Author	:	**K.DAMODARA CHARI**
Title of the thesis	:	**SCREENING AND CHARACTERIZATION PLANT GROWTH PROMOTING RHIZOBACTERIA AND INFLUENCE ON MAIZE**
Degree	:	**MASTER OF SCIENCE IN AGRICULTURE**
Faculty	:	**AGRICULTURE**
Discipline	:	**AGRICULTURAL MICROBIOLOGY**
Major Advisor	:	**Dr.N.TRIMURTULU**
University	:	**ACHARYA N. G. RANGA AGRICULTURAL UNIVERSITY**
Year of submission	:	**2014**

ABSTRACT

Plant growth promoting rhizobacteria are the soil inhabiting bacteria around/on the root surface and are directly or indirectly involved in promoting plant growth and development by production and secretion of various regulatory chemicals in the vicinity of rhizosphere. Generally, plant growth promoting rhizobacteria facilitate the plant growth directly by either assisting in resource acquisition (nitrogen, phosphorus and essential minerals) or modulating hormone levels, or indirectly by decreasing the inhibitory effects of various pathogens on plant growth and development in the forms of biocontrol agents.

These PGPR are eco-friendly in nature, low-cost effective, certainly help in improving profitability and livelihoods of small and marginal farmers. Therefore their use in crop production makes agriculture profitable and sustainable. Keeping in view the importance of PGPR and such biodiversity available an experiment entitled “Screening and characterization of Plant Growth Promoting Rhizobacteria (PGPR) and influence on maize.” was conducted at Department of Agricultural Microbiology and Bioenergy and college Farm, College of Agriculture, Rajendranagar, Hyderabad for characterizing efficient PGPR isolates with multiple beneficial activities.

A total sixteen pure PGPR bacterial isolates were collected from different resource laboratories of Andhra Pradesh and Amaravathi. These isolates were screened *in vitro* for plant growth promoting attributes like phosphate solubilization, production of Indole acetic acid (IAA), ammonia, hydrogen cyanide (HCN) and siderophores. Bacterial isolates were identified as *Bacillus* (PGP-7, PGP-12, PGP-14) *Pseudomonas* (PGP-1, PGP-8, PGP-13, PGP-16), *Azotobacter* (PGP-6, PGP-9, PGP-11), *Rhizobium* (PGP-2, PGP-3, PGP-4) and

Azospirillum (PGP-5, PGP-10, PGP-15) and were further studied for their antagonism against two soil borne pathogens *viz., Rhizoctonia solani* and *Sclerotium rolfsii.*

All isolates were further tested for their compatibility with the commonly used agrochemicals, *i.e.* fungicides (metalaxyl, ziram, propiconazole and carbendazim), insecticides (chloripyriphos, dimethoate, imidachloprid and thiodicarb) and herbicides (atrazine, butachlor, propaquizafop and pendimethalin). Efficient free living nitrogen fixers were molecular characterized with *nif* gene amplification. Efficient PGPR isolates, phosphate solubilizers [*Pseudomonas* (PGP-1), *Bacillus* (PGP-14)], and free living nitrogen fixers *[Azotobacter* (PGP-9)*, Azospirillum* (PGP-15)]. The efficient PGPR isolates were selected for maize field experiment.

The results revealed that 75% isolates showed positive results for phosphate solubilization, 100% for ammonia, 80 % for IAA , 62.5% for siderophores and 62% isolates showed for HCN productions. All the sixteen PGPR isolates were examined for the potential to inhibit two fungal pathogens viz., *Rhizoctonia solani* and *Sclerotium rolfsii* under *in vitro* conditions. Out of sixteen isolates, eleven isolates exhibited inhibition potential against two soil borne plant phytopathogens.

From compatibility studies the potential compatibility of sixteen isolates with recommended dosage of fungicides, insecticides and herbicides could be assessed. Among all isolates PGP-1(*Pseudomonas*), PGP-9(*Azotobacter*), PGP-15(*Azospirillum*) were not affected by recommended dosage of all fungicides, insecticides and herbicides. Molecular characterization of nitrogen fixing ability of efficient PGPR nitrogen fixers such as PGP-9 (*Azotobacter* sp) and PGP-15(*Azospirillum* sp) cultures was carried out. Their banding pattern of DNA on agarose gel electrophoresis was compared with that of reference *Azotobacter* strains and *Azospirillum* strains. The PCR amplification was carried out for cultures in order to check the presence of *nif* H1, *nif*H2 genes for *Azotobacter* and *Azospirillum* characterization.

These efficient isolates (PGP-1, PGP-8, PGP15) were formulated as carrier based microbial inoculants and tested for purity (10^8 cells/gram) prior to sowing. These formulated microbial inoculants were applied to soil along with chemical fertilizers and farm yard manure (all plots including control) with recommended dosages. T1: 100 % RDF (N2: P_2O_5: K_2O ratio: 120 kg:50 kg:60 kg ha^{-1}),T2: 75 % RDN + nitrogen fixer *Azospirillum* ,T3: 75 % RDN+ nitrogen fixer *Azotobacter* ,T4: 75 % RDP + phosphate solubilizers, T5: 75 % RDN&P + nitrogen fixer *Azospirillum* + Phosphate solubilizers,T6: 75 % RDN & P + nitrogen fixer *Azotobacter* + phosphate solubilizers, T7: 75 % RDN & P + *Azotobacter* + *Azospirillum* + phosphate solubilizers.

The combination of microbial inoculants and 75% reduced chemical fertilizers could improve the growth and yield attributes of maize. Particularly combination of three efficient microbial inoculants and 75% chemical fertilizer treated plots (T7 75%RDN & P + *Azospirillum* + *Azotobacter* + PSB) were highly significant in all components followed by

two microbial inoculants combination with 75%reduced chemical fertilizers. The nutrient status such as available nitrogen, phosphorus, potassium of the soil was improved in microbial inoculants applied treatments significantly compared to non inoculated 100% RDF treatment(T1). Economics results reveal that the combination of PGPR inoculants and 75% reduced chemical fertilizers will increase the net returns over 100% RDF without inoculation of microbial inoculants. Microbial population was varied and improved because of reduced use of chemical fertilizers.

Chapter I

INTRODUCTION

In the recent years, India faced so many problems such as several folds rise in petroleum prices there by its striking influence on the prices of chemical nitrogenous fertilizers. The prices of nitrogenous fertilizers have nearly doubled during the last three years which has necessitated searching for cheaper source of nitrogen to meet the needs of crops. Generally maize crop needs huge amount of fertilizers. Farmers use chemical fertilizers to increase production to get higher yields, but the excessive use of fertilizers leads to contamination of soil and ground water and reduce the soil fertility. Especially in India purchase of chemical fertilizers by marginal farmers is difficult and expensive. Hence alternate use of plant growth promoting rhizobacteria as microbial inoculants can replace chemical fertilizers partially.

Maize is an exhaustive crop belonging to family graminaceae. It is an important cereal and has immense importance as food grain in India. In India, maize (*Zea mays* L.) is the third most important cereal after rice and wheat that provides food, feed, fodder, and serves as a source of raw material for developing hundreds of industrial products *viz*., starch, protein, oil, alcoholic beverages, food sweeteners, pharma, cosmetics, bio-fuel etc. India is the fifth largest producer of maize in the world contributing to 3% of the global production.

Maize is cultivated in an area of about 8.78 M ha with a production and productivity of 21.76 M t and 2.48 t ha^{-1} respectively (DMR, 2012). Among the major producing states, Andhra Pradesh leads the list in maize production with the contribution of 17% to the total maize production in India. The demand for maize is expected to touch 45 MT by 2030, of which 24-25 % will be used for human consumption, 60% as poultry and livestock feed and remaining as industrial raw material. The productivity of maize mainly depends on its nutrient management (Kumar *et al.*, 2007). It needs higher doses of nutrients and considered as a better indicator crop for evaluating plant growth promoting rhizobacteria. Hence it is necessary to screen different isolates obtained from different areas of the growth rate for their PGPR/multiple beneficial activity for further exploration for better crop growth & yield in maize.

In the rhizosphere of crop plants bacteria are present abundantly. These rhizoshere bacteria are collectively called rhizobacteria. Plant growth promoting bacterial strains are rhizospheric competent which can survive and colonize the rhizosphere soil (Cattelan *et al.*, 1999). These rhizosphere bacteria that positively stimulate the plant growth are called plant growth promoting rhizobacteria (PGPR). The effect of rhizosphere colonization by PGPR received attention on potato plant development by Kloepper *et al.* (1980). They reported that inoculation of potato seed pieces with *Pseudomonas fluorescens* and *Pseudomonas putida* reduced the disease incidence and increased the yield.

A wide group of free living soil bacteria is considered to be PGPR which includes *Pseudomonas, Azotobacter, Azospirillum, Klebsiella, Enterobacter, Alcaligens, Arthobacter ,Gluconacetobacter*, *Burkholderia*, *Rhizobium* , *Bradyrhizobium* etc. Among them *Pseudomonas* species are more dominant rhizobacteria (Kloepper *et al*., 1989; Okon and Labandera-Gonzalez, 1994; Glick,1995)

PGPR may improve the plant growth and yield by direct or indirect mechanisms. Direct mechanisms involve asymbiotic nitrogen fixation (Boddey and Dobereiner, 1995) that may be utilized by the plants, solubilisation of minerals including phosphorus (De Freitas *et al*., 1997; Gaur, 1990) or production of plant growth regulators such as auxins (indole-3-acetic acid (IAA), gibberillins, cytokinins or ethylene (Arshad and Frankenberger,1993; Glick,1995) synthesizing inhibitors, such as 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Rashedul *et al*., 2009), which act directly on plant itself and affect growth. Indirect mechanisms of growth promotion include production of iron sequestering siderophores (Scher and Baker, 1982) or production of antibiotics (Shanahan *et al*., 1992) and hydrogen cyanide (Flaishman *et al*., 1996) that serve to protect the plants from soil phytopathogens.

Azospirillum known for many years as PGPR was isolated from the rhizosphere of many grasses and cereals all over the world, in tropical as well as in temperate climates (Steenhoudt and Vanderleyden, 2000) This bacterium was originally selected for its ability to fix atmospheric nitrogen (N_2) and since the mid-1970s, it has consistently proven to be a very promising PGPR and recently the physiological, molecular, agricultural and environmental advances made with this bacterium were thoroughly reviewed by Bashan *et al.* (2004).

Azotobacter represents the main group of heterotrophic, non symbiotic gram negative, free living nitrogen-fixing bacteria. They are capable of fixing an average 20 kg N/ha/year. The genus *Azotobacter* includes 6 species, with *A.chroococcum* most commonly inhabiting in various soils all over the world (Mahato *et al*., 2009). Jadhav (1981) stated that dry matter and nitrogen content of maize increased by additive nitrogen fertilizer application.

Pseudomonads are able to improve plant health and are implicated in the natural suppressiveness of certain soils to many soil borne diseases. Some *Pseudomonas* have been recognized as antagonist to plant pathogens, and antibiotic producers. Siderophores, including salicylic acid, pyochelin and pyoveridine, which chelate the iron and other metals, also contribute to the disease suppression by conferring a competitive advantage to biocontrol agents(Kloepper et al.,1989) for the limited supply of essential trace minerals in natural habitats5

In the context of increasing international concern for food and environmental quality, the use of PGPR for reducing chemical inputs in agriculture is a potentially important issue. PGPR have been applied to various crops to enhance growth, seed emergence and crop yield, and some have been commercialized. PGPRs are the potential tools for sustainable agriculture and trend for the future.

In this study mainly selected efficient strains of free living nitrogen fixers such as *Azospirillum* sp and *Azotobacter* sp and phosphate solubilizing *Pseudomonas* sp and *Bacillus* sp. used for carrier based microbial inoculants and studied the effect on maize crop yield attributes.

Objectives of investigation:

1. Collection and screening of rhizobacterial isolates from different resource labs of Andhra Pradesh for PGPR activities.
2. To find efficient PGPR isolates with biocontrol activity.
3. To study the molecular characterization of nitrogen fixing ability of efficient PGPR strains.
4. To study the compatibility of efficient PGPR isolates with agro chemicals.

5. To study the performance of selective efficient PGPR strains on maize crop in the field experiment.

Chapter II

REVIEW OF LITERATURE

The present investigation was carried out by screening and characterization of sixteen plant growth promoting rhizobacterial isolates from different resource laboratories of Andhra Pradesh, India and their influence on maize crop. The literature pertaining to these aspects is reviewed and presented below under the appropriate headings, which will provide an overview of the current status of the research work on the these aspects.

2.1 SCREENING OF PURE ISOLATES FOR PGPR PROPERTIES

Screening of PGPR characters of bacterial isolates is an essential step for selection of efficient strains for microbial inoculants preparation. These PGPR characters includes such as mineral phosphate solubilization, nitrogen fixation, growth regulators production, biocontrol activity etc.

2.1.1 Mineral Phosphate Solubilization

Fekadu Alemu (2013) isolated twelve *Pseudomonas fluorescens* species from rhizospheric soil of faba bean and tested for phosphate solubilization. All tested isolates of *Pseudomonas fluorescens* species have a potential of phosphate solubilization on Pikovskaya's media. He summarized that all *Pseudomonas fluorescens* spp. can be used as bio-fertilizers for soil fertility improvement.

Singh and Ghosh (2012) isolated a total of 93 isolates. Out of 93 isolates 30 isolates were selected for their evaluation of phosphate solubilizing potential on pikovskaya's media. Out of 30 isolates five isolates showed positive result on pikovskaya's medium producing clear zones. Further, out of five isolates, three isolates individually, and as well as consortia were tested on *Zea mays* in pot under natural environmental conditions. The observations reveals that C (32) D, C (32) E were efficient strains by improving the growth of pot experiments. Further these isolates were used for biofertilizer production.

Qureshi *et al.* (2012) isolated the PSB and conducted field trial of PSB with chemical fertilizer and revealed that bacterial inocula produced significantly higher seed cotton yield 1630kg.ha^{-1} as compared to 1511 kg ha^{-1}. Also reported that solubilizing efficiency and solubilizing index of bacterial isolates ranged between 233 to 275 and 3.3 to 3.8 respectively.

Phosphate solubilizing microorganisms *Bacillus subtilis* and *Bacillus cereus* isolated by Maheshwar and Sathyavani (2012) from the groundnut rhizosphere soil, showed maximum solubilization around 40^0C. Screening of PSB was done in *Bacillus subtilis, Bacillus cereus* in both solid & liquid medium. *Bacillus subtilis* is the most efficient for phosphate solubilising activity, at pH 7 with carbon sources (0.35+0.02), nitrogen sources (0.28+0.03) and potash sources (0.12+0.09).

Manivannan *et al*. (2011) reported six different Phosphate solubilizing microorganisms (*Pseudomonas* spp. *Bacillus* spp. *Penicillium* sp. and 3 strains of *Aspergillus* spp.) from the rhizosphere soils of chilli and tomato plants. He found that *Penicillium* spp. solubilized tricalcium phosphate 1045 $\mu g.ml^{-1}$ and *Pseudomonas* spp. 985 $\mu g.ml^{-1}$ after 14th days of incubation in Pikovskaya's broth medium and in all cultures phosphate solubilization ability was decreased due to organic acid production.

Mahantesh and Patil (2011) isolated Phosphate solubilizing bacteria (PSB) from the area around Bidar region and screened on the basis of their solubilization of inorganic tricalcium phosphate in liquid cultures. Ten strains that had higher solubilization potential were selected and characterized.

Two stress tolerant phosphate solubilizing rhizobacteria, *Arthrobacter spp.* and *Bacillus spp.* isolated by Banerjee *et al.* (2010) from tomato rhizosphere and characterized using morphological and biochemical tests. In addition to phosphate solubilizing ability these strains also demonstrated various Plant growth promoting and biocontrol activities including indole acetic acid (IAA) production. These two strains had the potential to be used as Plant growth promoting rhizobacteria (PGPR).

Keneni *et al.* (2010) reported five efficient PSB (Phosphate Solubilizing Bacteria) isolates based on their ability in forming a higher clear zone diameter than the other isolates. The phosphate solubilizing efficiency of these five isolates along with Jim-41 isolate were studied using different P sources [Tricalcium Phosphate (TCP), Egyptian Rock Phosphate (ERP), Bikilal Rock Phosphate (BRP) and Old Bone meal (OB)]. The PSB isolates significantly (P£ 0.01) solubilized a higher amount of TCP, ERP and OB over the uninoculated control.

Eighty *Pseudomonad* strains were screened for phosphate solubilization. Out of 80, three isolates (*Pseudomonas aeruginosa*, *P. plecoglossicida* and *P. mosselii*) showed the ability to solubilize tri-calcium phosphate Jha *et al.* (2009). They reported that, *P. plecoglossicida and P. mosselii* can be used as biofertilizers because of the innate potential of phosphate solubilization, production of siderophores, IAA, protease, cellulase and HCN.

Oliveira *et al.* (2009) reported several phosphate solubilizing microorganisms from maize rhizospheric soil. The strains of bacteria, fungi and most of the actinobacteria were confirmed as PSM with high ‘P’ solubilization activity. Among the bacteria isolates, B17 and B5, identified as *Bacillus* sp. (B17) and *Burkholderia* sp. (B5) respectively, were the most efficient ‘P’ solubilizing strains from P-Ca source culture solution solubilizing 67% and 58.5% of the total ‘P’ respectively. The B5 isolate also had the highest reduction in pH in the growth solution to 4.46.

2.1.2 Nitrogen fixation

Shrivastava (2013) isolated diazotrophic bacteria from the rhizosphere of rice plants. The rate of nitrogenase enzyme activity based on acetylene reduction assay showed remarkable variation in these isolates which ranged from 0.69 to 1.63 μmol C_2H_4.mg protein.h^{-1}. Based on biochemical characterization and carbon source utilization they were clustered in 6 clusters. It is hereby reported that species of *Pseudomonas, Klebsiella, Azotobacter* and *Agrobacterium* were predominantly present in this region especially in the rhizosphere of rice plants.

Gothwal *et al.* (2007) showed nitrogen fixing ability both in solid and liquid culture conditions. The maximum coloring zone was developed in T-1 (22mm) where as minimum was in T-3 (4 mm) in case of the *C. polygonoides* associated bacterial community. The coloring zone was found maximum in TS-2 (27 mm) and minimum in TS-1 (11 mm), in case of isolates associated with rhizosphere of *L. sindicus*. The highest and lowest acetylene reduction activity (ARA) was detected in TS-13 (8303 nmoles.24 h^{-1}) and T-10 (1658 nmoles.24 h^{-1}), respectively.

Azin *et al.* (2005) isolated rhizobacteria from oil palm rhizosphere, rhizoplane and inner root tissues. Identification of isolates was done by morphological biochemical and physiological characterization. The isolates were successfully identified as *Paenibacillus durus, Paenibacillus polymyxa, Azospirillum lipoferum, Herbaspirillum, Acetobacter.* Acetylene reduction assay was used for quantifying the isolates for fix atmospheric N_2 fixation. The nitrogen fixing capacities of the isolates ranged from 7.0 $x10^{-12}$ to 1.0 $x10^{-8}$ C_2H_4 $cfu^{-1}h^{-1}$.

David *et al.* (1980) studied acetylene reduction assay for Nitrogenase activity by using Gas Chromatography. Nitrogenase was assayed with the cyanobacterium

Anabaena L-31. In addition, nodules excised from a local variety of peanut *(Arachis hypogea* var. TG-1) were incubated for 30 min at 25^0C in 5-ml serum vials containing a gas phase of air and C_2H_2 (0.1atm) to determine nitrogenase activity. They used ammonical silver nitrate (10 $mg.ml^{-1}$) to terminate acetylene reduction assay to measure nitrogenase activity. Silver nitrate quantitatively precipitated acetylene as the carbide salt, but did not affect the ethylene form.

Murumkar *et al*. (2013) isolated 93 *Azospirillum* isolates and reported the *A. lipoferum* isolates exhibited a higher average of nitrogenase activity compared to *A. brasilense* isolates (105.9 vs. 20.8 nmol C_2H_4.mg $protein^{-1}.h^{-1}$, respectively). Based on the nitrogenase activity, seven highly efficient *Azospirillum i*solates along with MPKV strain *Asp*-BNF were studied for their genetic variability by employing RAPD-PCR technique. On the basis of UPGMA clustering analysis, the *A.lipoferum* isolates were classified into four broad clusters and the genetic variation observed among *A.lipoferum* isolates was due to domestication of the isolates in different agro-ecological regions as well as their nitrogenase activity. Based on nitrogen-fixing ability, highly efficient *Azospirillum* isolates may be further exploited in biofertilizer production.

2.1.3 Production of Plant Growth Promoting Substances (PGPS)

Kaur and Sharma (2013) isolated 35 rhizobacterial isolates. Ten isolates were identified and characterized as *Pseudomonas* sp. These were screened for growth promotion activities,production of indole acetic acid (IAA), ammonia (NH3), hydrogen cyanide (HCN), siderophore, phosphate (P) solubilization, catalase, antibiotic resistance spectra and seed germination on water agar medium along with reference strain PGPR LK884 (*Pseudomonas diminuta*). Maximum amount of IAA was produced by PGPR-3 (70.05 $\mu g.ml^{-1}$) followed by PGPR-2 (66.79 $\mu g.ml^{-1}$) as compared to PGPR LK 884 (61.58 µg/ml) in the presence of L-Tryptophan as precursor of IAA. 70% of isolates showed capacity for Phosphate solubilization in the range of 5.08 to 13.45 mg/100 ml. Maximum P-solubilization was noticed with PGPR-3 (13.45 mg /100 ml) followed by PGPR-2 (13.15 mg/100 ml). Two isolates of *Pseudomonas* sp. PGPR-2 and PGPR-3 also produced siderophores, HCN, NH_3 and improved seed germination in *kabuli* and *desi* chickpea**.**

Twenty four PGP Rhizobacterial isolates were isolated by Yasmin *et al.* (2013) from rhizosphere soil of maize. Most efficient bacterial isolates were screened on the basis of their positive activity for siderophore production, P-solubilization and bacteriocin production. The PGPR isolate 9K showed maximum P-solubilization index.

Siderophore production was exhibited by 1K and KB. The PGPR isolates1K, KB and 9K were selected for re-inoculation studies on maize under induced drought stress condition. The PGPR isolate 9K increased drought tolerance in maize plants by enhancing root proliferation and improving relative water content of leaves, the root to shoot dry weight ratio increased significantly by 26% as compared to inoculated control.

Hussain and Srinivas (2013) isolated 35 isolates of fluorescent *Pseudomonas* and *Azotobacter* each from rhizosphere of *Acacia nilotica* and *Albizia lebbeck*. These isolates were studied for their ability to produce different plant growth promoting traits. More than 70% of the isolates of *Azotobacter* and fluorescent *Pseudomonads* produced IAA. High Gibberillic acid production was detected in *Azotobacter* (71.42%) isolates. Higher phosphate solubilization was detected in the isolates of *Azotobacter* (74.28%) followed by *Pseudomonas* (63.00%). Siderophore production was seen in 81.42% of isolates. HCN production was higher in traits of *Azotobacter* (77.00%). On the basis of multiple plant growth promoting activities, nine bacterial isolates (six *Azotobacter* and three *Pseudomonas)* were evaluated for their quantitative IAA production using different substrates. IAA production was highest in the *Pseudomonas* and *Azotobacter* when tryptophan was used as substrate and IAA production was least when ethanol was used.

Singh *et al.* (2013) had reported that most PGPR belong to gram negative genera, and the greatest numbers of strains are members of the *fluorescent Pseudomonads* and isolated *Pseudomonas spp.* from nine different rhizospheric soils of wheat & pigeon pea. Among the 21 *Pseudomonas spp.* four different *Pseudomonas spp.* isolates (YSY-13, YSY-15, YSY-17 and YSY-19) exhibited maximum Plant growth promoting and heavy metal tolerant activities.

Thirty *Bacillus* cultures were isolated by Sarvani and Reddy (2012) from the rhizospheric soils of groundnut and red gram crops growing in Rangareddy district, India and identified as *Bacillus* spp. based on their colony morphology, cell morphology and biochemical characteristics. These were screened for PGP attributes and antagonism against soil borne phytopathogens viz., *Sclerotium rolfsii*, *Rhizoctonia solani* and *Fusarium solani*. Results revealed that 50% (15/30 isolates) reacted positively for one or more PGP properties. A high prevalence of antagonists was found against the three fungal pathogens. Majority of the bacterial isolates (33%) displayed antagonism through the production of siderophores / HCN and the remaining isolates showed antagonism with negative results for siderophores and HCN indicating that, the rhizospheric soils are the rich source of *Bacillus* fungal antagonists, which have a potential to be used in the future as PGP inoculants.

Rhizobacteria were found in the rhizosphere of plants and are beneficial for plant development, they promote plant growth by increasing the availability of nutrients, increasing the uptake of nitrogen, phosphorus etc. described by Ram and Singh (2012). PGPR help plants directly by secreting phytohormones and some other plant growth-promoting substances and are used as bio fertilizers. PGPR also protects plants against pathogens by exploiting their antagonistic potential. Some species of PGPR help plants by exploring root growth for their stability in stressed conditions.

Sixty five bacteria were isolated and characterized by their morphological, cultural, and staining and biochemical characteristics, of which 35 were selected for the screening of PGPR isolates. Sixteen isolates were successfully characterized for the PGPR traits like indole acetic acid (IAA) production, phosphorus solubilization and production of enzymes like urease, chitinase, amylase, cellulase, protease and β-1,3 glucanase (Rani *et al*. 2012). These were further investigated to show the PGPR traits in pigeon pea seedling emergence, increase of shoot length, root length, dry matter production of shoot, nodule number and nodule mass.

Twenty *Pseudomonad* strains were isolated by Noori and Saud (2012) from the rhizosphere soils of paddy and were screened for their plant growth promoting activity. All the 20 tested isolates of *Pseudomonads* were positive for the production of siderophores and HCN, while of the 20 antagonist bacteria strains, 15 strains (75%) showed positive for the production of Plant growth promoting hormone, IAA.

IAA producing bacterium was isolated by Sudha *et al.* (2012) from rhizosphere soil and identified as *Rhizobium spp.* and *Bacillus spp.* Optimization of indole acetic acid production was carried out at different cultural conditions, such as pH, temperature and substrate with *Rhizobium spp. Bacillus spp.* and *Rhizobium spp*. produced higher amount of indole acetic acid (6.1 mgmL^{-1}) than the *Bacillus spp*. (4.4mg mL^{-1}) at pH 7 and 37^{0}c in the Bengal gram substrate. Partial purification of indole acetic acid was done by thin layer chromatography (TLC). *Rhizobium spp*. appears to be a suitable soil microorganism for high level of IAA production.

Sakthivel and Karthikeyan (2012) obtained 30 bacterial isolates from *Coleus forskohlii* rhizospheric soil. All the isolates were identified as *Azospirillum* spp., *Bacillus* spp., *Pseudomonas* spp. and *Azotobacter* spp. These bacterial strains were tested on morphological, biochemical and screened for their direct growth promoting activities (IAA production, production of ammonia and phosphate solubilization) and indirect growth promoting activities (HCN production, Siderophore production).

Ponmurugan *et al.* (2012) isolated ten *Azotobacter spp.* from 150 rhizospheric soils of vegetable plants and screened multiple plant growth promoting activities of *Azotobacter* isolates were determined in terms of IAA production, NH_3 release, PO_4 solubilization, HCN and siderophore production. Antifungal activity of the *Azotobacter* was determined against *Aspergillus flavus, Cercospora* spp. and *Fusarium oxysporum* and found higher zone of inhibition (18-26 mm) at higher concentration of culture suspension.

Ashraf *et al.* (2011) isolated twelve bacterial strains from root and rhizosphere samples collected from different sugarcane growing areas. Ten strains were identified as *Pseudomonas* and two as *Azotobacter* on the basis of colony and cell-morphology. All isolates showed IAA production in growth medium containing tryptophane as a precursor. Though IAA production ($4.49 mg.l^{-1}$) was detected in isolate A17 where as IAA production in strains A4 and A11 was also significant. Most of the strains showed beneficial effects on root length, root area and plant dry weight which were comparable to those observed in treatments where confirmed PGPR were used as positive control.

Supraja *et al.* (2011) isolated fifteen fluorescent *Pseudomonas* isolates from rhizospheric soils of redgram and maize crops in the Rangareddy district. These test isolates were biochemically characterized and screened *in vitro* for their plant growth promoting traits like phosphate solubilization, production of indole acetic acid (IAA), Hydrogen cyanide (HCN) and siderophore. Due to production of HCN and siderophores, fluorescent *Pseudomonas* isolates inhibited the growth of *Fusarium moniliforme*. All the 15 isolates inhibited the growth of fungal pathogen except MPF-1. Among the 14 isolates, RPF-5 (46.30%) showed highest percent inhibition zone. This study suggested that a few isolates of the fluorescent *Pseudomonas* could be potential biocontrol agents against *F. moniliforme* based on the studies conducted *in vitro*.

Six bacteria were isolated from different rhizospheric soils and were tested for the production of IAA in a medium with tryptophan. A low amount of IAA production was recorded by *Azotobacter* strain without tryptophan addition. Production of IAA in *Azotobacter* increased with increase in tryptophan concentration from 1 to 5 mg/ml. Production of IAA was further confirmed by 3 isolates of *Azotobacter* (Azb3, Azb5 Azb7) and subsequently by TLC analysis. *Azotobacter* isolates (Azb3, Azb5 Azb7) showed inhibitory effects on the growth of root elongation at all concentrations of tryptophan compared to control. On the other hand, high concentration of exogenous tryptophan could exhibit toxic effects on plant growth (Patil, 2011).

Number of bacterial species inhabiting rhizosphere are known to exert beneficial effects on plant growth (Ahemad *et al.* 2011). Such rhizobacteria must colonize the root surface efficiently and facilitate the plant growth through nitrogen fixation, solubilization of insoluble phosphorus, production of siderophores and production of phytohormones, lowering of ethylene concentration, production of antibiotics, antifungal metabolites and induced systemic resistance.

Yadav *et al.* (2010) reported five isolates of PGPR designated as *Pseudomonas aeruginosa* strain BHUPSB02, *Pseudomonas putida* strain BHUPSB04, *Bacillus subtilis* strain BHUPSB13, *Paenibacillus polymyxa* strain BHUPSB17 and *Bacillus boronophilus* strain BHUPSB19 were successfully isolated. An experiment was conducted under plant growth chamber where chickpea plants were grown in plastic cups containing soil and mixed with isolates of PGPR to investigate the effect of PGPR on the growth of chickpea plant. Isolates of PGPR induced production of plant hormones (indole acetic acid), phosphate solubilization and ammonia production and enhanced plant growth. Most of isolates resulted in a significant increase in shoot length, root length and dry matter production of shoot and root of chickpea seedlings.

Psedomonas fluorescens strain was isolated by Upadhyay and Srivastava (2010) and it was reported that this strain solubilized complexed phosphates and also synthesized phytohormone (IAA).

Verma *et al.* (2010) reported that co-inoculation of the *Rhizobium* sp. BHURCO1 and *Pseudomonas fluorescens* could be an effective biofertilizer for chickpea (*Cicer arietinum* L.) production as both isolates showed PGP properties.

Isolated ten isolates of bacteria designated as PGB1, PGB2, PGB3, PGB4, PGB5, PGT1, PGT2, PGT3, PGG1 and PGG2. Mishra *et al.* (2010) reported that isolates PGPB4, PGT1, PGT2, PGT3, PGG1 and PGG2 induced production of indole acetic acid, but PGT3 was able to solubilize phosphorus.

Mehta *et al.* (2010) selected a highly efficient P-solubilizing strain, presumptively, identified as *Bacillus circulans* MTCC 8983 and screened for plant growth promoting traits. They reported that the strain solubilised tricalcium phosphate and produced substantial amount of soluble phosphorus (957.3 mg lit^{-1}) in Pikovskaya's (PVK) broth and exhibited the production of indole acetic acid (15.13μg ml^{-1}), siderophores (57.80%) and growth inhibition against *Dermatophora necatrix* (46.57%).

Plant growth promoting activities of forty different strains of *Pseudomonas fluorescens* and *Pseudomonas putida,* isolated from the rhizosphere of wheat (*Triticum aestivum* L.) and canola (*Brassica napus* L.) were reported by Abbas *et al*. (2010). Most of the bacterial isolates were able to produce IAA, HCN, siderophores and also solubilised phosphorus.

Ashrafuzzamen *et al.* (2009) isolated ten isolates of bacteria and characterized to investigate the effects of PGPR isolates on the growth of rice. They conducted a pot culture experiment, prior to seeds grown in plastic pots; seeds were treated with PGPR isolates. PGB4 and PGG2 were found almost equally better in all aspects such as dry matter production, plant height and root length of rice and IAA production. Isolate PGT3 was also found to be promising in IAA production having an additional property of phosphate solubilization.

Eight bacteria isolated by Gangwar and Kaur (2009) from sugarcane (*Saccharum spp.*), seven from rye grass (*Lolium perenne*) and identified as *Azospirillum, Bacillus, E. coli* and *Pseudomonas*. These isolates were screened *in vitro* for plant growth promoting traits and they reported that maximum IAA production was recorded in S5 (*E. coli)* and R7 (*Bacillus*) i.e. 19.3 and 20.0 mg ml^{-1} respectively. The maximum siderophore production was observed in S6 (*E.coli*) and R6 (*Pseudomonas*) i.e. 2.4 and 3.0 mg ml^{-1} respectively. Only S5 (*E.coli*) was observed to solubilize phosphate (21 mg P).

Karnwal (2009) isolated 30 *fluorescent Pseudomonads* from different plants rhizosphere and were characterized on the basis of plant growth-promoting activities. These isolates were tested for their ability to produce indole acetic acid in pure culture in the absence and presence of L-tryptophan at 50, 100, 200 and 500µg.ml^{-1}. For both strains, indole production increased with increases in tryptophan concentration (0.5, 1.2, 4.3 and 9.3 µg/ml; and 0.2, 0.7, 3.8, and 8.3 µg.ml^{-1}, respectively). *P. aeruginosa* AK2 was less effective in production of indole acetic acid than *P. fluorescens* AK1. Inoculation of rice seeds with *P. fluorescens* AK1 and *P. aeruginosa* AK2 showed a good level (2.30 pmol.ml^{-1} and 2.1 pmol.ml^{-1}) of indole acetic acid compared to uninoculated seeds (1.6 pmol.ml^{-1}).

Farah and Iqbal (2008) isolated a total of 72 bacteria belonging to *Azotobacter, Pseudomonas, Mesorhizobium* and *Bacillus* from different rhizospheric soils. More than 80% of the isolates of *Azotobacter, Pseudomonas* and *Mesorhizobium ciceri* produced IAA, whereas only 20% of *Bacillus* isolates were IAA produces. These test isolates were biochemically characterized and were screened *in vitro* for their plant growth promoting traits like production of indole acetic acid, ammonia, hydrogen cyanide, siderophore, phosphate solubilization and anti fungal activity.

2.2 EFFICIENT PGPR ISOLATES WITH BIO CONTROL ACTIVITY

2.2.1 Production of Siderophores

Sreedevi *et al.* (2014) isolated ten *Pseudomonas* sp. Among isolated strains, three *Pseudomonas* isolates P1, P2 and P3 showed siderophore production on succinic acid medium and chromo azural S agar plate medium. The ability of *Pseudomonas* to grow and to produce siderophores is dependent on the iron content and the type of carbon sources in the medium. Four basal media, supplemented with different concentration of iron, were employed to study the effect of iron and different organic carbon sources on siderophore production in *Pseudomonas* isolates. Maximum siderophore production was 94, 88, 83 units for P1, P2and P3 isolates respectively. All three isolates have showed both type of siderophore production i.e. wine red color formation in supernatant indicated production of hydroxamate type (pyoverdine) while yellow color formation in supernatant showed presence of catecholate or phenolate type (pyochelin) siderophore.

Tailor and Joshi (2012) isolated seven bacterial isolates from sugarcane rhizosphere. All the isolates were found to produce more than 85% siderophore units. Among them S-11 was found be the most efficient siderophore producer (96 % SU). S-11 was further characterized and identified as *Pseudomonas fluorescens.* Physico-chemical parameters were evaluated for optimum production of siderophores by *Pseudomonas fluorescens* strain.

Twenty four hydrogen cyanide (HCN) producing *fluorescent Pseudomonads* isolated by Jayaprakashvel *et al.* (2010) from the rhizosphere of sand dune vegetation from Chennai coastal area and were subjected to spectrophotometric assay to relatively quantify the amount of hydrogen cyanide (HCN) produced by them. Five FP strains designated as AMET1039, AMET1041, AMET1042, AMET1055 and AMET1064 produced more amount of HCN in their volatile fraction.

2.2.2 HCN Production

Konineeka and Chandan (2011) maintaining marketable status of fruits using Plant Growth Promoting Rhizobacteria (PGPR) in combination with kinetin and gibberellic acid to ensure biosafe food from the health standpoint that has yielded significant results. They isolated three wild type PGPR strains viz. PGPR1, PGPR2 and PGPR3 from the endophytic regions of pointed gourd crop. The strains were capable of producing Indole -3-acetic acid and HCN. Isolate 1 produced about 16µg.ml^{-1} IAA and was a weak producer of HCN ,while isolate 2 produced 23 g.ml^{-1} IAA and was capable of producing large amount of HCN (filter paper turned dark brown) whereas PGPR 3 released 30µg.ml^{-1} IAA and moderate amount of HCN.

Out of 144 bacteria from cucumber rhizosphere, eight isolates were identified as *Pseudomonas fluorescens*. Maleki *et al.* (2010) reported that these isolates were selected for root colonization and PGP properties. Among these CV-6 strain produced considerable amounts of siderophore, indole acetic acid and also shown positive reactions for HCN, catalase, protease and phosphatase.

Ahmadzadeh and Sharirifi-tehrani (2009) detected the production of HCN by six isolates out of the 41 selected fluorescent *Pseudomonads* having good antagonistic activity against *Rhizoctonia solani* under *in vitro* conditions.

Jha *et al.* (2009) reported the production of HCN by some new *fluorescent Pseudomonad* strains having biocontrol activity against phytopathogenic fungi in addition to the P-solubilization efficiency and stated that they can be used as biofertilizers as well as biocontrol agents.

Seven *fluorescent Pseudomonads* isolated from the roots, shoots and rhizosphere soil of sugarcane were shown to produce HCN which had biocontrol ability against *Colletotrichum falcatum* inciting red rot disease in sugarcane in addition to their ability to plant growth (Mehnaz *et al.* 2009).

2.2.3 Antagonistic Activity

Kumar *et al.*(2012) isolated seven bacterial isolates from rhizosphere of common bean growing at Uttarakhand, Himalaya and based on 16S rRNA gene sequence the isolate BPR7 was identified as *Bacillus* sp. BPR7. Strain BPR7 strongly inhibited the growth of several phytopathogens such as *Macrophomina phaseolina, Fusarium oxysporum, F. solani, Sclerotinia sclerotiorum, Rhizoctonia solani* and *Colletotricum* sp. *in vitro*. Cell-free culture filtrate of strain BPR7 also caused colony growth inhibition of all test pathogens. PGP and antifungal activities of *Bacillus* sp. BPR7 suggest that it may be exploited as a potential bioinoculant agent for *P. vulgaris*

Plant Growth Promoting Rhizobacteria (PGPR) consist a wide range of beneficial soil bacteria inhabiting rhizosphere of plant. *Pseudomonads* were the most important Plant growth promoting rhizosphere bacteria in different crop plants. Soltani *et al.* (2012) reported three isolates (PA24, PA1 and PA18) of *Pseudomonads* showed *in vitro* antifungal activity against *Rhizoctonia solani*. Results of this study showed that *Pseudomonads* which are native to soils of Iran had the potential to be used for promotion of plant growth and suppression of soil-borne plant pathogens.

Singh and Yami (2010) studied 103 macroscopically different bacterial isolates from 21 different rhizosphere soil samples. Isolates were screened for antagonism against five fungal phytopathogens, viz, *Fusarium oxysporum, Alternaria solani, Sclerotium rolfsii, Exserohilum turcicum* and *Phytophthora infestans* by dual culture technique on Potato dextrose agar with diffusion assay. Based on chitinolytic potential of *Bacillu subtilis* isolate showed maximum % inhibition of 53.29% with *Fusarium oxysporum* , 41.23 % with *Alternaria solani* , 28.41% with *Sclerotium rolfsii,,* 15.24 % with *Exserohilum turcicum* and no inhibition with *Phytophthora infestans*. Among the phytopathogens tested, sensitivity of *Bacillus subtilis* to fungi containing chitin on their cell wall demonstrates the possible role of chitinase in the antifungal activity.

Aris Tri Wahyudi et *al.* (2011) isolated fourteen isolates of *Pseudomonas* sp from soybeans rhizosphere and identified with various tests for the determination of the growth promoter were based on IAA production, phosphate solubilization and growth promoter of length of root and stems and number of lateral roots of soybean sprouts. Test of siderophore, chitinase, as well as anti anti-fungal compounds productions to inhibit the growth of *Fusarium oxysporum, Rhizoctonia solani* and *Sclerotium rolfsii,* were used as a biocontrol agent determination. On the basis of excellent growth promotion and biocontrol activities they recommended 5 isolates of *Pseudomonas* sp which were Crb-3, Crb-16, Crb-17, Crb-44 and Crb-94 as potential isolates of *Pseudomonas* sp that could be applied as inoculants of soybean plant.

Pseudomonas spp. isolated by Nicolas (2011) from rhizospheric soil of tomato and pepper plants, was evaluated as potential antagonist of fungal pathogens and tested against the causal agents of stem canker and leaf blight (*Alternaria alternata* f. *spp. lycopersici*), southern blight (*Sclerotium rolfsii*) and root rot (*Fusarium solani*). All strains significantly inhibited *Alternaria alternata* f. *spp. lycopersici*, particularly in 25% TSA medium. antagonistic effect on *Sclerotium rolfsii* Sacc. and *Fusarium solani* was greater on Kings B medium.

Eleven *Pseudomonas* spp. were isolated by Rakh *et al.* (2011) from rhizospheric soil and tested for their antagonistic activity against *Sclerotium rolfsii*. A soil bacterium identified as, *Pseudomonas cf. monteilii 9,* showed highest antagonistic activity against *Sclerotium rolfsii*. In dual cultures, the *Pseudomonas cf. monteilii 9* inhibited the *Sclerotium rolfsii* up to 94 % in terms of dry weight. *Pseudomonas cf. monteilii 9* produced diffusible antibiotic, volatile metabolites, hydrogen cyanide and siderophores which affected *Sclerotium rolfsii* growth.

Singhai *et al.* (2011) used four *Pseudomonas* strains with or without vermin compost amendment to see their performance on potato plant growth and yield along with suppression of common scab of potato. The *Pseudomonas* strain R1 when applied with vermicompost gave the best plant growth and yield along with maximum reduction in scab incidence and scab index and it was later identified as *Pseudomonas mosselii*. The results revealed the potential of *P. mosselii* strain R1 in promoting plant growth as well as inducing antimicrobial mechanisms systemically in the host plants facilitated by the organic amendment in the form of vermicompost.

Manjunatha *et al.* (2010) total of 92 isolates of fluorescent *Pseudomonads* were collected from the rhizosphere soil of chilli, sunflower, redgram, groundnut, field bean, greengram, brinjal, tomato, burmuda grass, beans, sorghum, paddy and sesame .Among two potential antagonistic isolates viz., *P. fluorescens* (Pf4 and Pf6) used for evaluating biocontrol efficacy. Among two isolates *P. fluorescens* (Pf4 and Pf6), Pf4 showed maximum inhibition of mycelial growth of *Macrophomina phaseolina* (21.30%), *Rhizoctoinia bataticola* (24.07%), *Rhizoctonia solani* (32.96%), *F. oxysporum*.f. sp.*udum* (48.14%), *C. gloeosporioides* (41.67%), *F.solani* (24.07%), *Sclerotium rolfsii* (40.74%), *Cercospora capsici* (21.67%), *Alternaria sesami* (20.00%) and *Xanthomonas axonopodis pv. punicae* (1.84 mm) through dual plate technique thus proved to be broad spectrum.

Akhtar *et al.* (2010) studied the effects of *Bacillus pumilus, Pseudomonas alcaligenes,* and *Rhizobium spp.* on wilt disease caused by *Fusarium oxysporum f. spp. lentis* and on the growth of lentil. They reported that combined application of *B. pumilus and P. alcaligenes* with *Rhizobium spp.* resulted in the greatest increase in plant growth, number of pods, nodulation and root colonization by rhizobacteria, and also reduced wilting in *Fusarium*-inoculated plants.

Abeysinghe (2009) used a combination of two compatible biological agents, *Bacillus subtilis* CA32 and *Trichoderma harzi*anum RU01, both antagonistic to the pathogen *Rhizoctonia solani*, to control damping off in *Solanum melongena* and *Capsicum annuum*.

Seven PGPR strains were isolated by Fatima *et al.* (2009) from the rhizoplane and rhizosphere of wheat. These strains were analyzed for production of indole acetic acid (IAA), phosphorous solublization capability and inhibition of *Rhizoctonia solani*. Strains WPR-51, WPR-42 and WM-30 were selected to test antagonistic activity on two wheat varieties infected with *R. solani*. These three strains belonged to *Azotobacter* and *Azospirillum*. Out of these three strains, WPR-51 and mixture of all three strains showed maximum inhibition of *R. solani* growth.

2.3 MOLECULAR CHARACTERIZATION OF EFFICIENT PGPR STRAINS

Mahbouba *et al.* (2013) isolated 10 strains (Azo4, Azo5, Azo6, Azo7, Azo8, Azo10, S1, S2, S3 and S9) and identified. These isolates were screened *in vitro* for their growth promoting traits. The molecular identification was done by simple PCR to amplification for 16S rDNA gene using primers AZ16S-D. Some strains (Azo4, Azo5, Azo6, Azo7, Azo8 and Azo10) have high identity with genus *Azospirillum* which indicated that these isolates belong to the *Azospirillum* genus, especially to species of *Azospirillum brasilense* and nested PCR approach performed with degenerate primers was used to amplify *nif*H gene fragments from the bulk DNA. The amplification product bands at the expected *nif*H gene fragment size are about 370 bp. Some isolates (S1, S2, S3, Azo4, Azo5, Azo6, S9 and Azo10) were considered by the nitrogen-fixing gene *nif*H detection of their genome and *A. brasilense* inoculated durum wheat shows good growth and yield.

Patel *et al.* (2013) isolated 35 free living nitrogen fixing bacterial isolates from different regions of North Gujarat. One individual isolate was selected for the molecular characterization and phylogenetic relatedness. The biochemical and molecular characterization of the isolate was carried out. The bacterial isolate was identified as *Azotobacter salinestris*. The phylogenetic tree was constructed and it showed strong homology of the isolate with *Azotobacter salinestris*.

Khider *et al*. (2012) compared classical approach with molecular based method for identification of *Azotobacter chroococcum* from soil samples. *A. chroococcum* was isolated from soil source in Erbil city, Iraq. All the soil samples showed similar biochemical, morphological characters of *A.chroococcum* and molecular characterization based on detection of *nif genes* have been successfully applied to describe *A. chroococcum isolated* from soil. The PCR products for *nif* H1 1102bp, *nif*H2 246bp, *nif*H3 128bp, *nif*U 930bp, *nif*V 1146bp and VF gene 594bp were detected on gel electrophoresis, and revealed that the isolated bacteria *A. chroococcum* belonged to genus *Azotobacter*.

Gosal *et al*. (2011) isolated 72 diazotrophic bacteria from wheat rhizospheric soil. The isolates were identified on the basis of cultural, morphological and biochemical characterization. These isolates showed amplification with two *nifH* primers (*nifH1*and *nifH2*), there by confirming their diazotrophic potential. The positive *nifH* isolates were further characterized using restriction fragment length polymorphism of 16S rDNA to reveal diversity among them based on UPGMA clustering and partial sequencing of 16S rDNA the isolates were identified as *Azotobacter* sp., *Azospirillum* sp., *Rhizobium larrymoorei*, *Pseudomonas aeruginosa* and *Xanthomonas oryzae.*

Shiva Reddy *et al.* (2010) isolated *Bacillus megaterium* from soil of different Agro climatic zones of Karnataka and identified. Molecular characterization of the *Bacillus megaterium* isolates was done using RAPD technique and investigates the effect of *Bacillus megaterium* on seed germination of *Sesamum indicum* and on plant growth was carried out.

Rajeswari and Kasthuri (2009) studied the *nifH* gene sequence of the nitrogen-fixing bacterium *Azotobacter* spp. which was determined with the use of polymerase chain reaction (PCR). The *Azotobacter* species was isolated from marine source in two different seasons. They were cultivated under laboratory conditions using nitrogen free *Azotobacter* specific medium. The phylogenetic tree revealed that isolated *Azotobacter* spp. was distantly related to uncultivated and uncultured organisms. They did not form any branch with other *Azotobacter* spp. in the data base.

Sajjad Mirza *et al.* (2006) isolated a nitrogen-fixing phytohormone producing bacterial isolate from kallar grass (strain K1). It was was identified as *Pseudomonas* spp. by 16S ribosomal RNA gene sequence analysis and rrs identity level was high with an uncharacterized marine bacterium (99%), *Pseudomonas* sp. PCP2(98%), uncultured bacteria (98%), and *Pseudomonas alcaligenes* (97%). Partial *nifH* gene amplified from strainK1 showed 93% and 91% sequence similarities to those of *Azotobacter chroococcum* and *Pseudomonas stutzeri*, respectively.

Potrich *et al.* (2001) studied *Azospirillum amazonense* genomic organization patterns of the nitrogen fixation genes similar to those of the distantly related species *A. brasilense*. Our work suggests that *A. brasilense nifHDK*, *nifENX*, *fixABC* operons and *nifA* and *glnB* genes may be structurally homologous to the counterpart genes of *A. amazonense*. This was the first analysis revealing homology between *A. brasilense nif* genes and the *A. amazonense* genome. Sequence analysis of PCR amplification products revealed similarities between the amino acid sequences of the highly conserved *nifD* and *glnB* genes of *A. amazonense* and related genes of *A. brasilense* and other bacteria. However, 16S ribosomal RNA gene-based PCR system for specific detection of the *A.amazonense* non-coding regions (the upstream activator sequence region and the region between the *nifH* and *nifD* genes) differed from related regions of *A. brasilense* even in nitrogenase structural genes which are highly conserved among diazotrophic bacteria.

Kumar And Kannaiyan were investigated a substantial molecular diversity of N_2 fixing bacteria has been detected in field grown rice based on retrieval of *nif H* or *nif D* gene fragments from root DNA. Targeted PCR finger printing of heterotrophic and endophytic diazotrophs from rice, using *nif H* primer, generated specific replicon with a molecular weight of approximately 750 bp. However, multiple replicons with molecular weight ranging from 500-1500 bp were observed in some isolates. The existence of considerable genetic and molecular diversity in the diazotrophic bacteria of rice and the scope for its better exploitation to achieve sustainable rice production.

2.4 TO ASSESS COMPATIBILITY OF PGPR ISOLATES WITH AGROCHEMICALS

Maheswari (2013) isolated fifteen fluorescent *Pseudomonads* isolates from the rhizosphere and rhizoplane of plant roots. A compatibility of isolates with fungicides is of concern. Except FP-I isolate at 500 and 750 ppm concentrations of propiconazole, growth of all the fifteen isolates was observed in all the evaluated concentrations of systemic fungicides. Whereas, the detrimental effect on growth of isolates was found in non systemic fungicides. Copper oxychloride checked the growth of eleven isolates at 3000 ppm concentration and seven isolates at 2000 ppm. Growth of isolate FP-VI was checked at all the three concentrations of mancozeb and copper oxychloride. Only isolates FP-III, FP-IV, FP-XII and FP-XIII were found to be grown on all the tested fungicides and their respective concentrations. All the isolates were found to be compatible with thiram fungicide, commonly recommended for seed treatment.

Hefnawy *et al.* (2012) concluded that *Aspergillus niger* and *Aspergillus fumigatus* were not affected at lower concentrations of tested herbicides i.e. glyphosate and putraline up to 200 mg l^{-1} and at 800 mg l^{-1} phosphate solubilization by *Aspergillus niger* and *A. fumigates* decreased approximately 30% and 85% respectively.

Sarvani *et al*.(2011) isolated and characterized bacterial isolates as *Rhizobium, Pseudomonas* and *Bacillus* isolates based on cultural, morphological and biochemical characters. These were previously studied for PGP attributes including biocontrol activity. The PGP isolates having biocontrol activity were selected further to know the compatibility against commonly used agrochemicals like fungicides (copper oxy chloride, carbendazim, thiram and captan) insecticides (phorate, carbofuran, imidachloprid and chlorpyriphos) and herbicides (alachlor, butachlor, pendimethalin and oxy fluorofen) at their recommended and half the recommended dosages. Results revealed that, majority of the isolates found to be compatible with the agrochemicals used at their recommended and half the recommended dosages.

Vijay Krishna Kumar *et al.* (2011) studied the growth promoting activities of commercial formulation of a bioagent, *Bacillus subtilis* MBI 600(Integral®) and its compatibility with rice fungicides were evaluated. Integral has good tolerance to hexaconazole, propiconazole, and validamycin; moderate tolerance to tricyclazole; and poor tolerance to benomyl and mancozeb at 1000 ppm. Integral showed compatibility to carbendazim and azoxystrobin up to 400 ppm. Overall, our results suggest that Integral produces siderophores, promoted rice seedling emergence and growth, and is compatible with rice fungicides.

Vimal and Patel (2011) reported that pesticides viz., carbendazim, monocrotophos and chlorpyriphos increased the phosphate solubilizing activity of *Bacillus sphaericus* and *Burkholderia cepacia* at their recommended dose.

Kumar *et al.* (2011) reported that *Bacillus subtilis* showed good tolerance to hexaconazole, propiconazole, and validamycin, moderate tolerance to tricyclazole and poor tolerance to benomyl and mancozeb at 1000 ppm. Integral showed compatibility to carbendazim and azoxystrobin up to 400 ppm.

Koche (2011) reported that the incorporation of metalaxyl, Fosetyl–Al, COC and Bordeaux mixture in growth medium of *P. fluorescens* affected its growth. COC and B.M. was less compatible with all *Pseudomonas* isolates which may be due to the presence of antibacterial properties in both fungicides as compared to metalaxyl and Fosetyl-Al. Maximum growth of Pf_{15} was observed in metalaxyl (0.2%) (35.33 $cfu.ml^{-1}$). Whereas Pf_9 (37.33 $cfu.ml^{-1}$) was most compatible to Fosetyl-Al followed by Pf_{10} (35.00 $cfu.ml^{-1}$).

Ingle and Desmukh (2010) isolated plant growth promoting rhizobacteria from cotton rhizospheric soils and see the sensitivity of rhizobacteria with commonly used agrichemicals such as carbendazim, carbaryl, copper oxy chloride, thiram, vitavax, paushamycin was tested turbidometrically at 620 nm. PGPR was found to be sensitive to carbaryl 50WP%@0.3 % (CoRb- 1, 12.0%), vitavax @ 0.25% (CoRb-8, 20.4%), thiram @ 0.3% (CoRb-3, 44.6%), carbendazim (CoRb -1, 0.1%), copper oxychloride@ 0.3% (CoRb-6, 27.5%). Antibiotic, pausharnycin @ 250ppm showed maximum inhibition of growth of PGPR isolates. Maximum suppression in growth was observed in CoRb -2 (48.2%) at 72 hrs in COC at 0.3%.

Balamuragan *et al.* (2010) isolated 25 phosphate solubilising bacteria of tea garden soil, screened *in vitro* and studied its bioecology. The biochemical characterization of the isolates showed more closeness to *Pseudomonas* sp. Isolates tested with agrochemicals, hexaconazole fungicide treated soil and registered higher PSB population followed by untreated soil. It indicates PSB might have utilized the compounds present in the fungicide for its establishment in soil. This study revealed the compatibility or tolerance level of PSB beneficial organisms with different agrochemicals in tea soil.

Ahemad and Khan (2010) studied the effect of 4 herbicides Quizalfop-p-ethyl, clodinafop, metribuzin and glyphosate on plant growth promoting activities like phosphate solubilization, siderophore, IAA, and HCN, ammonia by herbicide tolerant *Enterobacteria asburial* strain PS2 isolated from mustard rhizosphere. The herbicide at recommended dose had less inhibitory effect while the dose higher than the recommended one adversely affected the plant growth promoting traits of *Enterobacteria asburial* strain PS2. Among all herbicides Quizalfop-p-ethyl generally showed maximum toxicity to plant growth promoting activities of this bacterium.

Insecticides endosulfan @ 15 ml kg^{-1} and chlorpyriphos @ 10 ml kg^{-1} were reported to be compatible with recommended fungicide captan and *Rhizobium* inoculant when applied as seed treatment for the control of termites in chickpea (*Cicer arietinum* L.) (Cheema *et al.* 2009).

Nongthombam *et al.* (2008) reported that application of systemic pre emergence herbicides, viz., anilofos and pendimethalin alone and in combination at their recommended rates increased efficiency of phosphate solubilizing microorganisms resulting in increased availability of phosphorus in soil.

Ahmed *et al.* (2007) studied the effect of different concentrations (0, 10, 20, 50, 100, 200, 500 and 1000 micro g lit^{-1}) of the fungicides Captan, Thiram, Luxan, Fernasan-D and Milcurb on inhibition of growth and colony sizes of seven *Rhizobium* strains and ten *Bradyrhizobium* strains.They concluded that Captan at the concentrations of 100 and 1000 micro g lit^{-1} was the most toxic, followed by Thiram, Milcurb, Luxan then Frernasan-D. All strains tolerated low fungicide concentrations (<=100 micro g lit^{-1}) but they were sensitive to high concentrations (>=500 micro g lit^{-1}) with varying degrees of sensitivity. It was Concluded *Rhizobium* strains were more tolerant than *Bradyrhizobium* strains.

The survival of *Mesorhizobium ciceri* (SP4) and *Azotobacter chroococcum* (CBD-15 and M4) on chickpea seeds treated with fungicides Bavistin and Thiram, was studied by Sunitha *et al.* (2007). The survival of phosphate solubilizing bacteria (PSB), *Pseudomonas striata* (27) and *Bacillus polymyxa* (H5), was examined on two cultivars

(Arkel and BV) of pea seeds treated with Thiram. The viability of *A. chroococcum* (W5) was also examined on wheat seeds treated with Bavistin, Captan and Thiram under laboratory conditions using standard dilution and the plate count technique.

Madhavi (2006) evaluated compatibility of different agrochemicals against *Pseudomonas fluorescens* strains *in vitro* and stated that Carbendazim, Fipronil and Fluchloralin were found compatible with the *P. fluorescens* at their recommended and half the recommended dosages.

The bio efficacy of Pendimethalin and Fluchloralin was tested by Sarkar *et al.* (2005) in mustard and concluded that the populations of fluorescent *Pseudomonas* and *Azotobacter* were improved with the application of these herbicides.

The higher pod yield in groundnut (*Arachis hypogaea*) was reported by Jhala *et al.* (2005) when four herbicides Fluchloralin, Pendimethalin, Butachlor and Metalachlor were applied along with *Rhizobium* inoculation than the treatments without inoculation.

Kishore *et al*. (2005) reported that the *Pseudomonas aeruginosa* GSE-18 and GSE-19 were tolerant to the recommended field application rate for chlorothalonil. Fungicide tolerance is not uncommon in *P. fluorescens*.

Durai (2004) evaluated systemic and non-systemic fungicides for their compatibility with *Pseudomonas fluorescens*. Of the fungicides tested, Thiram @ 1000ppm showed the highest inhibition (4.7%). Mancozeb was found to be compatible with *P. fluorescens* even @ 500ppm, as the growth of inhibition was nil and the inhibition was only 0.7% even at 1000ppm.

A field experiment was conducted Deshmukh *et al.* (2004) to evaluate the effect of *Rhizobium japonicum* (*Bradyrhizobium japonicum*) with different herbicides (Alachlor, Pendimethalin, Fluchloralin, Chlorimuron-ethyl and Trifluralin) and concluded that maximum grain yield was obtained by the treatment *R. japonicum* alone (2862 kg ha^{-1}) followed by *R. japonicum* + Pendimethalin at 1 kg. a.i ha^{-1} (2763 kg. ha^{-1}).

Ghosh *et al.* (2003) evaluated the lethal concentrations of Chlorpyrifos, Monocrotophos, Fenvalerate and Phorate in green gram and concluded that nitrogen fixation and grain yield were highest with *Rhizobium* + Chlorpyrifos and *Rhizobium* + Phorate treatments when *Rhizobium* was supplied simultaneously or 24h after insecticide application, respectively.

Mathew (2003) showed that *Pseudomonas fluorescens* was compatible with all the nine pesticides used. Amongst them imidacloprid gave best compatibility.

De *et al.* (2003) conducted pot and field experiments to evaluate the biological control of *Trichoderma harzianum, Gliocladium virens* and *Pseudomonas fluorescens* against *Fusarium oxysporum* f.sp. *lentis* infecting lentil and their compatibility with fungicides. Seed treatments with Carbendazim + Thiram and *Gliocladium virens* + *Pseudomonas fluorescens* + Carboxin were effective in the field in controlling 48.8 and 44.2% lentil wilt.

Kutcher *et al.* (2002) studied the effect of seed applied liquid- or soil-applied granular rhizobium (*Rhizobium leguminosarum* bv. *viciae*) inoculants and seed applied fungicide treatments with metalaxyl (Apron FL) Apron FL + Thiram 75 WP and Thiram 75 WP. Results showed that there is compatibility between the seed-applied fungicide and seed-applied liquid or soil-applied granular *Rhizobium* inoculants.

The effect of *Rhizobium* with Captaf (at 3 g kg^{-1} of seed) in chickpea variety GL769 was studied by Khurana and Sharma (2002) and reported that there is compatibility between the fungicide and *Rhizobium* as there was no decrease in the yield and yield attributing characters.

2.5 TO STYDY THE INFLUENCE OF EFFICIENT PGPR STRAINS WITH MAIZE CROP

Hussain *et al.* (2013) isolated seventy two bacterial isolates from maize rhizosphere. All bacterial isolates were tested for P-solubilizaton . Out of thirty bacterial isolates, fifteen showing higher phosphatase activity, ACC-deaminase activity and auxin production in liquid culture were selected for further evaluation for their growth promoting activities under axenic conditions. Inoculation with selected bacteria significantly increased shoot length, root length, shoot fresh and dry weight, and root fresh and dry weights up to 39.7, 58.9, 99, 69.4, 97.7 and 87%, respectively over uninoculated control.

Baral and Adhikari (2013) conducted one field experiment to study the effect of *Azotobacter* on growth and yield of maize. The treatments were control, 120:60:40kg N P_2O_5 K_2O(RDF) ha^{-1},*Azotobacter* seed inoculation, *Azotobacter* soil application, Azotobacter+10 t FYM ha^{-1}, 10 t FYM ha^{-1}, RDF +*Azotobacter,* RDF+ *Azotobacter* + 10 t FYM ha^{-1}. Analysis of variance showed that grain yield, plant height, ear height, ear length, kernel per rows and 1000 grain weight were significantly affected with treatments. Only inoculation of *Azotobacter* increased 15 to 35% grain yields over non inoculated treatments. The benefit of *Azotobacter* inoculation was higher in the absence of chemical fertilizer application.

Qudsia bano *et al.*(2013) studied the effects of *Azospirillum lipoperum* inoculation on biochemical attributes and growth of maize plant under drought stress in two varieties which were subjected to drought stress at vegetative stage. Water deficiency affected accumulation of free amino acids, soluble sugars, proline and soluble protein contents. Seed inoculated plants had an increased accumulation free amino acids and soluble sugars compared to rhizosphere inoculated plants. The plants growth aspects i.e. shoot and root fresh weight, shoot and root dry weight, shoot length and root length, also showed results in consistence with the biochemical attributes. Thus *Azospirillum* strain showed promising effects and can be a potent inoculants for maize that can help the crop to endure limited water availability.

Noumavo *et al.* (2013) were studied the effects of PGPR either singly or in combination on maize growth under laboratory and greenhouse conditions. The highest germination percentage was obtained with the combination of *Pseudomonas fluorescens* and *Pseudomonas putida*. This combination also recorded the best vigor index, plants circumferences, number of leaves and the leaf area. The maximal heights of plants were observed with seeds treated with *Azospirillum lipoferum* with an increase of 37.32%. The highest rates of underground dry matter were recorded with *A. lipoferum*, with an increase of more than 56% comparative to control, while the combination *P. fluorescens* and *P. putida* increased the aerial dry matter of 59.11%. Finally, the highest value of the aerial biomass was obtained with the plants treated with the combination of *P. fluorescens* and *P. putida* and the highest underground biomass was obtained with plants treated only with *A. lipoferum*. These results suggest that specific combinations of PGPR can be considered as efficient alternative biofertilizers to promote maize seed germination, biomass and crop yield.

Namazari *et al.* (2012) studied the effect of Biosuper biofertilizer and mineral fertilizer on yield and yield components of corn separately and collectively. The results showed that using Biosuper biofertilizer had positive effect on 100 kernel weight, ear weight and grain yield. Also, using mineral fertilizer led to increase 100 Kernel weight, ear weight and grain yield. Application of fertilizers was effective on the traits and collective application of mineral fertilizer with Biosuper biofertilizer increased yield components, as this enhancement led to increase yield by 30.69% related to when the biofertilizer not applied

Karnwal (2012) reported that, inoculation of *Bacillus lentus, Bacillus* sp., *Azospirillum brasilense, Bacillus subtilis, Pseudomonas fluorescens, Azotobacter diazotrophicus, Bacillus halodurans* and *Pseudomonas* strains as biofertilizer will be clearly showed greater root and shoot dry weight response in maize and wheat compared to control by different plant growth substances of these rhizospheric bacteria.

Ramakrishnan and Selvakumar (2012) studied the effect of biofertilizers on growth and yield of tomato. Seedlings of tomato were treated with T_0-Control, T_1-*Azotobacter*, T_2-*Azospirillum* and T_3-*Azotobacter* with *Azospirillum*. Observations showed that significantly high performance in whole plant dry weight (g plant^{-1}), plant height (cm), number of leaves per plant, number of fruits per plant, yield per plant (g), average fruit weight per plant (g), chlorophyll and protein content. In all the treatments, *Azotobacter* with *Azospirillum* treated plants showed significantly ($P<0.05$) maximum yield when compared with single inoculations and control. The overall results suggest that biofertilizer inoculation improves plant mineral concentration through nitrogen fixation and thereby alters fruit production in tomato plants.

Dhanasekar and Dhandapani (2012) studied the effect of biofertilizers *Azotobacter, Azosprillium, Phosphobacter and Rhizobacter* on the growth of *Helianthus annuus*. *Azotobacter, Azosprillium, Phosphobacter, Rhizobacter* were isolated from soil and root nodules. The efficiency of biofertilizer was checked by treating them with hybrid seeds of *Helianthus annulus* (TCSH-1 and SSH-48). In terms of growth and yield parameters, it was observed that when compared with control, microbial biofertilizer application showed 90% increase yield and 45.87% in growth in TCSH-1 and 60% increased yield and 40.95% increased growth was observed in SSH-48.

Usha Rani *et al.* (2012) isolated, enumerated and characterized the PGPR from the rhizosphere soil of pigeon pea for the enhancement of growth of pigeon pea. Sixty five isolates were identified and characterized, of which 35 were selected for the screening of PGPR isolates. Sixteen isolates were successfully characterized for the PGPR traits, dual plate culture method and HCN production technique, and the best one was selected. These were further investigated to show the PGPR traits in pigeon pea seedling emergence, increase of shoot length, root length, dry matter production of shoot, nodule number and nodule mass. Furthermore, PGPR isolates remarkably increased seed germination of pigeon pea.

Adjanohoun *et al*. (2011) reported that *Azospirillium lipoferum, Pseudomonas fluorescens* and *Pseudomonas putida* are the best PGPR candidates for maize crop improvement on reddish ferrous field. An increased root biomass of 59.57% and 23.40% was recorded with *Pseudomonas fluorescens and P.aeruginosa,* respectively, while other members of the 15 identified PGPR showed little or no significant growth promoting effect on maize crops compared to non-PGPR colonized maize field

Sharifi and Khavazi (2011) evaluated the effects of seed priming with PGPR on dry matter remobilization and yield of maize hybrids, in the factorial experiment based on randomized complete block design with three replications. Factors included seed priming of maize hybrids (SC-404, SC-410 and SC-434) with PGPR in three levels without priming (as control), priming with *Azotobacter* or *Azospirillum* and *Azotobacter* + *Azospirillum*. The results showed that seed priming with PGPR affected grain yield, plant height, number of kernels per cob and number of grains per row of cob significantly.

Naserirad *et al.* (2011) studied the response of maize cultivars(SC604, SC704 and SC807) with integrated application of biofertilizer factor as main plots and bio-fertilizer factor (non-inoculation, inoculation with *Azotobacter*, *Azospirillum* and double inoculation of *Azotobacter* and *Azospirillum*) as sub plots. The effect of cultivar on plant height, stem diameter, number of grain per row, 1000-grain weight, grain yield, biological yield and protein contents were found significant. Cultivar of SC704 had the highest plant height (201.1 cm), number of grains per row (42.8 grains), grain yield (10850 kg ha $^{-1}$) and biological yield (22040 kg ha $^{-1}$) compared with other cultivars. The effect of plant growth promoting rhizobacteria on all traits was significant. Double-inoculation of *Azotobacter* and *Azospirillum* had the highest plant height (212.4 cm), stem diameter (2.5 cm), number of rows per ear (14.5 row), number of grains per row (44.2 grain), 1000-grain weight (315.4 g), grain yield (10190 kg ha 1),biological yield (21320 kg ha 1) and protein content (10.7%) when compared with other treatments. The interaction effect of cultivar × plant growth promoting rhizobacteria (PGPR) on grain yield, biological yield and protein content was significant ($p<0.01$). The highest and lowest grain yield obtained from SC704 with double inoculation of *Azotobacter* and *Azospirillum* (12,320 kg ha $^{-1}$) and SC 604 with non inoculation treatment (12,320kg ha $^{-1}$)$_{,}$ respectively.

Marques *et al.* (2010) isolated six bacterial isolates from a metal contaminated site, screened *in vitro* for their PGP characteristics and their effects on the growth of maize were assessed. Isolates were identified as 3A10T, ECP37T, corresponding to

Chryseobacterium palustre and *Chryseobacterium humi*, and 1ZP4, EC15, EC30and1C2, corresponding to strains within the genera *Sphingobacterium, Bacillus, Achromobacter,* and *Ralstonia,* respectively. Plants inoculated with1C2 generally outperforming the other treatments. Two other bacterial isolates, 1ZP4 and ECP37T also led to increased plant growth in the green house. These three species, corresponding to strains within the genera *Ralstonia* (1C2), *Sphingobacterium* (1ZP4), and to a strain identified as *C. humi* (ECP37T) can thus be potential agents to increase crop yield in maize plants.

Sachin (2009) studied the effect of bioinoculants on the growth of bamboo (*Bambusa bamboo*) and maize seedlings. Seed germination test was conducted to determine the effect of PGPR on the germination percentage of maize seeds. It was found that *Azotobacter chroococcum* at concentration of 10^{8}cfu ml^{-1} increased germination of maize seeds. Effect of PGPR on growth of bamboo seedlings and maize seedlings was studied by transferring one week old bamboo and maize seedlings in the nitrogen free Hoagland’s medium and in soil pots. Seedlings harvested in 25 days were found to have significant increase in root length and plant height and also in dry weight of root and shoot.

Susana *et al*. (2009) studied and reported the growth promotion effect of PGPR in the field when applied on maize and wheat seeds at the sowing time. *Pseudomonas aurantiaca* SR1 colonized the root system of both crops and it persisted at appropriate population densities. It also showed a significant plant growth promoting effect that was reflected in the yield. Another relevant finding was that both crops when inoculated with *P. aurantiaca* SR1, showed higher yields with lower doses of fertilizers than those conventionally applied.

Gholami *et al.(*2009) studied the effect of plant growth-promoting rhizobacteria (PGPR) on seed germination, seedling growth and yield of field grown maize were evaluated in three experiments. In these experiments six bacterial strains include *P.putida* strain R-168*, P.fluorescens* strain R-93*, P.fluorescens* DSM 50090*, P.putida* DSM291*, A.lipoferum* DSM 1691*, A.brasilense* DSM 1690 were used. Results showed that seed Inoculation significantly enhanced seed germination and seedling vigour of maize. The leaf and shoot dry weight and also leaf surface area significantly were increased by bacterial inoculation in both sterile and non-sterile soil. The results showed that inoculation with bacterial treatments had a more stimulating effect on growth and development of plants in non sterile than sterile soil. Inoculation of maize seeds with all bacterial strains significantly increased plant height, 100 seed weight, number of seed per ear and leaf area .The results also showed significant increase in ear and shoot dry weight of maize.

Swedrzynska and Sawicka (2000) studied influence of inoculation with *Azospirillum brasilense* strain, on two levels of nitrogen fertilizer, and seed treatment with fungicidal seed dressing on the development and yield of maize which were determined in field experiments. In these studies nitrogenase activity, chlorophyll content in plants, yield and quality were used as control parameters. The results indicated that inoculation of maize crop with an active strain of *Azospirillum brasilense* had a beneficial effect on maize vigour and yield.

Chapter III

MATERIAL AND METHODS

The present study was carried out at the Department of Agricultural Microbiology & Bioenergy, College of Agriculture, Rajendranagar, Hyderabad and Agricultural research station, Amaravathi, Guntur dist. Pure cultures of Plant growth promoting rhizobacterial isolates collected from different resource laboratories of Andhra Pradesh. Attempts were also made to assess the screening and characterization of of those isolates with multiple beneficial properties like biocontrol potential against plant pathogens, nitrogen fixation, mineral phosphate solubilization and production of plant growth promoting substances. The efficient PGPR isolates were selected for PGPR microbial inoculants and their influence on maize crop yield attributes. The material used and methods employed in the investigation are outlined below.

The general laboratory techniques followed in the present study were those described by Cappuccino and Sherman (1992), Nene and Thapliyal (1993) and Aneja (2001) for preparation of media, sterilization, isolation and maintenance of bacterial cultures, with slight modifications wherever necessary.

3.1 COLLECTION OF PLANT GROWTH PROMOTING RHIZOBACTERIA

Efficient plant growth promoting isolates are collected from different resource laboratories of Andhra Pradesh and these isolates were tested for their purity and preservation in Dept.of Agricultural Microbiology & Bioenergy, College of Agriculture, Rajendranagar, Hyderabad (Table.3.1)

3.2 GLASSWARE

Petriplates, Test tubes, Microscopic slides, conical flasks of different capacities *i.e.*, 1000ml, 500ml, 250ml, pipettes of 1, 2.2, 5, 10 and beakers and measuring cylinders of 50,100,500 and 1000ml ,eppendorf tubes of 1.5ml, PCR tubes, eppendorf micropipettes of 0.5-10µl,10-100µl,100-1000µl were used. All the glassware used was of Borosil make.

3.2.1 Cleaning of Glassware

Glassware were first washed with a detergent, then cleaned with tap water and finally placed in the chromic acid solution prepared with following composition:

Potassium dichromate	:	60g
Conc.H_2SO_4	:	60ml
Distilled water	:	1000ml

The glassware were kept in the cleaning solution for 24h and then thoroughly washed with running tap water before its final cleaning with distilled water and dried.

3.2.2 Sterilization of glassware

Glassware was wrapped in butter paper and sterilized in hot air oven at 160^0C for 2h before use. Media, distilled water, etc., were sterilized in an autoclave at 15lb psi (121^0C) for 20 min.

3.2.3 Precaution to avoid contamination

The inoculation work of microbial cultures were carried out in laminar air flow. The laminar bench and air flow was disinfected using U.V lamp prior to comencement of work.

3.3 EQUIPMENT AND APPARATUS USED

Hot air oven and autoclaves were used for sterilization of heat stable glassware and media respectively. BOD incubators were used for incubating cultures at different temperatures. Cultures were stored and maintained in a refrigerator. The pH was measured by using digital pH meter. Cyclomixer was used for homogenization during serial dilution. Plate mixer was used for spread plate technique. Centrifuge was used for making cell-free cultures and DNA pellet formatting protocols. Eppendorf thermocycler(PCR), gelelectrophoresis, Bio rad UV trans illuminator . Agilent 7820 A Gas Chromatography was used for Acetylene reduction assay for nitrogen fixing ability of bacterial isolates. Hi-media zonal scale was used to measure the zone around the colonies during phosphate solubilization and biocontrol activity. Samples were weighed using a single pan electric balance. Compound electron microscope was used to observe the morphology of bacterial cultures.

3.4 CHEMICALS USED

The chemicals used in the present investigation were of analytical and laboratory grades. The pH of the media was adjusted to the required level using 10N NaOH and 10N HCL. Formaldehyde 10% solution was used to fumigate the Laminar air flow chamber and BOD incubators for disinfection.

3.5 IDENTIFICATION OF BACTERIAL ISOLATES

3.5.1 Morphological Characterization

All the sixteen isolates were checked for their purity and then studied for the colony morphology and pigmentation. The cell shape and gram rection were also recorded as per the standard procedures given by Barthalomew and Mittewar (1950) and Anonymous (1957).

3.5.2 Colony Morphology

Morphological characteristics of the colony of each isolate were examined on Nutrient agar and specialized medium and incubated for according to isolate. Cultural characterization of isolates observed by different characteristics of colonies such as shape, size, elevation, surface, margin, color, odor, pigmentation, etc were recorded.

3.5.3 Gram’s Staining

A drop of sterile distilled water was placed in the center of glass slide. A loopful of inoculm from young culture was taken, mixed with water, and placed in the center of the slide. The suspension was spread out on slide using the tip of inoculation needle to make a thin suspension. The smear was dried in air and fixed through mild heating by passing the the slide 3 to 4 times over the flame. The smear was then flooded with crystal violet solution for 1 min and washed gently with flow of tap water. Then the slide was flooded with iodine solution . After incubation at room temperature for 1 min, iodine solution was drained out followed by washing with 95% ethanol. After that, it was washed with water within 15 to 30 sec and blot carefully. The smear was incubated with safranin solution for 1 min. The slide was washed gently in flow of tap water and dried in air. The slide was examined under microscope at 100X power with oil immersion and data were recorded.

3.5.4. Motility test

This test was done using the hanging drop method . A drop of the test organisms in asaline suspension was placed on a cover slip. The cover slip was inverted and placed on cavity slide,this was viewed under the microscope.

3.5.5 Pellicle test

The active *Azospirillum* isolates were inoculated at subsurface level in screw cap tubes containing sterilized semisolid N- free malate medium(Okon *et al*.,1977) under aseptic conditions.The tubes were incubated at 30^0C for a period of one week and observed for growth of *Azospirillum* as subsurface pellicle.

3.5.6 Cyst formation

Azotobacter sp have ability to form cysts under adverse conditions.Presence of cyst is as one of the criterion for identification of these isolates.The *Azotobacter* isolates were grown om Waksman No.77 N free agar medium for 7 days. These isolates were stained with a mixture of neutral red and light green SF yellowish , observed under oil immersion.

3.5.7 Biochemical and Physiological Characterization

Different biochemical tests performed and the protocols followed are briefly outlined below.

3.5.8 Starch Hydrolysis

Sterile starch agar plates were spotted with 10 μl overnight broth cultures of the isolates and incubated at 28 ± 2^o C for 24-48 h. After incubation, the plates were flooded with iodine solution . The formation of a transparent zone around the colony was taken as positive reaction for the test.

3.5.9 Hydrogen Sulfide Test

Sterilized Hydrogen sulfide- Indole-Motility agar (SIM agar-Appendix I) stabs were inoculated along the wall of the tubes with overnight cultures of the isolates and incubated for 48 h at 28 ±2^o C. Visualization of black colour along the line of inoculation indicated a positive reaction for the test.

3.5.10 Indole Production

Sterilized SIM agar slants were inoculated with the overnight cultures of the isolates and incubated for 48 h at 28 ±2^o C. Following incubation, 10 drops of Kovac's indole reagent were added to each tube. The isolates showing production of red colour were recorded as positive for indole production.

3.5.11 Catalase Test

This test was performed to study the presence of catalase enzyme in bacterial colonies. Fresh cultures of Pure isolates were taken on glass slides and one drop of H_2O_2 (30 %) was added. Appearance of gas bubble indicated the presence of catalase enzyme.

3.5.12 Oxidase Test

The overnight cultures of the test isolates were spotted on plates poured with sterile trypicase soy agar (TSA-Appendix I) and the plates were incubated for 24 h at 28 ±2^o C. After incubation, 2-3 drops of N, N, N', N'- tetramethyl- p-phenylenediamine dihydrochloride (Wurster's reagent) were added onto the surface of growth of each test organism. The isolates showing change of colour to maroon were noted as oxidase positive.

3.5.13 Gelatin liquefaction

The overnight cultures of the test isolates were inoculated to sterilized nutrient gelatin (Appendix I) deep tubes and incubated for 24 h at 28 ±2^o C. Then the tubes were kept in the refrigerator for 30 minutes at 4^oC. The isolates showing liquefied gelatin were taken as positive and those which resulted in solidification of gelatin on refrigeration were recorded as negative for the test.

3.5.14 Methyl Red Test

Sterilized glucose- phosphate broth tubes were inoculated with the test culture and incubated at 28±2^0C for 48h. After incubation five drops of methyl red indicator was added to each tube and gently shaken. Red colour production was taken as positive and yellow colour production was taken as negative for the test.

3.5.15 Voges Prausker's Test

To the presterilized glucose-phosphate broth tubes, test cultures were inoculated and incubated at 37^0C for 48h. After incubation ten drops of Baritt's reagent A was added and gently shaken followed by addition of 10 drops of Baritt's reagent B. Development of pink colour in the broth was taken as positive for the test.

3.5.16 Citrate Utilization

Isolates were streaked on Simmon's citrate agar slants and incubated at 28±2^0C for 24h. Change in colour from green to blue indicates the positive reaction for citrate utilization.

3.5.17 Denitrification test

Sterilized nitrate broth (Appendix I) tubes inserted with Durham's tube in inverted position were inoculated with overnight grown cultures of the test organisms and incubated at 25^oC for 10-15 days. After incubation, the isolates which showed accumulation of gas in the Durham's tubes were scored as positive for denitrification.

3.5.18 Carbohydrate Utilization

All pure bacterial isolates were screened for the carbohydrate fermentation abilities using 4 different carbohydrates (lactose, sucrose, dextrose and mannitol) in Peptone broth medium. Bacterial isolates were inoculated in broth containing specific carbohydrate. The change in colour of Peptone broth was observed for utilization of particular carbohydrate present in broth.

3.5.19 Biotin requirement:

Two sets of test tubes containing 10 ml of sterile nitrogen free semisolid medium were prepared One with biotin(100mg l^{-1}) other set without biotin were inoculated with 0.1ml of the standard inoculms of *Azospirillum* isolates, incubated at 37^0C for three days and observed the tubes for growth of *Azospirillum* isolates.Incase were growth occurred in the medium without biotin, a second transfer was made to fresh medium without biotin and biotin requirement was confirmed.

3.6 SCREENING OF ISOLATES FOR PLANT GROWTH PROMOTING PROPERTIES

Pure isolates were isolated by streaking isolates on respective media plates and screened for following Plant growth promoting properties.

3.6.1 Phosphate Solubilization

For this test sterilized Pikovskaya's agar was poured as a thin layer on to the sterilized petri plates and incubated for 24h . After incubation the Pikovskaya's plates were spot inoculated with sixteen isolates and incubated at 28 ± 1^0C for 4-5 days. Formation of a clear zones around the colonies were considered as positive result for phosphate solubilization.

PSE (Phosphate Solubilization Efficiency) = Z / C x 100

Z- Clearance zone including bacterial growth

C- Colony diameter

3.6.2 Nitrogen fixation efficiency byAcetylene reduction assay(ARA)

The nitrogen fixing capacity of the test organisms were evaluated by using acetylene reduction assay(ARA) following the standard procedure (Bergersen,1980).Twent five ml of semisolid N-free sodium malate medium (*Azospirillum*), JNFb- medium (*Azotobacter*) were prepared in 100 ml vials. The vials were inoculated with 25µl of respected PGP isolates (PGP-5, PGP-6, PGP-9, PGP-10, PGP11, PGP-15) and incubated in an incubator at 28 ± 1^oC . After 5 days of growth, cotton plugs were replaced by Suba-seal septa and tightened with aluminium cap. The air in the vial was replaced with nitrogen gas.Ten percent (v/v) of the inert gas was removed and ten percent pure acetylene gas was injected. The vials were incubated for 24h at room temperature . After incubation,1 ml of gas sample was withdrawn and injected into the gas chromatograph(Agilent 7820 A ,India) fitted with Porapak R column and Flame ionization detector(FID). The column temperature was maintained at 80^{o}c . Nitrigen gas was used as carrier gas at the flow rate of 20ml.min^{-1})

The acetylene reduction activity of the strains was calculated using the formula:

$$\frac{\text{Sample peak length of ethylene(mm)} \times \text{Attenuation} \times \text{volume of gas phase of flask} \times 0.0006}{\text{Incubation time (h)} \times \text{volume of gas sample injected into gas chromatograph(ml)}}$$

The acetylene reduction activity of the sample was expressed as nmoles of ethylene formed mg of protein h^{-1}. At the end of experimental period the cell protein content of the cultures were determined following the method described by Lowery *et al*.(1951)

3.6.3 Indole Acetic Acid Production

Indole acetic acid production was tested according to Gorden and Weber (1951). The active culture of each test isolate was raised in 5ml respective broth tubes and incubated at determined temperature and time. After incubation these cultures were centrifuged at recommended rpm and time. Two drops of O- phosphoric acid was added to 2ml of supernatant and incubated for 30 min to develop the colour. Development of pink colour considered as positive for IAA production.

3.7 SCREENING OF EFFICIENT PGPR ISOLATES FOR BIOCONTROL ACTIVITY

3.7.1 Siderophore Production

Siderophore production was estimated qualitatively. Chrome Azurol S (CAS) Agar medium (Schwyn and Neilands, 1987): For the detection of siderophores, each *Pseudomonas* isolate was grown in synthetic medium,containing 0.5 µM of iron and incubated for 24 h on arotary shaker at room temperature. Chrome Azurol S (CAS) assay is used to detect the siderophores. The CAS plates were used to check the culture supernatant for the presence of siderophores. Culture supernatant was added to the wells made on the CAS agar plates (mannitol, 10.0g; sodium glutamate, 2.0g; K_2HPO_4, 0.5g; $MgSO_4.7H_2O$, 0.2g; NaCl, 0.1g; distilled water, 1000 ml, pH- 6.8-7.2) and incubated at room temperature for 24 h. Formation of yellow to orange coloured zone around the well indicates siderophore production.

3.7.2 Hydrogen Cyanide Production(HCN)

The HCN production was tested by the method of Castric and Castric (1983). First respective media plates i.e., YEMA (*Rhizobium*), Nutrient agar (*Bacillus*), Succinate agar (*Pseudomonas*), *Azotobacter* medium *(Azotobacter*) were prepared separately and incubated for 24h. After that, 1ml of culture of each test isolate was inoculated on respective media plates separately. A disc of whatman filter paper No.1 of the diameter equal to the petri plate size, impregnated with alkaline picric acid solution (0.5% picric acid (w/v) in 1% sodium carbonate) was placed in the upper lid of the inoculated petri plates under aseptic condition. The control plate did not receive the inoculum. The plates were incubated-upside up at 28 ± 2^0C for 48-72h. Change in colour from yellow to light brown, moderate or strong reddish brown was taken as indication of HCN production.

3.7.3 Antagonistic Activity

Pure isolates of common disease causing soil phytopathogens viz., *Sclerotium rolfsii, Rhizoctonia solani* were obtained from the Dept. of Plant Pathology, College of Agriculture, Rajendranagar

Antagonistic activity was verified by following dual culture technique (Skidmore and Dickinson, 1976). First, the bacterial isolates were streaked on respective media plates and incubated at respective temperature and time. Loopful of each bacterial isolate was streaked on the potato dextrose agar plate at one end, which was pre-inoculated with 5 days old, 5mm mycelial disc of test pathogen at the other end. Control plate was maintained by placing only pathogen mycelial disc in the centre without bacteria.

The assay plates were incubated at 28 ± 1^0C for 5 days and observations were made on inhibition of mycelial growth of the test pathogens. For each bacterial isolate three replications were maintained with suitable controls.

The per cent growth inhibition over control was calculated by using the formula:

Percent Inhibition =

$$\frac{\text{Growth of pathogen in control(mm)-growth of pathogen in treatment(mm)}}{\text{Growth of pathogen in control(mm)}} \text{x100}$$

Note: In this the percent inhibition in control is taken as zero percent.

3.8 MOLICULAR CHRACTERIZATION OF PGPR

In order to analyze the molecular characterization of efficient PGPR isolates with *Nif* gene specific primers such as *Nif* H1 and *Nif* H2. Plant growth promoting rhizobacterial isolates were included in this study.

3.8.1 Chemicals

All the chemicals used in this project were of molecular biology grade and obtained from Sigma, Amersham Biosciences. USB, Bangalore Genei, Life Technologies, Invitrogen, etc. other consumables like plastic ware were obtained from Axygen and Tarson. Standard solutions and buffers were prepared according to the procedures given by Ausubel *et al*. (1999)

3.8.2 Equipment

The instruments used in the study are Centrifuges, Electrophoresis Units, Thermal Cycler and Gel documentation system.

3.8.3 Primers

*Nif*H1 and *Nif* H2 primers employed in PCR reactions are listed in table 3.2 which were obtained from PRR Biotech solutions Pvt Ltd, Hyderabad, India

The primers were all dissolved in sterile distilled water, stored at -20^{0}C and when required, dilution to get the working stock of required concentration as recommended in the respective PCR procedures. Decamer primers with their sequences (Setubal *et al*., 2009).

3.8.4 Methods

Different PCR reactions were carried out as per the standard procedures given by Ausubel *et al*. (1999), brief account of the same has been presented here.

3.8.4.1 Bacterial Genomic DNA Isolation

Genomic DNA was isolated by following the standard method. 6ml of bacterial culture grown overnight was used for isolation of DNA. The culture was centrifuged in 1.5 ml eppendorf tube for 2 minutes at 10000 rpm. The pellet was resuspended in 567µl of TE buffer by repeated pipetting. To this suspension 30µl of 10% SDS and 3µl proteinase K (20 $mgml^{-1}$) were added. Mixed well and incubated at 37^0C for 1 hour followed by addition of 100µl of 5M NaCl. To this mixture 80µl of CTAB (10%) and NaCl (0.7M) solution was added, mixed well and incubated at 65^0C for 10 minutes. Equal volume of phenol/chloroform(isoamyl alcohol) was added, gently mixed and centrifuged at 12000 rpm for 7 minutes. The upper phase was taken to a fresh eppendorf tube. Equal volume of chloroform(isoamyl alcohol) was added, mixed well and centrifuged at 12000 rpm for 5 minutes. This step was repeated twice. Finally, the supernatant was taken in a fresh 1.5ml eppendorf tube and the DNA was precipitated with 0.6 volumes of isopropanol. After incubation at room temperature for 30 minutes, the precipitate was centrifuged at 12000 rpm for 7 minutes to pellet the DNA. The supernatant was decanted. The pellet was washed with 70% alcohol twice, air dried and dissolved in TE [10Mm Tris-HCL, 1Mm EDTA (Ph 8.0)]. The chemicals and reagents used for DNA isolation are listed in Appendix II.

3.8.4.2 Polymerase Chain Reaction

Genomic DNA (25-50$ngµl^{-1}$) of the Plant growth promoting rhizobacterial isolates was used as template and PCR amplification was performed in a 20µl reaction mixture as constituted . PCR reaction was carried out in a DNA Thermocycler with heated lid. Each of 20 µl reaction. The melting and annealing temperature were calculated following method of Womble (2000).

Table.3.3 PCR mixture for DNA amplification

PCR mixture	Concentration
Primer(F)	1.0µl
Primer(R)	1.0µl
dNTPS(2mM)	2.0 µl
10xBuffer	2.0 µl
Taq polymerase	0.1 µl
Sterile distilied water	11.9 µl
Template DNA	2.0µl
TOTAL	**20.0 µl**

3.8.4.3 Gel electrophoresis

DNA amplification was checked by electrophoresis of each PCR product in a 1.5% (w/v)agarose gel, in TBE buffer for 1 h at 3.2 V/cm. Gels were stained in ethidium bromide for 15 min and thereafter washed for 5 min. the samples were loaded along with a standard ladder of 100 bp for comparision of the PCR products. DNA fragments were visualised at 312 nm with a UV-transilluminator ImageMaster VD (Helmut *et al*.,2004).

Table 3.4 Optimization conditions for PCR amplification

Cycle	Stage	Temperature (^oC)	Duration (min)	No. of cycles
	Lid temperature	105	-	-
I	Initial denaturation	96	1	1
II	Denaturation	96	1	30
	Annealing	55	1	
	Extension	72	1	
III	Extension Dump	72	6	1

3.9 TO ASSESS COMPATIBILITY OF PGPR ISOLATES WITH AGROCHEMICALS

Compatibility of bacterial test isolates with commonly used agrochemicals like **fungicides** (metalaxyl, ziram, propiconazole, carbendazim),**insecticides** (chlorpyriphos, dimethoate, imidachloprid, thiodicarb) and **herbicides (**atrazine, butachlor, propaqizofop, pendimethalin) (Table 3.5) was tested by following inhibition zone technique (Nene and Thapliyal, 1993) at recommended dosages by maintaining three replications.

Five ml of water agar seeded with bacterial suspension was poured in to the petri plates containing 10ml of warm nutrient agar and rotated gently for uniform distribution. Sterilized filter paper discs of 6mm in diameter were dipped in different chemicals at different concentrations dried and placed over nutrient agar medium plates seeded with bacterial isolates. Control was maintained by dipping the filter paper discs in sterile distilled water and plates were incubated at 28 ± 2 ^{0}C for 48 to72 h. The inhibition zone (in mm) around the disc was measured.

3.10 INFLUENCE OF EFFICIENT PGPR ISOLATES ON MAIZE CROP YIELD ATTRIBUTES

A field experiment was conducted to investigate the effect of efficient free living nitrogen fixers and phosphate solubilizing biofertilizers on yield attributes of maize crop.

3.10.1 Location of the experiment

The field experiment was carried out at College Farm, College of Agriculture, Rajendranagar, Hyderabad. The farm is geographically situated at an altitude of 542.3m above mean sea level on 17^{0}-19’ N latitude and 78^{0} -28’ E longitude and falls under the Southern Telengana Agroclimatic Regions of Andhra Pradesh.

3.10.2 Climate

The weather data during experimental crop growth period period was taken from the meteorological observatory located at Agricultural Research Institute, Rajendranagar.

The weekly mean maximum temperature during the crop growth period (10 Oct 2013 – 14 Feb 2014) ranged between 32.8^{0} C and 27.1^{0} C with an average of 29.1^{0}C in

2013-14, while the weekly mean minimum temperature ranged from 22.1°C to 7.5°C with an average of 14.3°C.

The weekly mean relative humidity in the morning (R.H-I) during the crop season ranged from 76.3 to 94 per cent with an average of 85.3 per cent while weekly mean relative humidity in the afternoon (R.H-II) varied from 26.7 per cent to 79.3 per cent with an average of 64 per cent.

The weekly mean sunshine hours fluctuated in between 2.6 hours to 9.4 h with an average of 7.6 h in 2013-14.

The weekly mean wind velocity ranged from 0.7 km per hour to 4.0 km per hour with an average of 2.1 km.h^{-1} during 2013-14.

The weekly mean pan evaporation during the cropping period ranged from 1.7 to 5.0 mm day^{-1} with an average of 3.1 mm day^{-1}. About 282.2 mm rain fall received during the crop growing season in 11 rainy days.

3.10.3 Physical and chemical properties of the soil

The soil of the experimental site was red clay loam with good drainage and fine bed. Composite soil samples were collected randomly from the experimental field prior to sowing and analysed for the physical, chemical and microbiological properties by adopting standard procedures at Department of Agricultural Microbiology and Bioenergy and Department of Soil Science and Agricultural Chemistry, College of Agriculture Rajendranagar, ANGRAU, Hyderabad and the results are summarized in Table.3.6

3.10.4 Microbiological properties of the experimental site

Viable population of bacteria, fungi, actinomycetes, *Rhizobium* and *Pseudomonas* was analyzed by the standard serial dilution plate count method (Vlassak *et al.* 1992). The microbial colonies appearing after the stipulated time period of incubation were counted as Colony forming units (CFU) g-1 fresh weight of the soil sample. The microbial populations were expressed as number of colony forming units per gram of soil and the results were summarized in table 3.7.

Table 3.7 Initial microbial population of the experimental site

S. No.	**Microbial group**	**Log CFU g^{-1} of soil**
1.	Bacteria	7.50×10^3
2.	Fungi	4.33×10^3
3.	*Azotobacter*	3.51×10^3
4.	*Rhizobium*	3.85×10^3
5.	Phosphate solubilizers	4.71×10^4
6.	*Azospirillum*	3.64×10^3

3.10.5 PREVIOUS CROP HISTORY

The cropping history of the experimental site for the previous three years is summarized below.

S. No.	**Year**	***Kharif***	***Rabi***
1	2010	Maize	Sunflower
2	2011	Sunflower	Maize
3	2012	Fallow	Fallow

3.10.6 DETAILS OF THE TREATMENTS USED IN THE FIELD EXPERIMENT

Crop	:	Maize(*Zea mays.L*)
Hybrid	:	DHM-117
Season	:	Rabi
No. of treatments	:	7

No. of replications : 3

Plot size : 4.5 m×4.3 m

Spacing : 60 cm x 20 cm

Experimental design : RBD

RDF : 120:50:60 NPK kg ha^{-1}

T1: 100 % RDF (Irrigated crop - N2: P_2O_5: K_2O ratio: 120kg:50kg:60kg/ha^{-1})

T2: 75 % RDN + Nitrogen fixer *Azospirillum*

T3: 75 % RDN+ Nitrogen fixer *Azotobacter*

T4: 75 % RDP + Phosphate solubilizers

T5: 75 % RDN&P + Nitrogen fixer *Azospirillum* + Phosphate solubilizers

T6: 75 % RDN&P + Nitrogen fixer *Azotobacter* + Phosphate solubilizers

T7: 75 % RDN&P + *Azotobacter* + *Azospirillum* + Phosphate solubilizers

When applying *Azotobacter, Azospirillum* inoculum, applied 75% RDF of N and P, K is 100% RDF.When applying phosphate solubilizers inoculum , apply 75% RDF of P was applied and N and K were applied 100% RDF.

3.10.7 Date of Sowing: 9th December 2013

Crop	Hybrid	Date of Sowing	Date of Harvesting
Maize	**DHM-117**	**09-12-2013**	**10-04-2014**

3.11 CULTURAL PRACTICES

3.11.1 Land preparation

The land was ploughed and harrowed twice after the harvest of previous crop, to bring the soil to fine tilth(Photo.3.1a)

3.11.2 Fertilizer and microbial inoculm

The fertilizers were applied as per the treatments combinations. The crop was supplied with recommended dose of fertilizers. Nitrogen @120 Kg ha^{-1}, phosphorus @ 50 Kg ha^{-1} and potassium @ 60 Kg ha^{-1} in the form of single super Phosphate, urea and muriate of potash, respectively were adopted. Chemical fertilizers applied according to different treatments described in table 3.8. Chemical fertilizers were applied @75% reduced amount of nitrogen and posphatic fertilizers . Entire dose of P and K was applied as basal at the time of sowing. Nitrogen was applied through Urea in three split applications and free living *Azotobacter* sp, *Azospiillum* sp, Phosphate solubilizing bacteria (PSB) of efficient PGPR carrier based microbial inoculants along with FYM were incorporated in soil.

Efficient strains of free nitrogen fixing *Azospirillum,Azotobacter*, phosphate solibilizing bacteria were inoculated into respective broth culures such as N- free sodium malate broth for *Azospirillum*, Jensen's broth for *Azotobacter*, Pikovaskaya's broth for phosphate solubilizers (*Pseudomonas,Bacillus*) incubated for 48h at 30^{0}C. *Azotobacter* and *Azospirillum* broth were incubated for seven days. Each broth culture flask tested for purity as 10^{8}-10^{9} cells ml^{-1}.

Lignite is sterilized and pH maintained at 7.0. Mixed with broth cultures aspetically and incubated for 24h . After incubation purity checked by serial dilution and plating method for cells viability and recommended count(10^{8}-10^{9}). For treatments with a combination of bacteria,inoculm of each PGPR was adjusted to the required concentration and then they were mixed in the ratio of 1:1 (w/w) prior to sowing.

3.11.3 Seed source and sowing

The seed material maize (DHM-117) was obtained from Maize research station, Agricultural research station, Rajendranagar, Hyderabad. Healthy and bold seeds were dibbled with a spacing of 60×10 cm at a depth of 3-5 cm on 9th Dec, 2013. Efficient *Azospirillum, Azotobacter* and PSB were screened and selected among the isolates and were prepared as powder formulation and inoculated in furrows @ 0.05 kg per ha^{-1}(Photo.3.1b)

3.11.4 Weed management

Atrazine 50% WP @ 1.0 kg a.i. ha^{-1} was applied one day after sowing (DAS). Hand weeding was done twice at 20 DAS and 35 DAS to keep the experimental plots weed free.

3.11.5 Water management

First irrigation was given immediately after sowing of the crop to ensure proper germination. Later irrigations were given at crop growth recomended stages.

3.11.6 Plant protection and intercultural operation

Intercultural operation was carried out thrice at 30 and 60 days after sowing (DAS) immediately after hand weeding. The crop was sprayed with pesticides chloripyriphos @ 2 ml per litre against stem borer . The crop was also sprayed with carbendazin @ 1 $g.ml^{-1}$ and ridomil @ 500 $g.acre^{-1}$ fungicide for the control of fungal disease of the crop.

3.11.7 Harvesting and shelling

Harvesting was done at physiological maturity, judged visually when about 95 per cent grains were turned into golden colour. Initially the border rows were harvested. Later the net plot cobbs were harvested and stover bundled. The post harvest observations were recorded from the harvest samples. The cobs from net plot area were shelled . After sun drying, the net plot grain yields and straw were recorded treatment wise and reported in kg ha^{-1}

3.12 Pre-Harvest Observations on Crop Growth

Observations on crop growth at 30, 60 DAS and at harvest were taken from five plants, which were randomly selected and labeled in each plot

3.12.1 Plant Height (cm)

Plant height (cm) was measured from the base of the plant to the terminal end of the unfolded leaf at knee height, flowering and harvesting stages.

3.12.2 Dryweight plant(g)

Five plants representing the population were randomly harvested at each observation for recording dry matter production. The plants were removed along with root system. The roots were separated from each selected plant, above ground parts were cleaned, transferred to properly labelled brown paper bags and partially dried in the sun for 2 days and then dried in hot air oven at a temperature of 60^{o}C to a constant weight. It was weighed and expressed as kg ha^{-1}.

3.12.3 Microbial population levels at different growth stages

Microbial population were counted at three different stages and estimated soil health by anlalsys microbial count in rhizospheric zone.

3.13 Yield attributes

3.13.1 Cob weight (g)

Five cobs were randomly selected from net plot and the cob weight and finally recorded the mean weight of cobs was achieved.

3.13.2 Number of rows cob^{-1}

Five cobs were randomly selected from net plot and in each cob, the number of rows was counted and finally the mean number of rows cob^{-1} was reported.

3.13.3 Number of grains row^{-1}

From the randomly selected five cobs, the number of grains row^{-1} was counted and finally the mean number of grain row^{-1} was determined.

3.13.4 Number of grains cob^{-1}

Grain number was counted from cob sub sample, averaged and expressed as number of grains cob^{-1}.

3.13.5 Test weight (100 seed weight)(g)

Five samples each of 100 grains were collected randomly from the net plot produce treatment wise and weighed, averaged and expressed in grams.

3.13.6 Grain yield (kg ha^{-1})

The kernels from the air-dried cobs of each net plot were separated, cleaned and dried to obtain at least 13 per cent moisture. Weight of grains of each plot was recorded separately and expressed as grain yield in kg ha^{-1}.

3.13.7 Stover yield (kg ha^{-1})

The stover obtained from each net plot was weighed after it was completely sun dried and expressed as stover yield in kg ha^{-1}.

3.14 Post-Harvest Soil Analysis

3.14.1 Available nitrogen

It was estimated by alkaline permanganate method as outlined by Subbiah and Asija (1956). It was expressed in kg ha^{-1}**.**

3.14.2 Available phosphorus

Available phosphorus content of soil was determined by Olsen's method as described by Jackson (1973). It was expressed in kg ha^{-1}.

3.14.3 Available potassium

Available potassium was determined by flame photometer after extracting the soil with neutral normal ammonium acetate as described by Jackson (1973). It was expressed in kg ha^{-1}.

3.15 Economics

The cost of cultivation ha^{-1} was calculated for the individual treatment on the basis of inputs used and prevailing market price of the produce.

Gross monetary returns were estimated by multiplying economic yield with prevailing market price of soybean seed.

Net monetary returns were calculated by deducting cost of cultivation from gross monetary returns for each treatment. Benefit - Cost (B: C) ratio was calculated by using the formula.

$$\text{Benefit - cost ratio} = \frac{\text{Net returns (Rs ha}^{-1}\text{)}}{\text{Cost of cultivation (Rs ha}^{-}}$$

3.16 STATISTICAL ANALYSIS

The data obtained in different experiments was transformed using angular transformations wherever necessary and was statistically analyzed using Completely Randomized Design (CRD) as per the procedures given by Snedecor and Cochran (1967) and Panse and Sukhatme (1985). Data on different characters viz., growth and yield components and yield, were subjected to analysis of variance procedures as outlined for randomized block design (Gomez and Gomez, 1984). Statistical. Statistical significance was tested by F–value at 0.05 level of probability and critical difference was worked out where ever the effects were significant.

Table 3.1 Collection of efficient Plant Growth Promoting Isolates

S.No	PGPR isolate code	Collection Source
1.	PGP-1	Biofertilizers laboratory, ARS, Amaravathi, Guntur (dist), A.P.
2.	PGP-2	Department of soil and biology, ICRISAT, Hyderabad. A.P.
3.	PGP-3	Department of soil and biology, ICRISAT, Hyderabad. A.P.
4.	PGP-4	Department of soil and biology, ICRISAT, Hyderabad. A.P.
5.	PGP-5	Biofertilizers laboratory, ARS, Amaravathi, Guntur (dist), A.P.
6.	PGP-6	Biofertilizers laboratory, ARS, Amaravathi, Guntur (dist), A.P.
7.	PGP-7	Biofertilizers laboratory, ARS, Amaravathi, Guntur (dist), A.P.
8.	PGP-8	Biofertilizers laboratory, ARS, Amaravathi, Guntur (dist), A.P.
9.	PGP-9	Biofertilizers laboratory, ARS, Amaravathi, Guntur (dist), A.P.
10.	PGP-10	Biofertilizers laboratory, ARS, Amaravathi, Guntur (dist), A.P.
11.	PGP-11	Biofertilizers laboratory, ARS, Amaravathi, Guntur (dist), A.P.
12.	PGP-12	Biofertilizers laboratory, ARS, Amaravathi, Guntur (dist), A.P.
13.	PGP-13	Dept of Agricultural Microbiology and Bioenergy, CAR' Nagar, Hyderabad, A.P.
14.	PGP-14	Dept of Agricultural Microbiology and Bioenergy, CAR' Nagar, Hyderabad
15.	PGP-15	Dept of Agricultural Microbiology and Bioenergy, CAR' Nagar, Hyderabad ,A.P.
16.	PGP-16	Dept of Agricultural Microbiology and Bioenergy, CAR' Nagar, Hyderabad, A.P.

Table 3.2 Details of *Nif* primer sequences and dilution

Sequence Name	**Sequence text(5'-3')**	**Length**	**Concentration (pm/µl)**	**Volume in µl to be added to get 100pm/ µl**	**Reference**
Nif H_1-F	CAGACACGAAGAAGCCGGGC	20 Tm58	174.42	348.84	Setubal *et al*. (2009)
Nif H_1-R	GACCAGCAGCTTGTTGTTGA	20 Tm52	170.24	340.48	Setubal *et al.* (2009)
Nif H_2-F	CGCCGGCGCAGTGTTTGCGG	20 Tm 62	323.81	647.62	Setubal *et al*. (2009)
Nif H_2-R	CACTCGTTGCAGCTGTCGGC	20 Tm53	324.82	651.54	Setubal *et al*. (2009)

Nif –Nitrogen fixing gene primers; pm-piko moles; Tm-melting temperature

Table 3.5 Details of Agrochemicals used for the compatibility studies with PGPR isolates

S. No.	COMMON NAME	TRADE NAME	FORMULATION	CHEMICAL NAME	RECOMMENDED DOSAGE
A. FUNGICIDES					
1.	Metalaxyl	Ridomil	25 % WP	2-[(2,6-dimethylphenyl)- (2-methoxy-1-oxoethyl) amino]propanoic acid	2g lit^{-1}
2.	Ziram	Hexazir	25 % WP	Zinc bis(dimethyldithiocarbamate)	1 g lit^{-1}
3.	Carbendazim	Bavistin	50% WP	Methyl benzamidazole 2-ethyl Carbamate	1 g lit^{-1}
4.	Propiconazole	Tilt	25% EC	-1-1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1,2,4-triazole	3ml lit^{-1}
B. INSECTICIDES					
5.	Chloripyrifos	Dursban	20%EC	*O*,*O*-Diethyl *O*-3,5,6-trichloropyridin-2-yl phosphorothioate	2ml lit^{-1}
6.	Thiodicarb	Larvin	75%WP	3,7,9,13-tetramethyl-5,11-dioxa-2,8,14-trithia-4,7,9,12-tetraazapentadeca-3,12-dien-6,10-dion	2 g lit^{-1}
7.	Imdachloprid	Confidor	17.8% SL	1-(6-Chloro-3-pyridyl methyl) N- nitroimidazolidin-2 cylideneamine.	0.25ml lit^{-1}
8.	Dimethoate	Cygon	30% EC	*O*,*O*-dimethyl *S*-[2-(methylamino)-2-oxoethyl] dithiophosphate	0.2ml lit^{-1}
C. HERBICIDES					
9.	Atrazin	Atratof	50%WP	1-Chloro-3-ethylamino-5-isopropylamino-2,4,6-triazine	3 g lit^{-1}
10.	Butachlor	Machete	50%EC	*N*-(Butoxymethyl)-2-chloro-*N*-(2,6-diethylphenyl)acetamide	0.02ml lit^{-1}
11.	Propaquizafop	Falcon	100%EC	1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1,2,4-triazole	3ml lit^{-1}
12.	Pendimethalin 30EC	Stomp	30 %EC	N-(1-ethyl propyl) -2-6-dinitro-3, 4 oxylidine	6.6ml lit^{-1}

Table 3.6 Physico-chemical properties of the experimental site

Properties	**Results**	**Method adopted**
Physical properties		
Soil fraction	% composition	Bouyoucos hydrometer method (Piper, 1966)
Sand	28	
Silt	23	
Clay	49	
Textural class	**Sandy clay loam**	
Chemical properties		
Soil reaction (pH) (1:2.5 soil : water)	7.5	Glass electrode pH meter (Jackson, 1967)
Electrical conductivity (dS m^{-1})	0.34	Solubridge method & Digital EC meter (Jackson, 1967)
Organic carbon (%)	0.39	Walkley and Black’s modified method (Jackson, 1967)
Available Nitrogen (kg N ha^{-1})	232.7	Alkaline permanganate method (Subbaiah and Asija, 1956)
Available phosphorus (kg P_2O_5 ha^{-1})	25.9	Olsen’s method using colorimeter (Olsen *et al*., 1954)
Avalable potassium (kg K_2O ha^{-1})	272.5	Neutral ammonium acetate method using flame photometer (Muhr *et al*., 1963)

Table 3.8 Chemical fertilizer and PGPR Microbial inoculants application in Maize field experiment

TREATMENT	**BASAL DOSE(Kg.ha^{-1})**			**SPLITS (UREA) (Kg.ha^{-1})**		**MICROBIAL INOCULANTS FOR EACH@ (Kg.ha^{-1})**	**FARM YARD MANURE (Kg.ha^{-1})**
	SSP	**UREA (1st split)**	**MOP**	**2nd split**	**3rd split**		
T1(100% RDF)	250	86	100	86	86	-	1
T2(75%RDF N,100% P,K)	250	86	100	54	54	100	1
T3(75%RDF N,100% P,K)	250	86	100	54	54	100	1
T4(75%RDF P, 100% N,K)	188	86	100	86	86	100	1
T5(75%RDF N&P,100% K)	188	86	100	54	54	100	1
T6(75%RDF N&P,100% K)	188	86	100	54	54	100	1
T7(75%RDF N&P,100% K)	188	86	100	54	54	100	1

Fig 3.1 Field experiment photos of Maize

a) **Layout of Maize plot (400 sq.m)**

b) Sowing operation (09-12-2013)

Fig 4.1 Growth stages of maize crop

a)Crop growth at knee height stage

b) T_2(75%RDN) +Nitrogen fixer *Azospirillum* treated plot (milking stage)

c) T_7(75% RDN & P)+ Nitrogen fixer *Azsospirillum*+ Nitrogen fixer *Azotobacter* + phosphate solubilizer treated Maize plot

Chapter IV

RESULTS AND DISCUSSION

Plant growth promoting rhizobacterial strains collected from different resource laboratories of Andhra Pradesh state, were confirmed for purity and maintained in the department of Agricultural Microbiology & Bioenergy, College of Agriculture, Rajendranagar. The isolates were characterized, identified based on morphological, biochemical, and molecular characteristics and studied for their functional plant growth promoting activities and their influence on maize crop. The results obtained on these aspects are presented.

4.1 Collection and screening of bacterial isolates from different resource laboratories in Andhra Pradesh for their PGPR activities.

4.2 To find efficient PGPR isolates with biocontrol activity.

4.3 To study the molecular characterization of nitrogen fixing ability of efficient PGPR strains.

4.4 To study the compatibility of efficient PGPR isolates with agro chemicals.

4.5. To study the performance of PGPR strains with maize.

4.1 SCREENING OF RHIZOBACTERIAL ISOLATES FOR PLANT GROWTH PROMOTING ACTIVITIES

By the objective of research part collected unknown bacterial isolates are collected from different resource laboratories of Andhra Pradesh and these isolates were tested for their purity and preserved in the department of Agricultural Microbiology& Bioenergy, College of Agriculture, Rajendranagar, Hyderabad. Collected isolates were further screened for Plant growth promoting activities *in vitro*.

4.1.2 Screening of isolates for PGPR properties

Plant root colonizing bacteria can function as harmful, deleterious rhizobacteria (DRB) or beneficial, plant growth promoting rhizobacteria (PGPR). PGPR colonize roots of monocots and dicots, and enhance plant growth by direct and indirect mechanisms. Modification of root system architecture by PGPR implicates the production of phytohormones and other signals that lead, mostly to enhanced lateral root branching and development of root hairs. PGPR also modify root functioning, improve plant nutrition and influence the physiology of the whole plant.

For identification of efficient PGPR strains with multiple activities, microbial isolates (*Bacillus, Pseudomonas, Azotobacter, Rhizobium and Azospirillum*) were subjected to further studies to understand their Plant Growth Promoting Properties (PGPR) under *in vitro* conditions. Pure isolates were screened for mineral phosphate solubilization, nitrogen fixation, ammonia production and IAA production.

4.1.2.1 Phosphate solubilization ability of bacterial isolates

Among sixteen PGP Isolates 12 isolates were able to solubilize phosphate on Pikovskaya's media containing Tri calcium phosphate in the range of 10mm to 25mm and depicted in table. 4.1. Among 12 isolates PGP-1 recorded the highest solubilization zone (24.66mm) (Plate.4.1a) followed by PGP-7(21mm), PGP-9 (20.66mm), PGP-15(20.33mm), PGP-16 (18.66mm), PGP-13 (17.66 mm), PGP-8(17.33 mm), PGP-14 (15.66 mm),PGP-4 (15.66 mm),PGP-12 (14.33mm) and less solubilization by PGP-11 (13mm).

Studies revealed that inoculation of phosphate solubillizing microorgnism's enhanced the crop yields by solubilizing the soil fixed and applied phosphates (Zaidi, 1999; Gull *et al.,* 2004). Species of the genus *Bacillus*, *Pseudomonas, Rhizobium, Aspergillus* and *Penicillium* are the potential P-solubilzers commonly present in the soil (Rodriguez and Fraga, 1999). Asea *et al.* (1988) reported that *Bacillus megaterium* is the most effective phosphate solubilizer**.** Singh and Ghosh (2012) obtained similar results in evaluation of phosphate solubilizing potential on Pikovskaya's media. Out of 30 isolates, five isolate shown positive result on Pikovskaya's medium producing clear zones. Similar results were reported by Phosphate solubilizing microorganisms *Bacillus subtilis* and *Bacillus cereus* isolated by Maheshwar and Sathiyavani (2012) from the groundnut rhizosphere soil, shows maximum phosphate solubilizing zone.

Similarly, Ribeiro and Cardoso (2012) characterized several PGPR isolates for their phosphatase activities and found that majority of the isolates were phosphatase producers, Mahantesh and Patil (2011) isolated Phosphate solubilizing bacteria (PSB) from the area around Bidar region and screened on the basis of their solubilization of inorganic tricalcium phosphate in liquid cultures. Ten strains that had higher solubilization potential were selected and characterized and Keneni *et al.* (2010) reported five efficient PSB (Phosphate Solubilizing Bacteria) isolated based on their ability in forming a higher clear zone diameter than the other isolates. Oliveira *et al.* (2009) reported several phosphates solubilizing microorganism from maize rhizosphere soil.

4.1.2.2 Nitrogen fixation ability of bacterial isolates

Nitrogenase is a versatile enzyme capable of catalyzing the reduction of several substrates other than nitrogen ,including acetylene (C_2H_2), azide, nitrous oxide, nitriles and isonitriles, the observations that acetylene is an inhibitor of dintrogen(N_2) fixation (Schollhorn and Burris 1967),and is converted by the nitrogen fixing enzyme nitrogenase to ethylene(Dilworth 1966) provided the basis for the development of the first simple method for estimation of N2 fixation (Stewart *et al.* 1967,Hardy *et al* .1968).The C_2H_2 reduction method provides a simple, inexpensive, highly sensitive and non-destructive procedure for measuring rates of nitrogen fixation.

Acetylene Reduction Assay **(**ARA) was carried out to examine the nitrogen-fixing ability of the sixteen PGP isolates under laboratory conditions. The data revealed considerable variability in the nitrogen fixing ability among the studied strains that ranged from to 4.34 to 9.25 nmoles C_2H_4 mg protein h^{-1} (Table.4.1 and Fig no.4.1).

Among sixteen bacterial isolates only six isolates showed nitrogen fixing ability (PGP-5, PGP-6, PGP-9, PGP-10, PGP11, and PGP-15). Out of six isolates PGP-15 showed highest nitrogen fixing ability (9.25 nmoles C_2H_4 mg protein h^{-1}) followed by PGP-9 (8.55 nmoles C_2H_2 mg protein h^{-1}), PGP-6(6.58), PGP-10(6.37 nmoles C_2H_2 mg protein h^{-1}), PGP-5 (5.58 nmoles C_2H_4.mg protein.h^{-1}) and least by PGP-11(4.34 nmoles C_2H_4 mg protein h^{-1}). Similar kinds of results reported by Shrivastava (2013) isolated diazotrophic bacteria isolated from the rhizosphere of rice plants. The rate of nitrogenase enzyme activity based on acetylene reduction assay showed remarkable variation in these isolates which ranged from 0.69 to 1.63 nmol. C_2H_4 mg protein$^{-1}h^{-1}$.

Similar results were obtained by Murumkar *et al.* (2013) who isolated 93 *Azospirillum* isolates and reported the *A. lipoferum* isolates exhibited a higher average nitrogenase activity compared to *A. brasilense* isolates (105.9 vs. 20.8 nmol C_2H_4 mg protein$^{-1}h^{-1}$, respectively), similar results recorded by Gothwal *et al.* (2007) and Azin *et al.* (2005).

4.1.3 Production of plant growth promoting substances

Plant growth promoting substances such as auxins, gibberellins, ethylene substances produced by plant growth promoting rhizospheric bacteria. These are directly involved in plant growth promotion. In the present study sixteen PGPR isolates were screened for indole acetic acid production for selection of efficient growth promoting bacterial strain.

4.1.3.1 Indole acetic acid production

Effect of IAA in plants is significant and some of them are apical dominance, phototropism, gravitrophism, prevention of leaves and fruit abscission and induction of adventitious root system. Therefore IAA has profound influence on crops.

Among sixteen bacterial isolates IAA production varied with supplementation of L-tryptophan and without supplementation of L-tryptophan. Out of sixteen isolates PGP-15 showed maximum IAA production (4.4 µg ml^{-1}) without supplementation of tryptophan, followed by PGP-5 (3.51µg ml^{-1}), PGP-1(2.40 µg.ml^{-1}), PGP-16(2.26 µg ml^{-1}), PGP-9(1.39 µg ml^{-1}), PGP-6(1.37µg ml^{-1}) produced (Table.4.1 & Fig 4.2)

Enhancement in production of IAA was observed with the supplementation of L-Tryptophan @ 10 mg per liter by PGP-15 (7.76 µg ml^{-1}), followed by PGP-5(7.51 µg ml^{-1}), PGP-1(7.41 µg ml^{-1}), PGP-8(6.86 µg ml^{-1}), PGP-9(5.98 µg ml^{-1}), PGP-6(5.29 µg ml^{-1}), PGP-4(4.80 µg ml^{-1}), PGP-14(4.46 µg ml^{-1}), PGP-10(3.63 µg ml^{-1}), PGP-2(3.39 µg ml^{-1}) and some of the isolates produced IAA only in the presence of L-Tryptophan.PGP-2, PGP-4, PGP-7, PGP-8, PGP-10, PGP-12, PGP-14 were capable of IAA production with supplementation of L-Tryptophan. The isolates showed higher auxin production in the presence of precursor (L-tryptophan) as compared to without supplementation of L-TRP in the media. Asghar *et al.* (2004) reported a several fold enhancement in auxin production by rhizobacteria with L-TRP than without L-TRP (table .4.1 and Fig .4.2) and Kaur and Sharma (2013).

In the present study IAA production and phosphate solubilization by PGPR isolates were in agreement with the earlier reports available on PGPR strains which were isolated from wheat showed IAA production ranging from 5.5-31.0 µg ml^{-1} and (Abbasi *et al*. 2011). Hussain and Srinivas (2013) isolated 35 isolates *Pseudomonas* and *Azotobacter* each from rhizosphere of *Acacia nilotica* and *Albizia lebbeck* and reported 70% of the isolates of *Azotobacter* and *Pseudomonas* produced IAA. High Gibberillic acid production was also detected in *Azotobacter* (71.42%) isolates (Sudha *et al.,* 2012)

Similar kinds of results were also reported by Ponmurugan and Gopi (2006) who found that phosphate solublizing bacteria (PSB) isolated from the rhizosphere of different field crops including maize, were capable of producing auxin under *in vitro* conditions.

4.1.3.2 Ammonia production

All sixteen PGPR isolates were positive for ammonia production in peptone water tubes (Dye, 1962, Olivera *et al.* 2011). The isolate PGP-1 produced ammonia strongly (+++), PGP-3, PGP-4, PGP-5, PGP-7, PGP-8, PGP-9, PGP-10, PGP-12, PGP-13, PGP-14, PGP-15, PGP-16 were moderately (++) ammonia producers. PGP-6, PGP-11 were weak (+) ammonia producers. (Table.4.1).

4.2 IDENTIFICATION AND CHARACTERIZATION OF PGPR

4.2.1 Morphological and cultural characterization

The cell morphology, colony morphology, Gram reaction were studied for sixteen plant growth promoting isolates. Among sixteen PGP isolates, thirteen isolates were gram positive (PGP-1, PGP-2, PGP-3, PGP-4, PGP-5, PGP-6, PGP-6, PGP-8, PGP-9, PGP-10, PGP-11, PGP-14, PGP-15, PGP-16) and remaining three PGP isolates (PGP-7,PGP,-12,PGP-14) were gram negative (Table .4.2).

All isolates colony morphology and cultural characters were studied on basic nutrient agar medium. Similarly for all isolates colony morphology was studied on king's B agar medium, *Azotobacter* agar (Jensen's agar), *Azospirillum* agar (N- free sodium malate medium) and yeast extract mannitol agar (YEMA) medium. All isolates showed different colony morphology on different specific media (Table.4.3).

The bacterial isolates PGP-1, PGP-8, PGP-13, PGP-16 were greenish fluorescent pigmentation on King's-B agar medium (Vlassak,*et al*.,1992) (plate 4.4a), the isolates PGP-2, PGP-3, PGP-4 were formed whitish mucilaginous translucent colonies on yeast extract mannitol agar medium (plate.4.4c) (Vincent, 1970). PGP-7 (4.4b), PGP-12, PGP-14 were whitish spreading type colonies on nutrient agar medium (Plate.4.4 b).

PGP-5, PGP-9, PGP-11 were growth recorded on Jensen's agar medium (Jenson, 1954,Norris and Chapman,1968) with light brownish to brownish pigmentation (plate 4.5a).The isolate PGP-5, PGP-10, PGP-15 growth was fully recorded in N-free sodium malate medium (Okon *et al.*1977) with whitish growth and light pellicle type colonies observed (Plate .4.5c).

Further bacterial isolates were characterized on specialized media like BMS agar (Potato infusion agar) production of pigments after 2 weeks at 35 ◦C (Krieg and Holt, 1984), pellicle test and cyst formation. The bacterial isolates PGP-9, PGP-11 showed cyst formation in microscopic observation (Waksman No.77 N free agar medium grown cultures) (Vela and

Wyss,1964) . The isolates PGP-5, PGP-10, PGP-15 formed orange color (plate 4.5b) pellicle like colonies (Plate. 4.4g).

4. 2.2 Biochemical and characterization

The isolates were examined for biochemical characterization. All the PGP isolates tested for different biochemical tests like IMVIC tests, catalase test, oxidase test Starch hydrolysis test, Gelatin liquefaction test, H_2S production test, Carbohydrate utilization test and denitrification test.

Results presented in Table.4.4 and plate 4.6, results of IMVIC tests, PGP -6, PGP-9, PGP-11 and PGP-15 were positive for Indole production . For methyl red test only PGP-6, PGP-9, PGP-11 were positive. For Voges Prausker's test only PGP-1, PGP-8, PGP-13, PGP-16, PGP-6, PGP-9, PGP-11 were positive. For citrate utilization test all the sixteen isolates were positive. All the PGP isolates were oxidase positive, Catalase test is positive almost all isolates except PGP-7, PGP-12 and PGP-14. Starch hydrolysis test is positive only PGP-6, 9, PGP-7, PGP-12, PGP-14. Only PGP-1, PGP-8, PGP-1, PGP-16 isolates positive for Gelatin liquefaction.

For H_2S test only PGP-1, PGP-8, PGP-13, PGP-16, PGP-2, PGP-3, and PGP-4 were positive. Denitrification test was positive for PGP-1, PGP-8, PGP-13, PGP-16, PGP-7, PGP-12, and PGP-14.

In carbohydrate utilization test glucose, fructose, lactose, malate, mannitol sucrose sugars were used. Results were recorded and presented in table no.4.4 Isolate PGP-1 utilized glucose, fructose sugars; PGP- 8 utilized sucrose and glucose; PGP-13, PGP-16 were utilizes glucose and fructose; the three isolates PGP-2, PGP-3, PGP-4 were utilizes sucrose, glucose, mannitol sugars and lactose, fructose, malate sugars were not utilized. Isolates PGP-6, PGP-9, and PGP-11) were positive for utilization of sucrose, glucose, fructose, mannitol except malate sugar. The isolates PGP-5, PGP-10, PGP-15 utilized glucose, fructose, sucrose, malate except lactose.

The isolates could be identified based on gram reaction and colony morphology on different media. Sixteen isolates showed growth on nutrient agar but identification of PGP isolates is easy with growth on specialized media. Observations recorded after streaking and incubation on different medium. Both morphological, cultural biochemical characters were used for identification of the isolates using the criteria mentioned in Bergey's Manual of Determinative Bacteriology (1994). Probable identification of isolates upto genus level are presented in Table.no.4.5.

Free living nitrogen fixers *Azospirillum* sp (PGP-5, PGP-10.PGP-15), *Azotobacter* sp (PGP-6, PGP-9, and PGP-11) (Norris and Chapman,1968) were based on cultural, biochemical characters and nitrogen fixing ability (Acetylene Reduction Assay).The findings were in accordance with Attitalla *et al.*(2010). Phosphate solubilizing bacterial isolates *Pseudomonas sp* (PGP-1, PGP-8, PGP-13, PGP-16) were characterized morphologically (florescence under UV light, Suryakala *et.al.,* 2004), biochemically and on the basis of phosphate solubization. *Bacillus* spp (PGP-7, PGP-12, PGP-14) were characterized as rod shaped, gram positive, aerobic and facultative anaerobic, endospore forming bacteria (Todar,2004) and P-solubilization.

Based on microscopic examination and cultural characteristics, Preeti *et al.* (2011) identified four isolates as *Pseudomonas* spp. and others as *Bacillus subtilis.* The rhizobial isolates did not absorb the colour of congo red, which is a characteristic feature of rhizobia as reported by Somasegaran and Hoben (1994). Rhizobia form white, translucent, glistening colonies with varying quantity of slime production on congored YEM agar as described by the Vincent (1970). Sandeep *et al.* (2011) isolated *Azotobacter spp.* and identified based on morphological and biochemical characteristics.

Similarly Joseph *et al*. (2007a) isolated 150 bacterial isolates belonging to *Bacillus* (40 isolates), *Pseudomonas* (35 isolates), *Rhizobium* (35 isolates) and *Azotobacter* (40 isolates) from different rhizospheric soils of chickpea in the vicinity of Allahabad. One hundred and eighteen *Bacillus* spp. were isolated from the rhizosphere of soybean plant of Cirebon, Indonesia and further examined for plant growth promoting activities (Wahyudi *et al.,* 2011).

4.3 SCREENING OF EFFICIENT PGPR ISOLATES FOR BIO CONTROL ACTIVITY

PGPR are indigenous to soil and the plant rhizosphere and play a major role in the biocontrol of plant pathogens. Biocontrol may be defined as condition or practice where by survival or activity of pathogen is reduced through the agency of any other living organisms with the result that there was reduction in the incidence of disease caused by the pathogen. These microorganisms can also function as competitors of pathogens for colonization sites and nutrients

They can suppress a broad spectrum of bacterial, soil born fungal and nematode diseases**.** Greater application of PGPR is possible in agriculture for biocontrol of plant pathogens and biofertilization. Such microorganisms can produce substances that may limit the damage caused by phytopathogens, e.g. by producing antibiotics, siderophores and a variety of enzymes. Many rhizobacteria have been reported to produce siderophores, antifungal metabolites like, HCN, phenazines, pyrrolnitrin, 2, 4-diacetylphloroglucinol, pyoluteorin, viscosinamide and tensin (Bhattacharyya and Jha, 2012).

4.3.1 Siderophore production

Almost all organisms require iron for survival. However, under aerobic, neutral pH growth conditions soluble iron is rapidly oxidized and forms extremely insoluble oxides and oxyhydroxides . Most aerobic and facultative organisms must scavenge trace amounts of soluble iron and have to transport it into the cell by the production of small, soluble, high-affinity Fe (II1) - coordinating ligands, the siderophores .The production of siderophores and the specific transport of iron into the ligand-producing cell is an important adaptation for survival and success in a variety of iron limited environment. Siderophore production was included as one of the mechanism of biocontrol activity of the PGPR. Siderophore production was observed on 0.2 % aqueous ferric chloride solution.

Plant growth promoting rhizobacteria can also function as competitors of pathogens for colonization sites and nutrients these microorganisms produce siderophores and inhibit the root pathogens by creating iron limiting conditions in the rhizosphere and reduces probability of plant disease. Some siderophores are low molecular weight biomolecules secreted by microorganisms in response to iron starvation for acquisition of iron from insoluble forms by mineralization and sequestration.

Out of sixteen isolates, ten bacterial isolates produced siderophores. Among ten, four isolates (PGP-1, PGP-8, PGP-15, PGP-16) were strong (+++) for siderophore production and remaining six (PGP-2, PGP-7, PGP-9, PGP-10, PGP-13, PGP-14) isolates were moderate (++) producers of siderophores (Table .4.6) (Plates 4.1(d), 4.2(d), 4.3(d)).

Siderophores such as pseudobacin and pyoverdin (yellow green fluorescent pigment of *Pseudomonas* bacteria) present high antimicrobial activity and affinity to ions of trivalent iron (Maksimov, *et al*., 2011).

Tailor and Joshi (2012) isolated seven bacterial isolates from sugarcane rhizosphere. The isolates are found to produce more than 85% siderophore units. Amongst them S-11 was found be the most efficient siderophore producer (96 % SU). S-11 was further characterized and identified as *Pseudomonas fluorescens.* Physico-chemical parameters were evaluated for optimum production siderophores by *Pseudomonas fluorescens* strain.

4.3.2 Production of HCN

HCN production is attributed as one of the mechanisms of bio control activity of the PGPR, the ability of the 109 isolates to produce HCN was determined by the picric acid assay.

Out of sixteen PGP isolates nine isolates produced HCN (Table no.4.6 and Plate 4.1(c), 4.2(c), 4.3(c) of which PGP-1 PGP-8, PGP-7, PGP-9 proved strong (+++) for HCN production and remaining isolates produced HCN moderately (++) PGP-16, PGP-13 and PGP-4, PGP-6, PGP-11 produced weakly(+).

Ahmadzadeh and Sharirifi-tehrani (2009) detected the production of HCN by six isolates out of the 41 selected *Pseudomonads* having good antagonistic activity against *Rhizoctonia solani* under *in vitro* conditions.

Jha *et al.* (2009) also reported that the production of HCN by some new *Pseudomonad* strains having biocontrol activity against phytopathogenic fungi in addition to the P solubilization efficiency and stated that they can be used as biofertilizers as well as biocontrol agents. Similar results were observed by Maleki *et al.* (2010) reported the isolates *Pseudomonas fluorescens* CV-6 strain which produced considerable amounts of siderophore, indole acetic acid and also shown positive reactions for HCN, catalase.

Seven *Pseudomonads* isolated from the roots, shoots and rhizosphere soil of sugarcane were showed to produce HCN which had biocontrol ability against *Colletotrichum falcatum* inciting red rot disease in sugarcane in addition to their ability to plant growth (Mehnaz *et al.* 2009).

4.3.3 Antagonistic Activity

Antifungal activity of sixteen isolates (PGP-1 to PGP-16) was checked against *Rhizoctonia solani* and *Sclerotium rolfsii* under *in vitro* conditions using PDA media (Table .4.7). Based on both per cent inhibition and inhibition zone out of sixteen isolates, eleven isolates PGP-1(Plate 4.7) PGP-2, PGP-4, PGP-7, PGP-8, PGP-9 ,PGP-11 ,PGP-13, PGP-14, PGP-15, PGP-16)isolates exhibited inhibition potential against one or the other phytopathogen, while the remaining five (PGP-3, PGP-5, PGP-6, PGP-10, PGP-12) did not show any inhibitory activity against any of the pathogens tested. The antifungal activity of strains tested varied with percent inhibition from 20.50 to 36.05 with *Rhizoctonia solani* similarly antifungal activity of strains tested varied with percent inhibition from 17.17 to 35.17 with *Sclerotium rolfsii*.(Table no.4.5 and Fig no.4.3).

4.3.4 Antifungal assay with *Rhizoctonia solani*

Among sixteen Isolates PGP-1 induced large percent inhibition (36.10%) (Table .4.6 and Plate.4.7) followed by PGP-9 (35.50%), PGP-8 (35.15%), PGP-7 (34.49%), PGP-4 (35.72%), PGP-16 (34.23%), PGP-14 (34.66%), PGP-13 (33.45%), PGP-11 (30.84%), and PGP-15 (30.21%) the least inhibition percentage was showed by PGP-2 (20.53%).

4.3.5 Antifungal assay with *Sclerotium rolfsii*

Among sixteen isolates PGP -14 induced large percent inhibition (35.29%)(Table . 4.6 and plate. 4.7) and inhibition zone (4.00mm) followed by PGP-7 (35.47%), PGP-9 (33.89%), PGP-16 (33.74%), PGP-11 32.27%), PGP-4 (31.67%), PGP-13 (30.85%), PGP-1 (30.54%), PGP-8(29.74%), PGP-15(25.20%) and the least by PGP-2(17.62%).

Shaban and El-Bramaway (2011) studied the biological control of damping off and root rot causing fungi (*Fusarium oxysporum, F. solani, Macrophomina phaseolina, Rhizoctonia solani* and *Sclerotium rolfsii*) with antagonistic microorganisms (*Rhizobium* spp. and *Trichoderma* sp). Results revealed that combined effect of both *Rhizobium* spp and *Trichoderma* spp. were found to be beneficial in controlling the fungal diseases of legume crops.

Pankaj Kumar *et al.* (2012) reported that *Bacillus* spp. BPR7 strongly inhibited the growth of several phytopathogens such as *Macrophomina phaseolina*, *Fusarium oxysporum, F. solani, Sclerotinia sclerotiorum, Rhizoctonia solani* and *Colletotricum* spp. *in vitro*. Cell-free culture filtrate of strain BPR7 also caused colony growth inhibition of all test pathogens. PGP and antifungal activities of *Bacillus* sp. BPR7 suggested that it can be exploited as a potential bioinoculant agent for *Phaseolus vulgaris.*

Seven PGPR strains were isolated by Fatima *et al.* (2009) .These strains were analyzed for production of indole acetic acid (IAA), phosphorus solublization capability and inhibition of *Rhizoctonia solani*. Strains WPR-51, WPR-42 and WM-30 were selected to test antagonistic activity on two wheat varieties infected with *R. solani*. These three strains belonged to *Azotobacter* and *Azospirillum*. Out of these three strains, WPR-51 and mixture of all three strains showed maximum inhibition of *R. solani* growth.

4.3 MOLECULAR CHARACTERIZATION OF NITROGEN FIXING ABILITY OF EFFICIENT PGPR STRAINS

The molecular method based on detection of *nif*H1genes (nitrogenise genes), *nif* H_2 has been successfully applied.

4.3.1 DNA Isolation

Genomic DNA was isolated from two PGPR strains (efficient strains of nitrogen fixers) such as PGP-9 (*Azotobacter* spp.) and PGP-15 (*Azospirillum* spp.). Isolated DNA was quantified with Nano drop method (Fig .4.4).

4.3.2 Quantitative and Qualitative analysis of total genomic DNA

Quantification of genomic DNA was done using Nano Drop 8000 Spectrophotometer. The concentration of g DNA is shown in Table. 4.7.

4.3.3 Visualization of PCR Product

To confirm the targeted PCR amplification, 2 µl of PCR product from each tube was mixed with1 µl of 6X gel loading dye and electrophoreses on 1.5 % agarose gel. (Fig .4.5).100 bp DNA ladder size : 100,200,300,400,**500**,600,700,800,900,1000,1 kb DNA ladder size: 500, 1000, 2000, **3000,** 4000,5000,6000,8000.

The banding pattern of DNA of PGP-9 and PGP-15 on agarose gel electrophoresis was compared with that of reference *Azotobacter* strains and *Azospirillum* strains. The PCR production was carried out for cultures in order to check the presence of *nif* H1, *nif*H2 genes for *Azotobacter* and *Azospirillum.* The *nif*H1 gene was 500 bp *nif*H2 gene was 3000 bp. Similar results found by Mahbouba *et al.* (2013). They reported the amplification product bands at the expected *nif*H gene fragment size are about 370 bp. Some isolates (S1, S2, S3, Azo4, Azo5, Azo6, S9 and Azo10) were considered by the nitrogen-fixing gene *nif*H detection of their genome and *A. brasilense* inoculated durum wheat shows good growth and yield, Khider *et al.* (2012) compared classical approach with molecular based method for identification of *Azotobacter chroococcum* from soil samples.

Gosal *et al.* (2011) isolated 72 diazotrophic bacteria .The isolates were identified on the basis of cultural, morphological and biochemical characterization. These isolates showed amplification with two *nifH* primers (*nifH1*and *nifH2*), there by confirming their diazotrophic potential. The positive *nifH* isolates were further characterized using restriction fragment length polymorphism of 16S rDNA to reveal diversity among them based on UPGMA clustering and partial sequencing of 16S rDNA the isolates were identified as *Azotobacter* sp., *Azospirillum* sp., *Rhizobium larrymoorei*, *Pseudomonas aeruginosa* and *Xanthomonas oryzae*.

Kumar and Kannaiyan investigated substantial molecular diversity of N_2 fixing bacteria from field grown rice, based on retrieval of *nif H* or *nif D* gene fragments from root DNA. Targeted PCR finger printing of heterotrophic and endophytic diazotrophs from rice, using *nif H* primer, generated specific replicon with a molecular weight of approximately 750 bp. However, multiple replicons with molecular weight ranging from 500-1500 bp were observed in some isolates. The existence of considerable genetic and molecular diversity in the diazotrophic bacteria of rice and the scope for its better exploitation to achieve sustainable rice production

4.4 ASSESSING THE COMPATIBILITY OF EFFICIENT PGPR WITH AGRO CHEMICALS

Biological control approach will be successful, if the biocontrol agents are compatible with the fungicides, insecticides and herbicides which are commonly used for plant protection under field conditions. Therefore, the present investigation was undertaken to know the compatibility of local PGPR isolates against the commonly used agrochemicals like fungicides (metalaxyl, ziram, propiconazole and carbendazim), insecticides (chloripyriphos, dimethoate, imidachloprid and thiodicarb) and herbicides (atrazine, butachlor, propaquizafop and pendimethalin) at their recommended and half the recommended dosages (Table.4.8 and plate .4.8 to 4.10) under *in vitro* condition.

4.4.1 Effect of Fungicides on efficient PGPR Isolates

The PGPR bacterial isolates were screened for their compatibility with the four commonly used fungicides (metalaxyl, ziram, propiconazole and carbendazim.

Among sixteen PGPR isolates only PGP-1, PGP-9, PGP-13, PGP-14, PGP-15 isolates exhibited good compatibility (0 mm inhibition) with fungicides with recommended dosages. Whereas PGP-2, PGP-4, PGP-6, PGP-5, PGP-8, PGP-11, PGP-12 were affected by Ziram i.e. showed medium compatibility (1mm inhibition zone).PGP-3, PGP-10 were affected by Metalaxyl fungicide. Similarly PGP -10 is affected by all fungicides, which showing medium compatibility (1mm inhibition zone).PGP-7, PGP-12 affected by Proficonazole fungicide shown medium compatibility (1mm). PGP-16 had moderate compatibility with carbendazim.

4.4.2 Effect of Insecticides on efficient PGPR Isolates

The PGPR bacterial isolates were screened for their compatibility with the four commonly used insecticides (Chloripyriphos, Dimethoate, Imidachloprid and Thiodicarb).

Among sixteen PGPR isolates only PGP-1, PGP-9, PGP-11, PGP-12, PGP-14, PGP-15 were not affected by any insecticides, indication of high compatibility with insecticides at recommended dosages. Isolates PGP-2, PGP-4, PGP-8, PGP-10 with low compatibility were affected by Chloripyriphos with 1mm inhibition zone PGP-10 is affected by all insecticides. Isolates PGP-2, PGP-5, PGP6, PGP-7, PGP-13 were affected (medium compatibility with1mm inhibition zone) by Dimethoate insecticide with recommended dosage. Isolates PGP-3-, PGP-10, PGP-13, and PGP-16 were showing medium compatibility (1mm zone) with Thiodicarb insecticide with recommended dosage.

4.4.3 Effect of Herbicides on efficient PGPR Isolates

The sixteen PGPR bacterial isolates of were evaluated for their compatibility with the four commonly used herbicides (Butachlor, Atrazine, Propaquizafop and Pendimethalin).

Among all isolates seven isolates viz., PGP-1, PGP-2, PGP-9, PGP-11, PGP-13, PGP-14, PGP-15, had shown high compatibility (0mm inhibition zone) with all herbicides. Isolates PGP-3, PGP-4, PGP-7, PGP-10 were affected by Butachlor which showed moderate compatibility (0.5mm inhibition zone) with herbicide. Only PGP-3, PGP-8 isolates were affected by Atrazine insecticide. Only Isolate PGP-4 affected by Propaquizafop herbicide with medium compatibility and 1mm inhibition zone), PGP-5(low compatibility with 2 mm inhibition zone), PGP-6(low compatibility with 2 mm inhibition zone, PGP-12(medium compatibility) were affected by Pendimethalin herbicides with recommended dosage.

Among all isolates PGP-1.PGP-9.PGP-15 were not affected by recommended dosage of all fungicides, Insecticides and herbicides used in the present study.

Similar results have been reported by Maheswari (2013) for *Pseudomonas* isolates with systemic and non systemic fungicides, Sarvani (2011) reported similar results who studied the *Pseudomonas, Bacillus , Rhizobium* compatibility against commonly used agrochemicals like fungicides (Copper oxy chloride, Carbendazim, Thiram and Captan) insecticides (Phorate, Carbofuran, Imidachloprid and Chlorpyriphos), and herbicides (Alachlor, Butachlor, Pendimethalin and Oxyfluorofen) at their recommended and half the recommended dosages. Similar results were reported by Ghosh *et al.* (2003). He evaluated the lethal concentrations of Chlorpyrifos, Monocrotophos, Fenvalerate and Phorate in green gram and concluded that nitrogen fixation and grain yield were highest with *Rhizobium* + Chlorpyrifos and *Rhizobium* + Phorate treatments when *Rhizobium* was supplied simultaneously or 24h after insecticide application, respectively.

4.5 Selection of efficient strains for Maize field experiment

Among sixteen isolates, three efficient isolates selected based on multiple PGPR characters, biocontrol activity, and compatibility with different commonly used agrochemicals. For field experiment of maize crop, the selected isolates *Azotobacter* sp free-living nitrogen fixer, *Azospirillum* associative nitrogen fixer and phosphate solubilizing *Pseudomonas* sp, *Bacillus* spp.

These efficient isolates were used for formulates carrier based microbial inoculants and tested purity (10^8 cells/gram) prior to at the time of sowing. These microbial inoculants are applied as soil application along with chemical fertilizers and Farm Yard Manure with recommended dosages. The details of fertilizers and microbial inoculants were presented in table 4.9 , the combination of microbial inoculants with chemical fertilizers were T1: 100 % RDF (N2: P_2O_5: K_2O ratio: 120kg:50kg: 60kg ha^{-1}),T2: 75 % RDN + nitrogen fixer *Azospirillum* ,T3: 75 % RDN+ nitrogen fixer *Azotobacter* ,T4: 75 % RDP + Phosphate solubilizers, T5: 75 % RDN&P + nitrogen fixer *Azospirillum* + Phosphate solubilizers,T6: 75 % RDN & P + nitrogen fixer *Azotobacter* + Phosphate solubilizers, T7: 75 % RDN & P + *Azotobacter* + *Azospirillum* + Phosphate solubilizers.

4.6 INFLUENCE OF EFFICIENT PGPR STRAINS ON MAIZE CROP

The results of the maize experiment entitled "To study the influence of efficient PGPR strains with maize crop'' conducted during *rabi* 2013 with Randomized block design, at the College Farm, College of Agriculture, Rajendranagar, Hyderabad are presented and are discussed here under. Experimental data were statistically analyzed apportioned under various heads and subheads, furnished in tables and illustrated through figures wherever necessary. The experimental data on various growth parameters, yield attributes, and economics are interpreted in tables.

The potential PGPR strains such as nitrogen fixers and phosphate solubilizers are show significant results in maize growth and yield attributes with different combinations.

The soil of the experimental site was sandy clay loam with a low content of organic carbon (0.39%), low in nitrogen (232.7 kg ha^{-1}), medium in available phosphorus (25.9 kg ha^{-1}) and potassium status (272.5 kg ha^{-1}).

4.6.1 Influence of PGPR on plant growth parameters

4.6.1.1 Plant height

Data inscribed in Table .4.10 and depicted in Fig.4.6, Clearly shows that the maize plant height increased linearly with age up to 70 DAS and there afterwards the growth is rather slow as the crop reaches tassel initiation stage.

The plant height results reveal that in the initial knee height stage (40 DAS) plant height is varied with different combinations of chemical and microbial inoculants. The highest plant height (32.77cm) was recorded in treatment T7 (75 %RDN& P + *Azospirillum* + *Azotobacter* + PSB), followed by T6 (75 % RDN& P + *Azospirillum* + *Azotobacter* + PSB)30.98cm,T5(75 % RDN& P + *Azospirillum* + PSB)30.33cm, T2(75 % RDN+ *Azospirillum*) 28.20cm and lowest plant height was recorded in T1(100 % RDF)27.65cm(photo.4.1a).

The plant height in 70 DAS increased vigorously with different combination of treatments. The plant height varied 174.36 cm to 187.32cm. The highest plant height (187.32 cm) was recorded in T7 (75 % RDN & P + *Azospirillum* + *Azotobacter* + PSB), next highest plant height (184.57cm) was recorded in treatment T5 and T6 (75 % RDN& P + *Azospirillum* +*Azotobacter*+ PSB). The plant height in not much different in harvesting stage. Similar plant difference recorded in this stage as 70 DAS.

Similar results were reported in plant height and leaf area in different crops inoculated with *Pseudomonas*, *Azospirillum* and *Azotobacter* strains by Burd *et al.*(2000) reported that plant growth promoting rhizobacteria might enhance plant height and productivity by synthesizing phytohormones, increasing the local availability of nutrients, facilitating the uptake of nutrients by the plants decreasing heavy metal toxicity in the plants antagonizing plant pathogens. Similar results were also reported by Hussain *et al.* (2013)

The findings was of Yasari and Patwardhan(2007) reported that application of *Azotobacter* and *Azospirillum* strains increased canola yield (21.17%), pod per plant (16.05%), number of branches (11.78 %) and weight of 1000grain (2.92 %).

Usha Rani *et al.* (2012) isolated, enumerated and characterized the PGPR from the rhizosphere soil of pigeon pea. Further investigated to show the PGPR traits in pigeon pea seedling emergence, increase of shoot length, root length, dry matter production of shoot, nodule number and nodule mass. Furthermore, PGPR isolates remarkably increased seed germination of pigeon pea.

Similar results were reported by Karnwal (2012) the inoculation of *Bacillus lentus, Bacillus* spp., *Azospirillum brasilense, Bacillus subtilis, Pseudomonas fluorescens, Azotobacter diazotrophicus, Bacillus halodurans* and *Pseudomonas* strains as biofertilizer will be clearly showed greater root and shoot dry weight response in maize and wheat compared to control by different plant growth substances of these rhizospheric bacteria.

4.6.1.2 Root length

The results of root length depicted in table.4.10 and Fig .4.7. The root length are significantly influenced by different combinations of chemical fertilizers and microbial inoculants. The root length is varied in 40 DAS, 70 DAS, harvesting stages.

In 40 DAS the highest root length (14.06 cm) was recorded in T7 (75 %RDN& P + *Azospirillum* + *Azotobacter* + PSB) and lowest root length (11.9 cm) in control plot T1 (100 % RDF).

In 70 DAS the highest root length (35cm) was observed in T7 (75 %RDN& P + *Azospirillum* + *Azotobacter* + PSB). The root length was more increased in T5 (75 % RDN+ *Azospirillum*+ PSB), T6(75 % RDN& P + *Azospirillum* +*Azotobacter*+ PSB),T7(75%RDN& P + *Azospirillum* + *Azotobacter* + PSB) compared to control (T1).

In the harvesting stage not much difference was observed in root length in different treatments. But highest root length (33.66cm) was recorded in treatment T7 (75%RDN& P + *Azospirillum* + *Azotobacter* + PSB) followed by the treatment T6 (75 % RDF + *Azotobacter* + PSB (31.66cm), T5 (30cm). The lowest root length was recorded in control T1 (100%RDF) 28cm.

The root length was more at 40 DAS and 70 DAS significantly with combination of reduced chemical fertilizers (75% RDF) and microbial inoculants combination. This complies with the finding of Rasul *et al.* (1998) who reported that root elongation is associated with the production of indole acetic acid in the early stages of the crop. The indole acetic acid increased in inoculated plants as compared to control. Gomaa and Kholas (1999) reported that the root length in mungbean was increased with the combined effect of organic manure, vermicompost, chemical fertilizers and biofertilizers.

4.6.1.3 Dry weight of plant

The dry weight of the plant varied at different stages in different treatments depicted in table. 4.10 and Fig .4.8 and fig. In 40 DAS the highest dry weight of plant (37.33g) was recorded in T7 (75 %RDN& P + *Azospirillum* + *Azotobacter* + PSB) and lowest root length (30.05g) was found in control T1 (100% RDF).

In 70 DAS the highest dry weight of plant was observed in T7 (75 % RDN& P + *Azospirillum* + *Azotobacter* + PSB) 75g, followed by T5 (75% RDN+ *Azospirillum*+ PSB) 68.54g and T3 (75%RDN + *Azotobacter*) 68.33g.

In the harvesting stage dry weight of plant varied from 240.15g to 272.89 g. The highest dry weight of plant recorded (272.89 g) in treatment T7 (75 % RDN& P + *Azospirillum* + *Azotobacter* + PSB).Lowest dry weight (240.15 g) of plant was recorded in control T1.

Sharifi and Khavazi (2011) reported similar results. Evaluated when the effect of seed priming with PGPR on dry matter remobilization and yield of maize hybrids, seed priming of maize hybrids (SC-404, SC-410 and SC-434) with PGPR in three levels without priming (as control), priming with *Azotobacter* or *Azospirillum* and *Azotobacter* + *Azospirillum*. The results showed that seed priming with PGPR affected grain yield, plant height, number of kernels per cob and number of grains per row of cob significantly.

Similar results reported by Bashan *et al.*(2003) and Cakmake *et al.*(2004) reported that inoculation of seeds with *Azospirillum* resulted in significant changes in various growth parameters, increase in total plant biomass etc. The increase in dry matter accumulation with seed priming with PGPR indicated the favourable response of corn hybrids to seed priming with PGPR.

Adjanohoun *et al*. (2011) reported that *Azospirillium lipoferum, Pseudomonas fluorescens* and *Pseudomonas putida* are the best PGPR candidates for maize crop improvement on reddish ferrous field. An increased root biomass of 59.57% and 23.40% was recorded with *Pseudomonas fluorescens and P.aeruginosa,* respectively, while other members of the 15 identified PGPR showed little or no significant growth promoting effect on maize crops compared to non-PGPR colonized maize field. Similarly Tylak *et al.* (1989) reported *Azotobacter* inoculation positively affected maize and sorghum dry matter.

4.6.2 Effect of PGPR on yield attributes of maize

4.6.2.1 Cob weight

Cob weight at harvest stage differed significantly as influenced by application of microbial inoculants and chemical fertilizers in different combinations (Table.4.11 and Fig .4.9).

Cob weight varied in different treatments from 703.33g to 756.66g. All the treatments showed more cob weight compared to control plot T1 (100% RDF) 703.33g. Maximum cob weight was recorded in T_7 (75% RDN& P + *Azospirillum* + *Azotobacter* + PSB) 756.66g, followed by T5 (75% RDN& P + *Azospirillum*+ PSB) 723.33g, T6 (75% RDN&P + *Azotobacter* +PSB) 716.66g. Treatment T_2 (75% RDF+ *Azospirillum*) showed increased cob weight 725g.

Similar types of results were reported by Zahir et al. (1998). An increase of 18% in ear weight was showed when seed was inoculated by *Azotobacter* and *P. florescence*.

4.6.2.2 No. of rows per cob

No. of rows per cob maximum in treatment T_7 (75%RDN&P + *Azospirillum* + *Azotobacter* + PSB) 15.83 and treatment T_2 (75% RDN+ *Azospirillum*) 15.20, followed by treatment T6 (75 % RDN&P + *Azotobacter* +PSB) 15.03, T5 (75 % RDN & P + *Azospirillum*+ PSB) 15.00. Lowest No. of rows per cob was recorded in control plot T_1 (100%RDF) 14.00. (Table .4.11 and Fig .4.10).

4.6.2.3 No. of kernels per row

No.of kernels per row was maximum in treatment T_7 (75 %RDN& P + *Azospirillum* + *Azotobacter* + PSB) 32.45 compared to all other treatments. The lowest No. of kernels per row recorded in control T1 (100 % RDF) 28.46 (Table .4.11 and Fig .4.11). Similar results were reported by De Freitas (2000) and Cakmakı *et al.*(2007) in wheat and maize.

4.6.2.4 No. of kernels per cob

No.of kernels per cob were significantly increased with application of microbial inoculants and chemical fertilizers i.e. is maximum in treatment T7 (75 % RDN & P + *Azospirillum* + *Azotobacter* + PSB) 438.88, followed by T2 (75 % RDN+ *Azospirillum*) 431.66, T6 (75 % RDN & P + *Azotobacter* + PSB) compared to control T1 (100 %RDF) 416.66. The individual combination of microbial inoculants such as T2 (75% RDN+ *Azospirillum*) 431.66, showed more No. of kernals per cob (Table.4.11and Fig .4.12).

4.6.2.5 Test weight (100 seed weight)

Weight of 100 seeds at harvest stage differed significantly as influenced by application of microbial inoculants and chemical fertilizers in different combinations (Table no.4.11**).** The T7 (75 %RDN& P + *Azospirillum* + *Azotobacter* + PSB) treatment showed maximum test weight (26.31gm) followed by treatment, T2 (75 % RDN+ *Azospirillum*) 26.31g, T3 (75% RDN + *Azotobacter*) 25.76g.

Similar type of results were recorded by Namazari *et al.* (2012) who studied the effect of Biosuper biofertilizer and mineral fertilizer on yield and yield components of Corn separately and collectively. The results showed that using Biosuper biofertilizer had positive effect on 100 Kernel weight, ear weight and grain yield. Also, using mineral fertilizer led to increase in 100 Kernel weight, ear weight and grain yield. Application of fertilizers was effective on the traits and collective application of mineral fertilizer with Biosuper biofertilizer increased yield components, as this enhancement led to increase in yield by

30.69% related to when the biofertilizer not applied. Similar results were reported by Gholami *et al.* (2009) and indicated that *Azosprilum* and *Pseudomonas* caused an increase in 100 Kernel Weight.

Chabot et al. (1993) and Zahir *et al.* (1998) reported increased dry matter production of corn seed inoculated with bacteria by 32% and 18%, respectively. Javed et al. (1998) also showed increased biomass and dry weight with an application of plant growth bacteria.

4.6.2.6 Grain yield (kg.ha^{-1})

The grain yield significantly influenced by treatments of combination of chemical and microbial inoculants. The grain yield varied from 2653.33kg ha^{-1} to 3366 kg ha^{-1} depicted in table .4.12 and Fig. 4.13. The highest grain yield (3366 kg ha^{-1}) was recorded in treatment T7 (75 % RDN & P + *Azospirillum* +*Azotobacter* + PSB), followed byT6, T5. In contrast lowest grain yield (2653.00kg ha^{-1}) was in treatment T1 (75%RDF) (4.1(b) & 4.1 (c)).

Similar results reported by Baral and Adhikari (2013) when conducted one field experiment to study the effect of *Azotobacter* on growth and yield of maize. The treatments were control, 120:60:40kg N : P_2O_5 : K_2O(RDF) ha^{-1}, *Azotobacter* seed inoculation, *Azotobacter* soil application, *Azotobacter* +10 t FYM ha^{-1}, 10 t FYM ha^{-1}, RDF +*Azotobacter,* RDF+ *Azotobacter* + 10 t FYM ha^{-1}. Analysis of variance showed that grain yield, plant height, ear height, ear length, kernel per rows and 1000 grain weight were significantly affected with treatments. Only inoculation of *Azotobacter* increased 15 to 35% grain yields over non inoculated treatments. The benefit of *Azotobacter* inoculation was higher in the absence of chemical fertilizer application. Increase in the yield and improvement in growth related measurements due to inoculations with *Azotobacter* and *Azospirillum* were reported by Meshram and Shende (1982), Lin *et al.*(1983) and Fulchieri and Frioni (1994).

Swati Yadav *et al.*(2011) reported similar results by field experiment was implemented during the two successive seasons of 2009 and 2010 at the to evaluate the effects of application of *Azospirillum* with varying amounts of nitrogen fertilizer on growth and yield of maize. The application treatments included control (no fertilizer), chemical fertilizer and biofertilizer. Lin *et al.* (1983) reported, the *Azospirillum* increased yield due to promoting root growth which in turn enhanced nutrients and water uptake from the soil. Inoculation with *Azospirillum* strains resulted in yield increase in maize (Bashan and Levanon, 1990), Baral and Adhikari (2013) reported benefit of *Azotobacter* inoculation was higher in the absence of chemical fertilizer application.

Okon (1985) mentioned that increasing yield was attributed to the plant growth promoting substances by root colonizing bacteria more than the biological nitrogen fixation. Biari *et al.* (2008) found that growth stimulator bacteria such as *Azosprillum* and *Azotobacter* had positive effects on corn yield when grown at field conditions.

Naserirad *et al.* (2011) studied the response of maize cultivars with integrated application of biofertilizer as a factor in main plots and bio-fertilizer factor (non-inoculation, inoculation with *Azotobacter*, *Azospirillum* and double inoculation of *Azotobacter* and *Azospirillum*) as sub plots. The effect of cultivar on plant height, stem diameter, number of grain per row, 1000-grain weight, grain yield, biological yield and protein contents were found significant. Similar results were reported by Dhanasekar and Dhandapani (2012) on the effect of biofertilizers, *Azotobacter, Azosprillium, Phosphobacter and Rhizobacter* on the growth of *Helianthus annuus*. A 60% increased yield and 40.95% increased growth was observed in SSH-48. PGPR were applied to various crops to enhance growth, seed emergence and crop yield, and some have been commercialized (Dey *et al*., 2004; Herman *et al*., 2008).

4.6.2.7 Stover yield (kg.ha^{-1})

The stover yield also significantly influenced by treatments of combinations of chemical and microbial inoculants. The Stover yield varied from 3540 kg.ha^{-1} to 4530kg.ha^{-1}. The highest stover yield (4530 kg.ha^{-1}) was recorded in treatment T7 (75% RDN& P + *Azospirillum* +*Azotobacter* + PSB+ FYM) followed by T5 (4276.66kg.ha^{-1}), T6 (4272 kg.ha^{-1}). In contrast lowest Stover yield (3540 kg.ha^{-1}) was on treatment T3 (75%RDN+ *Azotobacter* +FYM).The individual microbial inoculants with combination of chemical fertilizers were recorded highest in treatment T2(75% RDN+ *Azospirillum*).The lowest stover yield was recorded in control T1(3540 kg.ha^{-1}) (Table .4.12 and Fig .4.14)

4.6.3 Soil Nutrient Status

The available major nutrients *viz*., nitrogen, phosphorus and potassium contents in soil as influenced by application of different combinations of microbial inoculants and chemical fertilizers were presented in the table .4.13.

4.6.3.1 Available nitrogen (kg ha^{-1})

Lowest available nitrogen at the time of harvest which was recorded in T1 (228.63 kg ha^{-1}) control plot. The available nitrogen is highest in T7, which is combinational application of *Azospirillum, Azotobacter,* PSB with 75% chemical fertilizers dose. There is a progressive increase in the balance with decrease in yield as registered in various treatments. Nitrogen fixers were enhancing the available nitrogen content in the experiment field plots.

4.6.3.2 Available phosphorus (kg ha^{-1})

Similarly phosphorus available in the soil at the time of harvest varied from 23.9 kg ha^{-1}) control and the highest in T_4 (36.33 kg ha^{-1}). The available phosphorus increased highest in T4 with PSB microbial inoculants application. Lowest available phosphorus recorded in control T1 (23.9kg ha^{-1}) Compared to all treatments. Similar views were expressed by Yazdani et al. (2009) who demonstrated that use of growth stimulating bacteria and phosphate solute in combination with chemical fertilizer was able to reduce phosphorus fertilizer application by 50% without of reduction in corn yield.

Sundara *et al.* (2002) found that the application of phosphate solubilizing biofertilizer, *Bacillus megaterium* var. *phosphaticum*, increased the phosphorus availability in the soil of sugarcane crop. This can be due to the synergistic action of the NBF, PSBF and possibly the NBF also has some phosphate solubilizataion activity. Rajeshwar and Khan (2010) reported that the phosphorus availability was increased in all the biofertilizers and in recommended dose of chemical fertilizer treatments in kharif rice.

Higher phosphorus content may be due to inoculation and availability of phosphorus in soil by microbes. Biofertilizer enhances soil fertility by solubilizing unavailable sources of bound phosphate into available forms in order to facilitate the plant to absorb them (Singh and Yadav 2008). The root system of legumes has capacity to solubilize soil phosphorous through the excretion of amino acids and encourage the growth and multiplication of phosphate solubilising bacteria

4.6.3.3 Available potassium (kg ha^{-1})

Soil available potassium differed significantly as influenced by application of microbial inoculants and chemical fertilizers with reduced (75%) application in different combinations after harvest of Maize crop. The basal potassium level in the soil was 235 kg ha^{-1}.

Lowest potassium available was (274.1 kg ha^{-1}) in control T1 (100%RDF) without microbial inoculants application. Highest available potassium was recorded (in treatment T7 (75% RDN& P + *Azospirillum* +*Azotobacter* + PSB+ FYM). by Naga Raju *et al.* (2009) reported similar results.

Selvakumar *et al.* (2012) reported that available soil nitrogen, phosphorus and potassium content in black gram crop was significantly increased by the application of biofertilizers (*Rhizobium* + phosphobacteria) application.

4.6.4 Microbial population dynamics (log CFU g^{-1} of dry soil)

Fluctuations in the microbial community in the maize rhizosphere, due to bacterial inoculations was recorded. Data interpreted with two factorial RBD.

The data on the microbial population of soil *viz*., Bacteria, Fungi, *Azotobacter*, *Azospirillum*, *Rhizobium* and PSB, at different intervals of crop as influenced by application of and chemical fertilizers in different combinations were synergistic in nature as bacteria provide soluble phosphate and plants supply root borne carbon compounds (mainly sugars), that can be metabolized for bacterial growth (Perez *et al*., 2007). The PSM along with other beneficial rhizospheric micro flora enhance crop production. Synergistic interactions on plant growth have been observed by co inoculation of PSB with N_2 fixers such as *Azospirillum* (Belimov *et al*., 1995) and *Azotobacter* (Kundu and Gaur, 1984), or with vesicular arbuscular mycorrhizae (Kim *et al*., 1998).

In the other hand, as suggested by *Roesti et al*. inoculum of the PGPR strains on the seeds may have shifted the bacterial community equilibrium at early stages of plant growth and favored growth of beneficial populations.

4.6.4.1 Bacterial population at different growth stages of crop (log10^6 CFU g^{-1} of dry soil)

An increasing trend in bacterial population was observed till 70 DAS of crop growth. Highest population was observed in T7 (75%RDN & P + *Azospirillum* + *Azotobacter* + PSB) at 70 DAS. Bacterial population was increased at flowering stage due to release of root exudates in the rhizosphere of the plant. Total bacterial count was significant in all inoculated treatments over T1 (100% RDF). A decline in population was observed at harvesting stage in all the treatments. The population depicted in Table. 4.14 and Fig .4.15).

4.6.4.2 *Azotobacter* population at different growth stages of crop (log10^3 CFU g^{-1} of dry soil)

Azotobacter population increased upto 70DAS of crop growth in all the treatments and maximum population was observed in T6 at 70 DAS followed by T7, T3 & T5 were on far. *Azotobacter* population was increased in T1 also because of the nature of the host plant, it being cereal crop.

Vivek and Singh (2001) reported that there was a significant increase in the inoculated bacterial (*A. chroococcum, A. lipoferum* and *P. striata*) populations in vermicompost by the second week. Maximum numbers were found between 45-60 days. After the 60th day there was a decline in count of microbes (Table .4.15 and Fig .4.16).

4.6.4.3 *Azospirillum* population at different growth stages of crop (log10^3CFU g^{-1} of dry soil)

In the *Azospirillum* microbial population gradually increased in all treatments till 70DAS irrespective of treatments, but maximum population was recorded in T7 & T2 where these two received inoculation of *Azospirillum*. Similarly the retention of population at harvesting stage was also higher in T2 &T7. (Table .4.16 and Fig .4.17).

4.6.4.4 PSB population at different growth stages of crop (log10^4 CFU g^{-1} of dry soil)

PSB population recorded an increasing trend in all the treatments upto 70DAS of crop growth. Population showed inclination towards harvest in all treatments except in T4.Incase of T4, phosphate solubilizing bacteria alone inoculated with 25% reduced chemical phosphatic fertilizer could support the significantly high population of PSB up to harvest. The current finding concerning earlier response of soil microbial population to mixed treatment was also reported by Balakrishnan *et al.* (2007). Available phosphorus was highest in available phosphorus in T4 with 36.66 kg.ha^{-1}(Table .4.17 and Fig .4.18)

Pseudomonas spp. are common rhizobacteria (Latouret *et al.*, 1996; Milling *et al.*, 2005). A large body of research has been devoted to the study their (often beneficial) interactions with the plant (Glick, 1995; Bloemberg and Lugtenberg, 2001; Dobbelaere *et al.*, 2003). Recent findings indicate that *Pseudomonas* populations may shift along the root system in relation to variations in the nature and quantity of root exudates (Di Battista-Leboeuf *et al.*, 2003). These results suggest that *Actinobacteria, Bacteroidetes* and *Pseudomonas* represent stable components of the microbial ecosystem whose within-group members and possibly cell numbers remain unaffected by changes in nutrients, or are less sensitive to such changes. These stable patterns may be explained by the oligotrophic nature of these rhizobacteria, which are commonly encountered soil bacteria (Sait *et al.*, 2002). These populations respond slowly to changes in the root environment, including those brought about by root exudates (Duineveld *et al.*, 1998).

4.6.4.5 *Rhizobium* population at different growth stages of crop (log10^3 CFU g^{-1} of dry soil)

The rhizobial population data reveals that at 0 DAS, across all the treatments, in the rhizosphere of maize *Rhizobium* population has been increased upto 70DAS and same was maintained till harvest. Increase may be due to root exudates and soil nutrients in the rhizosphere of maize. Retained population at harvesting stage, maximum in T4 followed by T3. The T3 & T4 treatments were supplemented with respective *Azotobacter* and phosphate solubilizing bacteria where in these PGPR isolates were known to produce various vitamins, siderophores, IAA. All these cofactors might have supported the saprophytic growth of *Rhizobium*. (Table .4.18 and Fig 4.19).

This retention of high population will be beneficial in crop rotation of cereal followed by legumes cropping system as natural beneficial population. Farmers' perceptions of crop importance in improving or depleting soil fertility were investigated in the NGS in Nigeria (Manyong et al., 2000). Cereals, particularly maize and sorghum, deplete soil nutrients while legumes (soybean, groundnut, and cowpea in that order) beneficial. Farmers thus correctly perceive the role the legumes play in enhancing soil fertility.

4.6.4.6 Fungal population (log 10^3 CFU g^{-1} of dry soil)

The fungal populations results show that, the application of PGPR inoculants suppress the soil fungal population from 0DAS to 70 DAS. At harvesting stage where the decrease in population of PGPR isolates with raise in the fungal population was observed. This indicates that the PGPR microbial inoculants were suppressing the fungal population. (Table.4.19 and Fig .4.20).

4.7 Economics

The data pertaining to the economics of maize crop as influenced by application of PGPR microbial inoculants and 75% reduced chemical fertilizers in different combinations were presented in the Table no.4.20.

The highest net return (27,266.58 Rs ha^{-1}) with high B:C ratio (1.65) was recorded in the treatment supplied with 75 %RDN & P+ *Azospirillum* +*Azotobacter* + PSB. Further it was followed by treatment supplied with 75 % RDN & P +*Azospirillum* + *Azotobacter* + PSB (T7) (21,266.58 Rs ha^{-1}) and with 75 % RDN+ *Azospirillum*(20,750 Rs ha^{-1}) and lowest net returns in treatment control T1 with 100% RDF(17,993.29 Rs ha^{-1}).

The results show that the combination of PGPR inoculants and 75% reduced chemical fertilizers will increase the net returns over 100% RDF without inoculation of microbial inoculants. Among all treatments T7 (75 % RDN & P+ *Azospirillum* +*Azotobacter* + PSB) improved yield better than all other treatments because of co inoculation of three microbial inoculants which could have improved the soil fertility and nutrient status, inhibition of the growth of phytopathogens, Production of growth promoting substances etc.

Table 4.2 Morphological and growth characteristics of bacterial isolates

PGPR isolate	Gram reaction	Shape	Motility	Cyst formation	Pigmentation	Pellicle formation	Spore production
PGP-1	-ve	rods	-	-	Greenish	-	-
PGP-2	-ve	rods	-	-	No	-	-
PGP-3	-ve	rods	-	-	No	-	-
PGP-4	-ve	rods	-	-	No	-	-
PGP-5	-ve	Curved/spiral	+	-	Yellow	-	-
PGP-6	-ve	rods	+	+	Light brown	+	-
PGP-7	+ve	rods	-	-	No	-	+
PGP-8	-ve	rods	-	-	Greenish	-	-
PGP-9	-ve	rods	+	+	Brown	-	-
PGP-10	-ve	Curved/spiral	+	-	Yellow	+	-
PGP-11	-ve	rods	+	+	Pale brown	-	-
PGP-12	+ve	rods	-	-	No	-	+
PGP-13	-ve	rods	-	-	Greenish	-	-
PGP-14	+ve	rods	-	-	No	-	+
PGP-15	-ve	Curved/spiral	+	-	Yellow	+	-
PGP-16	-ve	rods	-	-	Greenish	-	-

+ Production − No production +Ve gram positive reaction -Ve gram Negative reaction

Table.4.3 Cultural characteristics of PGPR isolates on different media

PGP isolate	**Colony morphology on Nutirent Agar**	**Colony morphology on Yeast mannitol agar**	**Colony morphology on king's B medium**	**Colony morphology on Jensen's medium**	**Colony morphology on N-free malate medium**
PGP-1	Yellow , round, non spreading		Yellow green, round, non spreading ,opaque	-	-
PGP-2	White ,gummy	Gummy white, round, non-spreading, smooth, raised, transluscent, mucoid	-	-	-
PGP-3	White gummy	Gummy white, round, non-spreading, smooth, raised, transluscent, mucoid	-	-	-
PGP-4	Whit gummy	Gummy white, round, non-spreading, smooth, raised, transluscent, mucoid	-	-	-
PGP-5	Pale white and raised	-	-	-	White dense small, undulate margins
PGP-6	Milky white	-	-	Milky white, irregular, non-spreading, smooth, raised, opaque,light brown	-
PGP-7	Off white, irregular, non-spreading, smooth, flat, opaque	-	-	-	-
PGP-8	Yellow , round, non spreading	-	Yellow green, round, non spreading ,opaque	-	-
PGP-9	Milky white	-	-	Milky white, irregular, non-spreading, smooth, raised, opaque,brown	-
PGP-10	Pale white and raised	-	-	-	Pale yellow ,dense small,undulated

					margins
PGP isolate	**Colony morphology on Nutirent Agar**	**Colony morphology on Yeast mannitol agar**	**Colony morphology on king’s B media**	**Colony morphology on Jensen’s media**	**Colony morphology on N-free malate medium**
PGP-11	Milky white	-		Milky white, irregular, non-spreading, smooth, raised, opaque	-
PGP-12	Off white, irregular, non-spreading, smooth, flat, opaque	-	-	-	-
PGP-13	Yellow , round, non spreading	-	Yellow green, round, non spreading ,opaque	-	-
PGP-14	Off white, irregular, non-spreading, smooth, flat, opaque	-	-	-	-
PGP-15	Pale white and raised	-	-	-	White dense small,undulated margins
PGP-16	Yellow , round, non spreading	-	Yellow green, round, non spreading ,opaque	-	-

Table .4.4 Biochemical and characteristics of PGPR isolates

S. No	Isolates	Indole test	MR	Vp test	Citrate utilization test	Catalase test	Oxidase test	Starch hydrolysis test	Gelatine liquefaction test	H_2 S test	Carbohydrate Utilization						Denitirfication test
											Lactose	**Sucrose**	**Glucose**	**Fructose**	**Malate**	**Mannitol**	
1	**PGP-1**	-	-	+	+	+	+	-	+	+	-	+	+	+	-	-	+
2	**PGP-2**	-	-	-	+	+	+	-	-	+	+	+	+	-	-	+	-
3	**PGP-3**	-	-	-	+	+	+	-	-		-	+	+	-	-	+	-
4	**PGP-4**	-	-	-	+	+	+	-	-	+	-	+	+	-		+	-
5	**PGP-5**	-	-	-	+	+	+	-	-	-	+	+	+	+	+	+	-
6	**PGP-6**	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	-
7	**PGP-7**	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	+
8	**PGP-8**	-	-	+	+	+	+	-	+	+	-	-	+	-	-	-	+
9	**PGP-9**	+	+	+	+	+	+	+	-	-	+	+	+	+	-	+	-
10	**PGP-10**	-	-	-	+	+	+	-	-	-	+	+	-		+	+	-
11	**PGP-11**	+	+	+	+	+	+	-	-	-	-	+	+	-	-	+	-
12	**PGP-12**	-	-	-	+	-	+	+	-	-	-	-	+	-	-	-	+
13	**PGP-13**	-	-	+	+	+	+	-	+	-	-	-	+	+	-	-	-
14	**PGP-14**	-	-	-	+	-	+	+	-	-	+	+	-	-	-	-	+
15	**PGP-15**	+	-	-	+	+	+	-	-	-	+	+	-	-	+	+	-
16	**PGP-16**	-	-	+	+	+	+	-	+	+	-		+	+	-	-	+

MR - Methyl red + positive result

VP - Voges Praskaur’s test, - Negative result

Table .4.5 Morphological and Biochemical characterization of isolates upto genus level

S.No	PGPR isolate code	Source	Probable genus
1.	PGP-1	Biocontrol laboratory, ARS, Amaravathi, Guntur (dist), A.P.	*Pseudomonas* spp
2.	PGP-2	Department of soil and biology, ICRISAT, Hyderabad.	*Rhizobium* spp
3.	PGP-3	Department of soil and biology, ICRISAT, Hyderabad.	*Rhizobium* spp
4.	PGP-4	Department of soil and biology, ICRISAT, Hyderabad.	*Rhizobium* spp
5.	PGP-5	Biofertilizers laboratory, ARS, Amaravathi, Guntur (dist), A.P.	*Azospirillum* spp
6.	PGP-6	Biofertilizers laboratory, ARS, Amaravathi, Guntur (dist), A.P.	*Azotobacter* spp
7.	PGP-7	Biofertilizers laboratory, ARS, Amaravathi, Guntur (dist), A.P.	*Bacillus* spp.
8.	PGP-8	Biofertlizers laboratory, ARS, Amaravathi, Guntur (dist), A.P.	*Pseudomonas* spp.
9.	PGP-9	Biofertilizers laboratory, ARS, Amaravathi, Guntur (dist), A.P.	*Azotobacter* spp.
10.	PGP-10	Biofertilizers laboratory, ARS, Amaravathi, Guntur (dist), A.P.	*Azospirillum* spp.
11.	PGP-11	Biofertilizers laboratory, ARS, Amaravathi, Guntur (dist), A.P.	*Azotobacter* spp.
12.	PGP-12	Biofertilizers laboratory, ARS, Amaravathi, Guntur (dist), A.P.	*Bacillus* spp.
13.	PGP-13	Dept of Agricultural Microbiology and Bioenergy, CAR’ Nagar, Hyderabad	*Pseudomonas* spp.
14.	PGP-14	Dept of Agricultural Microbiology and Bioenergy, CAR’ Nagar, Hyderabad	*Bacillus* sppp.
15.	PGP-15	Dept of Agricultural Microbiology and Bioenergy, CAR’ Nagar, Hyderabad	*Azospirillum* spp.
16.	PGP-16	Dept of Agricultural Microbiology and Bioenergy, CAR’ Nagar, Hyderabad	*Pseudomonas* spp.

Table 4.6 Biocontrol activity of PGPR isolates:

Isolate	Siderophore production	HCN production	Percent inhibition (%) of *Rhizoctonia solani*	Inhibition zone(mm)	Percent inhibition (%) of *Sclerotium rolfsii*	Inhibition zone(mm)
PGP-1	+++	+++	36.10	4.00	30.54	1.00
PGP-2	++	-	20.53	00	17.62	00
PGP-3	-	-	0.00	-	0.000	-
PGP-4	-	+	35.72	2.00	31.67	1.00
PGP-5	-	-	0.00	-	0.000	-
PGP-6	+	-	0.00	-	0.000	-
PGP-7	+	+	34.49	1.00	35.47	2.00
PGP-8	+++	++	35.15	2.00	29.74	2.00
PGP-9	++	+	35.50	3.00	33.89	1.00
PGP-10	++	-	0.00		0.000	-
PGP-11	-	+	30.84	1.00	32.27	1.00
PGP-12	-	-	0.00	-	0.000	-
PGP-13	+	+	33.45	1.00	30.85	1.00
PGP-14	+	+	34.66	1.00	35.29	4.00
PGP-15	+++	+	30.21	1.00	25.20	2.00
PGP-16	+++	++	34.23	1.00	33.74	1.00
Control			00		00	
CD @ 0.05 probability			**0.934**		**0.706**	

HCN- Hydrogen cyanide

+ Weak production	++ Moderate production
+++ Strong production	− No production

Table.4.8 Compatibility of PGPR isolates with agrochemicals

S. no	Isolates	Fungicides				Insecticides				Herbicides			
		Metalaxyl	Ziram	Propiconazole	Carbendazim	Chloripyriphos	Dimethoate	Imidacloprid	Thiodicarb	Butachlor	Atrazin	Propaquizafop	Pendimethalin
1.	PGP-1	H	H	H	H	H	H	H	H	H	H	H	H
2.	PGP-2	H	M	H	H	M	H	H	H	H	H	H	H
3.	PGP-3	M	H	H	H	H	M	H	M	MD	MD	H	H
4.	PGP-4	H	M	H	H	M	H	H	H	MD	H	M	M
5.	PGP-5	H	M	H	H	H	M	H	H	H	H	H	L
6.	PGP-6	H	M	L	H	H	M	H	H	H	H	H	L
7.	PGP-7	H	H	M	M	H	M	H	H	MD	H	H	H
8.	PGP-8	H	M	H	H	M	H	H	H	H	MD	H	H
9.	PGP-9	H	H	H	H	H	H	H	H	H	H	H	H
10.	PGP-10	M	M	M	M	L	M	M	M	MD	H	H	H
11.	PGP-11	H	M	H	H	H	H	H	H	H	H	H	H
12.	PGP-12	H	M	M	H	H	H	H	H	H	H	H	M
13.	PGP-13	H	H	H	H	H	M	H	M	H	H	H	H
14.	PGP-14	H	H	H	H	H	H	H	H	H	H	H	H
15.	PGP-15	H	H	H	H	H	H	H	H	H	H	H	H
16.	PGP-16	H	M	H	MD	H	H	H	M	H	H	H	H
	control	0	0	0	0	0	0	0	0	0	0	0	0

Low compatibility -H (2mm)   Medium compatibility -M (1mm)   Moderate compatibility -MD (0.5mm)   High compatibility -H (0mm)

Table .4.9 Based on all Multiple PGPR characteristics, biocontrol activity and compatibility with agrichemicals, efficient PGP isolates are selected for Maize field experiment

Efficient isolates	**Plant growth promoting characters**				**Biocontrol activity**				
	p-solubilization (PSE%)	**Ammonia production**	**Nitrogen fixation (nmol C_2H_4/mg protein/ h)**	**IAA production(µg/ml)**	**HCN production**	**Siderophore production**	**Antifungal activity**		**Compatibility with agrochemicals**
							S.rolfsi	***R.solani***	
PGP-1(*Psudimonas* spp	500	**+++**	**-**	7.41	+++	+++	4.00	1.00	H
PGP-9(*Azotobacter* spp)	190.9	**++**	8.55	5.98	+	+	3.00	1.00	MD
PGP-15(*Azospirillum* spp)	138.4	**++**	9.25	7.76	+	++	1.00	2.00	H

High compatibility -H (0mm) Moderate compatibility -MD (0.5mm) Medium compatibility -M (1mm)
+ Weak production ++ Moderate production +++ Strong production

Table 4.10 Influence of PGPR inoculants on Growth parameters of Maize

Treatments	**Plant height (cm)**			**Root length(cm)**			**Dry weight (g)**		
	40DAS	**70DAS**	**Harvesting stage**	**40DAS**	**70DAS**	**Harvesting stage**	**40DAS**	**70DAS**	**Harvesting stage**
T_1	27.65	174.36	176.66	11.9	26.66	28	30.05	66.66	240.15
T_2	28.20	182.75	185.00	13.03	29.00	28.33	31.93	67.42	244.23
T_3	27.75	177.66	184.33	12.03	28.00	30.00	30.33	68.33	245.00
T_4	28.21	175.02	175.66	12.00	29.00	30.66	30.25	68.00	249.33
T_5	30.33	184.57	184.97	12.6	31.66	30.00	31.436	68.54	246.50
T_6	30.98	184.54	184.33	12.53	35.33	31.66	30.76	66.40	252.66
T_7	32.77	187.32	192.32	14.06	35.00	33.66	37.33	75.00	272.89
CD (P=0.05%)	**2.04**	**7.563**	**9.913**	**1.175**	**5.45**	**2.618**	**4.358**	**4.782**	**18.59**
SED	**0.937**	**3.471**	**4.550**	**0.539**	**2.502**	**1.201**	**2.000**	**2.195**	**8.533**
SE(m)	**0.662**	**2.454**	**3.217**	**0.381**	**1.769**	**0.849**	**1.414**	**1.552**	**6.033**

T1: 100 % RDF, T2: 75 % RDN + Nitrogen fixer *Azospirillum*, T3: 75 % RDN+ Nitrogen fixer *Azotobacter*
T4: 75 % RDP + Phosphate solubilizers , T5: 75 % RDN & P + Nitrogen fixer *Azospirillum* + Phosphate solubilizers
T6: 75 % RDN & P + Nitrogen fixer *Azotobacter* + Phosphate solubilizers
T7: 75 % RDN & P + *Azotobacter* + *Azospirillum* + Phosphate solubilizers

Table .4.11 Influence of PGPR inoculants on yield attributes of Maize

Treatments	Cob weight(g)	Number of rows per cob	Number of kernels per row	Number of kernels per cob	Test weight(g)
T_1	703.33	14.00	28.46	405.55	25.43
T_2	725.00	15.20	29.77	431.66	26.15
T_3	706.66	14.43	28.82	416.66	25.76
T_4	713.33	14.33	28.65	410.66	26.13
T_5	723.33	15.00	31.51	405.55	25.78
T_6	716.66	15.03	30.98	417.88	25.31
T_7	756.66	15.83	32.453	438.88	26.86
CD (P=0.05%)	**31.358**	**1.010**	**2.510**	**22.760**	**0.407**
SED	**14.392**	**0.463**	**1.156**	**10.44**	**0.186**
SE(m)	**10.177**	**0.328**	**0.817**	**7.386**	**0.134**

T1: 100 % RDF, T2: 75 % RDN + Nitrogen fixer *Azospirillum*, T3: 75 % RDN+ Nitrogen fixer *Azotobacte*
T4: 75 % RDP + Phosphate solubilizers , T5: 75 % RDN & P + Nitrogen fixer *Azospirillum* + Phosphate solubilizers
T6: 75 % RDN & P + Nitrogen fixer *Azotobacter* + Phosphate solubilizers
T7: 75 % RDN & P + *Azotobacter* + *Azospirillum* + Phosphate solubilizers

Table 4.12 Influence of PGPR inoculants on kernel yield of Maize

Treatments	**Grain yield (kg.ha^{-1})**	**Stover yield(kg.ha^{-1})**
T_1	2653.33	4100.00
T_2	2750.00	4133.33
T_3	2676.66	3540.00
T_4	2683.33	4183.33
T_5	2820.00	4276.66
T_6	2866.66	4272.00
T_7	3366.66	4530.00
CD (P=0.05%)	**6.723**	**4.424**
SED	**155.422**	**203.071**
SE(m)	**109.901**	**143.59**

T1: 100 % RDF,
T2: 75 % RDN + Nitrogen fixer *Azospirillum*,
T3: 75 % RDN+ Nitrogen fixer *Azotobacter*
T4: 75 % RDP + Phosphate solubilizers ,
T5: 75 % RDN & P + Nitrogen fixer *Azospirillum* + Phosphate solubilizers
T6: 75 % RDN & P + Nitrogen fixer *Azotobacter* + Phosphate solubilizer
T7: 75 % RDN & P + *Azotobacter* + *Azospirillum* + Phosphate solubilizers

Table 4.13 Influence of PGPR microbial inoculants and chemical fertilizers on the available soil nitrogen, phosphorus and potassium after harvesting of Maize crop

Treatments	Available Nitrogen (Kg ha^{-1})	Available Phosphorus (Kg ha^{-1})	Available Potassium (Kg ha^{-1})
T1	228.63	23.9	276.36
T2	239.63	24.03	276.244
T3	242.20	25.50	264.30
T4	240.90	36.33	266.05
T5	247.60	28.366	264.04
T6	236.93	28.30	261.60
T7	244.13	31.06	275.28
SE(m)	**1.887**	**1.034**	**11.89**
CD (0.05%)	**5.814**	**3.186**	**37.06**

T1: 100 % RDF,
T2: 75 % RDN + Nitrogen fixer *Azospirillum*,
T3: 75 % RDN+ Nitrogen fixer *Azotobacter*
T4: 75 % RDP + Phosphate solubilizers ,
T5: 75 % RDN & P + Nitrogen fixer *Azospirillum* + Phosphate solubilizers
T6: 75 % RDN & P + Nitrogen fixer *Azotobacter* + Phosphate solubilizer
T7: 75 % RDN & P + *Azotobacter* + *Azospirillum* + Phosphate solubilizers

Table 4.17 Phosphate solubilizing bacterial population at different growth stages ($\log 10^4$ CFU g^{-1} of dry soil)

TREATMENTS	0 DAS	40DAS	70DAS	Harvesting stage	Mean
T1	4.736	4.923	5.076	4.906	4.906
T2	4.643	4.916	5.006	4.956	4.899
T3	4.733	4.956	4.986	4.836	4.874
T4	4.766	5.053	5.113	5.413	5.074
T5	4.706	5.003	4.996	4.680	4.8495
T6	4.770	5.060	5.043	4.930	4.938
T7	4.680	5.010	5.046	4.966	4.935
Mean	4.719	4.988	5.038	4.955	4.925
CD5%					
Factor A	0.088				
Factor B	0.060				
Treatments/(AxB)	0.166				

T1: 100 % RDF
T2: 75 % RDN + Nitrogen fixer *Azospirillum*
T3: 75 % RDN+ Nitrogen fixer *Azotobacter*
T4: 75 % RDP + Phosphate solubilizers
T5: 75 % RDN & P + Nitrogen fixer *Azospirillum* + Phosphate solubilizers
T6: 75 % RDN & P + Nitrogen fixer *Azotobacter* + Phosphate solubilizers
T7: 75 % RDN & P + *Azotobacter* + *Azospirillum* + Phosphate solubilizers
RDF: Recommended dose of fertilizer
RDN: Recommended dose of Nitrogen
RDN & P: Recommended dose of Nitrogen and Phosphorus
DAS: Days after sowing

Table 4.16 ***Azotobacter* population at different growth stages of crop**

($\log 10^3$ CFU g^{-1} of dry soil)

Treatment	**0 DAS**	**40DAS**	**70DAS**	**Harvesting stage**	**Mean**
T1	3.360	3.673	3.8	3.696	3.632
T2	3.633	3.76	3.93	3.800	3.780
T3	3.493	3.94	3.96	3.893	3.821
T4	3.553	3.826	3.906	3.793	3.769
T5	3.486	3.663	3.95	3.900	3.749
T6	3.576	3.946	3.996	3.946	3.866
T7	3.470	3.873	3.966	3.803	3.778
Mean	3.510	3.811	3.929	3.833	3.771
CD 5%					
Factor A	0.048				
Factor B	0.039				
Treatments/(AxB)	0.087				

T1: 100 % RDF
T2: 75 % RDN + Nitrogen fixer *Azospirillum*
T3: 75 % RDN+ Nitrogen fixer *Azotobacter*
T4: 75 % RDP + Phosphate solubilizers
T5: 75 % RDN & P + Nitrogen fixer *Azospirillum* + Phosphate solubilizers
T6: 75 % RDN & P + Nitrogen fixer *Azotobacter* + Phosphate solubilizers
T7: 75 % RDN & P + *Azotobacter* + *Azospirillum* + Phosphate solubilizers
RDF: Recommended dose of fertilizer
RDN: Recommended dose of Nitrogen
RDN & P: Recommended dose of Nitrogen and Phosphorus
DAS: Days after sowing

Table 4.15 ***Azospirillum*** **population at different growth stages of crop ($\log10^3$CFU g^{-1} of dry soil)**

Treatment	**0 DAS**	**40DAS**	**70DAS**	**Harvesting stage**	**Mean**
T1	3.583	3.893	3.900	3.856	3.808
T2	3.676	3.963	4.06	3.980	3.919
T3	3.650	3.870	3.933	3.836	3.822
T4	3.603	3.793	3.806	3.830	3.758
T5	3.680	3.936	4.006	3.873	3.873
T6	3.590	3.846	3.99	3.926	3.838
T7	3.716	3.950	4.066	3.956	3.922
Mean	3.642	3.893	3.965	3.893	3.848
CD 5%					
Factor A	0.024				
Factor B	0.012				
Treatments/(AxB)	0.059				

T1: 100 % RDF
T2: 75 % RDN + Nitrogen fixer *Azospirillum*
T3: 75 % RDN+ Nitrogen fixer *Azotobacter*
T4: 75 % RDP + Phosphate solubilizers
T5: 75 % RDN & P + Nitrogen fixer *Azospirillum* + Phosphate solubilizers
T6: 75 % RDN & P + Nitrogen fixer *Azotobacter* + Phosphate solubilizers
T7: 75 % RDN & P + *Azotobacter* + *Azospirillum* + Phosphate solubilizers
RDF: Recommended dose of fertilizer
RDN: Recommended dose of Nitrogen
RDN & P: Recommended dose of Nitrogen and Phosphorus
DAS: Days after sowing

Table 4.14 Bacterial population at different growth stages of crop (log10^6 CFU g^{-1} of dry soil)

Treatment	0 DAS	40DAS	70DAS	Harvesting stage	Mean
T1	7.480	7.553	7.590	7.540	7.540
T2	7.510	7.606	7.720	7.606	7.610
T3	7.480	7.620	7.656	7.630	7.596
T4	7.506	7.620	7.716	7.620	7.615
T5	7.510	7.663	7.736	7.666	7.643
T6	7.590	7.660	7.776	7.660	7.671
T7	7.490	7.640	7.796	7.660	7.646
Mean	7.509	7.623	7.712	7.626	7.617
CD 5%					
Factor A	0.021				
Factor B	0.010				
Treatments/(AxB)	0.043				

T1: 100 % RDF
T2: 75 % RDN + Nitrogen fixer *Azospirillum*
T3: 75 % RDN+ Nitrogen fixer *Azotobacter*
T4: 75 % RDP + Phosphate solubilizers
T5: 75 % RDN & P + Nitrogen fixer *Azospirillum* + Phosphate solubilizers
T6: 75 % RDN & P + Nitrogen fixer *Azotobacter* + Phosphate solubilizers
T7: 75 % RDN & P + *Azotobacter* + *Azospirillum* + Phosphate solubilizers
RDF: Recommended dose of fertilizer
RDN: Recommended dose of Nitrogen
RDN & P: Recommended dose of Nitrogen and Phosphorus
DAS: Days after sowing

Table 4.18 *Rhizobium* population at different growth stages of crop (log10^3 CFU g^{-1} of dry soil)

Treatment	**0 DAS**	**40DAS**	**70DAS**	**Harvesting stage**	**Mean of A**
T1	3.893	4.183	4.266	3.78	4.030
T2	3.780	4.146	4.160	4.160	4.061
T3	3.980	4.136	4.326	4.326	4.192
T4	3.750	4.113	4.353	4.353	4.142
T5	3.726	4.176	4.166	4.166	4.058
T6	3.860	4.023	4.236	4.236	4.088
T7	4.006	4.100	4.286	4.286	4.169
Mean of B	3.856	4.125	4.256	4.186	4.106
CD 5%					
Factor A	0.044				
Factor B	0.038				
Treatments/(AxB)	0.099				

T1: 100 % RDF ; T2: 75 % RDN + Nitrogen fixer *Azospirillum*
T3: 75 % RDN+ Nitrogen fixer *Azotobacter*
T4: 75 % RDP + Phosphate solubilizers
T5: 75 % RDN & P + Nitrogen fixer *Azospirillum* + Phosphate solubilizers
T6: 75 % RDN & P + Nitrogen fixer *Azotobacter* + Phosphate solubilizers
T7: 75 % RDN & P + *Azotobacter* + *Azospirillum* + Phosphate solubilizers
RDF: Recommended dose of fertilizer
RDN: Recommended dose of Nitrogen
RDN & P: Recommended dose of Nitrogen and Phosphorus
DAS: Days after sowing

Table 4.19 Fungal population at different growth stages of crop (log10^3 CFU g^{-1} of dry soil)

Treatment	**0 DAS**	**40DAS**	**70DAS**	**Harvesting stage**	**Mean(A)**
T1	4.320	4.096	3.993	4.163	4.143
T2	4.366	3.973	3.966	4.123	4.107
T3	4.276	3.983	3.973	4.016	4.062
T4	4.253	3.910	3.910	4.076	4.037
T5	4.380	3.990	3.953	4.146	4.117
T6	4.423	3.980	3.956	4.213	4.143
T7	4.296	4.050	4.000	4.226	4.143
Mean (B)	4.330	3.997	3.964	4.137	4.107
CD 5%					
Factor A	0.034				
Factor B	0.020				
Treatments/(AxB)	0.078				

T1: 100 % RDF
T2: 75 % RDN + Nitrogen fixer *Azospirillum*
T3: 75 % RDN+ Nitrogen fixer *Azotobacter*
T4: 75 % RDP + Phosphate solubilizers
T5: 75 % RDN & P + Nitrogen fixer *Azospirillum* + Phosphate solubilizers
T6: 75 % RDN & P + Nitrogen fixer *Azotobacter* + Phosphate solubilizers
T7: 75 % RDN & P + *Azotobacter + Azospirillum* + Phosphate solubilizers
RDF: Recommended dose of fertilizer
RDN: Recommended dose of Nitrogen
RDN & P: Recommended dose of Nitrogen and Phosphorus
DAS: Days after sowing

Table 4.20 Influence of microbial inoculants and chemical fertilizers on the economics of maize

Treatments	Gross returns (Rs ha $^{-1}$)	NET returns (Rs ha^{-1})	Cost of cultivation	B:C Ratio
T1	34493.00	17,993.50	17,000	1.02
T2	35750.00	20750.00	15000	1.38
T3	34796.00	19796.50	15000	1.31
T4	34883.00	18883.50	16000	1.20
T5	36660.00	20660.00	16000	1.30
T6	37266.00	21266.50	16000	1.32
T7	43766.00	27266.50	16500	1.65

T1: 100 % RDF
T2: 75 % RDN + Nitrogen fixer *Azospirillum*
T3: 75 % RDN+ Nitrogen fixer *Azotobacter*
T4: 75 % RDP + Phosphate solubilizers
T5: 75 % RDN & P + Nitrogen fixer *Azospirillum* + Phosphate solubilizers
T6: 75 % RDN & P + Nitrogen fixer *Azotobacter* + Phosphate solubilizers
T7: 75 % RDN & P + *Azotobacter* + *Azospirillum* + Phosphate solubilizers

Fig.4.1 Nitrogen fixing efficiency of bacterial isolates

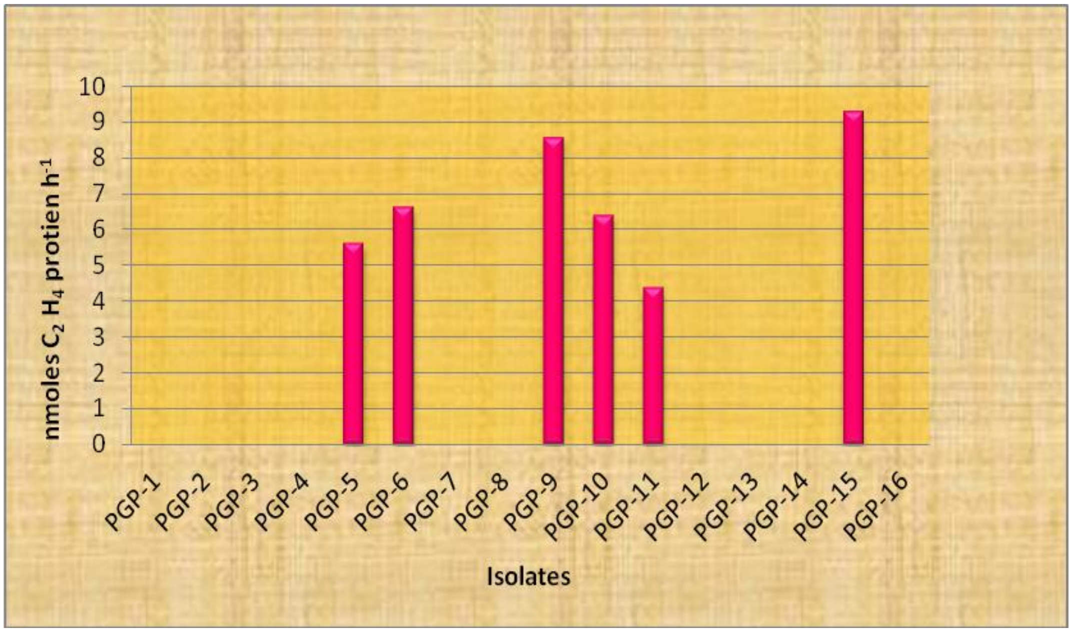


Fig .4.2 Indole Acetic Acid production of bacterial isolates

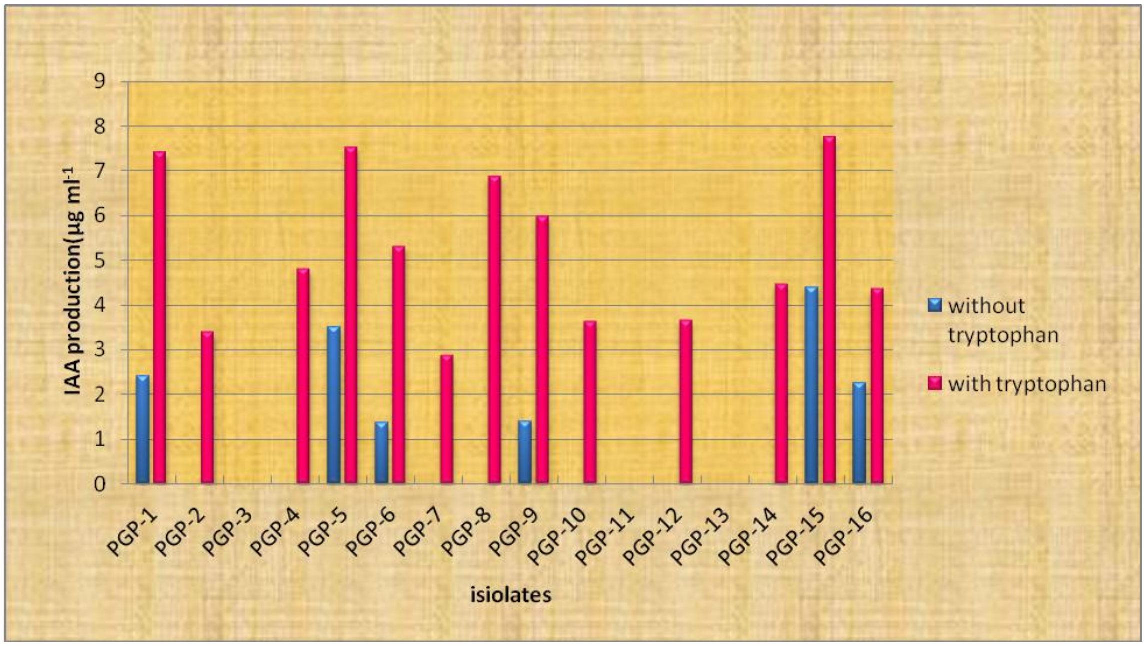


Fig .4.3 Antagonistic potential of efficient PGP isolates on the radial growth of *Rhizoctonia solani* and *Sclerotium rolfsii in vitro*

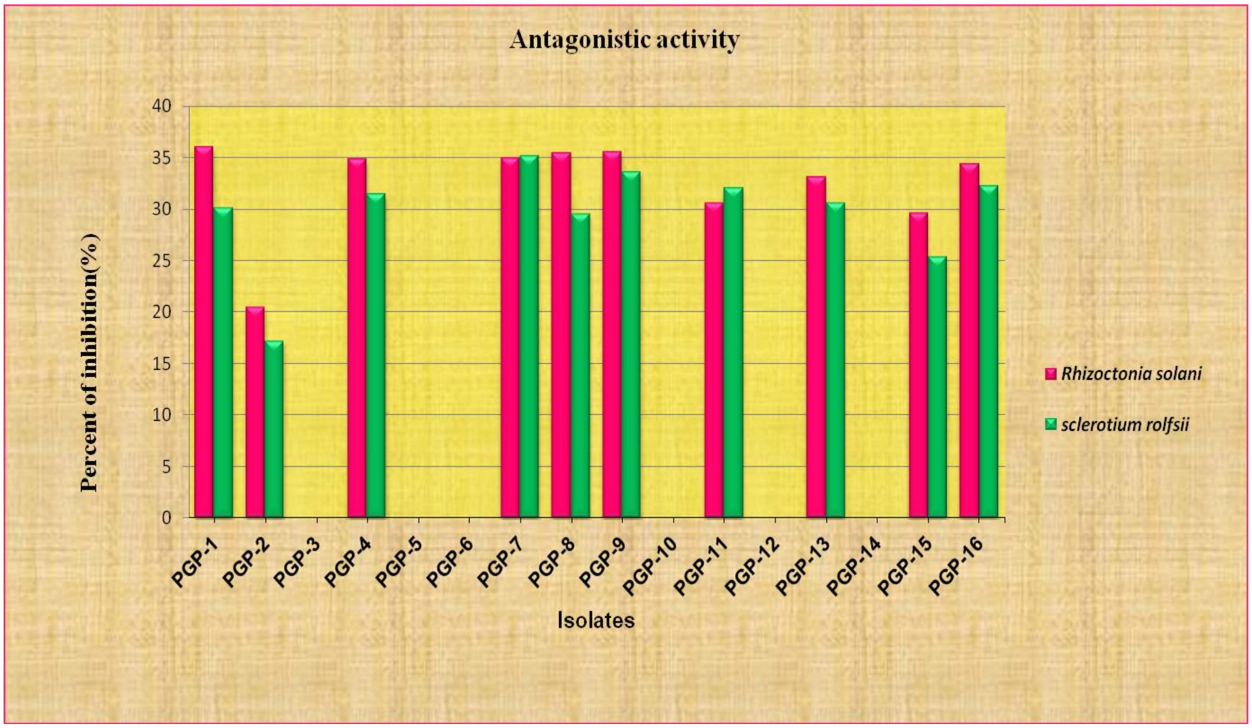
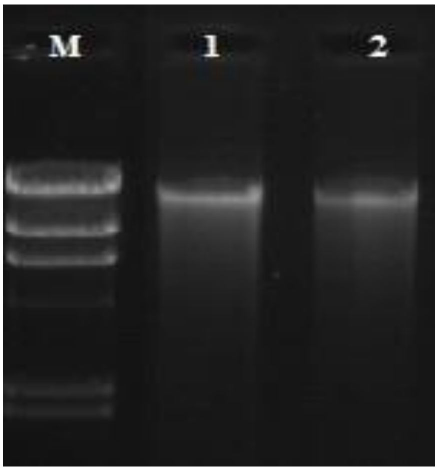


Fig 4.4 Gelelectrophoresis bands of extacted DNA samples of nitroen fixers



0.8 % agarose gel electrophoresis of gDNA

M: DNA Marker,

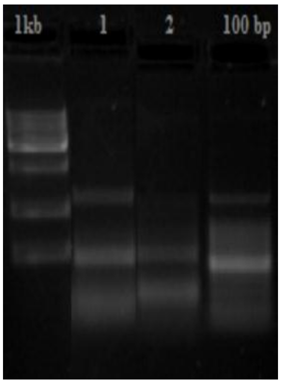
1 : *Azotobacter*

2 : *Azospirillum*

Table no.4.7 Quantification of DNA samples of efficient nitrogen fixers.

s.no	**Sample**	**Concentration (ng ml^{-1})**
1.	*Azotobacter*	33.32
2.	*Azospirillum*	34.98

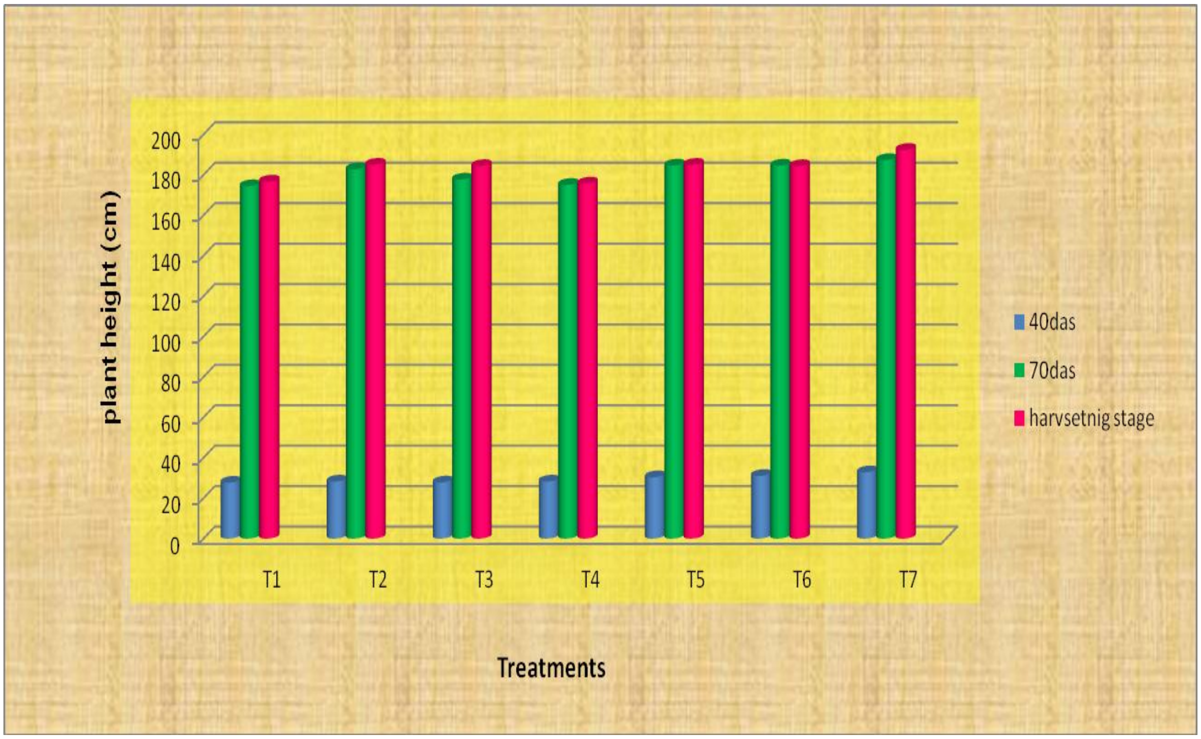
Fig .4.5 Agarose gel electrophoresis (1.5 %) of PCR Amplified products *nif* H1 & H2, 1 *Azotobacter(*PGP-9*)*, 2 *Azospirillum*(PGP-15)



100 bp DNA ladder size : 100,200,300,400,**500**,600,700,800,900,1000

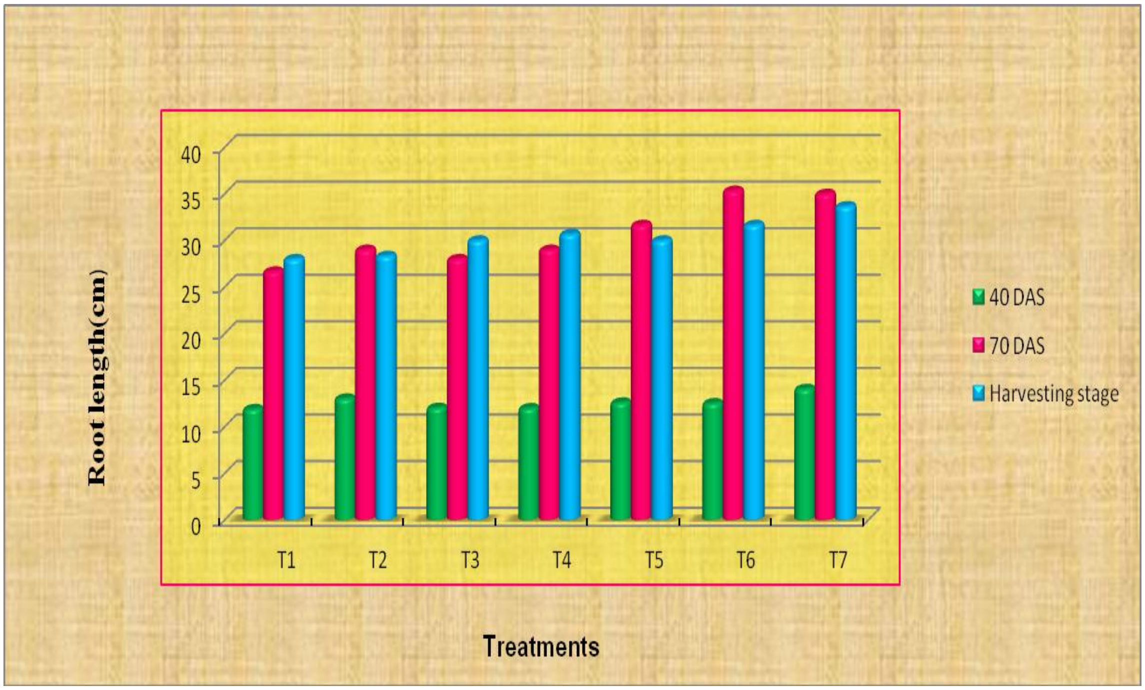
1 kb DNA ladder size : 500,1000,2000,**3000**,4000,5000,6000,8000

Fig 4.6 Influence of PGPR on Maize plant height at different growth stages of growth



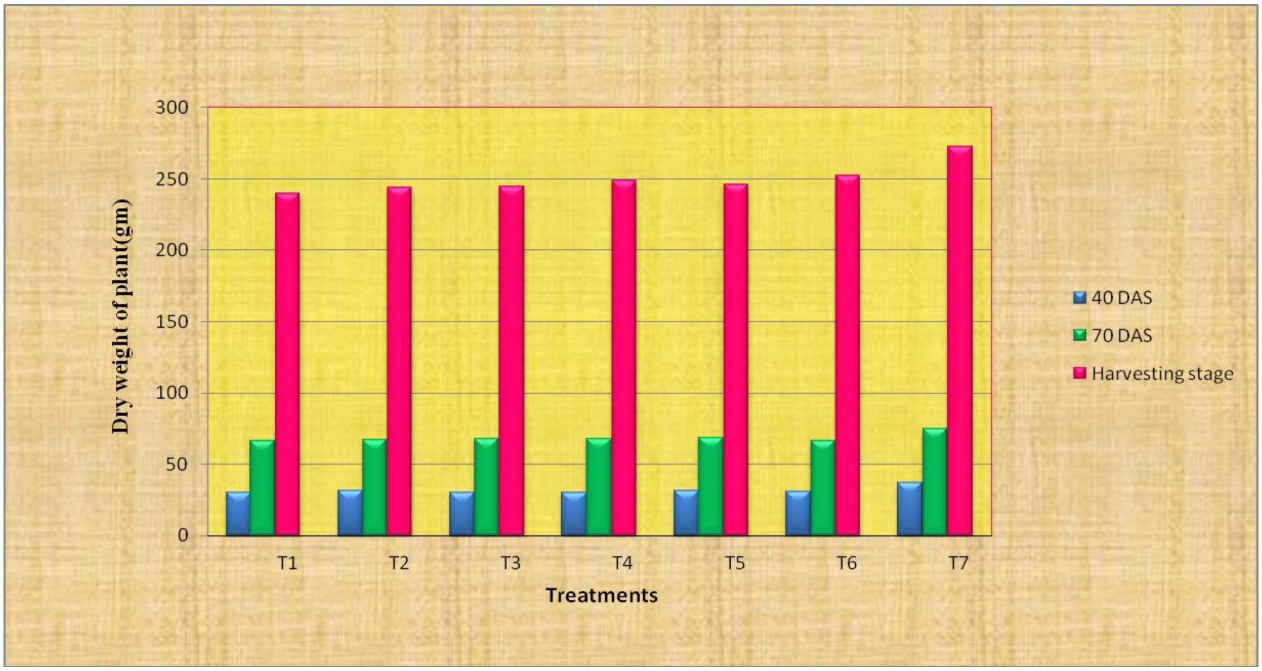
T1: 100 % RDF,
T2: 75 % RDN + Nitrogen fixer *Azospirillum*,
T3: 75 % RDN+ Nitrogen fixer *Azotobacter*
T4: 75 % RDP + Phosphate solubilizers ,
T5: 75 % RDN & P + Nitrogen fixer *Azospirillum* + Phosphate solubilizers
T6: 75 % RDN & P + Nitrogen fixer *Azotobacter* + Phosphate solubilizers
T7: 75 % RDN & P + *Azotobacter* + *Azospirillum* + Phosphate solubilizers
DAS: Days after sowing

Fig 4.7 Influence of PGPR on Maize root length at different growth stages of growth



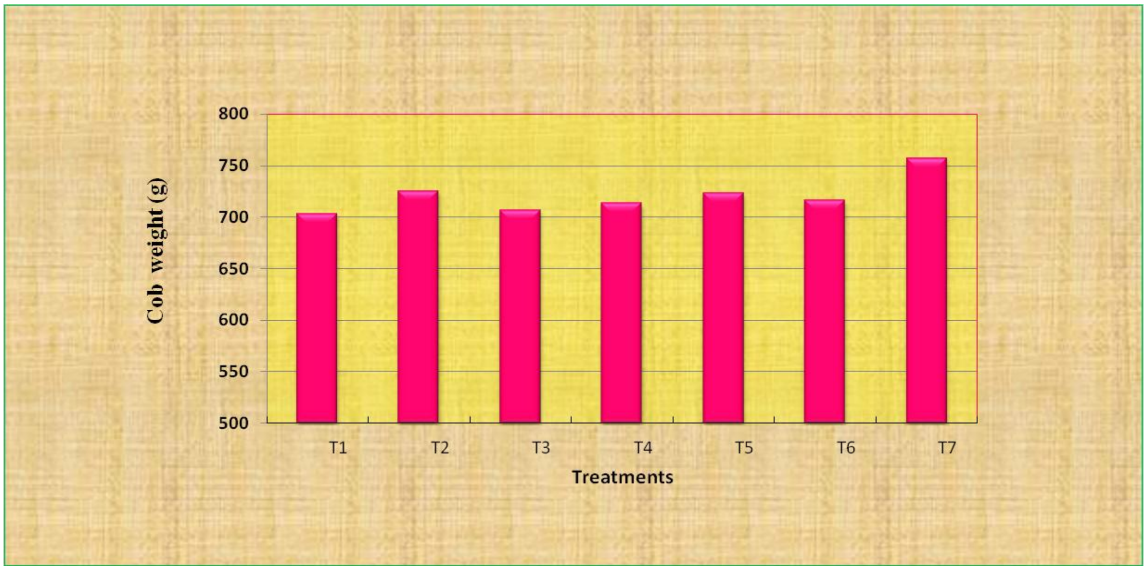
T1: 100 % RDF,
T2: 75 % RDN + Nitrogen fixer *Azospirillum*,
T3: 75 % RDN+ Nitrogen fixer *Azotobacter*
T4: 75 % RDP + Phosphate solubilizers ,
T5: 75 % RDN & P + Nitrogen fixer *Azospirillum* + Phosphate solubilizers
T6: 75 % RDN & P + Nitrogen fixer *Azotobacter* + Phosphate solubilizers
T7: 75 % RDN & P + *Azotobacter* + *Azospirillum* + Phosphate solubilizers
DAS: Days after sowing

Fig 4.8 Influence of PGPR on Maize plant dry weight at different growth stages



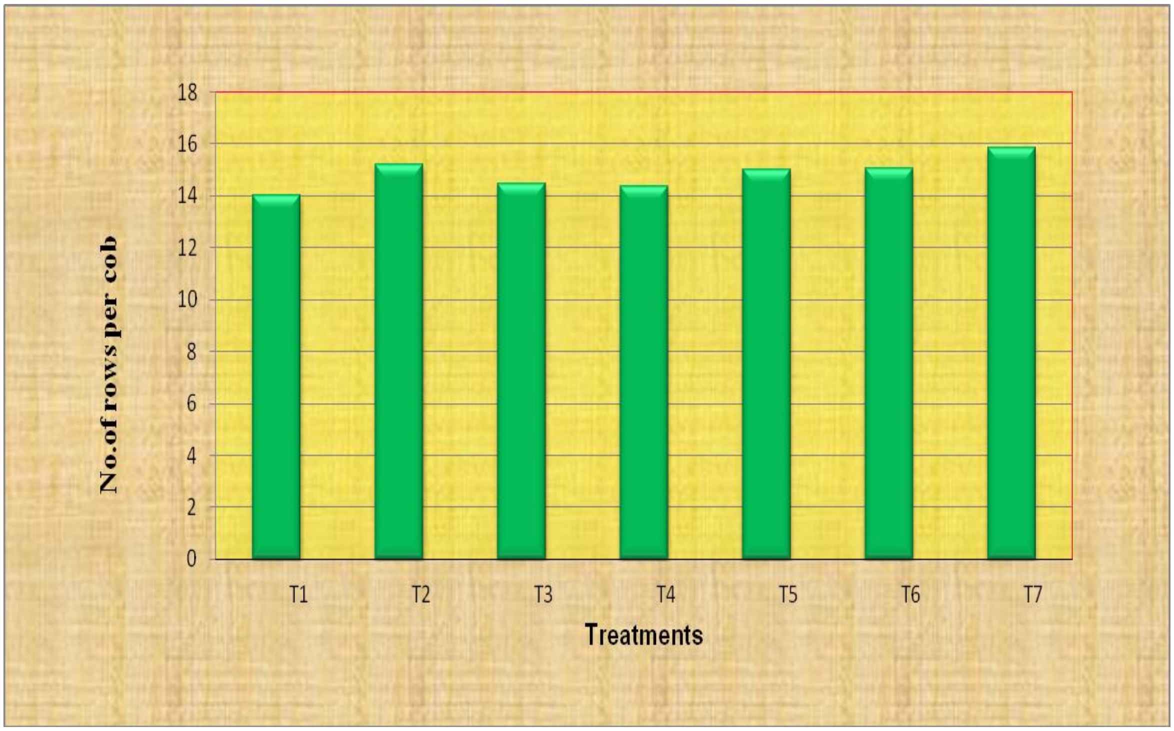
T1: 100 % RDF,
T2: 75 % RDN + Nitrogen fixer *Azospirillum*,
T3: 75 % RDN+ Nitrogen fixer *Azotobacter*
T4: 75 % RDP + Phosphate solubilizers ,
T5: 75 % RDN & P + Nitrogen fixer *Azospirillum* + Phosphate solubilizers
T6: 75 % RDN & P + Nitrogen fixer *Azotobacter* + Phosphate solubilizers
T7: 75 % RDN & P + *Azotobacter* + *Azospirillum* + Phosphate solubilizers
DAS: Days after sowing

Fig 4.9 Influence of PGPR on cob weight of maize



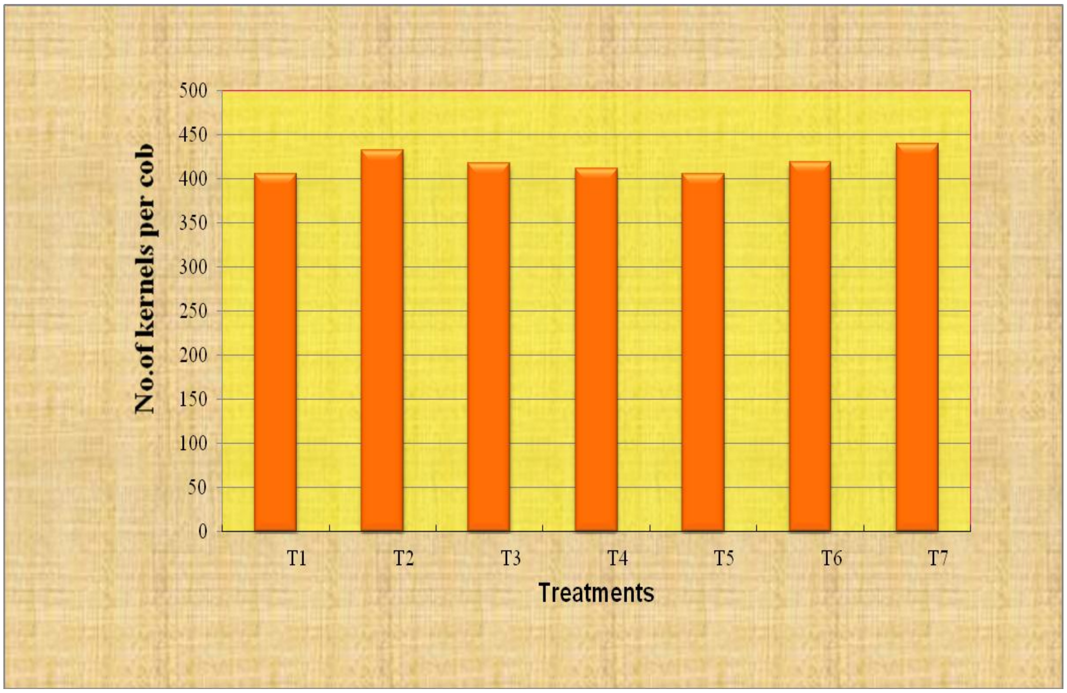
T1: 100 % RDF
T2: 75 % RDN + Nitrogen fixer *Azospirillum*
T3: 75 % RDN+ Nitrogen fixer *Azotobacter*
T4: 75 % RDP + Phosphate solubilizers
T5: 75 % RDN & P + Nitrogen fixer *Azospirillum* + Phosphate solubilizers
T6: 75 % RDN & P + Nitrogen fixer *Azotobacter* + Phosphate solubilizers
T7: 75 % RDN & P + *Azotobacter* + *Azospirillum* + Phosphate solubilizers
DAS: Days after sowing

Fig 4.10 Influence of PGPR on No. of rows per cob



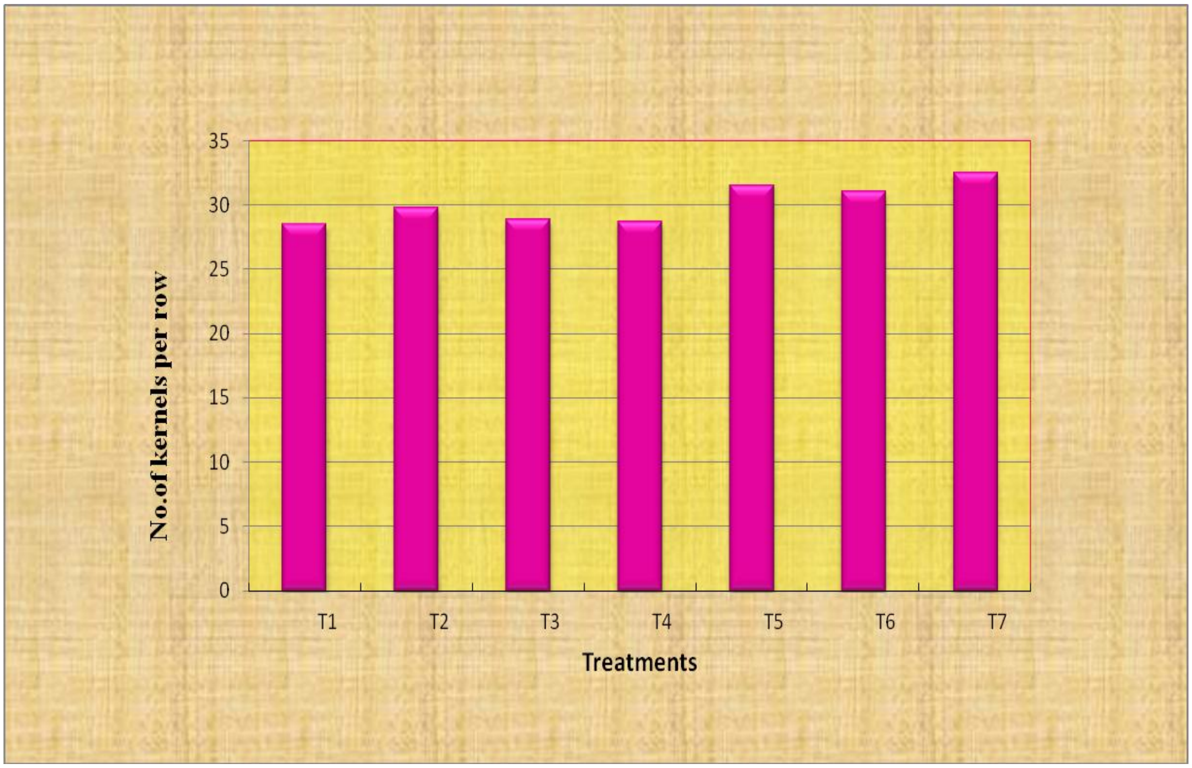
T1: 100 % RDF
T2: 75 % RDN + Nitrogen fixer *Azospirillum*
T3: 75 % RDN+ Nitrogen fixer *Azotobacter*
T4: 75 % RDP + Phosphate solubilizers
T5: 75 % RDN & P + Nitrogen fixer *Azospirillum* + Phosphate solubilizers
T6: 75 % RDN & P + Nitrogen fixer *Azotobacter* + Phosphate solubilizers
T7: 75 % RDN & P + *Azotobacter* + *Azospirillum* + Phosphate solubilizers
DAS: Days after sowing

Fig 4.12 Influence of PGPR on No.of kernels per cob



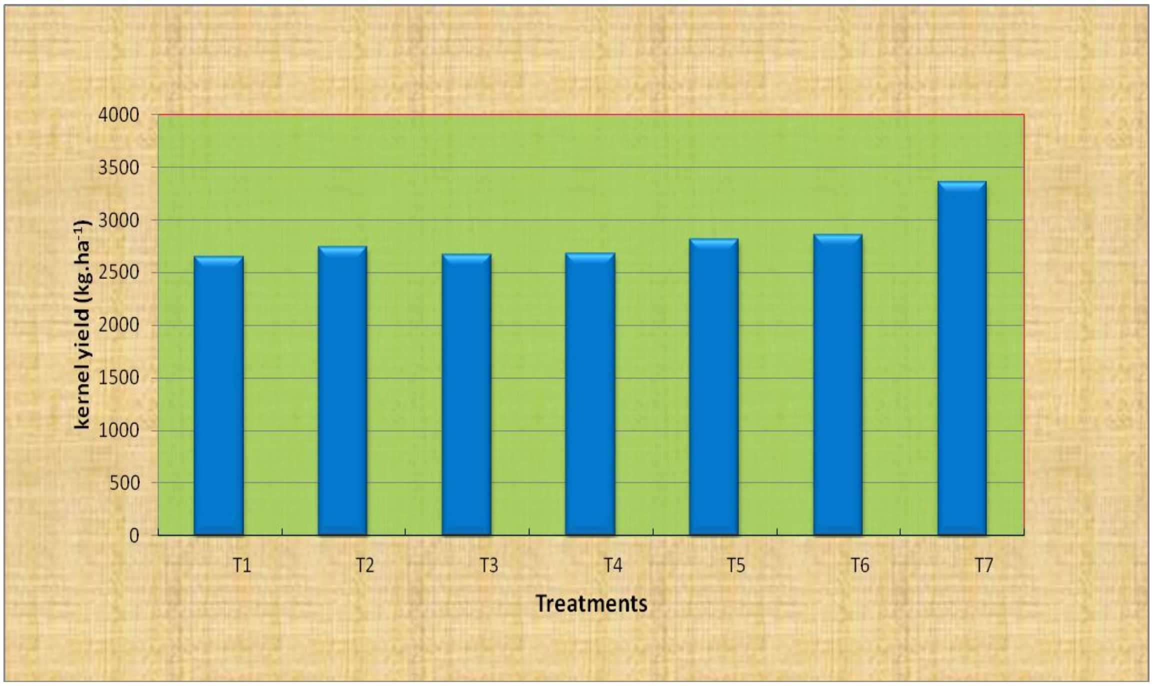
T1: 100 % RDF
T2: 75 % RDN + Nitrogen fixer *Azospirillum*
T3: 75 % RDN+ Nitrogen fixer *Azotobacter*
T4: 75 % RDP + Phosphate solubilizers
T5: 75 % RDN & P + Nitrogen fixer *Azospirillum* + Phosphate solubilizers
T6: 75 % RDN & P + Nitrogen fixer *Azotobacter* + Phosphate solubilizers
T7: 75 % RDN & P + *Azotobacter* + *Azospirillum* + Phosphate solubilizers

Fig 4.11 Influence of PGPR on no. of kernels per row



T1: 100 % RDF,
T2: 75 % RDN + Nitrogen fixer *Azospirillum*,
T3: 75 % RDN+ Nitrogen fixer *Azotobacter*
T4: 75 % RDP + Phosphate solubilizers ,
T5: 75 % RDN & P + Nitrogen fixer *Azospirillum* + Phosphate solubilizers
T6: 75 % RDN & P + Nitrogen fixer *Azotobacter* + Phosphate solubilizers
T7: 75 % RDN & P + *Azotobacter* + *Azospirillum* + Phosphate solubilizers
DAS: Days after sowing

Fig 4.13 Influence of PGPR on grain yield (kg ha^{-1})

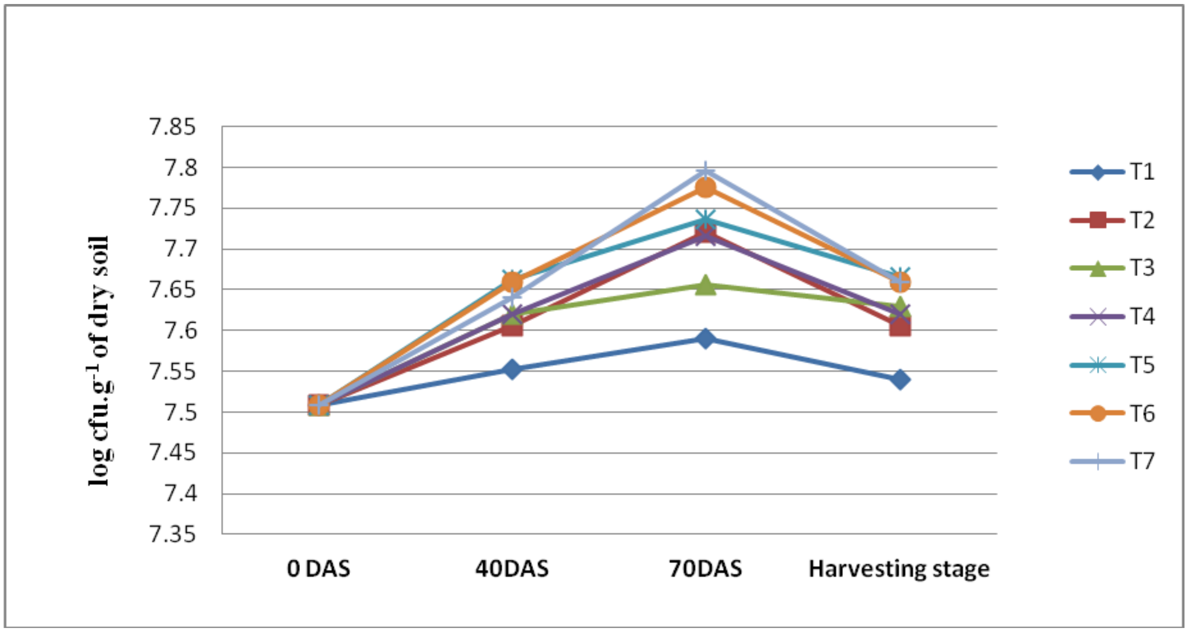


T1: 100 % RDF
T2: 75 % RDN + Nitrogen fixer *Azospirillum*
T3: 75 % RDN+ Nitrogen fixer *Azotobacter*
T4: 75 % RDP + Phosphate solubilizers
T5: 75 % RDN & P + Nitrogen fixer *Azospirillum* + Phosphate solubilizers
T6: 75 % RDN & P + Nitrogen fixer *Azotobacter* + Phosphate solubilizers
T7: 75 % RDN & P + *Azotobacter* + *Azospirillum* + Phosphate solubilizers

Fig 4.14 Influence of PGPR on Stover yield (Kg.ha^{-1})

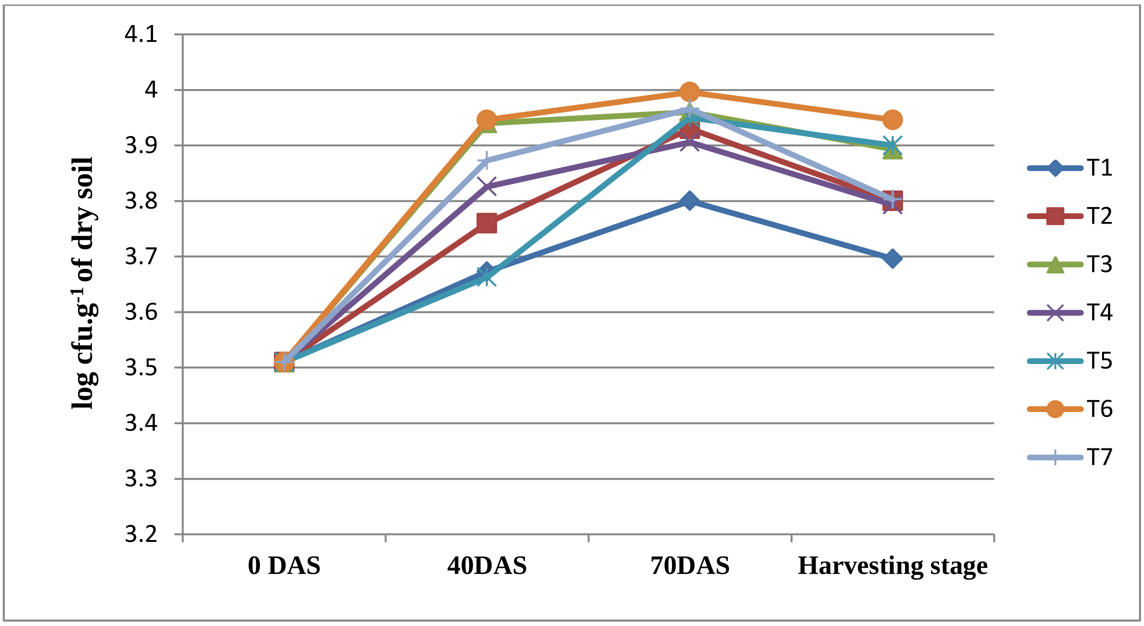
T1: 100 % RDF
T2: 75 % RDN + Nitrogen fixer *Azospirillum*
T3: 75 % RDN+ Nitrogen fixer *Azotobacter*
T4: 75 % RDP + Phosphate solubilizers
T5: 75 % RDN & P + Nitrogen fixer *Azospirillum* + Phosphate solubilizers
T6: 75 % RDN & P + Nitrogen fixer *Azotobacter* + Phosphate solubilizers
T7: 75 % RDN & P + *Azotobacter* + *Azospirillum* + Phosphate solubilizers

Fig.4.15 Bacterial population at different growth stages of crop (log10^{6} CFU g^{-1} of soil)



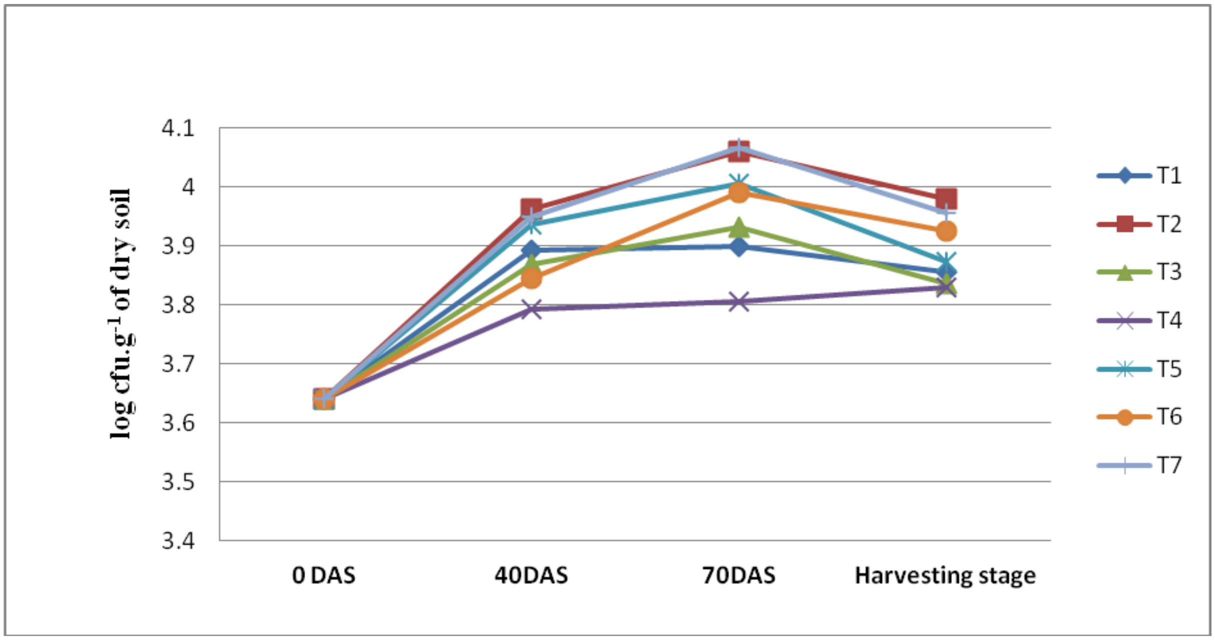
T1: 100 % RDF
T2: 75 % RDN + Nitrogen fixer *Azospirillum*
T3: 75 % RDN+ Nitrogen fixer *Azotobacter*
T4: 75 % RDP + Phosphate solubilizers
T5: 75 % RDN & P + Nitrogen fixer *Azospirillum* + Phosphate solubilizers
T6: 75 % RDN & P + Nitrogen fixer *Azotobacter* + Phosphate solubilizers
T7: 75 % RDN & P + *Azotobacter* + *Azospirillum* + Phosphate solubilizers
DAS: Days after sowing

Fig 4.16 ***Azotobacter*** **population at different growth stages of crop (log10^3 CFU g^{-1} of dry soil)**



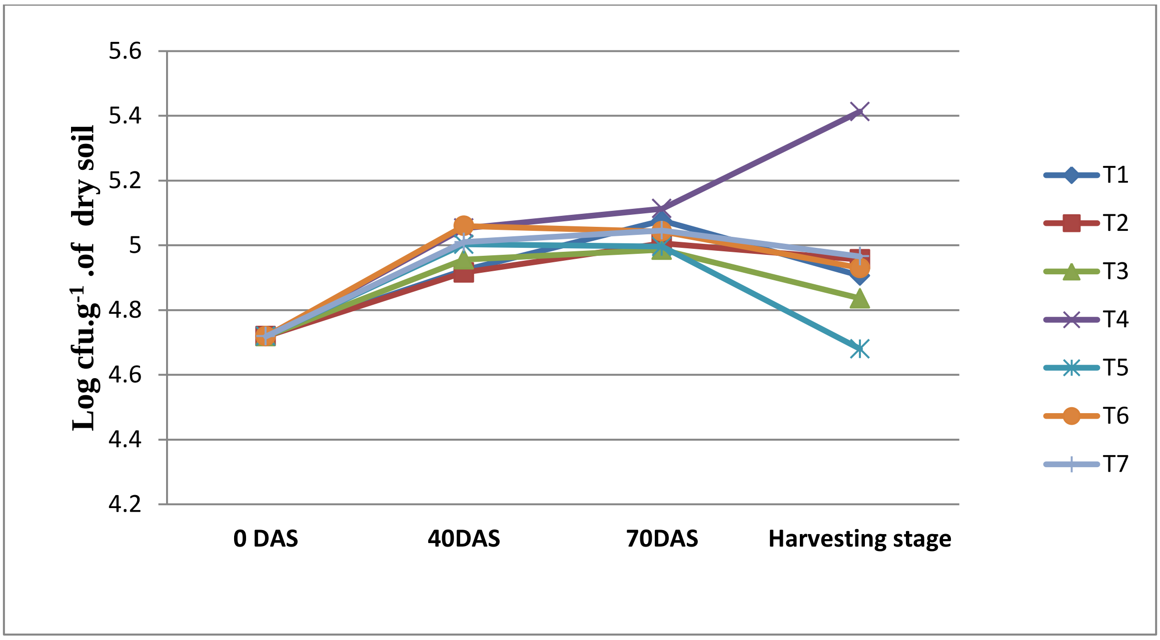
T1: 100 % RDF
T2: 75 % RDN + Nitrogen fixer *Azospirillum*
T3: 75 % RDN+ Nitrogen fixer *Azotobacter*
T4: 75 % RDP + Phosphate solubilizers
T5: 75 % RDN & P + Nitrogen fixer *Azospirillum* + Phosphate solubilizers
T6: 75 % RDN & P + Nitrogen fixer *Azotobacter* + Phosphate solubilizers
T7: 75 % RDN & P + *Azotobacter* + *Azospirillum* + Phosphate solubilizers
DAS: Days after sowing

Fig 4.17 *Azospirillum* population at different growth stages of crop (log10^3 CFU g^{-1} of dry soil)



T1: 100 % RDF
T2: 75 % RDN + Nitrogen fixer *Azospirillum*
T3: 75 % RDN+ Nitrogen fixer *Azotobacter*
T4: 75 % RDP + Phosphate solubilizers
T5: 75 % RDN & P + Nitrogen fixer *Azospirillum* + Phosphate solubilizers
T6: 75 % RDN & P + Nitrogen fixer *Azotobacter* + Phosphate solubilizers
T7: 75 % RDN & P + *Azotobacter* + *Azospirillum* + Phosphate solubilizers
DAS: Days after sowing

Fig 4.18 PSB population at different growth stages of crop (log10^4 CFU g^{-1} of dry soil)



T1: 100 % RDF

T2: 75 % RDN + Nitrogen fixer *Azospirillum*

T3: 75 % RDN+ Nitrogen fixer *Azotobacter*

T4: 75 % RDP + Phosphate solubilizers

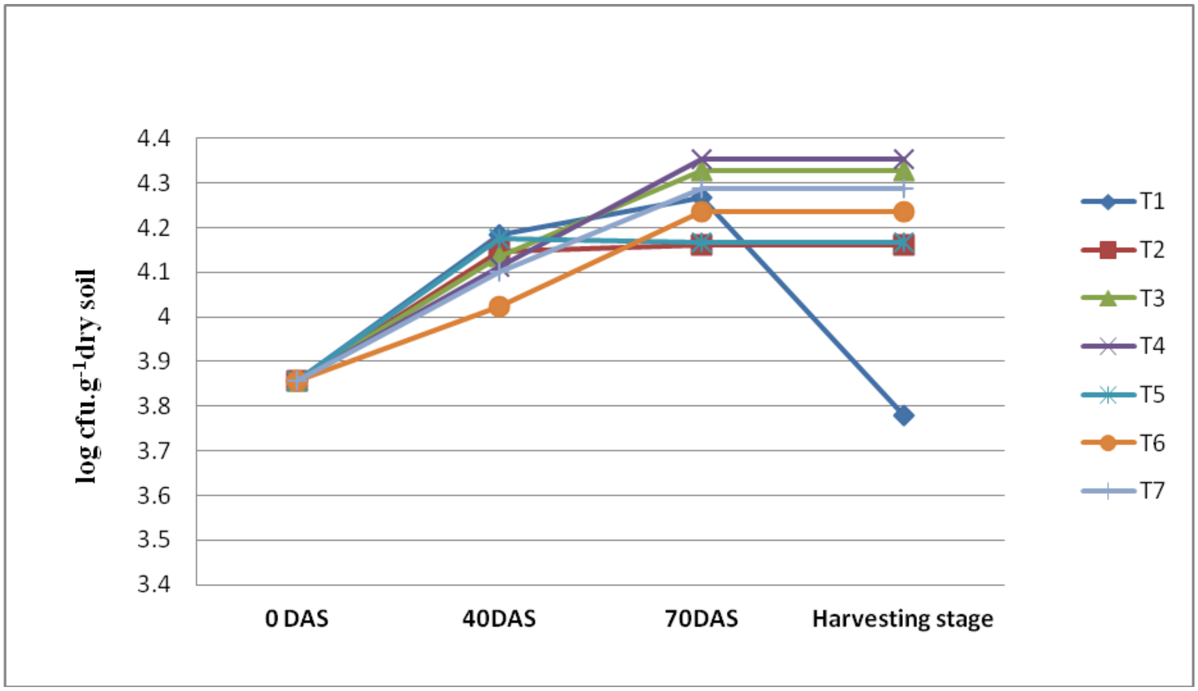
T5: 75 % RDN & P + Nitrogen fixer *Azospirillum* + Phosphate solubilizers

T6: 75 % RDN & P + Nitrogen fixer *Azotobacter* + Phosphate solubilizers,

T7: 75 % RDN & P + *Azotobacter* + *Azospirillum* + Phosphate solubilizers

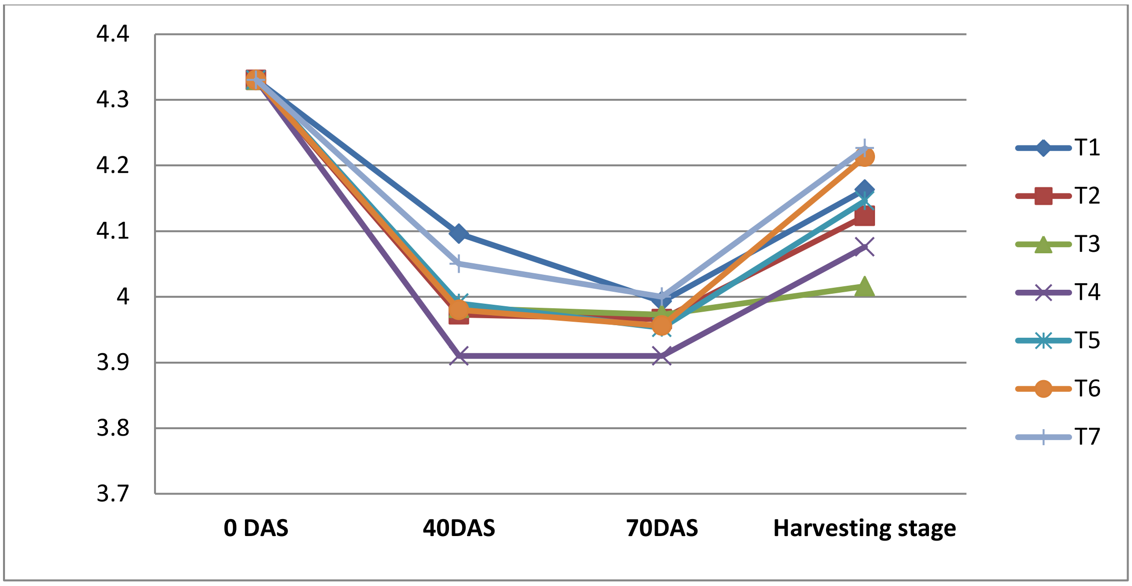
DAS: Days after sowing

Fig 4.19 ***Rhizobium*** **population at different growth stages of crop (log10^3 CFU g^{-1} of dry soil)**



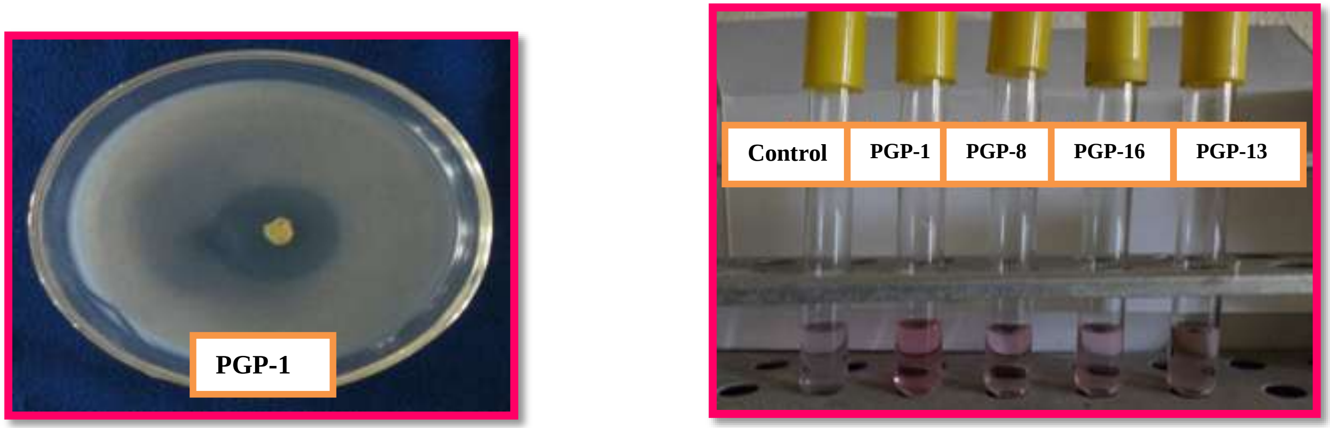
T1: 100 % RDF
T2: 75 % RDN + Nitrogen fixer *Azospirillum*
T3: 75 % RDN+ Nitrogen fixer *Azotobacter*
T4: 75 % RDP + Phosphate solubilizers
T5: 75 % RDN & P + Nitrogen fixer *Azospirillum* + Phosphate solubilizers
T6: 75 % RDN & P + Nitrogen fixer *Azotobacter* + Phosphate solubilizers
T7: 75 % RDN & P + *Azotobacter* + *Azospirillum* + Phosphate solubilizers
DAS: Days after sowing

Fig 4.20 Fungal population (log10^{3} CFU g^{-1} of dry soil)



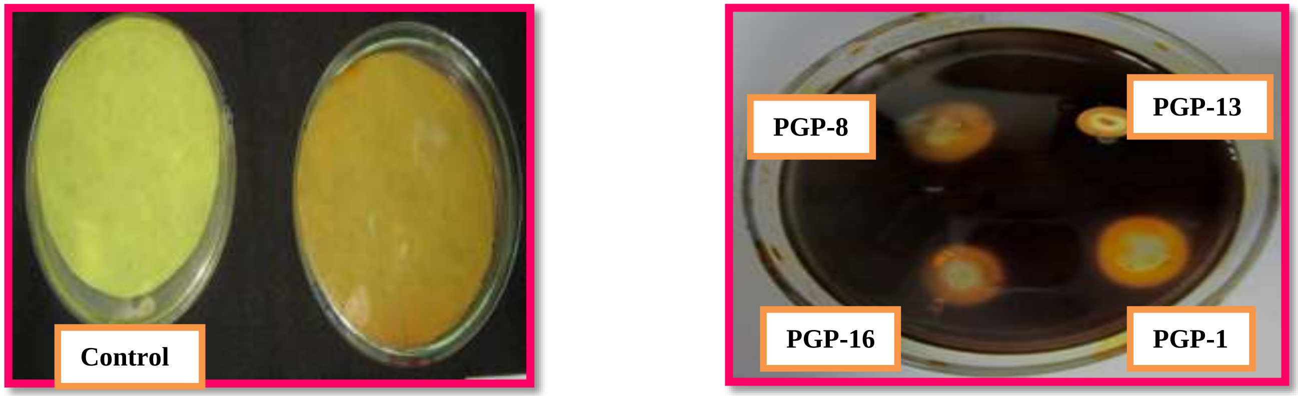
T1: 100 % RDF
T2: 75 % RDN + Nitrogen fixer *Azospirillum*
T3: 75 % RDN+ Nitrogen fixer *Azotobacter*
T4: 75 % RDP + Phosphate solubilizers
T5: 75 % RDN & P + Nitrogen fixer *Azospirillum* + Phosphate solubilizers
T6: 75 % RDN & P + Nitrogen fixer *Azotobacter* + Phosphate solubilizers
T7: 75 % RDN & P + *Azotobacter* + *Azospirillum* + Phosphate solubilizers
DAS: Days after sowing

Plate 4.1 Plant growth promoting characteristics of PGP-1(*Pseudomonas*) isolate



a) Phosphate solubilization

b) Indole Acetic Acid production(with L-tryptophan)



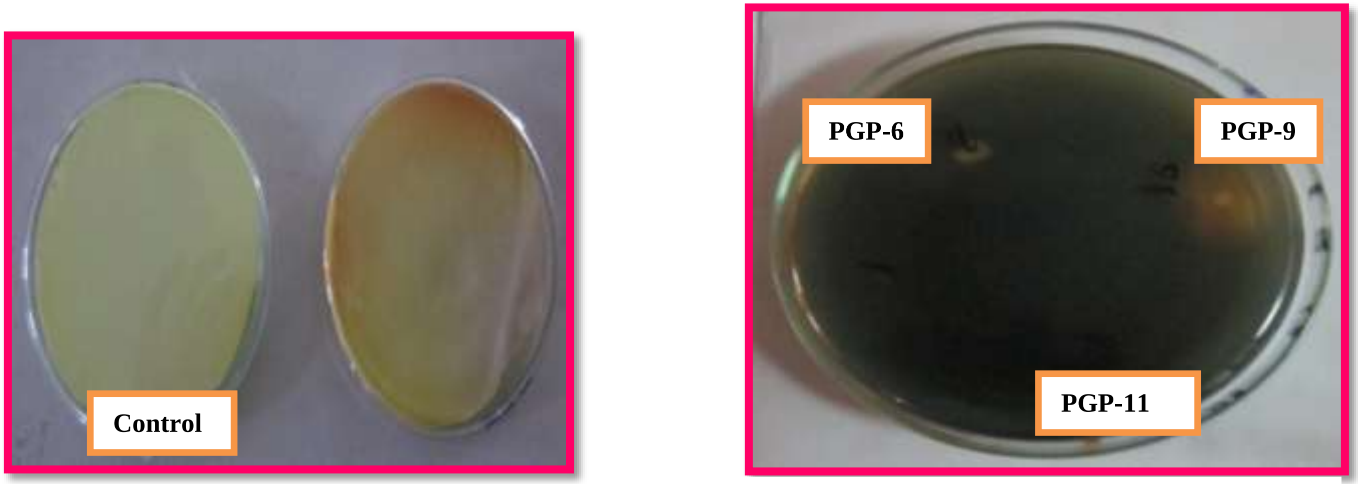
c) HCN production

d) Siderophore production

Plate 4.2 Plant growth promoting characteristics of PGP-9 (*Azotobacter*) isolate

a) Phosphate solubilization

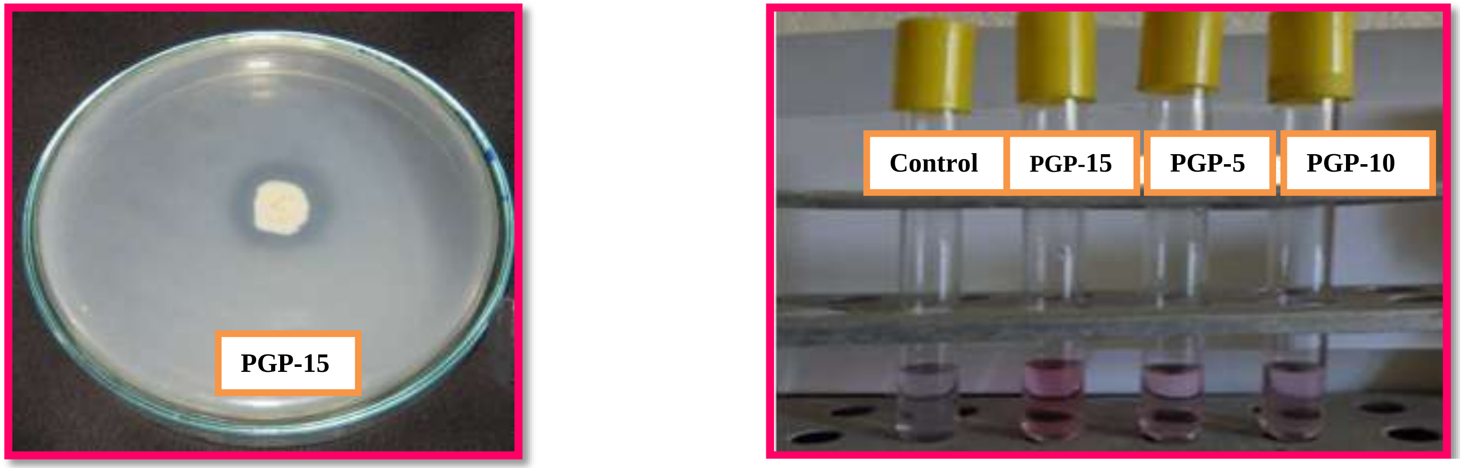
b) IAA production(with L-Tryptophan)



c) HCN production

d) Siderophore production

Plate 4.3 Plant growth promoting characteristics of PGP-15 (*Azospirillum*) isolate



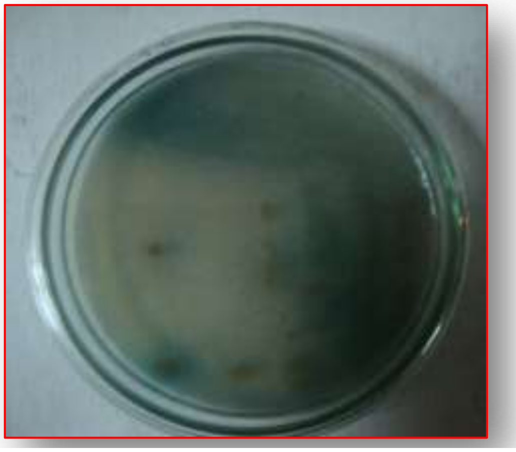
a) Phosphate solubilization

b) IAA production with (L-tryptophan)

c) HCN production

d) Siderophore production

Plate 4.4 Cultural characteristics of some PGPR isolates



a)PGP-1(*Pseudomonas)* on King's B Agar

b)PGP-7(*Bacillus*) on Nutrient Agar

c)PGP-4(*Rhizobium)* on Yeast Extract Mannitol Agar

d)PGP-9(*Azotobacter)*on Jensen's Agar

e)PGP-15(*Azospirillum*) on N-free Sodium Malate Agar

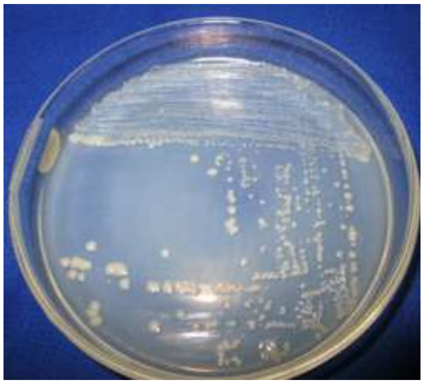
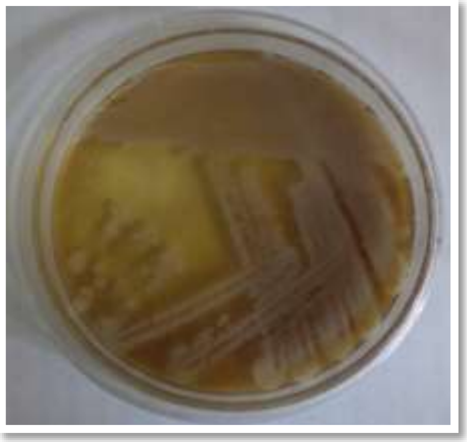
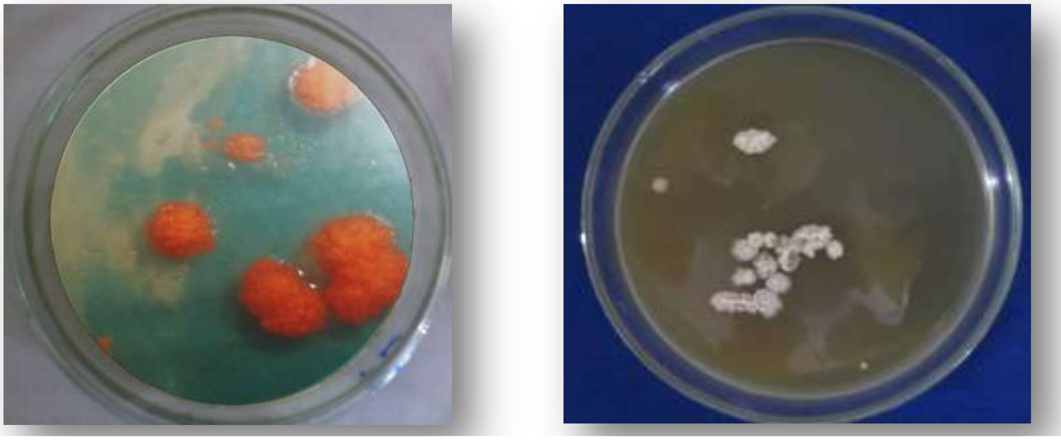


Plate 4.5 Characterization of *Azotobacter* and *Azospirillum* (Nitrogen fixers) isolates

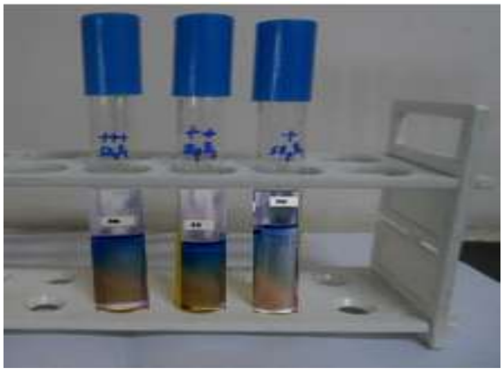


a) PGP-9(*Azotobacter*) brown pigmentation on Jensen's media



b)PGP-15(*Azospirillum*) orange colonies on Potato Infusion agar

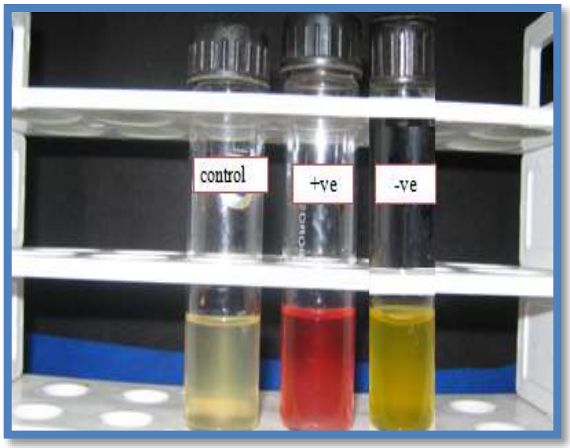
c) PGP-15(*Azospirillum*) pellicle like colonies on SIM Agar



d)Sub surface pellicle formation by *Azospirillum*(PGP-5,PGP-10,PGP-15) isolates

Plate 4.6 Biochemical tests for characterization of bacterial isolates

(a)Indole test



(b) Methyl red test

(c)Voges praskaur’s test

(d) Citrate utilization test

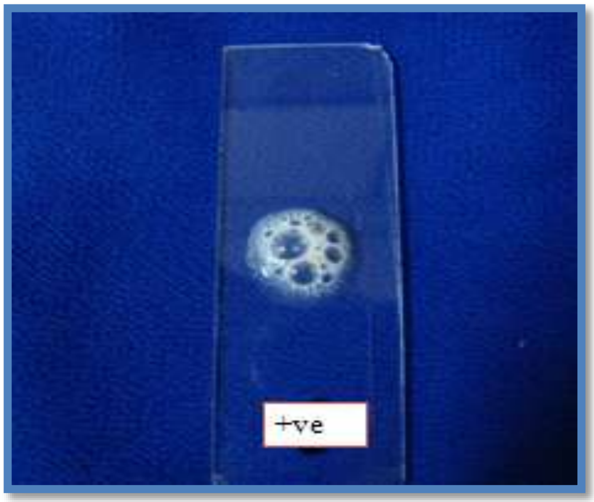
(e) Ammonia production

(f)Gelatin liquifaction test

(g) H_2S test

(h)Starch hydrolysis test

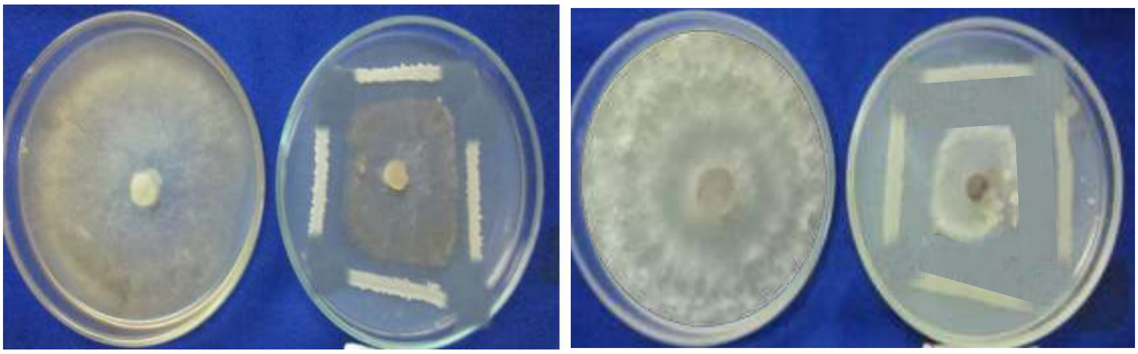
(i) Catalase test



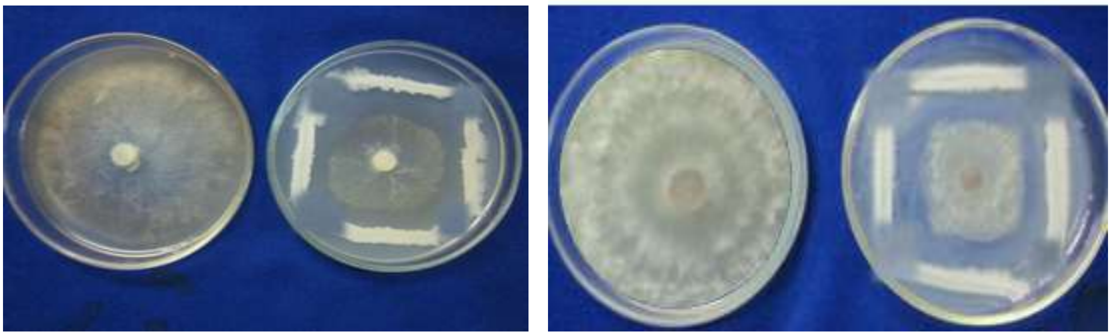
(j) Oxidase test

Plate 4.7 Antagonistic activity of PGP-isolates against soil born plant pathogens under *in vitro* conditions

a)PGP-1(*Pseudomonas*) With *Rhizoctonia solani* b)PGP-1 (*Pseudomonas*)with *Sclerotium rolfsii*

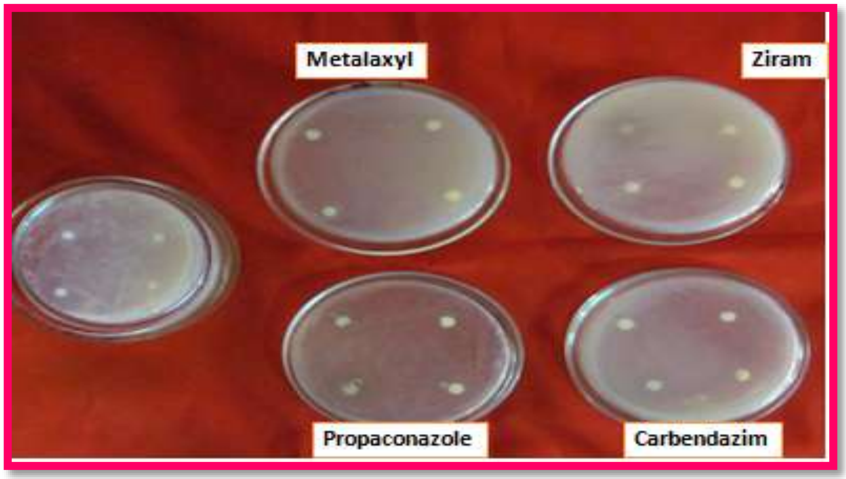


c) PGP-9(*Azotobacter*) With *Rhizoctonia solani* d) PGP-9 with (*Azotobacter*) *Sclerotium rolfsii*

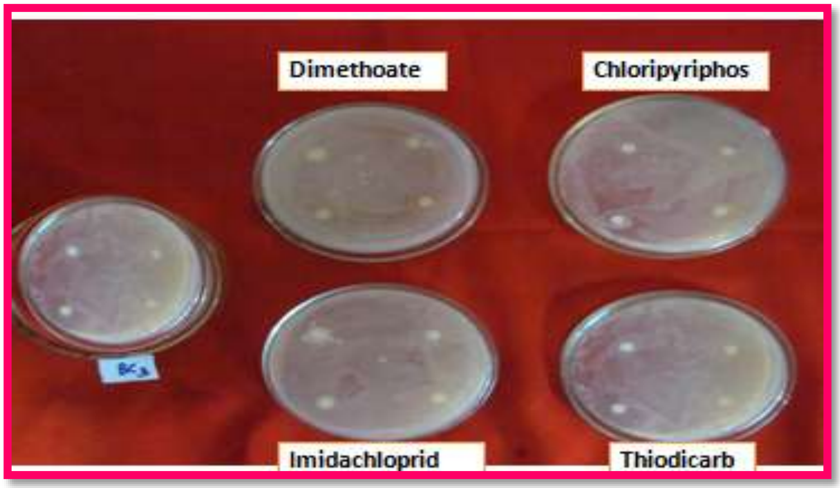


e) PGP-15(*Azospirillum*) with *Rhizoctonia solani* f) PGP-15(*Azospirillum*) with *Sclerotium rolfsii*

Plate 4.8 Compatibility of PGP-1 isolate with Agrochemicals



a) Compatibilty of PGP-1(*Pseudomonas* spp.) isolate with fungicides



b) Compatibilty of PGP-1(*Pseudomonas* spp.) isolate with insecticides

c) Compatibilty of PGP-1(*Pseudomonas* spp.) isolate with Herbicides

Plate 4.9 Compatibility of PGP-9 isolate with Agrochemicals

Compatibility of PGP-9(*Azotobacter* spp.) with fungicides

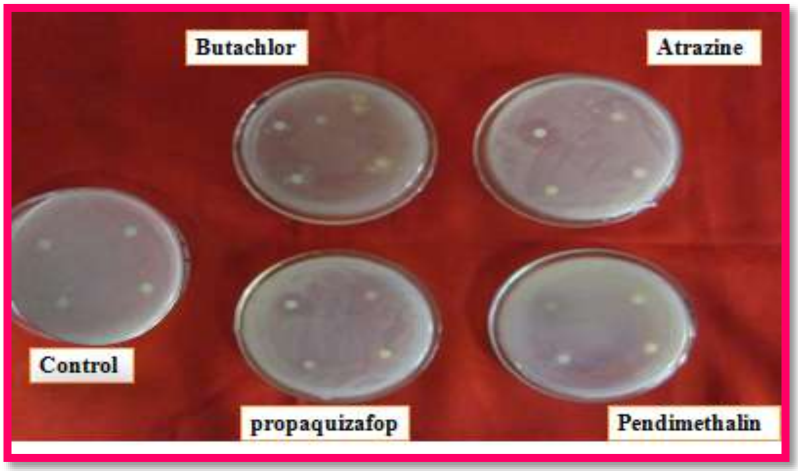
Comaptibility of PGP -9 (*Azotobacter* spp.) with insecticides

Comaptibility of PGP -9 (*Azotobacter* spp.) with herbicides

Plate 4.10 Compatiility of PGP-15 isolate with Agrochemicals

a)Comaptibility of PGP -15 (*Azospirillum* spp.) with fungicides

b)Compatibility of PGP -15 (*Azospirillum* spp.) with insecticides



c)Comaptibility of PGP -15 (*Azospirillum* spp.) with herbicides

Chapter V

SUMMARY AND CONCLUSIONS

The organisms that establish positive interactions with plant roots and show observable benefits on the plant growth are collectively called as Plant Growth Promoting Rhizobacteria (PGPR). The important traits of PGPRs include fixation of atmospheric nitrogen, solubilization of insoluble inorganic phosphates, production of plant hormones, siderophores, bacteriocins *etc.* These organisms also provide protection to plants against diseases by suppressing deleterious and pathogenic microorganisms. Plant growth-promoting rhizobacteria (PGPR) are free-living soil bacteria that colonize the rhizosphere and when applied to seed or crops enhance the growth of plants. The rhizosphere is the soil found around the root and under the influence of the root. It is a site with complex interactions between the root and associated microorganisms.

In the present study, an attempt has been made to collect bacterial isolates from different resource laboratories in Andhra Pradesh and screen the bacterial isolates for possession of multiple PGP activities, biocontrol activity. They characterized morphologically and biochemically and efficient strains were subjected to characterization. The bacterial isolates were tested for their compatibility against commonly used agrochemicals.

Bacterial isolates (16) were collected from Biofertilizers Laboratory, Agricultural research station, Amaravathi ,Guntur(dist), Department of soil and biology, ICRISAT, Hyderabad and Dept of Agricultural Microbiology and Bioenergy, College of Agriculture , Rajendranagar. Bacterial isolates were tested for their purity and preserved in Dept.of Agricultural Microbiology & Bioenergy, College of Agriculture, Rajendranagar, Hyderabad. Sixteen bacterial isolates were characterized by their cultural , morphological and biochemical characteristics. Among them, three isolates were identified as *Bacillus spp.*(PGP-7,12,14), four as *Pseudomonas spp.*(PGP-1,8,13,16), three as *Azotobacter* spp.(PGP-6, 9, 11), three as *Rhizobium* spp.(PGP-2, 3, 4) and three as *Azospirillum* spp.(PGP-5,10,15).

Further, all the isolates were screened for plant growth promoting attributes *viz.*, phosphate solubilization, nitrogen fixation, IAA production and biocontrol activity. Among sixteen PGP Isolates, 12 isolates were able to solubilise phosphate on Pikovskaya's media containing Tri calcium phosphate in the range of 10 mm to 25 mm. Among 12 isolates PGP-1 recorded the highest solubilization zone (25mm) immediately followed by PGP-7(22mm), PGP-9(21mm), PGP-15(20mm). The nitrogen fixing ability(Acetylene Reduction Assay) among the studied strains that ranged from to 4.22 to 9.15 nmoles ethylene/h/mg protein, Among sixteen bacterial isolates only six isolates are shown nitrogen fixing ability (PGP-5, PGP-6, PGP-9, PGP-10, PGP11, and PGP-15). Out of six isolates PGP-15 showed highest nitrogen fixing ability (9.15 nmoles C_2H_4 mg protein^{-1}.h^{-1}) and the least was PGP-11(4.22 nmoles mg protein^{-1}.h^{-1}).

Out sixteen isolates PGP-15 shows maximum IAA production (4.3 µg.ml^{-1}) without addition of tryptophan method, followed by PGP-5 (3.49 µg/ml). Enhancement in production of IAA was observed with the supplementation of L-Tryptophan @ 10mg per litre by PGP-15 (7.9 µg.ml^{-1}), followed by PGP-5(7.65 µg.ml^{-1}) and PGP-1(7.40 µg.ml^{-1}). All sixteen PGPR isolates were positive for ammonia production. The isolate PGP-1 produced ammonia strongly (+++) while PGP-6, PGP-11 were weak (+) ammonia production and other were produced ammonia moderately.

Out of sixteen isolates ten PGP isolates were produced siderophores. Among ten isolates PGP-1, PGP-8, PGP-15, PGP-16 were strong (+++) siderophore producers.

Out of sixteen PGP isolates, ten isolates produced HCN which PGP-1 PGP-8,PGP-7,PGP-9 proved strong(+++) HCN production and remaining isolates produce HCN moderately(++) by PGP-16,PGP-13.

Among sixteen Isolates PGP-1 induced large percent inhibition (36.10%) followed by PGP-9 (35.50%), PGP-8 (35.15%), PGP-7 (34.49%). Among sixteen isolates PGP -14 induced large percent inhibition (35.29%) and inhibition zone (4.00mm) followed by PGP-7 (35.47%), PGP-9 (33.89%), PGP-16 (33.74%), PGP-11 32.27%).

Compatibility of bacterial test isolates with commonly used agrochemicals like fungicides (metalaxyl, ziram, propiconazole, carbendazim), insecticides (chloripyriphos, dimethoate, imidachloprid, thiodicarb) and herbicides **(**atrazine, butachlor, propaqizofop, pendimethalin). From compatibility studies the potential compatibility of sixteen isolates with recommended dosage of Fungicides, Insecticides and Herbicides could be assessed. Among all isolates PGP-1.PGP-9.PGP-15 were not affected by recommended dosage of all fungicides, insecticides and herbicides.

Molecular characterization of nitrogen fixing ability of efficient PGPR nitrogen fixers such as PGP-9 (*Azotobacter* sp) and PGP-15(*Azospirillum* sp) cultures was carried out. Their banding pattern of DNA on agarose gel electrophoresis was compared with that of reference *Azotobacter* strains and *Azospirillum* strains. The PCR production was carried out for cultures in order to check the presence of *nif* H1, *nif*H2 genes for *Azotobacter* and *Azospirillum* characterization.

Among sixteen isolates, three efficient isolates were selected based on multiple PGPR characters, biocontrol activity, and compatibility with different commonly used agrochemicals. For field experiment of maize crop, The selected isolates for nitrogen fixer was *Azotobacter* spp. and associative nitrogen fixer was *Azospirillum* spp. and phosphate solubilizing *Pseudomonas* spp.

The results of the maize experiment entitled To study the influence of efficient PGPR strains with maize crop’’ conducted during *rabi* 2013 at the College Farm, College of Agriculture, Rajendranagar, Hyderabad.

These efficient isolates were formulated carrier based microbial inoculants and tested purity (10^8 cells/gram) prior to before sowing. These formulated microbial inoculants are applied soil application along with chemical fertilizers and Farm Yard Manure(all plots including control) with recommended dosages. T1: 100 % RDF (N2: P_2O_5: K_2O ratio: 120 kg:50 kg:60 kg.ha^{-1}), T2: 75 % RDN + Nitrogen fixer *Azospirillum* ,T3: 75 % RDN+ nitrogen fixer *Azotobacter* ,T4: 75 % RDP + Phosphate solubilizers, T5: 75 % RDN&P + nitrogen fixer *Azospirillum* + Phosphate solubilizers, T6: 75 % RDN & P + nitrogen fixer *Azotobacter* + Phosphate solubilizers, T7: 75 % RDN & P + *Azotobacter* + *Azospirillum* + Phosphate solubilizers.

The plant height, root length, dry weight recorded highest in treatment T7 (75%RDN& P + *Azospirillum* + *Azotobacter* + PSB) and significant in T6 (75% RDN& P + *Azospirillum* + *Azotobacter* + PSB)30.98cm,T5(75% RDN& P + *Azospirillum* + PSB)30.33cm ,T2 (75% RDN+ *Azospirillum*)28.20cm and lowest plant height was recorded in T1 (100% RDF)27.65cm in different growth stages of intervals (40 DAS,70 DAS, harvesting stage.

In the yield attributes of maize cob weight, more cob weight compared to control plot T1 (100% RDF) when compared to control plot T_1 (100% RDF) 703.33g. Maximum cob weight were recorded in T7 (75%RDN& P + *Azospirillum* + *Azotobacter* + PSB) 756.66g. No. of rows per cob were maximum in treatment T7 (75%RDN&P + *Azospirillum* + *Azotobacter* + PSB) 15.83 and treatment T2 (75% RDN+ *Azospirillum*) 15.20. No.of kernels per row is maximum in treatment T7 (75%RDN& P + *Azospirillum* + *Azotobacter* + PSB) 32.45 compared to all treatments. The lowest no.of kernels per row recorded in control T1 (100% RDF) 28.46. No.of kernels per cob significantly increased with application of microbial inoculants and chemical fertilizers i.e. is maximum in treatment T7 (75%RDN & P + *Azospirillum* + *Azotobacter* + PSB) 438.88, followed by T2 (75% RDN+ *Azospirillum*) 431.66.

The highest grain yield (3366 kg ha^{-1}) was recorded in treatment T7 (75% RDN& P + *Azospirillum* +*Azotobacter* + PSB), followed by T6, T5. In contrast lowest grain yield (2653.00kg ha^{-1}) in treatment T1 (75%RDF).The Stover yield varied from 3540 kg.ha^{-1} to 4530kg ha^{-1}. The highest stover yield (4530 kg ha^{-1}) was recorded in treatment T7 (75% RDN& P + *Azospirillum* +*Azotobacter* + PSB+ FYM) followed by T5 (4276.66kg ha^{-1}) ,T6(4272 kg ha^{-1}).

The nutrient status such as available nitrogen , phosphorus and potassium in the soil was improved with microbial inoculants applied treatments significantly compared to non inoculated 100% RDF treatment(T1).

Microbial population revealed total bacterial count was significant in all inoculated treatments over T1 (100% RDF). *Azotobacter* population increased upto 70 DAS of crop growth in all the treatments and maximum population was observed in T3 at 70 DAS followed by T6. *Azotobacter* population was increased in T1 also because of the nature of the host plant, it being cereal crop. In the *Azospirillum* microbial population gradually increased in all treatments till 70DAS irrespective of treatments, but maximum population was recorded in T2 & T7 where these two received inoculation of *Azospirillum*. *Rhizobial* population data reveals that at 0 DAS across all the treatments in the rhizosphere of maize *Rhizobium* population has increased upto 70 DAS and same was maintained till harvest. Increase may be due to root exudates and soil nutrients in the rhizosphere of maize. The application of PGPR inoculants at a great extent by suppressing the soil fungal population from 0 DAS to 70 DAS. At harvesting stage where we recorded the decrease in population of PGPR isolates raise in the fungal population was observed towards harvesting stage this indicating that the PGPR microbial inoculants presence would suppressing the fungal population.

Economics results indicate that the combination of PGPR inoculants and 75% reduced chemical fertilizers will increase the net returns over 100% RDF without inoculation of microbial inoculants. Among all treatments T7 (75%RDN & P+ *Azospirillum* + *Azotobacter* + PSB) was improved better yield than all treatments because of co inoculation of three microbial inoculants which improve the soil fertility and nutrient status, inhibit the growth of phytopathogens and production of growth promoting substances.

However, field studies showed that bacterial inoculants for the purpose of improving crop growth and yield due to their PGPR attributes as part of integrated nutrient and disease management. These isolates can be developed into effective biofertilizers either singly or in combination after further studies under different climatic conditions. These are eco-friendly in nature and cost effective. So use of efficient PGPR with multiple beneficial activities help in improving profitability in agriculture and improve livelihoods of small and marginal farmers.

FUTURE LINE OF WORK

1. To evaluate the persistence of PGPR inoculants in soil with different climatic situations like drought conditions and resistance.
2. To evaluate yield of cereal crops with the combination of PGPR bacteria with Vesicular Arbuscular Mychorrhizae(VAM).
3. The effect of agrochemicals on nitrogen fixing ability of PGPR isolates.

LITERATURE CITED

.

Abbasi, M.K., Sharif, S., Kazmi, M., Sultan, T and Aslam, M. 2011. Isolation of plant growth promoting rhizobacteria from wheat rhizosphere and their effect on improving growth, yield and nutrient uptake of plants. *Plant Biosystems*. 145 (1): 159-168.

Abbas, Z.P., Saleh, R.N., Asadi, R.H., Khavazi, K., Soltani, A., Shoary, N.A.R and Miransari, M. 2010. Plant growth-promoting activities of *fluorescent Pseudomonads* isolated from the Iranian soils. *Acta Physiologiae Plantarum*. 32: 281-288

Abeysinghe, S. 2009. Effect of combined use of *Bacillus subtilis* CA32 and *Trichoderma harzianum* RU01 on biological control of *rhizoctonia solani* on *Solanum melongena* and *Capsicum annuum*. *Pakithan Journal Nutrition*. 8: 9-16.

Adjanohoun, A., Allagbe, M., Noumavo, P.A., Hodonou, G.H., Sikirou, R., Dossa, K.K., GleleKaka, R., Kotchoni, S.O and Baba-Moussa, L. 2011. Effects of plant growth promoting rhizobacteria on field grown maize. *Journal of Animal & Plant Sciences*. 11(3): 1457-1465.

Ahemad, F., Husain, F.M and Ahmad, I. 2011. Rhizosphere and root colonization by bacterial inoculants and their monitoring methods: A critical area in PGPR research. *Microbes and Microbial Technology*. 363-391.

Ahemad, M and Khan, M. S. 2010. Influence of selective herbicide on plant growth promoting traits of phosphate solubilizing *Enterobacter asburia* strain PS2. *Research Journal of Microbiology*. 5(9): 849-857.

Ahmadzadeh, M and Sharifi-Tehrani, A. 2009. Evaluation of *fluorescent pseudomonads* for plant growth promotion, antifungal activity against *Rhizoctonia solani* on common bean, and biocontrol potential. *Biological Control*. 48: 101-107.

Ahmed, T.H.M., Elsheikh, E.A.E and Mahdi, A.A. 2007. The *in vitro* compatibility of some *Rhizobium* and *Bradyrhizobium* strains with fungicides. *African Crop Science Conference Proceedings*. 8: 1171-1178.

Akhtar, M.S., Shakeel, U and Siddiqui, Z.A. 2010. Biocontrol of *Fusarium* wilt by *Bacillus pumilus*, *Pseudomonas alcaligenes* and *Rhizobium spp*. on lentil. *Turkish Journal of Biology*. 34: 1-7.

Aneja, K.R. 2001. Experiments in Microbiology, Plant Pathology and Tissue culture. Viswaprakasham, New Delhi. 471.

Arshad, M and Frankenberger, Jr. W.T., 1993.plant growth promoting substances in the rhizosphere: Microbial production and functions.

Asea, P.E.A., Kucey, R.M.N. and Stewart, J.W.B. 1988. Inorganic phosphate solubilization by two Penicillium species in solution culture and soil. *Soil Biol. Biochem.* 20: 459-464.

Asghar, H. N., Z. A. Zahir, M. Arshad and A. Khaliq(2002). Relationship between in vitro productionof auxins by rhizobacteria and their growthpromoting activities in Brassica juncea L.*Biol.Fert. Soils* 35: 231-237.

Ashraf, M.A., Rasool, M and Mirza, M.S.2011. Nitrogen Fixation and Indole Acetic Acid Production Potential of Bacteria Isolated from Rhizosphere of Sugarcane (*Saccharum officinarum* L.). *Advances in Biological Research* 5 (6): 348-355.

Ashrafuzzaman, M., Hossen, F.A., Ismail, M.R., Hoque, Md. A., Islam, M.Z., 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.

Attitalla, I.H.,Abobaker, M.A.,Muftah, A.N.,Amir, H.G.,Latiffah, Z.,Hasnah, M.J.,Ibrahim, A.A and Baharuddin, S. 2010. Occurence and microbiological characteristics of *Azospirillum* strains associated with leguminous and non –leguminous plants in Jabal akhdar eco-region, Libya, *Amer,J.Agric.Enivorn.Sci.*8:617-625.

Azin, C.O., Amir, H.G and Chan, L.K. 2005. Isolation and characterization of Diazotrophic Rhizobacteria of oil palm roots. *Malasian Journal of Microbiology* .1(1):31-35.

Balamurugan, A., T. Princy, R. V. Pallavi, P. Nepolean, R. Jayanthi and R. Premkumar, 2010. Isolation and characterization of phosphate solubilising bacteria In tea (*Camellia sincsis*). *J. Bio. sci. Res.*1(4): 285-293.

Banerjee, S., Palit, P., Sengupta, C and Standing D. 2010. Stress induced phosphate solubilization by *Arthrobacter* sp. and *Bacillus* sp. isolated from tomato rhizosphere. *Australian Journal of Crop Science*. 4(6): 378-383.

Bano, Q., Ilyas, N., Bano, A., Zafar, N., Akram, A and Hassan, F. 2013. Effect of *Azospirillum* inoculation on maize (*zea mays* l.) under drought stress. *Pakistan Journal of Botany*. 45: 13-20.

Baral,B.R and Adhikari,P. 2013.Effect of *Azotobacter* on growth and yield of maize. *SAARC J. Agri.* 11(2): 141-147.

Barthalomew, J.W and Mittewer, T. 1950. A simplified bacterial strain. *Stain Technology*. 25: 153.

Bashan, Y and Levanony, H. 1990. Current status of *Azospirillum* inoculation technology. *Azospirillum* as a challenge for agriculture. *Canadian Journal of Microbiology* 36: 591.

Bashan, Y., Holguin, G and de-Bashan, L.E. 2004. *Azospirillum*-plant relationships: physiological, molecular, agricultural and environmental advances. *Canadian Journal of Microbiology*. 50: 521-577.

Bergerson, F.J.1980. Methods for evaluating biological nitrogen fixation.John wiley & sons, New York .702.

Bhattacharya, P.N and Jha. D.K .2012 . Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World J. Microbiol. Biotechnol*. 28:1327–1350.

Biari. A., Gholami, A and Rahmani, H.A. 2008. Growth promotion and enhanced nutrient uptake of maize (*Zea mays* L) by application of plant growth promoting rhizobacteria in Arid Region of Iran. *Journal of Biological Sciences*. 8(6): 1015-1020.

Bloemberg, G.V and Lugtenberg, B.J.J. (2001) Molecular basis of plant growth promotion and biocontrol by rhizobacteria. *Current Opinion in Plant Biology* 4: 343–350.

Boddey, R.M and Dobereiner, J. 1995. Nitrogen fixation associated with grasses and cereals: recent progress and perspectives for the future. *Fertilizer Research*. 42, 241–250.

Cakmakı, R., Erat, M., Erdo, U. G. and Dönmez, M. F. 2007. The influence of PGPR on growth parameters, antioxidant and pentose phosphate oxidative cycle enzymes in wheat and spinach plants. *J. Plant Nutr. Soil Sci*. **170**:288-295.

Cappuccino, J. C and Sherman, N., 1992. A Laboratory Manual for Microbiology: New York. 125–179.

Castric, K.F and Castric, P.A. 1983. Method for rapid detection of cyanogenic bacteria. *Applied and Environmental Microbiology*. 45: 700-702.

Cattelan, A.J., Hartel, P.G and Fuhrmann, J.J.1999. Screening of plant growth promoting rhizobacteria to promote early soybean growth. *Soil Sci. Soc. Am. J*. 63: 1670–1680.

Chanway, C.P., Hynes, R.K and Nelson, L.M. 1989. Mant growth promoting rhizobacteria: Effects on growth and nitrogen of lentil *(Lens escuknta* Moench) and pea *(Pisum sativum* L.). *Soil Biology and Biochemistry*. 21: 511-517.

Chebotar, V.K., Asis, J.C.A and Akao, S. 2001. Production of growth promoting substances and high colonization ability of rhizobacteria enhance the nitrogen fixation of soyabean when coinoculated with *Bradyrhizobium japonicum. Biology and Fertility of Soil.* 34: 427-432.

Cheema, H.K., Sharma, P., Singh, R., Taggar, G.K., Khanna, V and Kooner, B.S. 2009. Efficacy and compatibility of insecticides, fungicide and *Rhizobium* inoculant in combination for seed treatment in chickpea (*Cicer arietinum*). *Indian Journal of Agricultural Sciences*. 79(3): 190-194.

David, K. A. V. , Apte, S. K., Banerji, A and Joseph Thomas.1980. Acetylene reduction assay for Nitrogenase activity: chromatographic determination of ethylene for sample in less than one minute. *Applied and Environmental Microbiology.* 39(5):1078.

De Freitas, J.R., Banerjee, M.R., Germida, J.J., 1997. Phosphate solubilizing rhizobacteria enhance the growth and yield but not phosphorus uptake of canola (*Brassica napus* L.). *Biol. Fertil. Soil* 24:358–364.

De, R.K., Dwivedi, R.P and Narain, U. 2003. Biological control of lentil wilt caused by *Fusarium oxysporum* f.sp. lentis. *Annals of Plant Protection Sciences*. 11(1): 46-52.

Deshmukh, P.J., Potdukhe, S.R., Pardey, V.P and Ingle, Y.V. 2004. Effect of *Rhizobium japonicum* and chemical herbicides on nodulation, rhizobia survival and grain yield of soybean [*Glycine max (L) Merrill.*]. *Bioved Research & Agriculture & Tech*. 15(1/2): 89-93.

Dey, R., Pal, K. K., Bhatt, D. M. and Chauhan, S. M. 2004. Growth promotion and yield enhancement of peanut (Arachis hypogaea L.) by application of plant growth-promoting rhizobacteria. *Microbiological Research* .159: 371-394.

Dhanasekar, R and Dhandapani,R. 2012. Effect of biofertilizers on the growth of helianthus annuus. *International Journal of Plant, Animal and Environmental Sciences*. 2(4):143-147.

Di Battista-Leboeuf, C., Benizri, E., Corbel, G., Piutti, S., and Guckert, A. 2003. Distribution of *Pseudomonas sp*. populations in relation to maize root location and growth stage. *Agronomie* 23: 441–446.

Dilworth, M. J. 1966. Acetylene reduction by free living nitrogen fixing preparations from *Clostridium pasturianum. Biochim. Biophys. Acta*. 127: 285-294.

Dobbelaere, S., Croonen borghs, A., Thys, A., Ptacek, D.,Vanderleyden, J., Dutto, P., et al.(2001) Responses ofagrinomically important crops to inoculation with *Azospirillum*. *Aust J Plant Physiol* . 28: 871–879.

Dobbelaere, S., Vanderleyden, J., and Okon, Y. 2003. Plantgrowth-promoting effects of diazotrophs in the rhizosphere. *CRC Crit Rev Plant Sci*. 22: 107–149.

Duineveld, B.M., Rosado, A.S., Van elsas, J.D., and van Veen, J.A. 1998. Analysis of the dynamics of bacterial communities in the rhizosphere of the chrysanthemum via denaturing gradient gel electrophoresis and substrate utilization patterns. *Appl Environ Microbiol* .64: 4950–4957.

Durai, M. 2004. Management of charcoal rot of sesame (*Sesamum indicum* L.) incited by *Macrophomina phaseolina* Goid. M.Sc. (Ag.). Thesis submitted to Acharya N.G. Ranga agricultural University, Hyderabad.

Dye,D.W.1962. The inadequacy of the usual determinative tests for identification of *Xanthomonas* sp.*NZT sci.*5:393-416.

Farah, A and Iqbal, A. 2008. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiological Research.* 163: 173-181.

Fatima. Z., Saleemi, M., Zia, M., Sultan, T., Aslam, M., Rehma, R.U and Chaudhary, M.F. 2009. Antifungal activity of plant growth-promoting rhizobacteria isolates against *Rhizoctonia solani* in wheat. *African Journal of Biotechnology*. 2: 219-225.

Fekadu Alemu . 2013. Isolation of *Pseudomonas fluorescens* from rhizospheric soil of faba bean and assessment of their Phosphate solubility: in vitro study, Ethiopia . *Scholars Academic Journal of Biosciences (SAJB).* 1(7):346-351.

Flaishman, M.A., Eyal, Z.A., Zilberstein, A., Voisard, C., Hass, D., 1996. Suppression of *Septoria tritci* blotch andleaf rust of wheat by recombinant cyanide producing strains of *Pseudomonas putida*. *Mol. Plant MicrobeInteract*. 9: 642–645.

Frederick M. Ausubel , Roger Brent , Robert E. Kingston , David D. Moore , J. G. Seidman , John A. Smith , Kevin Struhl . Short Protocols in Molecular Biology, 4th Edition.

Gangwar, M and Kaur, G. 2009. Isolation and characterization of endophytic bacteria from endorhizosphere of sugarcane and rye grass. *The Internet Journal of Microbiology*. 7: 1.

Gholami, A.,Shahsavani, S and Nezarat, S.2009. The Effect of Plant Growth Promoting Rhizobacteria (PGPR) on Germination, Seedling Growth and Yield of Maize. *World Academy of Science, Engineering and Technology*.3 :01-16.

Ghosh, T.K., Tyagi, M.K and Duhan, J.S. 2003. *Rhizobial* compatibility against lethal doses of pesticides in green gram with different method of inoculation. *Indian Journal of Agricultural Research.* 37(2): 120-123.

Glick, B.R. 1995. The enhancement of plant growth by free-living bacteria. *Canadian Journal of Microbiology.* 41: 107-117.

Gomez , K.A and Gomez , A.A. Statistical Procedures for Agricultural Research, 2nd Edition.

Gorden, A.S and Weber, R.P. 1951. Colorimetric estimation of Indole Acetic Acid. *Plant Physiology*. 26: 192-195.

Gosal, S. K., Saroa, G. S., Vikal, Y., Cameotra, S.S., Pathania, N and Bhanot, A. 2011. Isolation and molecular characterisation of diazotrophic growth-promoting bacteria from wheat rhizospheric soils of Punjab. *Soil Research* .49(8): 725-732.

Gothwalv. R.K., Nigamm.K., Mohand.K., Sasmal, and Ghosh, P.2007. Screening of nitrogen fixers from rhizospheric bacterial isolates associated with important desert plants. *Applied ecology and environmental research* 6(2): 101-109.

Gull, M., Hafeez, F. Y., Saleem, M. and Malik, K. A. 2004. Phosphorus uptake and growth promotion of chickpea by co-inoculation of mineral phosphate solubilising bacteria and a mixed rhizobial culture. *Aust. J. Exp.Agric*. 44: 623-628.

Hardy, R.W.F., Holsten, R.D., Jackson, E.K., Burns, R.C. 1968. The acetylene-ethylene assay for N2 fixation: laboratory and field evaluation. *Plant Physiology*. 43:1185- 1207.

Hefnawy, M.A., A. E. Omima and A. A. Asmaa. 2012. Interaction Of some herbicides with phosphate solublization by *Aspergillus niger* and *Aspergillus fumigatus. Australian J. Basic and Applied Sci*., 6(10): 518-524.

Holt, J.G., Kreig, N.R., Sneath, P.H.A., Staley, J.T. and Williams, S.T. 1994. Bergey's Manual of systematic Bacteriology. Williams and Wilkins, Baltmore, USA.

Hussain, M. I., Asghar, H. N., Arshad, M and M. Shahbaz.2013. Screening of multi-traits rhizobacteria to improve maize growth under axenic conditions. *The Journal of Animal & Plant Sciences*. 23(2): 514-520.

Hussain, S.k., and Srinivas, P.2013. Evaluation of Plant Growth Promoting Traits by *Pseudomonas* and *Azotobacter* Isolated From Rhizotic Soils of Two Selected Agroforestry Tree Species of Godavari Belt Region, India. *Asian journal of experimental Biological sciences.* 4 (3): 2013.

Ingle,R, W and Deshmukh, V.V.2010. Antifungal activity of PGPR and sensitivity to Agrochemicals. *Annals of plant Protection Sciences.* 18 (2): 451-457.

Jackson M L. 1964. Soil chemical analysis. Englewood Cliffs, Prentice Hall, New York.

Jadhav, B and Joshi, R. 1981. Nitrate Content in Fiber Fractions Obtained from Some Crops Processed for Leaf Protein Extraction. *Progressive Research.* 7: 127-130.

Jayaprakashvel, M., Muthezhilan, R., Srinivasan, R., Hussain, A.J., Gobalakrishnan, G., Bhagat, J., Kaarthikeyan, C and Muthulakshmi, R. 2010. Hydrogen cyanide mediated biocontrol potential of *Pseudomonas spp*. AMET1055 isolated from the rhizosphere of coastal sand dune vegetation. *Advanced Biotech.* 9(10): 39-42.

Jenson,H.L.1954. The *Azotobactreriace*. *Bacteriology.Rev*.18:195-214.

Jha, B.K., Pragash, M.G., Cletus, J., Raman, G and Sakthivel, N. 2009. Simultaneous phosphate solubilization potential and antifungal activity of new *fluorescent Pseudomonad* strains, *P. aeruginosa*, *P. pelcoglossicida* and *P. mosselii*. *World Journal of Microbiological Biotechnology*. 25: 573-581.

Jhala, A., Rathod, P.H., Patel, K.C and Damme, P.V. 2005. Growth and yield of groundnut (*Arachis hypogaea* L.) as influenced by weed management practices and *Rhizobium* inoculation. *Communications in Agricultural and Applied Biological Sciences*. 70(3): 493-500.

Joseph, B., Patra, R.R and Lawrence, R. 2007. Characterization of plant growth promoting rhizobacteria with chickpea (*Cicer arietinum* L.). *International Journal of Plant Production*. 1(2): 141-151.

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.

Karnwal, A. 2012. Screening of plant growth promoting rhizobacteria from maize (*Zea mays*) and wheat (*Triticum aestivum*). *African Journal of Food, Agriculture, Nutrition and Development*.12 (3): 6170-6185

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 Microbilogy Research*.7(16):1465-1474.

Keneni, A., Assefa, F and Prabu, P.C. 2010. Isolation of phosphate solubilizing bacteria from the rhizosphere of faba bean of ethiopia and their abilities on solubilizing insoluble phosphates. *Journal of Agricultural Science Technology.* 12: 79-89.

Khider, A.K. 2012. Molecular Study of *nif*H1, *nif*H2, *nif*H3, *nif*U, *nif*V, VF Genes and Classical approach cared out to Identification of *Azotobacter chroococcum* from Soil. *Current Research Journal of Biological Sciences*. 4(5): 570-577.

Khurana, A.S and Sharma, P. 2002. Interaction of Captaf and *Rhizobium* inoculation in chickpea (*Cicer arietinum* L.). *Legume Research*. 25(4): 282-283.

Kishore, G. K., B. Pande and A. R. Podile, 2005. Manegement of late leaf spot of groundnut with chlorothalonil tolerant isolate of *Pseudomonas aueroginosa*. *Plant Pathology*. 54: 401-408.

Kloepper, J.W., Lifshits, R and Zablotowicz, R.W. 1989. Free living bacterial inoculate for enhancing crop productivity. *Trends in Biotechnology*. 7: 39-43.

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. *Phyto Pathology*. 70: 1078-1082.

Koche, M. D. 2011. Integrated disease management for root rot and gummosis in Nagpur mandarin. Ph.D thesis (unpub) Dr. PDKV., Akola.

Konineek, S and Chandan, S. 2011. Post harvest preservation of pointed gourd fruits with PGPR to ensure food safety. *Internet Journal of Food Safety*. 13: 69-76.

Kumar, K and Kannaiyan, S. Molecular characterization of *nif* genes of heterotrophic and endophytic diazotrophs in rice. National Biodiversity Authority, Neelankarai Chennai- 600 041.

Kumar, K., Amaresan, N., Bhagat, S., Madhuri, K and Srivastava, R.C. 2010. Isolation and characterization of rhizobacteria associated with coastal *agricultural* ecosystem of rhizosphere soils of cultivated vegetable crops. *World Journal of Microbiological Biotechnology*. 27:1625-1632.

Kumar, S., Upadhyay, J.P and Roy, S. 2002. Effect of pesticide seed dressing and *Rhizobium* inoculation on nodulation and yield of chickpea (*Cicer arietinum L.*). *Journal of Applied Biology*. 12(1/2): 81-83.

Kumar, V. K., Reddy, M. S., Kloepper, J. W., Yella reddy, S. K. Lawrence, K. S., . Zhou, H. Sudin, X. G., Miller, M. E., Apparao, P., Reddy, S. R., Niranjana, R and N. S. Chandra., 2011. Plant growth-promoting activities of *Bacillus subtilis* mbi 600 (integral) and its compatibility with commonly used fungicides in rice sheath blight management. *International Journal of Microbiology & Research*. 3(2): 120-130.

Kumar,P., Dubey, R.C and Maheshwari. D.K.2012. Bacillus strains isolated from rhizosphere showed plant growth promoting and antagonistic activity against phytopathogens. *Microbiological Research*. 167: 493– 499.

Kutcher, H.R., Lafond, G., Jhonston, A.M., Miller, P.R., Gill, K.S., May, W.E., Hogg, T., Johnson, E., Biederbeck, V.O and Nybo, B. 2002. *Rhizobium* inoculants and seed applied fungicide effects on field pea production. *Canadian Journal of Plant Science*. 82(4): 645-651.

Madhavi, M. 2006. Studies on biological control of fusarial wilt of chillies. M.Sc. (Ag.). Thesis submitted to Acharya N.G. Ranga Agricultural University. Hyderabad.

Mahantesh, P and Patil, C.S. 2011. Isolation and biochemical characterization of phosphate solubilizing microbes. *International Journal of Microbiology Research*. 3(1): 0975–9174.

Mahato , P., Badoni, A and Chauhan, J. S. 2009. Effect of *Azotobacter* and Nitrogen on Seed Germination and Early Seedling Growth in Tomato. *Researcher*, 1(4):62-66.

Mahbouba1, B., Christine, L.R., Nadir, B., Nadia, Y and Abdelhamid, D. 2013. Phenotypic and molecular characterization of plant growth promoting Rhizobacteria isolated from the rhizosphere of wheat (*Triticum durum*.) in Algeria. *African Journal of Microbiology Research.*7(23): 2893-2904.

Maheshwar, N.U and Sathiyavani, G. 2012. Solubilization of phosphate by *Bacillus spp*, from groundnut rhizosphere (*Arachishypogaea L). Journal of Chemical and Pharmaceutical Research*. 4(8): 4007-4011.

Maheshwari, M.N.2013.Compatibility of fluorescent *pseudomonads* isolates with fungicides. *International Journal of Food, Agriculture and Veterinary Sciences*. 3 (2):46-50.

Maksimov, I.V, Abizgildina, R.R., Pusenkova, L.I. 2011. Plant growth promoting rhizobacteria as alternative to chemical crop protectors from pathogens (Review) *Applied Biochemistry and Microbiology*. 47:333–345.

Maleki, M., Mostafaee, S., Mokhtarnejad, L and Farjaneh, M. 2010. Characterization of *Pseudomonas fluorescens* strain CV6 isolated from cucumber rhizosphere in Varamin as a potential biocontrol agent. *Australian Journal of Crop Science*. 4(9): 676-683.

Manivannan,M., Ganesh, P., Suresh Kumar, R., Tharmaraj, K and Shiney Ramya, B.2012. Isolation, Screening, Characterization and Antagonism Assay of PGPR Isolates from

Rhizosphere of Rice Plants in Cuddalore District. *International Journal of Pharmaceutical & Biological Archives.* 3(1):179-185.

Manyong, V. M., Makinde, K. O., Sanginga, N., Vanlauwe, B and Diels, J. 2001. Fertilizer use and definition of farmer domains for impactoriented research in the northern Guinea savanna of Nigeria. *Nutritional Cycle and Ecosystems*. 59: 129–141.

Marques. P.G., Pires,C., Moreira, H., Rangel, O.S.S and Castro, P.M.L.2010. Assessment of the plant growth promotion abilities of six bacterial isolates using *Zea mays* as indicator plant. *Soil Biology & Biochemistry*. 42:1229-1235.

Mathew, A.V., 2003. *Pseudomonas fluorescens* antagonism compatibility with pesticides and alternate media for mass multiplication, 6th Int. *PGPR Workshop, Calicut, India*. 159-164.

Mehnaz, S. 2011. Plant growth promoting bacteria associated with sugarcane. *Crop Ecosystems*. 165-187.

Mehta, P., Chauhan, A., Mahajan, R., Mahajan, P.K and Shirkot, C.K. 2010. Strain of *Bacillus circulans* isolated from apple rhizosphere showing Plant growth promoting potential. *Current Science*. 98: 4-25.

Shende, S.T and Meshram .1982. Response of maize to *Azotobacter chroococcum*. Plant&Soil 692:65-273.

Mishra, M., Kumar, U., Mishra, P.K and Prakash, V. 2010. Efficiency of Plant growth promoting rhizobacteria for the enhancement of *Cicer arietinum* L. growth and germination under salinity. *Advances in Biological Research*. 4(2): 92-96.

Murumkar D.R., Borkar S.G. and Chimote, V.P.2013. Diversity for Cell Morphology, Nitrogenase Activity and DNA Profile of *Azospirillum* species present in Rhizosphere Soils of Six Different Physiographic Regions of Maharashtra. *Research Journal of Biotechnology*. 8 (4):16-25.

Namazari,M., Rahimzadeh,F.,Yarnia,M and Babaoghli,F.2012.Effect of biological fertilizer and mineral fertilizer on yield and yield components of corn (*zea mays*) . *ARPN Journal of Agricultural and Biological Science.* 7(10):865-870.

Naserirad, H., Soleymanifard, A and Rahim, N.2011. Effect of Integrated Application of Bio-fertilizer on Grain Yield Components and Associated Traits of Maize Cultivars. *American-Eurasian J. Agric. & Environ. Sci*., 10 (2): 271-277.

Nene and Thapliyal. 1993. Fungicides in Plant Disease Control, Oxford and IBH Publishing House, New Delhi. 163.

Nicolas, P., Carlier, E., Andres, J., Rosas, S.B and Rovera, S. 2011. Characterization of rhizosphere bacteria for control of phytopathogenic fungi of tomato. www.ncbi.nlm.gov.

Nongthombam. S. Nayek. H. and A. C. Das, 2008. Effect of anilofos and pendimethalin herbicide on N_2 fixing and phosphate solubilizing microorganisms in relation to availability of nitrogen and phosphorus in a typic haplustept soil. *J. Crop Weed*. 4(2): 1-6.

Noori, M.S.S and Saud, H.M. 2012. Potential plant growth-promoting activity of *Pseudomonas spp.* isolated from paddy soil in Malaysia as biocontrol agent. *Journal Plant Pathology Microbiology*. 3: 2.

Norris, J.R. Chapman, H.M., 1968. Classification of *Azotobacter.* In Identification methods for microbiologists Gibbs B.M. and Shapton D.A. (Eds.) *Academic press London and New York*. 19-27.

Noumavo, P., Kochoni, E., Didagbe, Y., Adjanohoun, A., Allagbe, M., Sikirou, R., Gachomo, E.W., Kotchoni, S.O., Moussa, B.2013. Effect of Different Plant Growth Promoting Rhizobacteria on Maize Seed Germination and Seedling Development. *American Journal of Plant Sciences*.4: 1013-1021**.**

Okon, Y., Labandera-Gonzalez, C.A., 1994. Agronomic applications of *Azospirillum*-Improving Plant Productivity with Rhizosphere Bacteria. *Common wealth Scientific and Industrial Research Organization, Adelaide*, Australia. 274–278.

Okon, Y., Albrecht, S.L and Burris.1977.Methods for growing *Spirillum lipoperum* and for counting it in pure culture and in association with plants. *Applied and Environmental Microbiology*. 33:85-87.

Oliveiraa, 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. 2009. Phosphate solubilizing microorganisms isolated from rhizosphere of maize cultivated in an oxisol of the Brazilian Cerrado Biome. *Soil Biology and Biochemistry* .41: 1782–1787.

Olivera, S., Dusica, D., Dragana, J., Kuzmanovic, D., Natasa, R and Jelena, K.V. 2011. Improvement of common bean growth by co-inoculation with *Rhizobium* and Plant growth promoting rhizobacteria. *Romanian Biotechnological Letters*. 16(1): 5919-5926.

Patel, P. H., Patel, J.P and Bhatt,S. A. 2013. Characterization and phylogenetic relatedness of *Azotobacter Salinestris. Journal of Microbiology and Biotechnology Research*. 3(1):65-70.

Patil, V. 2011. Production of indole acetic acid by *Azotobacter spp*. *Recent Research in Science and Technology*. 3(12): 14-16.

Pikovskaya, R.I. 1948. Mobilization of phosphorus in soil connection with the vital activity of some microbial species. *Microbiologiya*. 17: 362–370.

Ponmurugan, K., Sankaranarayanan, A and Dharbi, N.A.A. 2012. Biological activities of Plant growth promoting *Azotobacter spp.* isolated from vegetable crops rhizosphere soils. *Journal of Pure and Applied Microbiology*. 6: 4.

Potrich, D. P., Passaglia, L. M. P and Schrank, I.S.2001. Partial characterization of *nif* genes from the bacterium *Azospirillum amazonense Brazilian journal Of Biological Research*. 34: 1105-1113.

Preeti, T., Ekka, S.R and Tripathi, R. 2011. *In Vitro* Study of *Pseudomonas spp*. isolated from Soil. *Journal of Phytology*. 3(4): 21-23.

Quan, C.S., Wang, X and Fan, S.D. 2011. Antifungal compounds of plant growth promoting rhizobacteria and its action mode. *Microbiology Monographs*. 18:117-156.

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

Rakh, R.R., Raut, L.S., Dalvi, S.M and Manwar, A.V. 2011. Biological controls of *Sclerotium rolfsii*, causing stem rot of groundnut by *Pseudomonas cf. monteilli*. *Recent Research in Science and Technology*. 3(3): 26-34.

Ram, H and Singh, V.P. 2012. Plant Growth-Promoting Rhizobacteria (PGPR) in plant productivity and protection. *Inroads Indian journal.com*.1(2):132-140.

Ramakrishnan, K and Selvakumar,G. 2012. Effect of biofertilizers on enhancement of growth and yield on Tomato. *International Journal of Research in Botany*. 2(4): 20-23.

Ramette, A., Moenne-Loccoz, Y and Defago, G. 2006. Genetic diversity and biocontrol potential of *flourescent pseudomonads* producing phloroglucinols and hydrogen cyanide from swiss soils naturally suppressive or conducive to *Thielaviopsis basicola*-mediated black root rot of tobacco. *FEMS Microbiology and Ecology*. 55: 369-381.

Rani, M.U., Arundhathi and Reddy, G. 2012. Screening of rhizobacteria containing Plant growth promoting (PGPR) traits in rhizosphere soils and their role in enhancing growth of pigeon pea. *African Journal of Biotechnology*. 11(32): 8085-8091.

Reddy, D.M.S., Mohan, B.K., Nataraja, S., Krishnappa, M and Abhilash, M. 2010. Isolation and molecular characterization of *Bacillus megaterium* isolated from different agro climatic zones of Karnataka and its effect on seed germination and plant growth of *Sesamum indicum*. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*. 6(1): 614-625.

Ribeiro, C. M. and E. J. B. N Cardoso (2012). Isolation,selection and characterization of root-associatedgrowth promoting bacteria in Brazil Pine(*Araucaria angustifolia*). *Microbiol. Res*. 167:69-78.

Rodriquez, H. and Farag, R. 1999. Phosphate solubilizingbacteria and their role in plant growth promotion.*Biotechnol. Adv*., 17:319- 339.

Saber, W.I.A., Ghanem K.M and El-Hersh, M.S. 2009. Rock phosphate solubilization by two isolates of *Aspergillus niger* and *Penicillium sp*. and their promotion to mung bean plants. *Research Journal of Microbiology*. 4: 235-250.

Sachin ,N.2009. Effect of *Azotobacter chroococcum* (PGPR) on the Growth of Bamboo (*Bambusa bamboo*) and Maize (*Zea mays*) Plants**.** *Biofrontiers*. 1(1): 24-31.

Sajjad Mirza, Mehnaz, S., Normand, P.,Combaret, C.P., Loccoz, Y.M., Bally, R.,Malik, K.A.2006. Molecular characterization and PCR detection of a nitrogen-fixing *Pseudomonas* strain promoting rice growth. *Biol Fertil Soils*.43: 163−170.

Sakthivel, U and Karthikeyan, B. 2012. Isolation and characterization of plant growth promoting rhizobacteria (PGPR) from the rhizosphere of coleus forskohlii grown soil. *International Journal of Recent Scientific Research*. 3(5):288 – 296.

Sandeep, C., Rashmi, S. N., Sharmila, V. R., Surekha, Tejaswini, R and Suresh ,C.K. 2011. Growth Response of Amaranthus gangeticus to *Azotobacter chroococcum* Isolated from Different Agroclimatic Zones of Karnataka. *Journal of Phytology*. 3(7): 29-34.

Sarkar, A., Mukherjee, P.K and Bhattacharya, P. M. 2005. Bio-efficacy of Pendimethalin and Fluchloralin in mustard. *Indian Journal of Weed Science*. 37(3/4): 275-276.

Sarvani, B and Reddy, R.S. 2012. *In vitro* screening of native *bacillus* isolates for Plant growth promoting attributes. *First International seminar on Bioresource and stress management.* Feb 6-9.

Sarvani, B. 2011. Isolation and study of Plant Growth Promoting Rhizobacteria from soils of Rangareddy district. M.Sc. (Ag.). Thesis submitted to Acharya N.G. Ranga Agricultural University. Hyderabad.

Scher, F.M., Baker, R., 1982. Effect of *Pseudomonas putida* and a synthetic iron chelator on induction of soil suppressiveness to *Fusarium* wilt pathogens. *Phytopathology*. 72, 1567–1573.

Schollhorn ,R and Burris,R.H. 1967. Reduction of azide by the N_2-fixing enzyme system. *Proceeding of Natural Academy Sciences. U S A*. 57(5): 1317–1323.

Schroth, M.N and Hancock, J.G. 1982. Disease-suppressive soil and root colonizing bacteria. *Science*. 216: 1376-1381.

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

Selvakumar, G., Lenin, M., Thamizhiniyan, P. and Ravimycin,T. 2009. Response of biofertilizers on the growth and yield of blackgram (*Vigna mungo* L.). *Recent Research in Science and Technology*. 1(4):169-175.

Setubal *et al*. 2009. Genome Sequence of *Azotobacter vinelandii*, an obligate aerobe Specialized to Support Diverse Anaerobic Metabolic Processes.

Shaban, W.I and El-Bramawy, M.A. 2011. Impact of dual inoculation with *Rhizobium* and *Trichoderm*a on damping off, root rot diseases and plant growth parameters of some legumes field crop under greenhouse conditions. *International Research Journal of Agricultural Science*. 1(3): 098-108.

Sharifi, R and Khavazi, K. 2011. Effects of seed priming with Plant Growth Promoting Rhizobacteria (PGPR) on yield and yield attributes of maize (*Zea mays* L.) hybrids. *Food, Agriculture and Environment.* 9(3&4): 496-500.

Shrivastava, U.P. 2013. Isolation and initial characterization of diazotrophic Plant Growth Promoting Rhizobacteria (PGPR) from rice rhizosphere of Parsa and Bara district of Nepal. *International Journal of Pharmacy & Life Sciences*. 4(3): 2481-2488.

Singh, A., Ghosh, A.K., Poonam. 2012. Screening and assessment of phosphate solubilising microbes as potential biofertilizer isolated from selected wetland and rain fed ecosystem of Bihar. *Asian Journal of Biological Science*. 3(2): 397-406.

Singh, Y., Ramteke, P.W and Shukla, P.K. 2013. Isolation and characterization of heavy metal Resistant *Pseudomonas spp.* And their Plant growth promoting activities. *Advances in Applied Science Research*. 4(1): 269-272.

Singhai, P.K., Sarma, B.K and Srivastava. J.S. 2011. Biological management of common scab of potato through *Pseudomonas spp*. and vermicompost. *Biological Control*. 57: 150-157.

Skidmore, A.M and Dickinson, C.H. 1976. Colony interaction and hyphal interference between *Septoria nodorum* and phylloplane fungi. *Transactions and Journal of the British Ceramic Society*. 66: 57-74.

Snedecor, G.W and Cochron, W.G. 1967. Statistical methods. Oxford and IBH Publishing Company. New Delhi. 593.

Soltani, A.A., Kazem, K., Asadi, H.R., Hossein, A.A., Mahtab, O and Payman, A.D. 2012. Evaluation of biological control traits in some isolates of *fluorescent Pseudomonads* and *flavobacterium*. *Journal of Agricultural Sciences*. 4: 1.

Somasegaran, P and Hoben, H. J. 1994. Hand book of rhizobia. Springer-verlag, New York. 79-158.

Sreedevi,B., Preethi,B. and Pramoda Kumari,J.Isolation, Production And Optimization Of Siderophore Producing *Pseudomonas* From Paddy Soil. *Int. J. Pharm. Res. Sci.*2014 02(1), 71-88.

Steenhoudt, O and Vanderleyden, J. 2000. *Azospirillum* a free-living nitrogen-fixing bacterium closely associated with grasses: genetic, biochemical and ecological aspects. *FEMS Microbiology Review*. 24: 487-506.

Stewart, W. D., Fitzgerald, G P and R H Burris. 1967. In situ studies on N2 fixation using the acetylene reduction technique. *Proc Natl Acad Sci U S A*. 58(5): 2071–2078.

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

Sudha, M., Shyamala, R.G., Prabhavathi, P., Astapriya, P., Yamuna, D.S and Saranya, A. 2012. Production and optimization of indole acetic acid by indigenous microflora using agro waste as substrate. *Pakistan Journal of Biological Sciences*. 15(1): 39-43.

Sundara, B., Natarajan, V and Arik. 2002. Influence of phosphorus solubilising bacteria on the changes in soil available phosphorus and sugarcane and sugar yields. *Field Crop Research.* 43-49.

Sunita, G., Rathi, M.S., Kaushik, B.D., Nain, L and Verma, O.P. 2007. Survival of bio-inoculants on fungicides-treated seeds of wheat, pea, chickpea and subsequent effect on chickpea yield. *Journal of Environmental Science and Health*. 42 (6): 663-668.

Supraja, Y., Reddy, R.S., Reddy, S.S and Rani, Ch.V.D. 2011. Plant growth promotion and biocontrol properties of local isolates of fluorescent Pseudomonads. *Journal of Research ANGRAU.* 39(3): 1-5.

Susana, B. R., Nicolas, A., Lorena, B. G., Javier, A. A, Carlier, E., Veronica, V., Bergesse, J and Rovera, M .2009. Efficacy of *Pseudomonas chlororaphis* subsp. *aurantiaca* SR1 for Improving Productivity of Several Crops. *Crop Production Technologies.* .199-210.

Swati Yadav, Juhi Yadav and Singh,S.G. 2011. Performance of *Azospirillum* for Improving growth, yield and yield attributing characters of maize (*Zea mays* L.) in Presence of Nitrogen Fertilizer. *Research Journal of Agricultural Sciences***.** 2(1): 139-141

Swedrzynska, D and Sawicka, A. 2000. Effect of Inoculation with *Azospirillum brasilense* on development and yielding of maize *(Zea mays sp. saccharata* L.) under different cultivation conditions. *Polish Journal of Environmental Studies.* 9(6): 505-509.

Tailor,J and Joshi,B.H. Characterization and optimization of siderophore production from *pseudomonas fluorescens* strain isolated from sugarcane rhizosphere. *Journal of Environmental Research and Development.* 6 (3): 688-694.

Thanuja, L and Ambika, S.R. 2010. Effect of bacterization of finger millet grains with the PGPRs isolated from the rhizoplane of *Holostemma ada-kodien* schutles on its germination and initial growth. *Advanced Studies in Biology*. 2(2): 89-97.

Todar,K.,2004.http://www.textbook of Bacteriology. net/*Bacillus*.html.304.

Tilak, K.V.B.R., Sigh, C.S., Roy, N.K. and Subba Rao, N.S.1982. *Azospirillum brasilense* and *Azotobacter chroococcum* inoculum effect on yield of maize *(Zea mays*), and sorghum (*Sorghum bicolor*). *Soil Biology and Biochemistry*.14: 417–418.

Upadhyay, A and Srivastava, S. 2010. Evaluation of multiple plant growth promoting traits of an isolate of *Pseudomonas fluorescens* strain Psd. *Indian Journal of Experimental Biology*. 48: 601-609.

Usha Rani, M., Arundhathi and Reddy, G. 2012. Screening of rhizobacteria containing Plant growth promoting (PGPR) traits in rhizosphere soils and their role in enhancing growth of pigeon pea. *African Journal of Biotechnology*. 11(32): 8085-8091.

Van, D. K and Nelson, E.B. 1998. Inactivation of seed exudates stimulants of *pythium ultimum sporangium* germination by biocontrol strains of *Enterobacter cloacae* and other seed associated bacteria. *Soil Biology and Biochemistry.* 30: 183-192.

Vela,G.R and wyss, O. 1964. Improved stain for visualization of *Azotobacter* encystment. *Journal of Bacteriol*ogy. 87:476–477.

Verma, J.P., Yadav, J and Tiwari, K.N. 2010. Application of *Rhizobium* sp.BHURCO1 and Plant growth promoting rhizobacteria on nodulation, plant biomass and yields of chickpea (*Cicer arietinum L.*). *International Journal of Agricultural Research.* 5(3): 148-156.

Vijay krishna kumar, K., Reddy, M.S., Kloepper, j.w., Reddy , R.S.Kr., Lawrence, K.S., Zhou ,X.G., Sudini ,H., Miller,M.E., Appa rao,P., Surendranatha reddy, E.C., Niranjana, S.R and Chandra nayaka. 2012. Efficacy of Bacillus subtilis MBI 600 Against Sheath Blight Caused by Rhizoctonia solani and on Growth and Yield of Rice. *Rice Science.*19(1): 55−63.

Vimal Ramani and H. H. Patel, 2011. Phosphate solubilization by *Bacillus sphaericus* and *Burkholderia cepacia* in presence of pesticides. *J of Agri. Tech*. 7(5): 1331-1337.

Vincent, J.M. 1970. A manual for the practical study of the root nodule bacteria. Blackwell Scientific publications. *Oxford and Edinburgh.* 1-3.

Vlassak, K.L., Van, H and Duchateau, L. 1992. Isolation and characterization of *fluorescent Pseudomonas* associated with the roots of rice and banana grown in Srilanka. *Plant and soil.* 145: 51-63.

Wahyudi, A. T., Astuti, R. P, Widyawati, A., Meryandini, A and Nawangsih, A.A. 2011. Characterization of *Bacillus* sp. strains isolated from rhizosphere of soybean plants for their use as potential plant growth for promoting Rhizobacteria. *Journal of Microbiology and Antimicrobials.* 3: 34-40.

Womble. D. D. 2000. The Wisconsin package of sequence analysis programs methods. *Molecular Biology*. 132: 3-22.

Yadav, J., Verma, J. P and Tiwari, K. N. 2010. Effect of plant growth promoting rhizobacteria on seed germination and plant growth Chickpea (*Cicer arietinum L.*) under *in vitro* conditions. *Biological Forum - An International Journal*. 2(2):15-18.

Yasmin, H., Bano, A and Samiullah. 2013. Screening of PGPR isolates from semi-arid region and their implication to alleviate drought stress. *Pakistan Journal of Botony*. 45: 51-58.

Yasari., E and Patwardhan, A.M. 2007. Effects of *Aztobacter* and *Azospirillium* inoculations and chemical fertilizers on growth and productivity of Canola. *Asian Journal of Plant Sciences*. 6 (1):77-82.

Yazdani, M., Bahmanyar, M. A., Pirdashti, H., Esmaili M.A . 2009**.** Effect of phosphate solubilization microorganisms and plant growth promoting rhizobacteria on yield and yield components of corn. *International Journal of Biological and Life Sciences*. 1: 2-6

Yeole, R.D and Dube, H.C. 2000. Siderophore mediated antibiosis of rhizobacterial *fluorescence Pseudomonads* against certain soil borne fungal plant pathogens. *Journal of Mycology and Plant Pathology*. 30: 333-338.

Zaidi, A., M.S., Khan and M. Aamil. 2004. Bioassociative effect of rhizospheric microorganisms on growth, yield and nutrient uptake of greengram. *Journal of Plant Nutrition*. 27: 599-610.

APPENDIX I

Composition of different growth media/reagents/indicators used

1. Glucose Broth

Peptone	5.00 g
Beef extract	3.00 g
Glucose	5.00 g
Distilled water	1000 ml
Bromo Cresol Purple (BCP) Solution	15 ml
pH	7.0 ± 0.2

2. Glucose Peptone Agar

Glucose	5.00 g
Peptone	10.00 g
Agar	15.00 g
Distilled water	1000 ml
pH	7.0 ± 0.2

3. **Hofer's Alkaline Medium**

Yeast extract	1.00 g
Mannitol	10.00 g
Dipotassium hydrogen phosphate	0.50 g
Magnesium Sulphate	0.20 g
Sodium chloride	0.10 g
Thymol blue	0.016 g
Distilled water	1000 ml
pH	11.0

4. Jensen's agar medium

Calcium hydrogen phosphate	1g
Dipotassium hydrogen phosphate	0.2g
Magnesium Sulphate	0.2g
Sodium chloride	0.2g
Ferric chloride	0.1g
Trace element solution	1ml
Agar	15g
pH	6.8 ± 0.2

5. **Lactose Agar Medium**

Lactose	10.00 g
Yeast extract	1.00 g
Dipotassium hydrogen phosphate	0.50 g
Magnesium Sulphate	0.20 g
Sodium chloride	0.10 g
Calcium carbonate	3.00 g
Agar	20.00 g
Distilled water	1000 ml
pH	7.0

6. MR – VP broth

Buffered peptone	7.00 g
Dextrose	5.00 g
Dipotassium hydrogen phosphate	5.00 g
Distilled water	1000 ml
pH	6.9 ± 0.2

7. Nutrient Agar

Peptone	5.00 g
Beef extract	3.00 g
Sodium chloride	5.00 g
Agar	18.00 g
Distilled water	1000 ml
pH	6.8 – 7.2

8. Nutrient Gelatin Agar

Peptone	5.00 g
Beef extract	3.00 g
Gelatin	120.00 g
Distilled water	1000 ml
pH	6.8 – 7.0

9. N – Free sodium malate medium

Mallic acid	5g
Dipotassium hydrogen phosphate	0.5g
Manganese sulphate	0.01g
Magnesium Sulphate	0.2g
Ferrous sulphate	0.5g
Sodium chloride	0.1g
Potassium chloride	4g
Bromo thymol blue	0.002g
Sodium molybdate	0.002g
Calcium chloride	0.02g
Agar	15g
pH	6.8 ±0.2

10. Pikovskaya’s Agar

Yeast extract	0.50 g
Dextrose	10.00 g
Calcium phosphate	5.00 g
Ammonium sulphate	0.50 g
Potassium chloride	0.20 g
Manganese sulphate	0.0001 g
Magnesium Sulphate	0.10 g
Ferrous sulphate	0.0001 g
Agar	20.00 g

Distilled water	000 ml
pH	7.0

11. Plate Count Agar

Casein enzymic hydrolysate	5.00 g
Yeast extract	2.00 g
Dextrose	1.00 g
Agar	0.00 g
Distilled water	1000 ml
pH	7.0 ± 0.2

12. Potato Dextrose Agar

Potato infusion	200.00 g
Dextrose	20.00 g
Agar	18.00 g
Distilled water	1000 ml
pH	5.6 ± 0.2

13. Potato infusion agar

Potato infusion	200g
Peptide digest of animal tissue	10g
Beef extract	5g
Dextrose	10g
Sodium chloride	5g
Agar	15g
pH	6.8 ±0.2

14. Simmons Citrate Agar

Magnesium Sulphate	0.20 g
Ammonium dihydrogen phosphate	1.00 g
Dipotassium phosphate	1.00 g
Sodium citrate	2.00 g
Sodium chloride	5.00 g
Bromothymol blue	0.08 g
Agar	18.00 g
Distilled water	1000 ml
pH	6.8 ± 0.1

15. Starch Casein Agar

Starch	10.00 g
Casein powder	1.00 g
Agar	18.00 g
Distilled water	1000 ml
pH	7.2 ± 0.2

16. Yeast Extract Mannitol Congored Agar

Yeast extract	1.00 g
Mannitol	10.00 g
Dipotassium hydrogen phosphate	0.50 g
Magnesium Sulphate	0.20 g
Sodium chloride	0.10 g
Congo red	0.025 g
Agar	20.00 g
Distilled water	1000 ml
pH	6.8 – 7.0

17. Yeast Extract Mannitol Broth

Yeast extract	1.00 g
Mannitol	10.00 g
Dipotassium hydrogen phosphate	0.50 g
Magnesium Sulphate	0.20 g
Sodium chloride	0.10 g
Calcium carbonate	1.00 g
Distilled water	1000 ml
pH	6.8 – 7.0

18. Pseudomonas Agar (For Fluorescein)

Pancreatic digest of casein	10 g
Peptide digest of animal tissue	10 g
Anhydrous dibasic potassium phosphate	1.50 g
Magnesium sulphate	1.50 g
Agar agar	15.0 g
pH	7.0

19. Peptone water

Peptic digest of animal tissue	10g
Nacl	5g
Distilled water	1000ml

20. Nitrate Broth

Peptone	5.0 g
Beef extract	3.0 g
Potassium nitrate	5.0 g
Water	1000 ml
pH	6.8-7.0

21. King’s B agar medium

Peptone	16.0 g
K2HPO4	1.6 g
MgSO4	1.6 g
Glycerol	10.0 g
Agar	18.00 g
Distilled water	1000 ml

22. Gram Stain Solutions

a) Crystal violet solution

Crystal violet	10.0 g
Ammonium oxalate	4.0 g
Ethanol	100 ml
Distilled water	1000 ml

b) Gram's Iodine solution

Iodine	1.0 g
Potassium iodide	2.0 g
Ethanol	25 ml
Distilled water	100 ml

c) Counter stain

2.5% safranin in ethanol	10 ml
Distilled water	100 ml

d) Ethyl alcohol (Decolouriser)

Ethanol	95 ml
Distilled water	5 ml

23. Salkowski Reagent

35% perchloric acid	50 ml
0.5M $FeCl_3$	1 ml

APPENDIX II

BUFFERS AND STOCK SOLUTIONS

DNA Extraction Buffer

2 % (w/v) CTAB (Nalgene)	10g
100 Mm Tris HCl, pH 8.0 (pH8.0)	100 ml of 0.5 M Tris HCl
20 mM EDTA, pH 8.0	20 ml of 0.5 M EDTA (pH 8.0)
1.4 M NaCl	140 ml of 5 M NaCl
PVP (Sigma)	200 mg

All the above ingredients except CTAB were added in respective quantities and final volume was made up to 500ml with double distilled water, the solution was autoclaved. The solution was allowed to attain room temperature and 10g of CTAB was dissolved by intense stirring, stored at room temperature.

EDTA (0.5M)

Dissolved 186.1 g of disodium EDTA .2 H_2O in 800 ml of H_2O and stirred using magnetic stirrer and adjusted pH to 8.0 with NaOH

Ethidium Bromide

Stock 20 mg/ml can be prepared by dissolving 1 gm of ethidium bromide in 50 ml of water.

RNase: (20 mg/ ml)

20 mg of RNase (Sigma) was dissolved in 500 µl of double distilled water + 500 µl of 50% Glycerol (Qualigens) and the solution was heated at 95˚C for 10 min and stored at -20˚C.

TAE Buffer (pH 8.0)

For 10X stock solution

400 mM Tris base

200 mM Glacial acetic acid

10 mM EDTA

Dissolve in appropriate amount of sterile water.

TBE (electrophoresis buffer- pH 8.4): To prepare a 10X solution 54g Trizma base, 22.5g of Boric acid along with 20ml of EDTA (0.5M, pH 8.0) IN 500 ml of water. For running the gel, 10X is made to 1X solution.

TE buffer (pH 8.0)

10 mM Tris HCl

1 mM EDTA

6X Gel loading buffer

0.25 % (w/v) bromophenol blue

0.25 % (w/v) xylene cyanol

40 % (w/v) sucrose in water

10 X PCR buffer

500 mM KCl and 100 mM Tris-Cl. pH 8.0