except T_2, T_7, T_{11} at Satkhira, respectively.

Rhizobium GR3 treated plantobserved the highest hundred nut weight (56.0 g at Cox' Bazar and 50.6 g at Noakhali) which was identical with all treatments except T_7, T_{10}, T_{11} at Cox' Bazarandexcept T_1, T_{11} at Noakhali, respectively (Table 29). However, hundred nut weight was recorded the highest (55.9 g at Kuakata and 52.0 g at Satkhira) which was identical with all treatments except T_7, T_{11} at Kuakata and except T_{10}, T_{11} at Satkhira, respectively.

Table 29. Yield and yield attributes of groundnut inoculated by *Rhizobium* strains

Treatment	100 nut weight (g)		Karnel weight of 100 nut (g)		Stover yield (t ha^{-1})	
Locations	Cox's Bazar	Noakhali	Cox's Bazar	Noakhali	Cox's Bazar	Noakhali
T_1: *Rhizobium* GR1	51.8ab	42.5b	35.7bcd	28.0bcd	3.87	4.53ab
T_2: *Rhizobium* GR2	52.2ab	45.3ab	42.1a	34.6a	3.40	3.10ef
T_3: *Rhizobium* GR3	56.0a	50.6a	43.2a	38.3a	3.70	4.13abcd
T_4: *Rhizobium* GR4	51.4ab	47.5ab	42.5a	36.9a	3.67	3.57cdef
T_5: *Rhizobium* GR5	54.9a	50.5a	41.6ab	34.2a	3.60	3.73bcde
T_6: *Rhizobium* GR6	50.1abc	47.1ab	38.3bc	32.6abc	3.57	3.33def
T_7: *Rhizobium* GR7	43.6cd	47.3ab	38.2bc	33.4ab	3.70	3.97abcde
T_8: *Rhizobium* GR8	48.9abcd	49.0a	40.2abc	36.7a	3.67	3.73bcde
T_9: *Rhizobium* GR9	54.2a	48.3ab	40.0abc	27.0cd	4.23	4.63a
T_{10}: *Rhizobium* GR10	46.3bcd	45.1ab	34.9cd	33.9ab	3.87	4.33abc
T_{11}: Control	41.5d	33.5c	30.4d	21.7d	2.71	2.80f
SE (±)	3.76	2.96	2.96	2.97	0.51	0.42
Level sig.	*	***	**	***	NS	**
CV (%)	9.20	7.87	9.35	11.2	17.2	13.7

Means followed by common letter are not significantly different at 5% level by DMRT

Table 30. Yield and yield attributes of groundnut inoculated by *Rhizobium* strains

Treatment	100 nut weight (g)		Karnel weight of 100 nut (g)		Stover yield (t ha^{-1})	
Locations	Kuakata	Satkhira	Kuakata	Satkhira	Kuakata	Satkhira
T_1: *Rhizobium* GR11	49.8ab	47.8ab	36.8bc	32.7c	3.42	3.72ab
T_2: *Rhizobium* GR12	53.2a	43.3b	40.4ab	32.9c	3.40	3.40b
T_3: *Rhizobium* GR13	50.6a	47.3ab	36.7bc	30.5c	4.00	3.80a
T_4: *Rhizobium* GR14	44.0abc	48.5ab	39.5ab	34.6c	3.56	3.63ab
T_5: *Rhizobium* GR15	50.4a	51.5a	37.9bc	31.7c	3.47	3.56ab
T_6: *Rhizobium* GR16	49.3ab	47.8ab	40.0ab	35.9c	3.56	3.56ab
T_7: *Rhizobium* GR17	40.5bc	48.3ab	36.5bc	36.3bc	3.50	3.63ab
T_8: *Rhizobium* GR18	48.9ab	48.0ab	40.7ab	42.0ab	3.46	3.60ab
T_9: *Rhizobium* GR19	55.9a	52.0a	44.0a	43.0a	3.84	3.79a
T_{10}: *Rhizobium* GR20	48.6ab	44.7b	33.0c	36.4bc	3.42	3.72ab
T_{11}: Control	36.5c	31.3c	21.4d	19.4d	2.72	2.71c
SE (±)	4.45	2.51	2.81	2.93	0.37	0.15
Level sig.	***	***	***	***	NS	**
CV (%)	11.8	6.65	9.33	10.5	13.2	5.34

Means followed by common letter are not significantly different at 5% level by DMRT

Similarly, kernel weight of hundred nut was recorded the highest (43.2 g at Cox's Bazar and 38.3 g at Noakhali) with Rhizobium GR3 treatment which was differed with all treatments except T1, T6, T7, T10, T11 at Cox's Bazarand except T1, T9, T11 at Noakhali, respectively (Table 29).Similarly, the kernel weight of hundred nut was recorded the highest (44 g at Kuakata and 43 g at Satkhira) which was differed with all treatments except T_4, T_6, T_8 at Kuakata and except T_{18} at Satkhira, respectively (Table 30).The stover yield was recorded non-significant in all treatments at Cox's Bazar but at Noakhali, it was recorded the highest (4.63 t ha^{-1}) with *Rhizobium* GR9 treatment which was differed with all treatments except T_1, T_3, T_7, T_{10} (Table

29).The highest stover yield (3.79 t ha^{-1} at Satkhira) was recorded with *Rhizobium* GR19 treatment which was identical with all treatments except T_2, T_{11} at Satkhira but it was non-significant with all treatment at Kuakata(Table 30).

The seed yield was recorded the highest (2.43 t ha^{-1} at Cox's Bazar and 2.60 t ha^{-1} at Noakhali) with *Rhizobium* GR9 treatment which was identical with all treatments except T_1, T_{10}, T_{11} at Cox's Bazarand T_2, T_3, T_5, T_{10}, T_{11} at Noakhali, respectively (Fig 13). The nut yield was recorded the highest (2.57 t ha^{-1} at Kuakata and 2.53 t ha^{-1} at Satkhira) was recorded with *Rhizobium* GR13 treatment which was differed with all treatments except T_2, T_6, T_7, T_9 at Kuakata andT_2, T_4, T_6 T_7, T_8, T_9 at Satkhira, respectively (Fig. 14).

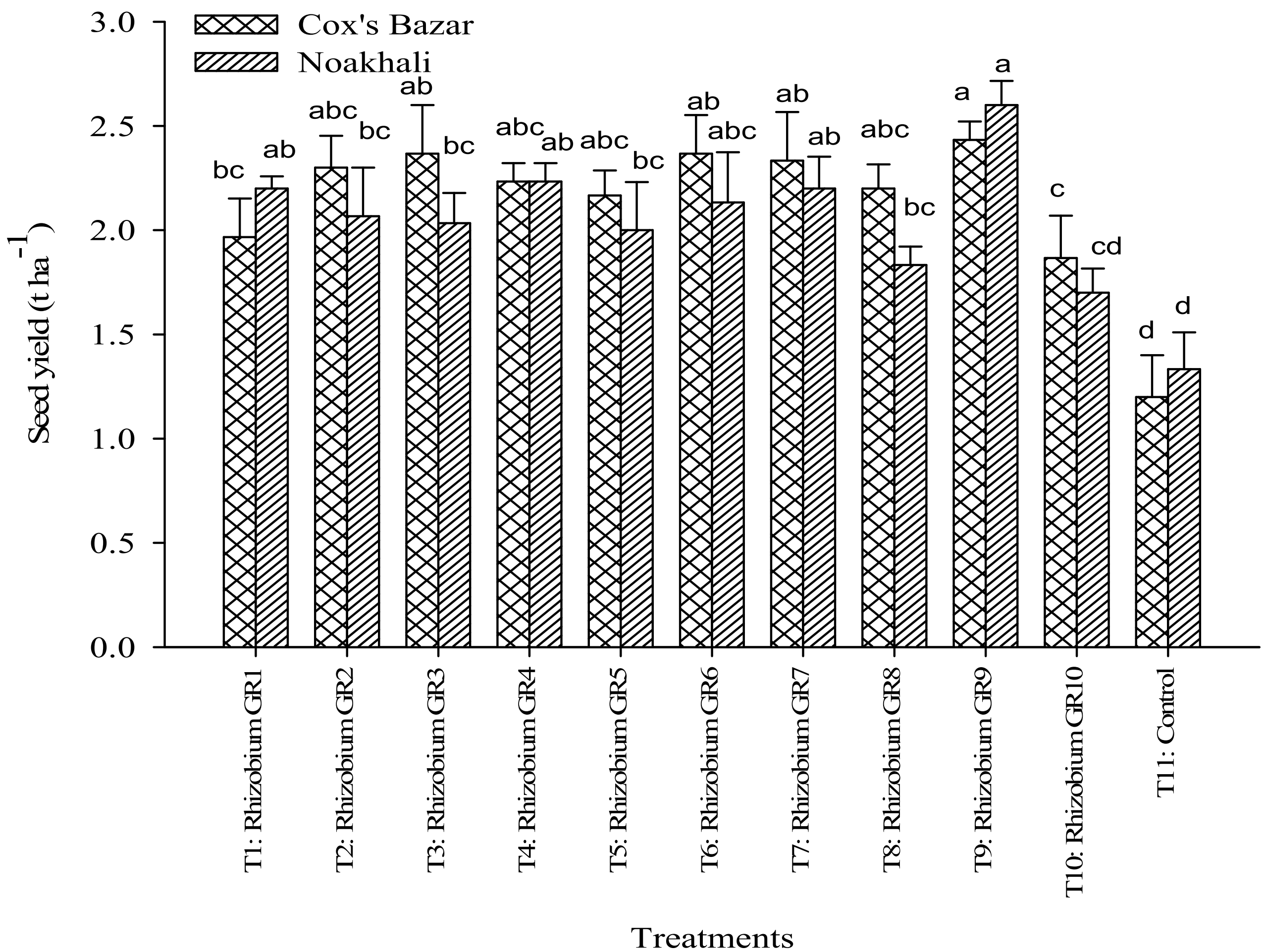


Fig.13. Nut yield of groundnut inoculated by *Rhizobium* strains at Cox's Bazar and Noakhali

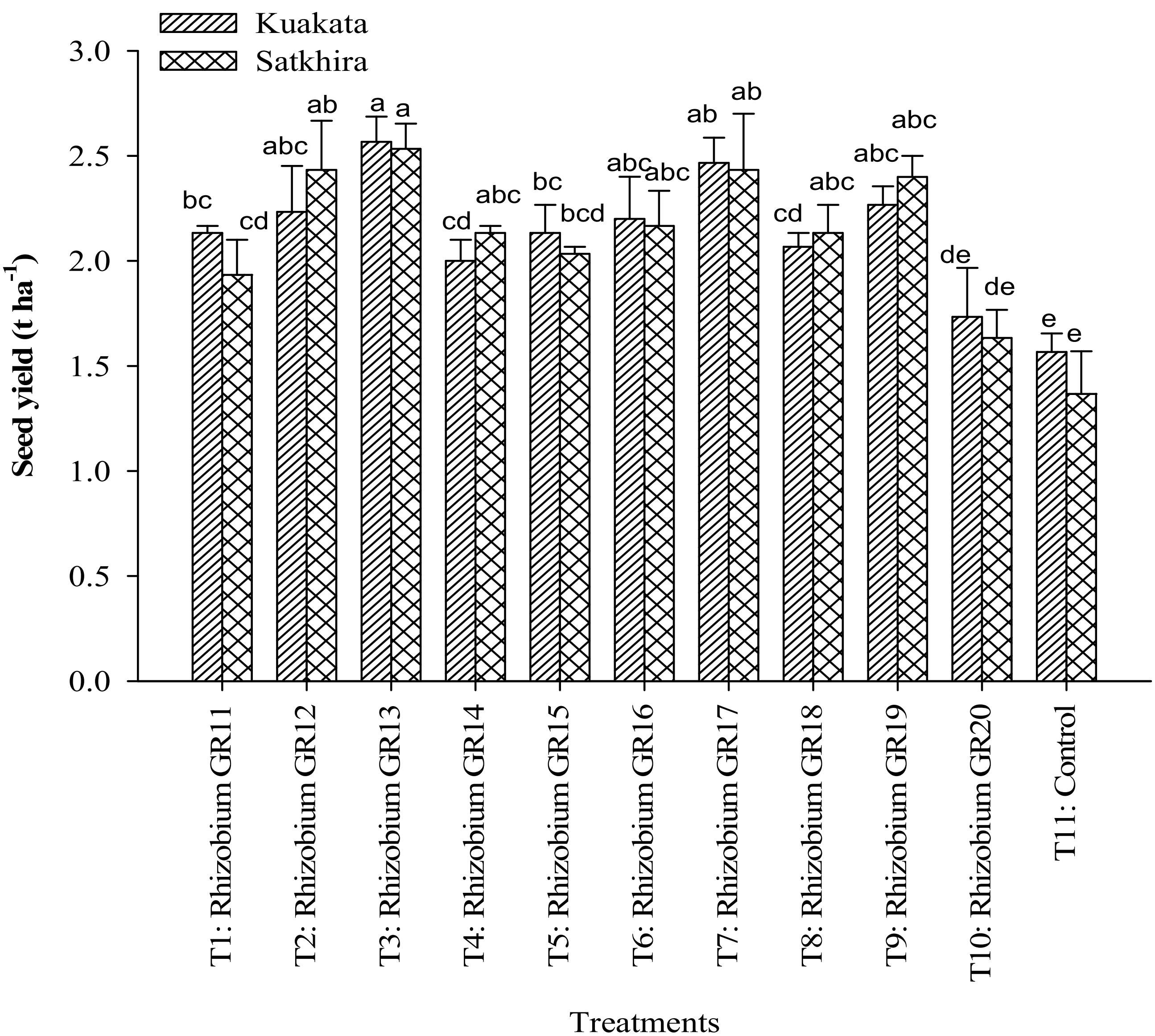


Fig.14. Nut yield of groundnut inoculated by *Rhizobium* strains at Kuakata and Satkhira

Conclusion:

Inoculation of *Rhizobium* biofertilizer on groundnut, the plants exhibited better performance in nodule numbers, nodule weights, aboveground biomass, root biomass, and plant height than non-inoculated plants, indicating that all *Rhizobium* sp. effectively enhanced nodulation and growth parameters on groundnut plant than non-inoculated groundnut in field native saline soil. Groundnut plants inoculated with *Rhizobium* sp. showed higher kernel yield, stover yield, and nut yield than non-inoculated plants, revealing the positive impact of *Rhizobium* sp. on yield parameters of groundnut. Groundnut plants inoculated with *Rhizobium* sp.GR1 to *Rhizobium* sp.GR10 strains at Cox's Bazar and Noakhali showed comparatively better performance with *Rhizobium* sp.GR9> *Rhizobium* sp.GR7>*Rhizobium* sp.GR3>*Rhizobium* sp.GR4>*Rhizobium* sp.GR3>*Rhizobium* sp.GR2>*Rhizobium* sp.GR5>*Rhizobium* sp.GR1>*Rhizobium* sp.GR8>*Rhizobium* sp.GR10 considering average nut yield of groundnut in both location than non-inoculated plants. Likewise, Groundnut plants inoculated with *Rhizobium* sp.GR11 to *Rhizobium* sp.GR20 strains at Kuakata and Satkhira showed comparatively better performance with *Rhizobium* sp.GR13> *Rhizobium* sp.GR17>*Rhizobium* sp.GR19>*Rhizobium* sp.GR12>*Rhizobium* sp.GR16>*Rhizobium* sp.GR18>*Rhizobium* sp.GR15>*Rhizobium* sp.GR14>*Rhizobium* sp.GR11>*Rhizobium* sp.GR20 considering average nut yield of groundnut in both location than non-inoculated plants. Therefore, *Rhizobium* sp. established an effective symbiotic association with groundnut and was responsible for increased growth and biomass, and improved yield characteristics of groundnut at all location in field levels.

Expt. 05: DNA isolation, PCR amplification, gene sequence of biofertilizer strains

Objective:

- **i.** To molecular characterize of saline stress tolerant effective rhizobial strains and gene sequence to know their taxonomy (family, genus and species).

Materials and methods:

DNA Isolation from Rhizobium bacteria

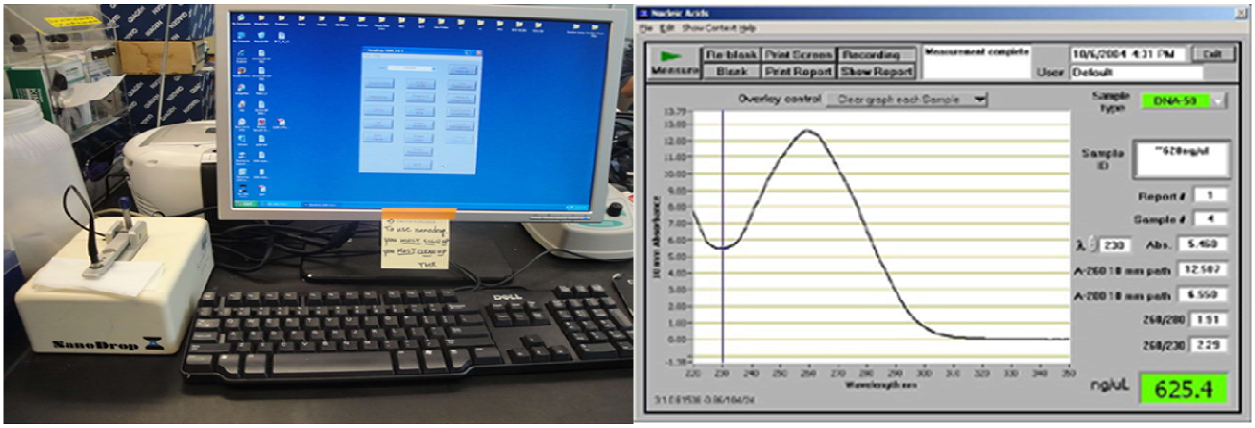
Pellet cell
Bacterial 1ml of overnight culture was centrifuged for 2 minutes at 13,000–16,000 × g. The supernatant was discarded for taking pellet cells.

Lyse Cells
1. Nuclei Lysis Solution was 600µl added and was pipeted gently to mix.
2. The sample was incubated for 5 minutes at 80°C, then cooled to room temperature.
3. RNase Solution of 3µl was added, mixed and incubated at 37°C for 15–60 minutes, then cooled to room temperature.

Protein Precipitation
4. Protein Precipitation Solution of 200µl was added and vortexed.
5. The sample was incubated on ice for 5 minutes.
6. It was centrifuged at 13,000–16,000 × g for 3 minutes.

DNA Precipitation and Rehydration
7. The supernatant was transferred to a clean tube containing 600µl of room temperature isopropanol and mixed.
8. The sample was centrifuged as in "Pellet Cells" above, and the supernatant was decanted.
9. Ethanol (70%) of 600µl was added at room temperature and mixed.
10. The sample was centrifuged for 2 minutes at 13,000–16,000 × g.
11. The Ethanol was aspirated and the pellet was air-dried for 10–15 minutes.
12. The DNA pellet was rehydrated by 100µl of Rehydration Solution for 1 hour at 65°C or overnight at 4°C.

DNA concentration measurements
In molecular biology, quantitation of nucleic acids was commonly performed to determine the average concentrations of DNA or RNA present in a mixture, as well as their purity. Reactions that use nucleic acids was required particular amounts and purity for optimum performance. To date, there were two main approaches used by scientists to quantitate, or establish the concentration, of nucleic acids (such as DNA or RNA) in a solution. These are spectrophotometric quantification and UV fluorescence tagging in presence of a DNA dye.



Photograph 7: DNA concentration measured by Nanodrop spectrophotometer

Polymerase chain reaction (PCR)

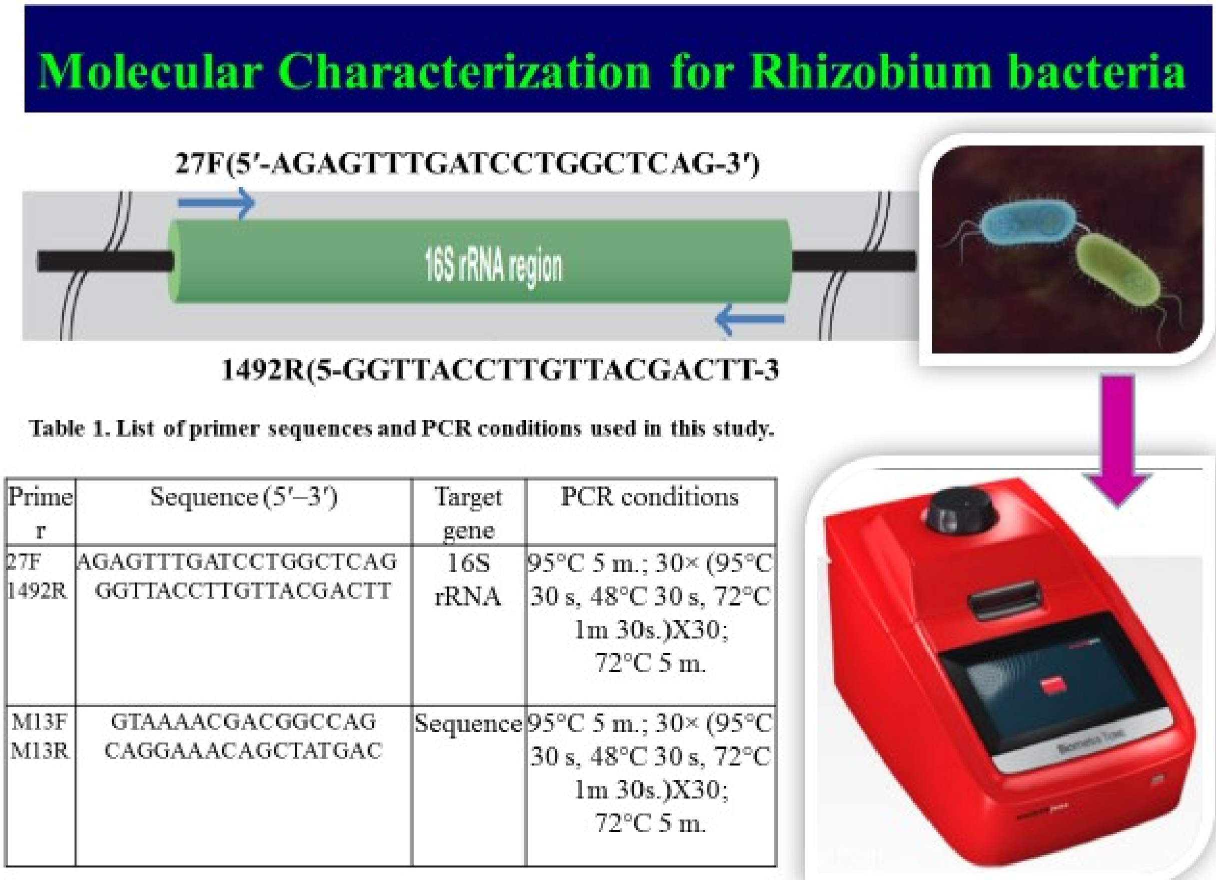
The DNA samples was amplified by polymerase chain reaction (PCR) widely used in molecular biology to make many copies of a specific DNA segment. Using PCR, a single copy (or more) of a DNA sequence was exponentially amplified to generate thousands to millions of more copies of that particular DNA segment. PCR reaction was made by following mixture

PCR mixture

PCR mixture= 25uL (TOTAL including DNA)	
1. Master mix	12.5 uL
2. Forward primer	1uL
3.Reverse primer	1uL
4.Template DNA	1uL
5. Water	9.5 uL
Total	25 ul

PCR amplification of 16S rDNA

Amplification of 16S rDNA fragments of extracted bacterial genomic DNA were conducted by PCR amplification. Bacterial 16S rDNA is amplified by PCR using the forward primer fD1 (5´-AGAGTTTGATCCTGGCTCAG-3´) and reverse primer rD1 (5´-AAGGAGGTGATCCAGC-3´). PCR amplifications were performed with approximately 50 ng of template DNA. The PCR conditions and primer sequences used for sequencing were shown in Figure16. The PCR products were gel purified with the QIAEX II Gel Extraction Kit (Qiagen GmbH, Hilden, Germany).



Primer	Sequence (5′–3′)	Target gene	PCR conditions
27F 1492R	AGAGTTTGATCCTGGCTCAG GGTTACCTTGTTACGACTT	16S rRNA	95°C 5 m.; 30× (95°C 30 s, 48°C 30 s, 72°C 1m 30s.)X30; 72°C 5 m.
M13F M13R	GTAAAACGACGGCCAG CAGGAAACAGCTATGAC	Sequence	95°C 5 m.; 30× (95°C 30 s, 48°C 30 s, 72°C 1m 30s.)X30; 72°C 5 m.

Photograph 8: Primer sequences and PCR conditions used in this study

Sequencing and phylogenetic analysis

DNA sequencing were performed using an Applied Biosystems 3730 automated sequencer and the M13 primer to obtain nearly full-length bacterial 16S rDNA sequences (approximately 1,500 bp). The bidirectional gene sequences were compiled using DNAMAN software (DNAMAN version 4.11, LynnonBiosoft, San, Ramon, CA, USA) and the sequences were analyzed with MEGA 5.2 software. The sequences were searched in the NCBI GenBank database using BLAST analysis. Phylogenetic analysis was conducted using MEGA version 5.2 and a neighbor-joining tree is constructed using Kimura 2-parameter distances with 1,000 replicates to estimate bootstrap support.

Results and Discussion:

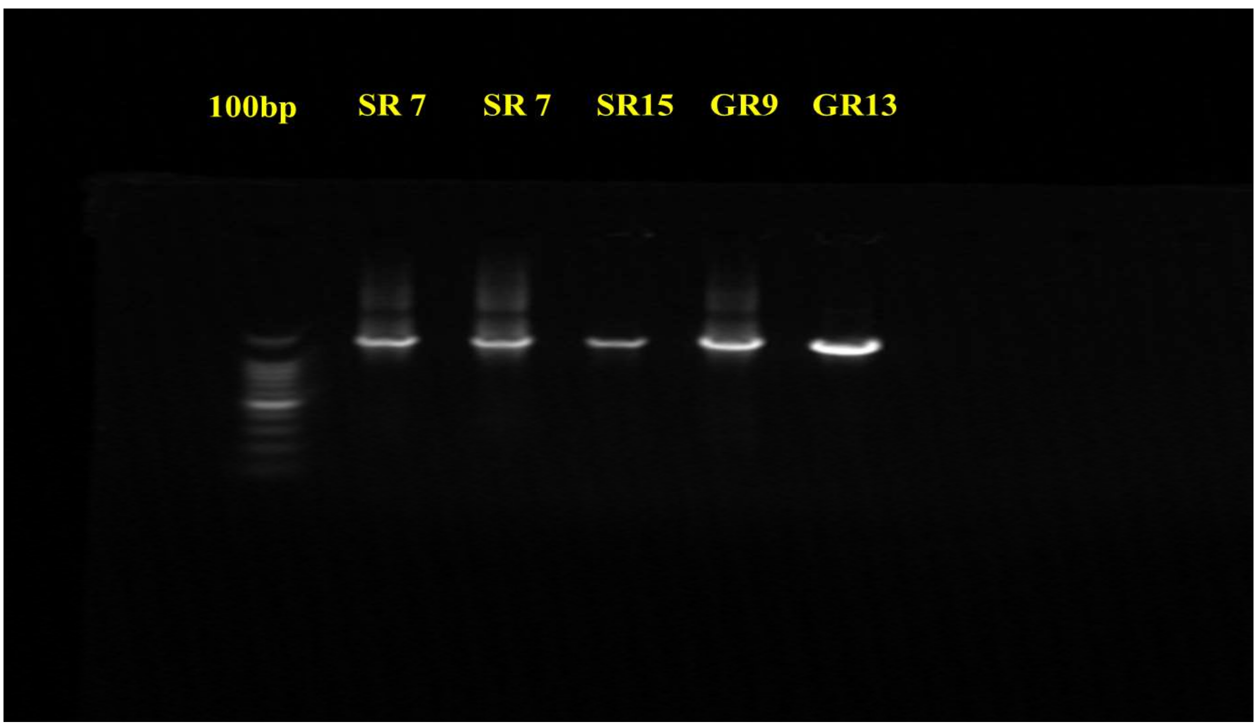
Cultivation of bacteria and DNA extraction

Selected effective *Rhizobium* sp. SR7, *Rhizobium* sp. SR15, *Rhizobium* sp. GR9 and *Rhizobium* sp. GR13 bacteria were cultured in test tubes containing 3 ml in YEM liquid broth by shaking in a rotary shaker at 180 rpm, 30°C, for 48 hours, and the cultures were centrifuged at 18,000 g for 5 minutes at 4°C. Genomic DNA was extracted from the pellet using DNA isolation kit

(Promega, USA) and DNA yield was quantified using a spectrophotometer. The extracted bacterial DNA were used as a template for PCR amplification.

Olegonucleotide primers:
One pair of primers, which were specific for housekeeping gene (16S rRNA) of every morphologically and biochemically identified bacteria, were used to amply a fragment of 16S rRNA gene by PCR. The specific primers were designed from 16S rRNA gene sequences.

PCR amplification:
About 50 ng of template DNA was used to amply fragments of the 16S rRNA gene by PCR. PCR was carried out in a final volume of 25 µL containing 1 µL template DNA, 12.5 µL master mix (including polymerase, buffer, dNPT, Mg^{2+}, promega company), 1 µl forward primer, 1 µl reverse primer, 9.5 µL sterile water. PCR amplification was performed staring with 5 min denaturing step at 95°C, followed by 30 cycle of denaturation at 95°C for 30 sec, annealing at 48°C for 30 sec, extension at 72°C for 1.5 min, and final extension 72°C for 5 min. The PCR products were assessed by electrophoresis on 1% agarose gel. DNA bands were visualized by UV illumination and photographed with gel documentation system. PCR products were purified using gel purification Kit and were kept for sequence.



Photograph 9: PCR amplification of Rhizobial 16S rDNA for rhizobial strains

Sequencing and phylogenetic analysis

Selected effective *Rhizobium* sp. SR7, *Rhizobium* sp. SR15, *Rhizobium* sp. GR9 and *Rhizobium* sp. GR13 bacterial DNA sequencing was performed using an Applied Biosystems 3730 automated sequencer with the M13 primer to obtain nearly full-length bacterial 16S rDNA sequences. The bidirectional gene sequences were compiled using DNAMAN software

(DNAMAN version 4.11, LynnonBiosoft, San, Ramon, CA, USA), and the sequences were analyzed using MEGA 5.2 software. The consensus sequences were used in a BLAST search of the NCBI Gene Bank database. Phylogenetic analysis was conducted using MEGA version 5.2, and a neighbor-joining tree were constructed using Kimura 2-parameter distances with 1000 replicates to estimate bootstrap support. The compiled sequence of the *Rhizobium* strains was deposited in the Gene Bank database and assigned accession number.*Rhizobium* sp. SR7, *Rhizobium* sp. SR15, *Rhizobium* sp. GR9 and *Rhizobium* sp. GR13 bacterial sequence were belonging to bacteria kingdom, proteobacteria phylum, alpha proteobacteria class, rhizobiales order, rhizobiaceae family, and genus *Rhizobium*(Fig. 18).

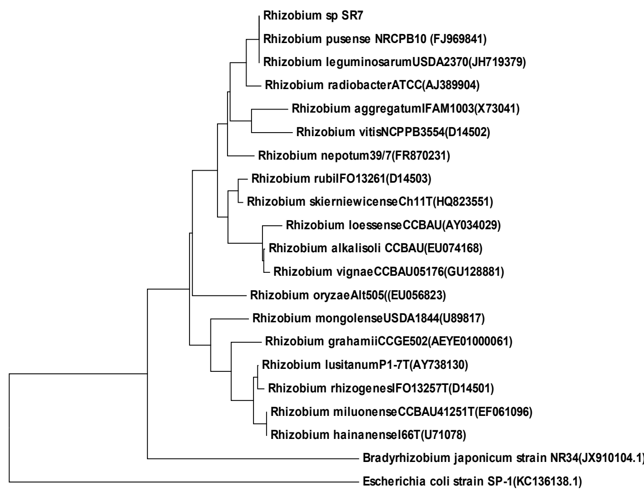


Fig.a Phylogenetic tree of *Rhizobium* sp. SR7

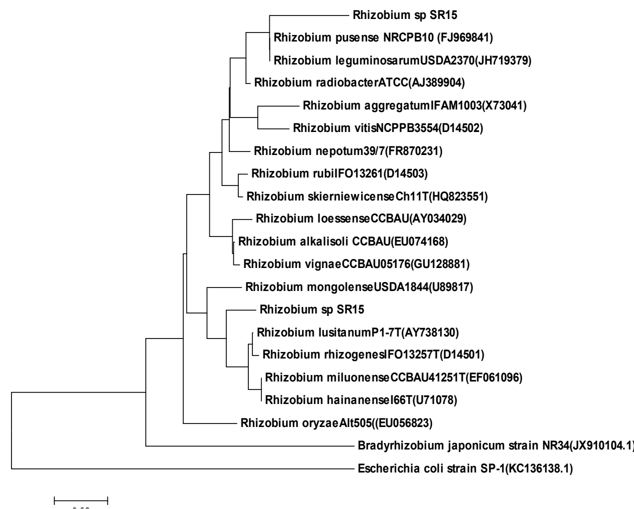


Fig.b Phylogenetic tree of *Rhizobium* sp. SR15

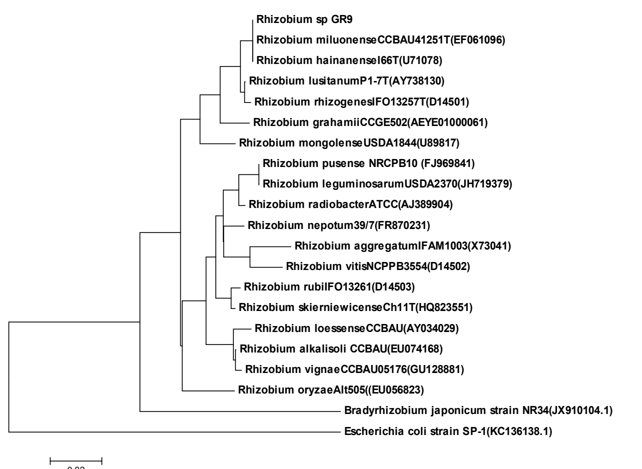


Fig.c Phylogenetic tree of *Rhizobium* sp. GR9

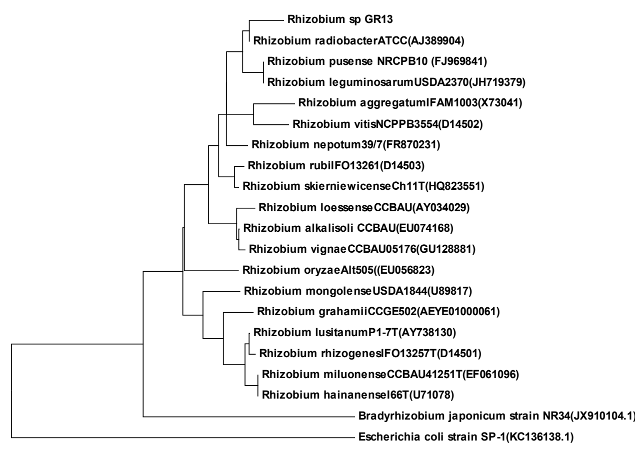


Fig.d. Phylogenetic tree of *Rhizobium* sp. GR13

Photograph 10. Phylogenetic analysis of salt tolerance *Rhizobium* bacteria (a,b,c,d)

Conclusion:

Rhizobium sp. SR7, *Rhizobium* sp. SR15, *Rhizobium* sp. GR9 and *Rhizobium* sp. GR13 bacterial sequence were found, and phylogenetic analysis were revealed that all *Rhizobium* strains were belonging to bacteria kingdom, proteobacteria phylum, alpha proteobacteria class, rhizobiales order, rhizobiaceae family, and genus *Rhizobium*.

Bibliography

Ahmed, B., Rasel, H.M., Hasan, M.R., and Miah, M.S.U. 2013. Effects of Soil Salinity on Crop Production rate of the South West Zone of Bangladesh. In International Conference on Mechanical, Industrial and Materials Engineering (ICMIME 2013). pp. 1-3.

Alam, F., Bhuiyan, M.A.H., Alam, S.S., Waghmode, T.R., Kim, P.J., and Lee, Y.B. 2015. Effect of *Rhizobium* sp. BARIRGm901 inoculation on nodulation, nitrogen fixation and yield of soybean (*Glycine max*) genotypes in gray terrace soil. Biosc. Biotechnol. Biochem. 79(10): 1660–1668.

Aneja K.R. 2003. Experiments in microbiology plant pathology and biotechnology, 4th edition,NewAge International Publishers, New Delhi, India.

Jackson, M.L. 1958. Soil Chemical Analysis, Constable and Co. Ltd., London.

JonesJBJ. Plant nutrition manual. 1st ed. Boca Raton (FL):CRC Press; 1998. p. 149.

Vincent JM (1970) A Manual for the Practical Study of Root-Nodule Bacteria, Blackwell, Oxford.

Wang E.T., Rogel M.A.and García-de los Santos A. 1999. Rhizobium etlibv. mimosae, a novel biovar isolated from Mimosa affinis. Int J Syst EvolMicr 49: 1479 – 1491.

12. Research highlight/findings (Bullet point – max 10 nos.):

- Rhizobia nodule samples (273) were collected from soybean and groundnut root nodules from saline stress areas of Bangladesh.
- Indigenous 273 bacterial species were isolated from different location. Forty salt tolerant *Rhizobium* species have been isolated, characterized for biofertilizer production and further used in pot and field trials.
- Salt-tolerance assay of selected *Rhizobium* bacteria was measured and all species were tolerated saline up to 48dS/m.
- Taxonomy and evolutionary relationship of potential strains have been characterized.
- Peat based low price biofertilizer technology packages have been developed.
- Biofertilizers have been prepared by potential salt tolerance *Rhizobium* species for soybean and groundnut.
- *Rhizobium* sp. inoculated plant showed the best performance by *Rhizobium* sp.SR15 in soybean and *Rhizobium* sp.GR13 in groundnut under pot trails
- Soybean plants inoculated with *Rhizobium* sp.SR1 to *Rhizobium* sp.SR10 strains tested in field of Cox's Bazar and Noakhali showed comparatively better performance with *Rhizobium* sp.SR7.Likewise, Soybean plants inoculated with *Rhizobium* sp.SR11 to *Rhizobium* sp.SR20 strains tested in the field of Kuakata and Satkhira showed comparatively better performance with *Rhizobium* sp.SR15,
- In the same way, groundnut plants inoculated with *Rhizobium* sp.GR1 to *Rhizobium* sp.GR10 strains tested in the field of Cox's Bazar and Noakhali showed comparatively better performance with *Rhizobium* sp.GR9. Likewise, Groundnut plants inoculated with *Rhizobium* sp.GR11 to *Rhizobium* sp.GR20 strains tested in the field of Kuakata and Satkhira showed comparatively better performance with *Rhizobium* sp.GR13.
- *Rhizobium* strains were belonging to bacteria kingdom, proteobacteria phylum, alpha proteobacteria class, rhizobiales order, rhizobiaceae family, and genus *Rhizobium*.

B. Implementation Position

1. Procurement:

Description of equipment and capital items	PP Target			Achievement			Remarks
	Phy (#)		Fin (Tk)	Phy (#)		Fin (Tk)	
	Unit	Quantity		Unit	Quantity		
Office equipments			**185000.00**			**184800.00**	100% of achievements
a) Desktop Computer	No.	01	60000.00	No.	01	60000.00	
b) Laser Printer	No.	01	20000.00	No.	01	20000.00	
c) UPS	No.	01	10000.00	No.	01	9800.00	
d) Laptop	No.	01	60000.00	No.	01	60000.00	
e) Scanner	No.	01	10000.00	No.	01	10000.00	
f) Digital Camera	No.	01	25000.00	No.	01	25000.00	
Office furniture			**48500.00**			**48400.00**	100% of achievements
a) Steel Almira	No.	01	24000.00	No.	01	23950.00	
b) Visitor/front chair	No.	04	16000.00	No.	04	16000.00	
c) Computer Table	No.	01	5000.00	No.	01	5000.00	
d) Computer Chair	No.	01	3500.00	No.	01	3450.00	
Lab/Field Equipments (Package 1)			**3,30,000.00**			**3,30,000.00**	100% of achievements
a). Soil Moisture Meter	No.	01	1,00,000.00	No.	01	1,00,000.00	
b). Hand Soil Penetrometer	No.	01	1,00,000.00	No.	01	1,00,000.00	
c). Pycnometer	No.	01	40,000.00	No.	01	40,000.00	
d). Sand Bath	No.	01	50,000.00	No.	01	50,000.00	
e). Dispersion cup	No	01	20,000.00	No	01	20,000.00	
f). Mechanical Stirrer	No.	01	20,000.00	No.	01	20,000.00	
Lab Equipment Thermo cycler (PCR)	No.	01	**499000.00**	No.	01	**498000.00**	100% of achievements
Lab Equipment Horizontal Gel Electrophoresis with power supplier	No.	01	**167500.00**	No.	01	**167000.00**	100% of achievements
Lab Chemicals			**462600.00**			**461220.00**	100% of achievements
Genomic DNA Purification Kit for Gram Negative Bacteria, (100 reaction)	pac.	8	144000.00	pac.	8	143600.00	
100bp DNA Ladder, 250ul (50 lanes)	pac.	5	45000.00	pac.	5	44900.00	
Hot Start Green Master Mix	pac.	6	54600.00	pac.	6	54300.00	
Agarose, pack size100gm	pac.	5	80000.00	pac.	5	79900.00	
DNA cleanup kit	pac.	5	50000.00	pac.	5	49950.00	
TBE buffer, 10 X	L.	2	14000.00	L.	2	13900.00	
Ethidium bromide	ml	60	30000.00	ml	60	29820.00	
Primer	pair	15	45000.00	pair	15	44850.00	
Lab Chemicals			**237476.00**			**235770.00**	100% of achievements
Microcentrifuge tubes, PCR tubes, PCR plates, Micro pipette Tips, glovesetc	LS	LS	46900.00	LS	LS	46880.00	
Mannitol	kg	5	45000.00	kg	5	44750.00	
K_2HPO_4	Kg	1	4500.00	Kg	1	4400.00	
$MgSO_4.7H_2O$	Kg	1	6000.00	Kg	1	5950.00	
NaCl	Kg	1	2076.00	Kg	1	2000.00	
Yeastextract	Kg	2	6000.00	Kg	2	5800.00	
Agar	Kg	10	60000.00	Kg	10	59800.00	
$CaHPO_4$	kg	1	4500.00	kg	1	4400.00	
$FeCL_3$	kg	1	6000.00	kg	1	5950.00	
K_2SO_4	Kg	1	5000.00	Kg	1	4900.00	
$Na_2B_4O_7.10\ H_20$	Kg	1	6000.00	Kg	1	5950.00	
$MnSO_4.H_2O$	Kg	1	1500.00	Kg	1	1490.00	
$ZnSO_4.7H_2O$	Kg	1	5000.00	Kg	1	4900.00	
$Na_2MoO_4.2H_2O$	g	500	7500.00	g	500	7400.00	
$(NH_4)_6Mo_7O25.H_2O$	g	500	7500.00	g	500	7450.00	
Isopropanol	L	4	14000.00	L	4	13800.00	
Polythene	LS	LS	10000.00	LS	LS	9950.00	

2. Establishment/renovation facilities:

Description of facilities	Newly established		Upgraded/refurbished		Remarks
	PP Target	Achievement	PP Target	Achievement	
-	-	-	-	-	-

3. Training/study tour/ seminar/workshop/conference organized:

Description	Number of participant			Duration (Days/weeks/ months)	Remarks
	Male	Female	Total		
(a) Training	-	-	-	-	-
(b) Workshop	-	-	-	-	-

C. Financial and physical progress

Fig in Tk

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
A. Contractual staff salary	274969	274969	274969	0	100.00	-
B. Field research/lab expenses and supplies	1185576	1173035	1173265	230	100.00	-
C. Operating expenses	300000	225000	223055	1945	99.14	-
D. Vehicle hire and fuel, oil & maintenance	220000	220000	220000	0	100.00	
E. Training/workshop/Field day etc.	0	0	0	0	0	-
F. Publications and printing	80000	0	0	0	0.00	PCR was printed by PIU-BARC.
G. Miscellaneous	39455	39455	39455	0	100.00	-
H. Capital expenses	900000	900000	898200	1800	99.80	-

D. Achievement of Sub-project by objectives: (Tangible form)

Specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output(i.e. product obtained, visible, measurable)	Outcome(short term effect of the research)
To isolate, identify and preserve of effective rhizobial strains collected from soybean and groundnut root nodules from saline stress areas of Bangladesh.	- Root samples from soybean and groundnut were collected from different fields of saline stress soils. - *Rhizobium* bacteria were isolated and preserved. - Bacterial culture mixed were for long time preservation.	- About 273 root samples from soybean and groundnut were collected from different fields of saline stress soils (Noakhali, Lakhmipur, Cox's Bazar, Satkhira, Bagerhat, Bhola, Borguna, Patuakhali) of Bangladesh using global positioning system (GPS) record along with crop history. - Isolation of all *Rhizobium* bacteria were done aseptically transferred to agar slants and preserved 4^0C in refrigerator. - The isolated strains were purified and transferred to agar slants and preserved as stock culture in a refrigerator at 4^0C. In addition, culture mixed with 50% (v/v) glycerol will be kept in -20^0C and -80^0C for long time preservation.	- Discovery of *Rhizobium* bacteria - *Rhizobium* bacterial short and long-time preservation - Bacterial gene bank establishment
To study symbiotic, biochemical and molecular characteristics of saline stress tolerant effective rhizobial strains and their taxonomy (family, genus and species).	- Growth observation - Morphology of cell - Gram negative test - Congo red test - Motility test - Salt stress test - Infectivity test -Molecular characterization were done	- Bacterial culture growth on 24 hours at 28±2°C. - All bacteria were rod shaped. - All bacteria were showed Gram negative. - All bacteria were not absorbed Congo red. =All bacteria were showed motile. - All Rhizobia bacteria were showed salt tolerant upto 48 dSm^{-1} salinity level. -All Rhizobia bacteria were found nodulation infectivity on soybean and groundnut seedlings. - *Rhizobium* strains were belonging to Rhizobiales order, Rhizobiaceae family, and genus *Rhizobium*.	Symbiotic, morphological, biochemical and molecular were characterized. Taxonomy (family, genus and species) were identified.
To use these effective strains for biofertilizer production for soybean and groundnut cultivation.	- Pot experimentation - Field experimentation	-*Rhizobium* sp.SR15 and *Rhizobium* sp.GR13 were found best biofertilizer strains for soybean and groundnut under pot trails, respectively. -*Rhizobium* sp.SR7 found best biofertilizer strains for soybean in field of Cox's Bazar and Noakhali. -*Rhizobium* sp.SR15 recorded best biofertilizer strains for soybean in field of Kuakata and Satkhira. - *Rhizobium* sp.GR9 showed best biofertilizer strains for groundnut in field of Cox's Bazar and Noakhali. - *Rhizobium* sp.GR13 receded best biofertilizer strains for groundnut in field of Kuakata and Satkhira.	Farmer can use low cost best biofertilizer

E. Materials Development/Publication made under the Sub-project:

Publication	Number of publication		Remarks (e.g. paper title, name of journal, conference name, etc.)
	Under reparation	Completed and published	
Technology bulletin/ booklet/leaflet/flyer etc.	-	-	-
Journal publication	-	-	-
Information development	-	-	-
Other publications, if any	-	-	-

F. Technology/Knowledge generation/Policy Support (as applied):

i. Generation of technology (Commodity & Non-commodity)

- Peat based low price biofertilizer technology packages have been developed.
- Biofertilizers have been prepared by potential salt tolerance *Rhizobium* species for soybean and groundnut.
- Forty salt tolerant *Rhizobium* species have been isolated, characterized and used for biofertilizer production.

ii. Generation of new knowledge that help in developing more technology in future

- Biofertilizer technology packages can be used for other new strains and peat soil also available in Bangladesh. So, local producer and entrepreneurs can easily adapt the technology with low price for biofertilizer production.
- Discovery of new potential salt tolerance *Rhizobium* species and same way biofertilizers production for soybean and groundnut.
- Symbiotic and molecular characterization *Rhizobium* species have been eventually applied for new identified bacteria.

iii. Technology transferred that help increased agricultural productivity and farmers' income

- Low price biofertilizer technology package increase yield and economic return in production for soybean and groundnutat different salt stress areas of Bangladesh.
- There is a great scope to improve the overall economic condition of farmers through adopting biofertilizer technology.

iv. Policy Support

- Recently the Ministry of Agriculture (MoA), Peoples Republic of Bangladesh has incorporated organic agriculture in the policies of agriculture and crop production. The findings of the present project may assist the policy makers of the agricultural sectors for planning and setting their future research directions in order to technology generation as well as increasing production and productivity for sustainable food and nutrition security in Bangladesh. It is important that policy makers come to a better understanding of the implications of biofertilizer.
- Biofertilizer is considered as a more sustainable and environmentally friendly organic fertilizer for cultivating crops. Crop production in the next decade will have to produce more food from less chemical fertilizer use by making more efficient use of natural resources and with minimal impact on the environment.
- The study concludes that biofertilizer production as a new resource saving organic farming practice that was appreciated and successfully adopted by the farmers in the study areas.

G. Information regarding Desk and Field Monitoring

i) Desk Monitoring [description & output of consultation meeting, monitoring workshops/seminars etc.):

Monitoring workshop	Date(s) of workshop	Remarks
NRM Unit, BARC & PIU-BARC, NATP-2	15/05/2018	There were some suggestions givenby experts for attaining better achievement of the sub-project activities and during final reporting, necessary steps were taken according to these suggestions.

ii) Field Monitoring (time& No. of visit, Team visit and output):

Monitoring team	Date(s) of visit	No. of visit	Remarks
Technical Division/Unit, BARC	14/03/2018	1	Satisfactory
PIU-BARC, NATP-2	17/01/2018 &14/03/2018	2	Satisfactory
Internal Monitoring (BARI)	06/02/2018	1	Satisfactory

H. Lesson Learned (if any)

- *Rhizobium* bacteria has adaptability tolerant saline up to 48dS/m.
- Saline coastal areas can used salt tolerance biofertilizers production for soybean and groundnut.
- *Rhizobium* sp.SR7and *Rhizobium* sp.SR15 might be used in soybean,and *Rhizobium* sp.GR9 and *Rhizobium* sp.GR13 might be usedin groundnut cultivation in saline stress areas of Bangladesh

I. Challenges (if any)

- Lack of information on biofertilizer technology.
- Existing soil conditions such as acidity, alkalinity, pesticide application and high nitrate level limiting nitrogen fixing capacity of biofertilizer.
- There is a great need of trained manpower and facilities to produce required amount of effective inoculum and maintain the quality of the products.
- There are no commercial inoculant producers for supplying sufficient amount of quality inoculum to farmers in collaboration with research organizationsfor quality control.
- There is no regular system to train extension workers and the farmers for field trials.

Signature of the Principal Investigator
Date ………………………………...........

Seal

Counter signature of the Head of the organization/authorized representative
Date ………………………….......……..
Seal