IMPACT OF INDIGENOUS PLANT GROWTH PROMOTING RHIZOBACTERIA AND CHEMICAL FERTILIZERS ON SOIL HEALTH AND PRODUCTIVITY OF CAPSICUM (*Capsicum annuum* L.)

Thesis

by

SHWETA GUPTA
(F-2012-25-D)

Submitted to

Dr. YASHWANT SINGH PARMAR UNIVERSITY OF HORTICULTURE & FORESTRY SOLAN (NAUNI) HP-173 230 INDIA

in

Partial fulfilment of the requirements for the degree

of

DOCTOR OF PHILOSOPHY
MICROBIOLOGY
DEPARTMENT OF BASIC SCIENCES
2016

Dr. Rajesh Kaushal
Senior Scientist
(Soil Microbiology)

Department of Soil Science and Water Management
College of Forestry
Dr Yashwant Singh Parmar University of Horticulture and Forestry (Nauni) Solan

CERTIFICATE-I

This is to certify that the thesis titled, "**Impact of Indigenous Plant Growth Promoting Rhizobacteria and Chemical Fertilizers on Soil Health and Productivity of Capsicum (*Capsicum annuum* L.)**" submitted in partial fulfilment of the requirements for the award of the degree of **DOCTOR OF PHILOSOPHY** in **MICROBIOLOGY (BASIC SCIENCES)** to Dr Yashwant Singh Parmar University of Horticulture and Forestry, (Nauni) Solan (HP) – 173 230 is a record of bonafide research work carried out by **Ms Shweta Gupta (F-2012-25-D)** daughter of Shri Desh Raj Gupta under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

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

Place: Nauni, Solan
Dated:

Dr. Rajesh Kaushal
Major Advisor

CERTIFICATE-II

This is to certify that the thesis titled, **"Impact of Indigenous Plant Growth Promoting Rhizobacteria and Chemical Fertilizers on Soil Health and Productivity of Capsicum (*Capsicum annuum* L.)"** submitted by **Ms Shweta Gupta (F-2012-25-D) daughter of Shri Desh Raj Gupta** to Dr Yashwant Singh Parmar University of Horticulture and Forestry, (Nauni) Solan (HP) – 173 230 India in partial fulfilment of the requirements for the award of degree of **DOCTOR OF PHILOSOPHY** in **MICROBIOLOGY (BASIC SCIENCES)** has been approved by the Advisory Committee after the thesis viva-voce examination of the student in collaboration with an External Examiner.

Dr. Rajesh Kaushal
Major Advisor

Dr. C.K. Sirkot
External Examiner

Dr. Nivedita Sharma
Dean's Nominee

Members of Advisory Committee

Dr. Rajnish Sharma
(Assistant Professor)

Dr. (Mrs) Anjali Chauhan
(Assistant Professor)

Mr R.S. Spehia
(Assistant Professor)

Professor and Head
Department of Basic Sciences
Dr. Yashwant Singh Parmar University of Horticulture and Forestry
Nauni-173 230, Solan (HP)

Dean
College of Forestry
Dr. Yashwant Singh Parmar University of Horticulture and Forestry
Nauni - 173 230, Solan (HP)

CERTIFICATE-III

This is to certify that all the mistakes and errors pointed out by the external examiner have been incorporated in the thesis titled**, "Impact of Indigenous Plant Growth Promoting Rhizobacteria and Chemical Fertilizers on Soil Health and Productivity of Capsicum (*Capsicum annuum* L.)"** submitted to Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (HP) by **Ms Shweta Gupta (F-2012-25-D)** in partial fulfilment of the requirements for the award of degree of **DOCTOR OF PHILOSOPHY** in **MICROBIOLOGY.**

_____

Dr. Rajesh Kaushal
Major Advisor

_____

Professor and Head
Department of Basic Sciences
Dr Yashwant Singh Parmar University of Horticulture and Forestry
Nauni-173 230, Solan (HP)

ACKNOWLEDGEMENTS

With immense servility, I must bow before Lord who sends galavance strength on me through the ups and downs of the life enabling me to see today "The joy of task done".

Every effort is motivated by ambitions and all ambitions have inspirations behind, I owe this pride place to my parents, sister (Sapna) and uncle (Mr. Ravi Sood) for their heartful blessings, selfless love, prudent persuasion and obstinate sacrifices.

Words betray to express my deep sense of gratitude to my esteemed teacher and chairman of my advisory committee, Dr. Rajesh Kaushal for his guidance, keen interest, critical analysis and valuable suggestions.

I emphatically owe my heartiest thanks Dr Rajnish Sharma, Dr. (Mrs.) Anjali Chauhan and Dr. R.S. Spehia worthy members of my advisory committee for their valuable suggestions and full cooperation throughout the course of investigation. Profound thanks are due to my faculty members Dr. (Mrs.) Nivedita Sharma, Dr. (Mrs.) Mohinder Kaur and Dr (Mrs.) Sunita.

I express my loyal and sincere thanks to Dr. P.K. Mahajan, (Dean) college of Forestry, Dr. (Mrs.) Veena Sharma (Professor and Head), Department of Basic Sciences for providing all the necessary facilities and means to carry out the experiments successfully.

Without mentioning the name of my senior's whole range of work would have been futile perhaps their unflinching support, guidance and timely help throughout my research work.

It is hard to find, difficult to leave and impossible to forget "Thanks" is too small word to express my heartfelt emotions to Ruchi mam, Gaurav, Shipu, Shruti Kirti, Bhawna, Sonal, Girish, Meenakshi, Shilpa and Saijal who stood by me throughout my research. With personal touch of fervently, I place on record my special thanks to Neha, Reena, Prerna, Shweta Sharma, Ritu, Panku, Subhash and Ankur for their continuous encouragement and moral support.

I have been fortunate in getting the intelligent guidance by my seniors and enthusiastic co-operation of my classmates and all my juniors are also acknowledged.

I am pleased to pay my ponderous thanks to Daya Ram ji, RHRS Bajaura, Joshi ji, Sukh Ram ji, Hemraj ji, staff members of Department of Basic Sciences and Birbal ji, Department of Soil Science and Water Management , Nauni for their cooperation in helping me to carry out my research.

Financial support from "Innovation in Science Pursuit for Inspired Research (INSPIRE) Programme", Department of Science and Technology, New Delhi is duly acknowledged.

Needless to say, errors and omissions are solely mine.

Place: Nauni, Solan
Date: **(Shweta Gupta)**

CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF FIGURES

ABBREVIATIONS USED

Abbreviation	Description
%	Per cent
^{0}C	Degree centigrade
µ	Micro
ACC	1-Aminocyclopropane-1-carboxylate
AMF	Arbuscular mycorrhiza fungi
APX	Ascorbate peroxidase
BNF	Biological Nitrogen Fixation
CAT	Catalase
CAS	Chrome-azurol-S
CF	Culture filtrate
cfu	Colony forming units
cm	Centimetre
conc.	Concentrated
CRD	Completely randomized design
df	Degree of freedom
DI	Disease incidence
DNA	Deoxy-ribonucleic acid
EC	Electrical conductivity
EDTA	Ethylene-di-aminotetra-acetic acid
FEMS	Federation of European Materials Societies
FYM	Farm yard manure
G	Gram
GR	Glutathione reductase
H	Hour
ha	Hectare
HCN	Hydrogen cyanide
IAA	Indole-3-acetic acid
JM	Jensen Medium
K	Potassium
kg	Kilogram
LSD	Least significant difference
MEA	Malt extract agar
min	Minute
ml	Millilitre
mm	Millimetre
MOP	Murate of potash
MSS	Mean Sum of Square
Mt	Metric tons
N	Nitrogen
NA	Nutrient agar
NC	Nutrient content
NFB	Nitrogen fixing bacteria
NHB	National horticulture board
nm	Nanometre
NU	Nutrient uptake
OC	Organic Carbon

OD	Optical density
P	Phosphorus
POP	Package of practices
PGPR	Plant growth promoting rhizobacteria
ppm	Parts per million
PSB	Phosphate solubilising bacteria
PSM	Phosphate solubilising microorganisms
PVK	Pikovskaya's medium
RBD	Randomized block design
RD	Recommended dose
RDF	Recommended dose of fertilizers
ROS	Reactive oxygen species
RP	Rock phosphate
rpm	Rotations per minute
rRNA	Ribosomal ribonucleic acid
RWC	Relative water content
SDS	Sodium do-decylphosphate
SOD	Superoxide dismutase
sp.	Species
SSP	Single super phosphate
TCA	Tri-carboxylic acid
TCP	Tri-calcium phosphate
USA	United States of America
UV	Ultra violet
Var.	Variety
VOCs	Volatile organic compounds
Vol.	Volume

Chapter-1

INTRODUCTION

Capsicum annuum L. commonly known as bell pepper or sweet pepper or shimla mirch is a domesticated species of genus *Capsicum*, belongs to nightshade family i.e. Solanaceae. This crop is native to Mexico with secondary centre of origin at Gautemela (Jean *et al*. 2013).

Bell pepper is the second-most consumed vegetable worldwide. In India, it is raised over an area of 32,000 hectares with annual production of 1,83,000 metric tons. In Himachal Pradesh, capsicum is being cultivated as an important summer season vegetable crop, providing rich dividends to the farmer with an annual production of 34,130 metric tons over an area of 2,070 hectares (NHB, 2015).

Bell pepper is an important agricultural crop, not only because of its economic importance, but also for the nutritional value of its fruits, as is characterized by its high levels of vitamin C (ascorbic acid), pro-vitamin A (carotene) and calcium (Mateos *et al*. 2013). Capsicum is consumed as salads, cooked, mixed, stuffed vegetable and is appreciated worldwide for its flavour, aroma and colour. It is also recommended for the treatment of dropsy, toothache and cholera (Peirce, 1987).

The crop requires warm, uniform soil moisture conditions and well drained sandy loam soil with pH range 5.5 to 6.8. A frost free period of about 130-150 days with temperature range of 15-35°C is optimum for its cultivation (Sood *et al*. 2007).

Rapid industrialization and urban development is the major threat to existing agricultural land and irrigation water (Tallapragada *et al*. 2011). In order to meet the growing demands of burgeoning population, the rampant use of chemical fertilizers contributes to the deleterious effects on agro-ecosystem as well as productivity of the crop (Zayed *et al*. 2013).

The ever increasing cost of pesticides or fertilizers coupled with consumer preference for organic food has led to supplementation and/or substitution of these inputs with organic or biodegradable inputs.

Several biotic and abiotic stresses contributes to lower yield of capsicum in our state. The pest resistance against applied pesticides and inoculum development due to continuous growing of same group of vegetable crops on a unit piece of land have led to the development of diseases like bacterial wilt (*Ralstonia solanacearum*) Phytophthora collar rot (*Phytophthora capsici*), anthracnose (*Colletotrichum capsici*) and dampimg off (*Pythium* spp.), which have turned out to be the major bottlenecks for its low productivity.

Water stress is among the most influential abiotic factors affecting the crop yield. Under stress conditions an increase in the generation of reactive oxygen species (ROS), usually takes place which lead to oxidative stress through the disturbance of redox homeostasis in plant cells (Hu *et al*. 2010). Long dry periods are likely to result in flower shedding and fruit fall. Thereby, using available water efficiently in order to get maximum profit from per unit area has become an important consideration.

In the frame of modern agriculture, micro flora of soil is of great significance because it has both beneficial and detrimental influence on mankind. Therefore, current trends in agriculture are focused on one of the most acceptable and environmentally conscious approach i.e. manipulation of beneficial microbial population in the rhizosphere (Mrkovacki and Bjelic, 2011).

Plant growth promoting rhizobacteria are therefore gaining importance as cost-effective, ecofriendly, non-hazardous and non-bulky agri-inputs in site specific integrated nutrient management system which can supplement about 25-50 per cent of chemical fertilizers and decreases the deleterious effects of environmental stresses through various modes of action such as biofertilization (increasing the availability of nutrients to plant), phytostimulation (production of phytohormones) and biocontrol (controlling diseases, mainly by the production of

antibiotics, antifungal metabolites and induction of plant defense responses) (Nihorimbere *et al*. 2011).

Many rhizospheric bacterial species have been tested and found to be beneficial for plant growth, yield and crop quality (Ahemad and Kibret, 2014). The most predominant rhizosphere colonizing bacteria belongs to *Pseudomonas* and *Bacillus* species because of their association with soil organic matter, nutritional diversity and rapid growth rate (Vijayapal *et al*. 1998).

The PGPR isolated from native rhizosphere are, in general, better adapt themselves as compared to non-indigenous bacterial strains. The studies on isolation and characterization of PGPR from capsicum rhizosphere (Ponmurugan and Gopi, 2006; Datta *et al*. 2011, Gupta *et al.* 2014; 2015) further, emphasized on beneficial effects of rhizospheric microbes on crop growth and yields. However, there is no, commercial biofertilizers/PGPR formulations for capsicum crop which may be used in different agro-climatic zones of Himachal Pradesh both under open and polyhouse conditions.

The integrated use of chemical fertilizers, organic manures and bio fertilizers hold great promise in securing high level of crop productivity with good quality and also to protect soil health from deterioration and pollution hazards. Therefore, there is an urgent need to develop an effective inoculum of plant growth promoting rhizobacteria (PGPR) for capsicum with multiple PGP traits along with optimum doses of chemical fertilizers, which may go a long way in building of soil fertility and productivity of bell pepper.

Keeping in view all the above points, the present investigations were undertaken to isolate indigenous isolates of PGPR with multiple plant growth promoting traits and to study the effects of inoculation of PGPR's on biotic/abiotic stress and with different levels of N and P fertilizers on growth of capsicum with the following objectives:

i) Screening and molecular characterization of indigenous PGPR isolates associated with capsicum.

ii) To study the effect of indigenous PGPR inoculant's for physiological efficacy under water stress conditions.

iii) To study the efficacy of indigenous PGPR to control major soil borne disease(s) of capsicum.

iv) To study the effect of PGPR and chemical fertilizers on soil health and productivity of capsicum.

Chapter-2

REVIEW OF LITERATURE

In modern cultivation process indiscriminate use of fertilizers exerts deleterious effects on soil microorganism, soil productivity and pollutes the environment (Youssef and Eissa, 2014). Long term application of chemical fertilizers often leads to reduction in pH and disturbance in equilibrium of exchangeable bases thus making them unavailable to crops which ultimately reduced the productivity of crop. To obviate this problem, researchers have been throwing up unique innovative interesting ideas for research in the thin layer of soil immediately surrounding plant roots known as rhizosphere, where, the physical, chemical and biological properties have been changed by root growth and their activities, thereby, providing the stimulating growth environment for microbial populations capable of exerting beneficial, neutral or detrimental effects on plant growth (Maji and Chakarbartty, 2014). The indispensable part of rhizosphere biota that competitively colonize plant roots, stimulates plant growth and reduces the incidence of plant diseases are termed as plant growth promoting rhizobacteria (Kloepper and Schroth, 1978).

The enhancement of plant growth by application of root colonizing microorganisms is well evident (Gray and Smith, 2005; Bhattacharya and Jha, 2012; Gupta *et al*. 2015). In recent years, the biological approaches for improving crop production are gaining strong status among agronomists and environmentalists following integrated plant nutrient management system. In this context, there is an ongoing rigorous research worldwide with greater impetus to explore a wide range of rhizobacteria possessing multiple plant growth promoting traits like phytohormone, siderophore, 1-amino-cyclopropane-1-carboxylate deaminase, hydrogen cyanate (HCN), ammonia production, nitrogenase activity, phosphate solubilization, salinity tolerance, biological control of phytopathogens etc. (Ahemad and Kibret, 2014). Various genera of PGPR like *Pseudomonas, Enterobacter, Bacillus, Variovorax, Klebsiella, Burkholderia, Azospirillum, Serratia* and *Azotobacter* have been reported worldwide to play a significant role in enhancing plant growth and development (Hayat *et al*. 2010).

The pertinent literature available is reviewed under the following heads:

2.1 PGPR: Root colonization and mode of action

2.2 PGPR as biofertilizers for enhanced growth and yields

2.3 PGPR as biocontrol agents

2.4 PGPR as drought stress alleviators

2.1 PGPR: ROOT COLONIZATION AND MODE OF ACTION

2.1.1 PGPR as root colonizers

Microbial attachment to and proliferation on roots is generally referred to as root colonization. Root colonization is an important factor in plant pathogenesis of soil-borne microorganisms as well as in beneficial interactions used for microbiological control, biofertilization, phytostimulation and phytoremediation (Thomas *et al*. 2008). The degree of proximity between the PGPR and host plant can vary depending on where and how they colonizes the host plant. The relationships can be categorized into two levels of complexicity:

1) Rhizospheric
2) Endophytic

In rhizospheric relationship, the PGPRs can colonize the rhizosphere, the surface of the roots or even the superficial intercellular spaces of plant roots (McCully, 2001). Rhizobacteria are rhizosphere competent bacteria that aggressively colonize all the ecological niches found on the roots at all the stages of plant growth and able to multiply in presence of competing microflora. During the process of root colonization bacteria multiply in the spermosphere (region around the seed) in response to seed exudates rich in nutrients (carbohydrate, amino acids etc.), get attached to root surface and colonize the developing root system (Kloepper *et al*. 1980).

The most predominant rhizosphere colonising bacteria belong to the genus *Pseudomonas* and *Bacillus* because of their association with soil organic matter, nutritional diversity and rapid growth rate (Vijaypal *et al*. 1998). It is only due to the changes in different soil physical and chemical properties (pH, moisture

content, temperature, redox, salinity, texture, stability of aggregates, fertility, organic matter content), presence or absence of pesticides/other xenobiotic substances, soil fauna, the nutrient status of rhizosphere, nature of root exudates as compared to the bulk soil that in turn can affect the ability of introduced PGPR strains to colonize and proliferate in the rhizosphere (Saharan and Nehra, 2011).

In endophytic relationship, endophytes entered the plant tissue primarily through the roots, however, aerial portions of plants such as flowers, stems, and cotyledons, are also used for their entry (Bell *et al.* 1995). PGPR enter into the plant roots by three putative pathways i.e. root tips, point of emergence of lateral roots and the axils of emerging or developing lateral roots (James, 1998). These endophytes, either become localized at the point of entry or consistently colonize the internal plant tissue of their host and can live within cells, in the intercellular spaces or in the vascular system, without causing harm to the host (Sharma *et al*. 2005). Endophytic bacteria have the advantage of being protected from high physical and chemical stress, competitive environment and induce greater growth promotion than the bacteria restricted to rhizosphere and/or to the root surface (Austin and Nobel, 2003). Bacterial endopohytes have also been shown to prevent the disease development by modifying biochemicals produced by plants and through endophyte mediated *de novo* synthesis of structural compounds such as fungitoxic metabolites (Sturz *et al*. 2000). Researchers have found bacterial endophytes in potato (Garbeva *et al.* 2001), maize (Fisher *et al.* 1992), rice (Stoltzfus *et al.* 1997) and cucumber (Mahafee and Kloepper, 1997).

2.1.2 PGPR: Mechanism of action

PGPR mediated plant growth promotion occurs by the alteration of the whole microbial community in rhizosphere niche either facilitating resource acquisition, modulating plant hormone levels or by decreasing the inhibitory effects of various pathogens through various direct/ indirect modes of action (Castro *et al*. 2009).

Direct stimulation includes biological nitrogen fixation, producing phytohormones (auxins, cytokinins, gibberellins), solubilizing minerals

(phosphorus, iron), production of enzymes while indirect stimulation is basically related to biocontrol, including antibiotic production, chelation of available Fe in the rhizosphere, synthesis of extracellular enzymes to hydrolyze the fungal cell wall, competition for niches within the rhizosphere and induction of systemic resistance (Loon Van, 2007).

2.1.2.1 Nitrogen fixation

Nitrogen (N) is the most vital nutrient for plant growth and productivity. The biological process responsible for reduction of molecular nitrogen into ammonia using nitrogenase complex is referred to as biological nitrogen fixation (BNF) which is contributing 180×10^6 metric tons/year globally (Rubio and Ludden, 2008). Out of which 80% comes from symbiotic associations and the rest from free-living or associative systems. The ability to reduce and siphon out such appreciable amounts of nitrogen from the atmospheric reservoir and enrich the soil is confined to bacteria and archaea (Tilak *et al*. 2005). These include symbiotic nitrogen fixing forms namely: *Rhizobium* in leguminous plants, *Frankia* in non-leguminous trees and non-symbiotic N_2-fixing forms (diazotrophs) such as *cyanobacteria, Azospirillum, Azotobacter, Acetobacter diazotrophicus, Azoarcus* etc. (Park *et al*. 2005).

The nitrogen-fixing bacterial community can be varied by various abiotic and biotic factors such as plant species, biomass, root exudates, soil pH, water content, microbial biomass and nutrients (Zhan and Sun, 2011). The colony forming units (cfu) of the cultured nitrogen-fixing bacteria have been found ranging from 5.0×10^3 to 1.5×10^7 cfu g^{-1} from soil samples of Wamena Biological Garden (Suliasih and Widawati, 2005); 0.97×10^6 to 12.78×10^6 cfu g^{-1} soil, in the *Potentilla parvifolia* shrubland of north-western China (Tai *et al*. 2013).

2.1.2.2 Phosphate solubilization

Phosphorus is among the primary essential nutrient elements required by the plant in optimum amount for its proper growth and development. Although average P content in soil is about 0.05% but only 0.1% of the total P present is available to the plants because of its immobilization, chemical fixation and low

solubility (Kundu *et al*. 2002). Under such conditions, several phosphate solubilizing microorganisms (PSMs) convert the insoluble form of phosphorus to soluble form through acidification, secretion of organic acids, protons chelation, exchange reactions and synthesis of enzymes like phosphatase, phosphohydrolases, phytase, phosphonoacetate hydrolase, D-α-glycerophosphatase and C-P lyase (Richardson *et al*. 2009).

Phosphate solubilizing bacteria (PSB) are commonly found in most soils but their establishment and performances are severely affected by soil and environmental factors (Ahemad and Khan, 2012). For instance, phosphate solubilization efficiency of the P-solubilisers from chickpea rhizosphere have been from 2.2 to 227.2 µg/ml (Kundu *et al*. 2009), whereas, in tea rhizosphere it ranges from 40.62 ± 1.1 to 136.73 ± 1.7 mg/l (Sharma *et al*. 2012).

Bacterial genera like *Azotobacter, Bacillus, Beijerinckia, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Microbacterium, Pseudomonas, Rhizobium* and *Serratia* are reported as the most significant phosphate solubilizing bacteria (Bhattacharyya and Jha, 2012). Muhammad *et al*. (2013) studied five promising PSB strains i.e. *Burkholderia* sp., *Bacillus* sp., *Pseudomonas* sp., *Flavobacterium* sp. and *Pseudomonas* sp. and reported significant increase in plant growth, grain yield, rhizosphere phosphatase activity, mineralization of organic P and soil available P over uninoculated control.

2.1.2.3 Siderophore production

Iron is required as a cofactor for a large number of enzymes and iron-containing proteins in microbial metabolism. In the aerobic environment, iron occurs principally as Fe^{3+} and is likely to form insoluble hydroxides and oxyhydroxides, thus making it inaccessible to both plants and microorganisms (Rajkumar *et al*. 2010).

Under iron limiting conditions, PGPR produces low molecular weight compounds called siderophores to acquire ferric ions (Whipps, 2001). Binding of the siderophore to a metal increases the soluble metal concentration. Plants assimilate iron from bacterial siderophores by means of different mechanisms, for

instance, chelate and release of iron, the direct uptake of siderophore-Fe complexes or by a ligand exchange reaction (Schmidt, 1999). Further, siderophores restrict the growth of pathogenic microorganisms by limiting the iron availability as it bind to the available form of iron (Fe^{2+}) in the rhizosphere (Ahmad *et al.* 2008). Numerous studies of the plant growth promotion and disease resistance as a result of siderophore producing rhizobacterial inoculations have been reported (Rajkumar *et al*. 2010). Sharma *et al*. (2003) assessed the role of the siderophore-producing *Pseudomonas* strain GRP3 on iron nutrition of *Vigna radiata*. After 45 days, reported decline in chlorotic symptoms and increased chlorophyll a and chlorophyll b content in Pseudomonas strain GRP3 inoculated plants over control.

Similarly, significant diseases reduction and plant growth in wheat (Munazza and Hafeez, 2012) and rice (Meera and Balabaskar, 2012) has been reported with inoculation of siderophore producing rhizospheric *Pseudomonas* isolates.

Not only iron, siderophores also form stable complexes with other heavy metals that are of environmental concern, such as Al, Cd, Cu, Ga, In, Pb and Zn as well as with radionuclides including U and Np (Neubauer *et al*. 2000). Hence, bacterial siderophores help to alleviate the stresses imposed on plants by high soil levels of heavy metals (El-Azeem *et al*. 2007).

2.1.2.4 Phytohormone production/ phytohormonal modification

IAA has been implicated in virtually every aspect of plant growth and development, as well as defense responses (Santner *et al.* 2009). Generally, indole-3-acetic acid (IAA) affects plant cell division, extension, differentiation, stimulates seed germination, control processes of vegetative growth, initiates lateral/adventitious root formation, mediates responses to light, gravity and resistance to stressful conditions.

Microbial synthesis of the phytohormone auxin (indole-3-acetic acid) has been known for a long time. It is reported that 80% of microorganisms isolated from the rhizosphere of various crops possess the ability to synthesize and release

auxins as secondary metabolites. *Azotobacter* and *Azospirillum* has been reported to produce IAA in range of 19.4 to 30.2µg/ml (Fatima *et al*. 2009).

IAA production by PGPR causes modifications in root system architecture by increasing the number of root tips and the root surface area, thus increasing water/nutrient acquisition (Egamberdieva and Kucharova, 2009). Plants inoculated with *Pseudomonas putida* were able to survive drought stress due to the production of IAA (Marulanda *et al*. 2009). It has also been reported that bacterial VOCs from *Bacillus subtilis* strain GB03 cause growth promotion in Arabidopsis by upregulating transcripts involved in auxin homeostasis (Zhang *et al*. 2010).

Also, biocontrol activity of IAA involves inhibition of spore germination and mycelial growth of pathogenic fungi (Uneo *et al*. 2004). Not only this, rhizobacterial IAA loosens plant cell walls and as a result facilitates an increasing amount of root exudation that provides additional nutrients to support the growth of rhizosphere bacteria (Spaepen and Vanderleyden, 2011).

The amino acid tryptophan, identified as the main precursor for IAA stimulates the level of IAA biosynthesis (Zaidi *et al*. 2009), while, anthranilate, a precursor for tryptophan, reduces IAA synthesis. This can be illustrated by increased indole production by *Pseudomonas fluorescens* AK1 and *Pseudomonas aeruginosa* AK2 with increase in tryptophan concentration (Karnwal, 2009).

2.1.2.5 Aminocyclopropane-1-carboxylate (ACC) deaminase

Generally, ethylene is an essential metabolite for the normal growth and development of plants (Khalid *et al*. 2006). Under stress conditions like salinity, drought, water logging, heavy metals and pathogenicity, the endogenous level of ethylene is significantly increased due to the activity of indole acetic acid (IAA) induces the activity of ACC synthase to convert S-adenosylmethionine to 1-aminocyclopropane-1-carboxylic acid (ACC), an immediate precursor of ethylene biosynthetic pathway (Zapata *et al*. 2007). Increased ethylene induces defoliation and other cellular processes that may lead to reduced crop performance (Bhattacharyya and Jha, 2012).

Plant growth promoting rhizobacteria which possesses the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, take up the ACC upon its exudation by the plant roots and convert it into 2-oxobutanoate and NH_3 (Arshad *et al*. 2007). As the concentration of ACC outside the root decreases due to its degradation, more ACC is exuded by the plant roots, so deleterious effect of ethylene is abated by the removal of ACC, thus ameliorating plant stress and promoting plant growth (Glick, 2007). Currently, bacterial strains exhibiting ACC deaminase activity conferring the tolerance towards draught stress and enhanced plant growth have been reported worldwide such as *Enterobacter cloacae* and *Pseudomonas putida* in tomato (Grichko and Glick, 2001), *Achromobacter piechaudii ARV8* in pepper and tomato (Mayak, 2004) *Pseudomonas fluorescens* TDK1 in groundnut (Govindasamy *et al*. 2008), *Bacillus* spp. in *Brassica campestris* (Ghosh *et al*. 2003) and pepper (Hui and Kim, 2013) etc.

2.1.2.6 HCN production

A secondary metabolite produced commonly by rhizosphere microorganisms is hydrogen cyanide (HCN), a gas known to negatively affect plant root metabolism and growth except host plants. For instance, HCN producing *P. fluorescens* has been reported as potential biological weed control reducing more than 90% of root and shoot growth in weeds (Ramette *et al*. 2003). HCN affects the sensitive organisms by inhibiting the synthesis of ATP-mediated cytochrome oxidase (Knowles, 1976). Thereby, participating tremendously in the reduction of phytopathogens and deleterious rhizobacteria (Rudrappa *et al*. 2008). The role of cyanide production in Pseudomonad virulence against fungal pathogen (*F. moniliforme*) (Supraja *et al*. 2011), *Ralstonia solanacearum* antagonism (Zhou *et al*. (2012) and enhanced plant growth (Wani *et al*. 2007) has been well documented.

2.1.2.7 Antibiotis and lytic enzyme production

The antibiotics and lytic enzymes production are among the major defence mechanisms used by plant growth promoting bacteria against different types of microbes. The production of antibiotics such as polymyxin, pyrrolnitrin, pyoluteorin, viscosinamide (Glick *et al*. 2007), lipopeptide biosurfactants

(Raaijmakers *et al*. 2010), 2,4-diacetylphloroglucinol (Zhou *et al*. 2012; Asadhi *et al*. 2013) by various *Bacillus* and *Pseudomonads* strains against various phytopathogens has been well documented.

Lytic enzymes production such as chitinase and chitobiase by *Mucor, Trichoderma* and *Pseudomonas* (Uthao and Peberdy, 1991), β-1,3-glucanases, cellulases, lipase, chitinases and proteases by *Paenibacilllus* strains (Budi *et al*. 2000), chitinase by *B. subtilis* BS2 (Loganathan *et al*. 2014) pectinase by *Rhizobium*, *Azospirillum*, *Yersinia* and *Frankia* (Guo *et al*. 2015) etc. has been well related to their antagonistic behavior against various phytopathogens.

Several abiotic factors such as oxygen, temperature, carbon and nitrogen sources and micro-elements have been identified to influence antibiotic production by bacterial biocontrol agents (Raaijmakers *et al*. 2010). Kumar *et al*. (2012) reported that among twenty eight hydrolytic enzymes producers (*Bacillus* sp.) isolated from tomato rhizospheric soil isolate DPNSB-18 exhibited the highest (4.65 IU/ml) chitinase activity, isolate DPNSB-15 produce highest (0.79 IU/ml) protease activity, isolate DPNSB-20 exhibited the highest (0.39 IU/ml) β-glucanase activity and highest (0.75 IU/ml) cellulase production was made by isolate DPNSB-3.

2.1.2.8 Induced systemic resistance

One of important method of indirect mechanism used by PGPR is the induced systemic resistance (ISR), which results from the specific recognition of bacterial determinant(s) like lipopolysac-charides (LPS), flagella, siderophores, cyclic lipopeptides (2,4-diacetylphloroglucinol, homoserine lactones and volatiles like acetoin, 2,3-butanediol) by plant receptor(s) on the roots and is manifested as a reduction of the number of diseased plants or in disease severity as stimulate the host plant's defense responses by jasmonate and ethylene signaling pathways which induce pathogenesis-related proteins in plants such as phenylalanine ammonia lyase, peroxidase, polyphenol oxidase [catechol oxidase], beta -1,3 glucanase and phenolics (Loganathan *et al*. 2014) upon subsequent infection by a pathogen (Ongena *et al*. 2007; Lugtenberg and Kamilova, 2009).

Reduced bacterial canker in tomato (Girish and Umesha, 2005), Fusarium wilt (Liu *et. al*. 1995) and angular leaf spot in cucumber (Wei *et al*. 1996), *Xanthomonas oryzae* pv. Oryzae invasion in rice (Chitrashree *et al*. 2011) has been reported due to enhanced production of various defence related proteins induced in host plant by pretreatment with PGPR.

2.1.2.9 Reduced activity of plant antioxidant defence and Increased accumulation of osmolytes in plants

Generally, during ideal growth conditions, generation of reactive oxygen species (ROS) such as super oxide radicals, hydrogen peroxide and hydroxyl radicals occurs at low levels (Apel and Hirt, 2004). Water deficit conditions disrupt photosynthetic machinery and increase photorespiration, thus altering the normal homeostasis of cells, subsequently resulting in amassed production of ROS. Excess ROS results in enhanced lipid peroxidation and subsequent damage to proteins, DNA and lipids (Pompelli *et al*. 2010), but ROS also act as a signal for the activation of stress-response and defense pathways involving the activation of enzymatic components like superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR) and non-enzymatic components like cysteine, glutathione, ascorbic acid etc. (Pitzschke *et al*. 2006). Therefore, significant relation exits between drought stress and antioxidant enzyme activity. PGPR have been reported to mitigate the adverse effect of drought stress by lowering the antioxidant enzyme activity in the plant (Han and Lee 2005; Liu *et al*. 2007). For instance, reduced activity of the antioxidant enzymes APX (Vardharajula *et al*. 2011), APX, CAT (Naseem and Bano, 2014) conferring protection against drought stress in maize plants inoculated with PGPR has been well documented.

Plants under water deficit conditions accumulates osmolytes such as proline, glycine betaine and trehalose in order to alleviate cell turgidity losses (Rodriguez *et al*. 2009; Farooq *et al*. 2009). Elevated levels of amino acids and sugars, due to inoculation of PGPR have been reported in maize (Vardharajula *et al*. 2011), pepper and wheat (Yadav *et al*. 2005).

2.2 PGPR AS BIOFERTILIZERS FOR ENHANCED GROWTH, YIELDS AND FRUIT QUALITY

Biofertilizers are the substances, prepared from living microorganisms which, when applied to the seeds or plant surfaces adjacent to soil can colonize rhizosphere or the interior parts of the plants and thereby promotes root growth.

PGPR inoculants currently commercialized as novel solution for plant growth enhancements. The various direct/indirect mechanisms involved have a significant plant growth promoting potential, retaining more soil organic N and other nutrients in plant–soil systems, thus reducing the need of N and P fertilizers (Kennedy *et al*. 2004) and enhancing the release of nutrients (Lynch, 1990).

The effect of PGPR in crop productivity varies under laboratory, greenhouse and field trials. Because, soil is an unpredictable environment and an intended result is sometimes difficult to achieve. Climatic variations also have a large impact on the effectiveness of PGPR but sometimes unfavorable growth conditions in the field are to be expected as normal functioning of agriculture (Zaidi *et al*. 2009). Evidences are there on the maintenance of soil fertility by the rhizobacterial isolates to increase the availability of nutrients for plants. Under both natural agro-ecological niches and controlled soil environments, significant increase in yields of different crop plants has been observed following PGPR applications (Minorsky, 2008).

Inoculation of Capsicum plants with *Bacillus* spp. and *Streptomyces* spp. has resulted in remarkable increase in total number of fruits, fruit-weight and yields over uninoculated control (Datta *et al*. 2011). Increased seed germination, seedling vigour, plant growth and yield of tomato plants by *Pseudomonas fluorescens* (Girish and Umesha, 2005; Minorsky, 2008) and *Bacillus* spp. (Girish and Umesha, 2005; Guillen *et al*. 2006) has also been reported widely. PGPR inoculation to plants not only increased the plant growth, yield parameters but also improve the fruit quality and plant nutritional status. For instance, improved lycopene content and texture of tomato with *B. subtilis* BS2 (Loganathan *et al*. 2014), antioxidant activity and potassium contents of tomato with *Pseudomonas* + *Azotobacter* + *Azosprillum* + AMF (Ordookhani *et al*. 2010),

enhanced upatake of Fe, Zn, Mg, Ca, K and P by rice plants on application of Pseudomonads and Acinetobacter (Esitken *et al*. 2006), has been well documented.

Fertilizers are essential in large quantities for plant growth and development in agricultural fields. However, excessive application of chemical fertilizers has led to a world-wide concern about environmental degradation. Therefore, eco-friendly PGPR can provide an alternative to chemical fertilizers (Ahanthem and Jha, 2008). Phosphate solubilising bacteria along with 100 per cent recommended dose of NPK in cabbage resulted in maximum plant height, number of leaves, total chlorophyll content, biological yield, volume of head, average weight of head, yield of head per hectare, NPK content and available nitrogen, phosphorous content in soil after harvest of crop over absolute control, 100%RD of chemical fertilizers and biofertilizers alone (Kumawat *et al*. 2012).

Biofertilizers helped to save 25 per cent NP, as treatment of cabbage cv. Golden Acre with PSB + 75 per cent P + recommended dose of N & K or Azospirillum/Azotobacter + 75 per cent N + recommended dose of P & K reported a significant increase in plant height, non wrapper leaves, head weight and head shape over recommended doses of fertilizers (Gill *et al*. 2011). Kaushal and Kaushal (2013) reported that the application of MK5 isolate at 75% recommended dose of NP fertilizers not only increased the yields of cauliflower by 24% but also saved 31kg N/ha and 8 kg P/ha fertilizers over control (recommended doses of NPK). Similarly saving of 20% N and P fertilizers by incorporating the developed INM module (80% RD of NP fertilizers and PGPR) in capsicum have also been reported by Gupta *et al*. (2015).

2.3 PGPR AS BIOCONTROL AGENTS

The resulting intensive application of pesticides on crops affects the environment and health of humans and animals (Oliveira *et al.* 2008). Biocontrol is an emerging trend aimed at reducing chemical input while increasing plant fitness, productivity and resistance to diseases in sustainable agriculture involving several mechanisms such as improved nutrient availability, production of cell wall

lytic enzymes, competition for nutrients and prevention of growth of pathogens or induction of systemic resistance (Nihorimbere *et al.* 2011). Co-inoculation of PGPR has been demonstrated as a sustainable approach in plant health management. Prudent application of PGPR inoculants can expand the spectrum of biocontrol activity (Datta *et al*. 2011).

Kloepper *et al.* (1993) treated cucumber seeds with rhizobacterial strains like *Pseudomonas putida 89B-27* and *Serratia marcescens* 90–166 and recorded a significant decrease in incidence of bacterial wilt. Cho and Chung (1998) investigated effects of rhizobacteria on the early growth of cucumber and tomato plug seedlings and reported that *Azospirillum* spp., *Pseudomonas* spp. inhibited the growth of *Fusarium* spp., *Pythium* spp. and *Rhizoctonia* spp. on both plants. Yuan and Zhou (2006) reported that the rhizospheric isolates collected from tomato and pepper growing areas showed antagonistic activity against *Botrytis cinerea, F. graminearum, Colletotrichum capsici, Alternaria solani, P. capsici* and *Mycosphaerella melonis.*

Inoculation of plants with *Pseudomonas* spp. has been reported with significant reduction in Fusarium wilt in radish (Bakker *et al*. 2007), halo blight in beans (Alstroem, 1991), bacterial wilt in cucumber (Kloepper *et al*. 1993), Fusarium wilt in tomato (Cho and Chung, 1998), Damping off in tomato (Cho and Chung, 1998), tomato spotted wilt virus (TSWV) incidence in tomato (Hanafi and Fellah, 2006; Kandan *et al.* 2005), bacterial wilt of eggplant (Ramesh *et al*. 2009) and bacterial wilt of tomato (Guo *et al*. 2004).

Plants treated with *Bacillus* spp. resulted in reduced soft rot incidence in pepper (Park *et al*. 2013), fruit rot caused by *B. cinerea* in tomatoes (Sadfi *et al*. 2007), anthracnose caused by *Colletotrichum gloeosporioides* in pepper (Hong *et al*. 2002) phytophthora blight caused by *Phytophthora* spp. in pepper (Park *et al*. 2013; Lin *et al*. 2010), infestation of root not nematode *M. incognita* infecting eggplant (Dai *et al*. 2009; Shalaby and Sedik, 2008), bacterial wilt of eggplant (Ramesh *et al*. 2009) and tomato (Guo *et al*. 2004).

2.4 PGPR AS DROUGHT STRESS ALLEVIATORS

Abiotic stresses are considered to be the main source of plant growth and yield reduction; however, the intensity of these stresses varies with a number of soil and plant factors. A water deficit causes diminished water potential and turgor loss which results in stomatal closure, decline in the rate of photosynthesis, disruption of membrane integrity, protein denaturation and osmotic stress (Bartels and Sunkar 2005; Alcazar *et al*. 2011). Drought stress apart from photosynthesis inhibition, causes changes in chlorophyll contents, damages the photosynthetic apparatus, increases ethylene concentration and reduces the root growth (Iturbe-Ormaetxe *et al*. 1998). Reactive oxygen species (ROS) such as superoxide, hydrogen peroxide and hydroxyl radicals are produced, which can cause cellular damage through oxidation of lipids and proteins, chlorophyll bleaching, damage to nucleic acids, ultimately leading to cell death (Ashraf, 2009).

Plants develop self defense mechanisms by producing antioxidant enzymes like superoxide dismutase, ascorbate peroxidase, glutathione reductase and catalase (Abdel Latef, 2011) and non-enzymatic antioxidants like cellular redox buffers, carotenoids, flavonoids, tocopherols, ascorbate, glutathione, etc. also protect the plant from stress-induced adverse effects (Amirjani, 2012). The efficiency of antioxidant defense systems is related to the degree of plant tolerance against a stress (Gill and Tuteja, 2010).

Inoculation of plants with PGPR can amplify productivity of crops under a drought stress environment (Chanway and Holl, 1994). PGPR mitigates the impact of drought stress on plants through a process called rhizobacterial-induced drought endurance and resilience (RIDER), which includes physiological and biochemical changes. Various RIDER mechanisms include modification in phytohormonal levels, antioxidant defense, bacterial exopolysaccharides (EPS), and those associated with metabolic adjustments encompass accumulation of several compatible organic solutes like sugars, amino acids, polyamines etc. Production of heat-shock proteins (HSPs), dehydrins and volatile organic compounds (VOCs) also plays significant role in the acquisition of drought tolerance (Kaushal and Wani, 2015). Increased length of lateral root as well as

density and length of root hairs with PGPR strain led to a greater exchange surface area with soil and thus a higher water flux through the whole root system up to the leaves of the plant (Kechid *et al*. 2013).

Tomato plants when inoculated with the bacterium *Achromobacter piechaudii* under water stress conditions, reported a significant increase in fresh and dry weight of inoculated plants (Mayak *et al*. 2004). Significantly reduced ethylene production and increase in the fresh and dry weights of both drought-treated tomato and pepper seedlings with PGPR strain *Achromobacter piechaudii* ARV8 has been reported (Kohler, 2008).

Similar increase in root dry matter and aerial parts in canola (*Brassica napus*) whose seeds were inoculated with *Pseudomonas asplenii* is reported by Reed and Glick (2005). Figueiredo *et al*. (2008) reported that seed inoculation with two strains of *P. polymyxa* singly or in mixture enhanced the plant growth, nitrogen content and nodulation of bean under drought stress compared to uninoculated control. Marcia *et al.* (2008) reported enhanced phosphatase, nitrate reductase, Peroxidase (POD), Catalase (CAT) activity and proline accumulation under moderate and severe drought stress with inoculation of lettuce plants with *Pseudomonas mendocina*. Inoculation of seeds of *Phragmites australis* with *Pseudomonas asplenii* improved germination and protect the plants from growth inhibition under drought stress (Bashan *et al.* 2008).

Wang *et al.* (2012) reported that inoculation of cucumber plants with consortium of three plant-growth-promoting rhizobacterium strains (*Bacillus cereus* AR156, *Bacillus subtilis* SM21 and *Serratia sp.* XY21), could induced systemic tolerance to drought stress in cucumber plants, by protecting plant cells, maintaining photosynthetic efficiency, root vigor and increasing superoxide dismutase (SOD) activity along with leaf proline content. Similarly, enhanced the proline, chlorophyll and water content in basil plants (*Ociumum basilicum* L.) inoculated with consortium of *Pseudomonades* sp, *Bacillus lentus* and *Azospirillum brasilens* under stress conditions have been reported by Heidari *et al.* 2011.

Chapter-3

MATERIALS AND METHODS

The present investigations entitled **"Impact of indigenous plant growth promoting rhizobacteria and chemical fertilizers on soil health and productivity of capsicum (*Capsicum annuum* L.)"** were conducted in the section of Microbiology (Basic Sciences) and Soil Microbiology Laboratory (Soil Science and Water Management) at Dr Y S Parmar University of Horticulture and Forestry, Nauni-Solan, Himachal Pradesh during the years 2013-2016. A brief accounts of the materials used and methodologies adopted are presented under the following heads:

3.1 SAMPLE COLLECTION

The rhizospheric soil samples and roots of capsicum (*Capsicum annuum* L.) plants were collected from the eleven locations of agro-climatic zone-I, eight locations of agro-climatic zone-II and nine locations of agro-climatic zone-III of Himachal Pradesh taken for my research work (Fig. 1). In each location two sites and two sub-sites of each site were selected for sampling purposes. The samples were placed in plastic bags and stored in Soil Microbiology Laboratory of Department of Soil Science and Water Management for further isolation and analysis work.

Details of soil / root samples for isolation:

1. Agro climatic zones = 3
2. Locations = 28
3. Sites = 56
4. Rhizospheric soil samples = 112
5. Root samples = 112

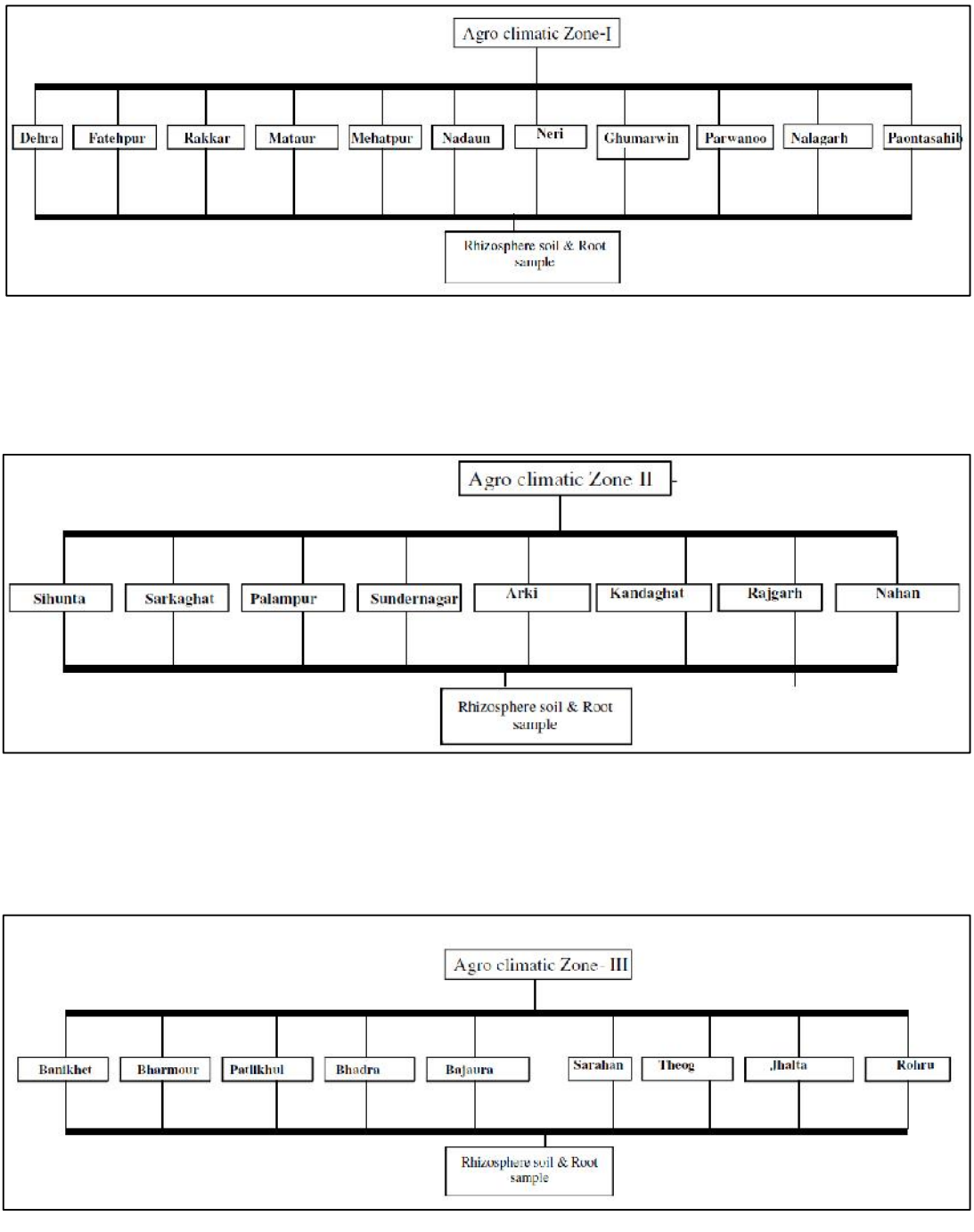


Fig. 1 Different locations of different agro-climatic zones of Himachal Pradesh used for sample (rhizospheric soil and capsicum root) collection

3.2 MEDIA

Composition of the media (Atlas, 1995) used for the study are as follows:

CONSTITUENTS	GRAM/LITRE
3.2.1 Nutrient Agar (NA)	
Beef extract	3.0
Peptone	5.0
NaCl	5.0
Agar	20.0
pH	6.5
3.2.2 Malt Extract Agar (MEA)	
Malt extract	20.0
Agar	20.0
pH	5.6 ± 0.01
3.2.3 Pikovskaya's (PVK) Broth	
Glucose	10.0
$Ca_3 (PO_4)_2$	5.0
$(NH_4)_2 SO_4$	0.5
KCl	0.2
$MgSO_4.7H_2O$	0.1
$MnSO_4$	0.004
$FeSO_4$	0.002
Yeast extract	0.5
Bromocresol purple	0.1
3.2.4 Pikovskaya's (PVK) Agar	
Pikovskaya's broth + 20 g agar	
3.2.5 Jensen's Medium (N-free medium)	
K_2HPO_4 anhydrous	1.0
$MgSO_4.7H_2O$	1.0
NaCl	0.5
$FeSO_4$	0.1

	Sucrose	20.0
	$Ca(CO_3)_2$	2 .0
	Agar	20.0

3.2.6 Chrome-azurol-S Agar

CAS	0.06
HDTMA	0.07
HCl	0.02
$FeCl_3$	0.2
Agar	20.0

3.2.7 King's Medium B

Proteose peptone	20.0
K_2HPO_4(anhydrous)	1.5
$MgSO_4.7H_2O$	1.5
Glycerol	15.0
Agar	20.0
pH	7.2

3.2.8 Potato Dextrose Agar Medium (PDA)

Dextrose	20.0
Potatoes extract	40.0
Agar	20.0

3.2.9 Luria Bertani (LB) Agar

Tryptophan	10.0
Yeast extract	5.0
NaCl	5.0
Agar	20.0
pH	7.5

3.2.10 Minimal Agar

KH_2PO_4	3.0
Na_2HPO_4	6.0
NaCl	5.0
NH_4Cl	2.0

	$Mg(SO_4)_2$	0.1
	Glucose	8.0
	Agar	15.0
	pH	7.0
3.2.11	**Tributyrin Agar**	
	Peptone	5.0
	Beef extract	3.0
	Tributyrin	1.0
	Agar	15.0
3.2.12	**Protease Medium**	
	Pancreatic digest of casein	5.0
	Yeast extract	2.5
	Glucose	1.0
	Skim milk	0.7g/10 ml (autoclaved separately)
	Agar	20.0
3.2.13	**Starch Agar Medium**	
	Starch (soluble)	20.0
	Peptone	5.0
	Beef extract	3.0
	Agar	20.0
3.2.14	**Czapek Mineral Salt Agar Medium**	
	$NaNO_3$	2.0
	KH_2PO_4/K_2HPO_4	1.0
	$MgSO_4.7H_2O$	0.5
	KCl	0.5
	Carboxymethyl cellulose (CMC)	5.0
	Peptone	2.0
3.2.15	**Dworkin and Foster Minimal Salt Medium**	
	KH_2PO_4	4.0
	Na_2HPO_4	6.0

Citric acid	2.0
Gluconic acid	2.0
$MgSO_4.7H_20$	0.2
Glucose	2.0
Agar	15.0

3.2.16 Pectinase Screening Agar Medium

Pectin	10.0
Diammonium ortho-phosphate	3.0
KH_2PO_4	2.0
$MgSO_4$	0.1
Agar	15.0
pH	7.5

3.3 CHEMICALS AND REAGENTS

Analytical grade (AR) chemicals and reagents obtained from standard company were used for different experiments under present investigations.

3.4 MICROBIOLOGICAL METHODS

3.4.1 Sterilization

Glasswares used were thoroughly washed in detergent water, running tap water followed by rinsing in distilled water. Glasswares were sterilized in hot air oven at 170^oC temperature for 20 min. All the media, water blanks were sterilized in autoclave at 15 pounds per square inch pressure of pure steam for 25 minutes unless mentioned otherwise. Laminar airflow chamber was sterilized by disinfectant followed by ultra violet (UV) radiation for atleast 30 min before start of the work.

3.4.2 Isolation and enumeration of rhizospheric and endophytic rhizobacteria

Isolation of rhizospheric and endophytic rhizobacteria from soil and root samples of capsicum was done by serial dilution method on nutrient agar medium.

3.4.2.1 Preparation of serial dilution

One gram of soil from each sample was taken and transferred to 9 ml sterilized water blank under aseptic condition and then agitated intermittently for 5-10 minutes on a shaker, which provided 10^{-1} dilution and further dilutions were prepared using sterile 9 ml blanks.

3.4.2.2 Detection of microbial count

Microbial count was performed by standard plate count technique (Wollum, 1982) by employing different media for different groups of microorganisms.

Suspension of 0.1ml from dilution blank was spread over pre-poured solid media *viz.*, Nutrient Agar, Jensen medium (Jensen, 1987) and Pikovskaya's medium (Pikovskaya, 1948) with the help of glass spreader under aseptic conditions for enumeration of bacteria, free nitrogen fixing bacteria and phosphate solubilizing bacteria, respectively. Plates were incubated in inverted position at 28±2°C for 48 hours. After the incubation period, the microbial count was expressed as colony forming unit per gram of soil ($cfug^{-1}$ soil).

3.4.2.3 Isolation of endophytic rhizobacteria

The root sample was surface sterilized by 0.2 per cent mercuric chloride ($HgCl_2$) for two min followed by repeated washing at least 6-7 times in sterilized distilled water. The surface sterility of roots was cross checked by incubating the surface sterilized roots in sterilized nutrient agar medium for 24 h at 28±2°C. One g of surface sterilized root sample was crushed in some amount of distilled water to produce slurry by using pestle and mortar under aseptic conditions and then the volume was to made to 10ml with sterilized distilled water. A known amount (0.1ml) of suspension was spread on pre-poured solid agar medium *viz.*, Nutrient Agar, Jensen medium and Pikovskaya's medium with the help of glass spreader under aseptic conditions as in case of rhizosphere and the microbial counts were expressed as colony forming unit per gram of root ($cfug^{-1}$ root).

3.4.3 Maintenance of the cultures

The isolated bacterial isolates were purified by streak plate method and maintained on the slants of respective medium at 4°C in refrigerator. The culture of *Phytophthora capsici,*

Fusarium oxysporum, *Rhizoctonia solani* and *Pythium* spp. were procured from Department of Plant Pathology, Dr. Y S Parmar University of Horticulture and Forestry, Nauni, Solan. The culture of *Colletotrichum capsici* (Sydow) (ITCC no. BI0004) and *Ralstonia Solanacearum* (Smith) (ITCC no. 6490) were procured from Division of Plant Pathology, Indian Type Culture Collection (ITCC), IARI, New Delhi. The fungal cultures were maintained on malt extract agar at 4°C. The bacterial culture was maintained on nutrient agar at 4°C. Sub-culturing of all cultures was done once in fortnight on respective medium at incubation temperature of 28±2°C.

3.5 MEASUREMENT OF GROWTH

3.5.1 Preparation of inoculum

A bacterial cell suspension (OD 1.0 at 540 nm) of 48 h old culture grown on nutrient broth at the rate of 10 per cent was used as inoculum in all experiments, unless mentioned otherwise.

3.5.2 Turbidity

Growth was monitored by measuring the change in absorbance of the inoculated broth at 540 nm using un-inoculated broth as blank.

3.5.3 Viable count

Appropriate dilutions of bacterial cell suspension were used to seed nutrient agar plates. The number of viable cells in the initial population was obtained by counting the number of colonies developed after incubating the plates and multiplying this figure by dilution factor.

3.6 SCREENING OF BACTERIAL ISOLATES FOR MULTIFARIOUS PLANT GROWTH PROMOTING TRAITS UNDER *IN VITRO* CONDITIONS

3.6.1 Phosphate solubilization

3.6.1.1 Qualitative estimation of phosphate solubilization

The P-solubilization ability of the isolates was carried out by method outlined by Pikovskaya's (1948).

The ability of bacteria to solubilize phosphorus was tested by streaking on the PVK agar plates containing known amount of tri-calcium phosphate ($Ca_3(PO_4)_2$) and was incubated for 72 h at 35 ± 2°C. Colonies showing solubilization halos (>0.1mm diameter) were selected. The halo diameter around the colony was calculated by subtracting colony size from total size.

3.6.1.2 Quantitative estimation of phosphate solubilization

PVK broth was used to study the solubilization of phosphorous. 50 ml of medium was dispensed in 250 ml of Erlenmeyer flask containing 0.5 per cent tri-calcium phosphate (TCP) and autoclaved at 15 pound per square inch of pure steam for 20 min. The bacterial inoculum was prepared by streaking 48 h old growth of bacterial inoculum on nutrient agar slants. The flasks were inoculated with 10 per cent (5 ml) of the bacterial suspension (OD 1.0 at 540 nm) and incubated at 30±2°C on rotating shaker at 100 rpm for 72 h. Flasks were withdrawn after 72 h and contents were centrifuged at 15,000 rpm for 20 min at 4°C. The culture supernatant was used for determination of the phosphorous as per method described by Bray and Kurtz (1945).

The procedure essentially consists of estimating soluble phosphorus formed by the action of phosphate solubilizing bacteria on tri-calcium phosphate. The soluble phosphorus formed was estimated spectrophotometrically and the results were extrapolated by standard curve drawn using di-hydrogen phosphate (Appendix 1.1). An aliquot (1.0 ml) from the culture supernatant was taken in 25 ml volumetric flask and diluted to about 5 ml with distilled water. Then 5 ml of ammonium molybdate was added and the mixture was thoroughly shaken and evolved CO_2 is released. The contents of the flasks were diluted to 20 ml. Added 1.0 ml of working solution of $SnCl_2$ and immediately the volume made to 25 ml. Then after 5-10 minutes as color develop, the color intensity was measured on spectrophotometer after 10 minutes at 660 nm using red filter.

3.6.2 Nitrogen fixing ability

Each of the purified isolate were seeded in a straight line on Jensen's medium and was incubated for 72 hr at 28±2^{o}C and the plates showing growth of bacteria in the form of bacterial colony were selected.

3.6.3 Siderophore production

The siderophore production was carried out by method outlined by Schwyn and Neilands (1987).

3.6.3.1 Qualitative estimation of siderophore by Chrome–azurol-S (CAS) plate assay

Siderophore production was detected by CAS plate assay method. Sterilized CAS blue agar was prepared by mixing CAS (60.5 mg/50ml distilled water) with 10 ml iron solution (1mM $FeCl_3.6H_2O$ in 10 mM HCl). This solution was slowly added to hexadecyltrimethyl ammonium bromide (HDTMA) solution, (HDTMA was prepared by dissolving 72.9 mg HDTMA in 40 ml distilled water). Thus, 100 ml CAS dye was prepared. 750 ml nutrient agar was mixed with 1,4 piperazine diethane sulphonic acid (30.24 g) and pH 6.8 was adjusted with 0.1N NaOH. It was autoclaved separately and then mixed with Chrome azurol- S (100 ml) under aseptic conditions and then the plates were prepared for further experiments.

A bit of 72 h old culture of each test bacterium was placed on prepoured blue coloured chrome-azurol-S agar (CAS) plates. Plates were incubated at 30 ± 2°C for 24 h and observed for production of orange halo around the bit.

Per cent siderophore production efficiency = $\frac{Z-C}{C} \times 100$

Where,

Z = Size of halozone (mm)

C = colony size (mm)

3.6.3.2 Quantitative estimation of siderophore using Chrome-aszurol-S (CAS) liquid assay method

Cell free extract of culture supernatant (0.1ml) was mixed with 0.5 ml Chrome-aszurol-S (CAS) assay solution along with 10µl of shuttle solution (0.2 M 5-Sulfosalicyclic acid). It was kept at room temperature for ten minutes and absorbance was recorded at 630 nm. The minimal medium was used as a blank and the reference (r) was prepared using exactly the same component except the cell free extract of culture supernatant. The siderophore units were calculated using formula.

Per cent siderophore unit = $\frac{Ar - As}{Ar} \times 100$

Where,

Ar is defined as absorbance at 630 nm of reference

As is absorbance at 630 nm of the test

3.6.4 Quantitative estimation of Indole-3-acetic acid (IAA)

The quantitative estimation of IAA production was carried out by method outlined by Gorden and Palleg (1957). Bacterial cultures were grown in modified Luria Bertani broth amended with (5 mM L-tryptophan, 0.065per cent sodium dodecyl sulphate and 1per cent glycerol) and incubated at 35°C under shaking conditions for 72 hours. The cultures were centrifuged at 15,000 rpm for 20 minutes and supernatant were collected and stored at 4°C.

In measuring IAA equivalents, 3 ml of supernatant was pipette into test tube and 2 ml Salkowski reagent (2 ml 0.5 M $FeCl_3$ + 98 ml 35 per cent $HClO_4$) was added to it. The tubes containing the mixture were left for 30 minutes (in dark) for color development. Intensity of color was measured spectrophotometrically at 535 nm. Similarly, color was also developed in standard solution of IAA (10-100 µg/ml) and a standard curve was established by measuring the intensity of this color (Appendix 1.2).

3.6.5 HCN Production

The HCN production was carried out by method outlined by (Bakker and Schippers, 1987). Bacterial cultures were streaked on pre-poured plates of King's B medium amended with 1.4 g/l glycine. Whatman No.1 filter paper strips were soaked in 0.5 per cent picric acid in 2 per cent sodium carbonate and were placed in the lid of each petriplates and plates were sealed with parafilm and were incubated at 35±2°C for 1-4 days. Un-inoculated control was kept for comparison of results. Plates were observed for change in color of filter paper from yellow to orange brown.

3.6.6 Ammonia production

The ammonia production was carried out by method outlined by (Lata and Saxena, 2003). Bacterial cultures were grown in peptone water (5 ml) in tubes. Tubes were incubated

at 30ºC for 4 days. After 4 days, 1ml of Neissler's reagent was added to each tube and observed for presence of yellow to brown color which indicated ammonia production.

3.6.6 ACC (1-aminocyclopropane-1-carboxylic acid) - deaminase activity

Screening for ACC deaminase activity of bacterial isolates was done based on their ability to use ACC as a sole nitrogen source. The bacterial isolates were spot inoculated on petri plates containing modified Dworkin Foster minimal salts medium (Dworkin and Foster, 1958) as amended with 10 ml micronutrient solution ($CaCl_2$- 200mg; $FeSO_4.7H_2O$- 200mg; H_3BO_3- 15mg; $ZnSO_4.7H_2O$- 20mg; Na_2MoO_4- 10mg; KI- 10mg; NaBr- 10mg; $MnCl_2$- 10 mg; $CoCl_2$- 5mg; $CuCl_2$- 5mg; $AlCl_3$- 2mg; $NiSO_4$- 2 mg; distil. water- 1000 ml). 3mM ACC was used as sole nitrogen source. Plates containing only DF salts minimal medium without ACC as negative control and with ammonium sulphate $(NH_4)_2SO_4$ (0.2per cent w/v) as positive control. The plates were incubated at 28ºC for 72 h. Growth of isolates on ACC supplemented plates was compared to negative and positive controls and was selected based on growth by utilizing ACC as nitrogen source.

3.6.7 Antagonistic activity of bacterial isolates against test pathogens

Agar streak plate method was used to test the efficacy of bacterial isolates against the test fungus. A loop full of 48 h old culture of each isolate were streaked a little below the centre of the prepared MEA petri plate and incubated at 35°C for 24 h to check contamination. Mycelial disc of 7 days old culture of the test fungal pathogen (*Fusarium oxysporum* (Martius), *Rhizoctonia solani*, *Pythium* spp., *Phytophthora capsici* and *Colletotrichum capsici* (Sydow) was placed separately on one side of the streak in each plate. A check inoculated with the test pathogen only was kept for comparison. Then plates were incubated at 28±1°C for 7 days and per cent growth inhibition was calculated as described by Vincent (1947).

$$I = \frac{C-T}{C} \times 100$$

Where;

I	=	Per cent growth inhibition
C	=	Growth of fungus in control
T	=	Growth of fungus in treatment

The anti-microbial activity against the bacterial pathogen *Ralstonia solanacearum* was evaluated by measuring the inhibition zones around the antagonistic bacteria, in the lawn of the pathogen prepared by spreading 24h old culture of the pathogen on the nutrient agar plate. A bit of 24 hr old culture of each test bacterium was placed on the prepared lawn of the test bacterial pathogen. Plates were incubated at 28±1°C for 72hr and observed for production of clear zone around the bit.

3.7 MORPHOLOGICAL, PHYSIOLOGICAL AND METABOLIC CHARACTERIZATION OF SELECTED BACTERIAL ISOLATES

The most efficient bacterial isolates selected on the basis of plant growth promoting traits and *in vitro* antagonistic activity against test pathogens were subjected to morphological, physiological and biochemical characterization by criteria of Bergey's Manual of Systematic Bacteriology (Claus and Berkley, 1986).

3.7.1 Morphological characterization

Morphological characteristics of isolates including colony morphology, Gram's reaction and cell shape were investigated.

3.7.2 Physiological characterization of selected isolates

Separate experiments were performed for optimization conditions (pH, temp. and salinity) for growth of selected bacterial isolates.

3.7.2.1 Effect of pH on growth

3 ml nutrient broth was taken in 5 ml test tubes. The medium was adjusted to various pH (2, 3, 4, 5, 6, 7, 8 and 9) using 0.1 N NaOH or 0.1 N HCl as the case may be. Each tube was inoculated with 0.1 ml of 48 h old bacterial cell suspension (OD 1.0 at 540 nm) of selected isolates and observed for turbidity.

3.7.2.2 Effect of temperature on growth

3 ml of nutrient broth was taken 5 ml test tubes and inoculated with 0.1 ml of 48 h old bacterial cell suspension (OD 1.0 at 540 nm). Each culture is incubated at various temperatures (20, 25, 30, 35, 40 and 45°C) and observed for turbidity.

3.7.2.3 Effect of salinity on growth

Nutrient broths with various concentration of NaCl (2, 4, 6, 8 and 10 per cent) were prepared. 3 ml of nutrient broth was taken 5 ml test tubes, separately and inoculated with 0.1 ml of 48 h old bacterial cell suspension (OD 1.0 at 540 nm) and observed for turbidity.

3.7.3 Metabolic characterization of selected isolates

3.7.3.1 Cell wall degrading enzyme production

3.7.3.1.1 Chitinase assay

The Chitinase assay was carried out by method outlined by Robert and Selitrennikoff (1988).

Preparation of colloidal chitin

Colloidal chitin was prepared by the method (Berger and Reynolds, 1958) given below:

1. Powdered chitin was digested overnight with conc. HCl at 4°C.
2. After digestion, distilled water was added carefully and mixed thoroughly, centrifuged and the supernatant was removed carefully (the first two-three wash is highly acidic).
3. Washing was continued with distilled water until the pH of solution reaches around 4.0.
4. pH of the colloidal chitin solution was adjusted to pH around 6-6.5 by using 2N NaOH.
5. The chitin suspension in water was centrifuged and the pellet was collected, dried and used at 0.3 per cent in minimal salt media.

The bacterial cultures were spotted on to the prepared minimal agar medium amended with 0.3per cent colloidal chitin and the plates were incubated at 30°C for 7 days. Development of halo zone around the colony after addition of iodine was considered as positive for chitinase enzyme production.

3.7.3.1.2 Proteolytic enzymes

The proteolytic activity was carried out by method outlined by Fleming *et al*. (1975). All the bacterial isolates were screened for proteolytic activity by plate assay method on skim milk agar (nutrient agar (100 ml) supplemented with separately sterilized skim milk). Spot inoculation with 24 h old bacterial culture was done on skim milk agar plate. Plates were incubated at 37°C for 24-48h and observed for proteolysis i.e. clear zone (diameter, mm) produced around the spot.

3.7.3.1.3 Amylolytic enzymes

The amylolytic activity was carried out by method outlined by Shaw *et al*. (1995). All bacterial isolates were screened for amylolytic activity by plate assay method. Spot inoculation with 24h old bacterial culture was done on starch hydrolysis agar plate. Plates were incubated at 37°C for 24-48h. Iodine was poured on the agar plates after incubation. Agar plates were observed for starch hydrolysis i.e. clear zone (diameter, mm) produced around the spot.

3.7.3.1.4 Cellulolytic enzymes

The cellulolytic activity was carried out by method outlined by Ghose *et al*. (1987). All bacterial isolates were screened for cellulolytic activity by plate assay method. Spot inoculation with 24h old bacterial culture was done on Czapek Mineral Salt Agar medium. Plates were incubated at 37°C for 24-48h. HDTMA was poured on agar plates after incubation. Agar plates were observed for starch hydrolysis i.e. clear zone (diameter, mm) produced around the spot.

3.7.3.1.5 Lipase enzymes

Lipase activity was carried out by method outlined by Kumar *et al.* (2012). All bacterial isolates were screened for lipase production by plate assay method. Spot inoculation with 24h old bacterial culture was done on TBA medium. Plates were incubated at 37°C for 24-48h. Agar plates were observed for clear zone (diameter, mm) produced around the spot.

3.7.3.1.5 Pectinase enzymes

Pectinase activity was carried out by method outlined by Raju and Divakar (2013). All bacterial isolates were screened for pectinase production by plate assay method. Spot inoculation with 24h old bacterial culture was done on pectinase screening agar medium. Plates were incubated at 37°C for 24 h. The plates were flooded with 50mM iodine solution and incubated for 15 min at 37°C. Agar plates were observed for clear zone (diameter, mm) produced around the spot.

3.7.3.2 Biochemical characterization

Biochemical characteristics of the isolates like methyl- red test, citrate utilization, starch, casein, gelatin hydrolysis, hydrogen sulphide production, catalase test and ability to ferment sugars were carried out by standard methods.

3.8 PREPARATION OF LIQUID FORMULATION AND SEED TREATMENT

3.8.1 Procurement of seeds

Seeds of capsicum (*Capsicum annuum* L.*)* variety California Wonder were procured from Seed Technology and Production Centre of Dr Y S Parmar University of Horticulture and Forestry Nauni, Solan (H.P.).

3.8.2 Seed surface sterilization

Capsicum seeds were surface sterilized in 0.2 per cent mercuric chloride ($HgCl_2$) solution for five minutes and rinsed several times with sterilized distilled water. Sterilization was checked by placing surface sterilized and unsterilized seeds on nutrient agar plates. The bacterial growth, if any, around the seeds was recorded after 48 h of incubation.

3.8.3 Preparation of liquid formulation

The population density (1.5 OD at 540 nm) that resulted in formation of 10^8 cfu/ml of Bacterial isolate was used for preparation of liquid formulation.

3.8.4 Seed inoculation

Seeds were soaked in liquid culture of different bacterial formulations in sterilized petri plate and thereafter the seeds were sown as per different experimental details.

3.9 NET HOUSE STUDIES

3.9.1 Preparation of potting mixture

Soil obtained from a furrow slice (0 to 15 cm depth) from a forest block of Department of Silviculture and Agroforestry, UHF, Solan was sieved through 2 mm sieve and used for pot culture experiment. The potting mixture was prepared by mixing sand, soil and farm yard manure (FYM) in a ratio of 1:2:1 and mixture was sterilized by 3 successive autoclave cycles of 1h each at 121°C. 1.8 Kg of potting mixture was filled in 15cm diameter pots.

The soil mixture was neutral in reaction (pH 6.6), EC in normal range (0.42 dSm^{-1}), medium organic carbon (0.92 per cent) content with sandy loam texture having available N (298.7 kg ha^{-1}) and K (194.9 kg ha^{-1}) contents in medium range, whereas, P content (24.6 kg ha^{-1}) in high range.

3.9.2 To study the efficacy of selected bacterial antagonists for the control of Capsicum bacterial wilt under net house conditions

Five selected strains of bacteria with the greatest inhibition under *in vitro* test were further tested in net house on capsicum plants to evaluate their ability to control bacterial wilt *in planta.*

A) At seedling stage

1.8 Kg of the sterilized potting mixture, was mixed with 100 ml of *R. solanacearum* (10^8 cfu/ml) by culturing *Ralstonia solanacearum* in nutrient broth for 48h at 28°C (10^9 cfu/ml) at 120 rpm on rotary shake and placed in 15cm diameter pots. One week after incorporation of the pathogen into the soil and one day before transplanting the seedlings, antagonists were incorporated in the soil at the rate of 75 ml per pot at 10^9cfu ml^{-1}. Four weeks old capsicum seedlings were root dipped in antagonistic bacterial suspension (10^9cfu/ml) for 60 min and transplanted into pathogen-antagonist mixture soil (Lemessa and Zeller, 2007).

B) At maturity stage

Capsicum plants at the maturity stage in all treatments were inoculated with the pathogen by punching each plant with sterilized needle at the base of stem above upper

secondary root, subsequently 80 ml of the suspension were poured in every pot over wounded area. After inoculation, all pots were covered by polyethylene bags for 24 h to maintain high humidity as suggested by Algam *et al*. (2010).

Treatments were replicated four times with three plants per pot with only pathogen inoculated as control.

Treatments:

T_1: Only Pathogen

T_2: Inoculated with PGPR1

T_3: Inoculated with PGPR2

T_4: Inoculated with PGPR3

T_5: Inoculated with PGPR4

T_6: Inoculated with PGPR5

Total treatments: 6

Replications: 3

Design: Completely Randomized Design (CRD)

Observations:

1. Seed germination
2. Vigour index
3. Disease severity
4. Total biomass and NPK content

3.9.3 To study the effect of indigenous PGPR inoculum for physiological efficacy under water stress conditions

Field capacity was determined by using tensiometer method and also confirmed by draining the soil for 72 h after saturation. Three levels of water stress i.e. 80, 60 and 40 per cent of the field capacity (FC) was determined and maintained as described by Ghorbanpour *et al*. (2013). Two efficient PGPR isolates of capsicum were used for seed inoculation. The control seeds will be treated with sterilized culture broth. The experiment will be carried under net house conditions out by taking the following treatments:

Treatments:

T_1: 100 per cent of field capacity

T_2: 80 per cent of field capacity

T_3: 80 per cent of field capacity + PGPR 1

T_4: 80 per cent of field capacity + PGPR2

T_5: 60 per cent of field capacity

T_6: 60 per cent of field capacity + PGPR1

T_7: 60 per cent of field capacity + PGPR2

T_8: 40 per cent of field capacity

T_9: 40 per cent of field capacity + PGPR1

T_{10}: 40 per cent of field capacity + PGPR2

Total treatments: 10

Number of replications: 3

Experimental Design: Completely Randomized Design (CRD)

Observations:

1. Root/shoot length and biomass
2. N, P content and their uptake
3. Relative water content in leaves
4. Total chlorophyll content
5. Total amino acid content
6. Antioxidant enzyme assays: Superoxide dismutase, Peroxidase and Catalase

3.9.4 To study the influence of selected PGPR at varying levels on N and P on productivity of capsicum and soil properties

The applications of N and P fertilizers were made through urea (46 per cent N) and single super phosphate (16 per cent P_2O_5) as per treatment. For 100 per cent, 80 per cent and 60 per cent recommended doses of fertilizers (RDF) urea was applied @ 165.95 mg, 132.76 mg and 99.57 mg per pot, respectively, whereas, single super phosphate was applied @ 362.62 mg, 290.09 mg and 217.57 mg/pot, respectively. A blanket application of full doze of K i.e @ 68.70 mg/pot was applied through muriate of potash (60 per cent K_2O).

Treatments:

T_1: 100 per cent recommended dose (RD) of N and P

T_2: 80 per cent RD of N and P

T_3: 60 per cent RD of N and P

T_4: 40 per cent RD of N and P

T_5: 80 per cent RD of N and P + PGPR1

T_6: 80 per cent RD of N and P + PGPR2

T_7: 80 per cent RD of N and P + PGPR3

T_8: 80 per cent RD of N and P + PGPR4

T_9: 60 per cent RD of N and P + PGPR1

T_{10}: 60 per cent RD of N and P + PGPR2

T_{11}: 60 per cent RD of N and P + PGPR3

T_{12}: 60 per cent RD of N and P + PGPR4

T_{13}: 40 per cent RD of N and P + PGPR1

T_{14}: 40 per cent RD of N and P + PGPR2

T_{15}: 40 per cent RD of N and P + PGPR3

T_{16}: 40 per cent RD of N and P + PGPR4

Total treatments: 16

Replications: 3

Experimental Design: Completely Randomized Design (CRD)

Observations:

A: Plant parameters

1) Plant traits such as root\shoot length and biomass.
2) Total NPK content in plant and their uptake.
3) Yield contributing factors like number of fruits per plant and fruit weight.

B: Soil properties

1) Available NPK content of soil
2) pH and EC
3) Organic carbon
4) Total microbial counts
5) Microbial biomass

3.10 FIELD STUDIES

3.10.1 Studies on the efficacy of best PGPR isolates and with the optimum NP doses as recorded from net house studies

To study the effect of selected PGPR with optimum doses of N and P chemical fertilizers on productivity of Capsicum field trials at Dharja, (Solan) was carried out by taking the following treatments:

Treatments:

T1: Control (100 per cent recommended dose (RD) of NPK + FYM*)

T2: 80 per cent RD + PGPR1

T3: 80 per cent RD + PGPR2

T4: 80 per cent RD + PGPR1 + PGPR2

T5: 60 per cent RD + PGPR1

T6: 60 per cent RD + PGPR2

T7: 60 per cent RD + PGPR1 + PGPR2

Total treatments: 7

Replications: 3

Experimental Design: Randomized Block Design (RBD)

(*Recommended dose of N, P_2O_5 and K_2O was 100, 76 and 45 Kg ha^{-1}, respectively. FYM (20-25 t ha^{-1}) and dose of K will be applied as per recommended level given in package of practices).

The soil was neutral in reaction (pH 7.1), EC in normal range (0.32 dSm^{-1}), medium organic carbon (0.42 per cent) content having available N (290.7 kg ha^{-1}) and K (214.6 kg ha^{-1}) contents in medium range, whereas, available P content (35.3 kg ha^{-1}) was in high range.

Observations:

A: Plant parameters

1) Plant traits such as root\shoot length and biomass.
2) Total NPK content in plant and their uptake.
3) Yield contributing factors like number of fruits per plant and fruit weight.

B: Soil Properties

1) Available NPK content of soil
2) pH and EC
3) Organic carbon
4) Total microbial count
5) Microbial biomass
6) Soil enzymes: Dehydrogenase, phytase and phosphatase

3.11 PLANT PARAMETERS

3.11.1 Germination per cent

Germination per cent was calculated as the number of seeds sown and the number of seeds germinated. It was expressed in percentage.

3.11.2 Vigor index

The vigor index was calculated using the formula as described by Abdul Babi and Anderson (1973).

Vigor index = (Mean root length + Mean shoot length) × Germination (per cent)

3.11.3 Analysis of seedling traits

One month old, capsicum seedlings (three from each pot of each replication) were obtained for the analysis of different traits such as: shoot and root characteristics.

3.11.3.1 Shoot characteristics

3.11.3.1.1 Shoot length

The shoot length of the seedlings was measured in centimeters (cm) from soil line to the highest tip of the seedling.

3.11.3.1.2 Shoot biomass

The seedlings were washed with water. Excess of water was wiped out by placing it between the folds of filter paper. Then the seedlings were cut at collar with a secateurs and shoot fresh weight was taken and expressed in mg/plant.

3.11.3.2 Root characteristics

3.11.3.2.1 Root length

The length of tap root was recorded in centimeters (cm) using measuring scale by placing it horizontally on the ground.

3.11.3.2.2 Root biomass

The seedlings were washed with excess of water was wiped out by placing it between the folds of filter paper. Then the seedlings were cut at collar with a secateurs and root fresh weight was taken and expressed in mg/plant.

3.11.4 Analysis of plant parameters

3.11.4.1 Plant characteristics

Shoot/root length and biomass were observed in similar manner as those of seedlings.

3.11.4.2 Fruit characteristics

3.11.4.2.1 Number of fruits per plant

Numbers of fruits harvested from plants at each harvest were counted and freshly summed up to work out the average number of fruit per plant.

3.11.4.2.2 Fruit weight (g)

The weight of the fruits from each plant at each picking was recorded and expressed in grams (g). The average of each treatment is worked out to calculate average fruit weight.

3.11.4.3 Yield per plant (kg)

The weight of the fruits from each plant at each picking was recorded and the total yield from all the pickings gave the fruit yield/plant, expressed as kg plant^{-1}.

3.11.5 Leaf characteristics

3.11.5.1 Leaf area

Total leaf area of three randomly selected leaves from each treatment was measured using leaf area meter (LI-Cor-3100) and average area per leaf expressed in cm^2.

3.11.5.2 Estimation of Chlorophyll

The chlorophyll content was determined by following the methods given by Withem *et al*. (1971). 1 gm of fresh leaves both from treatment and control were dipped in 10 ml of 80 per cent acetone and kept at room temperature overnight. Then absorbance was read at 663 and 645 nm for chlorophyll a and b, respectively. The quantity of chlorophyll content was calculated by using standard formulae and expressed as mg of chlorophyll per gram fresh weight of leaf tissues.

Chlorophyll a = 12.70 (A663) – 2.69 (A 645) × V/w × 1000

Chlorophyll b = 22.9 (A645) – 4.68 (A 663) × V/w × 1000

Total chlorophyll = 20.0 (A645) + 8.02 (A 663) × V/w × 100

where, A 663 =optical density at 663

A645= optical density at 645

V=Final volume of 80 per cent acetone chlorophyll extract

W=Fresh weight in gm of tissue extract

3.11.5.3 Leaf relative water content (RWC)

The 4^{th} fully expanded leaf (from the top) was weighed immediately after cutting (FW), hydrated to full turgidity by floating in distilled water, kept at room temperature (22°C) for 24 h to obtain turgid weight (TW) and finally oven dried for 48 h at 70°C to measure dry weight (DW). RWC was calculated by the following formula as described by Jeon *et al*. (2006):

RWC = [(FW − DW) / (TW − DW)] × 100.

3.11.5.4 Antioxidant enzyme extraction and assays

3.11.5.4.1 Superoxide dismutase (SOD, EC 1.15.1.1)

Superoxide dismutase activity was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) as described by Beauchamp and Fridovich (1971). One gram of samples were homogenized in 10 ml of 0.1M Tris-HCl buffer (pH 7.5) containing 1 mmol EDTA, 3 per cent polyvinyl pyrrolidone and 1 mmol $CaCl_2$ in a

pre-chilled pestle and mortar. The homogenates were filtered through four layers of cheese cloth and centrifuged at 10,000 rpm for 20 minutes at 4°C. Supernatants thus obtained were used as crude extract for assay.

The reaction mixture contained 1.98 ml of 50 mmol Tris-HCl (pH 7.8), 0.2 ml of 14 mmol methionine, 0.2 ml of 0.1 mmol EDTA, 0.1 ml of 60 mmol nitroblue tetrazolium (NBT) and 0.02 ml of enzyme extract. 0.1 ml of 3 mmol Riboflavin was added just before initiation of the reaction by switching on light (60 W-flurescent lamps). After 5 minutes of incubation the reaction was terminated by switching off the light. Identical solutions that were kept under dark, did not develop color served as blanks. The absorbance was read at 560 nm in the spectrophotometer against the blank. One enzyme unit is defined as the amount of enzyme, which could cause 50 per cent inhibition of the photochemical reduction of NBT. The activity was expressed as unit/gm fresh weight.

Per cent inhibition = {(V-v) × 100 × dilution} /V

Where,

V = Rate of non-irradiated reaction mixture

v = Rate of irradiated reaction mixture

Activity (units/ml) = (percent inhibition) / (50 percent × Vs)

Activity (units/gm fresh weight) = (units/ml) / {gm sample/total enzyme extract (ml)}

Where,

Vs = sample volume (ml)

50 per cent = inhibition of the rate of NBT reduction as per the unit definition

3.11.5.4.2 Catalase (CAT, EC 1.11.1.6) activity

Total CAT activity was assayed according to the method of Chandlee and Scandalios (1984). Five hundered mg of frozen leaves were homogenized in 5 ml of ice cold 50mM sodium phosphate buffer (pH 7.5). The extracts were centrifuged at 12,500 rpm for 20 minutes at 4°C. Supernatants thus obtained were used for measuring enzyme activity. The assay mixture contained 2.6 mL of 50 mmol L^{-1} potassium phosphate buffer (pH 7.0), 0.4 mL of 15 mmol L^{-1} H_2O_2 and 0.05 mL of enzyme extract. Decline in absorbance at 240 nm

per minute was observed. Unit activity was taken as the amount of enzyme, which decomposes 1 mole of H_2O_2 in 1 min. The enzyme activity was expressed in U g^{-1} fresh weight.

Activity (units/ml) = (ΔA / min × 1000) / (ε × Vs)

Where,

ε = 43.6

Vs = sample volume (ml)

Activity (units/gm fresh weight) = (units/ml) / {gm sample/total enzyme extract (ml)}

3.11.5.4.3 Peroxidase (POX, EC 1.11.1.7) activity

The assay was carried out as described by Addy and Goodman (1972). One hundered mg of frozen leaves were homogenized in 2 ml of 50 per cent ethanol in pre-chilled pestle and mortar and the homogenate was centrifuged at 10,000 rpm for 10 minutes at 4°C. The supernatant thus obtained were used within 4 hours for estimation of enzyme activity.

The reaction mixture contained 3 ml of buffered pyrogallol (0.05 M of pyrogallol in 0.1 M phosphate buffer, pH 7.0), 0.1 ml of H_2O_2 (0.1 per cent v/v) and 0.05 ml of enzyme extract. The change in optical density was measured at 430 nm after every 30 seconds for 2 minutes. One enzyme unit is defined as that amount of enzyme which catalyses the oxidation of one mole of pyrogallol per minute. The enzyme activity was expressed in U g^{-1} fresh weight.

Activity (units/ml) = (ΔA / min × Vt × dilution factor) / (ε × Vs)

Where,

Vt = assay mixture volume (3.15 ml)

Vs = sample volume (ml)

ε = 25.5

4 = derived from unit definition and principle

Activity (units/gm fresh weight) = (units/ml) / {gm sample/total enzyme extract (ml)}

3.11.5.5 Estimation of soluble protein

Soluble protein was estimated in the above enzyme extracts as described by Lowry *et al.* (1951). 5 ml of freshly prepared reagent (50 ml sodium carbonate (2per cent w/v) in 0.1 N sodium hydroxide, 0.5 ml of copper sulphate (1per cent, w/v) and 0.5 ml of sodium potassium tartrate (2per cent w/v) was added to 1 ml of suitably diluted enzyme extract and mixed immediately. After 10 minutes, 0.5 ml of 1 N Folin's reagent was added and the test tubes were shaken vigorously to ensure proper mixing. Intensity of the color developed was measured after 10 minutes at 660 nm against the reagent blank. The amount of protein was calculated from the standard curve prepared using bovine serum albumin (20-200 µg ml^{-1}) (Appendix 1.3).

3.11.6 Total nutrients analysis (N, P and K) in plant material

3.11.6.1 Sample collection and preparation

The samples collected were immediately weighed and brought to the laboratory in paper bags. All the samples were washed in series, first with tap water then 0.1 N HCl followed by distilled water. The washed samples were allowed to dry in air subsequently in oven at 60°C till constant weight. Oven dried samples of shoots and roots were ground and sieved (40 meshes) and stored in butter paper bags for chemical estimation.

3.11.6.2 Digestion of samples

3.11.6.2.1 Total N

The digestion of 0.50 g samples for estimating nitrogen was carried out in the concentrated H_2SO_4 in presence of digestion mixture having following composition:

Potassium sulphate (K_2SO_4)	=	400 parts
Mercuric oxide (HgO)	=	3 parts
Copper sulphate ($CuSO_4.5H_2O$)	=	20 parts
Selenium powder (Se powder)	=	1 part

3.11.6.2.2 Total P and K

For the estimation of P and K, 0.5 g plant sample was digested in diacid mixture prepared by mixing nitric acid and perchloric acid (4:1) taking all relevant precautions as suggested by Piper (1966).

3.11.6.3 Estimation of nutrient elements

The nitrogen was estimated in Kel Plus Classic Dx. Auto Analyzer. Phosphorus was determined by Vanado molybdo-phosphoric yellow colour method and potassium was determined by flame-photometer (Jackson, 1973).

3.11.6.4 Nutrient uptake

The total nutrient uptake (NU) by plant on dry weight basis was worked out by using the formula:

$$\text{NU (mg/plant)} = \frac{\text{per cent NC} \times \text{Biomass (g) (root)} + \text{per cent NC} \times \text{Biomass (g) (shoot)} + \text{per cent NC} \times \text{Biomass (g) (fruit)}}{100} \times 1000$$

3.11.6.5 Disease assessment (Bacterial wilt caused by *R. solanacearum*)

Disease ratings were recorded two weeks after pathogen challenge according to the scale given by Kempe and Sequeira (1983).

Scale		Rating
0	-	No symptoms
1	-	1-25 per cent wilting
2	-	26-50 per cent wilting
3	-	51-75 per cent wilting
4	-	76 -100 per cent wilted or dead

The disease incidence and biocontrol efficiency were calculated as follows:

$$\text{DI} = \frac{\text{Total no. of diseased plants}}{\text{Total no. of plants observed}} \times 100$$

Disease severity = {∑(rating × no. of plants rated)/Total no. of plants observed × highest rating} × 100

Biocontrol Efficacy = {Disease incidence of control - Disease incidence of plants treated with antagonist/Disease incidence of control} × 100

3.12 PHYSICO-CHEMICAL PROPERTIES, AVAILABLE NUTRIENTS, SOIL ENZYMES AND MICROBIOLOGICAL STATUS OF POTTING MIXTURE AND FIELD SOIL

3.12.1 Soil physico-chemical properties

The physico-chemical properties were recorded before and at the end of the experiment. The details of methods adopted for different physico-chemical properties are as:

3.12.1.1 pH and electrical conductivity

The soil pH was determined in 1:2.5::soil: water suspension and the electrical conductivity of the supernatant liquid was recorded and expressed in dSm^{-1} (Jackson, 1973).

3.12.1.2 Organic carbon

Organic carbon (OC) was determined by chromic acid titration method of Walkley and Black (1934).

3.12.1.3 Available Nitrogen

Available N was determined by alkaline potassium permanganate method of Subbiah and Asija (1956).

3.12.1.4 Available Phosphorous

0.5 N $NaHCO_3$ at 8.5 pH was used to extract available P (Olsen *et al*. 1954) and determined spectrophotometrically.

3.12.1.5 Available Potassium

Available K was extracted by normal neutral ammonium acetate (Merwin and Peech, 1951) and determined on flame photometer.

3.12.2 Soil Enzyme

3.12.2.1 Phosphatase

The phosphtase enzyme estimation was carried out by method given by Tabatabai and Bremner (1969). One gram of soil taken in test tube was incubated with 1ml of 5mM

buffered sodium p-nitrophenyl phosphate in acetate buffer (pH 5.2) and 0.3ml toluene at 37 °C for 1 hour. Determination of p-nitrophenol involved the colorimetric analysis of the extract obtained by treating the incubated soil sample with 4 ml water, 10 ml of 0.5 M NaOH and by filtering it through Whatman no. 42 filter paper. Asorbance of yellow colour of p-nitrophenol released was determined spectrophotometrically at 420 nm wavelength. The standard curve was prepared by p-nitrophenol (10-100 ppm) (Appendix 1.4). The result was expressed as µ mole of p-nitrophenol released per gram soil per hour (µmole p-nitrophenol g^{-1}soil h^{-1}).

3.12.2.2 Dehydrogenase

The dehydrogenase enzyme estimation was carried out by method given by Casida L E (1977). One gram of air-dried soil was taken in air-tight screw capped test tube, was incubated with 0.2 ml of 3 per cent 2,3,5 – Triphenyl tetrazolium chloride (TTC) and 0.5 ml of 1per cent glucose solution at 28 ± 0.5°C for 24 h, followed by addition of 10 ml of methanol and allowed to stand for 6 h. Clear pink coloured supernatant liquid was withdrawn and readings were taken spectrophotometerically at a wavelength of 485 nm (blue filter). Extrapolate Triphenyl formazen (TPF) formed from the standard curve drawn in the range of 10 to 90 µg TPF ml^{-1} (Appendix 1.5). The results were expressed as TPF (mg) formed per h per g soil.

3.12.2.3 Phytase

The phytase enzyme estimation was carried out by method given by Boyce *et al*. (1964).

3.12.2.3.1 Inorganic phosphate standard curve

Phosphate standard solutions are conveniently prepared by initially making a 1 mM stock solution of KH_2PO_4 in water. This may then be diluted to prepare a range of phosphate standard concentrations ranging from 200 to 1,000 nmol/ml phosphate. To 1.0 ml of each reference standard is added 1.0 ml of 0.5 M tri-chloroacetic acid (TCA) solution and 1 ml of reagent A (prepared fresh by dissolving 5.0 g of $FeSO_4.7H_2O$ in 90 ml of distilled water and adding 10.0 ml of 8.0per cent (w/v) ammonium molybdate. The 8.0per cent ammonium

molybdate solution is prepared by dissolving 8.0 g of ammonium molybdate in 50 ml of distilled H_2O. Then, 27.0 ml of 10 M H_2SO_4 is added and the solution is brought to 100 ml with distilled water). After standing at room temperature for 5 min, the absorbency of each was determined at 660 nm (Appendix 1.5).

3.12.2.3.2 Phytase assay

0.5 gm soil was inoculated with 0.5 ml of freshly prepared sodium phytate solution (0.1per cent (w/v) sodium phytate made up in 200 mM sodium acetate buffer, pH 5.5) for 15 min at 50°C. The individual assay component solution was pre-equilibrated to 50°C immediately prior to assay commencement. Assay was terminated by the addition 1.0 ml of 0.5 M TCA solution. Blank was prepared by separate incubation of substrate and enzyme at 50°C over the 15-min assay period, with subsequent addition of TCA followed by substrate addition. Color development was achieved by adding 1.0 ml of reagent A to all tubes (assay and blank) after assay termination and absorbency at 660 nm was determined after standing for 5 min at room temperature. The number of nanomoles of inorganic phosphate produced under the assay conditions used was determined using the standard curve generated (Appendix 1.6).

Enzyme unit $\mu mol\ ml^{-1}\ min^{-1} = \{y / (1000 \times 15)\} \times 2$

3.12.3 Total microbial count

1g of soil mixture was taken in 9 ml of sterilized water blank and the soil suspension was diluted in 10 fold series, then microbial count was determined by standard pour plate technique on nutrient agar media as described by (Subba Rao, 1999). The population was expressed as colony forming units per gram of soil (cfu/g soil).

3.12.4 Microbial biomass

Microbial biomass-C was determined by soil fumigation extraction method detailed by Vance *et al.* (1987). In this method, 20g of soil was fumigated with 50 ml chloroform in vacuum desicator for 24 hr in dark and other 20g soil sample was refrigerated, then both the samples (fumigated and un-fumigated) were extracted with 80 ml of 0.5M K_2SO_4, for half an hour and filtered through Whatmann no.1 filter paper. To 8 ml filter added 66.5 mM $K_2Cr_2O_7$

and 5 ml digestion mixture containing H_2SO_4 and ortho-phosphoric acid (2:1) and heated on hot plate at 120ºC for 30 min. After that final volume was made to 250ml with distilled water. 2-3 drops of ferroin indicator was added and titrated against 0.005 N Ferrous ammonium sulphate (FAS).

MB-C (µg/g soil) = {EC (F) – EC (UF)} / K

Where,

K = 0.25 ± 0.05 (factor which represents the efficiency of extraction of microbial biomass carbon)

EC (F) = Total amount of extractable carbon in fumigated soil samples

EC (UF) = Total amount of extractable carbon in un-fumigated soil samples

3.13 MOLECULAR CHARACTERIZATION OF SELECTED ISOLATE BY 16S rRNA SEQUENCING

3.13. 1 Genomic DNA extraction by conventional method (Sambrook *et al.* 1989)

Bacterial isolates was grown overnight at 37ºC in nutrient broth at 200 rpm. The cells were harvested and processed for DNA isolation.

Requirements:

96-100 per cent Ethanol

Sterile, DNase- free pipette tips and microcentrifuge tubes

RNase A (50 mg/ml)

10 per cent SDS

Phenol:Chloroform (1:1)

Extraction buffer (20 mg/ml lysozyme, 100 mM Tris HCl, 50 mM EDTA, 500 mM NaCl)

TE buffer (10 mM Tris HCl, 1 mM EDTA)

Procedure

1. 5 ml of overnight grown culture was transferred to a microcentrifuge tube and centrifuged at 13,000 rpm for 1 min and supernatant was discarded.
2. Bacterial pellet was suspended in 500 µl of extraction buffer and 50 µl of 10 per cent SDS. Cell pellet was resuspended by vortexing or pipetting.

3. Incubation at 65ºC water bath was done for 30 min until the sample lysate becomes clear. During incubation, tube was inverted at every 3 min.
4. After 65ºC incubation, 2 µl of RNase A (50 mg/ml) was added to sample lysate and mixed by vortexing. Then incubated at room temperature for 5 min.
5. To the lysate equal volume of phenol:chloroform (1:1) was added and mixed well.
6. Centrifuged the above mixture at 10,000 rpm for 5 min at room temperature. Two layers were formed. Upper aqueous layer was collected in new eppendorf tube with the help of pipette.
7. The phenol:chloroform extraction step was repeated.
8. Centrifuged the above mixture at 10,000 rpm for 5 min at room temperature. Two layers were formed. Upper aqueous layer was collected in another new eppendorf tube with the help of pipette.
9. 1/10 volume of 5M NaCl and 2.5 volume of absolute ethanol was added to aqueous phase collected in eppendorf tube.
10. Incubation was done at -20ºC overnight.
11. Centrifuged the above mixture at 12,000 rpm for 20 min at room temperature and supernatant was discarded.
12. The DNA pellet was washed with 1 ml of 70 per cent ethanol.
13. Centrifuged the above mixture at 12,000 rpm for 5 min at room temperature and supernatant was discarded.
14. DNA pellet was air dried for about 15 min until all the residual ethanol got evaporated.
15. Finally the DNA pellet was suspended in appropriate amount of TE and quantify.

3.13.2 Gel electrophoresis

The isolated DNA was finally suspended in 100 µl of elution buffer and quantified on 1per cent agarose gel.

3.13.3 Primer Designing

Universal primers were used for the amplification of 16S rDNA from bacterial isolate.

(1375-F 5’-GCAAGTCGAGCGGACAGATGGGAG3’)

(1375-R 5’-AACTCTCGTGGTGTGACGGGCGGT3’)

The primers were synthesized from IDT, USA.

3.13.4 PCR amplification of 16S rDNA

PCR reaction was carried out in 20 µl reaction containing ~50ng of template DNA, 20 pmoles of each primers, 0.2 mM dNTPs and 1 U Taq polymerase (Genei, Banglore) in 1xPCR buffer. Reaction were cycled 35 times at 94ºC for 30 s, 58ºC for 30 s, 72ºC for 1 min 30 s followed by final extension at 72ºC for 10 min. The PCR products were analyzed on 1per cent agarose gel in 1xTAE buffer, run at 100V for 1 h. Gels were stained with ethidium bromide and photographed. Amplified PCR products were eluted from the gel using gel extraction kit (Hi Yield Gel/ PCR DNA Extraction Kit from Real Genomics).

3.13.5 Gel elution was done by Hi Yield Gel/ PCR DNA Extraction Kit Gel dissociation

Agarose gel slice containing relevant DNA fragments was excised and extra agarose was removed to minimize the size of the gel slice. 300 mg of the gel slice was transferred into a microcentrifuge tube. 500 µl of DF buffer was added to the sample and mixed by vortexing. Incubation was done at 55 ^{o}C for 10-15 min until the gel slice gets completely dissolved. During incubation, the tube gets inverted at every 2-3 min and dissolved sample mixture get cool down to room temperature.

3.13.6 DNA binding

A DF column was placed in 2 ml collection tube. 800 µl of sample mixture (from above step) was applied into the DF column and centrifuged at 13,000 rpm for 30 seconds. Flow through was discarded and DF column was placed back in 2 ml collection tube.

3.13.7 Wash

600 µl of wash buffer (ethanol added) was added into DF column and centrifuged at 13,000 rpm for 30 seconds. Flow through was discarded and DF column was placed back in 2 ml collection tube. Centrifuged again for 3 min at 13,000 rpm to dry the column matrix.

3.13.8 DNA elution

Dried column was transferred into a new microcentrifuge tube. 15-30 µl of elution buffer or distilled water was added into the center of column matrix. Stand for 2 min until elution buffer or distilled water was absorbed by the matrix. Centrifugation was done for 2 min at 13,000 rpm to elute purified DNA. Eluted fragment was then sequenced using PCR primers.

The sequence of 16S rDNA from bacterial isolates were then analyzed using BLAST analysis (http://www.ncbi.nlm.nih.gov/blast) and maximum homology with bacterial strain is determined.

3.13.9 Sequence analysis

Sequencing was done from commercial sequencing facility Xcleris, Ahemdabad according to the protocol of manufacturer (Xcleris lab). The sequence was aligned with corresponding sequences of 16S rDNA from the database using BLAST from the website http://www.ncbi.nlm.nih.gov/blast (Altschul *et al.* 1997). Multiple alignments were generated by the MULTALIN program from the web site: http://prodes.toulouse.inra.fr/multialin/multialin.html (Corpet, 1988).

3.14 STATISTICAL ANALYSIS

The data recorded under laboratory and net house conditions on various parameters was subjected to statistical analysis as per methods outlined by Gomez and Gomez (1984). The LSD at 5 per cent level was used for testing the significant differences among the treated means.

Chapter-4

RESULTS AND DISCUSSION

The results obtained during the course of investigations are presented in this chapter under the following heads:

4.1 Isolation and enumeration of microbial population.

4.2 Screening of bacterial isolates for multifarious plant growth promoting activities.

4.3 Morphological, physiological and biochemical characterization of selected bacterial isolates.

4.4 To study the efficacy of indigenous PGPR to control bacterial wilt of capsicum.

4.5 To study the effect of PGPR and chemical fertilizers on soil health and productivity of capsicum.

4.6 To study the effect of indigenous PGPR inoculum for physiological efficacy under water stress conditions.

4.7 Molecular characterization (16S rRNA gene sequencing) of selected bacterial isolates.

4.1 ISOLATION AND ENUMERATION OF MICROBIAL POPULATION

Isolation of microorganisms was carried out from the rhizosphere and roots of the capsicum (*Capsicum annuum* L.) collected from different locations of agro-climatic zones (I-III) of Himachal Pradesh using modified replica plate method (Plate 1). The population capable of growth on different media was counted and reported as cfu/g of rhizosphere soil or cfu/g root. A summary of microorganisms colonizing the capsicum rhizosphere and roots of three agroclimatic zones of Himachal Pradesh is presented in Table 1.

Among various locations, Sarkaghat had the highest count (118.50×10^5 cfu/g soil), whereas, Bhadra had the lowest count (61.00×10^5 cfu/g soil) on the Nutrient Agar (NA) medium. The highest (70×10^5cfu/g soil) count of free living nitrogen fixers on Jensen's media were recorded from Paontasahib, whereas,

minimum (30.67 × 10^5 cfu/g soil) count were recorded from Arki. On the PVK medium highest count (84.00 × 10^5 cfu/g soil) were recorded from Sirmour, whereas, minimum count (43.50 × 10^5 cfu/g soil) were recorded from Dehra and Bhadra. Among all the locations, the maximum phosphate solubilisers (93.74 per cent) were recorded from the Nahan.

Maximum endophytes (96.00×10^2 cfu/g root) were recorded from the capsicum root samples from Mehatpur and Neri, whereas, Bhadra had the lowest count (62.50×10^2 cfu/g root) on the Nutrient Agar (NA) medium. Maximum (46.00×10^2 cfu/g root) free living nitrogen fixers on the Jensen's medium were recorded from Nalagarh, whereas Fatehpur had the lowest count (27.00×10^2 cfu/g root). On the PVK medium highest count (71.67 ×10^2 cfu/g root) were recorded from Rajgarh, whereas, minimum count (33.50 ×10^2 cfu/g root) were recorded from Paontasahib. Among all the locations, maximum endophytic phosphate solubilisers (91.18 per cent) were recorded from the Sundernagar.

The bacterial community can be affected by a number of abiotic and biotic factors. The total microbial count in general were more in rhizosphere (118.50 to 61.00 × 10^5 cfu/g soil) as compared to endophytic count (96.00 to 62.50 × 10^2 cfu/g root). This variation in rhizosphere and roots may be attributed due to positive influence exerted by root exudates, environmental conditions, age of plant, variety/cultivar type, time of sampling and physico-chemical properties of soil. The results are in confirmation with Gupta *et al.* (2014), who also reported that under natural conditions, the microbial count in capsicum rhizosphere (94.67 to 84.33 × 10^5 cfu/g soil) and plant roots (86.00 to 70.33 × 10^2 cfu/g root) varied significantly with location. Sharma (2009) and Kaushal (2011) have also reported greatest variation in microbial population with respect to location/plant parts used for isolation purpose. Gupta (2012), also reported that the population of phosphate solubilizing microorganisms varied from 20-24 per cent of the total population and in some soils it may be up to 85 per cent of the total population.

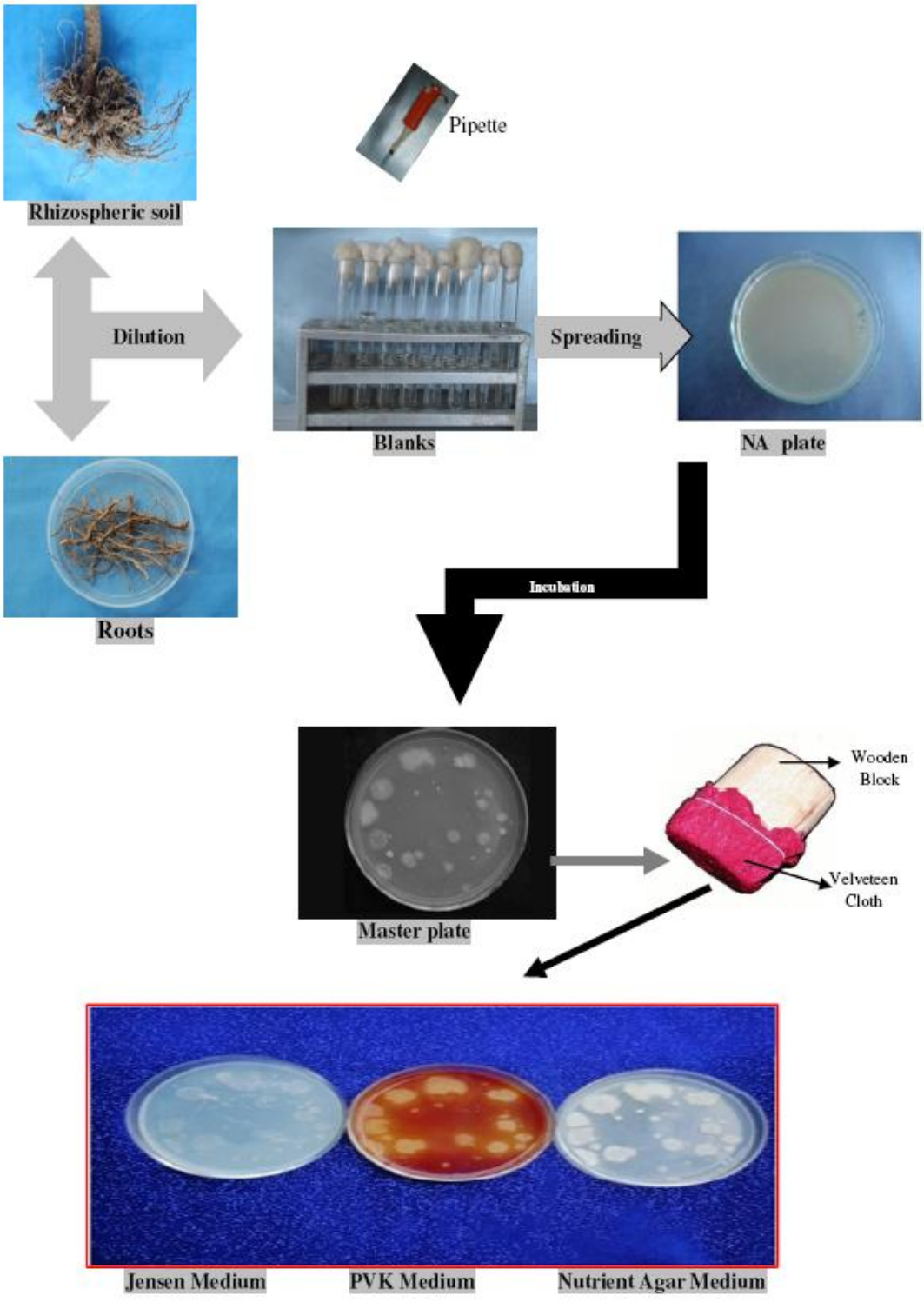


Plate 1. Isolation of bacterial isolates on different medium (Modified Replica Plate Method)

Table 1. Enumeration of rhizospheric and endophytic bacterial population associated with capsicum under different agro-climatic zones of Himachal Pradesh

Locations, Distt	Rhizospheric soil bacterial population ($\times 10^5$cfu/gsoil)				Endophytic bacterial population ($\times 10^2$cfu/g root)			
	Nutrient Agar (NA)	Jensen's Medium (JM)	Pikovskaya's Medium (PVK)	P-Solubilizing bacteria (percent)**	Nutrient Agar (NA)	Jensen's Medium (JM)	Pikovskaya's Medium (PVK)	P-Solubilizing bacteria (percent)**
Agroclimatic-zone I								
Dehra,Kangra	76.00	35.00	43.50 (29.0)*	66.67	65.00	29.50	36.00 (22.0)*	61.11
Fatehpur,Kangra	71.25	34.50	46.00 (30.0)	65.22	63.50	27.00	36.50 (26.0)	71.23
Rakkar,Kangra	81.50	45.00	52.50 (33.0)	62.86	75.50	36.00	43.50 (31.0)	71.26
Mataur,Kangra	92.00	57.00	70.00 (64.0)	91.43	69.00	34.00	45.00 (35.0)	77.78
Mehatpur, Una	75.00	44.00	55.00 (34.0)	61.82	96.00	34.50	55.00 (43.0)	78.18
Nadaun, Hamirpur	103.00	42.00	66.50 (39.0)	58.65	80.50	36.00	51.50 (36.5)	70.87
Neri,Hamirpur	86.00	43.00	63.50 (44.5)	70.08	96.00	36.50	51.00 (36.5)	71.57
Ghumarwin,Bilaspur	104.00	44.00	65.00 (41.0)	63.08	90.00	30.50	60.00 (45.5)	75.83
Parwanoo,Solan	97.00	35.00	70.50 (57.0)	80.85	69.50	29.00	46.00 (35.0)	76.09
Nalagarh, Solan	100.00	35.50	57.00 (41.5)	72.81	65.00	46.00	55.00 (44.0)	80.00
Paontasahib, Sirmour	104.00	70.00	81.50 (65.5)	80.37	67.50	30.50	33.50 (27.5)	82.09
Agroclimatic-zone II								
Sihunta, Chamba	89.00	52.50	51.00 (45.5)	89.22	67.00	28.00	36.00 (30.5)	84.72
Palampur,Kangra	96.50	59.00	68.50 (61.5)	89.78	71.00	32.50	47.00 (39.0)	82.98
Sarkaghat,Mandi	118.50	57.50	80.00 (74.0)	92.50	94.00	33.00	57.50 (50.5)	87.83
Sundernagar,Mandi	101.50	59.00	76.50 (69.5)	90.85	83.00	31.00	51.00 (46.5)	91.18
Arki,Solan	84.33	30.67	68.67 (63.5)	92.47	70.33	30.33	61.67 (44)	71.35
Kandaghat,Solan	86.67	40.67	79.00 (73.5)	93.04	75.67	34.67	56.67(47.5)	83.82
Rajgarh,Sirmour	94.67	42.33	84.00 (76.5)	91.07	86.00	44.33	71.67(45.0)	62.78
Nahan,Sirmour	89.33	33.33	74.67 (70.0)	93.74	83.67	36.67	58.00 (46.0)	79.31
Agroclimatic-zone III								
Banikhet,Chamba	89.5	50.50	61.50 (53.0)	86.18	78.50	34.00	42.50 (35.5)	83.53
Bharmour,Chamba	68.50	42.00	45.50 (33.5)	73.63	94.00	29.00	43.00 (38.5)	89.53
Bhadra,Kullu	61.00	34.50	43.50 (24.5)	56.32	62.50	29.00	37.50 (25.5)	68.00
Bajaura, Kullu	75.50	44.00	49.50 (36.0)	72.73	64.00	29.00	38.00 (22)	57.89
Patlikhul,Kullu	68.50	46.00	52.00 (35.0)	67.31	66.50	38.00	41.50 (24.5)	59.04
Sarahan,Kullu	71.00	41.00	68.0 0 (46.0)	67.65	63.50	32.00	42.50 (26)	61.18
Theog,Shimla	95.50	42.50	64.50 (43.5)	67.44	63.50	31.50	51.50 (31.5)	61.17
Jhalta,Shimla	81.00	37.00	56.00 (41.0)	73.21	91.00	30.50	48.50 (34.0)	70.10
Rohru,Shimla	80.50	40.00	64.50 (38.0)	58.91	70.50	33.00	48.00 (36.0)	75.00

*Number of colonies forming halozone on PVK medium

** P-solubilisers (per cent) = (Number of colonies forming halozone on PVK medium / Total number of colonies on PVK medium) × 100

4.1.1 Selection of morphotypes on the basis of plant growth promoting traits

Among all three agroclimatic zones of Himachal Pradesh, total of 157 most predominant, morphological distinct isolated colonies were screened for various plant growth promoting traits (PGPTs) viz. P-solubilisation, N-fixation and siderophore production.

Percentage of PGPR possessing various PGPTs among different agroclimatic-zones has been depicted in Fig. 2. Among the total isolates, 53.5 per cent (84/157) were able to solubilise phosphorus, 72.61 per cent (114/157) were able to fix nitrogen on N-free medium and 47.77 per cent (75/157) were able to synthesize siderophores on CAS medium. The highest (60.0 per cent) P-solubilisers was recorded from agroclimatic-zone III and lowest (45.0 per cent) from agroclimatic-zone I. The highest (95.0 per cent) N-fixers were recorded from agroclimatic-zone III and lowest (61.4 per cent) from agroclimatic-zone II. However, highest (63.3 per cent) siderophore producers was recorded from agroclimatic-zone I and lowest (32.5 per cent) from agroclimatic-zone III. The results are in confirmation with Antoun *et al*. (1998) who observed that out of 266 strains of rhizobia 54 per cent were able to solubilize insoluble phosphates while 58 per cent and 83 per cent were N-fixers and siderophores producers, respectively.

As, frequencies of PGPRs possessing plant growth promoting traits vary with respect to agroclimatic-zones of Himachal Pradesh, so, PGPTs are expressed in terms of single, binary and triple traits (Table 2). It was observed that combination of binary traits of P-solubilisation and N-fixation was highest (32.5 per cent) for agroclimatic-zone III, whereas, combination of binary traits like P-solubilisation and siderophore production was highest (7.0 per cent) for agroclimatic-zone II. However, combination of binary trait like N-fixation and siderophore production was found highest (23.3 per cent) for agroclimatic-zone I. Ratios of PGPR showing triple traits of P-solubilisation, N-fixation and siderophore production were highest among the rhizobacterial isolates of agroclimatic-zone-III (30.0 per cent).

This variation may be attributed to the varied soil characteristics and climatic conditions at each agroclimatic zone of Himachal.

Table 2. Characterization of bacterial isolates from different agroclimatic zones of Himachal Pradesh

AGROCLIMATIC ZONES	No. of isolates with single PGP activity			No. of isolates with binary PGP traits			No. of isolates with triple PGP traits
	P	N	S	P+N	P+S	N+S	P+N+S
Agroclimatic- zone I	9/60 (15.0)	10/60 (16.67)	9/60 (15.0)	3/60 (5.00)	1/60 (1.67)	14/60 (23.33)	12/60 (20.00)
Agroclimatic- zone II	11/57 (19.29)	14/57 (26.31)	7/57 (12.28)	8/57 (14.03)	4/57 (7.01)	3/57 (5.26)	10/57 (17.54)
Agroclimatic- zone III	1/40 (2.50)	13/40 (32.50)	0/40 (0.0)	13/40 (32.50)	1/40 (2.50)	3/40 (7.50)	12/40 (30.00)
Total	21/157 (13.37)	37/157 (23.57)	16/157 (10.19)	24/157 (15.29)	6/157 (3.82)	20/157 (12.74)	34/157 (21.65)

Figure in parentheses are per cent of isolates with plant growth promoting (PGP) traits.

4.2 SCREENING OF BACTERIAL ISOLATES FOR MULTIFARIOUS PLANT GROWTH PROMOTING ACTIVITIES

A total of 34 morphological distinct isolates (12 isolates from agroclimatic-zone I, 10 isolates from agroclimatic-zone II and 12 isolates from agroclimatic-zone III) possessing triple traits viz. P-solubilisation, N-fixation and siderophore production were further screened for multifarious plant growth promoting traits viz. Indole-3-acetic acid (IAA), ammonia, 1-aminocyclopropane-1-carboxylic acid (ACC)-deaminase, hydrogen cyanide production (HCN) production (Table 3, Plate 2) and antagonistic activity against six test pathogens i.e. *Fusarium solani* (Martius), *Rhizoctonia solani*, *Pythium* spp., *Ralstonia solanacearum*, *Phytophthora capsici* and *Colletotrichum capsici* (Sydow) (Table 4 and Plate 3). Similar to Ruchi *et al.* (2012), it was found that the majority of the rhizospheric isolates regularly produced IAA, siderophores, ammonia, HCN and solubilized tri-calcium phosphate.

Phosphorus and nitrogen are among the essential nutrients of the crop. Available form of phosphorus to plants is phosphate anions, which are mostly trapped via precipitation with cations such as Mg^{2+}, Ca^{2+}, Al^{3+} and Fe^{3+} and so become insoluble and unavailable to plants in these forms. The plants depend on biological nitrogen fixation (BNF) for available nitrogen as plant species is capable for fixing atmospheric dinitrogen into ammonia and expend it directly for

its growth. The results presented in Table 3 revealed that P- solubilisation efficiency of the selected isolates ranged from 26.96 to 95.24 per cent. Maximum phosphate solubilisation efficiency (95.24 per cent) was recorded for isolate ROH_{14}, whereas, minimum activity (26.96 per cent) was noted for FAT_9 and BHA_{23} isolates. All the isolates are able to grow on N-free media. Similar results of P-solubilisation by bacterial isolates of capsicum in range of 5.9 to 123.8 per cent in solid Pikovskaya's medium have been reported by Gupta (2012).

Iron is a vital nutrient for almost all forms of life. In the aerobic environment, iron occurs principally as Fe^{3+} and is likely to form insoluble hydroxides and oxy-hydroxides, thus making it generally inaccessible to both plants and microorganisms. Commonly, bacteria acquire iron by the secretion of low-molecular mass iron chelators referred to as siderophores which have high association constants for complexing iron. The siderophore production of the bacterial isolates was estimated qualitatively on the basis of colored zone on CAS medium. Variation was observed in per cent siderophore unit which ranged between 86.67 per cent to 28.97 per cent. Maximum siderophore unit was produced by isolate JHA_6 (86.67 per cent), whereas, minimum siderophore unit (28.97 per cent) was produced by isolate BAJ_{16}. Our findings are in line with those of Joseph *et al.* (2007), Kirti (2013) and Bhardwaj (2013) who also reported that bacteria produce siderophores.

PGPR are well known to stimulate the plant growth by acting as phytostimulators. IAA has been implicated in virtually every aspect of plant growth and development, as well as defense responses. This diversity of function is reflected by the extraordinary complexity of IAA biosynthetic, transport and signaling pathways. Perusal of data embedded in Table 3 revealed that 52.94 per cent (18/34) exhibited IAA production activity on Luria Bertani Broth. The level of IAA production in the selected isolates ranged from 31.33 µg/ml to 10.33 µg/ml. The isolate $SARA_9$ produced maximum concentration of IAA (31.33 µg/ml) and the minimum IAA (10.33 µg/ml) production was recorded for isolate RAK_9 after 72 hours of incubation. Comparable amount of IAA and siderophore for *Bacillus* and *Pseudomonas* sp. have been reported by Jarak *et al.* (2012). Further, the results are in line with the findings of Kaur and Sharma (2013) who also reported IAA production in range of 2.09 to 33.28 µmol/ml.

Apart from being a plant growth regulator, ethylene has also been established as a stress hormone as the endogenous level of ethylene is significantly increased during stress which negatively affects the overall plant growth. Plant growth promoting rhizobacteria which possess the enzyme, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, facilitate plant growth and development by decreasing ethylene levels, as take up the ethylene precursor ACC and convert it into 2-oxobutanoate and NH_3 inducing salt tolerance and reducing drought stress in plants. Among all, 55.88 per cent (19/34) isolates were able to grow on DF minimal salt medium having 1-aminocyclopropane- 1-carboxylic acid (ACC) as sole source of nitrogen. Similar ACC deaminase activity by bacterial isolates has been reported by Grichko and Glick, 2001 and Hui and Kim, 2013.

Microbial production of ammonia and HCN has been suggested as an important biofertilizer and biocontrol feature to enhance the plant growth. Only twelve (35.29 per cent) isolates were ammonia producers and five (14.70 per cent) isolates were HCN producers. The production of HCN by different isolates of *Bacillus* sp. from Cauliflower has been reported by Kaushal (2011) and Bhardwaj (2013).

Bacterial isolates exhibited variation in antifungal and antibacterial activity against the test pathogens (Table 4; Plate 3). Except THE_{17} isolate, all the tested isolates were able to inhibit the growth of one or more test pathogens. However, none of the tested isolates was able to inhibit the growth of all the test pathogens.

Among all, 50 per cent (17/34), were found to inhibit mycelia growth of *Fusarium oxysporum* Maximum (60.74 per cent) mycelia growth inhibition against *Fusarium oxysporum* was recorded for MAT_8 isolate and minimum (20.74 per cent per cent) for NAH_7 isolate. 17.65 per cent (6/34) isolates were able to inhibit the growth of *Rhizoctonia solani*. Maximium (33.33 per cent) growth inhibition was noted for isolate PAR_2, whereas, minimum (24.44 per cent) for RAK_9 isolate. Twelve isolates (35.29 per cent) were found to possess antagonistic activity against *Pythium* spp. Maximum (57.04 per cent) mycelia growth inhibition was recorded for PAL_7 isolate and minimum (17.78 per cent) for FAT_7

and BHA_{23} isolate 44.12 per cent (15/34) were found to inhibit the growth of *Ralstonia solanacearum.* Maximum (84.85 per cent) bacterial pathogen growth inhibition was recorded for ROH_6 isolate and minimum (18.18 per cent) for RAK_9 isolate. 50.0 per cent (17/34) were found to inhibit mycelia growth of *Phytophthora capsici*. Maximium (51.85 per cent) growth inhibition was noted for isolate JHA_6 which was statistically at par with SIH_6 and NER_4 isolates, whereas minimum (20.00 per cent) for two isolates i.e. BAN_{11} and ARK_9 isolate. Twelve isolates (35.29 per cent) isolates were found to have antagonism against *Colletotrichum capsici.* Maximum (57.04 per cent) mycelia growth inhibition was recorded for PAL_7 isolate and minimum (20.74 per cent) for $SARA_9$ isolate. The formation of zone may be due to secretion of antifungal substances that might have diffused in the medium and inhibited the fungal growth. The results are in line with that of Sharma (2009), Duffy *et al.* (2004), Wen *et al.* (2010) and Kirti (2013) also reported micro-organisms as effective biological control agents against pests/diseases.

4.2.1 Quantitative estimation of plant growth promoting traits

Out of 34 isolates only fifteen isolates i.e. RAK_9, MAT_8, NER_4, PAR_2, PAO_2, SIH_6, PAL_7, KAN_{11}, $BHAR_4$, PAT_9, PAT_{13}, $SARA_9$, JHA_6, ROH_6 and ROH_{14} possessing maximum PGP traits were selected for further studies.

Selected fifteen bacterial isolates were assessed for quantitative estimation of PGP traits after 72h of incubation at 37ºC and pH 7 under shaking conditions (110 rpm) and were compared on the basis of their P-solubilization activity (µg/ml), per cent siderophore unit and lytic enzyme activity.

From the data presented in Appendix 2.1 and Fig.3, it is clear that in PVK broth, TCP solubilisation ranged from 169.84 µg/ml to 59.00 µg/ml. Maximum (169.84 µg/ml) P-solubilization was recorded for isolate JHA_6 with corresponding viable count (30.27 $\times 10^7$ cfu/ml) and decrease in final pH to 5.17 from initial pH 7.0., however, P-solubilisation activity which was at par with isolate ROH_{14} (145.77 µg/ml). The minimum (59.00 µg/ml) TCP-solubilization was recorded for isolate PAO_2 with corresponding viable count (11.96 $\times 10^7$ cfu/ml) and final pH 6.77. The results are also in line with those of Sharma *et al.* (2012) who also recorded the P-solubilization in Pikovskaya's broth ranged from 34.47 to 128.32

µg/ml by different isolates. Similar results have also been recorded by (Dipta, 2013) while working with PGPR isolates belonging to *Bacillus* sp.

Table 3. Screening of selected PGPR isolates for their multifarious plant growth promoting traits

Isolate	P-solubilisation efficiency (per cent)	Siderophore production efficiency (per cent)	IAA production (µg/ml)	ACC-deaminase activity	Ammonia	HCN
Agroclimatic Zone I						
FAT_9	26.96 (31.11)	75.30 (60.35)	0.00	-	-	-
FAT_7	38.03 (38.02)	73.50 (59.65)	0.00	-	+	-
RAK_2	65.08 (53.82)	41.03 (39.80)	0.00	-	+	-
RAK_9	36.47 (37.09)	85.71 (76.34)	10.33	+	+	+
MAT_8	93.17 (77.66)	55.56 (48.23)	23.67	+	+	-
*MEH_4	36.06 (36.58)	33.24 (35.03)	0.00	-	-	-
NER_4	86.19 (68.55)	40.94 (39.61)	13.00	+	+	+
*GHU_6	45.32 (41.97)	65.17 (54.28)	0.00	-	+	-
PAR_2	78.02 (62.21)	80.59 (64.10)	19.00	-	-	-
PAR_4	31.87 (34.19)	56.03 (48.57)	0.00	-	-	-
PAO_2	82.59 (61.03)	75.40 (60.40)	0.00	-	-	-
PAO_8	33.33 (35.25)	33.73 (35.37)	0.00	-	-	-
Agroclimatic Zone II						
SIH_6	84.92(67.14)	85.71 (71.59)	12.33	+	-	-
BAN_{11}	43.38 (41.16)	43.38 (41.16)	15.00	+	-	-
PAL_7	58.52 (50.08)	77.98 (62.54)	21.67	+	+	-
ARK_1	72.94 (59.17)	75.18 (60.56)	13.67	+	+	-
ARK_5	42.07 (40.34)	30.92 (33.67)	0.00	-	-	-
ARK_9	43.56 (41.19)	34.03 (35.54)	19.00	+	-	-
KAN_{10}	74.76 (60.30)	73.89 (59.25)	0.00	-	-	-
KAN_{11}	90.11 (74.87)	78.57 (62.94)	25.00	+	-	-
NAH_4	43.96 (41.51)	55.56 (48.23)	0.00	-	-	-
NAH_7	78.02 (62.21)	58.52 (50.08)	0.00	-	+	-
Agroclimatic Zone III						
$BHAR_4$	81.06 (64.23)	81.62 (64.67)	22.33	+	-	+
BHA_{23}	26.96 (30.98)	33.24 (35.03)	25.67	+	-	-
BAJ_{16}	58.52 (50.08)	28.97 (32.51)	24.67	+	-	-
PAT_9	72.94 (59.17)	78.27 (66.98)	0.00	+	-	-
*PAT_{13}	34.34 (35.86)	79.80 (63.42)	0.00	-	+	-
$SARA_9$	82.74 (69.98)	74.90 (60.32)	31.33	+	-	-
THE_{17}	81.27 (64.66)	49.21 (44.47)	0.00	-	-	-
THE_{18}	45.71 (41.00)	61.59 (51.77)	13.00	+	+	-
JHA_6	94.87 (82.27)	86.67 (76.89)	21.00	+	-	+
JHA_9	30.16 (33.28)	30.92 (33.67)	0.00	+	-	+
ROH_6	92.31 (80.40)	37.82 (37.92)	21.33	+	-	-
ROH_{14}	95.24 (82.56)	84.44 (66.84)	23.67	+	+	-
$CD_{0.05}$	**22.50 (17.14)**	**20.74 (15.89)**	**1.44**	-	-	-

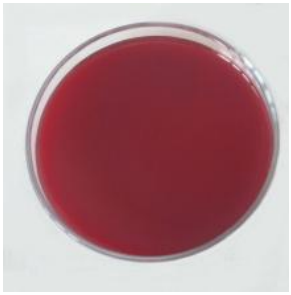
Figures in the parentheses are arc sin transformed values.

Table 4. Screening of selected PGPR isolates for antagonism against various test pathogens under laboratory conditions

Antagonistic activity against various pathogens						
Isolates	*Fusarium oxysporum*	*Rhizoctonia solani*	*Pythium* sp.	*Ralstonia solanacearum*	*Phytophthora capsici*	*Colletotrichum capsici*
Agroclimatic Zone I						
FAT_9	0.00 (0.00)	0.00 (0.00)	18.52 (25.47)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
FAT_7	28.89 (32.46)	0.00 (0.00)	17.78 (24.90)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
RAK_2	23.70 (29.06)	0.00 (0.00)	0.00 (0.00)	24.24 (29.39)	0.00 (0.00)	0.00 (0.00)
RAK_9	24.44 (29.62)	24.44 (29.62)	0.00 (0.00)	18.18 (24.75)	31.85 (34.30)	42.22 (40.50)
MAT_8	60.74 (51.19)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	24.44 (29.56)
$*MEH_4$	45.19 (42.20)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
NER_4	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	41.67 (40.19)	51.11 (45.62)	42.96 (40.94)
$*GHU_6$	0.00 (0.00)	31.11 (33.88)	0.00 (0.00)	33.33 (35.20)	0.00 (0.00)	0.00 (0.00)
PAR_2	35.56 (36.55)	33.33 (35.22)	0.00 (0.00)	41.67 (40.19)	0.00 (0.00)	0.00 (0.00)
PAR_4	0.00 (0.00)	30.37 (33.41)	24.44 (29.60)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
PAO_2	36.30 (37.02)	29.63 (32.94)	53.33 (46.89)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
PAO_8	33.33 (35.22)	0.00 (0.00)	28.89 (32.49)	0.00 (0.00)	0.00(0.00)	0.00 (0.00)
Agroclimatic Zone II						
SIH_6	45.93 (42.64)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	51.11 (45.62)	24.44 (29.60)
BAN_{11}	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	20.00 (26.53)	26.67(31.07)
PAL_7	43.70 (41.36)	0.00 (0.00)	57.04 (49.03)	0.00 (0.00)	0.00 (0.00)	57.04 (49.03)
ARK_1	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	36.36 (36.97)	26.67 (29.60)	0.00 (0.00)
ARK_5	22.96 (28.54)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
ARK_9	22.22 (28.04)	0.00 (0.00)	0.00 (0.00)	36.36 (37.07)	20.00 (27.58)	0.00 (0.00)
KAN_{10}	23.70 (29.06)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
KAN_{11}	0.00 (0.00)	0.00 (0.00)	27.41 (31.55)	30.30 (33.34)	24.44 (27.07)	0.00 (0.00)
NAH_4	22.96 (28.54)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
NAH_7	20.74 (27.07)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Agroclimatic Zone III						
$BHAR_4$	47.41 (43.50)	0.00 (0.00)	18.52 (25.47)	36.36 (36.97)	0.00 (0.00)	0.00 (0.00)
BHA_{23}	0.00 (0.00)	0.00 (0.00)	17.78 (24.90)	36.36(37.07)	21.48 (27.58)	0.00 (0.00)
BAJ_{16}	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	20.74 (27.07)	0.00 (0.00)
PAT_9	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	66.67 (54.76)	26.67 (31.07)	26.67 (31.07)
$*PAT_{13}$	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	48.48 (44.11)	46.67 (43.07)	31.85 (34.33)
$SARA_9$	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	36.36 (36.97)	20.74 (27.07)	20.74 (27.07)
THE_{17}	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
THE_{18}	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	21.48 (27.59)	21.48 (27.59)
JHA_6	54.81 (47.75)	28.89 (32.49)	0.00 (0.00)	0.00 (0.00)	51.85 (46.04)	54.07 (47.32)
JHA_9	0.00 (0.00)	0.00 (0.00)	24.44 (29.60)	0.00 (0.00)	22.96 (28.58)	0.00 (0.00)
ROH_6	0.00 (0.00)	0.00 (0.00)	53.33 (46.89)	84.85 (67.30)	24.44 (29.60)	53.33 (46.89)
ROH_{14}	0.00 (0.00)	0.00 (0.00)	28.89 (32.49)	72.73 (59.40)	41.48 (40.08)	0.00 (0.00)
$CD_{0.05}$	**5.16 (3.19)**	**2.02 (1.26)**	**2.09 (1.35)**	**7.62 (5.14)**	**3.14 (1.97)**	**2.56 (1.59)**

Figures in the parentheses are arc sin transformed values.

Results of quantitative estimation of siderophore using CAS liquid assay presented in Appendix 2.1 and Fig. 4, revealed that maximum (56.36) percent siderophore unit corresponding to maximum viable count (16.14×10^7 cfu/ml) and final pH 4.64 was obtained for isolate JHA_6 after 72 h of incubation which was

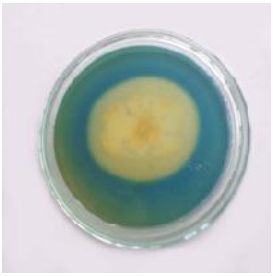


Control

Zone of Clearance

P-solubilisation by PGPR isolate on PVK medium

Control



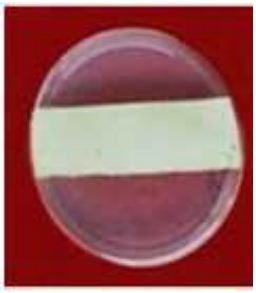
Yellowish Orange Zone

Sidrophore production by PGPR isolate on CAS medium

ACC Control

Growth of PGPR Isolates on ACC medium

ACC-deaminase production by PGPR isolate



control

Color change of filter paper (yellow to orange)

HCN Production by PGPR Isolate

IAA production by PGPR isolates

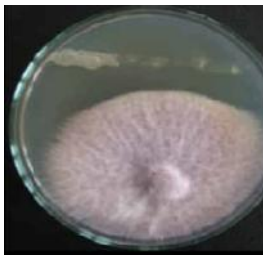
Ammonia production by PGPR isolates

Control – N free medium

Growth on Nitrogen free medium

Plate 2. Screening of bacterial isolates for multifarious plant growth promoting traits

Control (*Fusarium oxysporum*)



Fusarium oxysporum + PGPR isolate

Antifungal activity of bacterial isolates against *Fusarium oxysporum*

Control (*Rhizoctonia solani*)

Rhizoctonia solani + PGPR isolate

Antifungal activity of bacterial isolates against *Rhizoctonia solani*

Control (*Pythium spp*.)

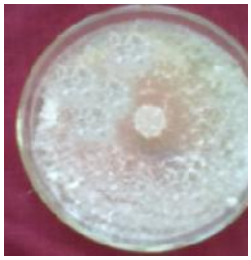
Pythium spp.+ PGPR isolate

Antifungal activity of bacterial isolates against *Pythium spp*.

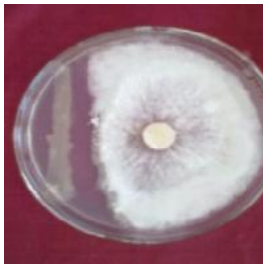
Control (*Ralstonia solanacearum*)

Ralstonia solanacearum + PGPR isolate

Antibacterial activity of bacterial isolates against *Ralstonia solanacearum*

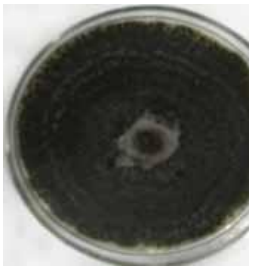


Control (*Phytophthora capsici*)

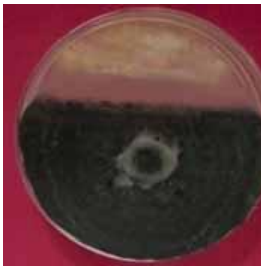


Phytophthora capsici + PGPR isolate

Antifungal activity of bacterial isolates against *Phytophthora capsici*



Control (*Colletotrichum capsici*)



Colletotrichum capsici + PGPR isolate

Antifungal activity of bacterial isolates against *Colletotrichum capsici*

Plate 3. Screening of selected PGPR isolates for antagonism against various test pathogens

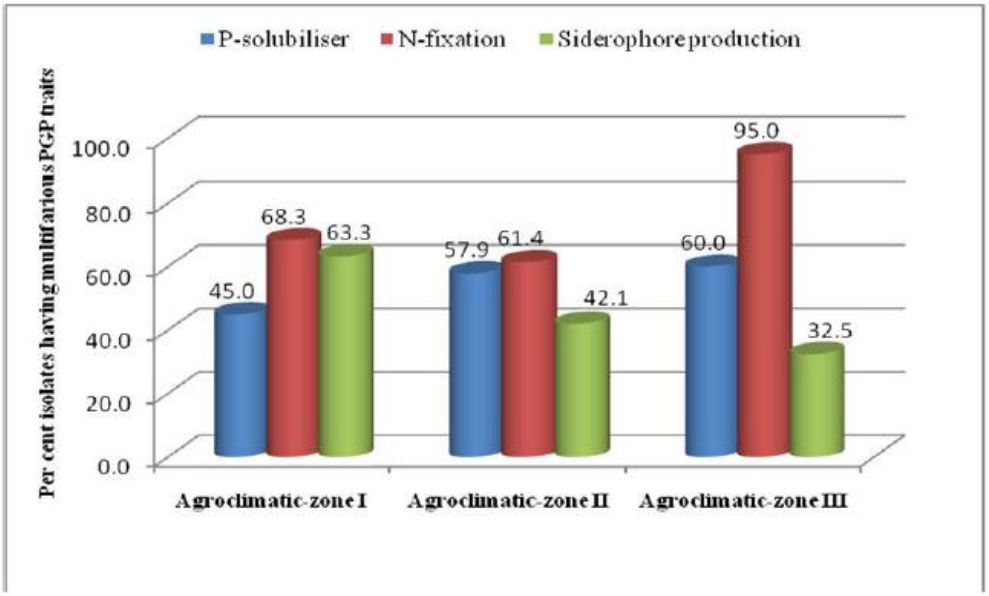
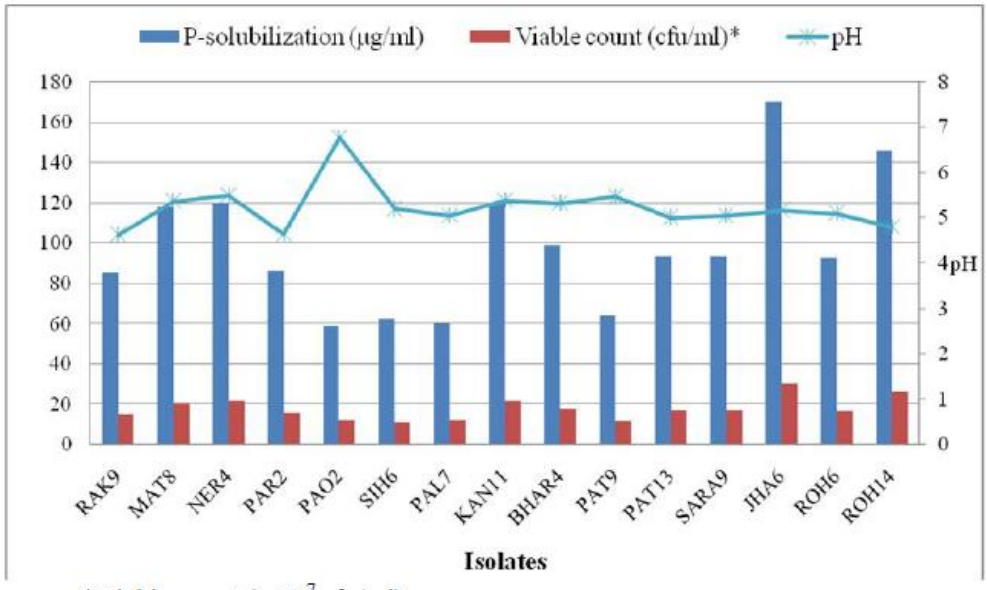


Fig. 2 Percentage of rhizobacterial isolates with different plant growth promoting traits in agroclimatic-zones of Himachal Pradesh.



*Viable count ($\times 10^7$ cfu/ml)

Fig. 3 Phosphate-solubilization, viable count and changes in pH of medium by selected bacterial isolates

significantly higher than all other isolates. The minimum (12.79 per cent) siderophore unit was recorded for isolate PAR_2 with viable count of 8.30×10^7 cfu/ml and final pH 4.53 as compared to initial pH of 7.0.

The selected fifteen isolates were also screened for production of different lytic enzymes viz. amylase, pectinase, protease, cellulase, lipase and chitinase (Plate 4, Appendix 2.1 and Fig 5). Out of fifteen isolates, eight (53.33 per cent) exhibited amylase activity. Significantly higher amylase enzyme activity (1.95) was recorded for isolate PAO_2 followed by the enzyme activity recorded for isolate ROH_6 (1.45). Pectinase enzyme activity was exhibited by four isolates (26.67 per cent) with enzyme activity index (EAI) ranging from 1.82 to 1.36. Maximum (1.82) pectinase activity was recorded for isolate JHA_6 followed by KAN_{11} (1.45). Among all nine isolates (60.00 per cent) were protease producers, maximum (1.95) enzyme activity was recorded for isolate PAO_2 which was found significantly at par with PAL_7 and ROH_4 isolates, whereas only two isolates (PAO_2 and $BHAR_4$) was found to exhibit cellulase activity. Seven (46.67 per cent) isolates exhibited lipase activity in range of 1.86 to 1.27, maximum (1.86) being possessed by PAL_7 isolate, which was statistically at par with $BHAR_4$ isolate. For chitinase activity five isolates (33.33 per cent) showed the enzyme index ranging from 1.27 to 1.95. Maximum EAI (1.95) was recorded for isolates PAL_7 and ROH_6, whereas, minimum (1.27) was observed for RAK_9 isolate. The production of lytic enzyme has been considered with defense related mechanisms which has been documented by Jetiyanon (2007) who found that a mixture of *B. amyloliquefaciens* strain IN937a and *B. pumilus* strain IN937b induced the production of defense related enzymes against the pathogen.

4.3 MORPHOLOGICAL, PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF SELECTED BACTERIAL ISOLATES

The selected fifteen isolates were subjected to morphological, physiological and biochemical characterization.

4.3.1 Morphological characterization of selected isolates

The results in Table 5 depicts the colony morphology, Gram's reaction and cell shape of selected isolates. The isolates showed variation w.r.t. Gram's reaction and were rods, cocci and cocco-bacillus in shape. From the results it was

revealed that all the selected isolates possess variable morphological features with respect to their form, elevation, margin, surface and color and appeared as circular, undulate, flat, creamish, irregular, umbonate, raised, curled, white, entire, lobate, convex and erose.

Table 5. Morphological characterization of selected bacterial isolates from different agro-climatic zones of Himachal Pradesh

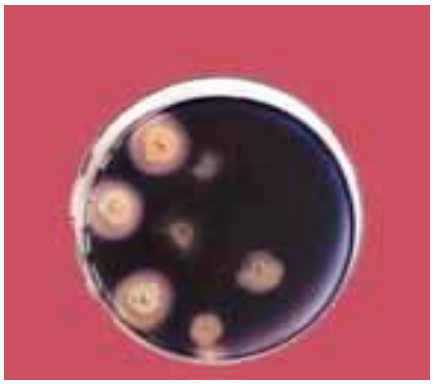
Isolates	Form	Elevation	Margin	Surface	Colour	Gram's Reaction	Cell shape	Arrangement
RAK_9	Punctiform	Flat	Entire	Smooth	Cream	+	Cocco-bacillus	Single
MAT_8	Irregular	Flat	Entire	Smooth	Yellowish Brown	-	Rod	Chains
NER_4	Punctiform	Raised	Entire	Smooth	Cream	+	Cocci	Single
PAR_2	Circular	Flat	Entire	Smooth	Cream	+	Cocco-bacillus	Single
PAO_2	Rhizoid	Flat	Erose	Smooth	Cream	-	Cocco-bacillus	Single
SIH_6	Irregular	Raised	Curled	Rough	Cream	+	Rods	Single
PAL_7	Irregular	Flat	Undulate	Smooth	Cream	+	Cocco-bacillus	Single
KAN_{11}	Irregular	Flat	Undulate	Smooth	Cream	-	Rods	Single
$BHAR_4$	Circular	Convex	Entire	Smooth	Cream	+	Cocco-bacillus	Single
PAT_9	Irregular	Flat	Entire	Smooth	Yellowish Brown	-	Cocci	Single
PAT_{13}	Irregular	Flat	Undulate	Smooth	Yellowish Brown	-	Cocco-bacillus	Single
$SARA_9$	Circular	Flat	Entire	Smooth	Cream	+	Rods	Single
JHA_6	Irregular	Flat	Entire	Smooth	Yellowish Brown	-	Rods	Single
ROH_6	Irregular	Flat	Undulate	Smooth	Cream	+	Coccus	Single
ROH_{14}	Irregular	Flat	Undulate	Smooth	Cream	+	Rods	Single

4.3.2 Physiological characterization of selected isolates

The results in Table 6 indicated the growth of selected fifteen isolates at varied levels of temperature (20 – 45ºC), pH (5.0 - 9.0) and salinity (2 per cent to 10 per cent). All the isolates were able to grow in a temperature range of (20 – 40 ºC), however, were unable to grow at 45 ºC. All the isolates grew well from pH (5.0 - 8.0), however, none was found to grow in the acidic condition of pH 2.0 and alkaline conditions of pH 9.0. Further, all the selected isolates grew well at 2-6 per cent salt concentration, eight isolates were able to grow at salt concentration of 8 per cent, however, none was in a position to grow at 10 per cent salinity.

4.3.3 Biochemical characterization of selected isolates

Biochemical characterizations of the selected fifteen isolates were carried out on the basis of biochemical tests (Table 6). Except four i.e. SIH_6, KAN_{11}, ROH_6 and ROH_{14} isolate, all isolates were able to hydrolyse gelatin. Eleven (73.3 per cent) isolates showed positive response for oxidase test, only five (33.33 per

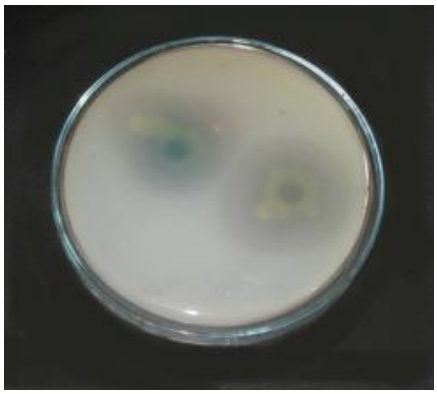


Zone of clearance

Amylase production by bacterial isolates on starch hydrolysis agar medium

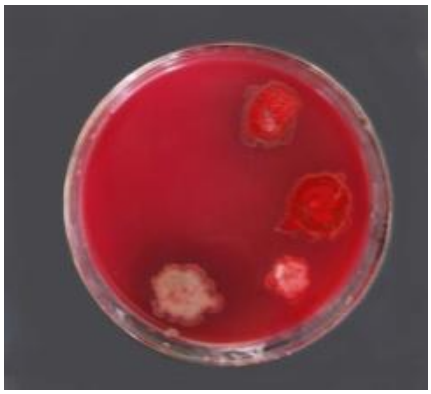
Zone of clearance

Pectinase production by bacterial isolates on pectinase screening agar medium



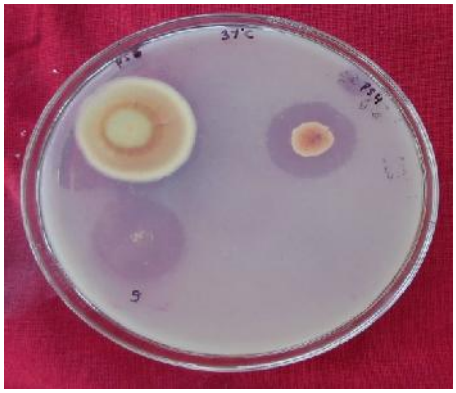
Zone of clearance

Protease production by bacterial isolates on skim agar medium



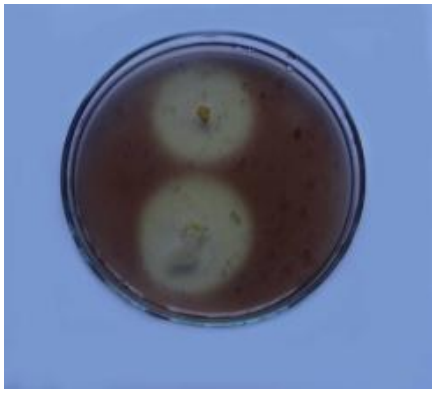
Zone of clearance

Cellulase production by bacterial isolates on Czapek Mineral salt agar medium



Zone of clearance

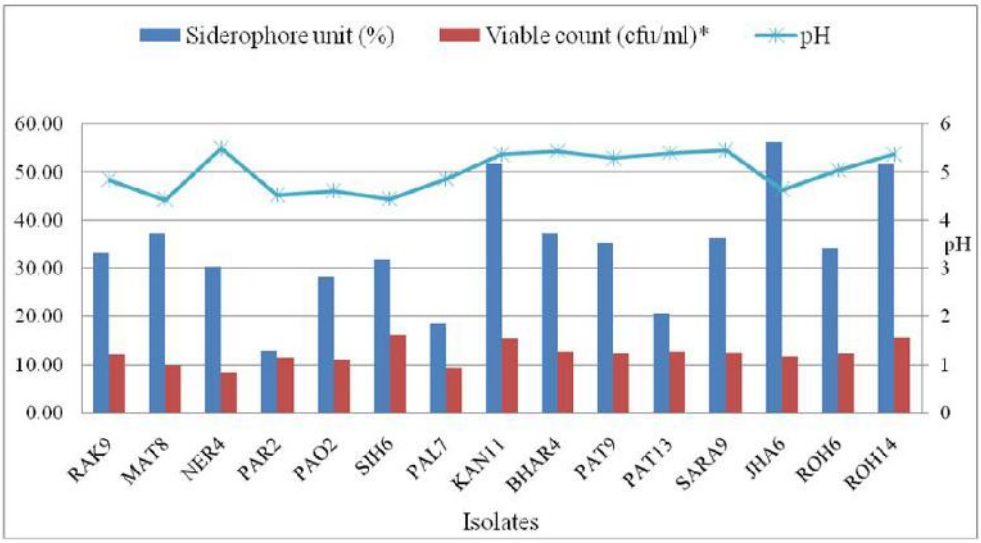
Lipase production by bacterial isolates on tributyrin agar medium



Zone of clearance

Chitinase production by bacterial isolates on colloidal chitin agar medium

Plate 4. Screening of selected bacterial isolates for various lytic enzyme production



*Viable count ($\times 10^7$ cfu/ml)

Fig. 4 Siderophore production unit (%), viable count and changes in pH of medium by selected bacterial isolates

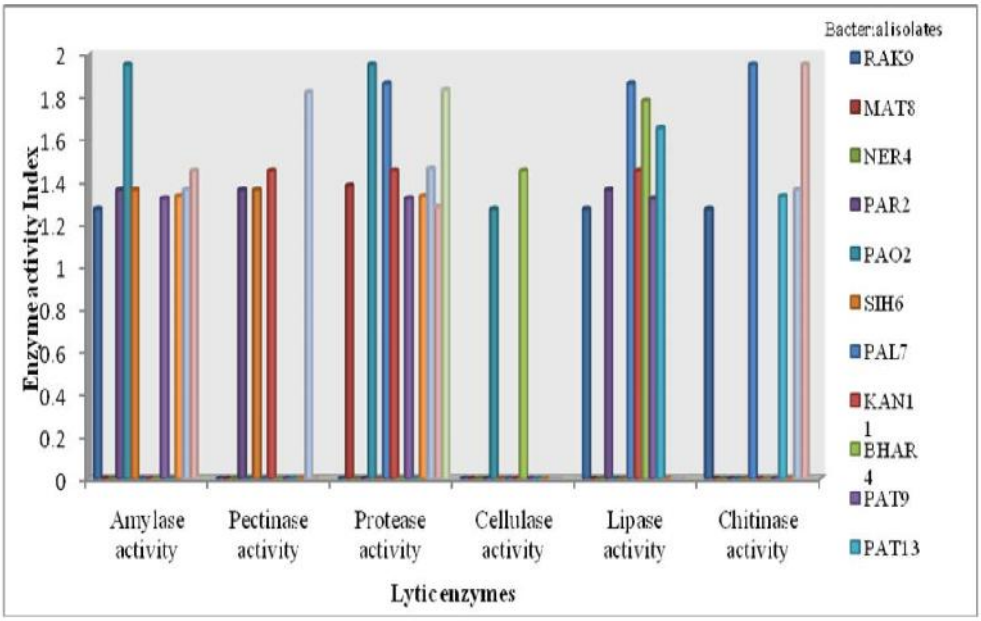


Fig. 5 Lytic enzyme production by selected bacterial isolates

cent) isolates gave positive response for citrate utilization, seven (46.67 per cent) isolates were found to be H_2S producers. All the isolates gave positive results for catalase test except ROH_{14}. Expect three ($BHAR_4$, JHA_6 and ROH_6) isolates all exhibited urease activity, four (26.67 per cent) isolates gave positive response for methyl red test and except one isolate i.e. ROH_{14} all were found negative for Voges Proskauer test. In case of carbon utilization, all the isolates were observed to ferment dextrose except JHA6, ten (66.67 per cent) isolates were able to ferment sucrose, lactose and fructose as carbon source. Among all only six isolates i.e. NER_4, SIH_6, PAL_7, $BHAR_4$, $SARA_9$ and ROH_{14} were able to ferment all the test carbohydrates (sucrose, dextrose, lactose and fructose).

Similar morphological, physiological and biochemical characteristics of PGPR isolates have been reported by Datta *et al*. (2011). The utilization of diverse carbon sources by bacterial isolates might indicates their metabolic and ecological diversity, presupposing their success of survival and competency in the environment when applied or introduced as a biofertiliser/biocontrol agent.

4.4 TO STUDY THE EFFICACY OF INDIGENOUS PGPR TO CONTROL BACTERIAL WILT OF CAPSICUM

Out of fifteen previously selected and characterized isolates (Table 5, 6) ten isolates possessing antagonistic activity against the bacterial pathogen *Ralstonia solanacearum* were selected for further studies. Data appended in Table 7 revealed that out of ten selected isolates, maximum (84.85 per cent) growth inhibition was recorded for ROH_6 isolate which was at par with isolate $SARA_9$ (72.73 per cent) and minimum (18.18 per cent) growth inhibition was recorded for isolate RAK_9.

4.4.1 Antibacterial activity of selected antagonistic isolates against *Ralstonia solanacearum* at different concentrations of cell free supernatant

Antibacterial metabolite production against the pathogen i.e. *Ralstonia solanacearum* was tested at different concentrations of cell free supernatant (Table 7) viz. 0.125 per cent (v/v), 0.250 per cent (v/v), 0.375 per cent (v/v) and 0.5 per cent (v/v). A large difference was observed in terms of zone of inhibition at concentration of 0.125 per cent ranged from 1.0 to 6.67 mm with maximum (6.67 mm) inhibition zone by ROH_6 isolate and minimum (1.0 mm) inhibition zone by isolate PAR_2.

Table 6. Physiological and metabolic characterization of selected bacterial isolates from different agroclimatic zones of Himachal Pradesh

Tests	RAK_9	MAT_8	NER_4	PAR_2	PAO_2	SIH_6	PAL_7	KAN_{11}	$BHAR_4$	PAT_9	PAT_{13}	$SARA_9$	JHA_6	ROH_6	ROH_{14}
Temperature (°C)*															
20	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
25	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
30	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
35	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
40	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
45	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
pH*															
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
4	+	+	-	-	-	+	+	-	+	+	+	-	-	+	-
5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Salinity (per cent**)***															
2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	-	+	-	-	+	-	+	-	-	+	+	-	+	+	+
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Biochemical Characteristics**															
Gelatin hydrolysis	+	+	+	+	+	-	+	-	+	+	+	+	+	-	-
Oxidase test	+	+	+	-	+	+	+	+	-	+	+	-	+	-	+
Citrate utilization	-	-	-	-	-	-	-	+	-	-	+	-	+	+	+
Catalase test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
H_2S production	-	+	-	+	+	-	+	-	+	-	-	-	-	+	+
Urease test	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+
Methyl red	-	-	+	+	+	-	-	-	-	-	-	-	-	+	-
Voges Prausker	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Sucrose	+	-	+	+	+	+	+	+	+	-	-	+	-	-	+
Dextrose	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
Lactose	-	+	+	-	-	+	+	+	+	+	+	+	-	-	+
Fructose	+	-	+	+	+	+	+	-	+	-	-	+	-	+	+

*** Growth present (+), Growth absent (-); **Activity present (+), Activity absent (-)**

At concentration of 0.25 per cent, zone of inhibition ranged from 2.67 to 10.0 mm whereas maximum (10.0 mm) zone of inhibition was exhibited by ROH_6 isolate and minimum (2.67 mm) inhibition zone was exhibited by KAN_{11}. At concentration of 0.375 per cent, the inhibition zone ranged from 2.00 to 12.33 with maximum (12.33 mm) zone of inhibition by ROH_6 isolate and minimum (2.00 mm) zone by RAK_9 isolate. For concentration of 0.5 per cent zone inhibition range was found to be 2.67 to 15.67 mm having maximum (15.67 mm) inhibition zone by ROH_6 isolate and minimum (2.67 mm) inhibition zone by isolate RAK_9. It was observed that antibacterial metabolite production was not much affected by further increase in (v/v) concentration of supernatant for all the isolates, hence 0.5 per cent (v/v) concentration could be further used for the study.

Table 7. Evaluation of antibacterial metabolite from selected antagonistic bacterial isolates against *Ralstonia Solanacearum*

Isolates	Per cent growth inhibition	Inhibition zone at conc. 0.125 per cent (v/v) in mm	Inhibition zone at conc. 0.250 per cent (v/v) in mm	Inhibition zone at conc. 0.375 per cent (v/v) in mm	Inhibition zone at conc. 0.5 per cent (v/v) in mm	Inhibition zone at conc. 0.625 per cent (v/v) in mm
RAK_9	18.18 (24.75)*	-	-	2.00	2.67	2.68
NER_4	41.67 (40.19)	2.33	5.00	11.00	12.67	12.67
PAR_2	41.67 (40.19)	1.00	3.67	8.33	8.67	8.67
KAN_{11}	30.30 (33.34)	-	2.67	7.33	8.00	8.01
$BHAR_4$	36.36 (36.97)	2.00	4.67	6.67	6.67	6.73
PAT_9	66.67 (54.76)	2.33	5.67	10.00	10.67	10.87
PAT_{13}	48.48 (44.11)	2.67	5.33	11.00	12.00	12.02
$SARA_9$	72.73 (59.40)	2.33	5.67	11.33	13.67	13.75
ROH_6	84.85 (67.30)	6.67	10	12.33	15.67	15.73
ROH_{14}	36.36 (36.97)	6.00	7.3	9.00	9.00	9.01
$CD_{0.05}$	**13.26 (9.09)**	**0.79**	**1.04**	**1.16**	**0.98**	**0.86**

*Figure in parenthesis () are arc-sin transformed values

Similarly, Lemessa and Zeller (2007) and Alyie *et al.* (2008) found growth inhibition of *R. solanacearum* by *Pseudomonas* spp., *Bacillus* spp. and *Paenibacillus macerans* under *in vitro* conditions.

4.4.2 Net house studies

4.4.2.1 Biocontrol studies

Evaluation of best five antagonistic strains (NER_4, PAT_9, PAT_{13}, ROH_{14} and ROH_6) against *R. solanacearum* revealed that the tested strains decreased disease incidence of bacterial wilt of pepper under net house conditions (Plate 5). The pathogen inoculation was done separately at the two stages i.e. one set pot

treatments were inoculated with the pathogen at the time of the transplantation and the other set of PGPR inoculated plants received the pathogen after six weeks of the transplantation.

The perusal of data embedded in Table 8 revealed that maximum (93 per cent and 734.11) germination per cent and vigor index were recorded in treatment T_5 whose seeds were inoculated with ROH_{14} isolate, followed by treatment T_4 receiving PAT_{13} isolate. At the seedling stage, all the antagonistic bacterial isolates showed no significant decrease in disease incidence over control.

On inoculation of the pathogen after six weeks of transplantation, the disease incidences of all treatments were significantly lower than the control (Fig 6). The minimum disease incidences (25.0 per cent) were found in treatment T_6 receiving ROH_6 isolate which was at par with the treatment T_3, T_4 and T_5 receiving PAT_9, PAT_{13} and ROH_{14} isolates, respectively. However, maximum disease incidence (83.3 per cent) and disease severity (79.2 per cent) were recorded with the treatment receiving only pathogen (control). The biocontrol efficacy of antagonist bacterial isolates towards the pathogen strain ranged from 29.2 per cent to 70.8 per cent. A strong *in vitro* antagonistic activity of the antagonist towards the different *R. solanacearum* strains correlated with a high biocontrol efficacy. The highest biocontrol efficacies of 70.8 per cent were observed for isolate ROH_6, which was at par with isolate ROH_{14} (58.3 per cent).

The *in vivo* inhibitory activity of the bacterial isolates against *R. solanacearum* in this study is in line with that of Hu *et al*. (2010) and Almoneafy *et al*. (2012) who also reported the antibacterial activity of suspension of *B. amyloliquefaciens* against capsicum bacterial wilt in green house and field. Similarly, Feng *et al*. (2013) also reported the 84.5 and 50.0 per cent control effect of two bacterial antagonistic isolates, X-3 and X-6, respectively, isolated from Xiahong-1 in tomato soil against *R. Solanacearum*. Ramesh *et al*. (2009) recorded 80 per cent and 70 per cent reduction of the same disease on eggplant (*Solanum melongena* L.) under greenhouse condition using *Pseudomonas* and *Bacillus* strains, respectively. Also, Lemessa and Zeller (2007) also reported that application of *B. subtilis* B2G and fluorescent pseudomonads APF1 significantly

Plate 5. Biocontrol potential of antagonistic bacterial isolates against *Ralstonia solanacearum* (net house conditions)

reduced bacterial wilt in tomato by 56 per cent and 60 per cent besides increasing the plant biomass.

4.4.2.2 Growth promotion

Studies on effect of antagonistic bacterial isolates on plant biomass and NPK content were carried after the pathogen inoculation at the fruit set stage of the plant (Table 8). Treatments receiving the bacterial inoculum significantly increased plant biomass over the pathogen control. Maximum plant biomass (31.90 g) was recorded for treatment T_5 receiving ROH_{14} isolate which was found statistically at par with treatment T_6 receiving ROH_6 isolate. Seedling inoculation with the bacterial isolates significantly increased the nutrient content of the plant over the control. Maximum N (1.47 per cent), P (0.30 per cent) and K (2.05 per cent) content was recorded for the treatment T_6 receiving ROH_6 isolate. The disease suppression by antagonistic bacterial isolates might be due to induction of systemic resistance in tomato plant which is mediated by jasmonic acid (JA) and ethylene (ET) hormones.

Table 8. Effect of antagonistic bacterial isolates on plant growth parameters and nutrient content of capsicum infested with *Ralstonia Solanacearum*

Treatment	Germination (per cent)	Vigour index	Plant biomass (g)	N content (per cent)	P content (per cent)	K content (per cent)
T_1 (Control)	58 (49.59)*	375.13	19.40	1.12 (1.06)**	0.20 (0.45)**	0.89 (0.94)**
T_2 (NER_4)	86 (68.26)	660.01	24.64	1.45 (1.20)	0.28 (0.53)	1.71 (1.30)
T_3 (PAT_9)	83 (65.65)	695.80	25.67	1.34 (1.16)	0.25 (0.50)	1.76 (1.33)
T_4 (PAT_{13})	91 (72.85)	709.06	24.83	1.36 (1.17)	0.26 (0.51)	1.84 (1.35)
T_5 (ROH_{14})	93 (74.76)	734.11	31.90	1.44 (1.20)	0.28 (0.53)	2.02 (1.42)
T_6 (ROH_6)	80 (63.60)	712.69	30.87	1.47 (1.20)	0.30 (0.53)	2.05 (1.43)
$CD_{0.05}$	**5.65 (4.87)**	**58.05**	**2.90**	**0.26 (0.11)**	**0.06 (0.07)**	**0.42 (0.16)**

*Figure in parenthesis () are arc-sin transformed values

**Figure in parenthesis () are square root transformed values

Almoneafy *et al*. (2012) had documented similar findings where antagonistic isolates supported high values of plant height and biomass when compared with the control treatment. Similar results were recorded by Wei *et al.* (2011) and Xue *et al.* (2012) who suggested antagonistic activity of the bacterial isolate as the major mechanism of inhibition of *R. Solanacearum*. Amaresan *et al.* (2012), Walia *et al*. (2012) also suggested the production of siderophore, HCN

and other plant growth promoting substances e.g. IAA by test antagonist as among the major elicitors of induced systemic resistance (ISR) in plants. Furthermore, Baysal *et al.* (2003), Jetiyanon (2007) reported breakdown products of the pathogen and/or plant cell wall components released by the activity of lytic enzymes as elicitors of plant defence responses.

4.5. TO STUDY THE EFFECT OF PGPR AND CHEMICAL FERTILIZERS ON SOIL HEALTH AND PRODUCTIVITY OF CAPSICUM

4.5.1 Screening of selected PGPR isolates under net house conditions

4.5.1.1 Effect of PGPR on growth parameters

Six isolates (MAT_8, NER_4, KAN_{11}, $BHAR_4$, JHA_6 and ROH_{14}) possessing maximum P-solubilising ability revealed a significant increase in various seedling parameters over control (un-inoculated absolute control) under net house conditions (Table 9; Plate 6(a)).

The maximum (92.0 per cent) seed germination was recorded for JHA_6 isolate, however, was statistically at par with MAT_8, KAN_{11} and ROH_{14} isolates. Similarly, maximum (768.20) value of vigour index was recorded for JHA_6 isolate treated seeds, followed by MAT_8 isolate (666.06) and the minimum value (57.33 per cent) seed germination and (366.15) vigour index, respectively was obtained with un-inoculated absolute control. Our findings are in conformity with those of Sakthivel *et al.* (2009) who has reported that seed bacterization with *Pseudomonas fluorescens* improved the seed germination and vigour index of tomato. Similarly, Ashrafuzzaman *et al.* (2009) also reported that seed treated with bacterial isolates have better germination and emergence of rice seedlings.

The application of bacterial isolate registered a significant increase in seedling length and biomass over un-inoculated control. The maximum (7.6 cm) seedling shoot height and (76.8 mg/plant) biomass was noted for JHA_6 isolate treated seeds, which was at par with ROH_{14} isolate. The maximum (6.0 cm) root length and (6.6 mg/plant) biomass was noted for ROH_{14} isolate treated seeds, whereas, the minimum (6.0 cm and 60.2 mg/plant) seedling height and biomass, (2.8 cm and 3.6 mg/plant) root length and biomass, respectively, was recorded for

un-inoculated absolute control. The 54.55 and 30.72 per cent increase in seedling length and biomass, respectively, with PGPR isolates over absolute control are in accordance with Suryanto *et al*. (2010) who have recorded increased seedling height and seedling biomass with bacterial isolates over control.

Table 9. Effect of bacterial inoculum on capsicum seedling growth parameters (net house conditions)

Treatments	Germination (per cent)*	Vigour index	Shoot length (cm)	Shoot biomass (mg/plant)	Root length (cm)	Root biomass (mg/plant)
Absolute Control	57.33 (49.20)	366.15	6.0	60.2	2.8	3.6
MAT_8	86.67 (68.60)	666.06	7.0	71.2	3.1	4.0
NER_4	77.33 (61.56)	549.60	6.6	67.0	3.2	4.2
KAN_{11}	88.00 (69.88)	645.38	6.9	67.3	5.5	5.8
$BHAR_4$	62.67 (52.32)	364.15	6.2	62.9	4.9	5.2
JHA_6	92.00 (73.89)	768.20	7.6	76.8	5.7	5.4
ROH_{14}	86.67 (68.88)	661.14	7.5	72.1	6.0	6.6
$CD_{0.05}$	**6.30 (5.45)**	**92.50**	**0.5**	**5.6**	**0.4**	**0.3**

*Figure in parenthesis () are arc-sin transformed values

4.5.1.2 Efficacy of selected PGPR at different doses of N and P on productivity of capsicum

On the basis of germination percentage and seedling growth parameters, four best performers bacterial isolates (MAT_8, KAN_{11}, JHA_6 and ROH_{14}) were further evaluated for capsicum productivity in combination along with varied levels (40, 60 and 80 per cent) of Recommended Doses (RD) of N and P fertilizers under net house conditions (Plate 6(b)). The periodic data pertaining to growth, yield of capsicum, soil physico-chemical and microbiological properties due to co-inoculation of bacterial isolates at varying doses of N and P fertilizers have been presented under the following sub heads:-

4.5.1.2.1 Plant growth parameters

The various growth and yield characters of capsicum were, in general, significantly influenced by the application of bacterial isolates along with N and P fertilizers (80, 60 and 40 per cent of recommended doses) levels over control treatment (Table 10).

Height of the plant is one of the important factors determining yield and harvest duration. Taller plants are considered to be more desirable because they lead to more number of branches which ultimately bear more number of fruits and result in increased productivity. The maximum (77.0 cm, 39.27g and 2.48g) shoot length, shoot biomass and root biomass, respectively, were recorded with T_8 (80 per cent NP + ROH_{14}) treatment, which was statistically at par with T_1 (100 per cent RDF) and T_7 (80 per cent NP + JHA_6) treatments. Maximum (31.02 cm) root length was recorded with T_7 (80 per cent NP + JHA_6) followed by T_8 treatment receiving isolate ROH_{14} along with 80 per cent NP, whereas, minimum (44.9 cm) shoot length, (22.35 g) shoot biomass, (16.8 cm) root length and (1.21 g) root biomass were recorded with T_4 treatment (40 per cent RDF).

The T_8 treatment (80 per cent NP + ROH_{14}) produced 5.62 per cent taller plants as compared to treatment T_1 (100 per cent RDF). The possible reasons for better shoot/root length and their biomass might be due biofertilization (increasing availability of the soil nutrients particularly N (asymbiotic N-fixation), P (P-solubilisation) and Fe (siderophore production) to plant by the direct mechanisms of application of PGPR inoculum in the rhizosphere. The increased availability might have contributed for more absorption of the same, which further resulted in enhanced cell division, cell enlargement, development of greater amount of fine roots and ultimately the better plant stands. Further, PGPR applied with inorganic fertilizers improved the effectiveness of chemical fertilizers. Application of PGPR helps in improving number of biological activities of desired microorganisms in soil, suppression of soil borne pathogens and improves growth of bell pepper plants. Similar results were also reported by Hernandez and Chailloux (2004), who recorded that the dry weight of tomato transplants grown in the greenhouse with 75 per cent fertilizer plus two co-inoculated PGPR was significantly greater than those with full fertilizer rate without PGPR. These findings are in conformity with Jackson *et al.* (1964), Mandal *et al*. (2003) and Poinkar *et al.* (2006).

The main objective of cultivation of a crop is to have maximum marketable yield for better returns. An examination of Table 10 indicated that various fruit parameters are also influenced significantly with the treatments under investigation. Maximum (11.3 cm, 22.9cm and 94.57 g) fruit length, breadth and

weight, respectively, was recorded in T_7 (80 per cent NP + JHA_6) treatment which was statistically at par with T_1 (100 per cent RDF) and T_8 (80 per cent NP + ROH_{14}) treatments. The minimum (6.11 cm) fruit length, (13.2 cm) fruit breadth and (56.45 g) fruit weight were recorded with T_4 treatment (40 per cent RDF).

There was a significant and linear increase recorded in capsicum yield by increase in chemical fertilizers levels from 60 to 80 per cent NP with conjoint application of PGPR isolates. The maximum (13.8) number of fruits per plant and hence, yield (1.27 kg/plant) was recorded for T_8 (80 per cent NP + ROH_{14}) treatment which was statistically at par with T_1 (100 per cent RDF) and T_7 (80 per cent NP + JHA_6) treatments and the minimum (8.4) number of fruits per plant and (0.49 kg/plant) yield was recorded under T_4 treatment (40 per cent RDF).

Table 10. Effect of PGPR and chemical fertilizers on capsicum plant growth parameters and yield (net house conditions).

Treatments	Shoot length (cm)	Shoot biomass (g/plant)	Root length (cm)	Root biomass (g/plant)	Fruit length (cm)	Fruit breadth (cm)	Fruit weight (g)	Fruit number (no./plant)	Fruit yield (kg/plant)
T_1 (100 per cent RDF	72.9	37.91	29.3	2.40	10.7	22.1	90.28	13.3	1.20
T_2 (80 per cent RDF)	57.7	29.41	23.2	1.86	8.5	17.2	71.35	10.3	0.74
T_3 (60 per cent RDF)	51.7	20.35	20.8	1.67	7.6	15.4	62.99	9.2	0.58
T_4 (40 per cent RDF)	44.9	22.35	16.8	1.21	6.1	13.2	56.45	8.4	0.49
T_5 (80 per cent RDNP + MAT_8)	63.3	32.30	25.5	2.04	9.3	18.9	79.24	11.3	0.90
T_6 (80 per cent RDNP + KAN_{11})	65.3	33.32	26.3	2.11	9.6	19.5	82.03	11.7	0.96
T_7 (80 per cent RDNP + JHA_6)	74.7	38.06	31.0	2.43	11.3	22.9	94.57	13.0	1.23
T_8(80 per cent RDNP + ROH_{14})	77.0	39.27	29.9	2.48	10.9	21.6	92.25	13.8	1.27
T_9(60 per cent RDNP + MAT_8)	56.7	28.90	22.8	1.83	8.3	16.9	69.95	10.1	0.71
T_{10}(60 per cent RDNP + KAN_{11})	53.3	27.20	21.5	1.72	7.8	15.9	65.31	9.5	0.62
T_{11}(60 per cent RDNP + JHA_6)	60.3	30.77	24.3	1.94	8.8	18.0	75.06	10.8	0.81
T_{12}(60 per cent RDNP + ROH_{14})	59.3	30.26	23.9	1.91	8.7	17.7	73.67	10.6	0.78
T_{13}(40 per cent RDNP + MAT_8)	48.0	24.48	19.3	1.55	7.0	14.3	57.88	8.6	0.50
T_{14}(40 per cent RDNP + KAN_{11})	48.7	24.82	19.6	1.57	7.1	14.5	58.81	8.7	0.51
T_{15}(40 per cent RDNP + JHA_6)	51.3	26.18	20.7	1.65	7.5	15.3	62.52	9.2	0.57
T_{16}(40 per cent RDNP + ROH_{14})	51.7	26.35	20.8	1.67	7.6	15.4	62.99	9.2	0.58
$CD_{0.05}$	**4.2**	**2.84**	**2.2**	**0.18**	**0.8**	**1.7**	**7.76**	**1.0**	**0.13**

It is pertinent to note that T_8 treatment recorded 5.83 per cent higher fruit yield as compared to T_1 (100 per cent NPK) treatment. Superimposition of PGPR over the inorganic fertilizers had a spectacular effect on the crop yield which was higher as compared to other treatments. This increase in yield and other fruit parameters with PGPR and NPK could be attributed to improve vegetative growth, better availability of nutrients at vital growth period, better absorption and mobilization of nutrients within the plant and increase in number fruits per plant. Similarly, Datta *et al.* (2011) also reported that inoculation of capsicum plants with rhizospheric *Bacillus* spp. results in increased number of fruits/plant, fruit length, width and weight as compared to control. Further, results are in conformation with Kaushal and Kaushal (2013) who also observed significantly higher curd yield by inoculation of PGPR with 75 per cent doses of fertilizers over control (un-inoculated plants).

4.5.1.2.2 Total nutrient (N, P and K) content and their uptake

Significant variations among total nutrient content and uptake were observed due to various treatment combinations (Table 11). The maximum (5.46 per cent and 78.0 mg/plant) N content and uptake was recorded under T_8 (80 per cent NP + ROH_{14}) which was at par with T_1 (100 per cent RDF) and T_7 (80 per cent NP + JHA_6). The maximum (0.59 per cent and 8.7 mg/plant) P content and uptake, was recorded under T_7 (80 per cent NP + JHA_6) treatment which was at par with T_1 (100 per cent RDF) and T_8 (80 per cent NP + ROH_{14}) treatments. Treatment comprising 100 per cent recommended doses of chemical fertilizers (T_1) resulted in maximum (3.79 per cent and 52.7 mg/plant) K content and uptake followed by T_8 (80 per cent NP + ROH_{14}), whereas, minimum N and P content (2.57 per cent and 0.32 per cent) and their uptake (13.1 and 1.6 mg/plant), respectively, were recorded under T_4 treatment (40 per cent RDF).

The increased the N content (6.8 per cent) and uptake (6.7 per cent) in T_8 and P content (7.3 per cent) and uptake (12.9 per cent) in T_7 over T_1 (100 per cent NPK) treatment might be attributed to microbial inoculation, inducing significant changes in the rhizosphere and creates favorable conditions for mineral uptake by plants. In general, PGPR promote the growth of the plant and increase the root surface area or the general root architecture. Plants growing better in turn release higher amounts of C in root exudates. The release of more C prompts increase in

microbial activity and this process continues in a cycle. The whole process makes more N available from the soil pool, influencing N flux into plant roots and the plant is able to take up more available N. The increase in P uptake may be attributed to increase in available P content that might have resulted due to P-solubilization activity by the applied bacterial isolates.

Table 11. Effect of varied levels of NP on NPK content and their uptake in capsicum (net house conditions)

Treatments	Nutrient content (%)			Nutrient uptake (mg/plant)		
	N	P	K	N	P	K
T_1 (100 per cent RDF	5.11	0.55	3.79	73.10	7.70	52.70
T_2 (80 per cent RDF)	4.10	0.47	2.85	39.50	4.50	25.50
T_3 (60 per cent RDF)	3.39	0.40	2.16	24.20	2.80	15.10
T_4 (40 per cent RDF)	2.57	0.32	2.05	13.10	1.60	11.00
T_5 (80 per cent RDNP + MAT_8)	4.35	0.49	2.99	47.30	5.30	31.10
T_6 (80 per cent RDNP + KAN_{11})	4.51	0.51	2.86	52.20	5.80	34.60
T_7 (80 per cent RDNP + JHA_6)	5.25	0.59	3.38	77.10	8.70	48.20
T_8(80 per cent RDNP + ROH_{14})	5.46	0.57	3.40	78.00	8.20	50.00
T_9(60 per cent RDNP + MAT_8)	3.53	0.41	2.39	26.90	3.10	20.50
T_{10}(60 per cent RDNP + KAN_{11})	3.80	0.44	2.46	32.60	3.80	16.50
T_{11}(60 per cent RDNP + JHA_6)	4.02	0.46	2.57	37.10	4.20	21.60
T_{12}(60 per cent RDNP + ROH_{14})	3.88	0.45	2.64	34.00	3.90	23.80
T_{13}(40 per cent RDNP + MAT_8)	3.09	0.37	1.84	18.90	2.30	11.80
T_{14}(40 per cent RDNP + KAN_{11})	3.14	0.38	1.80	20.10	2.40	14.40
T_{15}(40 per cent RDNP + JHA_6)	3.36	0.40	2.05	24.10	2.80	14.50
T_{16}(40 per cent RDNP + ROH_{14})	3.39	0.40	2.02	24.90	2.90	14.60
$CD_{0.05}$	**0.46**	**0.04**	**0.38**	**5.42**	**1.43**	**2.31**

The results are in confirmation with Han *et al.* 2006 who also reported that integrated rock P with inoculation of PSB increased the availability of P and K in soil, the uptake of N, P and K by plant and the growth of pepper and cucumber. Similar findings were observed by Srivastava and Verma (1984), who have also reported that inoculation with P-solubilizers increase the P concentration in plant. Sridevi and Mallaiah (2009), Zabihi *et al*. (2011) also reported significant increase in nutrient availability, their uptake and yield components in wheat by inoculation of PGPR over the un-inoculated control

4.5.1.2.3 Soil physico-chemical properties

The data presented in Table 12 explicit that none of the tried treatment influenced significantly the physico-chemical properties (pH, EC and OC) of soil,

however, registered significant increase in available nutrient (N and P) of soil over recommended doses of chemical fertilizers.

The probable reason for non significant changes in most of the physico-chemical properties might be shorter time period (4 - 5 months) between sowing and harvesting of the crop. The studies conducted by Badhulkar *et al.* (2000), Selvi *et al.* (2005) and Bajpai *et al.* (2006) also clearly revealed that only long-term experimentation brought changes in fundamental physico-chemical properties of soil.

Increasing fertilizer levels and integration with PGPR isolates resulted in higher available nutrient contents of soil. The available N and P content under net house conditions increased significantly with the incorporation of 80 per cent chemical fertilizers in combination with PGPR over 100% recommended doses of chemical fertilizers (control).

The perusal of data appended in Table 12 revealed that the maximum (405.6 kg ha^{-1}) amount of available N and (49.8 kg ha^{-1}) available P was observed in treatment T_8, which was statistically at par with T_1, T_5 and T_7. ROH_{14} inoculated plants receiving 80 per cent NP doses recorded 106.9 kg $ha^{-1}$and 25.2 kg/ha more available N and P over initial value. The maximum (256.7 kg ha^{-1}) available K content of soil after the harvest of crop was recorded with T_1 which was 61.8 kg ha^{-1} more than initial value and minimum (249.80, 25.60 and 166.10 kg ha^{-1}) available N, P and K content of soil was registered with T_4 treatment (40 per cent RDF).

The T_8 treatment receiving ROH_{14} isolates with 80 per cent NP doses resulted in increased available N (6.01 per cent) and P (7.52 per cent) contents over T_1 treatment receiving 100 per cent RDF. This increase in available N may be attributed to higher microbial activity of integrated nutrient management (INM) treatments which favored the conversion of atmospheric nitrogen by asymbiotic nitrogen fixation phenomenon and partially by mineralization of organically bound nitrogen to inorganic forms. Similar increase in available N in soil due to addition of microbial inoculum was observed by Gupta *et al.* (2015) in capsicum crop. Our results are further in conformation with those of Asokan *et al.*

(2000), Smith and Read (1997) who also reported accumulation of N in soil by mineralization of organic matter and fixation of atmospheric nitrogen by soil microbes.

Table 12. Effect of PGPR and chemical fertilizers on soil physico-chemical characteristics (net house conditions)

Treatments	pH	EC (dSm^{-1})	OC (%)	Available N ($kg\ ha^{-1}$)	Available P ($kg\ ha^{-1}$)	Available K ($kg\ ha^{-1}$)
T_1 (100 per cent RDF)	6.75	0.46	1.12	382.63 (83.9)	46.28 (21.7)	256.7 (61.8)
T_2 (80 per cent RDF)	6.92	0.56	1.36	369.69 (70.9)	40.41 (15.8)	213.72 (18.8)
T_3 (60 per cent RDF)	6.97	0.53	1.30	339.73 (41.0)	35.1 (10.5)	171.36 (-23.5)
T_4 (40 per cent RDF)	6.95	0.52	1.26	249.8 (-48.9)	25.66 (1.1)	166.1 (-28.7)
T_5 (80 per cent RDNP + MAT_8)	6.97	0.52	1.27	379.63 (80.9)	44.28 (19.7)	213.72 (18.8)
T_6 (80 per cent RDNP + KAN_{11})	6.77	0.51	1.24	371.68 (73.0)	42.6 (18.0)	228.82 (33.9)
T_7 (80 per cent RDNP + JHA_6)	6.84	0.50	1.21	390.65 (91.9)	47.93 (23.4)	235.79 (40.9)
T_8(80 per cent RDNP + ROH_{14})	6.60	0.52	1.26	405.64 (106.9)	49.76 (25.2)	236.95 (42.1)
T_9(60 per cent RDNP + MAT_8)	6.83	0.51	1.24	319.74 (21.0)	31.45 (6.9)	182.36 (-12.5)
T_{10}(60 per cent RDNP + KAN_{11})	6.82	0.51	1.24	345.72 (47.0)	36.2 (11.6)	193.98 (-0.9)
T_{11}(60 per cent RDNP + JHA_6)	6.80	0.50	1.23	355.71 (57.0)	38.02 (13.5)	195.75 (0.9)
T_{12}(60 per cent RDNP + ROH_{14})	6.82	0.49	1.18	361.71 (63.0)	39.12 (14.6)	206.75 (11.9)
T_{13}(40 per cent RDNP + MAT_8)	6.75	0.47	1.15	287.77 (-10.9)	26.6 (2.0)	169.2 (-25.7)
T_{14}(40 per cent RDNP + KAN_{11})	6.82	0.48	1.16	307.75 (9.0)	29.26 (4.7)	170.1 (-24.8)
T_{15}(40 per cent RDNP + JHA_6)	6.78	0.47	1.14	309.75 (11.0)	29.62 (5.1)	168.7 (-26.17)
T_{16}(40 per cent RDNP + ROH_{14})	6.80	0.48	1.16	291.76 (-6.94)	26.33 (1.8)	170.4 (-24.47)
$CD_{0.05}$	**NS**	**NS**	**NS**	**33.41**	**6.11**	**15.80**

*Figure in parenthesis () represents increase/decrease in ($kg\ ha^{-1}$) over initial value

Increase in the P availability might be due to activities of phosphate solubilising microorganisms, might have brought some P from unavailable pool to available pool. The release of various organic acids might have solubilized the insoluble P fractions and thus, resulting into a significant increased content of available P in the soil. The increase in phosphorus availability from insoluble sources by application of P-solubilizer *Pseudomonas striata* has also been reported by Singh and Gupta (2001). These findings are in conformation with Richardson (2001) and Qureshi *et al*. (2012) who also reported that phosphate solubilizing bacteria are common in the rhizosphere and facilitates the conversion of insoluble forms of P to plant available forms and ultimately increased availability to host plants.

4.5.1.2.4 Microbiological parameters

4.5.1.2.4.1 Total bacterial count in soil

Total bacterial count in soil microbial population viz. rhizospheric and endophytic bacterial population was significantly enhanced by the application of

conjoint use of PGPR and chemical fertilizers after harvest of crop (Appendix 2.3 and Figure 7). The maximum count (121.06 × 10^6 cfu/g soil) on NA medium was noted in rhizosphere of plants whose seeds were treated with T_8 (ROH_{14} + 80 per cent NP) treatment. For endophytic bacterial population maximum (99.27 × 10^2 cfu/g root) colonies on NA medium were recorded for seed bacterisation with T_7 (JHA_6 + 80 per cent NP) treatment. Whereas, the respective minimum value (53.4 × 10^6 cfu/g soil and 38.4 × 10^2 cfu/g root) was recorded in T_4 (40 per cent RDF) treatment.

The application of efficient inoculum of PGPR with high viable count (10^8 –10^{10} cfu/ml) coupled with adequate amounts of N and other nutrients might have contributed to increase in the total microbial population. These results are in conformation with those of Patil and Varade (1998), Selvi *et al*. (2004) and Qureshi *et al*. (2005). They have reported that 100 per cent N alone and un-inoculated control treatment had minimum microbial population over balanced fertilization i.e. 100 per cent NPK + FYM and/or biofertilizer.

4.5.1.2.4.2 Microbial biomass-carbon

The conjoint application of bacterial inoculant with chemical fertilizers significantly influenced the microbial biomass-carbon over chemical fertilizers alone (Appendix 2.3 and Figure 7). The maximum (68 mg MB-C/100g soil) microbial biomass was recorded under treatment T_8 (ROH_{14}+ 80 per centNP), whereas, the minimum (46.8 mg MB-C/100g soil) microbial biomass-C was recorded with T_4 treatment (40 per cent RDF). These findings are in accordance with those of Anderson and Domsch (1978) who reported the microbial biomass carbon in agricultural soil from 15 to 240 mg MB-C/100 g soil.

4.5.2 EFFICACY OF SELECTED BACTERIAL ISOLATES ALONG WITH CHEMICAL FERTILISERS ON PRODUCTIVITY OF CAPSICUM (FIELD CONDITIONS)

The periodic data during the year 2014 and 2015 pertaining to growth and yields of capsicum, soil nutrient status and microbiological properties by the co-inoculation of best two bacterial isolates (JHA_6 and ROH_{14}) alone and in consortia with two levels 60 and 80 per cent of RD doses of N and P fertilizers have been presented under the following sub heads:-

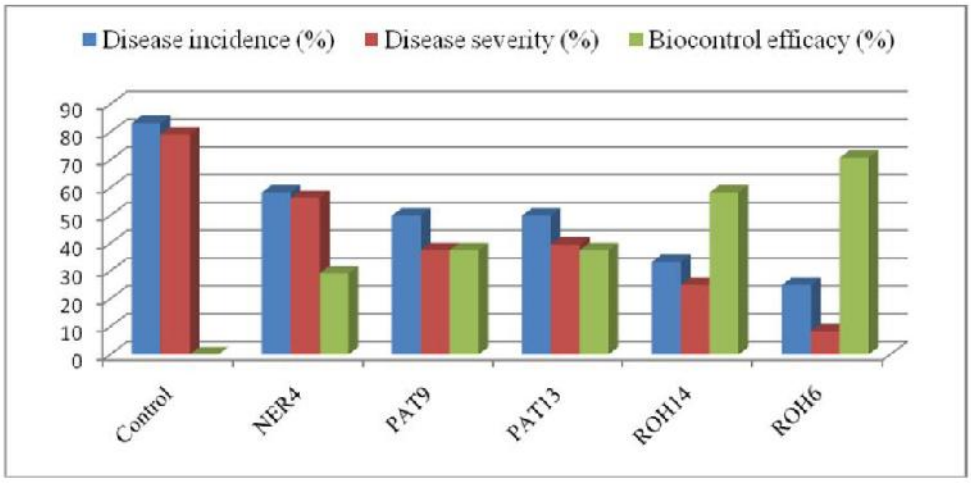
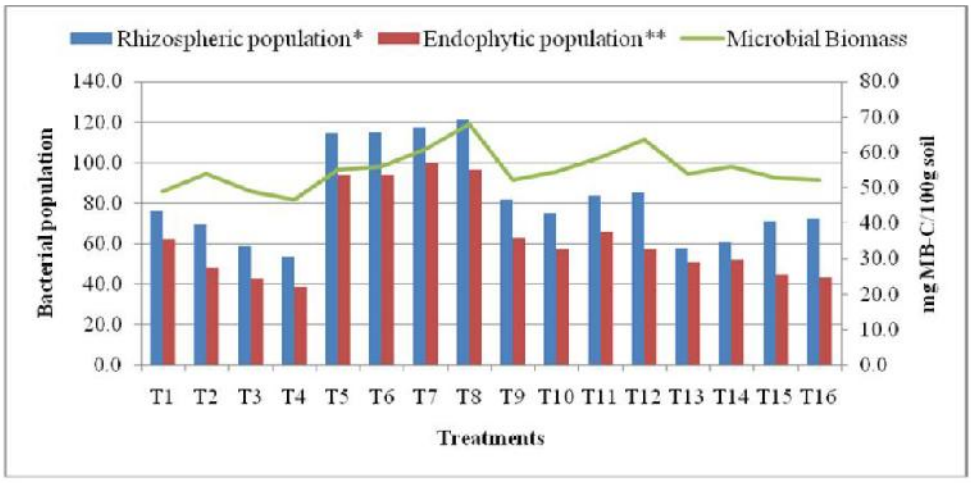


Fig. 6 Biocontrol potential of antagonistic bacterial isolates agaist *Ralstonia solanacearum* (at fruit set stage)



*Rhizospheric population ($\times 10^6$ cfu/g soil); **Endophytic population ($\times 10^2$ cfu/g root)

Fig. 7 Effect of bacterial inoculation at varied levels of NP on rhizospheric, endophytic microbial count and microbial biomass (net house conditions)

4.5.2.1 Effect on plant growth parameters

The application PGPR isolates at different levels of N and P significantly influenced the plant growth and yield parameters (Plate 6). A perusal of data (Table 13) revealed maximum (71.7 cm) plant height and (36.2 qha^{-1}) dry matter accumulation was recorded for T_4 treatment (80 per cent NP+PGPR consortia), which was statistically at par with T_1, T_2 and T_3 treatments. Increased plant height and dry matter accumulation in treatments receiving 80 per cent recommended doses of N and P with bacterial isolates either singly (T_2, T_3) or in consortia (T_4) over T_1 (unicoluated control with 100 per cent RDF) may be due to increased availability of phosphorus and nitrogen by inoculation of the bacterial isolates and added fertilizers, which might have resulted in better plant growth, improved photosynthetic and metabolic activity.

The conjoint application of PGPR isolate at 80 per cent NP fertilizers significantly influenced plant growth and yield over T_1 treatment i.e. 100 per cent recommended dose of NPK ($N_{(100)}$, $P_2O_{5(76)}$ & $K_2O_{(45)}$). Maximum (18.0 t ha^{-1}) yield was recorded under T_4 treatment, followed by T_3 (16.0 t ha^{-1}) treatment. Higher yield under these treatments may be attributed to more number of fruits per plant (9.9 and 9.5) and average fruit weight (76.1 and 70.6 g), respectively.

The increased yield parameters due to application of chemical fertilizers in conjunction with PGPR may be attributed to improved vegetative growth, better availability of nutrients, enhanced photosynthetic activity and improvement in soil physical properties which led to better soil physical health. The results are also in conformity of the findings of Sharma (1986), Paramaguru and Natarajan (1993), Dekha *et al*. (1996), Chandrappa *et al*. (2007), Khan and Parari (2012), and Gupta *et al.* (2015) who have also recorded increased plant height, biomass yield of chilli due to biofertilizer application including NPK.

4.5.2.2 Effect on plant nutrient content and their uptake

Perusal of data presented in Table 14 revealed that N, P and K content in plant was significantly influenced by conjoint application of bacterial isolate (s) and 80 per cent NP in comparison to recommended doses of fertilizers. The maximum (5.2 per cent) N content was recorded in T_4 (PGPR consortia + 80 per cent NP) which was statistically at par with T_3, while minimum (3.8 per cent) N

Table 13. Effect of PGPR and chemical fertilizers on plant growth parameters and yield of capsicum (field conditions)

Treatments	Plant height (cm)			Plant biomass (q ha^{-1})			No. of fruits (fruit $plant^{-1}$)			Fruit weight (g)			Yield (t ha^{-1})		
	2014	**2015**	**Mean**	**2014**	**2015**	**Mean**	**2014**	**2015**	**Mean**	**2014**	**2015**	**Mean**	**2014**	**2015**	**Mean**
T_1 (100 per cent RDF)	62.0	60.6	66.3	27.7	26.9	33.1	9.2	9.3	9.3	64.0	64.3	64.1	14.2	14.4	14.3
T_2 (80 per cent RDNP + JHA_6)	63.8	65.4	69.0	30.6	28.4	35.5	9.3	9.4	9.3	69.7	70.0	69.9	15.5	15.8	15.7
T_3 (80 per cent RDNP + ROH_{14})	68.4	69.2	68.8	32.7	36.0	34.4	9.4	9.5	9.5	70.2	70.9	70.6	15.9	16.2	16.0
T_4 (80 per cent RDNP JHA_6 + ROH_{14})	70.2	73.3	71.7	34.3	38.0	36.2	9.8	9.9	9.9	74.8	77.4	76.1	17.7	18.3	18.0
T_5 (60 per cent RDNP + JHA_6)	53.5	55.4	54.4	23.6	25.1	24.4	8.1	8.4	8.3	42.1	45.0	43.6	8.2	9.1	8.7
T_6 (60 per cent RDNP + ROH_{14})	54.4	56.9	55.6	24.4	25.3	24.8	8.2	8.3	8.2	44.7	46.7	45.7	8.7	9.3	9.0
T_7 (60 per cent RDNP JHA_6+ ROH_{14})	56.8	58.0	57.4	25.7	26.2	26.0	8.3	8.4	8.4	45.8	47.9	46.9	9.2	9.7	9.4
$CD_{0.05}$	**6.8**	**6.7**	**6.2**	**3.7**	**3.7**	**3.3**	**0.5**	**0.4**	**0.3**	**3.0**	**3.1**	**2.3**	**1.0**	**1.0**	**0.7**

content was recorded in T_6 treatment. The T_4 treatment had maximum (0.48 per cent) P content which was statistically at par with T_2 and T_3 (either of two PGPR with 80 per cent NP) treatments, whereas, minimum (0.41 per cent) P content was recorded in treatment T_5 (JHA_6 with 60 per cent NP). The maximum (4.78 per cent) K content was recorded with T_1 treatment (100 per cent RDF), whereas, minimum (3.71 per cent) K content was observed with treatment T_5 (ROH_{14} + 80 per cent NP).

Similarly, application of N and P nutrients at 80 per cent levels of recommended doses of chemical fertilizers in combination with PGPR recorded significantly higher uptake of N, P and K over sole application of 100 per cent recommended doses of fertilizers. The treatment T_4 resulted in maximum N, P and K uptake (189.3, 16.32 and 172.83 kg ha^{-1}, respectively), whereas, the respective minimum value was recorded in T_5 (JHA_6 + 60 per cent NP) treatment.

The application of consortia of two PGPR (JHA_6 and ROH_{14}) with 80 per cent NP doses increased the N and P content by 10.6 and 47.1 per cent and their uptake by 6.7 and 34.0 per cent, respectively, over sole application of 100 per cent recommended doses of chemical fertilizers (RDF). This increase may be attributed to more availability of N (atmospheric N fixation) and P (converting insoluble phosphate to soluble form through secretion of organic acids in soil) as a result of inoculation with efficient isolate(s) and increase in root length, root hairs development which might have explored the virgin horizons of soil for absorption and uptake of these nutrient elements.

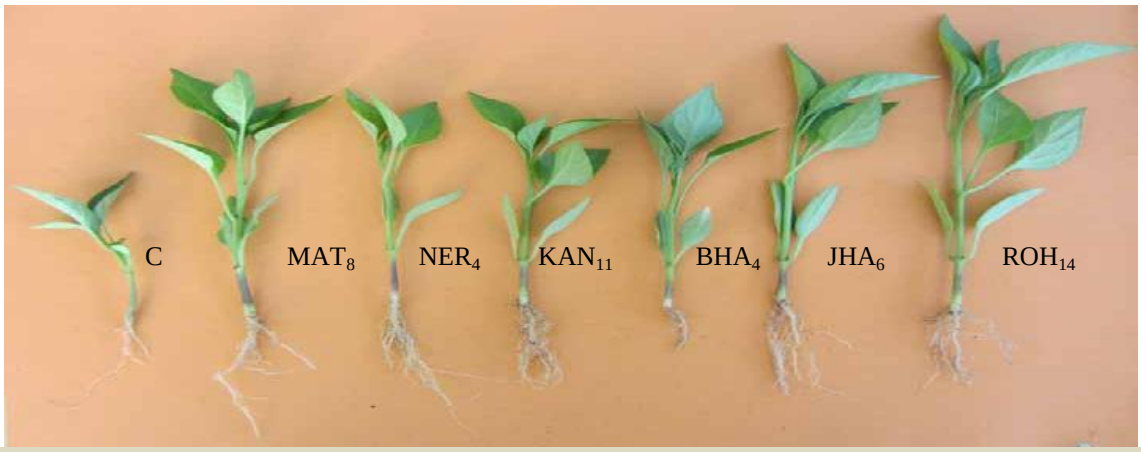
Further, the enhanced uptake by increase in specific ion fluxes at the root surface in the presence of plant growth promoting rhizobacteria has also been reported by (Kim *et al.* 1998), Bertrand *et al*. (2000). The findings are in line with those of Han *et al*. (2006), Tayeb Rezvani (2013) who also reported that the conjoint application of biofertilizers and chemical fertilizers increases N and P content in pepper plant over un-inoculated plants.

4.5.2.3 Effect on soil physico-chemical properties and available nutrient contents

The data pertaining to effect of 60 and 80 per cent recommended levels of N and P fertilizers along with bacterial isolate (s) on physio-chemical properties of soil at Nauni (Dharza) at the termination of experiment have been presented in Table 15.

Table 14. Effect of PGPR and chemical fertilizers on total nutrient content (NPK) and their uptake in capsicum (field conditions)

Treatments	Nutrient content (%)									Nutrient uptake (mg/plant)								
	N			P			K			N			P			K		
	2014	2015	Mean	2014	2015	Mean	2014	2015	Mean	2014	2015	Mean	2014	2015	Mean	2014	2015	Mean
T_1 (100 per cent RDF)	4.7	4.7	4.7	0.45	0.45	0.45	4.93	4.63	4.78	130.5	127.0	128.7	12.57	11.79	12.18	136.73	124.60	130.67
T_2 (80 per cent RDNP + JHA_6)	4.8	4.8	4.8	0.47	0.46	0.47	4.15	4.50	4.32	146.3	137.8	142.1	14.39	13.14	13.76	127.00	127.20	127.10
T_3 (80 per cent RDNP + ROH_{14})	5.2	5.1	5.1	0.47	0.48	0.47	4.12	4.52	4.32	170.8	181.6	176.2	15.48	14.60	14.44	134.58	163.08	148.83
T_4 (80 per cent RDNP JHA_6 + ROH_{14})	5.3	5.2	5.2	0.48	0.49	0.48	4.38	4.54	4.46	182.1	196.5	189.3	16.49	16.15	16.32	150.25	172.83	161.54
T_5 (60 per cent RDNP + JHA_6)	3.8	3.9	3.9	0.41	0.41	0.41	3.72	3.70	3.71	109.1	112.7	110.9	10.11	9.40	9.76	87.79	92.97	90.38
T_6 (60 per cent RDNP + ROH_{14})	3.7	3.8	3.8	0.42	0.41	0.42	3.81	3.72	3.76	112.2	115.5	113.8	10.18	9.94	10.06	92.78	94.30	93.54
T_7 (60 per cent RDNP JHA_6+ ROH_{14})	4.2	4.2	4.2	0.44	0.42	0.43	3.94	3.77	3.85	119.3	121.5	120.4	11.34	10.71	11.03	101.50	98.86	100.18
$CD_{0.05}$	**0.2**	**0.5**	**0.23**	**0.03**	**0.05**	**0.03**	**0.13**	**0.42**	**0.19**	**11.4**	**14.2**	**10.1**	**1.32**	**2.31**	**1.78**	**12.90**	**16.01**	**10.18**



a) Effect of selected PGPR on seedling growth of capsicum

b) Efficacy of selected PGPR (MAT_8, KAN_{11}, JHA_6 and ROH_{14}) and chemical fertilizer on capsicum (net house studies)

c) Efficacy of selected bacterial isolates (JHA_6 and ROH_{14}) and chemical fertilizers on capsicum (field conditions)

Plate 6. Effect of PGPR and chemical fertilizers on soil characteristics and productivity of capsicum

The minimum amount (274.72 and 34.64 kg ha^{-1}) of available N and P was obtained in T_5 treatment. Further, treatment T_1 registered maximum (283.72 kg ha^{-1}) available K and minimum (222.86 kg ha^{-1}) available K was obtained in T_5 treatment.

Apparently, synergistic effect of chemical fertilizers and PGPR could have brought significant improvement in soil available nutrients. The significant increase in available N and P may be attributed to more asymbiotic nitrogen fixation, phosphate solubilization by bacterial isolates that might have brought some P from unavailable pool to available pool. The enhanced nutrient availability especially N and P in the presence of PGPR has also been reported by Gopinath *et al*. (2009).

4.5.2.4 Effect on microbiological parameters

4.5.2.4.1 Total bacterial count in soil

The soil harbors a dynamic population of microorganisms. Their abundance in rhizosphere gives an indication of their possible role in decomposition of organic matter, fixation of atmospheric nitrogen, phosphate solubilization, transformations of nutrient elements, etc.

The results presented in Appendix 2.4 and Figure 9 revealed that the application of chemical fertilizers along with PGPR inoculants registered a significant increase in total microbial population over 100 per cent recommended levels of chemical fertilizers. The maximum (140.7×10^7 cfu/g soil) count on NA medium were noted in rhizosphere of plants whose seeds were treated with consortium of two PGPR isolates i.e. JHA_6 and ROH_{14} and receiving 80 per cent recommended doses of NP, whereas, the respective minimum value (89.7×10^7 cfu/g soil) was recorded in control (RDF).

Endophytic bacterial population was found to be significantly higher in case of seeds treated with PGPR in comparison with un-inoculated control (RDF). Maximum bacterial count (78.6×10^2 cfu/g root) on NA medium was recorded

Table 15. Effect of PGPR and chemical fertilizers on soil physico-chemical characteristics and available nutrient (NPK) content (field conditions)

Treatments	pH			EC (dSm^{-1})			OC (per cent)			Available Nitrogen ($kg\ ha^{-1}$)			Available Phosphorus ($kg\ ha^{-1}$)			Available Potassium ($kg\ ha^{-1}$)		
	2014	2015	Mean	2014	2015	Mean	2014	2015	Mean	2014	2015	Mean	2014	2015	Mean	2014	2015	Mean
T_1 (100 per cent RDF)	6.73	6.71	6.72	0.43	0.42	0.43	0.43	0.45	0.44	310.11	329.23	319.67	41.21	42.42	41.82	284.96	282.47	283.72
T_2 (80 per cent RDNP + JHA_6)	6.90	6.73	6.82	0.42	0.41	0.42	0.45	0.44	0.44	318.27	330.87	324.57	45.38	45.90	45.64	240.81	254.54	247.68
T_3 (80 per cent RDNP + ROH_{14})	6.86	6.75	6.81	0.42	0.42	0.42	0.45	0.45	0.45	325.96	344.90	335.43	48.50	47.28	47.89	244.79	260.01	252.40
T_4 (80 per cent RDNP JHA_6 + ROH_{14})	6.88	6.80	6.84	0.44	0.44	0.44	0.46	0.46	0.46	341.76	352.82	347.29	52.34	48.64	50.49	252.96	262.00	257.48
T_5 (60 per cent RDNP + JHA_6)	6.75	6.73	6.74	0.42	0.41	0.41	0.44	0.45	0.45	276.70	272.73	274.72	34.18	35.10	34.64	221.18	224.54	222.86
T_6 (60 per cent RDNP + ROH_{14})	6.82	6.68	6.75	0.42	0.42	0.42	0.44	0.44	0.44	277.46	274.62	276.04	36.67	37.42	37.04	223.37	228.88	226.12
T_7 (60 per cent RDNP JHA_6+ ROH_{14})	6.58	6.63	6.61	0.43	0.43	0.43	0.45	0.45	0.45	291.83	278.87	285.35	38.65	38.41	38.53	227.03	233.56	230.29
$CD_{0.05}$	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**24.01**	**28.72**	**28.41**	**5.35**	**4.62**	**3.98**	**27.20**	**26.71**	**25.02**

in T_4 (PGPR consortia + 80 per cent NP) treatment, whereas, minimum count (55.2×10^2 cfu/g root) was recorded in T_1 (RDF) treatment.

The application of efficient inoculum of PGPR with high viable count (10^8–10^{10}cfu/ml) coupled with adequate amounts of N and other nutrients might have contributed to increase in the total microbial population. Boominathan and Shivakumar (2015) recorded high microbial population level in the rhizosphere of the inoculated plants over control. These results are in conformation with those of Hong *et al*. (2002) who also reported that seed and soil treatment with bacterial isolates resulted in significant increase in bacterial population in rhizosphere and in the roots of plants. Results are also in line with the observation of Lal Bahadur *et al*. 2012.

4.5.2.4.2 Microbial biomass-carbon

Perusal of data appended in Appendix 2.4 and Figure 9 revealed that application of PGPR inoculants with 60 or 80 per cent NP chemical fertilizers recorded significantly higher microbial biomass over control (100 per cent RDF).

The microbial biomass carbon ranged from 45.96 to 108.66 mg MB-C/100 g soil. The treatment T_4 (PGPR consortia + 80 per cent NP) recorded maximum (108.66 mg MB-C/100 g soil) microbial biomass-carbon, whereas, the minimum (45.96 mg MB-C/100 g soil) microbial biomass-carbon was recorded under treatment T_1 (100 per cent RDF).

These results are in confirmation with Saini *et al*. (2005) and Sparling (1985) who also reported significant influence of integrated nutrient management on microbial biomass carbon.

4.5.2.4.3 Soil enzymes (Dehydrogenase, phytase and phosphatase) activity

The conjoint application of bacterial inoculants with chemical fertilizers singnificantly influenced the soil enzymes (dehydrogenase, phytase and phosphatase) activity over un-inoculated control (Appendix 2.5 and Fig. 10, 11 and 12). The maximum (73.26 µg TPF g^{-1}soil h^{-1}) dehydrogenase activity was recorded under T_4 treatment which was statistically at par with T_3 treatment whereas, minimum dehydrogenase activity (45.57 µg TPF g^{-1}soil h^{-1}) was recorded in T_1 treatment. Similar significant increase in dehydrogenase activity

due to more microbial activity in plant rhizosphere have been reported by (Gopinath *et al.* 2008).

Phosphatases play an important role in P cycling, where organic P is more due to limited biological mineralization of organic matter as a result of formation of complexes of organic P with active Al and Fe and the amount of available P is low. The soil enzyme phosphatase activity varied considerably among the treatments. The maximum (102.41 µg pNP g^{-1}soil h^{-1}) phosphatase activity was recorded in T_4 treatment which was statistically at par with T_2 treatment, whereas, minimum (44.21 µg PNP g^{-1}soil h^{-1}) dehydrogenase activity was recorded in T_1 treatment. Our result are consistent with Garcia-Gill *et al.* (2000), Turrion *et al.* (2000) who also reported similar phosphatase activity in mineral fertilized and control soil.

The soil enzyme phytase activity varied considerably among the treatments. The maximum (131.78 Enzyme unit µmol ml^{-1} min^{-1}) phytase activity was recorded in T_4 treatment followed by T_3 treatment, whereas, minimum (33.56 Enzyme unit µmol ml^{-1} min^{-1}) phytase activity was recorded in T_1 treatment. The results are in collaboration with Bennitez (2000) and Gopinath *et al*. (2009) who also reported the enhanced dehydrogenase, urease, phosphatase and phytase activity with organic amendments over chemical fertilizers, in capsicum rhizosphere. Similarly, Kaur and Reddy (2014) also demonstrated that inoculation of PSB together with rock phosphate fertilization increased the activities of soil enzymes such as dehydrogenase, acid phosphatase, alkaline phosphatase and phytase as compared to diammonium phosphate treatment in maize.

4.5.2.5 Economics of developed PGPR technology for capsicum

The economics of capsicum production as affected by various treatments (Appendix 2.6) revealed that maximum net return (Rs. 448417.0) and Benefit cost ratio (2.73) was computed by the application of treatment T_4 (PGPR consortia + 80 per cent N & P + 100 per cent K), however, minimum net return (Rs. 216626.46) and lowest Benefit cost ratio (0.83) was calculated in treatment T_5 (ROH_{14} + 60 per cent NP) due to minimum yield and high cost of fertilizers.

Thus, by taking the cost of cultivation in the application of consortium of PGPR (ROH_{14} and JHA_6) at 80 per cent levels of NP, the farmers are getting a net

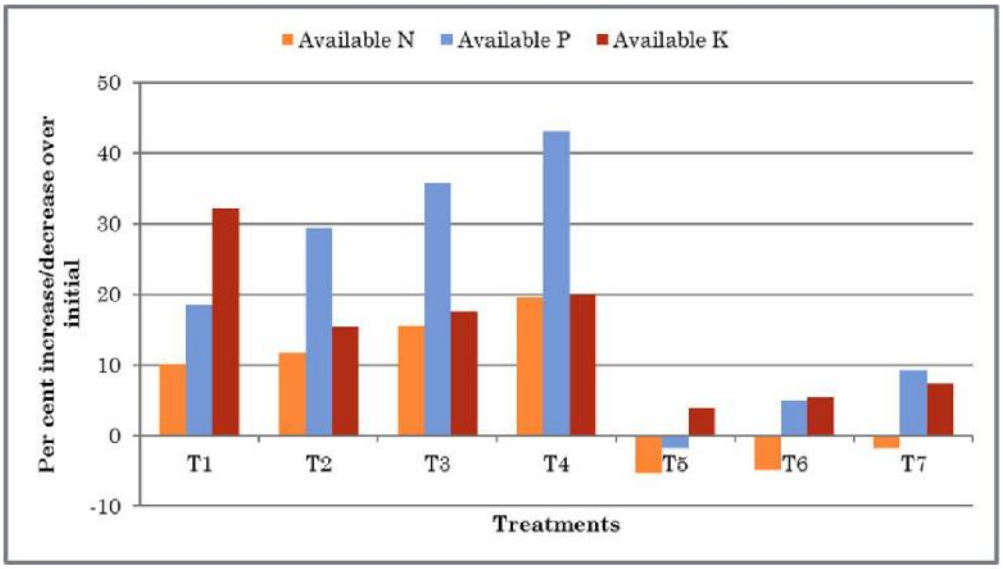


Fig. 8 Effect of conjoint use of PGPR and chemical fertilizers on available nutrient (NPK) content over initial values (field conditions)

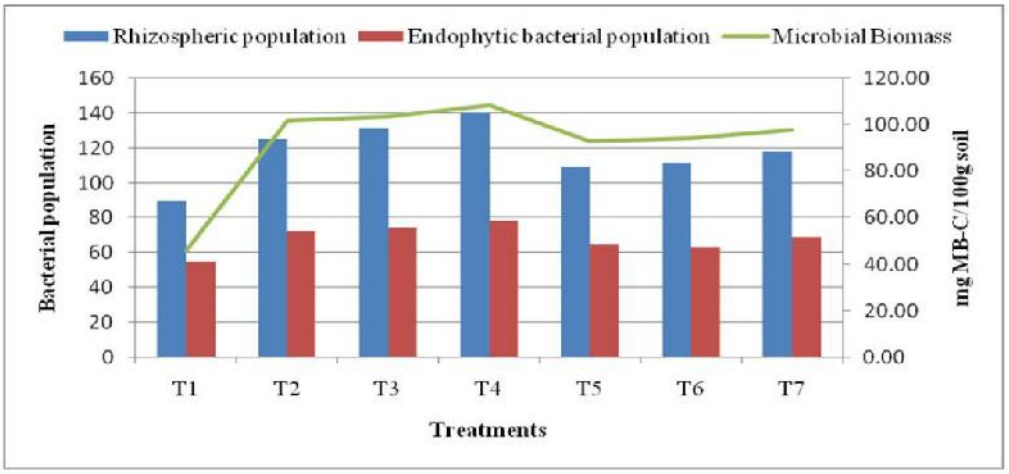


Fig. 9 Effect of bacterial inoculation at varied levels of NP on rhizospheric, endophytic microbial count and microbial biomass (field conditions)

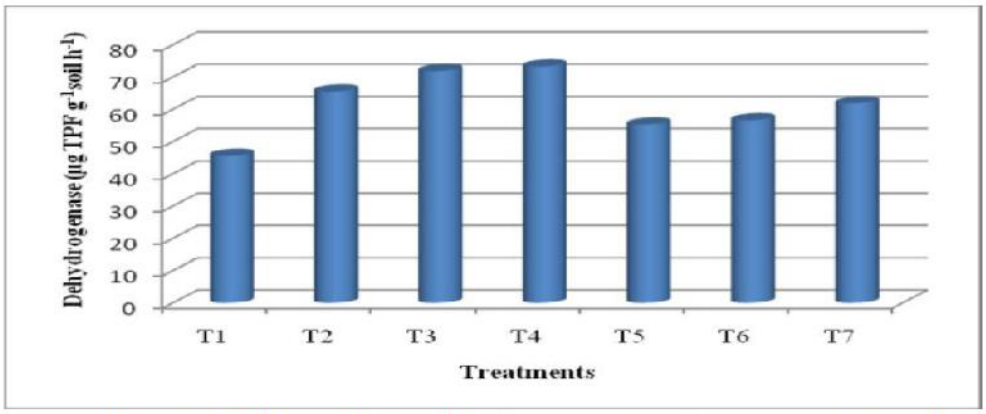


Fig. 10 Effect of PGPR and chemical fertilisers on dehydrogenase activity in the rhizosphere of capsicum

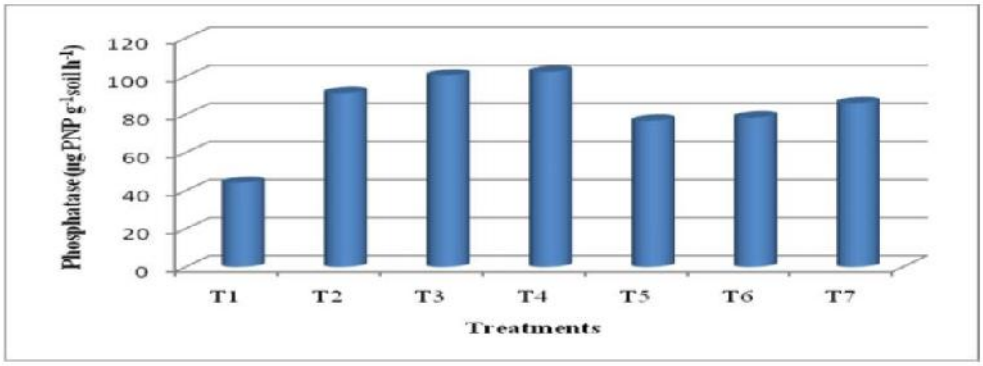


Fig. 11 Effect of PGPR and chemical fertilisers on phosphatase activity in the rhizosphere of capsicum

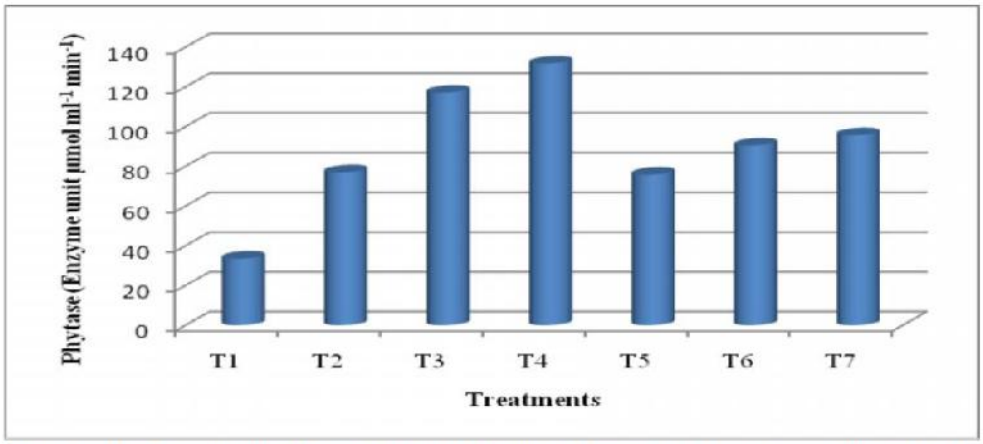


Fig. 12 Effect of PGPR and chemical fertilisers on phytase activity in the rhizosphere of capsicum

profit of Rs. 92,315/ha extra over recommended levels of chemical fertilizers besides saving 20 per cent dose of NP fertilizers.

The findings are in conformity with the results of the Khan and Pariari (2012) who also obtained highest value of cost:benefit ratio (1:3.02) with treatment combination of *Azospirillum* + N_{75} per cent + PK, followed by treatment receiving *Azotobacter* + N_{100} per cent + PK (1:2.85) while the least value (1:1.87) was found in *Azotobacter* + N_{50} per cent + PK, in *Capsicum annuum* L. Similarly, Amirthalingam (1988), Dekha *et al*. (1996) recorded higher net returns due to inoculation of *Azospirillum* in chilli.

4.6 TO STUDY THE EFFECT OF INDIGENOUS PGPR INOCULUM FOR PHYSIOLOGICAL EFFICACY UNDER WATER STRESS CONDITION

Two best isolates (ROH_{14} and JHA_6) possessing maximum PGP traits along with ACC-deaminase activity were selected further to study their effect on capsicum plants exposed to various levels of drought stress.

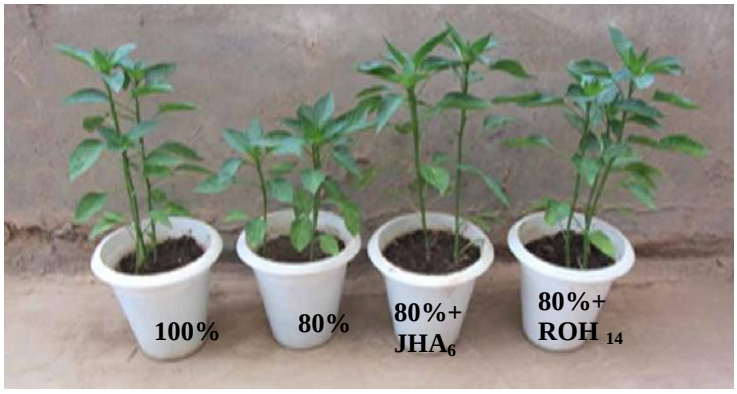
Perusal of data embedded in Table 16 revealed that inoculation of capsicum plants with both the PGPR strains resulted in a significant increase in shoot/root length, biomass of plants exposed to drought stress (Plate 7). Maximum (39.1 cm) shoot length and (10.55 g) shoot biomass was recorded in plants inoculated with ROH_{14} and subjected to 80 per cent FC soil moisture level, however, was statistically at par with T_1 (non-stressed, un-inoculated plants) and T_3 (plants inoculated with JHA_6 and grown under 80 per cent FC soil moisture level) treatments. Maximum (16.4 cm) root length was observed for treatment T_3, which was statistically at par with T_1 treatment. Maximum (10.9 mg) root biomass was observed for T_3 and T_4 treatments receiving either of two bacterial isolates and subjected to 80 per cent FC soil moisture level, which was statistically at par with the treatment T_1 treatment (non-stressed, un-inoculated plants). The results are in confirmation with Lim and Kim (2013), who reported that pepper plants treated with *Bacillus licheniformis* K11 and exposed to drought stress had 50 per cent higher shoot length and biomass than non-treated plants. Similarly, Mayak *et al.* 2004 reported increased drought tolerance of tomato (*Solanum lycopersicum* L.) and pepper (*Capsicum annuum* L.) seedlings with *Achromobacter piechaudii*

ARV8 inoculation. The reduced "drought stress imposed effects" on various growth variables with PGPR inoculation might be contributed due to asymbiotic N_2 fixation, solubilization of inorganic phosphate and mineralization of organic phosphate or other nutrients, modifying the phytohormone content like decreasing ethylene production by the ACC-deaminase activity and changing the balance of cytokinins and auxins or IAA signaling.

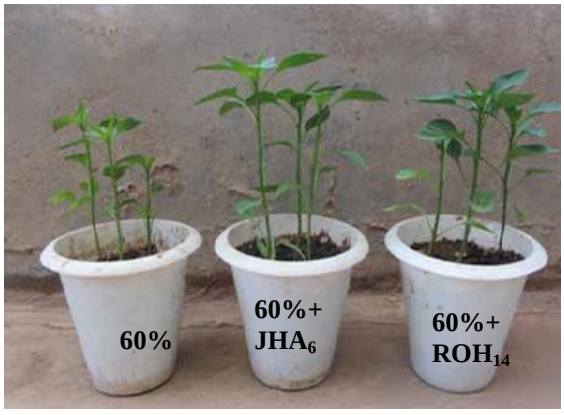
Data presented in Table 16 showed the effect of water stress, bacterial strain and their interactions on leaf area. The obtained data revealed that the drought stress substantially reduced the leaf area of the plants as compared to non-stressed plants. However PGPR inoculated plants, mitigated the drought stress effect by increasing leaf area (10-12 per cent; 16-22 per cent and 15-18 per cent) over un-inoculated treatments with 80 per cent, 60 per cent and 40 per cent FC soil moisture level, respectively.

On comparison of various un-inoculated water stress treatments (T_2, T_5 and T_8) with their inoculated counter-part treatments it can be concluded that PGPR has increased the relative water content (RWC), thereby improving drought tolerance of capsicum plants. Maximum (94.45 per cent) RWC has been noticed for T_3 treatment (plants inoculated with JHA_6 and subjected to 80 per cent FC soil moisture level), followed by T_4 treatment. Maximum (2.041 g/kg fresh leaves) total chlorophyll content has been noticed for T_4 treatment (plants inoculated with ROH_{14} and grown under 80 per cent FC soil moisture level), however, was statistically at par with treatment T_1 (un-inoculated non-stressed plants) and T_3 (plants inoculated with JHA_6 and grown under 80 per cent FC soil moisture level) treatments. Similar results have been reported by Vivas *et al.* 2003 who showed that inoculation of bacterial strain increased stomatal conductance and chlorophyll content of lettuce compared to a non-drought control. Grover *et al*. (2014) also reported that sorghum plants treated with PGPR, *Bacillus* spp. strain KB 129 under drought stress showed 24 per cent increase in RWC over plants that were not treated with PGPR. Higher RWC may help plants counteract the oxidative and osmotic stresses caused by drought stress, potentially contributing to greater productivity under stress.

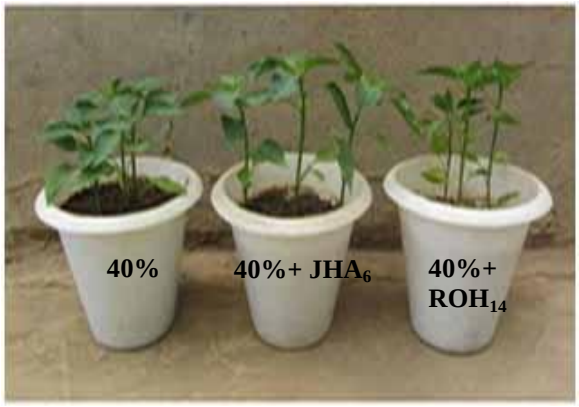
a) General view of drought stress experiment



b) Effect of bacterial inoculums at 80 per cent field capacity of soil moisture level



c) Effect of bacterial inoculums at 60 per cent field capacity of soil moisture level



d) Effect of bacterial inoculums at 40 per cent field capacity of soil moisture level

Plate 7. Effect of selected bacterial isolates at varied levels of drought stress

The obtained data (Table 16) revealed that the concentration of total soluble proteins was higher in plants grown under drought than well-watered conditions. Further the leaves of un-inoculated capsicum plants, which suffered from drought stress, had significant and substantial lower soluble proteins as compared to their respective PGPR inoculated stressed plants. This tended to occur regardless of bacterial strain. Maximum total soluble protein (0.384 mg/g fresh leaves) has been noticed in ROH_{14} inoculated plants subjected to 40 per cent of the field capacity. PGPR increased the active accumulation of organic and inorganic maintain cellular turgor and help plants lower water potential without decreasing actual water content. Thereby protects enzymes, proteins, cellular organelles and membranes against oxidative damage and helps plants tolerate drought-induced damage (Huang *et al.* 2014).

The water stress treatment caused a significant increase in the concentrations of antioxidant enzymes in all comparisons (Appendix 2.7 and Fig 13**)**. Maximum (82.62, 4.94 and 87.16 U/gm fresh weight) superoxide, peroxidase and catalase enzyme activities, respectively, was recorded for plants inoculated with ROH_{14} and subjected to 40 per cent FC soil moisture level followed by JHA_6 inoculated plants grown under same stress level. The results are in confirmation with Gururani *et al.* (2013) who also reported that treatment of potato plants with two PGPR strains i.e. *Bacillus pumilus* str. DH-11 and *Bacillus firmus* str. 40, induced an increase in the levels of ROS-scavenging enzymes including ascorbate peroxidase and catalase under drought stress in PGPR-treated plants compared with that non-treated plants. Similarly, Saravanakumar *et al*. (2011) reported an increase in the activity of catalase in green gram plants treated with *Pseudomonas fluorescens* Pf1 and *Bacillus subtilis* EPB. This increase was correlated with the observed drought tolerance. Wang *et al.* 2012 also reported an association of antioxidant enzyme production and drought tolerance in cucumber plants.

Growth and nutrient concentrations usually determine the performance of plants growing in any environment. The effects of water stress and bacterial strains on NPK content embedded in Table 16 and Appendix 2.8 (Fig 14) revealed that mineral content and their uptake under water stress treatments in capsicum was significantly decreased compared to the non-water stress treatment. Treatment with bacterial strains in the water stress treatment increased NPK

content and uptake per plant as compared to un-inoculated stressed plants. Maximum (5.08 per cent) N content and its uptake (5.79 mg/plant) was recorded in T_1 treatment (un-inoculated, non-stressed plants) followed by T_4 treatment. Similar, results were recorded for K content and its uptake. However, maximum (0.31 per cent and 0.36 mg/plant) P content and uptake, respectively, was recorded for T_3 and T_4 treatments followed by T_1 treatment.

PGPR inoculated plants subjected to various drought stress levels increased N-uptake (24-27, 15-24 and 33-45 per cent), P- uptake (27-28, 13-20 and 31-35 per cent) and K-uptake (18-19, 16-22 and 10-14 per cent) over respective control treatments (un-inoculated plants held at 80 per cent, 60 per cent and 40 per cent FC soil moisture level). Bacterial-induced alterations in root architecture may lead to an increase in total root surface area and consequently lead to improved water and nutrient uptake, with positive effects on plant growth as a whole. Similar improved water and nutrient uptake in PGPR inoculated plants under draught stress have been reported by Timmusk *et al*. (2014). The results are in confirmation with Vivas *et al*. (2003), who also reported increased N, P and K concentrations by 5, 70 and 50 per cent, respectively in lettuce inoculated by *Bacillus* sp. under drought stress conditions, as compared to the non-water stress control.

4.7 MOLECULAR CHARACTERIZATION (16S rRNA GENE SEQUENCING) OF SELECTED BACTERIAL ISOLATES

On the basis of 16S rRNA gene sequencing, JHA_6 isolate was identified as *Pseudomonas aeruginosa*, ROH_6 isolate was identified as *Providencia* spp. and ROH_{14} isolate was identified as *Bacillus amyloliquefaciens.*

4.7.1 Phylogenetic analysis of selected isolate by 16S rRNA gene sequence analysis

Universal 16S rRNA gene primers were successfully used for the amplification of 16S rRNA from bacterial isolates JHA_6, ROH_6 and ROH_{14}. As a result of amplication, an amplicon of expected size *i.e*. 1400 bp corresponding to

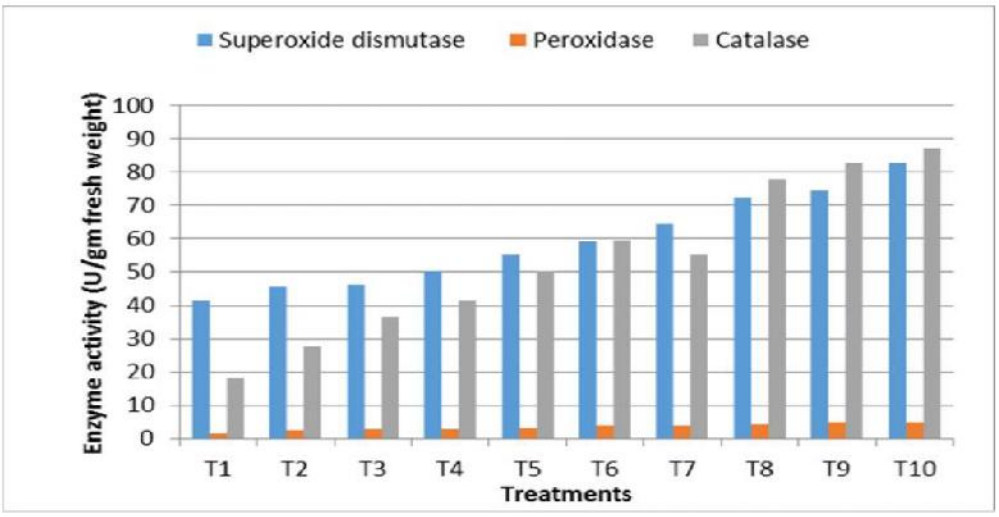


Fig. 13 Influence of selected PGPR isolates on antioxidant enzymes (SOD, POD and CAT) of capsicum at varied levels of drought stress (net house conditions).

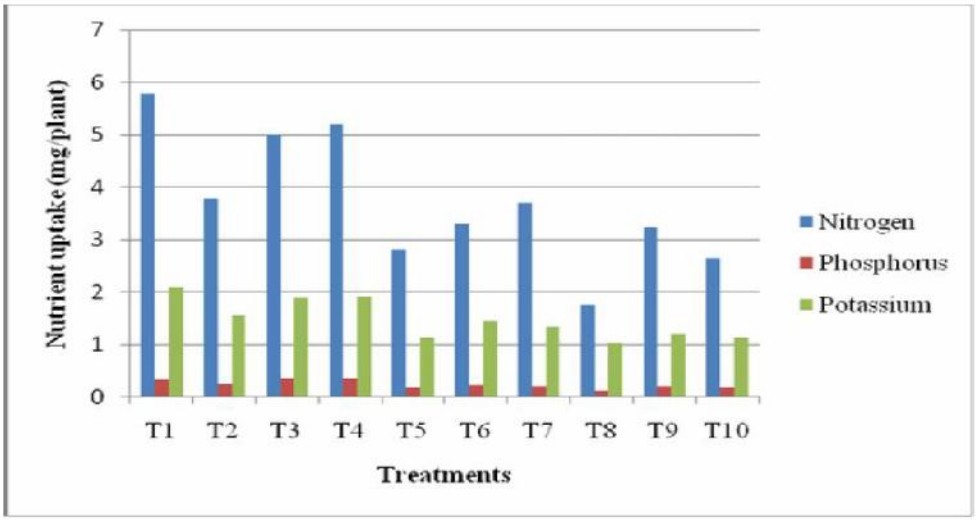


Fig. 14 Influence of selected PGPR isolates on NPK uptake of capsicum at varied levels of drought stress (net house conditions)

Table 16. Influence of PGPR isolates on growth parameters and nutrient (NPK) content of capsicum at varied levels of drought stress

Treatments	Shoot length (cm)	Root length (cm)	Shoot biomass (g/plant)	Root biomass (g/plant)	Leaf area (cm^2)	Relative water content in leaves (per cent)	Total chlorophyll content (g/kg fresh leaves)	Total soluble protein (mg/g fresh leaves)	Nutrient content (per cent)		
									N	P	K
T_1: 100 per cent of field capacity	38.3	16.1	10.33	1.07	22.14	92.47 (80.51)	2.027	0.233	5.08 (2.25)	0.31 (0.55)	1.84 (1.35)
T_2: 80 per cent of field capacity	32.9	13.8	8.88	0.92	19.90	76.13 (61.64)	1.848	0.254	3.85 (1.96)	0.26 (0.51)	1.58 (1.26)
T_3: 80 per cent of field capacity + JHA_6	38.9	16.4	10.51	1.09	22.73	94.45 (78.79)	2.036	0.273	4.32 (2.08)	0.31 (0.56)	1.63 (1.28)
T_4: 80 per cent of field capacity + ROH_{14}	39.1	15.2	10.55	1.09	24.07	91.90 (76.37)	2.041	0.274	4.48 (2.12)	0.31 (0.56)	1.64 (1.28)
T_5: 60 per cent of field capacity	28.1	11.8	7.59	0.79	13.10	62.27 (57.19)	1.689	0.294	3.36 (1.83)	0.22 (0.47)	1.35 (1.16)
T_6: 60 per cent of field capacity + JHA_6	31.8	13.4	8.60	0.89	16.83	68.41 (55.91)	1.713	0.317	3.50 (1.87)	0.25 (0.50)	1.53 (1.24)
T_7: 60 per cent of field capacity + ROH_{14}	30.6	12.9	8.26	0.86	15.60	71.15 (57.86)	1.747	0.347	4.07 (2.02)	0.24 (0.49)	1.47 (1.21)
T_8: 40 per cent of field capacity	23.3	7.8	6.28	0.65	11.27	50.87 (45.44)	1.657	0.352	2.54 (1.59)	0.19 (0.43)	1.47 (1.21)
T_9: 40 per cent of field capacity + JHA_6	28.8	10.1	7.79	0.81	13.83	54.17 (47.40)	1.671	0.384	3.77 (1.94)	0.23 (0.48)	1.38 (1.18)
T_{10}: 40 per cent of field capacity + ROH_{14}	28.2	9.9	7.62	0.79	13.23	51.76 (46.00)	1.685	0.379	3.17 (1.78)	0.23 (0.48)	1.36 (1.16)
$CD_{0.05}$	**2.02**	**1.0**	**0.55**	**0.06**	**1.21**	**28.82 (23.45)**	**0.068**	**0.02**	**0.57 (0.16)**	**0.016 (0.015)**	**0.10 (0.04)**

16S rDNA region of selected three strains were obtained and sequenced (Plate 8). The sequences so obtained were corrected after deleting the unreadable and ambiguous sequences. Finally, complete sequences of the selected three strains were obtained. The sequences of the selected three strains are presented in figure 15, 16 and 17 and BLASTn search of these sequences revealed the identity of strains with their closest match (Table 17).

The isolate JHA_6, ROH_6 and ROH_{14} showed 99 per cent homology with NR118644.1, NR042415.1 and NR075005.1 (*Pseudomonas aeruginosa* strain SNP0614, *Providencia vermicola* strain OP1165 and *Bacillus amyloliquefaciens* subsp. *plantarum* strain F2B42), respectively. The sequence of respective isolates were submitted to the NCBI under accession no. KX447673 (JHA_6), KX447672 (ROH_6) and KX447671 (ROH_{14}).

Table 17. Phylogenetic identification of the selected PGPR isolates

Isolates	Base pairs	Accession number	Highest similarity	Similarity (per cent)
JHA_6	1195	KX447673	*Pseudomonas aeruginosa*	99 per cent
ROH_6	633	KX447672	*Providencia vermicola*	99 per cent
ROH_{14}	238	KX447671	*Bacillus amyloliquefaciens subsp. plantarum*	99 per cent

To trace out the evolutionary patterns of the test isolate and to find out relationship of the same with other selected sequences at NCBI, phylogenetic tree was also constructed using Neighbour-Joining (J) method with 100 bootstrap samplings among 16S rRNA gene sequence of JHA_6, ROH_6 and ROH_{14}. These isolate were united with quite high statistical support by the boot strap method estimates for 1,000 replications and values inferred greater than 40 per cent phylogenetic tree (Fig. 18) further confirmed the identity of strains JHA_6 as *Pseudomonas aeruginosa*, ROH_6 as *Providencia* spp. and ROH_{14} as *Bacillus amyloliquefaciens*, as it clustered closely with already reported strains *Pseudomonas aeruginosa* NR074828.1, *Providencia vermicola* NR042415.1 and *Bacillus amyloliquefaciens* subsp. *plantarum* NR075005.1 with the boot strap value of 62, 73 and 46, respectively.

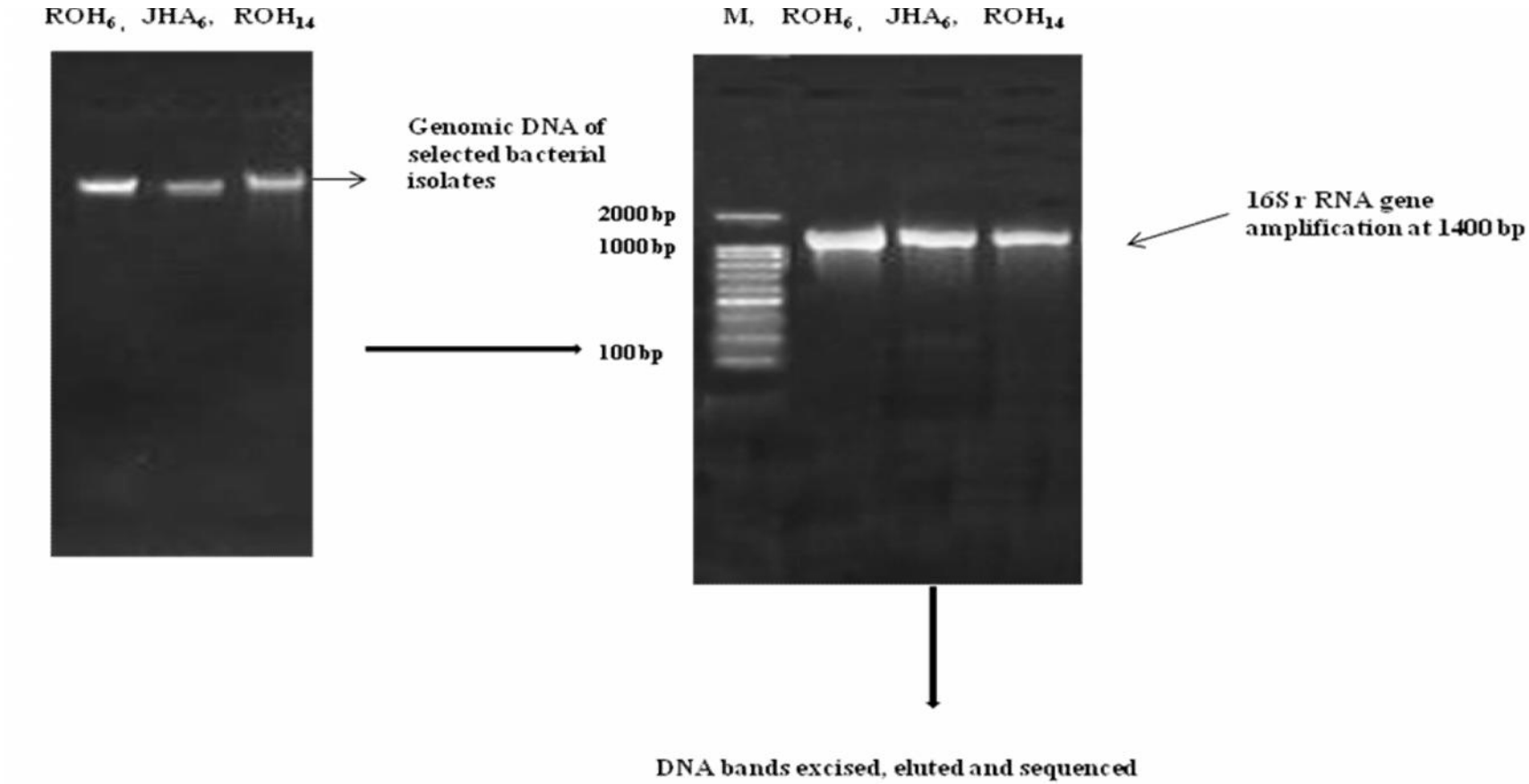


Plate 8. Molecular identification of selected bacterial isolates based on 16S rRNA gene sequencing.

GGGGGATAACGTCCGGAAACGGGCGCTAATACCGCATACGTCCTGAGGGAGAAAG
TGGGGGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTG
GTGGGGTAAAGGCCTACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCA
GTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAA
TATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCT
TCGRATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTG
TTTTGACGTTACCAACAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTA
ATACGAAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTG
GTTCAGCAAGTTGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAAC
TACTGAGCTAGAGTACGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGC
GTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGAC
ACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACG
CCGTAAACGATGTTGACTAGCCGTTGGGATCCTTGAGATCTTAGTGGCGCAGCTAA
CGCGATAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATT
GACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAG
AACCTTACCTGGCCTTGACATGCTGAGAACTTTCCAGAGATGGATTGGTGCCTTCGG
GAACTCAGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGG
TTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACCTCGGGTTGGCA
CTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCA
TCATGGCCCTTACGGCCAGGGCTACACACGTGCTACAATGGTCGGTACAAAGGGTT
GCCAAGCCGCGAGGTGGAGCTAATCCCATAAAACCGATCGTAGTCCGGATCGCAGT
CTGCAACTCGACTGCGTGAAGTCGGAATC

Fig. 15 ***Pseudomonas aeruginosa*** **(JHA_6) 16S rRNA gene, partial sequence**

CAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTTTGAAGAAGGCCCTTAGGGT
TGTAAAGTACTTTCAGTCGGGAGGAAGGCGTTGATGCTAATATCATCAAGATTGAC
GTTACCGACAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGG
AGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTGATT
AAGTTAGATGTGAAATCCCCGGGCTTAACCTGGGAATGGCATCTAAGACTGGTCAG
CTAGAGTCTTGTAGAGGGGGGTAGAATTCCATGTGTAGCGGTGAAATGCGTAGAGA
TGTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGG
TGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAA
CGATGTCGATTTGAAGGTTGTTCCCTTGAGGAGTGGCTTTCGGAGCTAACGCGTTAA
ATCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGG
GCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTAC
CTACTCTTGACATCCAGAGAA

Fig. 16 ***Providencia*** **sp. (ROH_6) 16S rRNA gene, partial sequence**

GGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGT
ACGGTCGCAAGACTGACTACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGG
ATCATGTGGTTTAATTCTAASCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCT
GACAATCCTAGAAATAGGACGTCCCCTTCGGGGGCAGAATGACAGGTGGTGATGGT
TGCCGTCACCTCTT

Fig. 17 ***Bacillus amyloliquefaciens*** **(ROH_{14}) 16S rRNA gene, partial sequence**

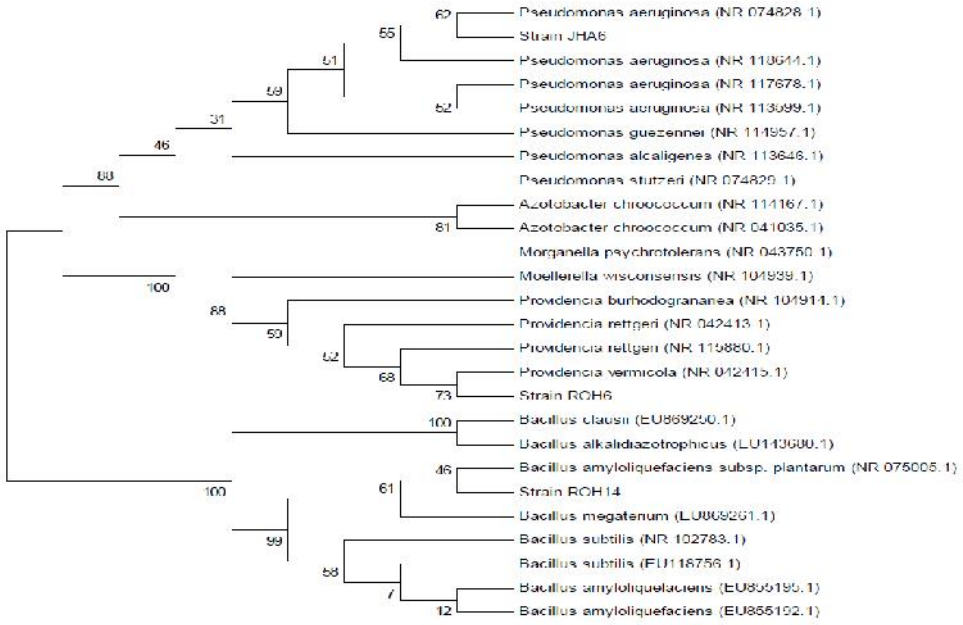


Fig. 18 **Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing relationships between bacterial isolates (JHA_6, ROH_6 and ROH_{14}) and related taxa**

Chapter-5

SUMMARY AND CONCLUSION

The present investigations entitled "**Impact of Indigenous Plant Growth Promoting Rhizobacteria and Chemical Fertilizers on Soil Health and Productivity of Capsicum (*Capsicum annuum* L.)**" was carried out during the year 2013-16. The studies were aimed to isolate and characterize rhizospheric, endophytic plant growth promoting bacteria and to test their bio-control efficacy against major soil borne diseases(s) of capsicum. Secondly, to study the efficacy of PGPR isolates at reduced doses of N and P fertilizers under net house and field conditions to have higher crop yield and improved soil health. Thirdly, to study the effect of indigenous PGPR inoculum for physiological efficacy under water stress conditions.

The salient features of the present investigations are as follows:

- Significant variations in rhizospheric and endophytic bacterial population were recorded in samples collected from different locations/sites. The bacterial count in rhizosphere ranged from 118.50 to 61.00 × 10^5cfu/g soil, however, the endophytic count varied from 96.00 to 62.50 × 10^2 cfu/g root.
- Among 157 isolates, 34 isolates (12 isolates from agroclimatic-zone I, 10 isolates from agroclimatic-zone II and 12 isolates from agroclimatic-zone III) possesses P-solubilising, N-fixation and siderophore production activity.
- Out of the selected 34 isolates, only 52.9 per cent (18/34) isolates were IAA producers with its content ranged from 31.33µg/ml to 10.33 µg/ml, 55.9 per cent (19/34) isolates were ACC- deaminase producers, 35.3 per cent (12/18) isolates were ammonia producers and only 14.7 (5/34) isolates were HCN producer.
- Except one isolate (THE_{17}) all the tested 34 isolates were able to inhibit the growth of one or more test pathogens. Seventeen isolates showed antagonism towards *Fusarium oxysporum,* six towards *Rhizoctonia solani,* twelve towards *Pythium* sp.*,* eleven towards *Ralstonia Solanacearum,* seventeen towards *Phytopthora capsici* and twelve isolates towards *Colletotrichum capsici.* However, no single isolate was able to inhibit the growth of all the six test pathogens.

- Fifteen isolates (RAK_9, MAT_8, NER_4, PAR_2, PAO_2, SIH_6, PAL_7, KAN_{11}, $BHAR_4$, PAT_9, PAT_{13}, $SARA_9$, JHA_6, ROH_6 and ROH_{14}) exhibiting the maximum plant growth promoting and protection traits were further assessed for quantitative estimation for P-solubilization (µg/ml), per cent siderophore unit and lytic enzyme activity.
- TCP solubilisation ranged from 169.84 to 60.16µg/ml and siderophore unit production from 56.36 to 12.79 per cent. Maximum (169.84 µg/ml) P-solubilization activity was recorded for isolate JHA_6 with corresponding viable count (30.3×10^7 cfu/ml) and decrease in final pH to 5.17 from initial pH 7.0, however, was at par with isolate ROH_{14} (145.8 µg/ml). Significantly higher siderophore unit (56.36 per cent) with viable count (16.1×10^7 cfu/ml) was obtained for isolate SIH_6 after 72 h of incubation which was significantly higher than all other isolates.
- Among all, eight isolates (53.3 per cent) were amylase producers, four (26.7 per cent) were pectinase producers, eight (53.3 per cent) were protease producers, two (PAO_2 and BHA_4) isolates were cellulase producers, seven (46.7 per cent) were lipase producers and five (33.3 per cent) were chitinase producers.
- All selected fifteen isolates varied for Gram's reaction (9 isolates (G +ve) and 6 isolates (G –ve)), were rods, cocci and cocco-bacillus in shape, able to grow in a temperature range of 20- 40ºC, pH 5.0-8.0 and salt concentration @ 2-6%.
- Among fifteen isolates, best five isolates possessing antagonistic activity against *R. solanacearum* (41.6-84.8 per cent) were selected to study their bio-control efficacy against *Ralstonia solanacearum* causing bacterial wilt in capsicum. The biocontrol efficacy of antagonist bacterial isolates towards the pathogen strain ranged from 29.2 to 70.8 per cent. Minimum disease incidences (25.0 per cent) were found in treatment receiving ROH_6 isolate which was statistically at par with the treatment T_3, T_4 and T_5 receiving PAT_9, PAT_{13} and ROH_{14} isolate, respectively. However, maximum (83.3 per cent) disease incidence and (79.2 per cent) disease severity were recorded with the treatment receiving only pathogen (control).
- Six isolates having maximum plant growth promoting traits were evaluated under net house conditions for germination percentage and seedling growth parameters, out of which four best performers (MAT_8, KAN_{11}, JHA_6 and

ROH_{14}) in combination along with varied levels (40, 60 and 80 per cent) of Recommended Doses (RD) of N and P fertilizers with un-inoculated plants receiving 100 per cent NPK as control were further evaluated for capsicum growth and yield under net house studies.

- Based on net house studies, two bacterial isolates (JHA_6 and ROH_{14}) alone and in consortia with variable (60 and 80 per cent) RD doses of N and P fertilizers were further evaluated under field conditions for enhanced growth and productivity of capsicum.
- The experimental soil was nearly neutral (pH 7.1), normal EC (0.32 dSm^{-1}) and low in organic carbon (0.42%), having medium available N (290.5 kg ha^{-1}), K (214.6 kg ha^{-1}) and high available P (35.3 kg ha^{-1}). The application of 80 per cent NP ($N_{80}P_{61}$) fertilizers in conjunction with PGPR isolates (JHA_6 or ROH_{14}) alone and in consortia, in general, recorded significantly higher growth and yields of capsicum over 100 per cent recommended level of chemical fertilizers (N_{100}, P_{76}& K_{45}).
- Maximum capsicum fruit yield (18.0 t ha^{-1}) was recorded under T_4 (PGPR consortia + 80 per cent NP), followed by T_3 (ROH14 + 80 per cent NP), which was 8.4% more compared to T_1 (100 per cent recommended level of chemical fertilizers).
- The total N, P and K uptake increased significantly with the conjoint application of either of the PGPR isolate(s) and 80 per cent recommended doses of chemical fertilizer over 100 per cent recommended doses of chemical fertilizers (RDF).
- The available nutrient status of soil increased significantly with combined application of PGPR and chemical fertilizers over recommended doses of chemical fertilizers. Maximum available N (347.3 kg ha^{-1}) and available P (50.5 kg ha^{-1}) was recorded in T_4 (PGPR consortia + 80 per cent NP), which was 56.8 kg ha^{-1} and 15.2 kg ha^{-1} higher over initial N and P soil test values, respectively.
- The conjoint application (chemical fertilizer + PGPR inoculant) significantly influenced the soil enzymes (dehydrogenase, phosphatase and phytase) activity, total bacterial count and microbial biomass-carbon over sole application of chemical fertilizers. However, none of the treatments significantly influenced the pH, EC and OC content of soil.

- Maximum net return (Rs 448417.0) and Benefit cost ratio (2.73) was noted for T_4 (PGPR consortia + 80 per cent NP + 100 per cent K) treatment, thus, providing net profit of Rs.92,315 ha^{-1} extra with over recommended levels of chemical fertilizers besides saving 20 per cent dose of NP chemical fertilizers.
- Two best isolates (ROH_{14} and JHA_6) possessing maximum plant growth promoting traits along with ACC-deaminase activity were further selected to study their effect on water stress on capsicum plants exposed to 40, 60 and 80 per cent field capacity.
- Maximum relative water content (94.45 per cent) has been recorded for T_3 treatment (plants inoculated with JHA_6 and subjected to 80 per cent field capacity soil moisture level). Maximum (2.041 g kg^{-1} fresh leaves) total chlorophyll content has been noticed for T_4 treatment (plants inoculated with ROH_{14} and grown under 80 per cent field capacity soil moisture level), however was statistically at par with treatments T_1 (un-inoculated non-stressed plants) and T_3 (plants inoculated with JHA_6 and grown under 80 per cent field capacity soil moisture level). Maximum SOD (82.62 U g^{-1} fresh weight), POD (4.94 U g^{-1} fresh weight) and CAT (87.16 U g^{-1} fresh weight) enzyme activities were recorded for plants inoculated with ROH_{14} and subjected to 40 per cent field capacity soil moisture level followed by JHA_6 inoculated plants grown under same stress level.
- PGPR inoculated plants subjected to various drought stress levels increased NPK-uptake over respective control treatments (un-inoculated plants held at 80, 60 and 40 per cent field capacity soil moisture level). Maximum (5.08%) N content and its uptake (5.79 mg $plant^{-1}$) was recorded in T_1 treatment (un-inoculated, non-stressed plants) followed by T_4 treatment (plants inoculated with ROH_{14} and grown under 80 per cent field capacity soil moisture level). Similar, results were recorded for K content and its uptake. However, maximum (0.31%) P content and uptake (0.36 mg $plant^{-1}$) was recorded for treatment T_3 (plants inoculated with JHA6 and grown under 80 per cent field capacity soil moisture level).

- On the basis of molecular characterization (16S rRNA gene sequencing), the efficient strains ROH_6, JHA_6 and ROH_{14} were identified as *Providencia* spp., *Pseudomonas aeruginosa* and *Bacillus amyloliquefaciens,* respectively.

CONCLUSION

From the present study, it may be concluded that the application of *Providencia* spp. (ROH_6) reduced the bacterial wilt disease incidence (*Ralstonia solanacearum*) by seventy percent. The application of PGPR consortia (JHA_6 + ROH_{14}) along with 80 per cent NP (N_{80} P_{61} kg/ha) brought a significant increase in yield by 10.89 q ha^{-1} (8.4%), available N (8.7%) and P (20.7%) content over recommended doses (N_{100} and P_{76} kg/ha) besides saving 20 per cent (20 kg N/ha and 15 kg P/ha) of chemical fertilizers. Further, application of *Pseudomonas aeruginosa* (JHA_6) and *Bacillus amyloliquefaciens* (ROH_{14}) induced the drought tolerance in capsicum by increasing antioxidant enzymes (SOD, CAT and POD) activities under drought stress.

LITERATURE CITED

Abdel Latef. 2011. Influence of arbuscular mycorrhizal fungi and copper on growth, acaccumulation of osmolyte, mineral nutrition and antioxidant enzyme activity of pepper (*Capsicum annuum* L.). *Mycorrhiza* **21**: 495–503.

Abdul Babi A A and Anderson J D. 1973. Vigour determination in soybean seed by multiple criteria. *Crop Science* **13**: 630-633.

Addy S K and Goodman R N. 1972. Polyphenol oxidase and peroxidase in apple leaves inoculated with a virulent or an avirulent strain of *Ervinia amylovora*. *Indian Phtopathological Society* **25**: 575-579.

Ahanthem S and Jha D K. 2008. Interactions between *Acaulospora* and *Azospirillum* and their synergistic effect on rice growth at different sources and regimes of soil phosphorus. *Mycorrhiza News* **20**(2):6–12.

Ahemad M and Khan M S. 2011. Functional aspects of plant growth promoting rhizobacteria: recent advancements. *Insight Microbiology* **1**(3): 39-54.

Ahemad M and Khan M S. 2012. Productivity of Greengram in tebuconazole-stressed soil, by using a tolerant and plant growth promoting *Bradyrhizobium* sp. MRM6 strain. *Acta Physiology Plant* **34**: 245–254.

Ahemad M and Kibret M. 2014. Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. *Journal of King Saud University-Science* **26**: 1-20.

Ahmad F, Ahmad I and Khan M S. 2008. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiological Research* **163**: 173–181.

Alcazar R, Altabella T, Marco F, Bortolotti C, Reymond M, Koncz C, Carrasco P and Tiburcio A F. 2011. Polyaminemetabolic canalization in response to drought stress in Arabidopsis and the resurrection plant *Craterostigma plantagineum*. *Plant Signaling and Behavior* **6**:243–250.

Algam S A E, Xie G L B, Yu S, Su T and Larsen J. 2010. Effects of *Paenibacillus* strains and chitosan on plant growth promotion and control of Ralstonia Wilt in tomato. *European Journal of Plant Pathology* **92**(3): 593- 600.

Almoneafy A A, Xie G L, Tian W X, Xu L H, Zhang G Q and Ibrahim M. 2012. Characterization and evaluation of *Bacillus* isolates for their potential plant growth and biocontrol activities against tomato bacterial wilt. *African Journal of Biotechnology* **11**(28): 7193-7201.

Alstroem S. 1991. Induction of disease resistance in common bean susceptible to halo blight bacterial pathogen after seed bacterization with rhizosphere pseudomonads. *The Journal of General and Applied Microbiology* **37**(6):495–501.

Altschul S F, Madden T L, Schaffer A A, Zhang J, Zhang Z, Miller W and Lipman D J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* **25**: 3389-3402.

Alyie N, Fininsa C and Hikias Y. 2008. Evaluation of rhizosphere bacterial antagonist for their potential to bioprotect potato (*Solanum tubersoum*) against bacterial wilt (*R. solanacearum*). *Biological Control* **47**: 282-288.

Amaresan N, Jayakumar V, Kumar K and Thajuddin N. 2012. Endophytic bacteria from tomato and chilli, their diversity and antagonistic potential against *Ralstonia solanacearum*. *Archives of Phytopathology and Plant Protection* **45**(3): 344-355.

Amirjani M A. 2012. Effects of cadmium on wheat growth and some physiological factors. *International Journal of Forest Soil and Erosion* **2**:50–8.

Amirthalingam S. 1988. Studies on the effect and Azospirillum, nitrogen and NAA on growth and yield of Chilli cv.K1. *South Indian Horticulture* **36**(4):218.

Anderson J P E and Domesch K H. 1978. A physiological method for the quantitative measurement of microbial biomass in soils. *Soil Biology and Biochemistry* **10**: 215-221.

Antoun H, Beauchamp C J, Goussard N, Chabot R and Lalande R. 1998. Potential of *Rhizobium* and *Bradyrhizobium* species as plant growth promoting rhizobacteria on non-legumes: effect on radishes (*Raphanus sativus* L.). *Plant Soil* **204**: 57-67.

Apel K and Hirt H. 2004. Reactive oxygen species: metabolism, oxidative stress and signal transduction. *Annual Review of Plant Biology* **55**:373–99.

Arshad M, Saleem M and Hussain S. 2007. Perspectives of bacterial ACC deaminase in phytoremediation. *Trends in Biotechnology* **25**: 356– 362.

Asadhi S, Reddy B V B, Sivaprasad Y, Prathyusha M, Krishna M, Kumar K V K and Reddy K R. 2013. Characterisation, genetic diversity and antagonistic potential of 2,4-diacetylphloroglucinol producing *Pseudomonas fluorescens* isolates in groundnut-based cropping system of Andhra Pradesh, India. *Archives of Phytopathology and Plant Protection* **46** (6):1966-1977.

Ashraf M. 2009. Biotechnological approach of improving plant salt tolerance using antioxidants as markers. *Biotechnology Advances* **27**:84–93.

Ashrafuzzaman M, Hossen F A, Razi I M, Hoque M A, Zahurul I M, Shahidullah S M and Meon S. 2009. Efficiency of plant growth-promoting rhizobacteria (PGPR) for the enhancement of rice growth. *African Journal of Biotechnology* **8**(7): 1247–1252.

Asokan R, Sukhada M and Lalitha A. 2000. Biofertilizers and pesticides for horticultural crops. *Indian Horticulture* **2**: 44-52.

Atlas R M. 1995. Handbook Media for environmental microbiology. CRL, Press Bocaratar, New York, pp. 43-371

Austin M B and Nobel A J P. 2003. The chalcone synthase superfamily of type III poly peptide synthases. National Production Report.pp.79-110

Badhulkar R M, Wandile W P, Badole and Balpande S S. 2000. Residual effect of long term application of FYM and fertilizer on soil properties (Vertisols) and yield of soybean. *Journal of the Indian Society of Soil Science* **48**: 89-92.

Bajpai R K, Chitale Shrikant, Upadhyay S K and Urkurkar J S. 2006. Long term studies on soil physico-chemical properties and productivity of rice wheat system as influenced by integrated nutrient management in inceptisol of Chattisgarh. *Journal of the Indian Societyof Soil Science* **54**(1): 24-29.

Bakker A W and Schippers B. 1987. Microbial cyanide production in the rhizosphere to potato yield reduction and *Pseudomonas* spp. mediated plant growth stimulation. *Soil Biology and Biochemistry* **19**: 451-457.

Bakker P A H M, Pieterse C M J and Van Loon L C. 2007. Induced systemic resistance by fluorescent *Pseudomonas* spp. *Phytopathology* **97**(2): 239–243.

Bartels D and Sunkar R. 2005. Drought and salt tolerance in plants. *Critical Review in Plant Sciences* **24**: 23–58.

Bashan Y, Puente M E, Bashan L E and Hernandez J P. 2008. Environmental uses of plant growth-promoting bacteria. In: Barka EA, Clement C (eds) Plant-microbe interactions. Trivandrum, Kerala, India, pp 69–93

Baysal O, Soylu E M and Soylu S. 2003. Induction of defence related enzymes and resistance by the plant activator acinebenzolar-S-methyl in tomato seedlings against bacterial canker caused by *Clavibacter michiganensis* ssp. *Michiganensis*. *Plant Pathology* **52**: 747-753.

Beauchamp C and Fridovich I. 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry* **44**: 276–287

Belimov A A, Dodd I C, Hontzeas N, Theobald J C and Safronova V I. 2009. Rhizosphere bacteria containing 1-aminocyclopropane-1-carboxylate deaminase increase yield of plants grown in drying soil via both local and systemic hormone signaling. *New Phytologist* **181**: 413–423.

Bell C R, Dickie G A, Harvey W L G and Chan J W Y F. 1995. Endophytic bacteria in grapevine. *Canadian Journal of Microbiology* **41**: 46-53.

Bennitez E, Melgar R, Sainz H, Gomez M and Nogales R. 2000. Enzyme activities in the rhizosphere of pepper (*Capsicum annuum*, L.) grown with olive cake mulches. *Soil Biology and Biochemistry* **32**: 1829–1835.

Berger L R and Reynolds D M. 1958. The chitinase system of a strain of *Streptomyces griseus*. *Biochimica et Biophysica Acta* **29(3)**: 522-534.

Bertrand H, Plassard C, Pinochet X, Touraine B, Normand P and Cleyet-marcel J C. 2000. Stimulation of the ionic transport system in *Brassica napus* by a plant growth promoting rhizobacterium (*Achromobacter* spp.). *Canadian Journal of Microbiology* **46**: 229-236.

Bhardwaj S. 2013. Integrated nutrient module for enhanced productivity of cauliflower under low and mid hills of himachal Pradesh. M.Sc. Thesis submitted to Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan (HP)

Bhattacharyya P N and Jha D K. 2012. Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World Journal of Microbiology and Biotechnology* **28**: 1327–1350.

Boominathan U and Sivakumar P K. 2015. Effect of seed with native PGPR on its vital seedling and antioxidant enzyme activities in *Curcuma longa* (L.). *International Journal of Advanced Research* **3**(8): 1211-1216.

Boyce A, Casey A and Walsh G. 1964. A phytase enzyme-based biochemistry practical particularly suited to students undertaking courses in biotechnology and environmental science. Biochemistry and molecular biology education printed in U.S.A. Vol. 32, No. 5, pp. 336–340

Bray R H and Kurtz L T. 1945. Determination of total organic and available forms of phosphorus in soils. *Soil Science* **59**: 39-45.

Budi S W, van Tuinen D, Arnould C, Gaudut E D, Pearson V G and Gianinazzi S. 2000. Hydrolytic enzyme activity of *Paenibacillus* sp. strain B2 and effect of antagonistic bacterium on cell wall integrity of two soil-borne pathogenic fungi. *Applied Soil Ecology* **15**: 191-199.

Casida L E. 1977. Microbial metabolic activity in soil as measured by dehydrogenase determinations. *Applied and Environmental Microbiology* **34** (6): 630-636.

Castro R O, Cornejo H A C, Rodriguez L M and Bucio J L. 2009. The role of microbial signals in plant growth and development. *Plant Signaling and Behavior* **4**(8):701–712.

Chandlee J M and Scandalios J G. 1984. Analysis of variants affecting the catalase development program in maize scutellum. *Theoretical and Applied Genetics* **69**: 71–77.

Chandrappa H, Venkatesh J, Sharanappa J and Prasad R. 2007. Influence of *Azotobacter*, *Azospirillum* and nitrogen on growth, yield and quality of Chilli. Proceedings of national seminar on production, development, quality and export of seed spices. NRCSS, Ajmer. pp. 278-283.

Chanway C P and Holl F B. 1994. Growth of outplanted lodepole pine seedlings one year after inoculation with plant growth promoting rhizobacteria. *Forest Science* **40**: 238–246.

Chitrashree C, Udayashankar A C, Nayak S C, Reddy M S and Srinivas C. 2011. Plant growth promoting rhizobacteria mediate induced systemic resistance in rice against bacterial leaf blight caused by *Xanthomonas oryzae*pv. *Oryza*e. *Biological Control* **59**: 114-122.

Cho Ja Y and Chung Soon J. 1998. Effect of rhizobacteria on the growth of cucumber and tomato plug seedlings. *Journal of the Korean Society for Horticultural Science* **39**(1): 18-23.

Claus D and Berkeley R. 1986. Genus *Bacillus* Cohn. 1872, 174. **In**: *Bergey's manual of systematic bacteriology*. Vol 2, Sneath P H A, Mair N S, Sharpe ME and Holt J G (Eds.).Baltimore M D, Williams and Wilkins, USA. pp.1105-1139

Cohen A C, Travaglia C N, Bottini R and Piccoli P N. 2009. Participation of abscisic acid and gibberellins produced by endophytic *Azospirillum* in the alleviation of drought effects in maize. *Botany* **87**: 455–462.

Contesto C, Milesi S, Mantelin S, Zancarini A and Desbrosses G. 2010. The auxin-signaling pathway is required for the lateral root response of Arabidopsis to the rhizobacterium *Phyllobacterium brassicacearum*. *Planta* **232**: 1455–1470.

Corpet F. 1988. Multiple sequence alignment with hierarchical clustering. *Nucleic Acids Research* **16** (22):10881-10890.

Dai M, Xu L, Wu X, Li M and Liu R J. 2009. Effects of plant growth-promoting rhizobacteria on root-knot nematode in tomato. *Chinese Journal of Biological Control* **25**(2): 181-184.

Datta M, Palit R, Sengupta C, Pandit M K and Banerjee S. 2011. Plant growth promoting rhizobacteria enhance growth and yield of chilli (*Capsicum annuum*.) under field conditions. *Australian Journal of Crop Science* **5**(5): 531-536.

Dekha B C, Bora G C and Shadeque A. 1996. Effect of *Azospirillum* on groth and yield of chilli (*Capsicum annuum* L.) cv.Pusa Jwala. *Haryana Journal of Horticuiltural Sciences* **25**: 44-46.

Dipta B. 2013.Phosphate solubilizing potential of plant growth promoting rhizobacteria of cauliflower. M.Sc. Thesis submitted to Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan (HP)

Duffy B, Keel C and Defago G. 2004. Potential role of pathogen signaling in multitropic plant microbe interactions involved in disease protection. *Applied and Environmental Microbiol*ogy **70**: 1836-1842.

Dworkin M and Foster J. 1958. Experiments with some microorganisms which utilize ethane and hydrogen. *Journal of Bacteriology* **75**: 592-601.

Egamberdieva D and Kucharova Z. 2009. Selection for root colonizing bacteria stimulating wheat growth in saline soils. *Biology and Fertility of Soils* **45**: 561–573.

El-azeem S A M A B D, Mehana T A and Shabayek A A. 2007.Some plant growth promoting traits of rhizobacteria isolated from Suez Canal region. *Egypt African Crop Science Conference Proceedings* **8**: 1517-1525.

Esitken A, Pirlak L, Turan M and Sahin F. 2006. Effects of floral and foliar application of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrition of sweet cherry. *Scientia Horticulturae* **110**: 324–327.

Farooq M, Wahid A, Kobayashi N, Fujita D and Basra S M A. 2009. Plant drought stress, effects, mechanisms and management. *Agronomy for Sustainable Development* **29**: 185–212.

Fatima Z, Saleem M, Muhammad Z, Sultan T, Aslam M, Rehman R and Chaudhary M F. 2009. Antifungal activity of plant growth promoting rhizobaterial isolates against *Rhizoctonia solani* in wheat. *African Journal of Biotechnology* **8**(2): 219-225.

Feng H, Li Y and Liu Q. 2013. Endophytic bacterial communities in tomato plants with differential resistance to *Ralstonia solanacearum*. *African Journal of Microbiological Research* **7**(15): 1311-1318.

Figueiredo M V B, Burity H A, Martìnez C R, Chanway C P. 2008. Alleviation of drought stress in the common bean (*Phaseolus vulgaris* L.) by co-inoculation with *Paenibacillus polymyxa* and *Rhizobium tropici*. *Applied Soil Ecology* **40**: 182–188.

Fisher P J, Petrini O and Scott H M L. 1992. The distribution of some fungal and bacterial endophytes in maize (*Zea mays* L.). *New Phytology* **122**: 299-305.

Fleming H P, Etchells J L and Costilus R H. 1975. Microbial inhibition by an isolate of *Pediococcus* from cucumber brines. *Applied Microbiology* **30**: 1040-1042.

Garbeva P, Van Overbeek L S, Van Vuurde J W L and Van Elsas J D**.** 2001. Analysis of endophytic bacterial communities of potato by planting and denaturing gradient gel electrophoresis (DGGE) of 16S rDNA based PCR fragments. *Microbial Ecology* **41**: 369-383.

Garcia-Gill J C, Plaza C, Soler-Rovira P and Polo A. 2000. Long-term effects of municipal solid waste compost application on soil enzyme activities and microbial biomass. *Soil Biology and Biochemistry* **32**: 1907–1913.

Ghorbanpour M, Hatami M and Khavazi K. 2013. Role of plant growth promoting rhizobacteria on antioxidant enzyme activities and tropane alkaloid production of *Hyoscyamus niger* under water deficit stress. *Turkish Journal of Biology* **37**: 350-360.

Ghose P K. 1987. Measurement of cellulose activities. *Pure and Applied Chemistry* **59**: 257-268.

Ghosh S, Penterman J N, Little R D, Chavez R and Glick B R. 2003. Three newly isolated plant growth-promoting bacilli facilitate the seedling growth of canola, *Brassica campestris*. *Plant Physiology and Biochemistry* **41**: 277–281. doi:10.1016/S0981-9428(03)00019-6

Gill N S, Bal J S and Khurana D S. 2011. Effect of biofertilizers on yield and quality traits of cabbage (*Brassica oleracia* var. *capitata* L.). *Indian Journal of Ecology* **38**(2): 82-85.

Gill S S and Tuteja N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry* **48**: 909–30.

Girish N and Umesha S. 2005. Effect of plant growth promoting rhizobacteria on bacterial canker of tomato. *Archives of Phytopathology and Plant Protection* **38**(3): 235-243.

Glick B R. 2007. Promotion of plant growth by bacterial ACC deaminase. *Critical Reviews in Plant Sciences* **26**: 227-242.

Gomez K A and Gomez A A. 1984. Statistical procedure for agricultural research. IInd *ed.* John Wiley and Sons, New York, pp. 357-427

Gopinath K A, Saha S, Mina B L, Kundu S, Pande H and Gupta H S. 2008. Influence of organic amendments on growth, yield and quality of wheat and on soil properties during transition to organic production. *Nutrient Cycling in Agroecosystems* **82**: 51–60.

Gopinath K A, Supradip S, Mina B L, Pande H, Srivastva A K and Gupta H S. 2009. Bell pepper yield and soil properties during conversion from conventional to organic production in Indian Himalayas. *Scientia Horticulturae* **122**: 339–345.

Gorden S A and Palleg L G. 1957. Quantitative measurement of IAA**.** *Plant Physiology* **10**: 37-38.

Govindasamy V, Senthilkumar M, Gaikwad K and Annapurna K. 2008. Isolation and characterization of ACC deaminase gene from two plant growth-promoting rhizobacteria. *Current Microbiology* **57**(4): 312–317.

Gray E J and Smith D L. 2005. Intracellular and extracellular PGPR: commonalities and distinctions inthe plant bacterium signaling processes. *Soil Biology and Biochemistr*y **37**: 395–412.

Grichko V P and Glick B R. 2001. Amelioration of flooding stress by ACC deaminase-containing plant growth-promoting bacteria. *Plant Physiology and Biochemistry* **39**: 11–17.

Grover M, Madhubala R, Ali S Z, Yadav S K and Venkateswarlu B. 2014. Influence of *Bacillus* spp. strains on seedling growth and physiological parameters of sorghum under moisture stress conditions. *Journal of Basic Microbiology* **54**: 951–961.

Guillen C R, Hernandez C F D, Gallegos M G, Rodriguez H R, Aguilar-Gonzalez C N, Padron C E and Reyes V M H. 2006. *Bacillus* spp. as biocontrol in an infested soil with *Fusarium* spp., *Rhizoctonia solani* Kuhn and *Phytophthora capsici* Leonian and its effect on development and yield of pepper crop (*Capsicum annuum* L.). *Revista Mexicana de Fitopatologia* **24**(2): 105-114.

Guo J H, Jiang C H, Xie P, Huang Z Y, Fa Z H. 2015. The plant healthy and safety guards plant growth promoting rhizo bacteria (PGPR). *Transcriptomics* **3**: 109. doi:10.4172/2329-8936.1000109

Guo J H, Qi H Y and Li S M. 2002. Biocontrol efficiency of three PGPR strains admixture to pepper bacterial wilt. *Bacterial Wilt Newsletter* **17**: 32-47.

Guo J H, Qi H Y, Guo Y H, Ge H L, Gong L Y, Zhang L X and Sun P H. 2004. Biocontrol of tomato wilt by plant growth promoting rhizobacteria. *Biological control* **29**: 66-72.

Gupta G, Parihar S S, Ahirwar N K, Snehi S K and Singh V. 2015. Plant growth promoting rhizobacteria (PGPR): Current and future prospects for development of sustainable agriculture. *Journal of Microbial and Biochemical Technology* **7**(2): 96-102.

Gupta S, Kaushal R, Spehia R S, Shashi Pathania and Sharma V. 2015. Productivity of capsicum as influenced by conjoint application of isolated indigenous PGPR and chemical fertilisers. *Journal of Plant Nutrition.* DOI: 10.1080/01904167.2015.1093139

Gupta S, Kaushal R, Spehia R S, Kaundal K and Chauhan A. 2014. Indigenous plant growth promoting rhizobacteria: potential green alternative for capsicum productivity under mid-hill conditions of north-western Himalayan region. *International Journal of Farm Sciences* **4**(3): 58-66.

Gupta S, Kaushal R, Spehia R S, Pathania Shashi and Sharma V. 2015. Productivity of capsicum as influenced by conjoint application of isolated indigenous PGPR and chemical fertilizers. *Journal of Plant Nutrition* - DOI: 10.1080/01904167.2015.1093139

Gupta S. 2012. Studies on selected plant growth promoting rhizobacteria on growth and yield of capsicum (*Capsicum annuum* L.). M.Sc. Thesis submitted to Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan (HP)

Gururani M A, Upadhyaya C P, Baskar V, Venkatesh J, Nookaraju A and Park S W. 2013. Plant growth-promoting rhizobacteria enhance abiotic stress tolerance in *Solanum tuberosum* through inducing changes in the expression of ROS-scavenging enzymes and improved photosynthetic performance. *Journal of Plant Growth Regulation* **32**: 245–258.

Han H S and Lee K D. 2005. Physiological responses of soybean-inoculation of *Bradyrhizobium japonicum* with PGPR in saline soil conditions. *Research Journal of Agriculture and Biological Sciences* **1**: 216–221.

Han H S, Supanjani and Lee K D. 2006. Effect of co-inoculation with phosphate and potassium solubilizing bacteria on mineral uptake and growth of pepper and cucumber. *Plant Soil and Environment* **52**: 130–136.

Hanafi A and Fellah K. 2006. Does the PGPR *Bacillus subtilis* induce plant resistance to whiteflies and *Pythium* spp. in greenhouse tomato? Bulletin OILB/SROP **29**(4): 105p.

Hayat R, Ali S, Amara U, Khalid R and Ahmed I. 2010. Soil beneficial bacteria and their role in plant growth promotion: a review. *Annals of Microbiology* **60**: 579–98.

Heidari M, Mousavinik S Mand Golpayegani A. 2011. Plant growth promoting rhizobacteria (PGPR) effect on physiological parameters and mineral uptake in Basil (O*ciumum basilicum* L.) under water stress. *Journal of Agricultural and Biological Science* **6**(5): 6-11.

Hernandez M I and Chailloux M. 2004. Las micorrizas arbusculares y las bacterias rizosfericas como alternativa a la nutricion mineral del tomate. *Cult Trop* **25**(2): 5–12.

Hong H, Qing C X, Yong C H, Xiong G and Fang P H. 2002. Selection of endophytic antifungal bacteria from capsicum. *Chinese Journal of Biological Control* **18**(4): 171-175.

Hu HQ, Li XS and He H. 2010. Characterization of an antimicrobial material from a newly isolated *Bacillus amyloliquefaciens* from mangrove for biocontrol of capsicum bacterial wilt. *Biological Control* **54**(3): 359-365.

Hu W H, Xiao Y A, Zeng J J and Hu X H. 2010. Photosynthesis, respiration and antioxidant enzymes in pepper leaves under drought and heat stresses. *Biologia Plantarum* **54**(4): 761-765.

Huang B, Da-Costa M and Jiang Y. 2014. Research advances in mechanisms of turfgrass tolerance to abiotic stresses: from physiology to molecular biology. *Critical Reviews in Plant Sciences* **33**: 141–189.

Hui L J and Kim S D. 2013. Induction of drought stress resistance by multifunctional PGPR *Bacillus licheniformis* K11 in Pepper. *The Plant Pathology Journal* **29** (2): 201–208.

Iturbe-Ormaetxe I, Escuredo P R, Arrese-Igor C and Becana M. 1998. Oxidative damage in pea plants exposed to water deficit or paraquat. *Plant Physiology* **116**: 173–81.

Jackson M L. 1973. Soil chemical analysis. Prentice Hall of India Limited, New Delhi. pp. 219-221.

Jackson R M, M E Brown and S K Burlinghum. 1964. Effects on tomato plant off *Azotobacter* application. *Nature* **192**: 575.

James E K and Olivares F L. 1998. Infection and colonization of sugarcane and other graminaceous plants by endophytic diazotrophs. *Critical Review of Plant Science* **17**: 77-119.

Jarak M, Mrkovacki N, Bjelic D, Josic D, Hajnal-Jafari T and Stamenov D. 2012. Effects of plant growth promoting rhizobacteria in green house and field trial. *African Journal of Mibrobiology Research* **6**: 5683-5690.

Jean S, Akoa A, Emmanuel Y and Nyoja J. 2013. Effect of chemical treatments on pests and diseases of pepper (*Capsicum annuum* L.). *Greener Journal of Agricultural Science* **3**(1): 012-020.

Jensen E S. 1987. Inoculation of pea by application of *Rhizobium* in planting furrow. *Plant and Soil* **97**: 63-70.

Jeon M W, Ali M B and Hahn E J. 2006. Photosynthetic pigments, morphology and leaf gas exchange during ex-vitro acclimatization of micropropagated CAM *Doritaenopsis* plantlets under relative humidity and air temperature. *Environmental and Experimental Botany* **55**: 183–194.

Jetiyanon K. 2007. Defensive-related enzyme response in plants treated with a mixture of *Bacillus* strain (In937a and In937b) against different pathogens. *Biological Control* **42**(2): 178-185.

Joseph B, Patra RR and Lawrence R. 2007. Characterization of plant growth promoting rhizobacteria associated with chickpea (*Cicer arietinum* L). *International Journal of Plant Production* **1**: 141-152.

Kandan A, Ramiah M, Vasanthi V J, Radjacommare R, Nandakumar R, Ramanathan A and Samiyappan R. 2005. Use of *Pseudomonas fluorescens* based formulations for management of tomato spotted wilt virus (TSWV) and enhanced yield in tomato. *Biocontrol Science and Technology* **15**(6): 553-569.

Karnwal A. 2009. Production of indole acetic acid by fluorescent *Pseudomonas* in the presence of L-tryptophan and rice root exudates. *Journal of Plant Pathology* **91**(1): 61-63.

Kaur G and Reddy M S. 2014. Role of phosphate-solubilizing bacteria in improving the soil fertility and crop productivity in organic farming. *Archives of Agronomy and Soil Science* **60**(4): 549-564.

Kaur N and Sharma P. 2013. Screening and characterization of native *Pseudomonas* sp. as plant growth promoting rhizobacteria in chickpea (*Cicer arietinum* L.) rhizosphere. *African Journal of Microbiology Research* **7**(16): 1465-1474.

Kaushal M and Kaushal R. 2013. Plant growth promoting rhizobacteria- impacts on cauliflower yield and soil health. *The bioscan* **8**(2): 549-552.

Kaushal M and Wani S P. 2015. Plant growth promoting rhizobacteria: drought stress alleviators to ameliorate crop production in drylands. *Annals of Microbiology* DOI 10.1007/s13213-015-1112-3.

Kaushal M. 2011.Effect of plant growth promoting rhizobacteria at varying levels of N and P on cauliflower (*Brassica oleracea* var. *botrytis* L.). Ph.D. Thesis submitted to Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan (HP)

Kechid M, Desbrosses G, RokhsiW, Varoquaux F, Djekoun A and Touraine B. 2013. The NRT2.5 and NRT2.6 genes are involved in growth promotion of Arabidopsis by the plant growth-promoting rhizobacterium (PGPR) strain *Phyllobacterium brassicacearum* STM196. *New Phytologist* **198**:514–524.

Kempe J and Sequeira L. 1983. Biological control of bacterial wilt of potatoes: attempts to induce resistance by treating tubes with bacteria. *Plant Disease* **67**: 499-501.

Kennedy I R, Choudhury A I M A and Kecskes M L. 2004. Non-Symbiotic bacterial diazotrophs in crop-farming systems: can their potential for plant growth promotion be better exploited? *Soil Biology and Biochemistry* **6**(8): 1229-1244.

Khalid A, Akhtar M J, Mahmood M H and Arshad M. 2006. Effect of substrate-dependent microbial ethylene production on plant growth. *Microbiology***75**: 231–236.

Khan S and Pariari A. 2012. Effect of N-fixing biofertilizers on growth, yield and quality of chilli (*Capsicum annuum* L.). *The Bioscan* **7**(3): 481-482.

Kim K Y, Jordan D and McDonald G A. 1998. Effect of phosphate solubilising bacteria and vesicular-arbuscular mycorrhizae on tomato growth and soil microbial activity. *Biology Fertility Soils* **26**: 79-87.

Kirti S. 2013. Characterization of plant growth promoting rhizobacteria and arbuscular mycorrhizae from cherry (*Prunus avium* L.). M.Sc. Thesis submitted to Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan (HP)

Kloepper J W and Schroth M N. 1978. Plant growth promoting rhizobacteria on radishes, In: Proceedings of the 4th international conference on plant pathogenic bacteria, Angers, France. pp. 879–882.

Kloepper J W, Schroth M N and Miller T D. 1980. Effects of rhizosphere colonization by plant growth promoting rhizobacteria on potato plant development and yield. *Phytopathology* **70**: 1078-1082.

Kloepper J W, Tuzun S, Liu L and Wei G. 1993. Plant growth-promoting rhizobacteria as inducers of systemic disease resistance. In: Lumsden RD, Waughn JL (eds) Pest management: biologically based technologies. American Chemical Society Books, Wash-ington, DC, pp 156–165

Knowles C J. 1976. Microorganisms and cyanide. *Bacteriological Review* **40:** 652-680.

Kohler J, Herna´ndez J A, Caravaca F, Rolda´N A. 2008. Plant-growth-promoting rhizobacteria and arbuscular mycorrhizal fungi modify alleviation biochemical mechanisms in water-stressed plants. *Functional Plant Biology* **35**: 141-151.

Krasensky J and Jonak C. 2012. Drought, salt, and temperature stressinduced metabolic rearrangements and regulatory networks. *Journal of Experimental Botany* **63**:1593–1608.

Kumar A, Devi S, Patil S, Payal C and Negi S. 2012. Isolation, screening and characterization of bacteria from rhizospheric soils for different plant growth promotion (PGP) activities: an *in vitro* study. *Recent Research in Science and Technology* **4**(1): 1-5.

Kumawat G, Soni A K and Kanwar J. 2012. Effect of different fertility levels and biofertilizers on growth and yield of cabbage (*Brassica oleracea* var. *capitata*). *Green Farming* **3**(6): 652-655.

Kundu B S, Gera R, Sharma N, Bhatia A and Sharma R. 2002. Host specificity of phosphate solubilizing bacteria. *Indian Journal of Microbiology* **42**: 19-2.

Kundu B S, Nehra K, Yadav R and Tomar M. 2009. Biodiversity of phosphate solubilizing bacteria in rhizosphere of chickpea, mustard and wheat grown in different regions of Haryana. *Indian Journal of Microbiology* **49**: 120-127.

Lal Bahadur, Tiwari D D, Mishra J and Gupta B R. 2012. Effect of integrated nutrient management on yield, microbial population and changes in soil properties under rice-wheat cropping system in sodic soil. *Journal of the Indian Society of Soil Sciences* **60**(4): 326-329.

Lata and Saxena A K. 2003. Characterization of plant growth promoting rhizobacteria. In: Training manual on biofertilizer technology (eds.) A K Saxena. IARI Delhi pp. 24-25.

Lemessa F and Zeller W. 2007. Screening rhizobacteria for biological control of *Ralstonia solanacearum* in Ethiopia. *Biological Control* **42**: 336-344.

Lim J H and Kim S D. 2013. Induction of drought stress resistance by multi-functional PGPR *Bacillus licheniformis* K11 in pepper. *The Plant Pathology Journal* **29**: 201–208.

Lin H F, Chen T H and Liu S D. 2010. Evaluation of *Bacillus subtilis* as a bio-control agent against pepper blight under greenhouse and field conditions. *Journal of the Agricultural Association of Taiwan* **11**(3): 210-22.

Liu H, He Y, Jiang H, Peng H, Huang X, Zhang X, Thomashow L S and Xu Y. 2007. Characterization of a phenazine-producing strain *Pseudomonas chlororaphis* GP72 with broad-spectrum antifungal activity from green pepper rhizosphere. *Current Microbiology*. **54**: 302–306.

Liu L, Kloepper J W and Tuzun S. 1995. Induction of systemic resistance in cucumber against Fusarium wilt by plant growth promoting rhizobacteria. *Phytopathology* **85**:695–698.

Loganathan M, Garg R, Venkataravanappa V, Saha S and Rai A B. 2014. Plant growth promoting rhizobacteria (PGPR) induces resistance against Fusarium wilt and improves lycopene content and texture in tomato. *African Journal of Microbiology Research* **8**(11): 1105-1111.

Loon L C van. 2007. Effects of beneficial microorganisms on plants. Bulletin-OILB/SROP **29**(2): 183-192.

Lowry O H, Rosebrough N J, Farr A L and Randall R J. 1951. Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* **193**(1): 265-275 pp.

Lugtenberg B and Kamilova F. 2009. Plant-growth-promoting rhizo-bacteria. *Annual Review of Microbiology* **63**: 541-556.

Lynch J M. 1990. Beneficial interactions between miroorganisms and roots. *Biotechnology Advances* **8**: 335-346.

Mahafee W F and Kloepper J W**.** 1997. Bacterial communities of the rhizosphere and endorhiza associated with field grown cucumber plants inoculated with a plant growth promoting rhizobacterium or its genetically modified deriative. *Canadian Journal of Microbiology* **43**: 344-353.

Maji S and Chakrabartty P K. 2014. Biocontrol of bacterial wilt of tomato caused by *Ralstonia solanacearum* by isolates of plant growth promoting rhizobacteria. *Australian Journal of Crop Science* **8**(2): 2008-2014

Mandal T, Ghanti P, Mahato B, Mandal A R and Thapa U. 2003. Effect of spacing and biofertilizer on yield and yield attributes of direct sown chilli (*Capsicum annuum* L. cv Bona Lanka). *Environmental Ecology* **21**(3): 712-715.

Marcia V B F, Burity H A, Martinez C R and Chanway C P. 2008. Alleviation of drought stress in the common bean (*Phaseolus vulgaris* L.) by co-inoculation with *Paenibacillus polymyxa* and *Rhizobium tropici*. *Applied Soil Ecology* **40**: 182-188.

Marulanda A, Barea J M and Azcon R. 2009. Stimulation of plant growth and drought tolerance by native microorganisms (AM fungi and bacteria) from dry environment. *Journal of Plant Growth Regulators* **28**: 115–124.

Mateos R M , Jimenez A, Roman P, Romojaro F, Bacarizo S, Leterrier M, Gomez M, Sevilla F, Rio L A D, Corpas F J and Palma J M. 2013.Antioxidant systems from pepper (*Capsicum annuum* L.): Involvement in the response to temperature changes in ripe fruits. *International Journal of Molecular Sciences* **14**: 9556-9580 doi:10.3390/ijms14059556

Mayak S, Tirosh T and Glick B R. 2004. Plant growth-promoting bacteria that confer resistance to water stress in tomatoes and peppers. *Plant Sciences* **66:** 525–530.

Mayak S, Tirosh T and Glick B R. 2004. Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. *Plant Physiology and Biochemistry* **42**: 565–572.

McCully M E. 2001. Niches for bacterial endophytes in crop plants: a plant biologist's view. *Functional Plant Biology* **28**: 983-990.

Meera T and Balabaskar P. 2012. Isolation and characterization of *Psuedomonas fluorescence* from rice fields. *International Journal of Food, Agriculture and Veterinary Sciences* **2**(1):113-120.

Merwin H D and Peech M. 1951. Exchange ability of soil potassium in the sand, silt and clay fractions as influenced by the nature and complementary exchangeable cations. *Soil Science American Proceedings* **15**: 125-128.

Minorsky P V. 2008. On the inside. *Plant Physiology* **146**: 323–324.

Mrkovacki N and Bjelic D. 2011. Plant growth promoting rhizobacteria (PGPR) and their effect on maize. *Field and Vegetable Crops Research.* **48**(2): 305-312.

Muhammad I, Roomi S, Muhammad Z A and T Sultan T. 2013. Integrated effect of plant growth promoting rhizobacteria, phosphate solubilizing bacteria and chemical fertilizers on growth of maize original research article. *International Journal of Agriculture and Crop Sciences* **6**(13): 913-921.

Munazza G and Hafeez. 2012. Characterization of siderophore producing bacterial strain *Pseudomonas fluorescens* Mst 8.2 as plant growth promoting and biocontrol agent in wheat. *African Journal of Microbiology Research* **6**(33): 6308-6318.

Naseem H and Bano A. 2014. Role of plant growth-promoting rhizobacteria and their exopolysaccharide in drought tolerance of maize. *Journal of Plant Interaction* **9**(1):689–701.

Neubauer U, Furrer G, Kayser A and Schulin R. 2000. Sidero-phores, NTA and citrate: potential soil amendments to enhance heavy metal mobility in phytoremediation. *International Journal of Phytoremediation* **2**: 353–368.

NHB. 2015. Nhb.gov.in/area-prod/Database-2015.pdf.

Nihorimbere V, Ongena M, Smargiassi M and Thonart P. 2011. Beneficial effect of the rhizosphere microbial community for plant growth and health. *Biotechnology, Agronomy,Society and Environment* **15**(2): 327-337.

Oliveira C A, Alves V M C, Marriel I E, Gomes E A, Scotti M R, Carneiro N P, Guimaraes C T, Schaffert R E and Sa N M H. 2008. Phosphate solubilizing microorganisms isolated from rhizosphere of maize cultivated in an oxisol of the Brazilian Cerrado Biome. *Soil Biology and Biochemistry* doi:10.1016/j.soilbio.2008.01.012

Olsen S R, Cole C V, Watenabe D S and Dean L A. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDS Circular. 939 p.

Ongena M, Jourdan E, Adam A, Paquot M, Brans A, Jori, B, Arpigny J L and Thonart P. 2007. Surfactin and fengycin lipopeptides of *Bacillus subtilis* as elicitors of induced systemic resistance in plants. *Environmental Microbiology* **9**: 1084-1090.

Ordookhani K, Khavazi K, Moezzi A and Rejali F. 2010. Influence of PGPR and AMF on antioxidant activity, lycopene and potassium contents in tomato. *African Journal of Agricultural Research* **5**(10): 1108-1116.

Paramaguru P and Natarajan S. 1993. Effect of *Azospirillum* on groth and yield of chilli (*Capsicum annuum* L.) grown under semi dry conditions. *South Indian Horticulture*. pp. 80-82.

Park J W, Balaraju K, Kim J W, Lee S W and Park K. 2013. Systemic resistance and growth promotion of chili pepper induced by an antibiotic producing *Bacillus vallismortis* strain BS07. *Biological Control* **65**: 246–257.

Park K H, Lee C Y and Son H J. 2005. Mechanism of insoluble phosphate solubilization *by Pseudomonas fluorescens* RAF15 isolated from ginseng rhizosphere and its plant growth promoting activities. *Letters in Applied Microbiology* **49**: 222-228.

Patil R B and Varade P A. 1998. Microbial population in rhizosphere as influenced by high input rates of fertilizer application to sorghum on a vertisol. *Journal of the Indian Society of Soil Science* **46**: 223-227.

Peirce L C. 1987. Vegetable characteristics, production and marketing. New York, John Wiley and Sons. pp. 323-325

Pikovskaya R I. 1948. Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. *Mikrobiologiya* **7**: 362-370.

Piper C S. 1966. Soil chemical analysis. Asia Publishing House, Bombay. 408 p.

Pitzschke A M, Forzani C and Hirt H. 2006. Reactive oxygen species signaling in plants. *Antioxidants and Redox Signaling* **8**: 1757–1764.

Poinkar M, Shembekar R Z, Neha C, Bhaladhare N, Khewale A and Dongarkar K. 2006. Effect of organic manure and biofertilizers on growth and yield of turmeric (*Curcuma longa* L.). *Journal of Soils and Crops* **16**(2): 417-420.

Pompelli M F, Barata-Luis R, Vitorino H, Gonclaves E, Rolim E, Santos M, Almeida-Cortez J and Endrez L. 2010. Photosynthesis, photoprotection and antioxidant activity of purging nut under drought deficit and recovery. *Biomass Bioenergy* **34**: 1207–1215.

Ponmurugan P and Gopi C. 2006. In vitro production of growth regulators and phosphatase activity by phosphate solubilizing bacteria. *African Journal of Biotechnology* **5**: 348-350.

Prathap M and Ranjitha Kumari B D. 2015. A critical review on plant growth promoting rhizobacteria. *Journal of Plant Pathology and Microbiology* **6**: 266-Doi:10.4172/2157-7471.1000266

Qureshi A Aziz, Narayanaswamy G, Chhonkar P K and Balasundaram V R. 2005. Direct and residual effect of phosphate rocks in presence of phosphate solubilizers and FYM on the available P, organic carbon and viable counts of phosphate solubilizers in soil after soybean, mustard and wheat crops. *Journal of the Indian Society of Soil Science* **53**(1): 97-100.

Qureshi M A , Ahmad Z A, Akhtar N, Iqbal A, Mujeeb F and Shakir M A. 2012. Role of phosphate solubilizing bacteria in enhancing P availability and promoting cotton growth. *The Journal of Animal & Plant Sciences* **22**(1): 204-210.

Raaijmakers J M, De Bruijn I, Nybroe O and Ongena M. 2010. Natural functions of lipopeptide from *Bacillus* and *Pseudomonads*: more than surfactants and antibiotics. *Federation of European Microbiological Societies - Microbiology Review* **34**(6): 1037-1062.

Rajkumar M, Ae N, Prasad M N V and Freitas H. 2010. Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. *Trends in Biotechnology* **28**: 142–149.

Raju E V N and Divakar G. 2013. Production of pectinase by using *Bacillus circulans* isolated from dump yards of vegetable wastes. *International Journal of Pharmaceutical Sciences and Research* **4**(7): 2615-2622.

Ramesh R, Joshi A A and Ghanekar M P. 2009. *Pseudomonads*: major antagonistic endophytic bacteria to suppress bacterial wilt pathogen, *Ralstonia solanacearum* in the eggplant (*Solanum melongena* L.). *World Journal of Microbiology and Biotechnology* **25**(1): 47-55.

Rammette A, Frapolli M, Defago G and Monne-Loccoz. 2003. Phylogeny of HCN synthase encoding hcnBC genes in biocontrol fluorescent Pseudomonas and its relationship with host plant species and HCN synthesis ability. *Molecular Plant Microbe Interactions* **16**: 525-535.

Reed M L and Glick B R. 2005. Growth of canola (*Brassica napus*) in the presence of plant growth-promoting bacteria and either copper or polycyclic aromatic hydrocarbons. *Canadian Journal of Microbiology* **51**: 1061–1069.

Richardson A E, Barea J M, McNeill A M and Prigent-Combaret C. 2009. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. *Plant Soil* **321**: 305–339 doi:10.1007/s11104-009-9895-2

Richardson A E. 2001. Prospects for using soil microorganisms to improve the acquisitions of phosphorus by plants. *Australian Journal of Plant Physiology* **28**: 897-906.

Robert W K and Selitrennikoff C P. 1988. Plant and bacterial chitinase differ in antifungal activity. *Journal of General Microbiology* **134**: 169-176.

Rodriguez-Salazar J, Suarez R, Caballero-Mellado J and Itturiaga G. 2009. Trehalose accumulation in *Azospirillum brasilense* improves drought tolerance and biomass in maize plants. *Federation of European Microbiological Societies – Microbiology Letters* **296**: 52–59.

Rubio L M and Ludden P W. 2008. Biosynthesis of the iron-molybdenum cofactor of nitrogenase. *Annual Review of Microbiology* **62**: 93–111.

Ruchi, Kapoor R, Kumar A, Kumar A, Patil S, Thapa S and Kaur M. 2012. Evaluation of plant growth promoting attributes and lytic enzyme production by fluorescent Pseudomonas diversity associated with Apple and Pear. *International Journal of Scientific and Research Publications* **2**(2): 1-8.

Rudrappa T, Splaine R E, Biedrzycki M L and Bais H P. 2008. Cyanogenic Pseudomonads influence multitrophic interactions in the rhizosphere. *Public Library of Sciences* (PloS ONE)**3**: 2073.

Sadfi Z N, Essghaier B, Hajlaoui M R, Achbani H and Boudabous A. 2007. Ability of the antagonistic bacteria *Bacillus subtilis* and *B. licheniformis* to control *Botrytis cinerea* on fresh market tomatoes. Bulletin-OILB/SROP **30**(6(1)): 63p.

Saharan B S and Nehra V. 2011. Plant growth promoting rhizobacteria: A Critical Review. *Life Sciences and Medicine Research* **21**: 36-48.

Saini V K, Bhadari S C, Sharma S K and Tarafdar J C. 2005. Assessment of microbial biomass under integrated nutrient management in soybean winter maize cropping sequence. *Journal of the Indian Society of Soil Science* **53**(3): 346-351.

Sakthivel U M and Karthikeyan B. 2009. Studies on isolation and characterisation of PGPR (*Pseudomonasfluorescens*) and its effect of seed inoculation on yield of tomato. *Journal of Phytology* **1**: 33–39.

Sambrook J, Fritsch E F and Maniatis T. 1989. Molecular cloning: a laboratory manual. New York: Cold Spring Harbor Laboratory.

Santner A, Calderon-Villalobos L I A and Estelle M. 2009. Plant hormones are versatile chemical regulators of plant growth. *Nature Chemical Biology* **5**: 301–307.

Saravanakumar D, Kavino M, Raguchander T, Subbian P and Samiyappan R. 2011. Plant growth promoting bacteria enhance water stress resistance in green gram plants. *Acta Physiologiae Plantarum* **33**: 203–209.

Schmidt W. 1999. Mechanisms and regulation of reduction-based iron uptake in plants. *New Phytologist* **141**: 1–26.

Schwyn B and Neilands J B. 1987. Universal chemical assay for the detection and determination of siderophore. *Analytical Biochemistry* **160**: 47-56.

Selvi D, Santhy P and Dhakshinamoorthy M. 2005. Effect of inorganic alone and in combination with farmyard manure on physical properties and productivity of vertic haplustepts under long-term fertilization. *Journal of the Indian Society of Soil Science* **53**(3): 302-307.

Selvi D, Santhy P, Dhakshinamoorthy M and Maheshwari M. 2004. Microbial population and biomass in rhizosphere as influenced by continuous intensive cultivation and fertilization in an inceptisol. *Journal of the Indian Society of Soil Science* **52**(3): 254-257.

Shalaby M E M and Sedik M Z. 2008. Biocontrol activity of some bacterial isolates against *Meloidogyne incognita*. *Egyptian Journal of Biological Pest Control* **18**(1): 119-125.

Sharma A, Johri B N, Sharma A K and Glick B R. 2003. Plant growth-promoting bacterium *Pseudomonas* sp. strain GRP3 influences iron acquisition in mung bean (*Vigna radiata* L. Wilzeck). *Soil Biology and Biochemistry* **35**: 887–894.

Sharma B C, Subba R and Saha A. 2012. *In vitro* solubilization of tricalcium phosphate and roduction of IAA by phosphate solubilizing bacteria isolated from tea rhizosphere of Darjeeling Himalaya. *Plant Sciences Feed* **2**(6): 96-99.

Sharma K. 2009. Selection and characterization of endophytic and rhizospheric microorganisms of chrysanthemum (*Dendranthema grandiflora* tzvelve). M.Sc. Thesis submitted to Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan (HP)

Sharma P K, Sarita S and Parel J. 2005. Isolation and characterization of an endophytic bacterium related to *Rhizobium*/*Agrobacterium* from wheat (*Triticum aestivum* L.) roots. *Current Sciences* **89**(4): 608-610.

Sharma R C. 1986. Nitrogen management of potatoes in the presence of farmyard manure and P K fertilizers on acid hill soils of Shimla. *Journal of Agricultural Sciences* **107**: 15-19.

Shaw F J, Lin P F, Chen C S and Chen C H. 1995. Purification and characterization of an extracellular α-amylase from thermos species. *Botanical Bulletin of Academia Sinica* **36**: 195-200.

Singh A K and Gupta R C. 2001. Enhancing phosphorus release from rock phosphate blending with phosphate solubilizers. **In:** Abstracts of fifth Agricultural Science Congress 139p.

Smith S E and Read D J. 1997. Mycorrhizal Symbiosis. Academic Press. San Diego. pp. 120-125.

Sood S, Bindal A, Sharma A and Chaudhary D R. 2007. Genetic architecture and relationship in bell pepper under sub temperate conditions of North Western Himalayas. *Indian Journal of Horticulture* **64**(2): 169-174.

Spaepen S and Vanderleyden J. 2011. Auxin and plant-microbe interactions. *Cold Spring Harbor Perspectives in Biology* http://dx.doi.org/ 10.1101/cshperspect.a001438

Sparling G P. 1985. The Soil biomass. **In**: Soil organic matter and biological activity. Vaughan D and Malcolm R E (eds.). Martinus Nijoff/Dr. W. Junk, Dordrecht. Boston, Lanchester. 223 p.

Sridevi M and Mallaiah K V. 2009. Phosphate solubilization by *Rhizobium* strains. *Indian Journal of Microbiology* **49**: 98-102.

Srivastava S N L and Verma S C. 1984. Effects of nitrogen, *Rhizobium* and techniques of phosphorus application on yield and quality of field pea (*Pisum sativum* L. var. *arvense* poir.). *Legume Research* **7**(1): 37-42.

Stoltzfus J R, Malarvithi P P, Ladha J K and Bruijn F J D. 1997. Isolation of endophytic bacteria from rice and assessment of their potential for supplying rice with biologically fixed nitrogen. *Plant Soil* **194**: 25-36.

Sturz A V, Christie B R and Nowak J. 2000. Bacterial endophytes: potential role in developing sustainable systems of crop production. *Plant Science* **19:** 1-30.

Subba Rao N S. 1999. Soil microorganism and plant growth. Oxford & IBH publishing Company, New Delhi. 252 p

Subbiah B V and Asija G L. 1956. A rapid procedure for the estimation of the available nitrogen in soils. *Current Science* **25**: 259-260.

Suliasih N and Widawati S. 2005. Isolation and identification of phosphate solubilizing and nitrogen fixing bacteria from soil in Wamena Biological Garden, Jayawijaya, Papua. *Biodiversitas* **6**(5): 175-177.

Supraja Y, Reddy R S, Reddy S S and Rani V D. 2011. Plant growth promotion and biocontrol properties of local isolates of *Fluorescent pseudomonas*. *The Journal of Research Acharya N G Ranga Agricultural University* **39**(3): 1-5.

Suryanto D, Patonah S and Munir E. 2010. Control of *Fusarium* wilt of chili with chitinolytic bacteria. *Hayati Journal of Biosciences* **17:** 5-8.

Tabatabai and Bremner. 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biology and Biochemistry* **1**: 301-317.

Tai X S, Mao W L, Liu G X, Chen T, Zhang W, Wu X K, Long H Z, Zhang B G and Zhang Y. 2013. High diversity of nitrogen-fixing bacteria in the upper reaches of the Heihe River, northwestern China. *Biogeosciences* **10**: 5589–5600.

Tallapragada P, Rajiv R, Anjanappa V , Sardesai S, Selvaraj S and Khan S. 2011. Comparing the potential of spent mycelium substrate of *Pleurotus florida* with biofertilizers to enhance growth of *Capsicum annuum*. *Asian Journal of Plant Science and Research* **1** (4):76-86.

Tayeb Rezvani H, Moradi P and Soltani F. 2013. The effect of nitrogen fixation and phosphorus solvent bacteria on growth physiology and vitamin C content of *Capsicum annum* L. *Iranian Journal of Plant Physiology* **3** (2): 673-682.

Thomas F C, Woeng C A and Lugtenberg B J J. 2008. Root colonisation following seed inoculation. *The Journal of Animal & Plant Sciences* **22**(1): 204-210.

Tilak K V B R, Ranganayahi N, Pal K N, Saxena A K, Nautiyal C S, Mital S, Tripathi A K and Joshi B N. 2005. Diversity of plant growth and soil health supporting bacteria. *Current Science* **89**(1): 136-150.

Timmusk S, Abd El-Daim I A, Copolovici L, Tanilas T, Kannaste A, Behers L and Niinemets U. 2014. Drought-tolerance of wheat improved by rhizosphere bacteria from harsh environments: enhanced biomass production and reduced emissions of stress volatiles. *Public Library of Sciences* **9**: e96086.

Turrion M B, Glaser B, Solomon D, Ni A and Zech W. 2000. Effects of deforestation on phosphorus pools in mountain soils of the Alay Range Khyrgyzia. *Biology and Fertility of Soils* **31**: 134–142.

Uneo M, Kihara J, Honda Y and Arase S. 2004. Indole acetic acid related compounds induce the resistance to rice blast fungus, *Magnaporthegriesea* in Barley. *Phytopathology* **152**: 606-61.

Uthao C J and Peberdy J F. 1991. Purification and characterization of an extracellular chitiobiase from *Trichoderma harzianum*. *Current Microbiology* **23**: 285-289.

Vance E D, Brookes P C and Jenkinson D S. 1987. An extraction method for measuring soil microbial biomass carbon. *Soil biology and Biochemistry* **19**: 703-707.

Vardharajula S, Zulfikar Ali S, Grover M, Reddy G and Bandi V. 2011. Drought-tolerant plant growth promoting *Bacillus* spp., effect on growth, osmolytes and antioxidant status of maize under drought stress. *Journal of Plant Interaction* **6**: 1–14.

Veera R T, Sundara R D, Jayashankar S, Vadivel E S and Shanmughavelu K G. 1988. Effect of nitrogen,phosphorus application and azospirillum inoculation on chilli (*Capsicum annuum* L.). *Proceedings of National Seminar on Chillies, Ginger and Turmeric*. pp. 65-70.

Vijaypal I, Jalalipal V and Jalali I. 1998. Rhizosphere bacteria for biocontrol of plant diseases. *Indian Journal of Microbiology* **38:** 187-204.

Vincent J M. 1947. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature* **150**: 850.

Vivas A, Marulanda A, Ruiz-Lozano J M, Barea J M and Azcon R. 2003. Influence of a *Bacillus* sp. on physiological activities of two arbuscular mycorrhizal fungi and on plant responses to PEG induced drought stress. *Mycorrhiza* **13**: 249-256.

Walia A, Mehta P, Chauhan A and Shirkot C K. 2012. Effect of *Bacillus* sp. strain CKT1 as inoculum on growth of tomato seedlings under net house conditions. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences* **84**(1):144-155. DOI 10.1007/s40011-013-0189-3

Walkley A and Black T A. 1934. An estimation of soil organic matter and purposed modification of chromic acid titration method. *Soil Science* **37**: 29-38.

Wang C, Yang W, Wang C, Gu C, Niu D, Liu H, Wang Y and Guo J. 2012. Induction of drought tolerance in cucumber plants by a consortium of three plant growth-promoting rhizobacterium strains. *Public Library of Sciences* **7**: e52565.

Wani P A, Khan M S and Zaidi A. 2007. Synergistic effects of the inoculation with nitrogen fixing and phosphate solubilizing rhizobacteria on the performance of field grown chickpea. *Journal of Plant Nutrition and Soil Science* **170**: 283–287.

Wei L, Kloepper J W, Tuzun S. 1996. Induced systemic resistance to cucumber diseases and increased plant growth by plant growth-promoting rhizobacteria under field conditions. *Phytopathology* **86**: 221–224.

Wei Z, Yang X, Yin S, Shen Q, Ran W and Xu Y. 2011. Efficacy of *Bacillus* fortified organic fertilizer in controlling bacterial wilt of tomato in the field. *Applied Soil Ecology* **48**: 152-159.

Wen H K, Tsou Y J, Lin M J and Chern L L. 2010. Activity and characterization of secondary metabolites produced by a new microorganism for control of plant diseases. *New Biotechnology* **27**(4): 397-402.

Whipps J M. 2001. Microbial interactions and biocontrol in the rhizosphere. *Journal of Experimental Botany* **52**: 487-511.

Winstead N N and Kelman A. 1952. Inoculation techniques for evaluating resistance to Pseudomonas solanacearum. *Phytopathology* **42**: 628-634.

Withem S H, Baldeys D F and Devila R M. 1971. Chlorophyll absorption spectrum and qualitative determination in experiment. (*In*) *Plant physiology*. Nostrand Van (Ed). Rein Hold Company, New York, pp 55-58.

Wollum II, A G. 1982. Cultural methods for soil microorganisms. **In***:* Methods of Soil Analysis, Part II, Chemical and Microbiological properties. American Society of Agronomy. Inc. Publisher Madison, Wisconsin, USA pp. 781-802

Xue Q Y, Li S M, Chen L F, Ding G C, Guo D W, Guo J H and Samalla K. 2012. Rhizocompetence and antagonistic activity towards genetically diverse Ralstonia solanacearum strains: an improved strategy for selecting biocontrol agents. *Applied Microbiological Biotechnology* **97**: 1361-1371.

Yadav S K, Jyothi L N, Maheswari M, Vanaja M, Venkateswarlu B. 2005. Influence of water deficit at vegetative, anthesis and grain filling stages on water relation and grain yield in Sorghum. *Indian Journal of Plant Physiology* **10**:20–22.

Youssef M M A and Eissa M F M. 2014. Biofertilizers and their role in management of plant parasitic nematodes. *European Journal of Biotechnology and Pharmaceutical Research* **5**: 1-6.

Yuan S Z and Zhou M G. 2006. Screening and root colonization of biocontrol agents against *Phytophthora capsici*. *Journal of Yangzhou University Agricultural and Life Sciences* **27**(4): 93-97.

Zabihi H R, Savaghebi G R, Khavazi K, Ganjali A and Miransari M. 2011. *Pseudomonas* bacteria and phosphorous fertilization, affecting wheat (*Triticum aestivum* L.) yield and phosphorus uptake under greenhouse and field conditions. *Acta Physiology Plant* **33**: 145–152.

Zaidi A, Khan M S, Ahemad M and Oves M. 2009. Plant growth promotion by phosphate solubilizing bacteria. *Acta Microbiologica et Immunologica Hungarica* **56**: 263–284.

Zapata P J, Botella M A, Pretel M T, Serano M. 2007. Responses of ethylene biosynthesis to saline stress in seedlings of eight plant species. *Journal of Plant Growth Regulation* **53**(2): 97-106.

Zayed M S, Hassanein M K K , Esa N H , Abdallah M M F. 2013. Productivity of pepper crop (*Capsicum annuum* L.) as affected by organic fertilizer, soil solarization and Endomycorrhizae. *Annals of Agricultural Science* **58**(2): 131–137.

Zhan J and Sun Q. 2011. Diversity of free-living nitrogen-fixing microorganisms in wastelands of copper mine tailings during the process of natural ecological restoration. *Journal of Environmental Sciences* **23**: 476–487, doi:10.1016/S1001-0742(10)60433-0

Zhang S, White T L, Martinez M C, McInroy J A, Kloepper J W and Klassen W. 2010. Evaluation of plant growth-promoting rhizobacteria for control of *Phytophthora* blight on squash under greenhouse conditions. *Biological Control* **53**: 129–135.

Zhou T, Chen D, Li C, Sun Q, Li F, Shen Q and Shen B. 2012. Isolation and characterization of *Pseudomonas brassicacearum* J12 as an antagonist against *Ralstonia Solanacearum* and identification of its antimicrobial components. *Microbial research* **167**: 388-394.

Department of Basic Sciences
Dr Yashwant Singh Parmar University of Horticulture & Forestry
Nauni, Solan (HP) – 173 230

Title of the thesis	**:**	**Impact of Indigenous Plant Growth Promoting Rhizobacteria and Chemical Fertilizers on Soil Health and Productivity of Capsicum (*Capsicum annuum* L.)**
Name of the student	**:**	Shweta Gupta
Admission Number	**:**	F-2012-25-D
Major Discipline	**:**	Microbiology
Minor Discipline	**:**	Biotechnology
Date of Thesis Submission	**:**	2016
Total pages of the Thesis	**:**	121+ IV
Name of Major Advisor	**:**	Dr Rajesh Kaushal

ABSTRACT

Capsicum is a renumerative crop to the farmers and is grown in both open fields and protective structures. The nutrient requirements and its sensitivity to extremes of environment coupled with higher incidence of diseases such as damping off (*Pythium* spp.), phytophthora collar rot (*Phytophthora capsici*), fruit rot (*Colletotrichum capsici*) and bacterial wilt (*Ralstonia solanacearum*) resulted in sizeable yield losses. The use of PGPR to supplement chemical fertilizers and pesticides is a potential alternative but no commercial formulation is available for use. So, the present investigations entitled "Impact of indigenous plant growth promoting rhizobacteria and chemical fertilizers on soil health and productivity of capsicum (*Capsicum annuum* L.)" was carried out during 2013-2016. Rhizosphere and root samples of capsicum were collected from twenty eight locations of agro-climatic zone I, II and III of Himachal Pradesh. Among 157 isolates, 34 morphological distinct isolates were selected for screening of possession of multifarious plant growth promoting traits. Among selected isolates, all were P-solubilizers, nitrogen fixers, siderophore producers, 18 isolates were able to produce IAA, 19 isolates were ACC- deaminase producers, 12 isolates were ammonia producers and only 5 isolates were HCN producer. Under laboratory conditions, except THE $_{17}$ isolate, all the tested isolates were able to inhibit the growth of one or more test pathogens i.e. *Fusarium solan*i, *Rhizoctonia solani*, *Pythium* spp., *Ralstonia solanacearum*, *Phytophthora capsici* and *Colletotrichum capsici*. Fifteen isolates possessing maximum PGP traits were characterized further. The TCP solubilisation by selected fifteen isolates ranged from 169.84 µg/ml to 60.16 µg/ml, siderophore production efficiency ranged from 56.36 percent to 12.79 per cent, able to grow in a temperature range of (20 - 40ºC), pH (5.0-8.0) and salt concentration (2-6%). On the basis of 16S rRNA gene sequencing three isolates (JHA_6, ROH_6 and ROH_{14}) possessing maximum multifarious PGP traits were identified as *Providencia* sp. (ROH_6), *Pseudomonas aeruginosa* (JHA_6) and *Bacillus amyloliquefaciens* (ROH_{14}). The application of isolated indigenous PGPR *Providencia* sp. (ROH_6) reduced the disease incidence of bacterial wilt (*Ralstonia solanacearum*) by 70% as compared to pathogen inoculated control. The conjoint application of PGPR isolate (JHA_6 and ROH_{14}) along with 80 per cent NP (N_{80} and P_{61} kg/ha) brought a significant increase in yield by 8.93%, available N and P contents by 8.64 and 20.73 per cent, over recommended doses (N_{100} and P_{76} kg/ha) besides saving 20 per cent chemical fertilizers. The study, therefore, indicates the potential of *Bacillus amyloliquefaciens* (ROH_{14}) and *Pseudomonas aeruginosa* (JHA_6) in partial replacement of N and P (~20%) applied through chemical fertilizers, besides higher productivity of crops. Further, the application of selected indigenous isolates (JHA_6 and ROH_{14}) can induce drought tolerance by increasing antioxidant enzymes (superoxide dismutase, catalase and peroxidase) activities. Thus, the conjoint application of PGPR isolates at 80 per cent doses of N & P has good prospects to be used as biofertilizer, biocontrol and biostimulant not only for enhanced growth and yield of capsicum but also to sustain soil health.

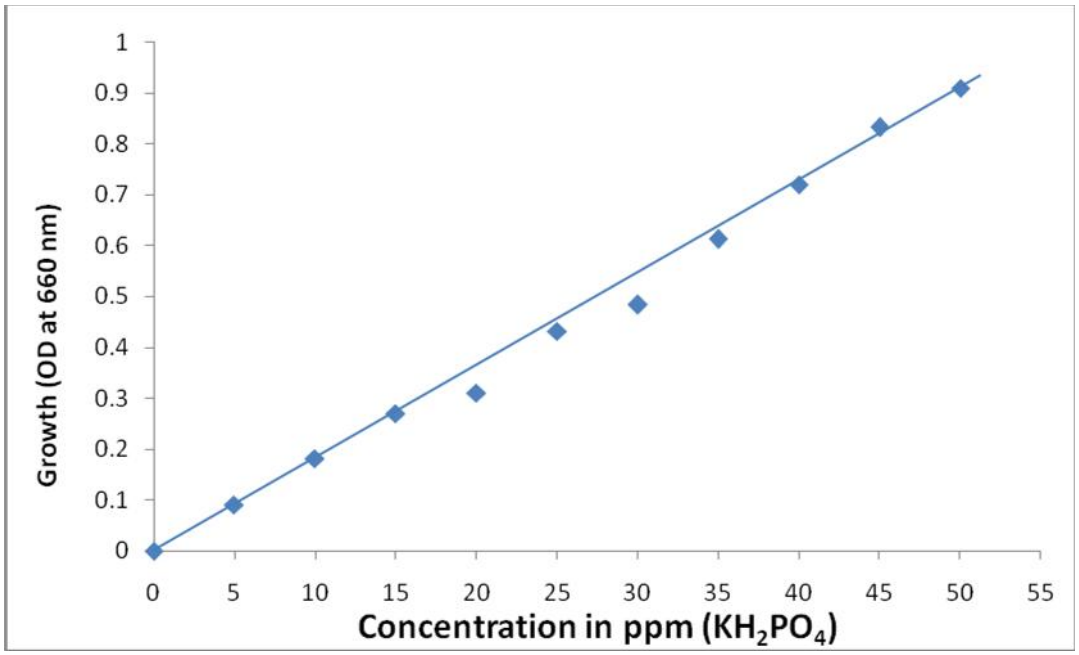
Signature of the Student
Name: Shweta Gupta
Date:

Signature of the Major Advisor
Name: Dr Rajesh Kaushal
Date:

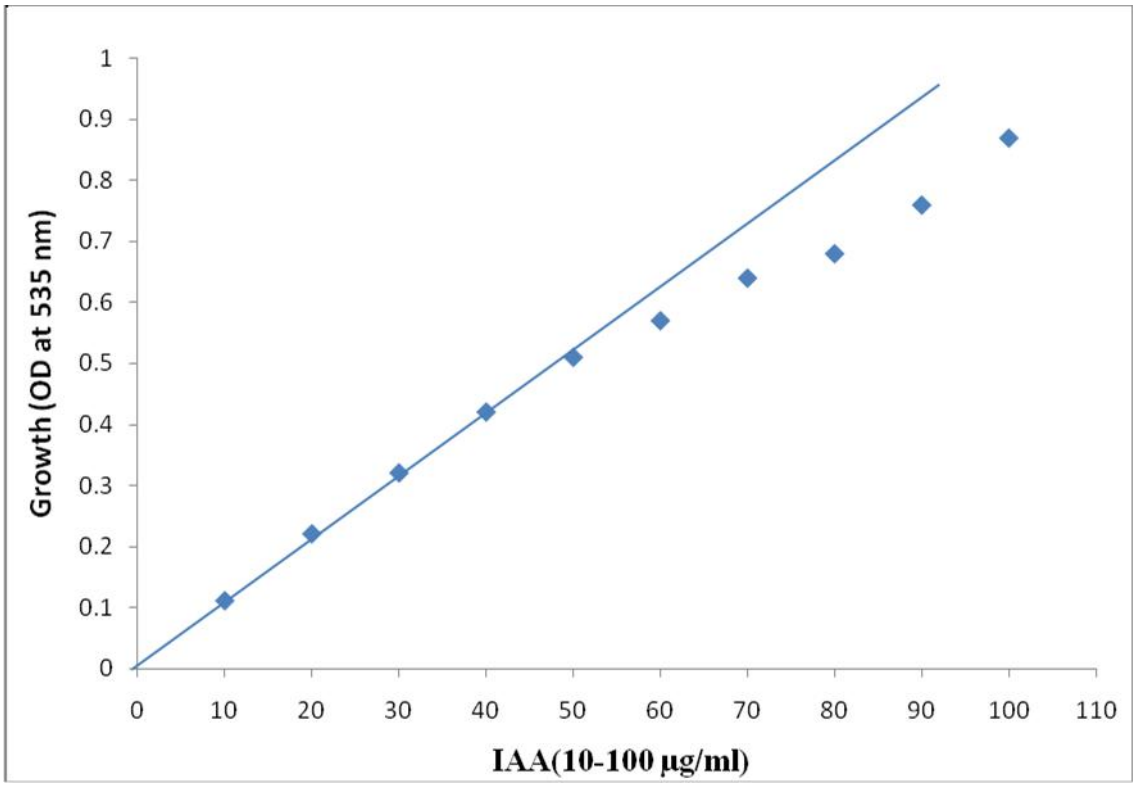
Countersigned

Professor and Head
Department of Basic Science
Dr YSP, UHF, Nauni, Solan (H P)

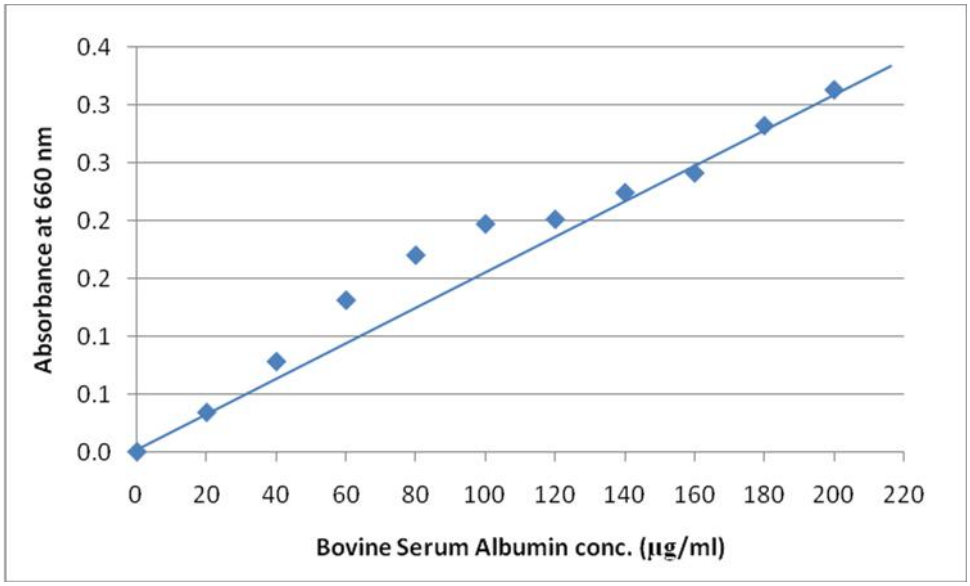
APPENDIX-I



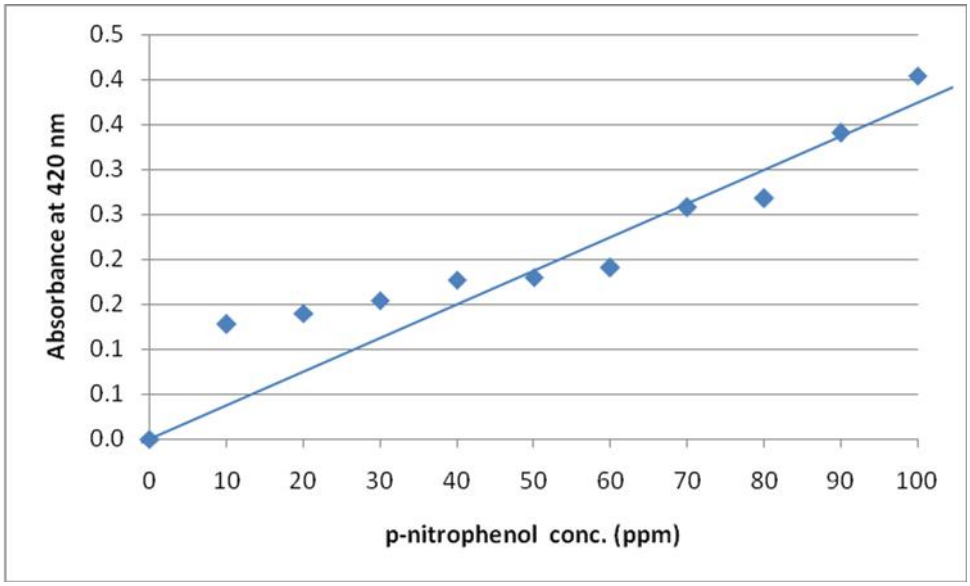
Appendix 1.1: Standard curve for phosphorus



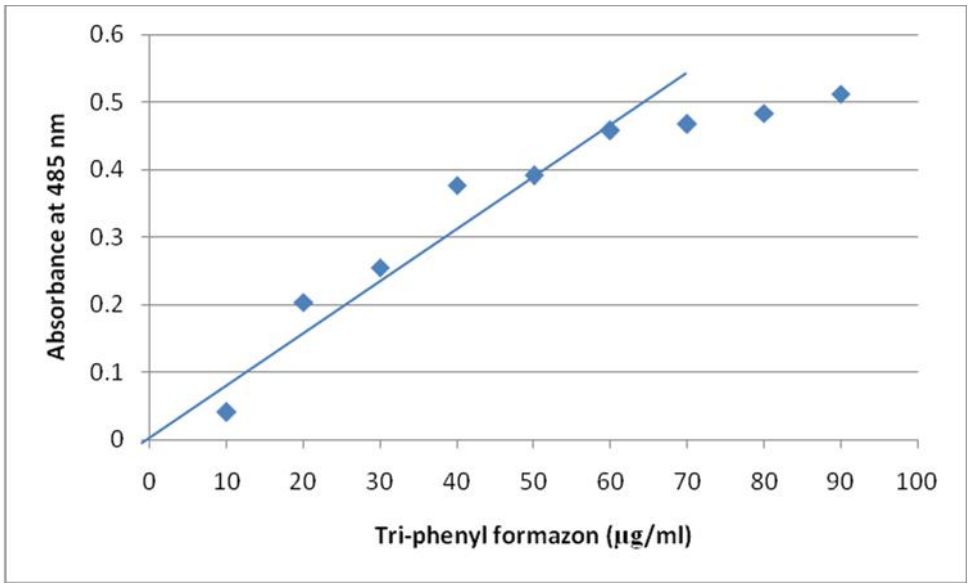
Appendix 1.2: Standard curve of indole-3-acetic acid



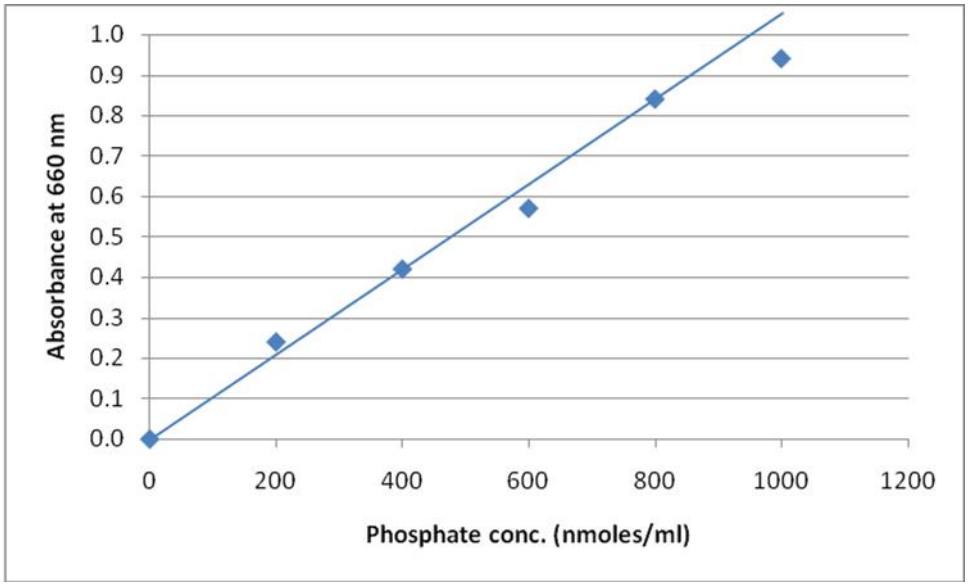
Appendix 1.3: Standard curve for soluble protein determination



Appendix 1.4: Standard curve for phosphatase determination



Appendix 1.5: Standard curve for dehydrogenase determination



Appendix 1.6: Standard curve for phytase determination

APPENDIX-II

Appendix 2.1.Quantitative characterization of plant growth promoting traits of selected bacterial isolates

Isolates	P-solubilization			Siderophore estimation			Lytic enzyme production (EAI)***					
	P-solubilization* (µg/ml)	Viable count ($\times 10^7$ cfu/ml)	Final pH	Siderophore unit (%)**	Viable count ($\times 10^7$ cfu/ml)	Final pH	Amylase activity	Pectinase activity	Protease activity	Cellulase activity	Lipase activity	Chitinase activity
RAK_9	85.25	15.34	4.63	33.19	11.97	4.85	1.27	0.00	0.00	0.00	1.27	1.27
MAT_8	117.83	20.87	5.37	37.40	9.70	4.43	0.00	0.00	1.38	0.00	0.00	0.00
NER_4	120.00	21.60	5.50	30.22	11.44	5.50	0.00	0.00	0.00	0.00	0.00	0.00
PAR_2	86.06	15.49	4.65	12.79	8.30	4.53	1.36	1.36	0.00	0.00	1.36	0.00
PAO_2	59.00	11.96	6.77	28.17	11.07	4.61	1.95	0.00	1.95	1.27	0.00	0.00
SIH_6	62.70	11.30	5.20	31.76	11.72	4.45	1.36	1.36	0.00	0.00	0.00	0.00
PAL_7	60.16	12.14	5.05	18.54	9.34	4.86	0.00	0.00	1.86	0.00	1.86	1.95
KAN_{11}	120.20	21.64	5.39	51.80	15.32	5.37	0.00	1.45	1.45	0.00	1.45	0.00
$BHAR_4$	98.94	17.81	5.33	37.43	12.74	5.44	0.00	0.00	0.00	1.45	1.78	0.00
PAT_9	64.27	11.57	5.47	35.29	12.35	5.29	1.32	0.00	1.32	0.00	1.32	0.00
PAT_{13}	93.25	17.11	5.02	20.57	12.73	5.40	0.00	0.00	0.00	0.00	1.65	1.33
$SARA_9$	93.25	17.11	5.05	36.35	12.54	5.45	1.33	0.00	1.33	0.00	0.00	0.00
JHA_6	169.84	30.27	5.17	56.36	16.14	4.64	1.36	1.82	1.46	0.00	0.00	1.36
ROH_6	92.56	16.66	5.10	34.35	12.18	5.05	1.45	0.00	1.28	0.00	0.00	1.95
ROH_{14}	145.77	26.26	4.80	51.77	15.50	5.37	0.00	0.00	1.83	0.00	0.00	0.00
$CD_{0.05}$	**34.89**	**2.12**	**0.40**	**4.23**	**0.76**	**0.46**	**0.13**	**0.12**	**0.15**	0.00	**0.13**	**0.06**

*P-solubilized = T-C; Where, T = P-solubilized in test, C = P-solubilized in control

**Per cent Siderophore Unit (%S.U.) = $\frac{Ar-As}{Ar} \times 100$; Where, Ar = Absorbance of reference at 630nm; As = Absorbance of test at 630nm.

***Enzyme activity index (EAI) = $\frac{A}{B}$; Where, A = Halozone diameter; B = colony diameter

Appendix 2.2 Effect of antagonistic bacterial isolates in controlling the capsicum bacterial wilt caused by *Ralstonia Solanacearum* (net house conditions)

Treatments	At seedling stage (6 days after transplantation)		At fruit set stage (six weeks after transplantation)		
	Disease incidence (%)	Biocontrol efficacy (%)	Disease incidence (%)	Disease severity (%)	Biocontrol efficacy (%)
Control	100.00 (89.96)*	0.00 (0.00)*	83.33 (72.34)*	79.17 (63.95)*	0.00 (0.00)*
NER_4	91.67 (81.15)	8.33 (8.81)	58.33 (49.85)	56.25 (48.61)	29.17 (28.87)
PAT_9	83.33 (72.34)	16.67 (17.63)	50.00 (44.98)	37.50 (37.48)	37.50 (33.74)
PAT_{13}	75.00 (67.47)	25.00 (22.49)	50.00 (44.98)	39.58 (38.68)	37.50 (33.74)
$SARA_9$	66.67(58.66)	33.33 (31.30)	33.33 (35.25)	25.00 (29.83)	58.33 (49.85)
ROH_6	58.33 (49.85)	41.67(40.12)	25.00 (26.44)	8.33 (14.41)	70.83 (61.09)
$CD_{0.05}$	**31.43 (27.64)**	**31.96 (27.84)**	**24.76 (19.86)**	**19.68 (13.60)**	**30.88 (26.79)**

***Figure in parenthesis () are arc-sin transformed values**

Appendix 2.3 Effect of varied levels of NP on microbiological soil parameters in capsicum (net house conditions)

Treatments	Rhizospheric population ($\times 10^6$ cfu/g soil)	Endophytic bacterial population ($\times 10^2$ cfu/g root)	Microbial biomass (mg MB-C/100g soil))
T_1 (100 per cent RDF	75.9	62.3	49.0
T_2 (80 per cent RDF)	69.3	47.5	54.0
T_3 (60 per cent RDF)	58.7	42.7	49.0
T_4 (40 per cent RDF)	53.4	38.4	46.8
T_5 (80 per cent RDNP + MAT_8)	114.2	94.1	55.1
T_6 (80 per cent RDNP + KAN_{11})	114.7	93.6	56.0
T_7 (80 per cent RDNP + JHA_6)	117.4	99.3	61.1
T_8(80 per cent RDNP + ROH_{14})	121.1	96.2	68.0
T_9(60 per cent RDNP + MAT_8)	81.6	62.8	52.4
T_{10}(60 per cent RDNP + KAN_{11})	74.7	57.1	54.7
T_{11}(60 per cent RDNP + JHA_6)	83.7	65.4	58.7
T_{12}(60 per cent RDNP + ROH_{14})	85.3	57.1	63.6
T_{13}(40 per cent RDNP + MAT_8)	57.5	50.5	54.0
T_{14}(40 per cent RDNP + KAN_{11})	60.7	51.8	56.2
T_{15}(40 per cent RDNP + JHA_6)	70.8	44.4	52.9
T_{16}(40 per cent RDNP + ROH_{14})	72.4	43.1	52.2

Appendix 2.4 Effect of varied levels of NP on microbiological soil parameters and available nutrients (field conditions)

Treatments	Rhizospheric count (cfu×10^7)			Endophytic count (cfu×10^2)			Microbial biomass (mg MB-C/100g soil)			Per cent increase in available NPK over initial		
	2014	2015	Mean	2014	2015	Mean	2014	2015	Mean	N	P	K
T_1 (100 per cent RDF)	88.9	90.5	89.7	54.7	55.8	55.2	45.33	46.58	45.96	10.04	18.47	32.21
T_2 (80 per cent RDNP + JHA_6)	123.1	127.1	125.1	71.3	73.7	72.5	100.78	103.05	101.91	11.73	29.29	15.41
T_3 (80 per cent RDNP + ROH_{14})	125.7	137.1	131.4	73.2	75.6	74.4	102.13	105.78	103.95	15.47	35.67	17.61
T_4 (80 per cent RDNP JHA_6 + ROH_{14})	137.1	144.3	140.7	76.3	81.0	78.6	107.92	109.40	108.66	19.55	43.03	19.98
T_5 (60 per cent RDNP + JHA_6)	106.8	111.1	109.0	63.9	66.0	65.0	92.47	93.96	93.22	-5.43	-1.87	3.85
T_6 (60 per cent RDNP + ROH_{14})	109.3	113.4	111.4	61.7	64.3	63.0	93.74	95.00	94.37	-4.98	4.93	5.37
T_7 (60 per cent RDNP JHA_6+ ROH_{14})	116.5	119.5	118.0	66.0	72.2	69.1	97.43	98.66	98.05	-1.77	9.15	7.31
$CD_{0.05}$	**10.7**	**10.7**	**6.81**	**8.6**	**8.7**	**5.28**	**5.46**	**4.70**	**3.44**	-	-	-

Appendix 2.5 Effect of varied levels of NP on soil enzymes in capsicum (field conditions)

Treatments	Dehydrogenase (µg TPF g^{-1}soil h^{-1})			Phosphatase (µg PNP g^{-1}soil h^{-1})			Phytase (Enzyme unit µmol ml^{-1} min^{-1})		
	2014	2015	Mean	2014	2015	Mean	2014	2015	Mean
T_1 (100 per cent RDF)	45.25	45.89	45.57	43.44	44.98	44.21	32.44	34.67	33.56
T_2 (80 per cent RDNP + JHA_6)	64.42	66.64	65.53	90.18	92.56	100.41	75.11	78.78	76.94
T_3 (80 per cent RDNP + ROH_{14})	70.98	72.73	71.86	99.37	101.45	91.37	116.43	117.77	112.10
T_4 (80 per cent RDNP JHA_6 + ROH_{14})	72.56	73.95	73.26	101.59	103.22	102.41	131.11	132.44	131.78
T_5 (60 per cent RDNP + JHA_6)	55.15	55.45	55.30	77.21	76.25	76.73	75.11	76.44	75.78
T_6 (60 per cent RDNP + ROH_{14})	56.32	56.90	56.61	78.85	78.35	78.60	89.78	91.11	90.44
T_7 (60 per cent RDNP JHA_6+ ROH_{14})	60.75	62.94	61.84	85.05	87.16	86.10	95.00	96.33	95.67
$CD_{0.05}$	**3.8**	**3.3**	**2.45**	**4.92**	**4.75**	**3.35**	**27.75**	**27.76**	**17.48**

Appendix 2.6 Effect of PGPR and chemical fertilizers on benefit cost ratio

Treatments	Yield of Capsicum ($KgHa^{-1}$)	Sale rate ($RsKg^{-1}$)	Total return	Total cost	Net profit	Benefit cost (B:C) ratio
T_1 (100 per cent RDF)	14266.63	25.00	356665.87	120921.40	235744.47	1.95
T_2 (80 per cent RDNP + JHA_6)	15656.05	25.00	391401.26	120358.46	271042.80	2.25
T_3 (80 per cent RDNP + ROH_{14})	16038.20	25.00	400954.93	120358.46	280596.47	2.33
T_4 (80 per cent RDNP JHA_6 + ROH_{14})	17936.68	25.00	448417.00	120358.46	328058.54	2.73
T_5 (60 per cent RDNP + JHA_6)	8665.06	25.00	216626.46	118571.64	98054.82	0.83
T_6 (60 per cent RDNP + ROH_{14})	9019.73	25.00	225493.27	118571.64	106921.63	0.90
T_7 (60 per cent RDNP JHA_6+ ROH_{14})	9444.61	25.00	236115.16	118571.64	117543.52	0.99

Appendix 2.7 Effect of PGPR on antioxidant enzymes (SOD, POD, CAT) of capsicum at varied levels of drought stress (net house conditions)

Treatments	SOD (U/gm fresh weight)	POD (U/gm fresh weight)	Catalase (U/gm fresh weight)
T_1: 100 per cent of field capacity	41.24	1.58	18.35
T_2: 80 per cent of field capacity	45.35	2.37	27.52
T_3: 80 per cent of field capacity + JHA_6	46.19	2.77	36.70
T_4: 80 per cent of field capacity + ROH_{14}	50.21	2.96	41.28
T_5: 60 per cent of field capacity	55.13	3.16	50.46
T_6: 60 per cent of field capacity + JHA_6	59.41	3.76	59.63
T_7: 60 per cent of field capacity + ROH_{14}	64.40	3.95	55.05
T_8: 40 per cent of field capacity	72.26	4.35	77.98
T_9: 40 per cent of field capacity + JHA_6	74.59	4.74	82.57
T_{10}: 40 per cent of field capacity + ROH_{14}	82.62	4.94	87.16
$CD_{0.05}$	**6.31**	**0.34**	**7.81**

Appendix 2.8 Effect of PGPR on nutrient (NPK) uptake of capsicum at varied levels of drought stress (net house conditions)

Treatments	Nitrogen uptake (mg/plant)	Phosphorus uptake (mg/plant)	Potassium uptake (mg/plant)
T_1: 100 per cent of field capacity	5.79	0.34	2.10
T_2: 80 per cent of field capacity	3.78	0.26	1.55
T_3: 80 per cent of field capacity + JHA_6	5.00	0.36	1.89
T_4: 80 per cent of field capacity + ROH_{14}	5.20	0.36	1.91
T_5: 60 per cent of field capacity	2.81	0.19	1.13
T_6: 60 per cent of field capacity + JHA_6	3.31	0.24	1.45
T_7: 60 per cent of field capacity + ROH_{14}	3.71	0.22	1.34
T_8: 40 per cent of field capacity	1.76	0.13	1.02
T_9: 40 per cent of field capacity + JHA_6	3.24	0.20	1.19
T_{10}: 40 per cent of field capacity + ROH_{14}	2.65	0.19	1.14
$CD_{0.05}$	**0.46**	**0.03**	**0.21**

APPENDIX- III

3.1 ANOVA of Table 3 (Screening of selected PGPR isolates for their multifarious PGP traits)

Source of Variation	df	Mean Sum of Squares		
		P-solubilization efficiency (%)	Siderophore production efficiency (%)	IAA production (µg/ml)
Treatment (T)	33	822.02	972.52	351.70
Error	68	110.65	95.16	0.79
Total	101			

3.2 ANOVA of Table 4 (Screening of selected PGPR isolates for antagonism against various test pathogens under laboratory conditions)

Source of Variation	df	Mean Sum of Squares					
		Fusarium oxysporum	*Rhizoctonia solani*	*Pythium sp.*	*Ralstonia solanacearum*	*Phytophthora capsici*	*Colletotrichum capsici*
Treatment (T)	33	1079.98	488.57	864.51	1439.24	941.72	995.15
Error	68	3.84	0.59	0.69	9.95	1.47	0.96
Total	101						

3.3 ANOVA of Appendix 2.1 (Quantitative characterization of plant growth promoting traits of selected bacterial isolates)

Source of Variation	df	Mean Sum of Squares											
		P-solubilization (µg/ml)	Viable count ($\times 10^7$ cfu/ml)	Final pH	Siderophore unit (%)	Viable count ($\times 10^7$ cfu/ml)	Final pH	Amylase activity	Pectinase activity	Protease activity	Cellulase activity	Lipase activity	Chitinase activity
Treatment (T)	14	3113.78	91.36	0.76	454.20	14.72	0.50	1.70	1.87	1.95	1.84	1.94	1.59
Error	30	437.89	1.62	0.06	6.42	0.21	0.07	0.01	0.01	0.01	0.01	0.01	0.001
Total	44												

3.4 ANOVA of Table 7 (Evaluation of antibacterial metabolite from selected antagonistic bacterial isolates against *Ralstonia Solanacearum*)

Source of Variation	df	Mean Sum of Squares				
		Per cent growth inhibition	Inhibition zone at conc. 0.125% (v/v) in mm	Inhibition zone at conc. 0.25% (v/v) in mm	Inhibition zone at conc. 0.375% (v/v) in mm	Inhibition zone at conc. 0.5% (v/v) in mm
Treatment	9	1290.74	14.90	21.26	27.71	42.92
Error	20	60.60	0.17	0.33	0.47	0.33
Total	29					

3.5 ANOVA of Table 8 (Effect of antagonistic bacterial isolates on plant growth parameters after the infestation by *Ralstonia Solanacearum* at fruit set stage)

Source of Variation	df	Mean Sum of Squares					
		Germination (%)	Vigour index	Plant biomass (g)	N content (%)	P content (%)	K content (%)
Treatment	5	323.002	87255.7	37.41	0.05	0.001	0.10
Error	18	10.73	1526.64	2.66	0.02	0.003	0.008
Total	23						

3.6 ANOVA of Appendix 2.2 Effect of antagonistic bacterial isolates in controlling the capsicum bacterial wilt caused by *Ralstonia Solanacearum*.

Source of Variation	df	Mean Sum of Squares				
		At seedling stage		At fruit set stage		
		Disease incidence (%)	Biocontrol efficacy (%)	Disease incidence (%)	Disease severity (%)	Biocontrol efficacy (%)
Treatment	5	972.22	972.22	966.45	1124.97	1732.67
Error	18	447.54	447.54	178.68	83.86	325.42
Total	23					

3.7 ANOVA of Table 9 (Effect of bacterial inoculums on capsicum seedling growth parameters (net house conditions)

Source of Variation	df	Mean Sum of Squares					
		Germination (%)	Vigour index	Shoot length (cm)	Shoot biomass (mg/plant)	Root length (cm)	Root biomass (mg/plant)
Treatment	6	268.44	73355.69	6.76	576.49	34.50	34.50
Error	14	9.70	2790.73	1.38	141.40	0.30	93.16
Total	20						

3.8 ANOVA of Table 10 (Effect of PGPR and chemical fertilizers on capsicum plant parameters and yield (net house conditions)

Source of Variation	df	Mean Sum of Squares								
		Shoot length (cm)	Shoot biomass (g)	Root length (cm)	Root biomass (g)	Fruit length (cm)	Fruit breadth (cm)	Fruit weight (g)	Fruit number	Fruit yield ($Kgplant^{-1}$)
Treatment (T)	15	306.86	79.75	49.77	0.31	6.59	27.17	523.86	9.78	0.22
Error	32	11.23	2.92	1.82	0.01	0.24	0.99	21.80	0.36	0.01
Total	47									

3.9 ANOVA of Table 11 (Effect of varied levels of NP on NPK content and their uptake in capsicum under (net house conditions)

Source of Variation	df	Mean Sum of Squares					
		Nitrogen (%)	Phosphorus (%)	Potassium (%)	NU (mg/plant)	PU (mg/plant)	KU (mg/plant)
Treatment	15	2.05	0.018	1.071	13.073	0.145	5.932
Error	32	0.08	0.0007	0.053	0.289	0.003	0.167
Total	47						

3.10 ANOVA of Table 12 (Effect of PGPR and chemical fertilizers on soil physico-chemical characteristics (net house conditions)

Source of Variation	df	Mean Sum of Squares		
		Available N (Kg ha^{-1})	Available P (Kg ha^{-1})	Available K (Kg ha^{-1})
Treatment (T)	15	5539.30	194.12	2624.76
Error	32	403.597	13.48	90.31
Total	47			

3.11 ANOVA of Table 13 (.Effect of PGPR and chemical fertilizers on plant growth parameters and yield of capsicum (field conditions))

Source of Variation	df	Mean Sum of Squares				
		Plant height (cm)	Plant biomass (q ha^{-1})	No. of fruits (fruit $plant^{-1}$)	Fruit weight (g)	Yield (t ha^{-1})
Treatment (T)	6	267.36	130.84	2.33	4140.93	57.76
Interval (I)	1	19.61	10.70	0.19	17.93	2.02
T × I	6	3.23	6.90	0.01	1.60	0.09
Pooled error	28	6.09	3.08	0.06	3.59	0.38
Total	41					

3.12 ANOVA of Table 14 (Effect of PGPR and chemical fertilizers on total nutrient content (NPK) and their uptake in capsicum (field conditions))

Source of Variation	df	Mean Sum of Squares					
		Nitrogen (%)	Phosphorus (%)	Potassium (%)	NU (Kg ha^{-1})	PU (Kg ha^{-1})	KU (Kg ha^{-1})
Treatment (T)	6	0.45	0.0045	0.99	5788.38	38.32	4669.28
Interval (I)	1	0.04	0.003	0.02	104.45	4.98	400.46
T × I	6	0.01	0.0001	0.11	91.60	0.17	309.58
Pooled error	28	0.04	0.0006	0.03	73.40	2.28	74.09
Total	41						

3.13 ANOVA of Table 15 (Effect of PGPR and chemical fertilizers on soil physic-chemical characteristics and available nutrient (NPK) contents (field conditions))

Source of Variation	df	Mean Sum of Squares		
		Available N (Kg ha^{-1})	Available P (Kg ha^{-1})	Available K (Kg ha^{-1})
Treatment (T)	6	5285.86	210.30	2774.99
Interval (I)	1	377.13	0.65	555.04
T × I	6	236.37	4.47	55.53
Pooled error	28	242.52	5.12	207.05
Total	41			

3.13 ANOVA of Appendix 2.4 (Effect of varied levels of NP on microbiological soil parameters and available nutrients (field conditions))

Source of Variation	df	Mean Sum of Squares		
		Rhizospheric count (cfu×10^7)	Endophytic count (cfu×10^2)	Microbial biomass (mg MB-C/100g soil)
Treatment (T)	6	1673.93	371.49	2681.84
Interval (I)	1	271.57	99.05	34.17
T × I	6	15.90	4.60	1.19
Pooled error	28	33.17	19.91	8.45
Total	41			

3.14 ANOVA of Appendix 2.5 (Effect of varied levels of NP on microbiological soil parameters and soil enzymes in capsicum (field conditions))

Source of Variation	df	Mean Sum of Squares		
		Dehydrogenase (µg TPF g^{-1}soil h^{-1})	Phosphatase (µg PNP g^{-1}soil h^{-1})	Phytase (Enzyme unit µmol ml^{-1} min^{-1})
Treatment (T)	6	577.94	2322.93	6059.90
Interval (I)	1	17.71	14.69	33.78
T × I	6	0.95	2.71	1.19
Pooled error	28	4.27	8.01	218.69
Total	41			

3.15 ANOVA of Table 16 (Influence of PGPR isolates on growth parameters and nutrient content of capsicum under varied levels of drought stress.)

Source of Variation	df	Mean Sum of Squares										
		Shoot length (cm)	Root length (cm)	Shoot biomass (g/plant)	Root biomass (g/plant)	Leaf area (cm^2)	Relative water content (%)	Total chlorophyll content (g/kg fresh leaves)	Total soluble protein (mg/g fresh leaves)	Nitrogen (%)	Phosphorus (%)	Potassium (%)
Treatment (T)	9	85.26	24.19	24.19	0.11	72.48	545.03	0.08	0.008	1.57	0.005	0.070
Error	20	1.41	0.37	0.37	0.001	0.50	189.55	0.002	0.0002	0.11	0.0001	0.004
Total												

3.16 ANOVA of Appendix 2.7 (Effect of PGPR on antioxidant enzymes (SOD, POD, CAT) of capsicum at varied levels of drought stress (net house conditions))

Source of Variation	df	Mean Sum of Squares		
		SOD (U/gm fresh weight)	POD (U/gm fresh weight)	CAT (U/gm fresh weight)
Treatment (T)	9	588.02	3.50	1656.01
Error	20	13.75	0.04	21.04
Total	29			

3.17 ANOVA of Appendix 2.8 (Effect of PGPR on nutrient (NPK) uptake of capsicum at varied levels of drought stress (net house conditions))

Source of Variation	df	Mean Sum of Squares		
		NU (mg/plant)	PU (mg/plant)	KU (mg/plant)
Treatment (T)	9	4.77	0.020	0.43
Error	20	0.07	0.0004	0.15
Total	29			

APPENDIX- IV

Cost of cultivation of capsicum

A. Fixed cost for all the treatments per hectare		
Nursery:		
1.	Cost of seeds 800g @ Rs 25000/Kg	Rs. 20000/-
2.	Soil fumigation formalin (1:7) 1L/bed @ Rs 125/L, for 10 beds	Rs. 1250/-
3.	FYM-25 Kg/bed, for 10 beds 250Kg@ Rs 1/Kg	Rs. 250/-
4.	PGPR 1 lt. @ Rs 50/lt	Rs. 50/-
5.	Plant protection	Rs. 100/-
6.	Labour charges @ Rs 230/day, for 25 labour	Rs. 5750/-
Preparatory tillage:		
1.	4 ploughings @ Rs 1000/ploughing	Rs. 4000/-
2.	3 plankings @ Rs 150/planking	Rs. 450/-
3.	Beds and channels making labour 40@ Rs 230/day	Rs. 9200/-
Transplanting:		
1.	25 labour @ Rs 230/day	Rs. 5750/-
2.	Irrigation 2 labours/day for 7 days or 14 labour @ Rs 230/day	Rs. 3220/-
Manures and fertilizer Application:		
1.	FYM 20-25 t/ha @ Rs 1/Kg	Rs. 25000/-
2.	15 labours for application @ Rs 230/day	Rs. 3450/-
Cultural operations:		
1.	Weeding, Hoeing, gap filling 40 labours @ Rs 230/day	Rs. 9200/-
2.	6 irrigations	Rs. 2760/-
Plant protection:		
1.	5 chemical spray	Rs 4000/-
2.	For 5 chemical spray 20 labours @ Rs 230/day	Rs 4600/-
Miscellaneous:		Rs 8000/-
Harvesting:		
	30 labour @ Rs 230/day	Rs 6900/-
Total Fixed cost		Rs 113930/-

B. Variable cost of cultivation of different doses of chemical fertilizers		
100% RDF of NPK		
Urea 217 Kg/ha @ Rs 4.70/Kg	=	1019.9
SSP 475 Kg/ha @ Rs 10.22/Kg	=	4854.5
MOP 90 Kg/ha @ Rs 11.30/Kg	=	1017
PGPR 2 lt. @ Rs 50/lt	=	100
Total	=	6991.4
PGPR + 80% RD of N and P+ 100% K		
Urea 173.6 Kg/ha @ Rs 4.70/Kg	=	815.92
SSP 380 Kg/ha @ Rs 10.22/Kg	=	3883.6
MOP 90 Kg/ha @ Rs 11.30/Kg	=	1017
PGPR 2 lt. @ Rs 50/lt	=	100
Total	=	6428.46
PGPR + 60% RD of N and P+ 100% K		
Urea 130.2 Kg/ha @ Rs 4.70/Kg	=	611.94
SSP 285 Kg/ha @ Rs 10.22/Kg	=	2912.7
MOP 90 Kg/ha @ Rs 11.30/Kg	=	1017
PGPR 2 lt. @ Rs 50/lt	=	100
Total	=	4641.64

C. Total cost of cultivation of different doses of chemical fertilizers		
Treatment	**Fixed cost + variable cost**	**Total cost of cultivation (Rs)**
100% RDF of NPK	113930 + 6991.4	120921
PGPR + 80% RD of N and P+ 100% K	113930 + 6428.6	120358
PGPR + 60% RD of N and P+ 100% K	113930 + 4641.64	118572

D. Benefit Cost ratio		
Yield of Capsicum	=	18.0 t/ha
Sale rate	=	Rs 25/Kg
Total return	=	Rs 448417 /-
Net Profit	=	Total return-Total cost
	=	448417-120358
	=	Rs 328058/-
Benefit Cost (B:C) Ratio	**=**	**Net Profit/ Total Cost**
	=	328058/120358
	=	2.73

BRIEF RESUME OF STUDENT

Name	**:**	**Shweta Gupta**
Father's Name	**:**	Sh. Desh Raj Gupta
Mother's Name	**:**	Smt. Ranjana Gupta
Date of Birth	**:**	07.04.1988
Permanent address	**:**	Murad Cottage near Ganju Niwas, Sanjauli, Shimla-171006

Educational qualifications:

Certificate/Degree	Month & Year	School	Board/University	Marks (%)	Division
Matriculation	04th June, 2004	Monal Public Senior Secondary School, Sanjauli	Himachal Pradesh Board of School Education	75.57	First
10+2	14thApril., 2006	St. Beeds College Shimla	Himachal Pradesh Board of School Education	71.20	First
B.Sc.	7th July, 2009	Hans Raj Mahila Maha Vidhyalaya, Jalandhar	G.N.D.U. Amritsar	79.05	First
M.Sc.	19th Jan., 2012	Dept. of Basic Sciences, College of Forestry	Dr. YS Parmar UHF, Nauni, Solan (HP)	80.60	First

Title of Thesis in M.Sc.: Studies on selected plant growth promoting rhizobacteria on growth and yield of capsicum (*Capsicum annuum* L.).

Scholarship/Stipend/Fellowship/Awards/Any other Distinction

- DST INSPIRE Scholarship for Ph.D. programme w.e.f 1st Feb. 2013.
- Best poster presentation award in the 2nd International conference on "Bio-resource and stress management" at PJTSAU, Rajendranagar, Hyderabad.
- First position in poster position "National symposium on modern agro-technologies for nutritional security and health".

Publications:
Research papers (in peered journals): 4

Scientific popular articles: 4

Others: 1

Visits abroad along with duration and purpose of visit: NA

(Shweta Gupta)