Application of Plant Growth Promoting Rhizobacteria in increasing the solubility and Bio-availability of Phosphorus

Submitted to

Lovely Professional University, Phagwara

IN FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF

Masters of Science

(M.Sc. Botany-Honours)

Submitted By

Pooja Claire

Reg. no. 11308791

Under Supervision of

Dr. Leena Parihar

Department of Biosciences & Biotechnology

CERTIFICATE

This is to certify that **Pooja Claire** bearing registration no. **11308791** has completed M.Sc. Dissertation titled ñApplication of Plant Growth Promoting Rhizobacteria in increasing solubility and Bio-availability of Phosphorusò under my guidance and supervision. To the best of my knowledge, the present work is her original investigation and study. No part of this dissertation has ever been submitted for any other degree at any university.

The dissertation is fit for submission and fulfillment of condition for the award of **Masters of Science in Botany- Honours.**

Signature and Name of Research Supervisor: Dr. Leena Parihar

Designation:

Lovely Faculty of Biosciences

Lovely Professional University, Phagwara

Date:

DECLARATION

I **Pooja Claire,** student of **M.Sc. Botany- Honours** in the department of Biosciences in Lovely Professional University, Phagwara, hereby declare that all the knowledge furnished in this dissertation report is based on my own intensive research and is genuine.

This dissertation report to the best part of my knowledge, contain part of my work which has not been submitted for the award of my degree either of this university or other university without proper citation.

Supervisor signature:

Signature and Name of the Student: Pooja Claire

Reg. no. 11308791

Lovely Professional University, Phagwara

Date:

ACKNOWLEDGEMENT

On the very outset of this report, I would like to extend my sincere & heartfelt obligation towards all the personages who have helped me in this endeavor. Without their active guidance, help, cooperation & encouragement, I would not have made headway in the project. I have acquired the new field of knowledge and developments from this work.

I am ineffably indebted to **Dr. Leena Parihar madam** for her valuable advice, splendid supervision and constant patience through which this work was able to take shape in which it has been presented. It was her valuable discussions and endless endeavor through which I have gained a lot.

I would like to extend my special thank you to **Dr. Ashish Sharma** who helped me in this project and gave me attention in his busy schedule.

I am extremely thankful and pay my gratitude to my faculty **of Biosciences** for valuable guidance and support on working of this project and constant supervision as well as for providing necessary information regarding the project & also for their support in my project.
I extend my gratitude to **Lovely Professional University, Phagwara** for giving me this opportunity.

I would like to thank all members of my Project Lab who helped me in the proceedings of my work.

I also acknowledge with a deep sense of reverence, my gratitude towards my parents and member of my family, who has always supported me morally as well as economically.

My thanks and appreciations also go to my friends in developing the project and people who have willingly helped me out with their abilities.

Finally, I wish to extend my warm thanks to everybody involved directly or indirectly in my work.

Thank you
Pooja Claire

ABSTRACT

The present study has been undertaken to study the effect of Plant Growth Promoting Rhizobacteria in increasing the solubility of Phosphorus. Phosphorus is the essential nutrient required by plants but due to soil fixation reactions P is considered major immobile compound and responsible for deficiency symptoms. Soil sample from *Aloe vera* plant was taken to isolate Phosphate solubilizing bacteria (PSB). Four bacterial isolates were selected i.e. B1, B2, B3 and B4 to study the plant growth promotion and Phosphorus uptake ability on Tomato plant (*Lycopersicon esculentum*). All the four bacterial isolates were able to solubilise P in the pikovskaya liquid media. Out of four bacterial isolates B4 bacteria solubilised 7.32 ppm P and mix consortium solubilised 7.59 ppm P. These gave best result for P solubilization ability when compared with standard bacteria i.e. *Pseudomonas putida*. All the four bacterial isolates shown IAA production when treated with tryptophan, but no IAA production was seen in the isolates containing no tryptophan. Mix bacterial consortium produced 33.6 mg/ml of IAA. The single bacterial isolates produced IAA in the range between 22-28 mg/ml. Inoculation of the above bacterial isolates in various treatments gave positive results for increased root and shoot and also increased relative water content. Mix consortium increased root length up to 42 cm. These bacteria have also helped in uptake of phosphorus. It was seen that mix bacterial consortium gave best results for increased plant growth (root and shoot length) and P uptake ability. Mix consortium containing four bacteria having ability to promote plant growth and P uptake can be used as bio-fertilizers in the agricultural land.

TABLE OF CONTENT

LIST OF FIGURES

LIST OF GRAPHS

LIST OF TABLES

Chapter – 1

INTRODUCTION

INTRODUCTION

1.1 Impact of chemical fertilizers

Soil is the basis for agricultural productions as it inhabitants beneficial micro-flora. Soil contains moisture and nutrients for plant growth and development. Therefore soil has played an important role in food production and meets the growing population needs. Increasing population in world has set an alarming situation to increase the food productivity to meet the growing population needs. In order to meet the food requirements agricultural land has been continuously supplied with chemical fertilizers to increase the yield and quality of food crops. Plant nutrition is the most important factors considered for increasing the productivity of agricultural products. Soil nutrients also help in affecting plant yield and quality. Therefore, farmers are continuously using chemical fertilizers to increase yield and increase their economic status (**Savci**, 2012).

Chemical based fertilizers are responsible for soil degradation. Changing climate and environmental factors including soil degradation has led to a challenging situation in increasing the food productivity. Inputs of chemical fertilizers more than required has led to the contamination of land with heavy metals and organic pollutants. As a result, heavy metal has been entered in food chain and deteriorated the food quality and nutrition level. The increasing indeterminate methods to increase the productivity have led to land degradation most likely agricultural land degradation. Most losses under land or soil degradation are soil alkalinity and salinity, water logging, soil erosion, nutritional loss of soil and ground water contamination. Use of chemical fertilizer in land is the major cause of losing soil fertility and provides soil and land degradation. Due to continuous use of fertilizers, excess fertilizers like Nitrogen and Phosphorus fertilizers resides in soil for long time then consequently led to water contamination with chemicals (**Banerjee and Sanyal,** 2013).

1.2 Plant Functions of soil nutrients

Plants require 16 essential mineral nutrients, out of which three (Carbon, Hydrogen and Oxygen) are available from external environment from air, water and other 13 are present in soil as both organic and inorganic forms (**Tucker,** 1999). These soil nutrients are absorbed by plants root and translocated to various plant parts. Soil mineral nutrients are of three types: Primary nutrients, secondary nutrients and micronutrients. These are essential to plants for

various physiological activities, leading to plant growth and development. Essential soil mineral nutrients are:

1.2.1 Macronutrients:

Macronutrients are required in large quantities. These are categorized as Primary nutrients and secondary nutrients. Primary nutrients: Nitrogen (N), Phosphorus (P) and Potassium (K) are needed in large amounts then secondary nutrients: Calcium (Ca), Magnesium (Mg) and Sulfur (S). Every plant requires optimum amounts of soil mineral nutrients (**Tucker,** 1999). **Nitrogen** is essential for synthesis of various amino-acids as it combines with C, O, H and S. It is also essential in synthesis of chlorophyll, vitamins and enzymes. It improves dry matter content in leafy vegetables and cereals. **Phosphorus** is required in photosynthesis and respiration. It is major component of energy storage and transfer processes in the form of ADP and ATP (adenosine di- and tri phosphate). It is major component of genetic composition ï DNA and RNA. P required in young stage of plant and induces root growth, flower and fruit development. It makes the plant susceptible for diseases and increases crop yield (**Uchida,** 2000). **Potassium** plays major role in Plantõs resistance to various stress conditions like drought. Potassium help in translocation of iron (alleviating iron toxicity) Involved in water use efficiency, maintaining turgor pressure and opening and closing of Stomata (**Cakmak,** 2005). **Calcium** has a role in cell wall composition, act as detoxifying agent and activator of several enzymes. Ca^{++} act as second messenger in plant growth and development and also maintains cell rigidity (**Helper,** 2005). **Magnesium** acts as central element in chlorophyll molecule. It is essential element in regulation plant growth and development since it is helpful in carbohydrate translocation and various other essential elements like potassium (**Gransee and Führs,** 2012)**.**

1.2.2 Micronutrients

Micronutrients required in traces to the plants, known as Trace Elements. **Boron** plays important role in various plant metabolic activities. Boron helps in maintaining cell-wall extensibility. It is involved in pollen tube growth and germination (**Ganie *et al*.,** 2013). **Molybdenum** is important activator of nitrogen-fixing enzymes (nitrate reductase). It is also involved in production of phytohormone - IAA (**Kaiser *et al*.,** 2005). **Iron** is important in photosynthesis electron transport chain and respiration. It is important molecule in heme

enzyme system **(Connolly and Guerinot,** 2002). **Zinc** is important activator molecule of various metabolic activities of plants. Needed in production of phytohormone auxin and participate in photosynthesis reactions. Increases water uptake efficiency. **Manganese** involved in production of amino acids and chlorophyll, essential in N metabolism. **Chlorine** involved in photosynthesis and respiration, maintains turgor pressure in guard cells (**IFAS,** 2002).

1.3 Phosphate solubility (Phosphorus - The Soul Compound for Plants)

Out of three most essential macro-nutrients (N, P, K), phosphorus is most growth- limiting essential nutrient important for plant growth and development. Phosphorus is mostly required for the transfer of energy in the form ATP in the cells and main constituent of genetic material: DNA and RNA, helpful in ripening and maturing of flower. Thus deficiency of P may lead to severe crop deficiency. Like all other mineral nutrients, phosphorus exists in soil, water, and living organisms but phosphate does not exists in elemental form as elemental P is highly reactive and combine with oxygen. Phosphorus exists as phosphate ions in which main compound P is surrounded by four oxygen atoms, chemically PO_4^{3-} , Orthophosphate. Organic phosphate from living organisms returns to the soil as inorganic phosphate. Besides all the characteristics, Phosphorus exhibit a unique feature as it is most immobile compound. It is not readily available to the plants. Phosphate in soil exists in three forms depending upon their solubility and availability to the plants. First, Solution P pool has very small phosphate content in the form of orthophosphate, the only form considered somewhat mobile and taken up by plants. Second, Active P pool available in water surrounding soil i.e. soil solution. As soon as the P from solution pool replenish, the P from active pool comes to the solution pool. P compound in active pool is thousands of pounds per acre of land. Active P pool is the main source of available P for plants. Third, fixed P pool is the most insoluble phosphate pool in the soil exists as solid crystalline form. Naturally occurring P in soil exists in fewer amounts. Phosphate fertilization is agriculturally adopted method of meeting the phosphate requirements for increasing plant yield and productivity (**UMN,** 2009).

After the application of phosphate fertilizers in the agricultural soils soon it becomes insoluble in the soil itself due to various reactions took place within the soil. Phosphorus molecule reacts to make complex compounds with the calcium, aluminium and iron and

absorbed to the soil particles. Phosphate existing as anionic form reacts with cationic molecules and gets fixed within the soil and stay as solid compounds. The extent of bond formation between these complex molecules depends upon the soil pH and texture. More the phosphate fertilizer added, more is the fixed compounds of phosphate (**FAO,** 2008).

When the phosphate fertilizers are added in agricultural land it soon gets turned into insoluble form due to phosphorus fixation reactions. Thus, its quantity decreases in soil and only small proportion is available for plant root uptake. Also some of the soluble components of phosphorus fertilizers leaches into water bodies due to the phenomenon of leaching and eutrophication and these will respond for algal and fungal growth in water reservoirs and responsible for water pollution. Also further frequent use of chemical fertilizers lower crop yield and led to soil erosion. Thus chemical farming practices have mismanaged the water reservoirs, degrade soil productivity and subvert ecology. All these above facts led the researchers to find some alternate methods to eliminate deleterious effects of chemical farming and also to meet the required quantity of phosphorus to the plants.

1.4 Bio-available Phosphorus

Commercial available Phosphate fertilizer prepared from Rock phosphate. The reserves of Rock Phosphate are non-renewable and is it estimated that 90% of rock phosphate has been extracted for agricultural practices. Considering this data rock phosphate reserves will get depleted within 50-100 years. Although continuous use of rock phosphate reserves had led to the depletion of quality of rock phosphate, as it contain Cadmium compound which is toxic to plants as well and living beings. The concentrations of orthophosphate in rock phosphate are continuously decreasing due to regular mining of these reserves (**Cordell,** 2008). Thus it is concluded that chemically available phosphorus in the form of fertilizers had been leading to deleterious effect on the soil fertility.

It is well known that soil micro-flora exhibit some beneficial characters for increasing the plant growth and yield. These beneficial micro-floras are collectively known as Plant Growth Promoting Rhizobacteria (PGPR). PGPRs have the efficiency to solubilise organic and inorganic forms of phosphorus and make it mobile for the uptake by plant roots. Increasing the solubility of phosphorus in the soil with the application of soil micro-flora phosphorus can be made BIO-AVAILABLE to the plants. In other words use of PGPRs to make

solubilise the phosphorus and make it available to the plant roots is known as Bio-available Phosphorus.

1.5 Uses of Tomato (*Lycopersicon esculentum*)

Tomato plant is second most cultivated fruit bearing crop after potato, belonging to family Solanaceae. The juicy fruit of tomato used as raw, in salads, juice; sauce (**Akintunde and Dairo,** 2012).

The root system of tomato plant is Tap root which grow up to 50 cm of the soil depth. The main roots i.e. tap root produces dense adventitious root system. The stem of tomato is hairy, solid and attains the height of maximum 2-3 meters. Flowers of tomato are bisexual. Both stamen and petals are 6 in number and yellow in color. Ovary is superior. Fruit is fleshy berry, oblong to globular in shape. Unripe fruit are green in color and ripe fruits are red to orange in color. Seeds are many and kidney shaped (**Naika *et al.*,** 2005).

Tomato is used in almost all vegetables cookingôs as an essential ingredient and flavoring agent. Tomato contains many nutritional qualities like high water content, calcium and lycopene (good for human health). It is considered cheap source of minerals and vitamin A, C and E. Tomato is frequently grown crop in Nigeria, since it met the demand of Vitamin A deficiency in infants of Nigeria and met other nutritional requirements in the year 1995. According to a study, Tomatoes are grown in 85% of gardens each year (**Adejumo,** 2010).

It is most widely grown horticultural crop with worldwide production of 120 million metric tons (**FAO,** 2007). Phosphorus requirements can be met by using these commercial grown vegetable having optimum concentrations of phosphate.

In this present study Tomato (*Lycopersicon esculentum*) plant has been used to study the Phosphorus uptake of the plant with the application of Plant Growth Promoting Rhizobacteria.

Keeping above facts in mind present study is conducted on the application of PGPRs for checking the solubility and bio-availabilty of Phosphorus.

TERMINOLOGY

PGPR	Plant Growth Promoting Rhizobacteria
P	Phosphorus
PSB	Phosphate Solubilising bacteria
Ppm	Parts per million
IAA	Indole Acetic Acid
ACC	1-aminocyclopropane- 1-carboxylate
Cd	Cadmium
Conc.	Concentration
Rpm	Revolutions per minutes
FW	Fresh Weight
TW	Turgid Weight
DW	Dry Weight
RWC	Relative water content
KH_2PO_4	Potassium di-hydrogen orthophosphate
$FeCl_3$	Ferric Chloride

CHAPTER- II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

2.1 Need to think on Chemical Fertilizers

Land degradation, the loss in soil nutritional levels due to human activities is the major point of concern in 21st century. Inappropriate use of chemicals in land is one of the main reasons for land degradation.

It was estimated that approximately 40-70% of agricultural production had been reduced to land degradation (**IFPRI,** 2000). ñThere was about 40-42 per hectare per year tons of total land productivity lost due to heavy land degradation in Ethiopia. It was found that loss of food productivity due to land degradation in the years 2000-2010 is about $7 billion in Ethiopiaò (**Baylis *et al*.,** 2012).

ñUnderestimated use of natural resources concluded to be the reason for soil salinization and consequently lead to 2,500-5000 km_2 of heavy loss of production. Land degradation has also resulted into soil nutrient depletion estimated in sub-Saharan Africa where soil nutrient depletion was found to be 22kg nitrogen, 3kg phosphorus and 15 kg potassium per ha and economic losses due to soil erosion and degradation in nutritional quality in South Asia was found to be US$600 million to US$1,200 millionò (**GRID-arindal,** 2014).

In the era of green revolution, soil nutritional levels are of utmost importance for increasing the yield of crops. Many studies have been done to explore the nutritional levels of soil in different geographical areas. These studies will be helpful to grow crops accordingly. The soil samples from Varanasi District took for studying the nutritional quality, it was found that levels of nitrogen, potassium, and phosphorus were low and soil was deficient in sulphur content (**Singh and Mishra,** 2012).

Soil salinity and toxicity are noted to be increased due to excess mineral nutrients in the soil. It was found that 40% of soil is acidic and toxic due to ï Al, Mn, Na and B (**White and Brown,** 2010). Soil mineral nutrient concentration has been found in Senapati District of Manipur. It was concluded that soil contain optimum concentration of iron, zinc, copper, manganese and boron mineral nutrients. The crops grown in this agricultural land are not deficient of discussed nutrients (**Athokpam *et al*.,** 2013). The soil samples from Tittakudi taluk of Tamilnadu state were tested for soil nutrients; results concluded that there was

decrease in the concentration of iron in the soils and led to the deficiency symptoms in the crop plants. There was a continuous decline in macro-nutrients amounts in the various soil samples of same region leading to various deficiency symptoms in the plants (**Srinivasan *et al.,*** 2013).

The concentration of Cadmium (Cd) in soil is increasing and harmful to living matter. As the cadmium is highly soluble and easily up taken by roots it is accumulating in plant tissues and other parts of plants, and entering into the food chain. In a study it was found that Cd accumulation in acidic soil is high than other soil pH. So by manipulating soil pH Cd uptake by plant roots can be managed (**Sarwar *et al.,*** 2010).

It was concluded that by correlating soil ecology and macronutrients soil fertility could be managed. Soil pH and electrical conductivity were found to be increasing the phosphorus content in the soil i.e. by increasing the soil pH (**Patel *et al.,*** 2014).

In case of calcareous soils with high pH, saline conditions, improper irrigation, drought conditions mostly in Asian continent it was estimated that soils micronutrients are declining and decreasing the crop yield. With the application of micronutrients 50% of crop yield and macronutrient use efficiency was increased in case of *Triticum durum* wheat (**Malakouti,** 2008).

In the earlier studies researchers found the interaction between the macronutrient uptake rates by the two different varieties of *Amaranthus* in two different seasons simultaneously. It was estimated that in the spring season *Amaranthus hypochondriacus Var. Cim* showed high N uptake and *Amaranthus hypochondriacus Var. Kharkofski* showed high K and Mg nutrient uptake and same genus showed maximum P and Ca uptake in summer season. It was concluded that different plants showed different nutrient uptake in different geological conditions (**Haghighi *et al.,*** *2012*).

India is the primary producer and consumer of chemical based fertilizers since the period of green revolution. Farmers use indeterminate amounts of chemicals their land to increase the plant growth and yield to make more money and thus increasing their economic status. It has been studied that 87 per cent of respondents feel that it is the chemical in their diet (from agricultural land) which lead to serious health issues like headache, indigestion, discomfort,

cough, nausea, eye disorders. Ecologically, chemical farming has lead to the decrease in the number of beneficial micro-organisms and made the soil poisonous by accumulating heavy metals and lost soil fertility (**Nishanthlalu and Sharon,** 2014).

Heavy use of chemical fertilizers leads to the decline in the population of soil micro-organisms. Although continuous use of chemical fertilizers works for few years but it might lead to express the deleterious effects on the beneficial soil micro-organisms. Thus indirectly soil nutrients reduce (**Aktar,** 2009). It was observed that Chemical fertilizer use in agricultural land raises plant growth and yield from successive years of application but it is resulted that use of chemical fertilizer and pesticides had increased the incidents of cancer in states where chemical fertilizers are used in many number especially in Punjab. Malwa region of Punjab accounts for 177 kg per hectare chemical fertilizer usage, which is above the margin (**Anand,** 2012).

In a recent study cancer patients in Malwa region of Punjab has been increased (1089 million per year) as compared to average national cancer patients (800 million per year). Hair sample was taken to study the metal toxicity of the cancer patients and comparison with the normal people of Malwa region. It was found that concentrations of Uranium, Barium, Manganese and Lead were high in cancer patients as compared to healthy people. ñUranium concentration in cancer patient (0.63µg U/g) was six times as high as healthy person (0.1µg U/g)ò (**Blaurock-Busch *et al.*,** 2014).

Phosphate fertilizers contain Cadmium metal as a contaminant because phosphate fertilizer extraction from rock sediments contains cadmium metal more than igneous rocks. Cd accumulate in soil and readily up taken by plant roots and thus entering in the food chain. Cd is toxic to humanôs health- kidney dysfunction, bone density reduction (**Grant,** 2011).

In India worldôs total phosphate rock production to be commercialised as phosphate fertilizer is 1,623 tons in year 1999. The production of same increased now due to continuous usage of phosphate fertilizer. The presence of metal contaminant was estimated and it was found that except Cd there were fluorides, mercury, lead and uranium present in the final product of phosphate fertilizer. In Asia ótai-Itai desease have been discovered due to presence of Cd in the human diet and many other ill effects of these metal contaminants were seen (**Dissanayake and Chandrajith,** 2009).

Phosphate fertilizers prepared from rock sediments reserves are declining and are going to replenish within a century or may be earlier if there is continuous usage of phosphate fertilizers in the agricultural land. Thus there is need to establish a method to replace the usage of phosphate fertilizer.

Phosphate fertilizer when applied in crops of plants soon get fixed in the soil and also some soluble portion of phosphate fertilizer leaches out in water reservoirs due to eutrophication and leaching phenomenon. It is mentioned that only 1% of the total soil P (400-4,000 kg P/ha in the top 30 cm) is taken up by the living plant biomass (10-30 kg P/ha), which in turn is said to be least quantity available to the plants (**Sharma *et al.*,** 2013). It is estimated that 30-40 ppm (parts per million) of phosphate is optimum for the growth of crop plants. If the concentration in soil below 30 ppm then additional phosphate compounds are added (**Douglas and durst,** 2002). Thus chemical based fertilizer usage is hazardous to living beings and damaging soil texture also.

Considering all the problems with regular usage of phosphate fertilizer and its cost effectiveness has led to search for new alternate ideas which are environment ï friendly, less costly and meet the phosphorus requirements to the crop plants.

2.2 Plant Growth Promoting Rhizobacteria

In 1904 German Scientists Hiltnes proposed the term órhizosphereô, which is the area around plant root actively colonized by beneficial bacteria fungi and actinomycetes. Plant root exudate is responsible to colonize beneficial micro flora in rhizosphere (**Maheshwar and Sathiyavani,** 2012). Soil pH, temperature, moisture, age and condition of plant are main factors which are responsible for bacterial colonization. These groups of beneficial micro-organisms are responsible to increase plant growth and yield and known as Plant Growth Promoting Rhizobacteria (PGPR). Several studies have shown that plant growth promotion is due to several mechanisms like phosphate solubilisation, biological nitrogen fixation, inhibition of pathogen attack, Siderophores production, phytohormone production (**Bhattacharya and Jha,** 2012).

Soil micro flora has beneficial and detrimental effects on plant growth promotion and yield directly and indirectly. Various mechanisms associated with the involvement of PGPRôs in

the plantôs growth and development is studied by various researches. Some of the direct and indirect mechanisms are: 1. ñPlant Growth Regulator has been produced such as IAA, Gibberellins and Cytokininsò 2. ñSoil micro flora is showing biological control on pathogenic micro-organisms via the phenomenon of competition. Siderophores and antibiotics are produced against pathogenò (**Zafar *et al.,*** 2012). 3. ñAsymbiotic nitrogen fixation is enhancedò (**Khan,** 2005). Several PGPR are known to produce Siderophores HCN (a kind of antibiotic) which solubilise mineral nutrients like phosphorus, zinc, iron, etc. in the soil and make them available to the plants. PGPR are associated with the production of plant hormone IAA (Indole Acetic Acid). 80% bacteria isolated from rhizosphere secrete IAA. This study was conducted to isolate Rhizobium species from leguminous plants. Isolated bacterial species tested for IAA production. Standard graph prepared. Tryptophan is the precursor of IAA i.e. IAA is produced in the presence of tryptophan. Effect of different tryptophan concentration studied. ñ*Rhizobium leguminosarum and Rhizobium loti* is efficient producer of IAA in bacterial isolatesò (**Sahasrabudhe,** 2011).

ñSome PGPR have the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase which hydrolyses ACC, the immediate precursor of ethylene in plants which hydrolysis ammonia and Ŭ-ketobutyrate. This hydrolysis reaction lowers the ethylene (inhibitory effect) level in plants, thus promote seedling root lengthò (**Bhattacharya and Jha,** 2012).

2.2.1 Phosphate Solubilizers

The Soil micro floras which are able to solubilise insoluble soil phosphorus are known as Phosphate Solubilising Microbes (PSMs). Few strains from genera such as *Alcaligenes, Acinetobacter, Arthrobacter, Azospirillum, Bacillus, Serratia, Rhizobium, Pseudomonas, Paenibacillus, Erwinia, Enterobacter, and Burkholderia* are known as phosphate solubilising microbes (**Esitken *et al.,*** 2010).

Pikovskaya (1948) has isolated phosphate solubilizing rhizobacteria from soil micro-flora. Pikovskaya agar media has been used to isolate phosphate solubilizing bacteria (**Sharma *et al.,*** 2013). Bacteria growing on pikovskaya agar media show clear halo zone indicating phosphate solubilization efficiency.

Both bacteria and fungi able are able to solubilise phosphate but bacteria are more efficient than fungi in phosphate solubilising (**Khan *et al.*,** 2009). ñThe most common phosphate solubilising bacterial strains belongs to genera *Pseudomonas, Bacillus, Enterobacter, Rhizobium"* (**Khan *et al.*,** 2009).

There are several ways by which these PSMs help in phosphorus solubilisation and plant growth and yield increase. The major mechanism of mineral phosphate solubilisation is through the action of organic acids produced by micro organisms. These organic acids secreted by Phosphate solubilising microbes lower the soil pH and thus mineralizing the organic phosphate compounds in soil. The other mechanisms associated phosphate solubilisation is production of ACC deaminase enzyme that modulates hormone level, production of IAA hormone.

PSBôs produce most important phytohormone IAA (Indole Acetic Acid), which is being produced in actively growing parts of plants like root, stem and leaves. Tryptophan is the precursor of IAA, carrying metabolic activities associated with the plant growth and development. Many bacterial strains have been studied showing effect of IAA on plant growth and better yield such as *Klebsiella pneumonia* (**Sachdev *et al.*,** 2009).

ñThe main principle associated with phosphate solubilisation is the production of organic acids. These are citric acid, lactic acid, succinic acid and so on, but 2-ketogluconic acid and gluconic acid are most actively participating in phosphate solubilizationò (**Stefan *et al.*,** 2012). The main property exhibited by PSB is the production of low molecular weight organic acids. Most Gram negative bacteria produce organic acids in periplasmic membrane through direct oxidation pathway of glucose whose physiological role remains uncertain. Enzymes of this pathway are oriented towards outer face cytoplasmic membrane and oxidize their substrate in periplasmic membrane (**Anthony,** 2004).

Many studies have been done in insoluble phosphate solubilization by PSM in harsh environments. It is studied that 80% of tropical soils in Latin America has ferric-iron rich, acidic and P-deficient soils. The study conducted in soils of Venezuelan region has this type of soil. Acidic soils are less fertile because available P make complexes with Al and Fe molecules and thus makes it deficient to plant. Many studies have concluded that bacteria colonizing roots of plants are able to contribute to P- nutrition of plants. However bacterial

growth in enriched media does not show any visible significance of phosphate solubilization in plates containing Al-Fe phosphate compounds which results from low amount of P solubilization (**Perez *et al.*,** 2007).

It has been demonstrated that many isolates not producing any visible halo on agar plates are indeed able to solubilise different types of insoluble phosphates in liquid medium (**Seshadri *et al.*,** 2000).

The use of phosphate solubilizing bacteria as inoculants simultaneously increases P uptake by the plant and crop yield. Strains from the genera *Pseudomonas, Bacillus and Rhizobium* are among the most powerful phosphate Solubilizers and noted to produce organic acids and acid phosphatases for mineralization of organic phosphates. Several phosphatases encoding genes have been cloned and characterized and a few genes involved in mineral phosphate solubilization have been isolated. Therefore, genetic manipulation of phosphate-solubilizing bacteria to improve their ability to improve plant growth may include cloning genes involved in both mineral and organic phosphate solubilization, followed by their expression in selected rhizobacterial strains. Chromosomal insertion of these genes under appropriate promoters is an interesting approach (**Rodriguez and Fraga,** 1999). Study conducted on isolation of 36 bacterial strains from subtropical soil of Central Taiwan. 36 bacterial strains characterization and phylogenatic analysis was done by 16S rDNA screening. ñTen isolates belonged to genus Bacillus, nine to genus Rhodococcus, seven to genus *Arthrobacter*, six to genus *Serratia* and one each to genera *Chryseobacterium, Delftia, Gordonia* and *Phyllobacterium.* In addition, four strains namely, *Arthrobacter ureafaciens, Phyllobacterium myrsinacearum, Rhodococcus erythropolis* and *Delftia sp*. are being reported for the first time as phosphate solubilizing bacteria (PSB) after confirming their capacity to solubilise considerable amount of tri-calcium phosphate in the medium by secreting organic acids.ò Eight different kind of organic acids detected by HPLC technique : citric acid, gluconic acid, lactic acid, succinic acid, propionic acid and three unknown organic acids from the cultures of isolated bacteria (**Chen *et al.*,** 2006). Endophytic microbes (inside intercellular spaces of plants) have been studied to show growth promotion activities via secretion like IAA, P solubilisation, siderophore production, enhanced mycorrhizal colonization. Two different endophytic *Penicillium* species from Tea (*Camellia sinensis* L.*)* leaves studied for phosphate solubilisation efficiency. Both species showed remarkable phosphate solubilisation efficiency

range between 80-86 µg/ml. Organic acids decrease soil or any medial pH, providing the facility to exchange the metal part of insoluble phosphates to potassium or sodium, resulting the formation of soluble phosphate salts. Inoculation of phosphate solubilising microorganisms to soil is a reliable technique for increasing soluble P in soil. Thus production of organic acids, chelate cations through their carboxylic acid group and convert it into soluble form (**Nath *et al.,*** 2012).

Many phosphate solubilizing bacterial strains isolated and characterized by employing Whole-cell fatty acids methyl ester (FAME) profile and16s rDNA sequence analysis from the rhizospheric soil of various crops of Korea. 13 best isolates identified and characterized on pikovskaya media on the basis of their solubility of insoluble phosphorus. ñThey were clustered under the genera *Enterobacter, Pantoea* and *Klebsiella* and the sequences of three representative strains were deposited in the GenBank nucleotide sequence data library under the accession numbers AY335552, AY335553, AY335554ò (**Chung *et al.,*** 2005).

Phosphate Solubilization efficiency have also been studied in bacteria isolated from rhizospheric soil of weed plants *viz. Chrysopogon aucheri*, *Lactuca dissecta*, *Solanum surattense* and *Sonchus arvensis* growing in saline soil of Khewra salt range and *Solanum surattense* growing in arid region of Attock. It was concluded that isolates from *Lactuca dissecta* showed maximum phosphate solubilization efficiency and can be commercialised to be used as bio-fertilizers (**Yasmin and Bano,** 2011).

2.2.2 Plant Growth Promotion

Plant growth promoting Rhizobacteria have the ability to promote plant growth and yield of plants and several studies have been done in plant growth promotion by PGPRs. All interactions in rhizosphere between and bacteria are due to plant mediated mechanisms.

PGPRôs possesses the ability to produce phytohormone which have direct role in plant growth promotion. Several PGPR produce IAA (Indole acetic acid), plant growth promoting hormone. IAA helps to increase root growth and increases root surface area, thus nutrient uptake efficiency increased. PGPRs exert disease control mechanisms, thus proved to be beneficial for sustainable agricultural practices (**Akhtar *et al.,*** 2012).

New bacterial species, *Acetobacter diazotrophicus* has been identified in the field of Sugarcane. This bacterium has been shown to produce to IAA in the presence of tryptophan

as a precursor of IAA biosynthesis. Inoculation of this bacterium in the field of maize plants has shown to increase root and shoot length and high IAA production value (**Patil *et al.*,** 2011).

Tryptophan is recognized as the primary precursor for the synthesis of IAA. There are various mechanisms proposed which shows that tryptophan is the precursor of IAA biosynthesis. IAA is the synthetic auxin, which various metabolic processes like root elongation, cell differentiation, etc. Various bacterial IAA biosynthesis mechanisms have been studied which concluded that tryptophan is necessary precursor for IAA biosynthesis. However, researchers have identified the bacterial tryptophan-independent pathway. Study was conducted on *Azospirillum brasilence* which showed the IAA production in the absence of tryptophan in the medium. But the enzymatic system for this pathway is still under consideration and also the existence of tryptophan-independent pathway is still a question. Although, IAA production alone cannot account for plant growth promotion. There could be some other physiological factors which are also helping in plant growth promotion. It has been analyzed that bacterial IAA produced and plantôs IAA together stimulate the activation of ACC synthase which produces ACC (1-aminocyclopropane- 1-carboxylate). ACC is precursor of ethylene, which inhibits the primary root elongation. Various PGPRs like *Pseudomonas putida* have the ability to produce ACC Deaminase enzyme which converts ACC to ammonia and Ŭ-ketobutyrate. Thus using this mechanism bacterium uses ACC as the nitrogen source and as the ACC levels in the root zone decreases automatically ethylene production reduces thus root elongates. Thus IAA together with ACC leads to root elongation thus plant growth increases (**Spaepen *et al***., 2007).

A study was conducted to check the inoculation of *Rhizobium* with tryptophan in the mung bean field. The results have shown that bacteria supplemented with Tryptophan have increased the nodulation, leaf area and plant height as compared with the control (**Zahir *et al.*,** 2010).

Application of different strains of plant growth promoting rhizobacteria in apple plant have concluded to increased plant growth, nutrient levels like P, Zn have also been increased (**Karakurt and Aslantas**, 2010).

Co-inoculation of different strains of PGPRs has been studied to see their effect on plant growth. Mix bacterial inoculation is more beneficial than single strain of bacteria. Bacterial consortia have been proved to enhance the beneficial characters like IAA production, P

solubilization, and siderophore production. In a study *Jatropha curcas* plant was grown in a barren land and inoculated with multiple bacterial strains (Consortium). Co-inoculation in field led to increased shoot length, root length, shoot and root weight, total biomass. Thus co-inoculation can prove as beneficial to plant growth promotion (**Jha and Saraf,** 2012).

It was reported that *Pseudomonas spp*. produce amino acids, IAA (growth hormone) and some organic acids which ultimately helpful in plant growth promotion (e.g. *Oryza sativa*) and P solubilization. Thus *Pseudomonas spp*. has been observed as effect Plant growth promoting rhizobacteria (**Meera and Balabaskar,** 2012).

2.2.3 PGPR as Bio-fertilizers

PGPRs can be used as bio-fertilizers. **Vessey** (2003) defines ñbio-fertilizers as a substance which contains living microorganisms which, when applied to seed, plant surfaces, or soil colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant.ò When bio-fertilizers are applied to the field, various activities enhanced like P solubilization, nitrogen fixation, phytohormone production, thus leading to enhanced yield of plants.

As the bio-fertilizers contain living organisms the viability of these organisms, productivity, storage and soil type to which they are applied must be kept under consideration. Shelf life to bio-fertilizers is considered to increase the viability and shelf life of bio-fertilizers. The formulations of bio-fertilizers include optimization of bio-fertilizer formulation, application of heat resistant, drought resistant and genetically modified strains and formulation of bio-fertilizer in liquid form. Also, carrier material can be used to maintain the viability of micro-organisms. Liquid formulation of bio-fertilizers is most efficiently used because its shelf life more than the solid forms of bio-fertilizer (**Brar *et al*.,** 2012).

Three isolates from rhizosphere of mung bean and soybean identified as *Pseudomonas spp.* and *Trichoderma spp.* have the ability to be used as bio-fertilizers and as bio-control agent. It was concluded that both the species have potential to be used as bio-control agent and as well as growth promoting agents (**Saraf *et al*.,** 2013).

2.2.4 Impact of PGPRs on Tomato plant

Tomato (*Lycopersicon esculentum*) is one of the most used vegetable in over the world not only because of its taste but its nutritional quality also. Tomato plant is the great source of

iron, vitamin A, B and C. it was studied that about 100 ml of tomato juice contain 20-25 % of total daily need of vitamin A. according to recent studies tomato contain an antioxidant, Lycopene which has the ability to reduce prostate cancer. The mineral constituents of tomato are heavily dependent on the nutritional levels of soil. Thus the soil, considered as growth medium must contain all the essential nutrients required by the plants. Like all other plants tomato also need heavy amounts of three macro-nutrients (N, P and K). Phosphorus in the tomato plant helps in root development and early setting of flower and thus the fruits. This will lead to increase in the yield of tomatoes. Thus it is advisable that the in the seedling stage heavy inputs of Phosphorus fertilizers are required. Phosphorus deficiency in the tomato plants is clearly visible due to changes in their morphology, in which the leaves develop deep green color with slight purple spots. Thus Phosphorus fertilization is essential to have good yield of tomatoes. In a recent study, Tomato plant requires ñ4000 mg/kg of the Phosphorus and leaves contain 5000 mg/kg after that much application of Phosphorus fertilizers. Also, tomato fruit would contain 27 mg of P in each 100 g of edible portion of tomatoò (**Sainju *et al.*,** 2003).

Several studies have been done on the use of PGPRs as bio-fertilizers to enhance the yield of tomato plants. Inoculation of the *Bacillus subtilis* strain to the roots of tomato plant have increased the weight of tomato fruit, shoot and root length, increased dry weight. Thus it was seen that application of PGPRs as the bio-fertilizers agents have shown positive results (**Mena-Violante and Olalde- Portugal,** 2007).

Several other PGPRs effect has been studied on tomato plants like *Pseudomonas, Azospirillum, Azotobacter*. Seven treatments with these bacteria have been studied on tomato plant. Out of those treatments best result was shown by co-inoculation of *Pseudomonas, Azospirillum* **(Sharafzadeh,** 2012). Two phosphate solubilization strains *Pantoea agglomerans* and *Burkholderia anthina* were studied for P solibilization efficiency and plant growth promotion in Tomato plants. Both strains gave positive result for increased root and shoot length, dry weight and optimum P uptake capability. Both strains proved best to be used as biocontrol agent and bio-fertilizer agents (**Walpola and Yoon,** 2013).

Chapter- III

RATIONALE & SCOPE OF STUDY

RATIONALE & SCOPE OF STUDY

Heavy use of chemical fertilizers have led to the ecological imbalance and in some cases essential nutrients gets fixed in the soil like phosphorus compound gets fixed into the soil due to various fixation reactions. Thus there is a need to combat with these problems coming in the path of changing paradigm of green revolution.

Plant growth promoting rhizobacteria have the property to combat with these problems. These rhizospheric bacteria secrete some organic acids which ultimately help to solubilise insoluble forms of phosphorus in the soil and make it available to the plants. PGPRs also help to promote plants growth and yield with the secretion of some special phytohormone like IAA (Indole acetic acid), Cytokinins and gibberellins.

Various studies have been done so far to check the effects of PGPRs on the growth of plants. The results were positive means they have the ability to promote plants growth and yield. They have potential to solubilise insoluble forms of phosphorus.

The beneficial microbial inoculants can fulfill diverse beneficial interactions in plants concerning the environment- friendly agricultural practices. The use of microbial inoculants as bio-fertilizers is eco friendly technique to reduce the negative effects of chemical farming. Moreover, in recent years mechanisms of PGPRs have been exploited to reduce negative effects of associated with food and fiber production.

There are some areas in world where soil is highly contaminated, with no vegetation, so by development of genetically engineered PGPRs contaminated soil can be mediated (**Denton 2007**). By the use of genetically engineered PGPRs crop productivity can also be improved and idea of research in recent decade.

Naturally occurring PGPR strains can also be led to produce some beneficial gene products to eliminate pest attack and disease on crop plants by the process of genome tinkering, where microbial gene can be inserted with that trait (gene) which can show positive effect against pest attack. This whole depends on business management, product marketing and extensive research. Thus PGPR studies can also led to advances in entrepreneurship and raise economy with positive effects on agricultural practices (**Bhattacharya and Jha,** 2012).

Adequate use of PGPR strains in agricultural practices can also led to increase in crop quality and yield, thus meeting the demand of growing population worldwide. Also, production of bio-fertilizers is beneficial to farmers in developed and developing countries.

3.1 Use of PGPR in Industries

In recent years in the period between 1897-1990 PGPR have been used on commercial purposes for the inoculation to the crops. Most prominent used strain is *Bacillus subtilis* which gave best results during the years 1990-2000 for the enhanced nutrients uptake, yield. Due to negative impact of pesticides in the fields, it was observed that use of bio-fertilizers in future will eliminate the usage of pesticides and fertilizers (Chemical based). Plants are prone to various types of diseases thus their yield is affected and in future use of PGPR as bio-control and plant growth promotion will give good results (**Niranjan *et al.*,** 2005).

Chapter – IV

OBJECTIVES OF STUDY

OBJECTIVES OF THE STUDY

The objective of present study was to assess the plant growth promotion and phosphate solubilization efficiency of Plant Growth promoting rhizobacteria. Phosphorus is the less mobile compound in the soil and its deficiency leads to many diseases and deficiency symptoms in the plants as well as deficiency symptoms in human beings. However, many farmers are using chemical based fertilizers in the excess amount which is creating serious problems in the human beings as well as other living genera. It was also noticed that phosphorus fertilizers contain Cd (Cadmium) compounds and other radioactive elements which is very dangerous to humans creating cancerous diseases.

Several plant growth promoting rhizobacteria have been studied which enhance the phosphate solubility and yield of plants especially when inoculated to the roots of the plants. Thus in the present study effect of Plant growth promoting rhizobacteria was seen to enhance the P uptake and growth of the Tomato plant (*Lycopersicon esculentum*). The main objectives of the present study are listed below:

- Isolation of Plant Growth Promoting Rhizobacteria from the soil
- Characterization of the bacteria for the Phosphorus solubilization potential
- Quantitative estimation of the IAA (Indole Acetic Acid), a plant growth hormone
- Inoculation of the Isolates in various treatments to the model plant (Tomato)
- Evaluation of the plant growth promotion ability and Phosphorus uptake by the bacterial isolates for the model plants

Chapter – V

MATERIAL & RESEARCH METHODLOGY

MATERIAL AND METHODOLOGY

In the present study following material and procedure has been used to study the effect of PGPRs on P solubilization and plant growth promotion.

5.1 Material used

Soil sample from *aloe vera* rhizospheric surface

Pikovskaya agar media

Pikovskaya broth

Nutrient broth

Standard bacteria *Pseudomonas putida* was used for comparison

Chemicals and Glassware

1. Characterization for Phosphorus Solubility: Potassium dihydrogen orthophosphate (KH_2PO_4) as a standard phosphorus compound.

   Tris-acid mix: Nitric acid (HNO_3), Sulphuric acid (H_2SO_4), perchloric acid in the ratio 9:4:1 (**Sharma *et al.*,** 2015).

   Bartonôs reagent: Ammonium molybdate, Ammonium vanadate and nitric acid.
2. Characterization for IAA: Indole acetic acid (IAA) and anhydrous ferric chloride ($FeCl_3$) and perchloric acid for preparation of Salkowski reagent (**Sahasrabudhe**, 2011).

Tomato (*Lycopersicon esculentum*) was used as model plant.

5.2 Isolation of PGPR

Pikovskaya agar media was dissolved in distilled water and heated in oven for 30 seconds and then autoclaved along with petri plates at 121 degree, 15 psi for 15 minutes. Soil sample from *Aloe vera* rhizosphere was dissolved in 10 ml of distilled water and serial diluted in the 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and10^{-5}. Media poured in petri plates and let it to solidify. From each serial dilution 0.1µl of sample was spread plated on individual petri plate. Then the plates were incubated at 28±2º c for 2 days.

5.3 Quantitative Estimation of phosphate solubilization efficiency

Four isolated bacteria B1, B2, B3, B4, mix bacteria consortium and standard bacteria *Pseudomonas putida* were grown in pikovskaya broth for 11 days at 28±2º c in shaker

incubator. After 11 days 5 ml of growth media was filtered through whattman filtered paper and centrifuged at 10,000 rpm for 20 min. Then the quantitative estimation of phosphorus was done by using the standard protocol given by **Sharma *et al.,*** 2015.

5.4 Quantitative estimation of IAA production by bacterial isolates

All the bacterial isolates were tested for IAA production in the presence and absence of Tryptophan. The bacterial isolates i.e. B1, B2, B3, B4, Mix consortium and *Pseudomonas putida* were grown in nutrient medium with tryptophan and without tryptophan. Then the samples were incubated for 2 days at 28±2º c. After incubation samples were then filtered and centrifuged at 7000 rpm for 10 minutes. The standard protocol given by **Sahasrabudhe**, (2011) was followed to determine the IAA concentration.

5.5 Pot experiment

Selected bacterial isolates were mass cultivated and then inoculated to tomato (*Lycopersicon esculentum*) plant with different treatments. Bacterial treatment was given to plant in the seeling stage. The various treatments given to tomato plants are mentioned below:

1. 2 Control plants with not any treatment
2. 2 plants inoculated with equal proportion of phosphate fertilizer
3. 2 plants treated with both bacteria and phosphate fertilizer
4. 2 plants inoculated with standard bacteria i.e. *Pseudomonas putida*
5. 2 with B1 bacteria
6. 2 with B2 bacteria
7. 2 with B3 bacteria
8. 2 with B4 bacteria
9. 7 plants with mix bacterial consortium

5.6 Measurement of relative water content and Shoot & root length

After one month of inoculation when the plants attained optimum height then the Fresh Weight (FW), Turgid Weight (TW) and Dry Weight (DW) of leaves were calculated to conclude for Relative Water Content (RWC). Also shoot and root length was also measured.

5.7 Quantitative estimation of soluble phosphorus

1 gram of plant leaves were taken and dissolved in 10 ml if tric-acid mixture. The mixture was kept overnight. Then the digestion of samples was done on hot plate at 100ºc till the mixture becomes colourless. Then the phosphate estimation was done using the standard protocol given by **Sharma *et al.*,** 2015. The unknown concentration of phosphorus was calculated from the standard graph of known phosphate concentration. The standard phosphate compound used was potassium di-hydrogen orthophosphate.

Chapter – VI

RESULT AND DISCUSSION

RESULTS

6.1 Isolation of Phosphate solubilizing bacteria

Bacterial colony grown in pikovskaya agar did not showed any clear halo zone but as they are growing in pikovskaya media which is particularly for isolation of phosphate solubilizing bacteria. Thus the resulted bacterial colony was said to be phosphate solubilizing bacteria. The resulted bacterial colony showed in figure 1. Four bacterial isolates were selected B1, B2, B3, and B4.

6.2 Quantitative estimation of phosphate solubilization potential of bacterial isolates

The four selected bacteria showed that in liquid media i.e. pikovskaya broth they are able to solubilise phosphorus as compared to the standard bacteria. The unknown concentration of phosphorus was calculated from standard graph (Graph no. 1) of phosphate compound, listed in table no. 1. It was observed that all the bacterial isolates showed optimum phosphate solubilization potential. Bacterial consortium showed high phosphate solubilization potential in liquid medium.

6.3 Quantitative estimation of IAA production by bacterial isolates

Isolated bacteria showed no IAA production in the absence of tryptophan. But in the presence of tryptophan all the bacteria showed optimum production of IAA as compared to standard bacteria. The slight pink colour appeared in culture containing tryptophan which is the indicator of IAA production by the bacterial isolates (Fig.2). The unknown concentration of IAA listed in table no. 2, was calculated from standard graph (Graph no. 2) of the IAA.

6.4 Inoculation of Bacterial isolates

The bacterial isolates inoculated to tomato plants in the seedling stage. All the plants were growing at the optimum rate. Comparison was done with the control plant. The figures showing optimum plant growth from day 5 of inoculation to day 40 of inoculation are shown in figure 3.1, 3.2, 3.3.

6.5 Measurement of relative water content and Shoot length and Shoot length

All the plants treated with various bacterial treatments showed optimum results for relative water content. The shoot length and root length of plants treated with bacterial isolates showed increased root length and shoot length as compared to control plant to which nothing is inoculated. The table showing relative water content is shown in table no. 4. Also table no. 5 indicates shoot length and root length. The figures showing increased plants shoot and root length is shown in figure no. 4.

6.6 Quantitative estimation of P uptake by Tomato plants (*Lycopersicon esculentum*)

All the plants treated with various different bacterial treatments had optimum P content. The P content of all the plant material was calculated from standard P graph and the concentrations are listed in table no. 5. All the treated plants had optimum P content as compared to control plant. Thus isolated bacteria considered as Plant growth promoting rhizobacteria have potential to solubilise Phosphorus and promoting plant growth.

EXPERIMENTAL WORK

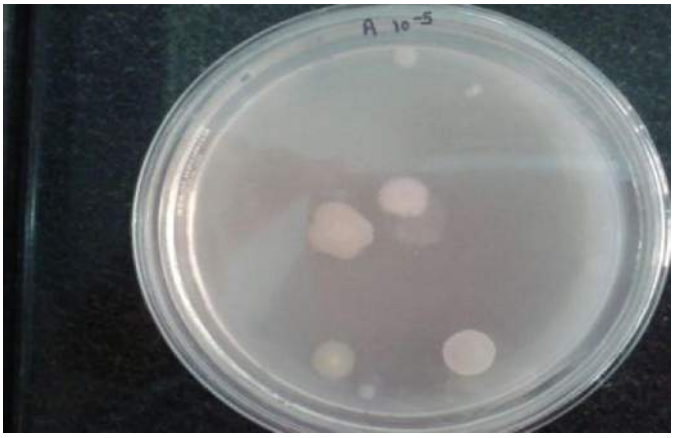
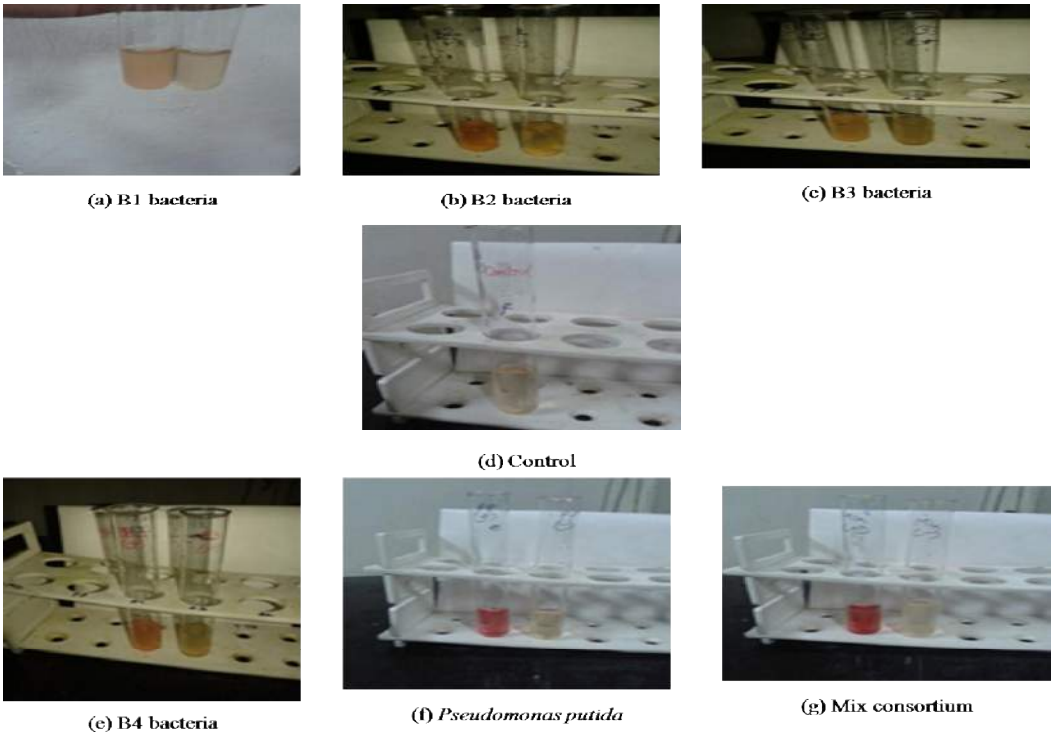


Figure 1: Showing isolated phosphate solubilizing bacteria



(a) B1 bacteria (b) B2 bacteria (c) B3 bacteria

(d) Control

(e) B4 bacteria (f) *Pseudomonas putida* (g) Mix consortium

Figure 2 (a,b,c,d,e,f,g): Showing IAA production indicated by pink colour appeared in the presence of tryptophan and no colour developed in cultures without tryptophan

Tomato plant showing growth results after bacterial inoculation

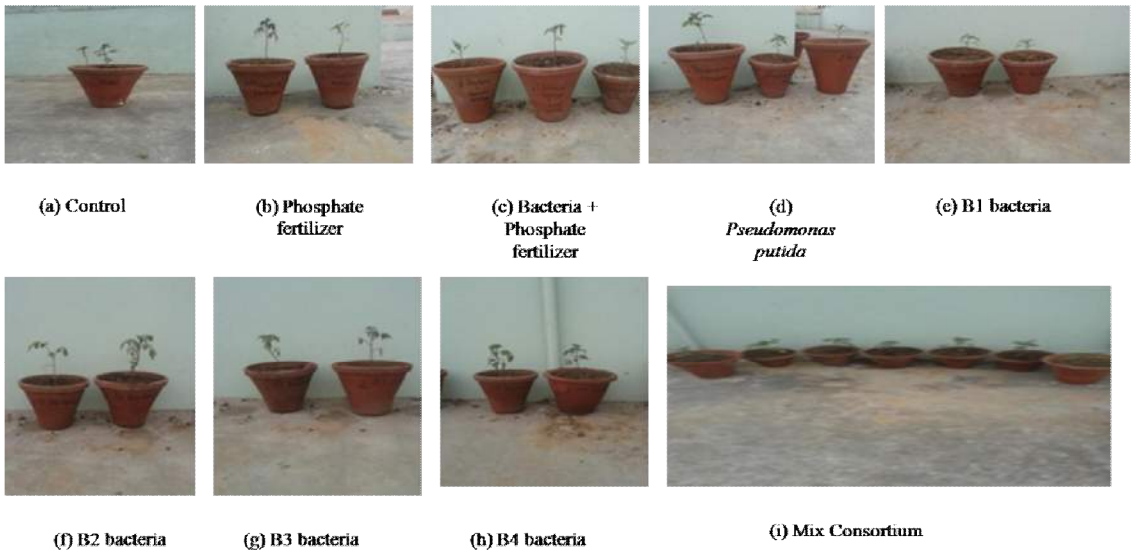


Figure 3.1 (a - i) Shows Tomato plants after 5 days of inoculation; Tomato plants given different treatments listed above

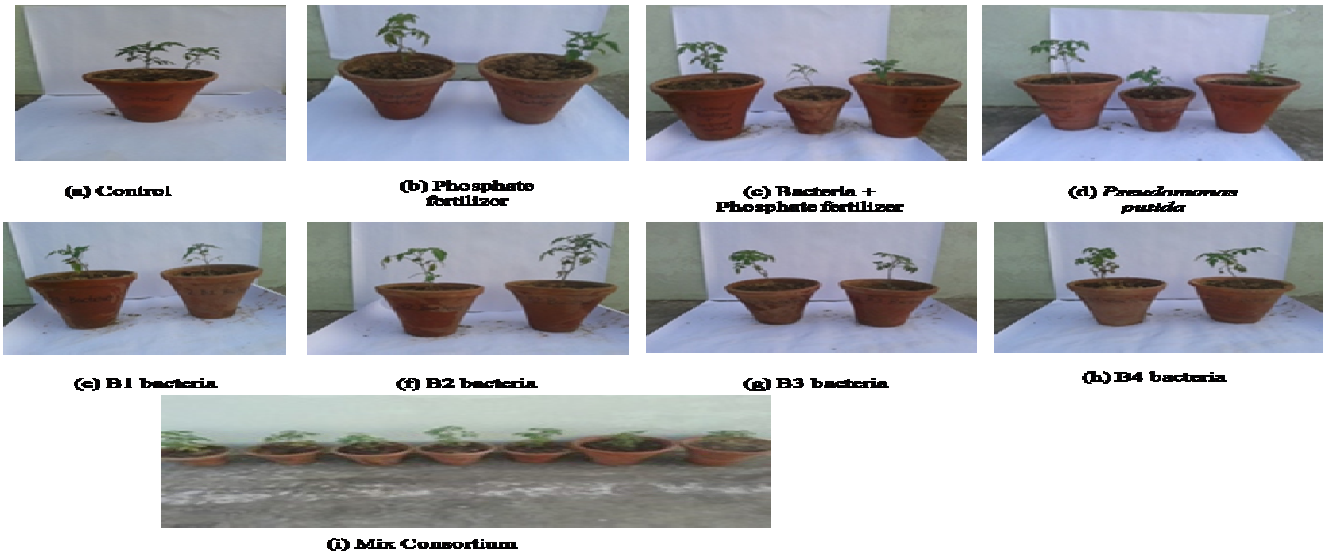


Figure 3.2 (a - i) Shows Tomato plants after 15 days of inoculation

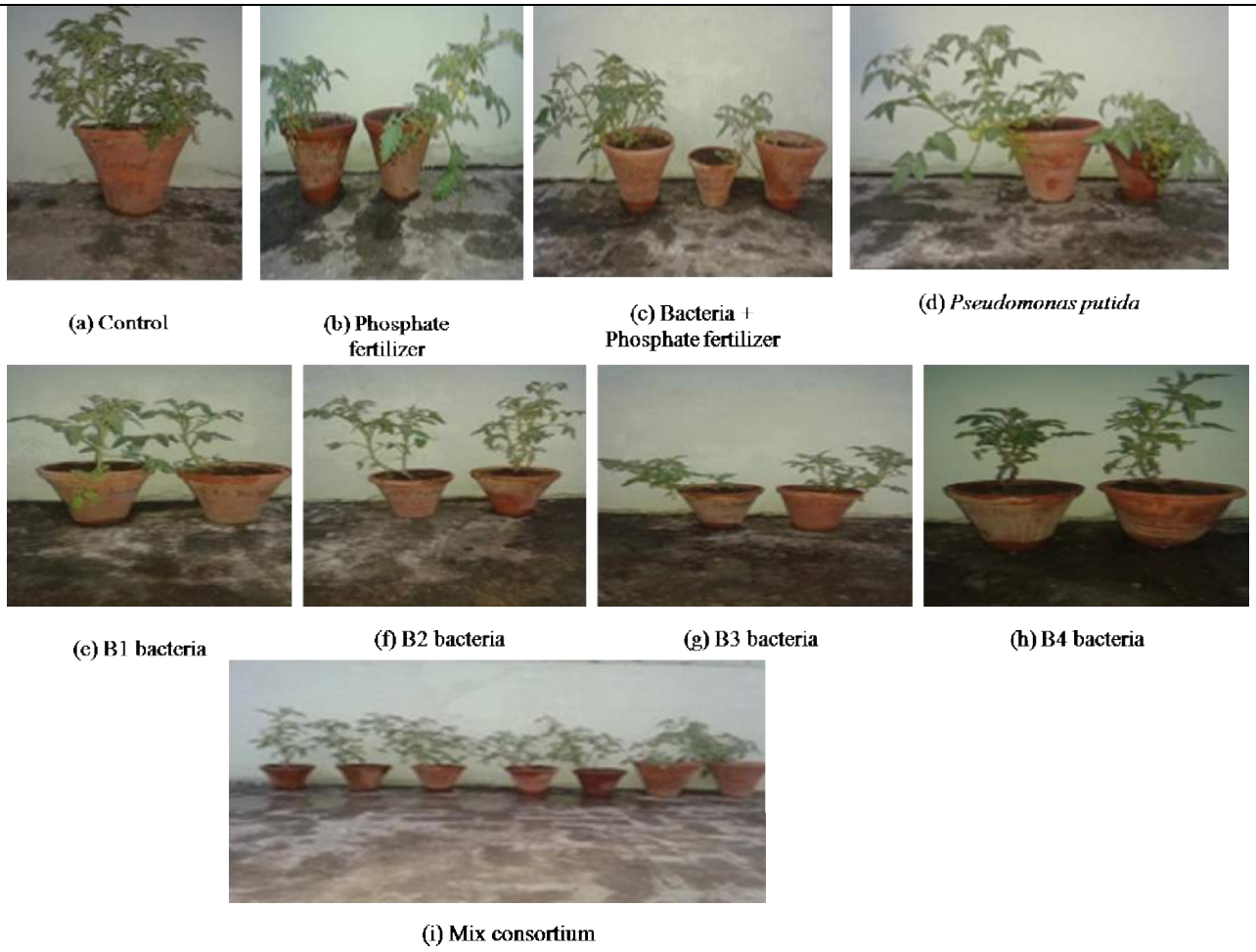


Figure 3.3 After 40 days of bacterial inoculation in several treatments, Tomato plants shows above shown results

Pictures showing Root length and shoot length

(a) Control

(b) Phosphate fertilizer (i)

(c) Phosphate fertilizer (ii)

(d) Bacteria + P fertilizer (i)

(e) Bacteria + P fertilizer (ii)

(f) *Pseudomonas putida (i)*

(g) *Pseudomonas putida (ii)*

(h) B1 bacteria (i)

(i) B1 bacteria (ii)

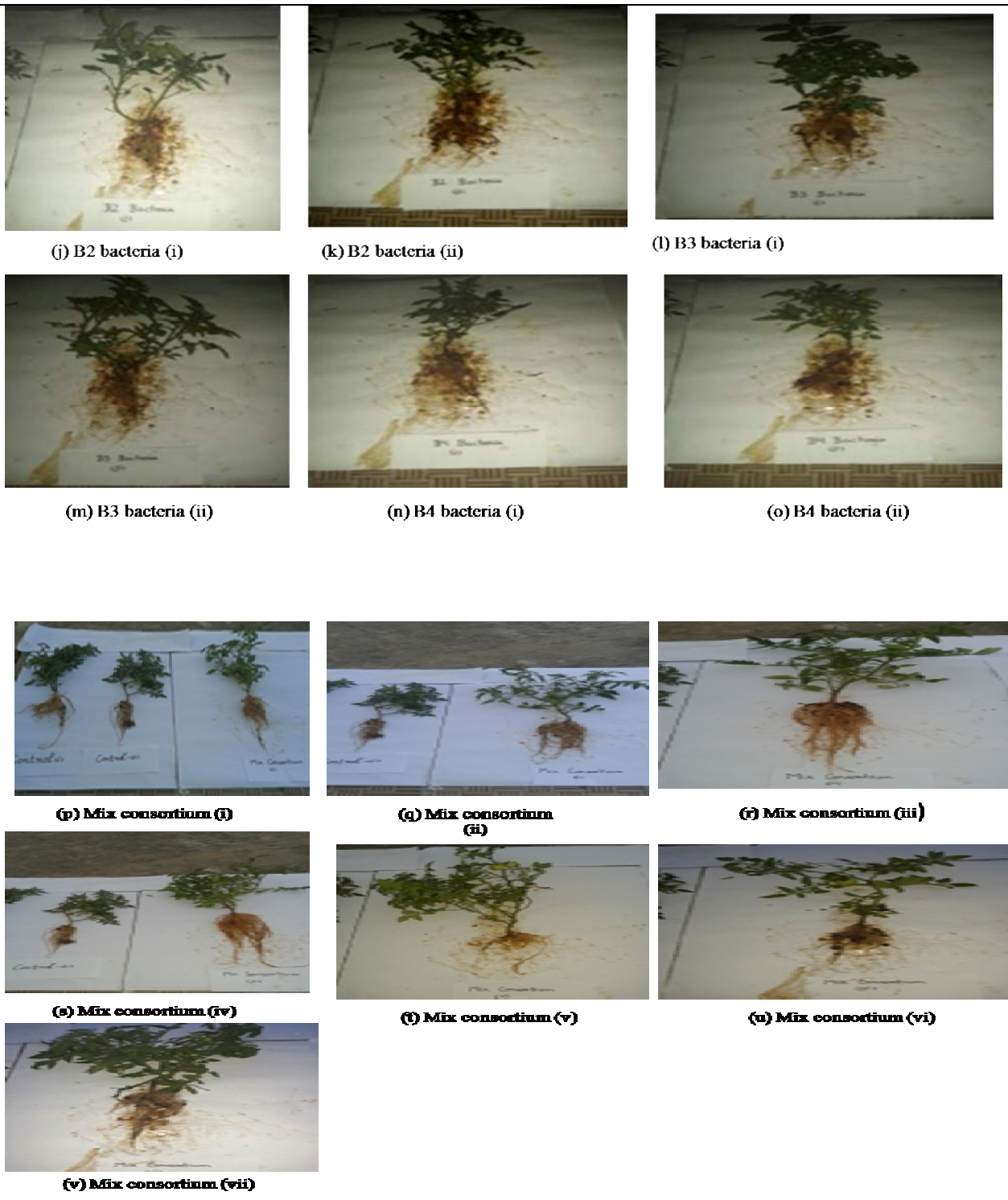
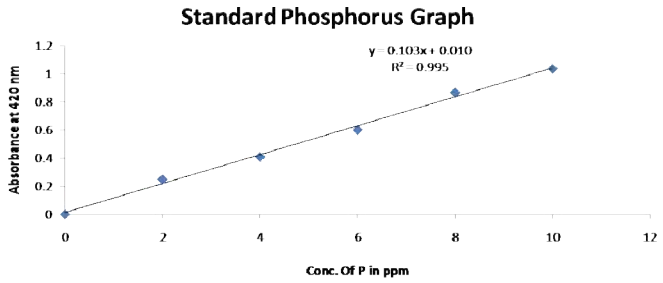
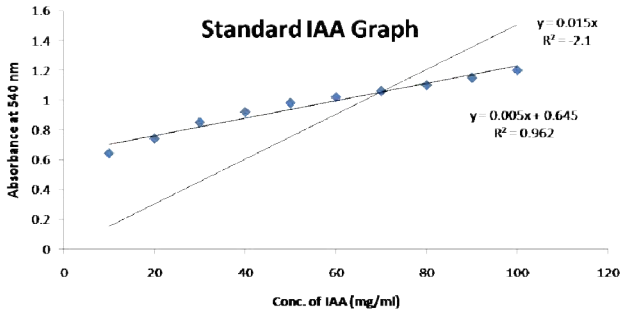


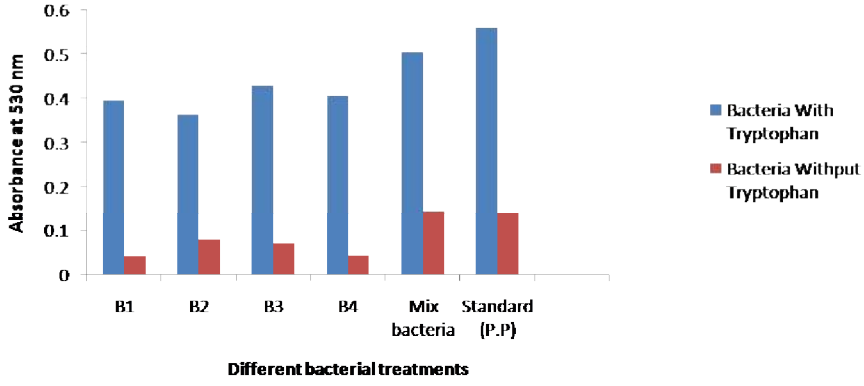
Figure 4: Showing root and shoots of Tomato plants



Graph 1: Standard Phosphate graph to calculate unknown conc. of P in ppm (Parts per million)



Graph 2: Standard IAA graph to calculate unknown conc. Of IAA in mg/ml for the bacterial isolates



Graph 3: Showing different bacterial isolates have high IAA content when treated with Tryptophan as compared to bacterial isolates without tryptophan content

Sr. no.	Bacterial Samples	Conc. In ppm (Parts per million)
1	B1	6.10
2	B2	6.63
3	B3	7.03
4	B4	7.32
5	Bacterial consortium	7.59
6	*Pseudomonas putida*	6.97
7	Control	0.77

Table 1: Showing the conc. Of soluble phosphorus by the bacterial samples calculated from standard graph in ppm (parts per million)

Bacterial Samples	IAA conc. (mg/ml) WITHOUT Tryptophan	IAA Conc. (mg/ml) WITH Tryptophan
B1	2.8	26.4
B2	5.4	26.66
B3	4.8	28.66
B4	3.0	27.06
Mix Consortium	9.66	33.6
Pseudomonas putida	9.26	37.33

Table 2: Showing IAA conc. In mg/ml in both without tryptophan treatment and with tryptophan treatment

Plant treatments with bacterial isolates	FW (Fresh weight) in grams	TW (Turgid weight) in grams	DW (Dry Weight) in grams	Relative Water Content (RWC) in %
Control (i)	0.755	0.85	0.11	64.88
Control (ii)	0.68	0.72	0.13	63.38
Pseudomonas putida (i)	1.45	1.53	0.12	74.02
Pseudomonas putida (ii)	1.37	1.45	0.17	65.75
Phosphate fertilizer (i)	1.15	1.31	0.12	66.62
Phosphate fertilizer (ii)	0.81	0.92	0.15	56.73
Bacteria + Phosphate fertilizer (i)	1.19	1.24	0.11	74.29
Bacteria + Phosphate fertilizer (i)	1.23	1.31	0.08	79.78
Mix Consortium (i)	1.58	2.0	0.34	28
Mix Consortium (ii)	0.94	0.99	0.13	68.81
Mix Consortium (iii)	1.40	1.50	0.24	53.33
Mix Consortium (iv)	1.81	2.2	0.23	48.36
Mix Consortium (v)	0.84	0.93	0.11	67.49
Mix Consortium (vi)	1.86	1.99	0.28	51.39

Mix Consortium (vii)	0.99	2.04	0.20	17.68
B1 bacteria (i)	0.78	0.91	0.09	69.02
B1 Bacteria (ii)	1.20	1.34	0.15	63.35
B2 Bacteria (i)	1.06	1.14	0.13	68.57
B2 Bacteria (ii)	1.11	1.21	0.11	71.64
B3 Bacteria (i)	1.23	1.31	0.07	81.41
B3 Bacteria (ii)	1.17	1.27	0.10	74.24
B4 Bacteria (i)	1.06	1.43	0.16	62.94
B4 Bacteria (ii)	1.31	1.39	0.11	74.61

Table 3: Showing the calculated relative water content from turgid weight, fresh weight and dry weight

Plant treatments with bacterial isolates	**Shoot length in cm**		**Root length in cm**	
Control (i)	36	35 ±1.41	22	21.5±0.70
Control (ii)	34		21	
Pseudomonas putida (i)	62	52±14.14	33	35.5±3.53
Pseudomonas putida (ii)	42		38	
Phosphate fertilizer (i)	28	29.5±2.12	13	16±4.24
Phosphate fertilizer (ii)	31		19	
Bacteria + Phosphate fertilizer (i)	46	38.5±10.60	27	20±9.89
Bacteria + Phosphate fertilizer (i)	31		13	
Mix Consortium (i)	45	48.28±5.02	26	27.28±8.69
Mix Consortium (ii)	41		22	
Mix Consortium (iii)	54		34	
Mix Consortium (iv)	55		42	
Mix Consortium (v)	50		18	
Mix Consortium (vi)	47		19	
Mix Consortium (vii)	46		30	
B1 bacteria (i)	20	25.5±7.77	19	20±1.41
B1 Bacteria (ii)	31		21	
B2 Bacteria (i)	24	25.5±2.12	12	13.5±2.12
B2 Bacteria (ii)	27		15	
B3 Bacteria (i)	33	35±2.82	20	21±1.41
B3 Bacteria (ii)	37		22	
B4 Bacteria (i)	25	23.5±2.12	16	15±1.41
B4 Bacteria (ii)	22		14	

Table 4: Showing shoot length and root length of treated Tomato plants with bacterial isolates

Samples	**Conc. In ppm (parts per million)**
Control (i)	0.398
Control (ii)	0.388
Pseudomonas putida (i)	1.601
Pseudomonas putida (ii)	1.543
Phosphate fertilizer (i)	1
Phosphate fertilizer (ii)	0.961
Bacteria + Phosphate fertilizer (i)	1.242
Bacteria + Phosphate fertilizer (ii)	1.271
Mix Consortium (i)	1.378
Mix Consortium (ii)	1.349
Mix Consortium (iii)	1.398
Mix Consortium (iv)	1.436
B1 Bacteria (i)	1.194
B1 Bacteria (ii)	1.145
B2 Bacteria (i)	1.233
B2 Bacteria (ii)	1.184
B3 Bacteria (i)	1.233
B3 Bacteria (ii)	1.174
B4 Bacteria (i)	1.145
B4 Bacteria (ii)	1.184

Table 5: Showing conc. of P uptake by treated Tomato plants with bacterial isolates

Chapter- VII

CONCLUSION AND FUTURE SCOPE

CONCLUSION

It was concluded that bacteria isolated from *Aloe vera* rhizospheric soil have the ability to solubilise insoluble Phosphorus in the liquid pikovskaya media containing tri-calcium phosphate. The four bacteria selected produced IAA only in the presence of tryptophan. Mix bacterial consortium produced IAA maximum as compared to single bacterial isolates. The bacterial isolates considered as phosphate solubilizing bacteria, were inoculated in nine different treatments with replicates so as to check their ability to promote plant growth and phosphorus solubility. After regular checking of growth it was observed that Tomato (*Lycopersicon esculentum*) plant inoculated with Mix bacterial consortium had attained increased root and shoot length as compared to control plant. Plants treated with *Pseudomonas putida* and bacteria + P fertilizer both also had proved to increase root and shoot length. The plants treated in single bacterial isolates had not given optimum results in increasing root and shoot length. All the bacterial isolates had helped in increasing P uptake as compared to control plants. Thus it was concluded that all the four bacterial isolates have the potential to solubilise insoluble Phosphorus and promoting plant growth. These bacterial isolates can be used as bio-fertilizers.

Chapter- VIII

REFERENCES

REFERENCES

1. **Aktar, M.D., Sengupta, D., and Chowdhury, A.,** (2009). Impact of Pesticides use in agriculture: their benefits and hazards. Interdiscip Toxicol, 2(1): 1-12.
2. **Akhtar, A., Hisamuddin., Robab, M.I., Sharf, A., and R.** (2012). Plant Growth Promoting Rhizobacteria: An Overview. J. Nat. Prod. Plant Resour., 2(1): 19-31.
3. **Akintunde, J.K., Dairo, K.P.,** (2012). Evaluation of Plant Growth-Promoting Rhizobacteria for the Control of Bacterial Wilt Disease of Tomato. Global Journal of Bio-Science and Biotechnology, 1(2): 253-256.
4. **Anthony, C.** (2004). The quinoprotein dehydrogenases for methanol and glucose. Archives of Biochemistry and Biophysics, 428: 2ï 9.
5. **Anand, V.** (2012). Rise in Cancer cases due to chemical fertilizers. Only My Health: daily dose for better living. http://www.onlymyhealth.com/rise-in-cancer-cases-due-chemical-fertilisers-1342549825.
6. **Athokpam, H., Wani, S.H., Kamei, D., Athokpam, H.S., Nongmaithem, J., Kumar, D., Singh, Y.,K., Naorem, B.S., Devi, T.,R., and Devi, L**. (2013). Soil macro- and micro- nutrient status of Senapati district, Manipur (India). African journal of Agricultural research, 8(39): 4932-4936.
7. **Banerjee, H., Sanyal, S.K.,** (2013). Emerging Soil Problems in Rice and their Amelioration. Rice knowledge management Portal (RKMP), 1-20.
8. **Bhattacharya, P.N., Jha, D.K.** (2012). Plant Growth Promoting Rhizobacteria: emergence in agriculture. World J Microbiol Biotechnol, 28: 1327-1350.
9. **Blaurock-Busch, E., Bush, Y.M., Friedle, A., Buerner, H., Parkas, C., Kaur, A.** (2014). Comparing the Metal Concentration in the Hair of Cancer Patients and Healthy People Living in the Malwa Region of Punjab, India. Clinical Medicine Insights Oncology: 8: 1-13.
10. **Brar, S.K., Sarma, S.J. and Chaabouni, E.** (2012). Shelf-Life of Bio-fertilizers: An accord between Formulations and Genetics. J Biofertil Biopestici., 3(5): 1-2.
11. **Cakmak, I**., (2005). The role of potassium in alleviating detrimental stresses. J. Plant Nutr. Soil Sci, 168: 521-530.
12. **Chen, Y.P., Rekha, P.D., Arun A.B., Shen F.T., Lai W.A. and Young C.C.** (2006). Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Applied Soil Ecology, 34: 33-41.

13. **Chung, H., Park, M., Madhaiyan, M., Seshadri, S., Song, J., Cho, H., and Sa, T.** (2005). Isolation and characterization of phosphate solubilizing bacteria from the rhizosphere of crop plants of Korea. Soil biology and biochemistry, 37, 1970-1974.
14. **Connolly, E.L., Guerinot, M.L.,** (2002). Iron stress in Plants. Genome Biology, 3(8): 1-4.
15. **Cordell, D.,** (2008). ñ8 reasons why we need to rethink the management of phosphorus resources in the global food systemò, The Story of P Information Sheet 1, Global Phosphorus Research Initiative (GPRI), Linkoping University, Sweden.
16. **Delvasto, P., Valverde, A., Ballester, A., Igual, J.M., Mun, oz, J.A., Gonza´ les, F., Bla´ zquez, M.L., Garcı´a, C., 2006**. Characterization of brushite as a re-crystallization product formed during bacterial solubilization of hydroxyapatite in batch cultures. Soil Biology & Biochemistry 38, 2645ï 2654.
17. **Denton, B.** (2007) Advances in phytoremediation of heavy metals using plant growth promoting rhizobacteria and fungi, Basic Biotechnol., 3: 1-5
18. **Dissanayake, C.B. and Chandrajith, R.** (2009). Phosphate mineral fertilizer, trace metals and human health. J. Natn. Sci. Foundation Sri Lanka, 37(3): 153-165.
19. **Douglas, B.B. and Durst, P.T.** (2002). Managing phosphorus for crop production. Penn State extension: Agronomy Facts, 1-6.
20. **Esitken, A., Yildriz, H.E., Ercisli, S., Donmez, M.F., Turan, M., Gunes, A.** (2010). Effects of Plant Growth Promoting Bacteria on yield, growth and nutrient contents of organically grown strawberry. Sci. Hort., 124: 62-66.
21. **FAO,** (2007). FAOStat, core production 2005. Available online: http://faostat.fao.org/site/340/default.aspx. Food and Agricultural Organization of the United Nations, Rome.
22. **FAO,** (2008). Efficiency of soil and fertilizer phosphorus use: Reconciling changing concepts of soils phosphorus behaviour with agronomic information, FAO Fertilizer and Plant Nutrition Bulletin 18. Food and Agricultural Organization of the United Nations, Rome.
23. **Ganie, M.A., Farida, A., Bhat, M.A., Malik, A.R., Junaid, J.M., Shah, A.M., Bhat, A.H. and Bhat, T.A.** (2013) Boron ï a critical nutrient element for plant growth and productivity with reference to temperate fruits. Current Science, 104(1): 76-85.

24. **Grant, C.A.** (2011). Influence of Phosphate fertilizer on Cadmium in Agricultural Soils and Crops. Agriculture and Agri-Food Canada, Brandon Research Centre, 143-155.
25. **Gransee, A., Führs, H.,** (2012)**.** Magnesium mobility in soils as a challenge for soil and plant analysis, magnesium fertilization and root uptake under adverse growth conditions. Plant and Soil, 368(1-2): 5-21.
26. **GRID-arindal** (2014). Impact on Environmental degradation on Yield and Area. The environmental Food Crisis, GRID-Arindal in collaboration with UNEP.
27. **Haghighi, M.L., Abbaszadeh, B. and Changaei**, **N.** (2012). Variation in Macronutrients Uptake by Two *Amaranthus* Varieties Planted in two different times. Annals of Biological Research, 3(8): 3949-3951.
28. **Helper, P.K.,** (2005). Calcium: A Central regulator of plant growth and development. Plant cell, 17(8):2142-2155.
29. **IFAS** (2002). Plant Nutrients in Citrus Tree. The Institute of Food and Agricultural Sciences (IFAS), University of Florida.
30. **IFPRI** (2000). Global study reveals new warning signals: degraded agricultural lands threaten world's food production capacity. International Food Policy Research Institute, IFPRI.
31. **Jha, C.K. and Saraf, M.** (2012). Evaluation of multispecies Plant-Growth Promoting Consortia for the growth promotion of *Jatropha curcas* L. J Plant Growth Regul, 31:588-598.
32. **Jolejole-Foreman, M.C., Baylis, K., and Lipper, L.** (2012). Land Degradationôs Implications in Agricultural Value of Production in Ethiopia: A look inside the bowl. International Association of Agricultural Economists (IAAE) Triennial Conference, Brazil, 1-64.
33. **Kaiser, B.N., Gridley, K.L., Brady, J.N, Philips, T. and Tyerman, S.D.** (2005). The Role of Molybdenum in Agricultural Plant Production. Annals of Botany, 96: 745-754.
34. **Karakurt, H. and Aslantas, R.** (2010). Effect of some Plant Growth Promoting Rhizobacteria (PGPR) strains on plant growth and leaf nutrient content of apple. Jounal of Fruit and Ornamental Plant Research, 18(1): 101-110.
35. **Khan, A.G.** (2005). Role of Soil microbes in the rhizosphere of the plant growing on trace metal contaminated soils in the phytoremediation. J. Trace Elem. Med. Biol., 18: 355-364.

36. **Khan, A.A., Jilani, G., Akhtar, S.M., Naqvi, S.M.S., Rashhed, M.** (2009). Phosphate solubilising Bacteria: Occurance, Mechanisms and their Role in Crop production, J. Agricul. Bio. Sci., 1(1): 48-58.
37. **Malakouti, M.J.** (2008). The effect of Micronutrients in ensuring efficient use of macronutrients. Turk J Agric For, 32: 215-220.
38. **Maheshwar, U.N. and Sathiyavani, G.** (2012). Solubilization of phosphate by *Bacillus sps*, from groundnut rhizosphere (*Arachishypogea L*). J. Chem. Pharm. Res., 4(8): 4007-4011.
39. **Meera, T. and Balabaskar, P.** (2012). Isolation and characterization of *Pseudomonas flourescencs* from Rice fields. International journal of food, agriculture and veterinary sciences, 2(1): 113-120.
40. **Mena-Violante, H.G. and Olalde-Portugal, V.** (2007). Alteration of Tomato fruit quality by root inoculation with palnt growth promoting rhizobacteria (PGPR): *Bacillus subtilis* BEB- 13bs. Scientia Horticulturae, 113: 103-106.
41. **Naika, S., Jeude, J.V.L.D., Goffau, M.D., Hilmi, M. and Dam, B.V.** (2005). Cultivation to Tomato: Production, Processing and Marketing. Agromisa Foundation and CTA, Wageningen, 1-92.
42. **Nath, R., Sharma, G.D., Barooah, M.** (2012). Efficieny of Tri-calcium phosphate solubilization by two different endophytic *Penicillium sp*. isolated from Tea (*Camellia sinensis* L.). European Journal of Experimental biology, 2(4): 1354-1358.
43. **Niranjan, R.S., Shetty, H.S. and Reddy, M.S.** (2005). Plant Growth Promoting Rhizobacteria: Potential green alternative for plant productivity. Spriger, 7: 197-216.
44. **Nishanthlalu, G.F.A. and Sharon, N.L.** (2014). A Study On Effects Of Chemical Farming On Farmers At Kilkundaiyar Village, Thiruvallur District, Tamilnadu. Indian Journal of Applied Research, 4(12): 95-97.
45. **Olaniyi, J.O., Akanbi, W.B., Adejumo, T.A., and Akande, O.G.** (2010). Growth, fruit yield and nutritional qulity of tomato varieties. Africam Journal of Food Science, 4(6): 398-402.
46. **Patil, N.B., Gajbhiye, M., Ahiwale, S.S., Gunjal, A.B., Kapadnis, B.P.** (2011). Optimization of indole 3-acetic acid (IAA) production by *Acetobacter diazotrophicus* L1 isolated from Sugarcane. International Journal of Biosciences, 2(1): 307-314.

47. **Patel, P.L., Patel, N.P., Patel, P.H. and Gharekhan, A.** (2014). Correlation Study of Soil Parameters of Kutch District Agriculture Land. International Journal of Scientific and Research Publications, 4(5): 1-5.
48. **Perez, E., Sulbaran, M., Ball, M. M., Yarzabal, L. A.** (2007). Isolation and characterization of mineral phosphate-solubilizing bacteria naturally colonizing a limonitic crust in the south-eastern Venezuelan region. Soil biology and Biochemistry, 39: 2905-2914.
49. **Rodriguez, H., Fraga, R.** (1999). Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnology advances, 17(4-5): 319-339.
50. **Sachdev, P.D., Chandhari, G.H., Kasture, M.V., Dhavale, D.D., Chopade, A.B.** (2009). Isolation and characterization of Indole Acetic Acid (IAA) producing *Klebsiella pneumonia* strains from rhizosphere of wheat (*Triticum aestivum*) and their effect on plant growth. Indian J. of Expt. Biol., 44: 993-1000.
51. **Sahasrabudhe, M.M.** (2011). Screening of Rhizobia for Indole Acetic Acid production. Annals of Biological Research, 2(4): 460-468.
52. **Sainju, U.M., Dris, R. and Singh, B.** (2003). Mineral nutrition of Tomato. Fort Valley state, USA. 176-183.
53. **Sarwar, N., Saifullah,, Malhi, S.S., Zia, M.H. Naeem, A., Bibi, S. and Farida, G.** (2010). Role of Mineral nutrition in minimizing cadmium accumulation by plants. J Sci. Food and Agric, 90: 925-937.
54. **Saraf, M., Thakkar, A., Pandya, U., Joshi, M., Parikh, J.** (2013). Potential of plant growth promoting microorganisms as biofertilizers and biopesticides and its exploitation in sustainable agriculture. J. Microbio. Biotech. Res., 3(5): 54-62.
55. **Savci, S.,** (2012). An Agricultural Pollutant: Chemical Fertilizer. International Journal of Environmental Science and Development, 3(1): 77-8.
56. **Seshadri, S., Muthukumarasamy, R., Lakshminarasimhan, C. and Ignacimuthu, S.** (2000). Solubilization of inorganic phosphate by *Azospirillum halopraeferans.* Current Science, 79 (5): 1-3.
57. **Sharma, B.S., Sayyed, Z.R., Trived, H.M., and Gobi, A.T.** (2013). Phosphate solubilising microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. Springerplus, 2: 587.

58. **Sharafzadeh, S.** (2012). Effects of PGPR on growth and nutrients uptake of Tomato. International Journal of Advances in Engineering and Technology, 2(1): 27-31.
59. **Sharma, A., Patni, B., Shankhdhar, D., Shankhdhar, S.C.** (2015). Evaluation of different PGPR strains for yield enhancement and higher Zn content in different genotype of rice (*Oryza sativa L.*). Journal of Plant Nutrition, 38(3): 456-472.
60. **Singh, R.P., and Mishra, S.K.,** (2012). Available Macro-nutrients (N,P,K and S) in the soils if Chiraigaon block of district Varanasi (U.P) in relation to soil characteristics. Indian J. Sci. Res., 3(1): 97-100.
61. **Spaepen, S., Vanderleyden, J. and Remans, R.** (2007). Indole- 3 Acetic Acid in microbial and micro-organism- Plant signalling. FEMS Microbiol Rev, 31: 425-448.
62. **Srinivasan, K. and Poongothai, S.** (2013). Macronutrients and micronutrients relation to Soil Characteristics of Wellington Reservoir, Tamilnadu, India. J. Chem. & Chemical Sciences, 3(3): 107-116.
63. **Tucker, M. R.,** (1999). Essential Plant Nutrients: their role in North Carolina soils and role in plant nutrition. Agronomist Division. 1-9.
64. **Uchida, R.,** (2000). Essential nutrients for plant growth: Nutrient Functions and Deficiency Symptoms. Plant Nutrient Management in Hawaiiôs Soils, Approaches for Tropical and Subtropical Agriculture. 31-55.
65. **UMN,** (2009). Nature of Phosphorus in soil. University of Minnesota Extension (UMN).
66. **Vessey, J.K.** (2003). Plant growth promoting rhizobacteria as biofertilizers. Plant Soil, 255: 571-586.
67. **Walpola, B.C., and Yoon, M.H.** (2013). Isolation and characterization of phosphate solubilising bacteria and their co-inoculation efficiency tomato plant growth and phosphorus uptake. African journal of microbiology, 7(3) : 266-275.
68. **White, P.J. and Brown, P.H.** (2010). Plant nutrition for sustainable development and global health. Annals of Botany. 105: 1073-1080.
69. **Yasmin, H. and Bano, A.** (2011). Isolation and Characterization of Phosphate Solubilizing Bacteria from Rhizospheric soil of weeds of Khewra Salt range and Attock. Pak. J. Bot., 43(3): 1663-1668.
70. **Zafar, M., Abbasi, M.K., Khan, M.A., Khaliq, T., Sultan, T., Aslam, M.** (2012). Effect of Plant Growth Promoting Rhizobacteria on growth, nodulation and nutrient,

accumulation of lentil under controlled condition. Elsevier B.V and Science press, 22(6): 848-859.

71. **Zahir, Z. A., Yasin, H.M., Naveed, M., Anjum, M.A. and Khalid M.** (2010). L-Tryptophan application enhances the effectiveness of *Rhizobium* inoculation for improving growth and yield of Mungbean (*Vigna radiata* (L.) Wilczek). Pak.J.Bot., 42(3): 1771- 1780.

APPROVAL PAGE

LOVELY PROFESSIONAL UNIVERSITY
Transforming Education, Transforming India

School of: Biotechnology

DISSERTATION TOPIC APPROVAL PERFORMA

Name of the Student: Pooja Claire — Registration No: 11308792

Batch: 2013-2015 — Roll No. 13402

Session: 2014 — Parent Section: 1342

Details of Supervisor: — Designation: A.P.

Name: Dr Ashish Saxena — Qualification: Ph. D.

U.ID: 18036 — Research Experience: 4 yrs

SPECIALIZATION AREA: Botany (pick from list of provided specialization areas by DAA)

PROPOSED TOPICS

1. Applications of Plant Growth Promoting Rhizobacteria for increasing the solubility & bioavailability of Phosphorus.
2. Biocontrol of plant pathogenic fungi by use of different PGPRs.
3. Solubilization studies on Phosphatic fertilizers by application of PGPRs.

Signature of Supervisor 18036

PAC Remarks: OK

APPROVAL OF PAC CHAIRPERSON: Signature: Date:

*Supervisor should finally encircle one topic out of three proposed topics and put up for approval before Project Approval Committee (PAC)
*Original copy of this format after PAC approval will be retained by the student and must be attached in the Project/Dissertation final report.
*One copy to be submitted to Supervisor.