STUDIES ON THE PHYLLOSPHERE MICROORGANISMS

Thesis submitted in part fulfllment of the requirements for the degree of **Master of Science (Agriculture)** in Agricultural Microbiology to the Tamil Nadu Agricultural University, Coimbatore

By

N.VIGNESHWARAN

I.D.No. 97-605-011

DEPARTMENT OF AGRICULTURAL MICROBIOLOGY
AGRICULTURAL COLLEGE AND RESEARCH INSTITUTE
TAMIL NADU AGRICULTURAL UNIVERSITY
COIMBATORE – 641 003

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CERTIFICATE

This is to certify that the thesis entitled **"STUDIES ON THE PHYLLOSPHERE MICROORGANISMS "** submitted in part fulfilment of the requirement for the degree of **MASTER OF SCIENCE (AGRICULTURE) IN AGRICULTURAL MICROBIOLOGY** to the Tamil Nadu Agricultural University, Coimbatore, is a *bona fide* research work carried out by Mr.**N.VIGNESHWARAN** under my supervision and guidance and that no part of this thesis has been submitted for the award of any other degree, diploma, fellowship or other similar titles and that the work has not been published in part or full in any scientific or popular journal or magazine.

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(Dr.T.NATARAJAN)
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Approved by

Chairman :

(Dr.T.NATARAJAN)

Members :

(Dr.G.PADMANABHAN)

(Dr.K.ILAMURUGU)

Date: 12.7.99

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N. Vigneshwaran.

(N.VIGNESHWARAN)

ABSTRACT

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N.VIGNESHWARAN

Degree : Master of Science (Agriculture)
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Chairman : **Dr.T.NATARAJAN**
Professor of Agricultural Microbiology

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The studies on phyllosphere microorganisms of selected agricultural crops *viz.*, rice, wheat, maize, soybean, cowpea, groundnut and cotton revealed the domination of bacteria over actinomycetes and fungi on leaves. The population of nitrogen fixing bacteria on the leaf surface varied from 8.0 to 50.0 per cent of the total heterotrophic bacteria. Among the various isolates, *Bacillus polymyxa, B.macerans* and *Pseudomonas* sp. showed maximum nitrogenase activity and also secreted more plant growth promoting substances like indole acetic acid and gibberellic acid.

Among the plant growth promoting phyllosphere (PGPP) bacterial isolates, gram negative PGPP 15 isolate from groundnut solubilised the insoluble phosphate by secreting more organic acids. The PGPP bacteria also exhibited variation in their natural resistance to antibiotics like

chloramphenicol and streptomycin. *Pseudomonas* sp. was highly resistant to chloramphenicol and streptomycin upto the concentration of 200 ppm than any other bacteria.

Under pot culture condition, foliar inoculation of *Bacillus macerans* significantly increased the growth and yield (15.50 % over control) of rice (Var. CO 43) apart from increasing total chlorophyll, nitrogen and phosphorus contents. The foliar inoculation of *Bacillus macerans* also increased the growth and yield of cotton (var. LRA 5166).

The field trial on soybean (var. CO 1) revealed that the foliar application of *Bacillus polymyxa* (PGPP 8) increased the grain yield by 32.23% over control besides increasing the total chlorophyll, nitrogen and phosphorus contents of soybean plants. The studies on the phyllosphere population of foliar inoculated bacteria showed higher population immediately after leaf spray followed by quick decline.

The phyllosphere bacterium *Pseudomonas* sp. (PGPP 1) was found to be antagonistic against the pathogens *viz.*, *Xanthomonas oryzae* and *Fusarium oxysporum*; whereas *Bacillus polymyxa* (PGPP 8) was antagonistic to *Helminthosporium oryzae* and *F.oxysporum*. *B.macerans* (PGPP 20) inhibited the growth of *X.oryzae* indicating their beneficial effect of controlling plant diseases.

Botanic pesticides like neem oil was found to be less harmful to the phyllosphere bacteria whereas synthetic pyrethroides and organophosphorus compounds are toxic to them. Cypermethrin inhibited all the three phyllosphere bacteria at its recommended level.

The survival study of PGPP bacterial inoculants showed that lignite is an ideal carrier material for inoculant production of PGPP bacteria *viz.*, *Pseudomonas* sp., *Bacillus polymyxa* and *B.macerans*. It supported a population of more than 10^8 cells/g even after 90 days of storage.

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INTRODUCTION

1. INTRODUCTION

Plant surfaces are the most attractive sites for the activity of various types of microorganisms such as saprophytes, parasites and symbionts. The micro-environments on the surfaces of various plant parts are described by the suffixes '-sphere' or '-plane'. Depending on the place of attachment, terminologies such as rhizosphere (Hiltner, 1904); rhizoplane (Clark, 1949), phyllosphere (Last, 1955; Ruinen, 1956); phylloplane (Last and Deighton, 1965) and spermosphere (Verona, 1958) were coined. The occurrence of extensive populations of microbial epiphytes on aerial living plant surfaces has inevitably led to the speculations about their ability to withstand the various climatic extremes to which they are exposed. In addition to the usual problems of nutrient supply, competition and antagonism, the phyllosphere microorganisms have to withstand drastic and repeated fluctuations in radiation levels, windspeed, temperature, humidity and various agricultural practices to which the leaf surface is exposed.

Studies on the role of non-pathogenic phyllosphere microorganisms was a neglected milieu, due to difficulty in experimentation. The realization in recent years that a good number of nitrogen fixing and plant growth promoting bacteria are harboured on the leaf surfaces and contribute substantially to the nitrogen economy and growth of the plant, stimulated the interest in the study of nonparasitic foliar microorganisms (Jones, 1976).

Leaf diffusates and exudates have been reported to encourage growth and multiplication of the phyllosphere microorganisms. Any change in the phyllosphere microbial activity of the host alters its physiological activity and the rhizosphere microflora, in turn the growth and productivity of the plants.

The nitrogen fixing phyllosphere microorganisms will be eco-friendly and cost effective. The saprophytes thriving in the phyllosphere using leaf exudates are the potential competent for the survival of leaf pathogens. Like plant growth promoting rhizobacteria (PGPR), plant growth promoting phyllobacteria (PGPP) are also reported to exist on the leaf surface by leading saprophytic/symbiotic life in the phyllosphere (Ruinen, 1970). These microorganisms may enhance the growth and yield of crop plants by secreting the growth promoting substances required for the plants.

Though complex, the phyllosphere is a valuable area in the hands of agricultural microbiologists to manipulate and influence the plant-microbe interrelationships to get higher returns from crop plants. With the above stated view, the present research work was carried out with the following objectives :

- To identify the microorganisms occurring on the phyllosphere of selected agricultural crops *viz.*, rice, wheat, maize, soybean, cowpea, groundnut and cotton.

- To isolate efficient strains of nitrogen fixing bacteria occurring on the phyllosphere.

- To identify the phyllosphere microorganisms secreting plant growth promoting substances like indole acetic acid (IAA) and gibberallic acid (GA) and also solubilising insoluble phosphates.

- To study the effect of inoculation of nitrogen fixing and plant growth promoting phyllosphere (PGPP) bacteria on growth and yield of cultivated crops like rice, cotton and soybean.

- To study the antagonistic activity of phyllosphere bacteria against the plant pathogenic microorganisms.

- To understand the interaction of phyllosphere microorganisms with pesticides.

- To asses the survival of phyllosphere microorganisms in lignite based inoculant preparation.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The term "PHYLLOSPHERE" was proposed independently by Last (1955) and Ruinen (1956) to indicate the micro-environment conditioned by the leaf. Ruinen's concept of the phyllosphere microorganisms included both pathogenic and saprophytic forms, whereas Last considered only the non-parasitic flora on the leaf surface as phyllosphere microorganisms.

2.1 PHYLLOSPHERE MICROORGANISMS

Ruinen (1961) reported that the phyllosphere of various trees were dominated by oligonitrophilic and nitrogen fixing organisms like *Beijerinkia, Azotobacter, Aerobacter, Pseudomonas,* and *Spirillum.*

Lactic acid bacteria were the dominant phyllosphere bacteria of tree leaves than that of proteolytic bacteria. Among fungi, species of *Aspergillus viz., A.niger* and *A.sydowii*, dominated the phyllosphere followed by *Alternaria* and *Curvularia* (Prasad and Edward, 1975).

The microorganisms forms a continuous layer on the leaves and shows considerable variation in thickness and species composition on the upper and lower surface. Pigmented bacteria were dominant on the leaf surface by their tolerance to ultraviolet (UV) light falling on the leaf surface (Good fellow and Austin, 1976).

Occurrence of epiphytes as the descendents of bacteria that immigrated to plants as aerosols with special adaptations to survive on leaf surface was observed by Lindemann and Upper (1985).

Romanovskaya *et al.* (1996) reported the pink pigmented facultative methanol using bacteria (PPFM-bacteria) *viz.*, *Methylobacterium extorquens*, *M. mesophilicum* and *M. fujiswaense* as the permanent inhabitors of phyllosphere of agricultural, drug and ornamental plants in Ukraine.

Behrendt et al. (1997) reported that the heterotrophic bacteria of grass phyllosphere was dominated by the genera *Pseudomonas, Clavibacter, Curtobacterium, Pantoea* and *Sterotrohomonas.*

2.2 NITROGEN FIXATION IN PHYLLOSPHERE

There is considerable evidence that atmospheric nitrogen is actually fixed on the leaf surface, although, the quantum of fixation varied considerably, depending on the leaf surface bacteria, type of plants and experimental conditions. In non-nodulated plants like *Coprosma robusta* and *Prunus armeniaca*, $^{15}N_2$ incorporation was observed in leafy shoots suggesting the occurrence of nitrogen fixing microorganisms (Stevenson, 1959).

Bhurat (1969) isolated the nitrogen fixing bacteria *viz., Pseudomonas atrofaciens, P. seminum, Cellulomonas galba, C. cellasea* and *Achromobacter*

iophagus from wheat leaves and *A. iophagus*, *A. xerosis*, *P.calcis*, *C.gola* and *Bacillus licheniformis* from pea leaves. The amount of nitrogen fixed varied from 7.6 to 13.4 mg g^{-1} of sucrose consumed by wheat isolates and from 6.9 to 15.0 mg g^{-1} of sucrose consumed by pea isolates.

Jones (1976) showed nitrogen fixation in Douglas fir (*Pseudotsuga douglasii*) leaves both in laboratory and field conditions by $^{15}N_2$ tracer methods. Ruinen (1970) suggested that the sheath region, being a closed system, seems to be an ideal environment for nitrogen fixers as they are not exposed to diurnal wetting and drying cycle and supposedly least affected by the photosynthetically generated oxygen.

Bessems' (1973) investigations on maize (*Zea mays*) and Guatemala grass *(Tripsacum laxum)* by acetylene reduction activity (ARA) and $^{15}N_2$ studies confirmed the nitrogen fixation by phyllosphere microflora. *Klebsiella spp.* occurring on leaf sheath fixed 260 to 400 kg N_2 ha^{-1} $year^{-1}$ and high C/N values in sheath water of guatemala grass favoured nitrogen fixation.

Surface counts of *Azotobacter* and *Beijerinckia* cells on cacao leaves were reported to be about 1 x 10^7 per cm^2 (Ruinen, 1974). Association of *Azotobacter* with slime formed around bases of *Eichhornia crassipes* has been reported by Purchase (1977).

Poor nitrogen fixation, (0.7 to 1.4 kg N_2 ha^{-1} $year^{-1}$) by epiphylls on coffee leaves was attributed to heavy nitrogen fertilization (Roskoski, 1980). Ito *et al.* (1980) also of the same opinion that nitrogen fixation occurred only when the plant suffered extreme nitrogen deficiency. They observed the lower portions of rice culm as an active sites of nitrogen fixation.

The nitrogen contribution made by the phyllosphere nitrogen fixing microorganisms of various plants from temperate latitudes was estimated to be 0.1 to 10 per cent of the total plant requirement for nitrogen (Sadykov and Umarov, 1980).

Murty (1983) demonstrated nitrogen fixation (ARA) on leaves of tropically grown sugarcane, sorghum, ragi, bamboo, mulberry and cotton, by naturally occurring *Beijerinckia* sp. He estimated 1.6 to 3.2 kg nitrogen ha^{-1} fixed during the entire growth period of cotton and observed no correlation between the rate of nitrogen fixation and types (C3 or C4) of plant species.

Murty (1984a) also observed more nitrogen fixation in cotton varieties having trichomes on leaves. The efficiency of *Beijerinckia* isolated from cotton was ten times lesser than the standard *Azotobacter* species, in terms of total nitrogen fixed per unit of carbon consumed.

The population density of *Beijerinckia* on leaves of cotton and rice was very low (10^3 cells/cm^2). Acetylene reduction activity (ARA) in leaf sheath of

rice was higher than the leaf surface but no positive correlation could be made between the *Beijernckia* population and ARA (Murty, 1984b)

Chaudhury and Sengupta (1991) estimated the nitrogen fixed by *Azospirillum lipoferum* and *Azotobacter* sp. isolated from leaves of *Avicennia officinalis* to be of 50 and 38 mg nitrogen l^{-1} of medium in seven days, respectively.

Brighigna *et al.* (1992) isolated and characterised the nitrogen fixers from phyllosphere of twelve species of *Tillandsia* from different Mexican habitats and observed that only *Bacillus megaterium* reduced acetylene in pure culture.

2.3 ISOLATION OF PHYLLOSPHERE MICROORGANISMS

Ruinen (1961) demonstrated the selective culturing of the free living phyllosphere diazotrophs like *Beijernckia* and *Azotobacter* in nitrogen free media.

Beech and Davenport (1971) suggested the four direct and indirect method of examination of leaf surface for quantitative estimations of yeast flora of apple and grape leaves.

Sharma (1974) used dilution plate and moist chamber techniques as the most reliable methods for getting quantitative and qualitative information of plant surface microorganisms, respectively.

Morris (1985) compared several techniques for removal of bacterial epiphytes and found that sonication of bean leaflets for twelve min removed more bacteria than other methods.

Helander and Rantio-Lehtimaki (1990) enumerated the phyllosphere microorganisms using leaf disc (5 mm diameter) culture plating method, whereas Ercolani (1991) estimated the phylloplane bacterial populations by leaf washing method.

Vijayakumar and Narasimham (1991) isolated the phylloplane bacteria of *Arachis hypogea* by leaf washing method and expressed the bacterial numbers per g of leaf.

Michereff *et al.* (1994) isolated the epiphytic bacteria of yam leaf by leaf washing and serial dilution method. Following direct and indirect techniques as such or with modifications were used to study the phyllosphere microorganisms by various workers:

	Techniques	Authors
Direct Method		
1.	Direct observation and Impression films	Ruinen (1961)
2.	Scanning microscopy	Pugh and Buckley (1971)
Indirect Method		
3.	Spore fall	Last (1955)
4.	Damp chamber (2 days)	Webster (1956)
5.	Plating and Damp chamber (7 days)	Ruinen (1961)
6.	Leaf washing	Kendrick and Burges (1962)
7.	Leaf maceration	Hislop and Cox (1969)
8.	Leaf impression	Lamb and Brown (1970)

2.4 METHODS AND TIME OF INOCULATION OF PHYLLOSPHERE MICROORGANISMS

Maiti and Sen (1990) adopted the foliar spray of *Rhizobium* cells in suspension with phosphate buffer, sucrose (0.1%) and citrate (0.1%) to inoculate soybean, lentil and pigeon pea.

Foliar inoculation of *Pseudomonas viridiflava* on tomato was made by spraying all plant surfaces to run off with a garden compression sprayer at a pressure of 1.8 - 2.0 kg after 3 weeks of planting. Inoculation at late afternoon will prevent the cell desiccation (Mariano and McCarter, 1993).

Michereff *et al.* (1994) suggested the foliar inoculation of yam with fungal (2 x 10^5 conidia/ml) and bacterial (10^8 cfu/ml) suspension in water amended with 0.05% Tween 80.

Belanger (1994) tried the foliar inoculation of fungi with five-day-old cultures containing 5x10^8 cfu/ml diluted in tap water to have 1 x 10^6 cfu/ml. Before use few drops (0.02%) of the surfactant Aqua-aid was added to the suspension for uniform distribution.

The cell pellet (at stationary phase) of *Pseudomonas fluorescens* suspended in 0.1M $MgSO_4$ was sprayed on wheat leaves by Fernando *et al.* (1994).

2.5 POPULATION DYNAMICS OF PHYLLOSPHERE MICROORGANISMS

Phyllosphere microorganisms are heavily influenced by type of host plant and environmental factors. The surface characteristics of leaves like wax deposits, silicification, venation and distribution of trichome may ultimately determine their suitability for trapping the microbial propagules (Ruinen, 1961).

Leben (1965) found that the depressions in epidermis favoured bacteria accumulation due to greater leaf exudation.

In the phyllosphere, bacteria and yeasts develop exponentially on newly unfolded leaves till a steady state is reached; sometimes the population density declines at the end of the season (Fokkema, 1971).

Many physical and environmental factors, such as temperature and relative humidity, can vary greatly in time spans of less than one minutes on leaf surfaces which inturn influence the phyllosphere organisms (Burrage, 1976).

In general, proportions of the total community comprised of filamentous fungi increased towards the latter part of the growing season, while yeasts and bacteria were relatively more numerous earlier in community development (Dickinson, 1976).

Shekhawat and Chakravarti (1977) reported that maximum population of epiphytic bacteria on surface of chilli leaves was found at the end of April and least in first week of June. Actinomycetes were rarely found in April and May however, a considerable number was observed at the end of June.

Buds might have a considerable population of microorganisms, which partly maintained itself on the developing leaves (Parbery *et al.*, 1981).

Leaf surface colonization observed as a variable of time, involves the following factors: trapping time, incubation time, chaining substrate as the leaf ages, pollen and dust deposition and changing weather conditions (Frossard, 1981).

Extracellular polysaccharides secreted by many epiphytic bacteria not only gives anchor to them but also modify their environment for better survival (Fett *et al.*, 1989).

The phylloplane is a dynamic environment with cyclic and noncyclici environmental variables, including temperature, relative humidity, dew, rain, wind and radiation. These factors alter the microorganisms very rapidly (Andrews, 1992).

2.6. INTRINSIC ANTIBIOTIC RESISTANCE OF PHYLLOSPHERE BACTERIA

The introduced phyllosphere bacteria has to compete with the existing population in the leaves. Serological typing and antibiotic marker techniques are generally used for differentiating the introduced strains from native strains.

Hopwood (1970) reported, that high resistance can be found as a result of single mutation to an antibiotic and when other things being equal, these are the compounds of choice as markers.

Andrews and Kenerley (1980) tagged the apple scale antagonist *Chaetomium globosum* with benomyl resistance (Ben^r) and studied its ability to persist on apple leaves. Fokkema (1983) used a benomyl resistant strain of *Sporobolomyces roseus* in an attempt to demonstrate natural biological control on wheat leaves.

Lindow (1983) studied the antagonists marked with streptomycin or rifampicin resistance against ice-nucleating bacteria on pear leaves.

Lin *et al.* (1984) reported that natural resistance to antibiotics in bacteria is commonly carried on plasmids (R-factors) rather than resulting from spontaneous mutation of the bacterial chromosome.

Lindemann (1985) tagged near-isogenic lines of *Pseudomonas syringae* pv. *tomato* with kanamycin or rifampicin resistance and subjected them to reciprocal competition studies on tomato leaves.

Rifampicin - resistance mutants of seven phyllosphere bacteria were developed by Mussaon *et al.* (1995) to study the efficiency of delivery systems into cotton.

2.7 PHOSPHATE SOLUBILISATION BY PHYLLOSPHERE BACTERIA

Pikovskaya (1948) made a pioneering attempt in isolating "Phosphorite", an organism capable of actively solubilising tricalcium phosphate (TCP) and coined the name 'Bacterium P'.

Solubilisation of insoluble phosphates by gram positive and gram negative rods and cocci shaped bacteria and several fungi including yeasts were reported by Ahmad and Jha (1968). The solubilisation of hydroxy apatite was maximum with *Aspergillus awamori* followed by *Pseudomonas striata* and *Bacillus polymyxa* (Arora and Gaur, 1978).

The phyllosphere bacterium *Bacillus megaterium* isolated from *Tillendsia* sp. was known to solubilise fixed form of phosphorus and also reduced the acetylene in pure culture (Brighigna *et al.*, 1992).

2.8 PRODUCTION OF PLANT GROWTH PROMOTING SUBSTANCES BY PHYLLOSPHERE BACTERIA

The formation of indole acetic acid (IAA) from tryptophan on leaf surfaces due to the actions of epiphytic microorganisms was reported by Libbert *et al.* (1996). Buckley and Pugh (1971) reported that some common plant fungi (*Cladosporium, Aureobasidium* and *Epicoccum*) produced auxin-like substances, but their effect on the plant was not known.

Varvaro and Surico (1984) reported that IAA production might have a role in the epiphytic fitness of *Pseudomonas syringae* pv. *savastanoi*. IAA - strains of *P.syringae* pv. *savastanoi* did not grow well as IAA+ strains on olive leaves. IAA production might have conferred changes in the plants that facilitated bacterial growth.

Auxin production by phyllosphere bacteria improved their habitats by increasing the rate of plant leaching and by local anatomical changes conducive for growth and survival (Loper and Schroth, 1986).

The excellent epiphytic nature of *Erwinia herbicola* on pear was attributed to its IAA production (Lindow, 1987).

2.9 CROP RESPONSE TO PHYLLOSPHERE BACTERIAL INOCULATION

Sharma and Mukerji (1976) reported that majority of epiphytic microorganisms are entirely neutral in their effect upon the host plant and their major role starts only after the onset of organ senescence.

Wani and Rai (1980) recorded 50 per cent yield increase and 88% increased leaf nitrogen content over control due to seed and foliage inoculation of sorghum with *Azotobacter* sp.

Pati and Chandra (1981) sprayed wheat at fortnightly intervals with suspensions of *Beijerinckia indica* and *Azotobacter chroococcum* and obtained significant yield increases of 70% over the unfertilized control, which was near to the yield obtained by normal nitrogen fertilization. Heat killed bacteria and the uninoculated medium had no effect on yield.

Sen Gupta and Sen (1982) compared the effect of spraying various isolates of *Klebsiella pneumoniae, Azotobacter* sp. and *Derxia gummosa* on

unfertilized wheat. Only *K.pneumoniae* increased the yield almost similar to that obtained with urea application (105 kg N ha^{-1}). SenGupta *et al.* (1982) also observed seven fold increase in yield of rice by spraying *K.pneumoniae*. Response to *Klebsiella* sp. inoculation was more with half the dose of fertilizer than the full dose.

Nandi *et al.* (1983) sprayed culture filtrates of *Klebsiella* sp. to get higher yield in cauliflower, cabbage, tomato, pumpkin, eggplant, potato, onion, radish and spinach. The absence of auxins, gibberellins or cytokinins in the culture filtrate confirmed the positive effect of nitrogen fixation by *Klebsiella* sp.

Sen *et al.* (1985) reported 39-680 per cent yield increase over uninoculated control in rice (var IR-8), 3-267 per cent yield increase in wheat (Sonalika) and 109-182 per cent yield increase in soybean (var. Bragg) due to foliar spray of diazotrophs.

Maiti and Sen (1990) reported that foliar spray of rhizobia, irrespective of cross inoculation groups, on the foliage of legume plants *viz.*, soybean, lentil and pigeon pea improved the growth and nitrogen content of the plants under laboratory condition. This beneficial effect was further increased to 30 per cent by supplementing the foliar spray with nutrients like sucrose and phosphate.

Ramarethinam *et al.* (1998) reported the diazotropic nature of *Bacillus subtilis* occurring on the phyllosphere of chilli crop. Foliar inoculation showed significant increase in net photosynthetic rate (NPR) and induced drought tolerance in host plants.

2.10 ROLE OF PHYLLOSPHERE BACTERIA IN THE CONTROL OF PLANT DISEASES

Swineburne (1981) showed that iron-chelating compounds (siderophores) in the phyllosphere inhibited the spore germination and appressoria formation of *Colletotrichum musae* and *Diaporthe perniciosa*.

Saprophytic bacteria are known to protect plants against bacterial diseases and certain phylloplane bacteria (PLB) have been shown to possess preinoculative protetive ability (PPA) for bacterial blight of cotton. The successful biological control of bacterial blight of cotton caused by *Xanthomonas campestris* pv. *malvacearum* by the PLBs *viz.*, *Flavobacterium* sp. *Aeromonas* sp. and *Pseudomonas* sp. was reported by Verma *et al.* (1983).

Spraying of common phyllosphere yeasts *Sporobolomyces roseus* and *Cryptococcus* was known to control *Colletotrichum graminicola* infection of maize leaves by preventing the appressorial penetration into the host (Williamson and Fokkema, 1985).

The antagonistic activity of *Erwinia herbicola* against the *Xanthomonas campestris* pv. *oryzae* was reported by Santhi *et al.* (1987). The

suppressive effect on the pathogen is due to its capability to lower the pH of the medium to levels unsuitable for the growth of *X.campestris* pv. *oryzae*.

The first report on the potential of yeast - like fungus (*Sporothrix flocculosa)* as a biocontrol agent of the wheat powdery mildew pathogen, *Erysiphe graminis* var. *tritici* was brought out by Hajlaoui and Belanger (1993).

Gliocladium roseum, Penicillum sp. and *Trichoderma viride* suppressed the conidiophores production of *Botrytis cinerea* by 97-100 per cent in strawberry leaves (Sutton and Peng, 1993).

The rice leaf-associated antagonistic *Pseudomonas aeruginosa* was isolated by *Rosales et al.* (1995) and known to inhibit the major rice pathogens *viz., Rhizoctonia solani, Pyricularia oryzae, Sarodadium oryzae* and *Fusarium moniliforme* in dual culture test.

Gould *et al.* (1996) demonstrated the biological control of *Botrytis cinerea* on petunia by the phylloplane bacteria using petal disk assay. This bacterium reduced the disease incidence by an average of 77 per cent on whole flowers inoculated with *B.cinerea* conidia in seven different trials.

2.11 INTERACTION OF PHYLLOSPHERE MICROORGANISMS WITH PESTICIDES

Bordeaux mixture caused a dramatic and prolonged reduction in the number of bacteria colonizing cherry leaves. Thiram seed treatment altered both the rhizosphere and the phylloplane microorganisms (Crosse, 1967).

Phyllosphere saprophytes are generally more tolerant to fungicides than the pathogens (Baker and Cook, 1974). The dose, frequency of application and persistence of toxic residues on plant surfaces obviously determine their effect on the miciroflora. Commercial pesticides are not screened for toxicity to non-pathogenic microorganisms (Hislop and Clifford, 1975). The older inorganic fungicides, e.g. copper and mercury compounds, have a broad spectrum of activity against phylloplane bacteria and fungi.

Foliar application of bavistin is known to increase the saprophytic bacteria and fungi both in the rhizophere and phylloplane, whereas number of pathogenic fungi decreased (Vijakumar and Narasimham, 1991).

Foliar application of propionazole (TILT 250 EC) had significant inhibitory effect on the filamentous fungi, especially on *Cladosporium*, whereas no significant effect was observed on yeasts and *Penicillium* sp (Elmholt, 1991).

Merilyn and Thomas (1991) reported that dithane M45 (100ppm) inhibited the growth and nitrogen fixation activity of *Beijerinckia* and bavistin had no such effect.

2.12 SURVIAL OF BACTERIA IN CARRIES USED FOR INOCULANT PRODUCTION

Among the various types of carriers, peat and lignite are extensively used for inoculant preparation of various biofertilizer organisms. The use of lignite amended with soybean powder as a carrier material for the *Rhizobium* sp. was suggested by Kandasamy and Prasad (1971).

Kloepper and Schroth (1981) reported that the dry powder inoculum of *Pseudomonas* sp. in talc powder with Xanthum gum was effective for seed tuber treatment of potato.

Gaur and Gaind (1984) reported that wood charcoal and soil mixture was found to be a good carrier material for the survial and maintenance of *Pseudomonas striat*a and *Bacillus polymyxa.* Favilli *et al.* (1987) observed high viability of *Azospirillum* sp. (10^9/g) in peat based inoculants upto 90 days of storage.

Narendranath (1995) recommended the use of lignite, alone or with various amendments like soymeal to increase the shelf life of groundnut rhizobial inoculant. Thangaraju (1996) recommended the use of decomposed coirpith with lignite or peat (1:1) for better survival of *Rhizobium* sp.

MATERIALS & METHODS

3. MATERIALS AND METHODS

3.1 COLLECTION OF LEAF SAMPLES

The leaf samples of selected agricultural crops *viz.*, rice, wheat, maize, soybean, cowpea, groundnut and cotton were collected at pre-flowering stage of the crop on non-rainy days in sterile polybags and taken to the laboratory immediately for the isolation of phyllosphere microorganisms.

3.2 ENUMERATION AND ISOLATION OF PHYLLOSPHERE MICROORGANISMS

Leaf samples were cut into 1.0 cm^2 pieces using sterile blade and 10 such pieces were placed in 100 ml sterile distilled water blank in 250 ml conical flask, shaken on a rotary shaker for 30 min at 100 rpm and serially diluted upto 10^{-4}. An aliquot of 0.1 ml of final dilution (10^{-4}) was spread on plates with nutrient glucose agar, Martin's rose bengal agar, Kenknight's agar and Jensen's N-free agar medium for bacteria, fungi, actinomycetes and nitrogen fixing bacteria respectively (Mariano and McCarter, 1993). Plates were incubated at 28°C in an incubator and the colonies formed were counted. Bacterial colonies growing on N-free medium were purified and transferred to N-free agar slants and stored under refrigerated condition until further use.

3.3 PRELIMINARY SCREENING OF PHYLLOSPHERE BACTERIAL ISOLATES FOR NITROGEN FIXATION BY ACETYLENE REDUCTION ACTIVITY (ARA) (Hardy *et al.*, 1968)

Jensen's nitrogen free agar slants were inoculated with the phyllosphere bacterial isolates and incubated at 28°C for 4 days. After good growth, the cotton plug was replaced with airtight serum stopper. Acetylene gas (10% by volume) was injected into the vial using a syringe after removing equal volume of air and incubated at 28°C in an incubator for 24h. From this 1.0 ml of gas sample was drawn and injected into gas chromatogram (Systronics MN 4010) fitted with Poropak column and FID detector for ethylene estimation. Similarly, the value for 1.0 ml standard ethylene gas was recorded. The bacterial cells on the slants were scrapped and collected in 2 ml of 2N NaOH and kept in a boiling water bath for 10 min. The sample was cooled, neutralised with 2 ml of 2N HCl and the total protein content was estimated by Bradford's method (Bradford, 1976).

The ethylene production was calculated using the following formula :

$$\text{nmoles of } C_2H_2 \text{ produced } h^{-1}\, mg^{-1} \text{ protein} = \frac{C \times P_s \times A_s \times V}{P_{std} \times A_{std} \times T \times P}$$

where,

C = Concentration of ethylene in the standard moles

P_S = Peak height of sample

A_S = Attenuation used for sample

V = Volume of airspace in assay vial (ml).

P_{std} = Peak height of standard

A_{std} = Attenuation used for standard

T = Time of incubation in h.

P = Protein content of bacterial growth in mg.

The ARA of authenticated strian of *Azotobacter chrococcum* (CZR1) and *Azospirillum lipoferum* (AZ 204) were estimated by growing the organism on Jensen's N-free agar medium and N-free malate semisolid medium, respectively.

3.4 SCREENING PHYLLOSPHERE BACTERIAL ISOLATES FOR THE PRODUCTION OF PLANT GROWTH PROMOTING SUBSTANCES

3.4.1 Extraction from culture filtrates (Tien *et al.*, 1979)

The organisms were grown in their respective media until good growth and centrifuged at 7700 rpm for 30 minutes. The cell free supernatants were collected and adjusted to pH 2.8 with 1N hydrochloric acid. To the culture filtrate in separating funnel, equal volume of peroxide free cold (4°C) diethyl ether was added, mixed and allowed to stand for 4 h at 4°C with intermittent shaking. The aqueous phase was separated from the organic phase and two more extractions were done at 4 h intervals and the fractions of organic phases were pooled and evaporated to dryness in the dark. The residue was

dissolved in 2.0 ml of distilled absolute methanol and used for bioassay and colorimetric assay.

3.4.2 Bioassay for plant growth promoting substances

3.4.2.1 Bioassay for indole acetic acid (IAA)

Rice root inhibition assay (Sircar and Chakraborty, 1957).

Five ml of autoclaved molten plain agar (3%) supplemented with 1.0 ml of culture filtrate was dispensed into 10.0 ml glass vials and allowed to solidify. Similarly, suitable control vials containing only plain agar and vials containing known concentration of IAA (25 to 10,000 ug/ml) were also maintained.

Rice seeds were surface sterilized with 0.1% mercuric chloride and washed well in sterile distilled water and then soaked in sterile distilled water for 24h at 28 $\pm$ 1°C in dark in an incubator. The soaked seeds were placed on moist filter paper in petri dish for another 24h in dark. The germinated seeds were transferred to IAA vials (2 seeds/vial) and allowed to grow for 48h in dark at 28$\pm$1°C. The mean root length after 48h was measured and compared with the control. The results were expressed as per cent inhibition over control. From a dosage response curve of IAA with known concentrations, IAA concentration in the sample was calculated.

3.4.2.2 Bioassay for gibberellic acid (GA)

Rice seedling test (Murakami, 1959).

The surface sterilized rice seeds were soaked for two days in sterile distilled water at room temperature. Sterile cotton of 3 mm thickness was placed at the bottom of petri dish (4 cm height) and to that 3 ml of culture filtrate was added and air dried. Uniformly germinated rice seeds (12) with 0.5 mm long coleoptiles were placed in each petri dish. To that 5.0 ml of sterile distilled water was added and the plates were incubated in light for 2 days, followed by a moist chamber incubation under light after adding further 3 ml of sterile distilled water.

After 5 days, the longest and shortest plants were rejected and the shoot length of other plants were measured from the mesocotyl to the second leaf sheath. From a dosage response curve with different concentrations of GA, the sample GA concentration was calculated.

3.4.3 Colorimetric assay

3.4.3.1 Spectrophotometric estimation of gibberellic acid (GA)

GA was estimated as per the method described by Holbrook *et al.* (1961).

Reagents required :

i. HCl (5% and 30%)

ii. Standard GA solution

iii. Zinc acetate solution (prepared by dissolving 21.9 g of zinc acetate in 80.0 ml distilled water and 1.0 ml glacial acetic acid. The final volume was made upto 100.0 ml with distilled water)

iv. Potassium ferrocyanide solution : Dissolved 106.0 g of potassium ferrocyanide in 100.0 ml distilled water.

Procedure

Fifteen ml of culture filtrate was taken in a test tube, to that, 2.0 ml of zinc acetate solution was added. After 2 min, 2.0 ml of potassium ferrocyanide solution was added. The content was centrifuged at low speed for 15 min. To 5.0 ml of supernatant solution, 5.0 ml of 30% HCl was added and incubated at 20°C for 75 min. The blank was treated with 5% HCl. The absorbance was measured at 254 nm in spectrophotometer. The concentration of GA in culture filtrate was calculated by comparing with standard curve prepared with known concentrations of GA.

3.4.3.2 Colorimetric estimation of IAA

IAA was estimated as per the method described by Gorden and Paleg (1957).

Reagents required :

a. Salper's reagent : One ml of 0.5 M FeCl3 dissolved in 50.0 ml of 35% $HClO_4$ (Freshly prepared)

b. IAA stock solution (100.0 ug/ml in 50% ethanol).

Procedure :

A loopful of bacterial culture was inoculated in 25.0 ml nutrient broth and incubated at 28°C on a rotary shaker. After 24h, the culture broth was centrifuged at 10,000 rpm for 15 min. To the 2.0 ml of supernatant, 3 drops of O-phosphoric acid was added followed by the addition of 4.0 ml of Salper's reagent and incubated for 25 min at room temperature. The absorbance was read at 530 nm in a colorimeter. Standard curve was prepared with various concentrations of pure IAA from which the IAA concentration in sample was calculated.

3.5 CHARACTERISATION OF PLANT GROWTH PROMOTING PHYLLOSPHERE (PGPP) BACTERIA

The phyllosphere bacterial isolates producing the plant growth promoting substances were characterized by morphological and biochemical tests.

3.5.1 Morphological Tests

Following morphological tests were carried out to characterize the PGPP bacterial isolates.

3.5.1.1 Bacterial shape

The bacterial shape was observed after simple staining with crystal violet.

3.5.1.2 Gram staining

The Gram's reaction was observed as per the method described by Barthomolew and Mittwer (1952).

3.5.1.3 Spore staining

The presence of endospore was observed by spore staining as per the method given by Aneja (1996).

3.5.1.4 Motility

The motility of bacteria was examined by hanging drop technique (Aneja, 1996).

3.5.2 Colony characters

The colony characters *viz.*, form, margin, elevation, size and colour were observed on agar medium and recorded.

3.5.3 Biochemical tests

Following biochemical tests were carried out as per the method described by Seeley *et al.* (1991).

3.5.3.1 Gelatin hydrolysis

The ability of the PGPP bacteria to produce gelatinase enzyme which liquefy the gelatin was tested. The cultures were inoculated into nutrient

gelatin deep tubes and incubated for 48h. Then, placed in a refrigerator at 4°C for 30 min and observed for gelatin liquification.

3.5.3.2 Starch hydrolysis

Cultures were streaked on nutrient agar plates containing 2% starch and incubated at 30°C. Hydrolysis of starch was tested using iodine solution. Plates were observed for the presence of clear zones surrounding the bacterial colonies.

3.5.3.3 Acid and gas production

The cultures were inoculated into broths with glucose, lactose and sucrose as carbon source along with Durham's tube and phenol red as indicator. After 48h of incubation, change of phenol red colour to yellow and presence of gas bubbles in Durham's tube were examined for acid and gas production, respectively.

3.5.3.4 Catalase activity

Catalase test was performed by adding H_2O_2 to agar slant culture. Release of free oxygen gas bubbles was a positive catalase test.

3.5.3.5 Voges-Proskauer test

The MRVP (Methyl-red and Voges-Proskauer) broth was inoculated with bacteria and incubated for 48h at 35°C. To that, 12 drops of V-P reagent I and 2 drops of V-P reagent II were added and shaken gently for

15 min. The positive reaction of acetyl methyl carbinol production was indicated by development of red colour.

3.5.3.6 Growth at 50°C and 60°C

Growth of PGPP bacteria at 50°C and 60°C was tested by incubating them in the incubator at desired temperature.

3.5.3.7 Growth in anaerobic agar

The bacteria were inoculated in anaerobic agar deep tubes and incubated and the presence of growth was recorded.

3.5.3.8 H_2S Production

Sulfide indole motility (SIM) agar deep tubes were stab inoculated with the bacteria and incubated at 35°C for 48h. Black colouration along the line of stab inoculation indicates the H_2S production.

3.6 PHOSPHATE SOLUBILISATION BY PGPP BACTERIA

The phyllosphere bacterial isolates were tested for phosphate solubilisation in the Sperber's hydroxy apatite medium as per the method described by Sperber (1958). Actively growing cultures were streaked on hydroxy apatite medium and incubated for 2 weeks. Bacterial growth surrounded by clear zones indicated the phosphate solubilisation.

The cultures which were positive for phosphate solubilisation were tested for:

i. pH change of the growth medium

ii. Change in titrable acidity of the growth medium

iii. release of soluble phosphate from tricalcium phosphate (TCP)

iv. acid phosphatase activity

3.6.1 Determination of pH change of the growth medium

The test cultures were inoculated to 50.00 ml Pikovskaya's broth (Pikovskaya, 1948) containing 250.0 mg of TCP and incubated for 7 days. An uninoculated control was also maintained. The changes in the pH of the culture broth due to bacterial growth was measured using pH meter after 10 days of incubation.

3.6.2 Estimation of titrable acidity in the culture filtrates

The culture medium was centrifuged at 10,000 rpm for 10 min to remove the cells and traces of TCP. Five ml of the supernatant was pipetted out into a clean beaker to which few drops of phenolphthalein indicator was added and titrated against 0.01N NaOH. The titrable acidity was expressed as ml of 0.01 N NaOH consumed per 5.0 ml of culture filtrate (Sperber, 1958).

3.6.3 Estimation of soluble phosphate in culture filtrate

The growth medium after ten days of incubation was centrifuged at 10,000 rpm for 10 min and the clear supernatant was analysed for the

presence of soluble 'P' as per the following method described by Olsen *et al.* (1954).

Reagents required

Reagent 'A'

A quantity of 12.0g of ammonium molybdate was dissolved in 250.0 ml of distilled water. Separately, 0.294 g of antimony potassium tartarate was dissolved in 100.0 ml of distilled water. Both the solutions were added to 100.0 ml of 5.0 N sulphuric acid. This solution was mixed thoroughly and the volume was made upto 2.0 l with distilled water.

Reagent 'B'

A quantity of 1.056 g of ascorbic acid was dissolved in 200.0 ml of reagent A.

Procedure

One ml of the culture filtrate was pipetted out into a 25.0 ml volumetric flask and diluted to 20.0 ml with distilled water. Four ml of reagent 'B' was added to the flask. The intensity of blue colour developed was read at 660 nm in colorimeter. The standard curve was prepared with potassium dihydrogen orthophosphate and the quantity of 'P' solubilised was determined by referring to standard graph.

3.6.4 Assay of acid phosphatase

Substrate solution

Dissolved 1.49 g EDTA, 0.84 g citric acid and 0.03 g p-nitrophenyl phosphate in 100.0 ml distilled water and adjusted to pH 5.3

Standard solution

Weighed 69.75 mg p-nitrophenol and dissolved in 5.0 ml distilled water (100 mM).

Enzyme extract

The test cultures were inoculated into nutrient broth and incubated for 10 days. After centrifuging at 10,000 g for 10 min, the supernatent was used.

Procedure

Three ml of the substrate was incubated at 37°C for 5 min. To that 0.5 ml of extract was added and mixed well. From this, 0.5 ml was removed immediately and mixed with 9.5 ml of 0.085N sodium hydroxide which corresponds to zero time assay (blank). The remaining solution (substrate + enzyme) was incubated for 15 min at 37°C. From this, 0.5 ml was drawn and mixed with 9.5 ml sodium hydroxide solution. The absorbance of incubated samples as well as the blank was measured at 405 nm. From the standard solution, 0.2 to 2.0 ml were taken and made upto 10.0 ml with sodium hydroxide solution and used for the preparation of standard curve. The acid

phosphatase activity was expressed as 'n' moles of p- nitrophenol released per min per mg protein.

3.7 DETERMINATION OF INTRINSIC ANTIBIOTIC RESISTANCE (IAR) OF PGPP BACTERIA

For the identification of PGPP bacteria, IAR character was determined as per the method designed by Josey *et al.* (1979). Antibiotics (Streptomycin and Chloramphenicol) were filter sterilized and added to molten nutrient agar in various concentrations (0, 5, 10, 15, 20, 25, 50, 100, 200, 300, 400 and 500 ppm) and dispensed in 20.0 ml quantity to sterile petri dishes. The plates were steaked with PGPP bacteria and incubated at 28 $\pm$ 1°C for 7 days. The growth of the organism in the antibiotic media was compared with growth on the media without antibiotic. Minimum inhibitory concentration (MIC) was judged as the lowest concentration of antibiotic that significantly reduced the growth compared to control plates, and maximum antibiotic concentration (MAC) was judged as the highest concentration of antibiotic that allowed the normal growth.

3.8 EFFECT OF INOCULATION OF PGPP BACTERIA ON GROWTH AND YIELD OF RICE (*Oryza sativa* L.) UNDER POT CULTURE CONDITIONS

A pot culture experiment was conducted at the Department of Agricultural Microbiology, AC & RI, Coimbatore to know the effect of inoculation of PGPP bacteria *viz.*, *Pseudomonas* sp. (PGPP 1) and *Bacillus*

macerans (PGPP 20) either alone or in combination with *Azospirillum lipoferum* (AZ 204) on rice variety CO 43. The organisms were inoculated by conventional application methods (seed treatment + seedling root dip + soil application) as well as through leaf spray.

Pots lined with polythene sheets were filled with clay soil collected from wetland, AC & RI, Coimbatore and the experiment was performed in completely randomised block design with three replications for each treatment.

3.8.1 Treatment details

T1 - Uninoculated control

Conventional methods of application (CMA)

T2 - *Azospirillum lipoferum* (AZ 204)

T3 - *Pseudomonas* sp. (PGPP 1)

T4 - *Bacillus macerans* (PGPP 20)

T5 - AZ 204 + PGPP 1

T6 - AZ 204 + PGPP 20

Leaf spray

T7 - AZ 204

T8 - PGPP 1

T9 - PGPP 20

T10 - AZ 204 + PGPP 1

T11 - AZ 204 + PGPP 20

CMA + Leaf spray

T12 - AZ 204

T13 - PGPP 1

T14 - PGPP20

T15 - AZ 204 + PGPP 1

T16 - AZ 204 + PGPP 20

The conventional methods of application *viz.*, seed treatment, seedling root dip and soil application were done as per the method described by Kalaivani (1998).

3.8.2 Seed treatment

Seeds (40 kg/ha) treated with lignite based inoculants (600 g/ha) were sown in the nursery bed.

3.8.3 Seedling root tip

Roots of rice seedlings were dipped in water slurry containing lignite based bacterial inoculants (1 kg/ha) for 15 min before transplanting.

3.8.4 Soil application

The lignite based bacterial inoculants (2 kg/ha) was prepared with soil and FYM (25 kg/ha each) and broadcasted uniformily before transplanting.

3.8.5 Dual inoculation

For dual inoculation, equal quantities of the two specified bacterial inoculants were mixed and used for seed treatment, seedling root dip and soil application as mentioned earlier.

3.8.6 Leaf spray

Bacterial inoculants were grown in their respective medium, the culture broth was centrifuged at 10,000 rpm for 10 min and the pellet of bacterial cells were suspended in distilled water amended with 0.05% Tween 80. The cell load was adjusted to 10^8 cfu/ml on the basis of turbidity measured with a spectrophotometer. Foliar inoculation was made four times at 30 days intervals by spraying on plant surfaces with a garden compression sprayer at a pressure of 1.8 - 2.0 kg/cm^2. Inoculation was made in late afternoon to prevent cell desiccation, as described by Mariano and McCarter (1993).

3.8.7 Growth parameters

Observation was made on growth at different stages of rice crop.

3.8.7.1 Shoot and root length

The shoot length was measured from the ground level to the tip of the plant and root length from ground level to root tip and expressed in cm.

3.8.7.2 Plant dry weight

Plant samples were dried in hot air oven at 70°C to a constant weight and the dry weight was recorded and expressed as g/pl.

3.8.8 Yield parameters

The yield parameters like number of tillers per hill, panicle numbers, panicle length, straw yield and grain yield were recorded.

3.8.9 Chemical analysis

3.8.9.1 Total chlorophyll content (Witham *et al.*, 1971)

Leaf chlorophyll was extracted in 80% acetone and the absorption coefficients, the total chlorophyll content was calculated and expressed as mg chlorophyll g^{-1} tissue.

3.8.9.2 Plant nitrogen (Humphries, 1956)

The total nitrogen content in plant was estimated by micro-kjeldahl method and expressed in per cent.

3.8.9.3 Plant phosphorus (Jackson, 1973)

The total phosphorus content in plant was estimated by vanedo molybdo phosphoric yellow colour development method and expressed in per cent.

3.8.10 Enumeration of PGPP bacterial population on leaf surface

The population of inoculated PGPP bacteria surviving on leaves were estimated by pour plate technique using N-free media incorporated with antibiotics. The samples were taken before the leaf spray as well as 2h after leaf spray and used for enumeration. The final count was taken before harvest.

3.9 EFFECT OF INOCULATION OF PGPP BACTERIA ON GROWTH AND YIELD OF COTTON (var. LRA 5166) UNDER POT CULTURE CONDITIONS

A pot culture experiment was conducted at the Department of Agricultural Microbiology, AC & RI, Coimbatore to know the effect of inoculation of PGPP bacteria *viz.*, *Pseudomonas* sp. (PGPP 1) and *Bacillus macerans* (PGPP 20) along with *Azospirillum lipoferum* (AZ 204) and *Azotobacter chroococcum* (CZR 1) on cotton variety LRA 5166. The pot soil mixture was prepared with field soil, farm yard manure and sand. Bacteria were inoculated by conventional methods (seed treatment + soil application) as well as through leaf spray. The experiment was performed in completely randomised block design with three replications for each treatment.

3.9.1 Treatment details

T1 - Uninoculated control

Conventional methods of application (CMA)

T2 - *Azospirillum lipoferum* (AZ 204)

T3 - *Azotobacter chroococcum* (CZR 1)

T4 - *Pseudomonas* sp. (PGPP 1)

T5 - *Bacillus macerans* (PGPP 20)

Leaf spray

T6 - AZ 204

T7 - CZR 1

T8 - PGPP 1

T9 - PGPP 20

CMA + leaf spray

T10 - AZ 204

T11 - CZR 1

T12 - PGPP 1

T13 - PGPP 20

3.9.2 Bacterial inoculation

The methods of seed treatment, soil application and leaf spray were the same as mentioned earlier.

3.9.3 Growth parameters

The plant growth parameters like root length, shoot length, leaves number and dry matter production were recorded.

3.9.4 Yield parameters

The yield parameters *viz.*, number of sympodial branches and bolls per plant, boll weight and seed cotton yield were recorded.

3.9.5 Chemical analysis

Total chlorophyll, plant nitrogen and phosphorus contents in plants were determined as described earlier.

3.9.6 Enumeration of PGPP bacterial population on leaf surface

The inoculated PGPP bacterial population on leaves was estimated by pour plate technique using antibiotics incorporated N-free medium. The type and concentration of antibiotic was decided based on IAR nature. The samples were taken before and 2h after leaf spray for enumeration. The final count was taken before harvest.

3.10 EFFECT OF INOCULATION OF PGPP BACTERIA ON GROWTH AND YIELD OF SOYBEAN (*Glycine max* (L.) Merr.)

A field trial was conducted at Agricultural Research Station (ARS), Aliyarnagar, TNAU to know the effect of inoculation of PGPP bacteria *viz.*, *Bacillus polymyxa* (PGPP 8) and *Pseudomonas* sp. (PGPP 1) either alone or in combination with *Rhizobium freidii* (CRS 1) and phosphate solubilising *Pseudomonas* sp. (PS 2) on growth and yield of soybean. The experiment was performed in randomized block design with three replications.

3.10.1 Treatment details

T1 - Uninoculated control

Conventional methods of application (CMA)

T2 - *Bacillus polymyxa* (PGPP 8)

T3 - *Pseudomonas* sp. (PGPP 1)

T4 - *Pseudomonas* sp. (PS 2)

T5 - *Rhizobium freidii* (CRS 1)

Leaf spray

T6 - PGPP 8

T7 - PGPP 1

T8 - PS 2

T9 - CRS 1

CMA + Leaf spray

T10 - CRS 1 + PGPP 8

T11 - CRS 1 + PGPP 1

T12 - CRS 1 + PS 2

3.10.2 Bacterial inoculation

The seed treatment, soil application and leaf spray were given as per the method described earlier.

3.10.3 Growth and yield parameters

The plant growth parameters *viz.*, root length, shoot length, dry matter production, nodule number, and pod number dry weight were recorded besides grain yield.

3.10.4 Chemical analysis

Total nitrogen, phosphorus and chlorophyll contents of leaves were determined as stated earlier.

3.10.5 Enumeration of PGPP bacterial population on leaf surface of soybean

The inoculated PGPP bacterial population on leaf surface was enumerated using media in corporated with antibiotic markers before and 2h after leaf spray as mentioned earlier.

3.11 COMPATIBILITY TEST FOR PGPP BACTERIA WITH OTHER DIAZOTROPHS UNDER *in vitro* CONDITIONS

The compatibility of the PGPP bacteria with dinitrogen fixers *viz.*, *Rhizobium freidii* (CRS 1), *Bradyrhizobium japonicum* (CRS 3), *Azospirillum lipoferum* (AZ 204) and *Azotobacter chroococcum* (CZR 1) and phosphate solubilizing bacterium *Pseudomonas* sp. (PS 2) were tested by cross streak assay.

The PGPP bacteria were first streaked in a line on nutrient agar medium in petri dish and incubated for 48h. Actively growing cultures of dinitrogen fixers and phosphate solubilisers were streaked perpendicular to the previous one and the plates were observed for growth after 48h of incubation.

3.12 STUDIES ON ANTAGONISTIC ACTIVITY OF PGPP BACTERIA ON FOLIAR PATHOGENS

The antagonistic activity of PGPP bacteria on the foliar pathogens *viz.*, *Xanthomonas oryzae*, *X. malvacearum*, *Helminthosporium oryzae*, *Fusarium oxysporum* and *Colletotrichum truncatum* were tested. Antagonistic activity

against bacterial pathogens were tested by cross streak assay as per the method described earlier.

The antagonistic activity against fungal pathogens were tested by paired culture test (Michereff *et al.*, 1994). Two agar discs (5 mm diameter and 2 mm thick) of fungal cultures were placed 70 mm apart in a petridish containing potato dextrose agar medium. Plates were incubated 12h at 28 ± 1°C. Each PGPP bacterium was streaked as a band at the centre of petri dish, except for the control. The formation of inhibition zone indicated the antagonistic activity.

3.13 EFFECT OF PESTICIDES ON PGPP BACTERIA (STANDARD FILTER PAPER METHOD BY Santhi *et al.*, 1987)

The effect of following pesticides on the PGPP bacteria were studied :

Pesticides	Recommended dose (Qty/600 l spray fluid)
Monocrotophos	1.25 l
Metasystax	0.50 l
Endosulfan	2.00 l
Neem oil	3.00 l
Dithane	2.00 l
Cypermethrin	3.30 l

The pesticides were used at different concentrations *viz.*, i) recommended dose, ii) double the recommended dose and iii) half of the recommended dose. The nutrient agar plates were seeded with the PGPP bacteria by spread plate technique and the agar surface was allowed to dry for 5 min.

About 10 µl of pesticide solution was added to the sterile filter paper disc (6 mm) and placed on the agar surface using sterile forceps and pressed gently to ensure firm contact. Two more discs with different concentrations were placed in the same plate. After 48h of incubation, the plates were observed for inhibition zone and the diameter was measured.

3.14 SURVIVAL OF PGPP BACTERIA IN CARRIER BASED INOCULANT

The PGPP bacteria grown in nutrient broth (4 x 10^{10} cells/ml) were mixed with sterilized and neutralised lignite and cured for 24h. They were stored in an opaque polythene bag of 300 gauze thickness at room temperature for 90 days. The viable number of bacterial cells were enumerated using nutrient agar medium at 15 days interval by conventional plate count method after serial dilution.

3.15 STATISTICAL ANALYSIS

The results of pot culture and field experiments were subjected to statistical scrutiny as per the methods detailed by Panse and Sukhatme (1978).

EXPERIMENTAL RESULTS

4. EXPERIMENTAL RESULTS

4.1 ENUMERATION AND ISOLATION OF PHYLLOSPHERE MICROORGANISMS FROM SELECTED AGRICULTURAL CROPS

Phyllosphere microorganisms occurring on the leaf surface of selected agricultural crops *viz.*, rice, wheat, maize, soybean, cowpea, groundnut and cotton were enumerated by serial dilution and plating technique using different media. The population of bacteria, fungi and actinomycetes occurring on the leaves is presented in Table 1. Besides total bacterial count, bacteria growing on Jensen's nitrogen free medium (Plate 1) was also enumerated, which constituted about 8.0 to 50.0 per cent of total heterotrophic bacteria (Fig.1).

The total bacterial population was higher on the cotton leaf surface (113.30 x 10^4 cfu/cm^2) followed by maize (106.70 x 10^4 cfu/cm^2), whereas bacteria growing on nitrogen free medium was more on wheat leaf (26 x 10^4 cfu/cm^2) followed by maize (19.33 x 10^4 cfu/cm^2). The fungal population was higher in wheat and soybean (9 x 10^4 cfu/cm^2) whereas actinomycetes population was more in cowpea (6 x 10^4 cfu/cm^2) followed by soybean (5.67 cfu/cm^2) phyllosphere. Groundnut leaf harboured the least population of bacteria (7.67 x 10^4 cfu/cm^2) growing on nitrogen free medium.

Table 1. Occurrence of phyllosphere microorganisms on selected agricultural crops

Crops	Microbial population (X 10^4 per cm^2)			
	Total heterotrophic Bacteria	Bacteria on N-free medium	Fungi	Actinomyetes
Rice	100.00	10.33	0.53	3.33
Wheat	50.00	26.00	0.90	2.33
Maize	106.70	19.33	0.87	3.00
Soybean	86.70	14.00	0.90	5.67
Cowpea	86.70	15.67	0.57	6.00
Groundnut	100.00	7.67	0.50	3.33
Cotton	113.30	12.33	0.87	2.33

Fig.1. Occurrence of phyllosphere microorganisms on selected agricultural crops

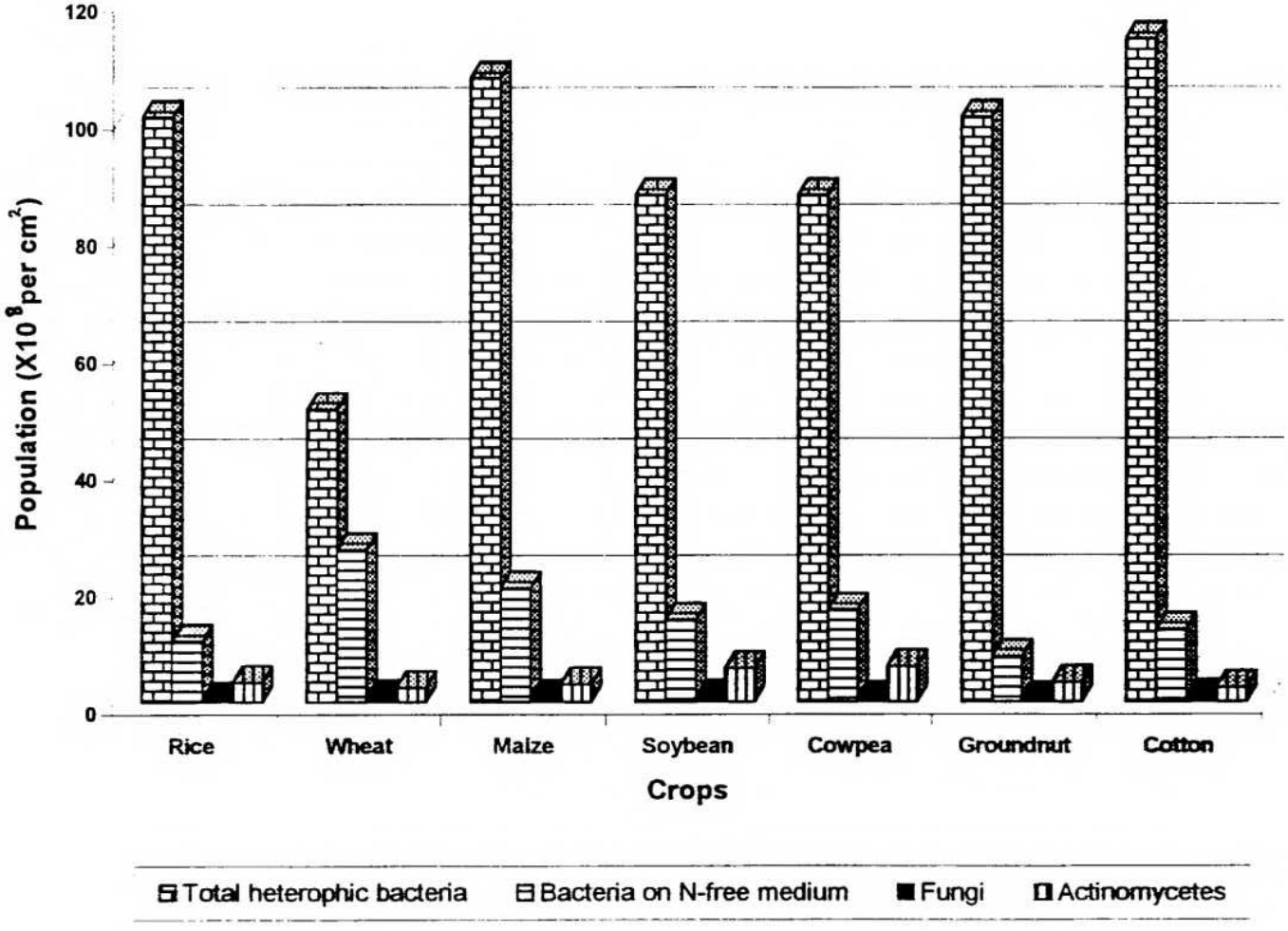
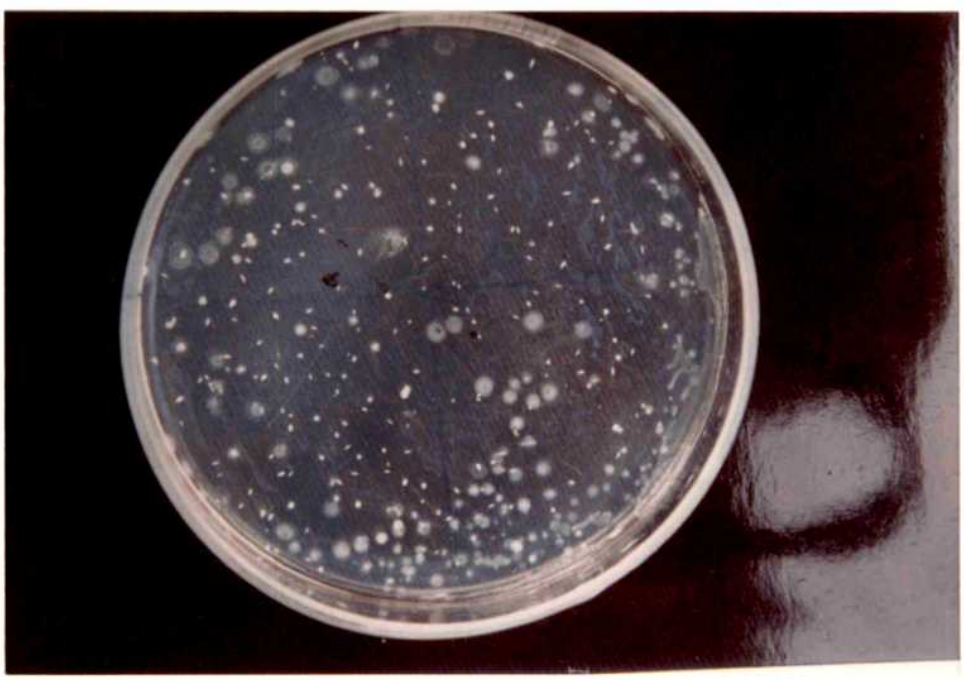


Plate 1. Growth of nitrogen fixing bacteria in Jensen's N-free agar medium



Twenty four morphologically distinct bacterial colonies growing on nitrogen free medium were selected, purified subcultured, and stored in nitrogen free agar slants under refrigerated condition for further studies.

4.2 PRELIMINARY SCREENING OF PHYLLOSPHERE BACTERIA FOR ACETYLENE REDUCTION ACTIVITY (ARA)

All the phyllosphere bacterial isolates growing on nitrogen free medium were examined for cell morphology, Gram's reaction and also for acetylene reduction activity. The results are presented in Table 2 and Fig.2.

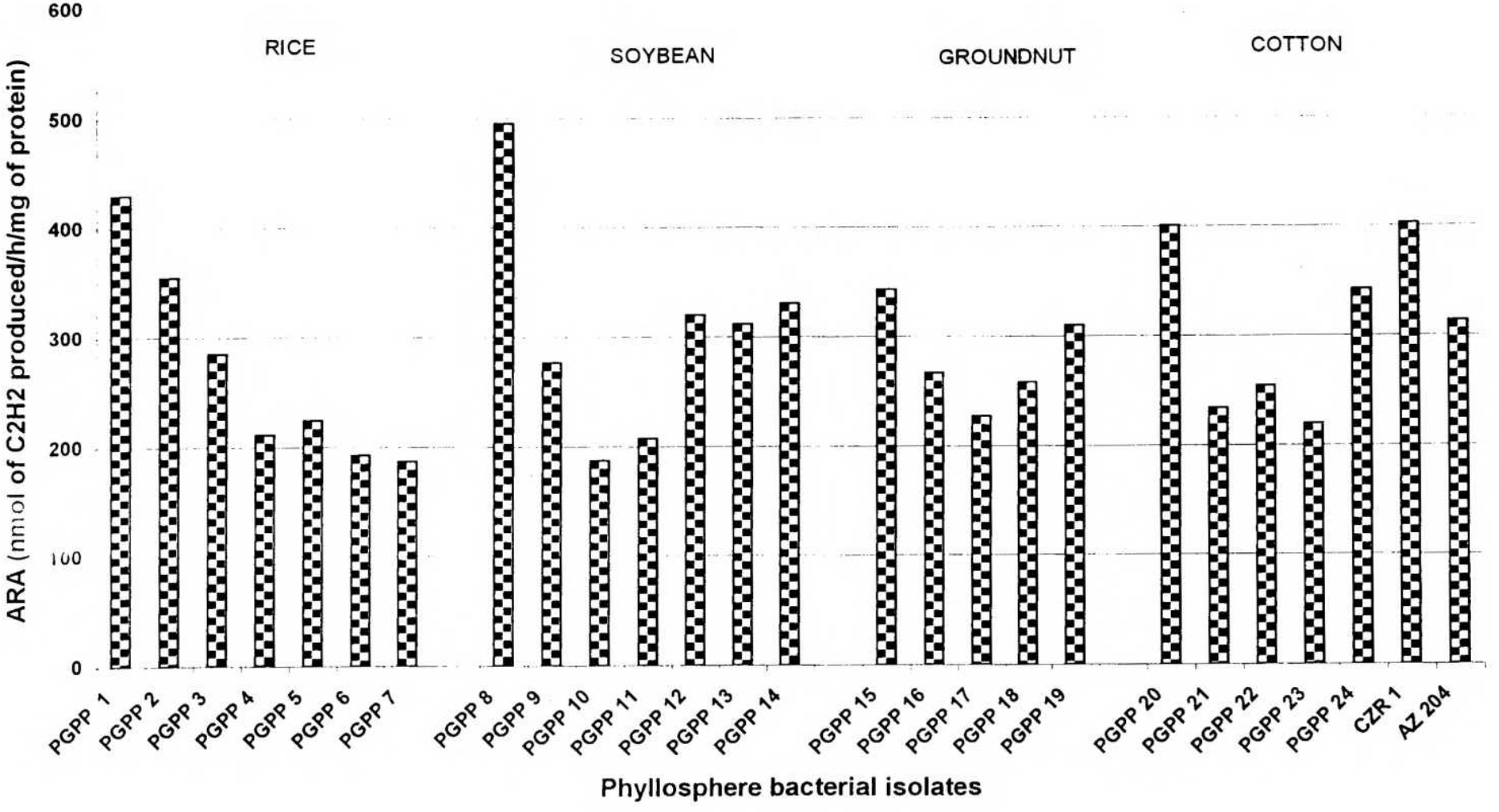
Among the 24 phyllosphere bacterial isolates, only 8 were Gram positive. Rod shaped bacteria were predominant on the leaves and the ARA of the isolates ranged between 186.56 to 495.06 nmol of C_2H_4 produced/h/mg of cell protein. The highest activity was recorded in the bacterium PGPP 8, isolated from soybean followed by PGPP 1 (429.77) isolated from rice. These two isolates recorded higher ARA than the standard diazotrophic cultures *viz.*, *Azotobacter chroococcum* (402.40) and *Azospirillum lipoferum* (313.00). Among the cotton isolates, PGPP 20 expressed the maximum ARA activity (400.77) followed by PGPP 24 (342.32).

Based on ARA, seven cultures were identified for further studies by selecting two isolates from each of rice (PGPP 1 and 2), groundnut (PGPP 15 and 19) and cotton (PGPP 20 and 24) and one from soybean (PGPP 8).

Table 2. Characterisation and acetylene reduction activity of phyllosphere bacterial isolates growing on N - free medium

Crops	Bacterial isolates	Cell shape	Gram reaction	Acetylene reduction activity (nmol of C_2H_4 produced /h/mg of cell protein)
Rice	PGPP – 1	Short rod	-	429.77
	PGPP – 2	Short rod	-	354.77
	PGPP – 3	Curved rod	+	284.90
	PGPP – 4	Short rod	-	211.02
	PGPP – 5	Short rod	-	223.95
	PGPP – 6	Short rod	-	192.50
	PGPP – 7	Long rod	-	186.56
Soybean	PGPP – 8	Long rod	+	495.06
	PGPP – 9	Cocci	+	276.54
	PGPP – 10	Short rod	-	187.23
	PGPP – 11	Long rod	-	207.14
	PGPP – 12	Long rod	+	320.11
	PGPP – 13	Short rod	+	311.70
	PGPP – 14	Short rod	-	330.41
Groundnut	PGPP – 15	Long rod	-	342.93
	PGPP – 16	Curved rod	-	266.85
	PGPP – 17	Curved rod	-	227.21
	PGPP – 18	Short rod	-	258.11
	PGPP – 19	Cocci	+	310.03
Cotton	PGPP – 20	Short rod	+	400.77
	PGPP – 21	Long rod	+	234.16
	PGPP – 22	Short rod	-	253.98
	PGPP – 23	Short rod	-	219.73
	PGPP – 24	Long rod	-	342.32
Azotobacter chroococcum (CZR 1)		Long rod	-	402.40
Azospirillum lipoferum (AZ 204)		Curved rod	-	313.00

Fig. 2. Acetylene reduction activity of phyllosphere bacteria



4.3 SCREENING PHYLLOSPHERE BACTERIA FOR PRODUCTION OF PLANT GROWTH PROMOTING SUBSTANCES

4.3.1 Indole acetic acid (IAA) production by phyllosphere bacteria

IAA production by seven phyllosphere bacterial isolates was tested by both chemical and plant bioassay method and compared with the IAA production by *Azotobacter chroococcum (CZR 1)*, *Azospirillum lipoferum* (AZ 204), *Rhizobium freidii* (CRS 1) and *Pseudomonas* sp. (PS 2) [Plate 2]. The results are presented in Table 3 and Fig.3. Among the phyllosphere bacteria, PGPP 1, PGPP 8, PGPP 15 and PGPP 20 produced equal amount of IAA (30.00 µg/ml) as determined by colorimetric method, whereas by plant bioassay technique, PGPP 8 recorded the highest amount (72.50 µg/ml) followed by PGPP 20 (57.50 µg/ml). Among the authenticated cultures, *Azospirillum lipoferum* produced the maximum amount of IAA as determined by both chemical (41.00 µg/ml) and plant bioassay methods (47.50 µg/ml). The overall IAA determination ranged from 23.00 to 41.00 µg/ml by colorimetric method and from 22.50 to 72.50 µg/ml by plant bioassay technique.

4.3.2 Gibberellic acid (GA) production by phyllosphere bacteria

Gibberellic acid production by phyllosphere bacteria and standard cultures were estimated by spectrophotometric and plant bioassay method (Plate 3). The results are presented in Table 4 and Fig.4. The phyllosphere bacterial isolate PGPP 8 produced highest amount of GA as estimated by both plant bioassay (4.75 µg/ml) and chemical (2.50 µg/ml) methods followed by PGPP 1 (4.50 µg/ml by plant bioassay and 2.50 µg/ml by chemical method).

Table 3. Indole acetic acid (IAA) production by phyllosphere bacteria (chemical and plant bioassay)

Bacteria	IAA (Colorimetric method)		IAA (Rice root inhibition assay)		
	OD Value (at 530 nm)	Concen-tration (µg/ml)	Mean root length (cm)	Per cent inhibition over control	Concen-tration (µg/ml)
PGPP 1	0.070	30.00	3.60	10.00	47.50
PGPP 2	0.060	23.00	3.90	2.50	22.50
PGPP 8	0.070	30.00	3.30	17.50	72.50
PGPP 15	0.070	30.00	3.80	5.00	32.50
PGPP 19	0.065	26.00	3.70	7.50	37.50
PGPP 20	0.070	30.00	3.50	12.50	57.50
PGPP 24	0.060	23.00	3.70	7.50	37.50
Azotobacter chroococcum (CZR 1)	0.065	26.00	3.70	7.50	37.50
Azospirillum lipoferum (AZ 204)	0.090	41.00	3.60	10.00	47.50
Rhizobium freidii. (CRS 1)	0.070	30.00	3.80	5.00	32.50
Pseudomonas sp. (PS 2)	0.060	23.00	3.70	7.50	37.50

Fig. 3. Indole acetic acid (IAA) production by phyllosphere bacteria (chemical and plant bioassay)

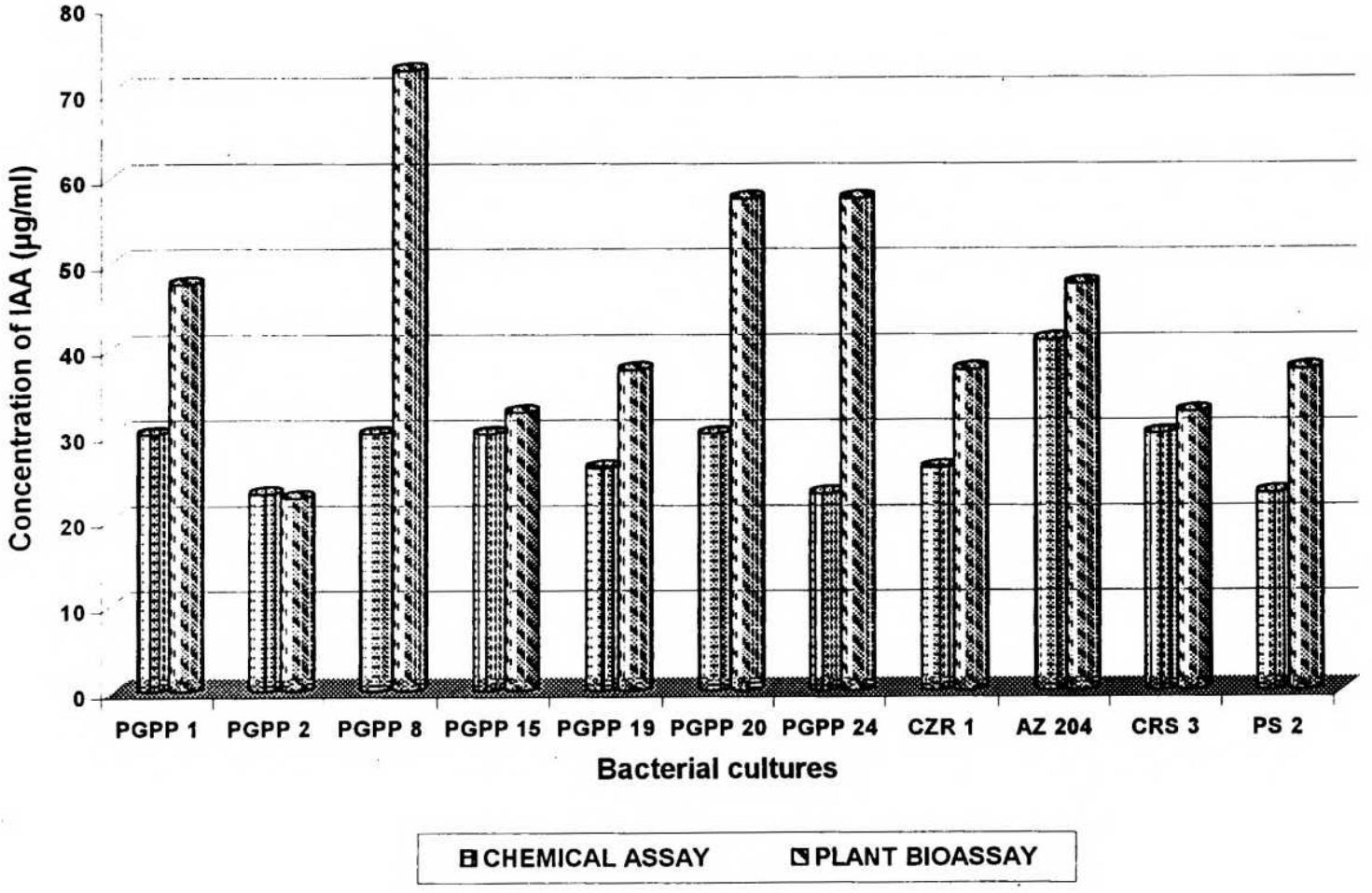


Plate 2. Indole acetic acid production by phyllosphere bacteria (Rice root inhibition assay)

Table 4. Gibberellic acid (GA) production by phyllosphere bacteria (chemical and plant bioassay)

Bacteria	GA (Spectrophotometric method)		GA (Rice seedling assay)	
	OD Value (at 254 nm)	Concentration (μg/ml)	Mean shoot length (cm)	Concentration (μg/ml)
PGPP 1	0.02	2.50	7.98	4.50
PGPP 2	0.01	1.25	7.78	3.50
PGPP 8	0.02	2.50	7.99	4.75
PGPP 15	0.01	1.25	7.63	3.00
PGPP 19	0.01	1.25	7.54	2.50
PGPP 20	0.02	2.50	7.96	4.25
PGPP 24	0.01	1.25	7.81	3.75
Azotobacter chroococcum (CZR 1)	0.01	1.25	7.64	3.05
Azospirillum lipoferum (AZ 204)	0.01	1.25	7.65	3.10
Rhizobium freidii (CRS 1)	0.02	2.50	7.60	2.95
Pseudomonas sp. (PS 2)	0.01	1.25	7.56	2.45

Fig. 4. Gibberellic acid (GA) production by phyllosphere bacteria (chemical and plant bioassay)

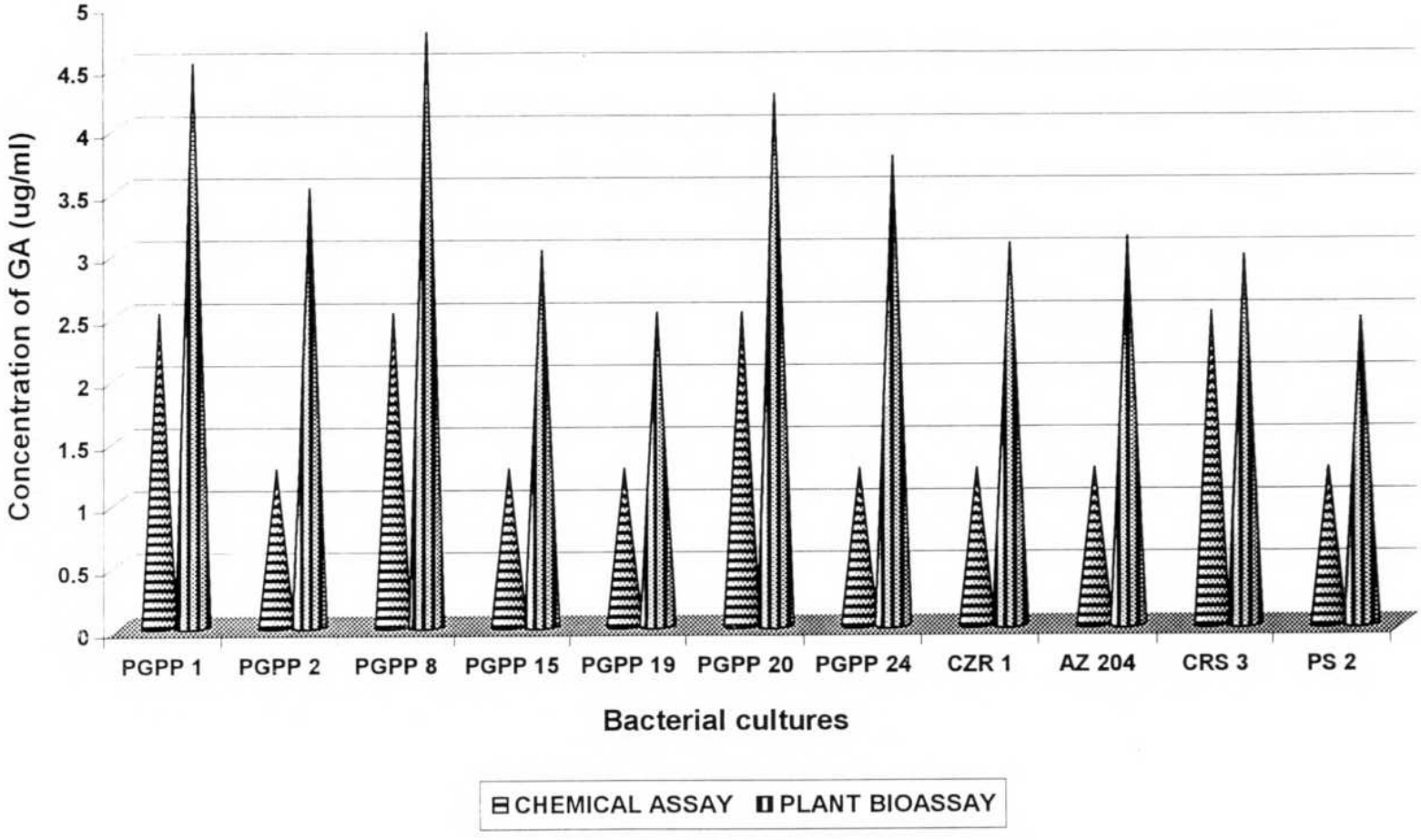
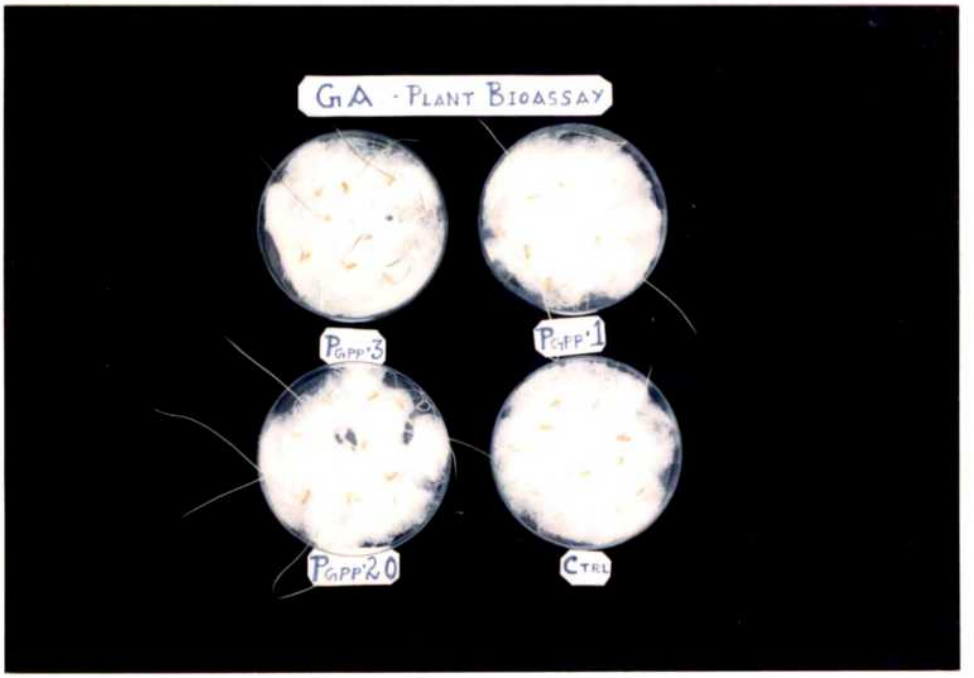
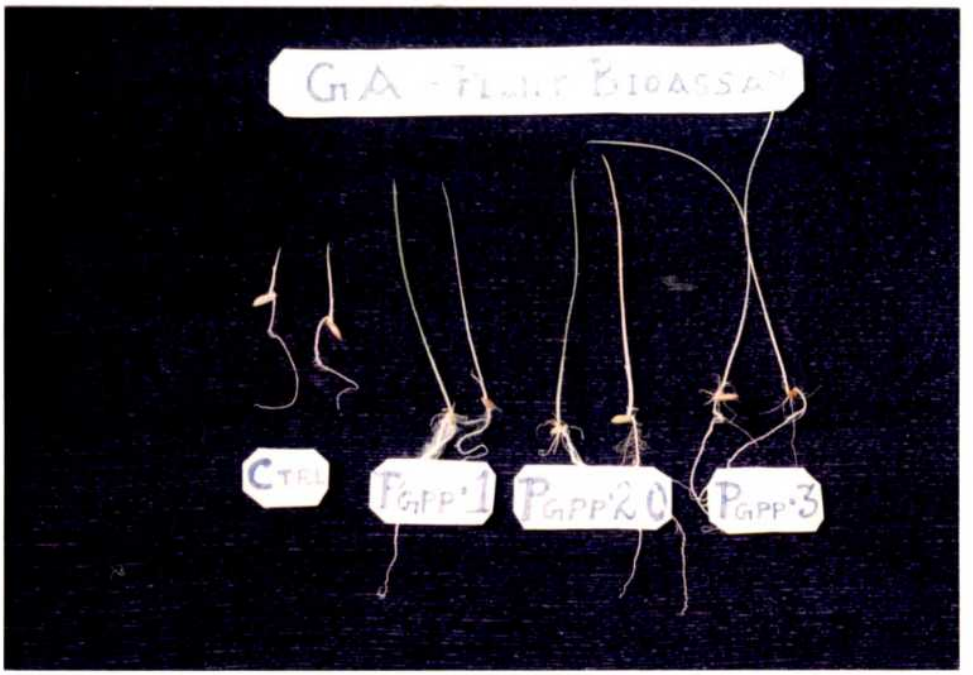


Plate 3. Gibberellic acid production by phyllosphere bacteria (Rice seedling assay)





Among the standard cultures, *Azospirillum lipoferum* (AZ 204) showed the maximum GA activity (3.10 µg/ml) followed by *Azotobacter chroococcum* (3.05 µg/ml)

Based on IAA and GA production, three phyllosphere bacterial isolates (PGPP 1, PGPP 8 and PGPP 20) were identified for further studies and these isolates were named as plant growth promoting phyllosphere (PGPP) bacteria.

4.4 CHARACTERISATION OF PLANT GROWTH PROMOTING PHYLLOSPHERE (PGPP) BACTERIA

The results of morphological, cultural and biochemical characters exhibited by the three PGPP bacteria *viz.*, PGPP 1 (rice), PGPP 8 (soybean) and PGPP 20 (cotton) which produced higher amount of plant growth promoting substances and higher ARA are presented in Table 5.

4.4.1 Morphological characters

Under microscope, the bacteria PGPP 1 and PGPP 20 appeared as short rods and PGPP 8 as long rods. PGPP 1 is gram negative in reaction whereas PGPP 8 and PGPP 20 were gram positive and produced endospores. The bacteria PGPP 1 and PGPP 8 were motile whereas PGPP 20 was non motile.

Table 5. Characterization of plant growth promoting phyllosphere (PGPP) bacteria having higher acetylene reduction activity

Characters	PGPP bacteria		
	PGPP 1 (rice isolate)	**PGPP 8 (soybean isolate)**	**PGPP 20 (cotton isolate)**
Morphological characters			
Shape	Short rod	Long rod	Short rod
Gram staining	Negative	Positive	Positive
Spore staining	Negative	Positive	Positive
Motility	Motile	Motile	Non motile
Cultural characters			
Form of the colony	Circular	Irregular	Irregular
Margin	Entire	Lobate	Lobate
Elevation	Raised, unwrinkled	Flat Wrinkled	Flat Wrinkled
Size (diameter in cm)	Small (0.3)	Small (0.2)	Large (0.6)
Colour	Dull brown	White	Light pink
Biochemical characters			
Gelatin hydrolysis	Negative	Negative	Positive
Starch hydrolysis	Negative	Positive	Positive
Acid and gas production	Negative	Positive	Positive
Catalase activity	Positive	Positive	Positive
Voges-Proskauer	Negative	Positive	Negative
Growth at 50°C	Negative	Negative	Positive
Growth at 60°C	Negative	Negative	Negative
Growth in anaerobic agar	Negative	Positive	Negative
H_2S production	Negative	Negative	Negative
Tentative identification	*Pseudomonas sp.*	*Bacillus polymyxa*	*B. macerans*

4.4.2 Colony characters

On agar plates, all the bacteria formed circular colonies with entire (PGPP 1) and lobate (PGPP 8 and PGPP 20) margins. The small colony (0.3 cm diameter) of gram negative PGPP 1 was raised, unwrinkled and dull brown in colour, whereas gram positive PGPP 8 colony was flat, wrinkled and 0.2 cm in diameter with white in colour and PGPP 20 was flat, wrinkled, large (0.6 cm diameter) and light pink in colour.

4.4.3 Biochemical characters

All the three bacterial isolates were positive for catalase activity and failed to grow at 50°C (except PGPP 20) and 60°C. Except PGPP 1, other two bacteria hydrolysed starch and produced acid and gas from sugars. The bacteria PGPP 20 alone liquefied the gelatin. Bacteria PGPP 8 which showed growth in anaerobic agar was positive for Voges-Proskauer test whereas other two bacteria were VP negative.

Based on the various morphological, cultural and biochemical characters, these three bacteria PGPP 1, PGPP 8 and PGPP 20 were tentatively identified *as Pseudomonas sp.*, *Bacillus polymyxa* and *B. macerans*, respectively.

4.5 PHOSPHATE SOLUBILISATION BY PGPP BACTERIA

The PGPP bacterial isolates were tested for phosphate solubilisation and compared with the authenticated phosphate solubilising bacterial strains *Pseudomonas sp.* (PS 2) and *Bacillus megaterium* var. *Phosphaticum.*

Among the seven PGPP bacteria tested, only PGPP 15 solubilised hydroxy apatite in the medium and formed clearing zone around the colony growth on the Sperber's hydroxy apatite medium (Table 6). The diameter of clearing zone (2.10 cm) produced by PGPP 15 was lower than that of *Pseudomonas* sp. PS 2 (2.20 cm) but higher than that of *B. megaterium* var. *phosphaticum* (1.80 cm).

4.5.1 Organic acid production and acid phosphatase activity of phosphate solubilising bacteria

The organic acid production and acid phosphatase activity of phosphate solubilising bacterium PGPP 15 was compared with the activity of *Pseudomonas* sp. (PS2) and *Bacillus megaterium* var. *Phosphaticum*. *Pseudomonas sp.* (PS 2) produced higher amount of organic acid (55.83 mg of lactic acid/50 ml broth) followed by PGPP 15 (48.55 mg) and *B.megaterium* var. *phosphaticum* (42.20mg). Similarly, *Pseudomanas* sp. (PS 2) showed the highest acid phosphatase activity (1.84 nmol of p-nitrophenol released/min/mg of protein) followed by PGPP 15 (1.65 nmol) and *Bacillus megaterium* var. *phosphaticum* (0.89 nmol).

4.5.2 Titrable acidity and pH reduction

The titrable acid production in the culture broth of phosphate solubilising bacteria as measured by the volume of 0.01N NaOH consumed ranged from 7.1 to 8.9 ml (Table 6). *Pseudomonas* sp. (PS2) produced the maximum titrable acidity (8.9 ml) followed by PGPP 15 (7.5 ml) and *Bacillus*

Table 6. Phosphate solubilisation, organic acid production, acid phosphatase activity and titrable acidity of selected phyllosphere bacteria.

Bacteria	Clearing zone in hydroxy apatite medium (diameter in cm)	Organic acid (mg of lactic acid /50 ml broth)	Acid phosphatase activity (nmol of p-nitrophenol released/min/mg of protein)	Titrable acidity (ml of 0.01 N NaOH consumed)	pH of the broth
PGPP 15	2.10	48.55	1.65	7.5	5.1
Pseudomonas sp.(PS 2)	2.20	55.83	1.84	8.9	5.0
Bacillus megaterium var.phosphaticum	1.80	42.20	0.89	7.1	5.3
Uninoculated Control	0.00	0.00	0.00	0.5	6.5

megaterium var. phosphaticum (7.1 ml). Growth of *Pseudomonas* sp. (852) in the culture caused the maximum pH reduction (pH 5.0) followed by PGPP 15 (pH 5.1) and *Bacillus megaterium var. phosphaticum* (pH 5.3).

4.6 INTRINSIC ANTIBIOTIC RESISTANCE (IAR) OF PGPP BACTERIA

The IAR of PGPP bacterial isolates (*Pseudomonas* sp. [PGPP 1], *Bacillus polymyxa* [PGPP 8] and *B. macerans* [PGPP 20]) to the antibiotics chloramphenicol and streptomycin were tested to know the maximum antibiotic concentration (MAC) that allowed the normal growth and minimum inhibitory concentration (MIC) that significantly reduced the growth (Plate 4) and the results are presented in Table 7 and Fig.5.

Among the three bacterial isolates, *Pseudomonas* sp. (PGPP 1) showed maximum resistance to both the antibiotics and was able to grow well in the media incorporated with 100 ppm (MAC) of both chloramphenical and streptomycin whereas at 200 ppm (MIC), these antibiotics reduced its growth. *Bacillus polymyxa* (PGPP 8) grow well with 20 and 25 ppm of chloramphenicol and streptomycin respectively whereas their respective concentrations at 25 and 50 ppm significantly reduced the growth. *Bacillus macerans* (PGPP 20) was found to grow well with 50 and 100 ppm of the antibiotics chloramphenicol and streptomycin respectively whereas their respective concentrations of 100 and 200 ppm significantly reduced its

Table 7. Intrinsic antibiotic resistance (IAR) of plant growth promoting phyllosphere (PGPP) bacteria.

Bacteria	Antibiotic concentration (ppm)			
	Chloramphenicol		Streptomycin	
	MAC	MIC	MAC	MIC
Pseudomonas sp. **(PGPP 1)**	100	200	100	200
Bacillus polymyxa **(PGPP 8)**	20	25	25	50
B. Macerans **(PGPP 20)**	50	100	100	200

MAC - Maximum antibiotic concentration that allowed normal growth

MIC - Minimum inhibitory concentration that significantly reduced the growth

Fig.5. Intrinsic antibiotic resistance of PGPP bacteria

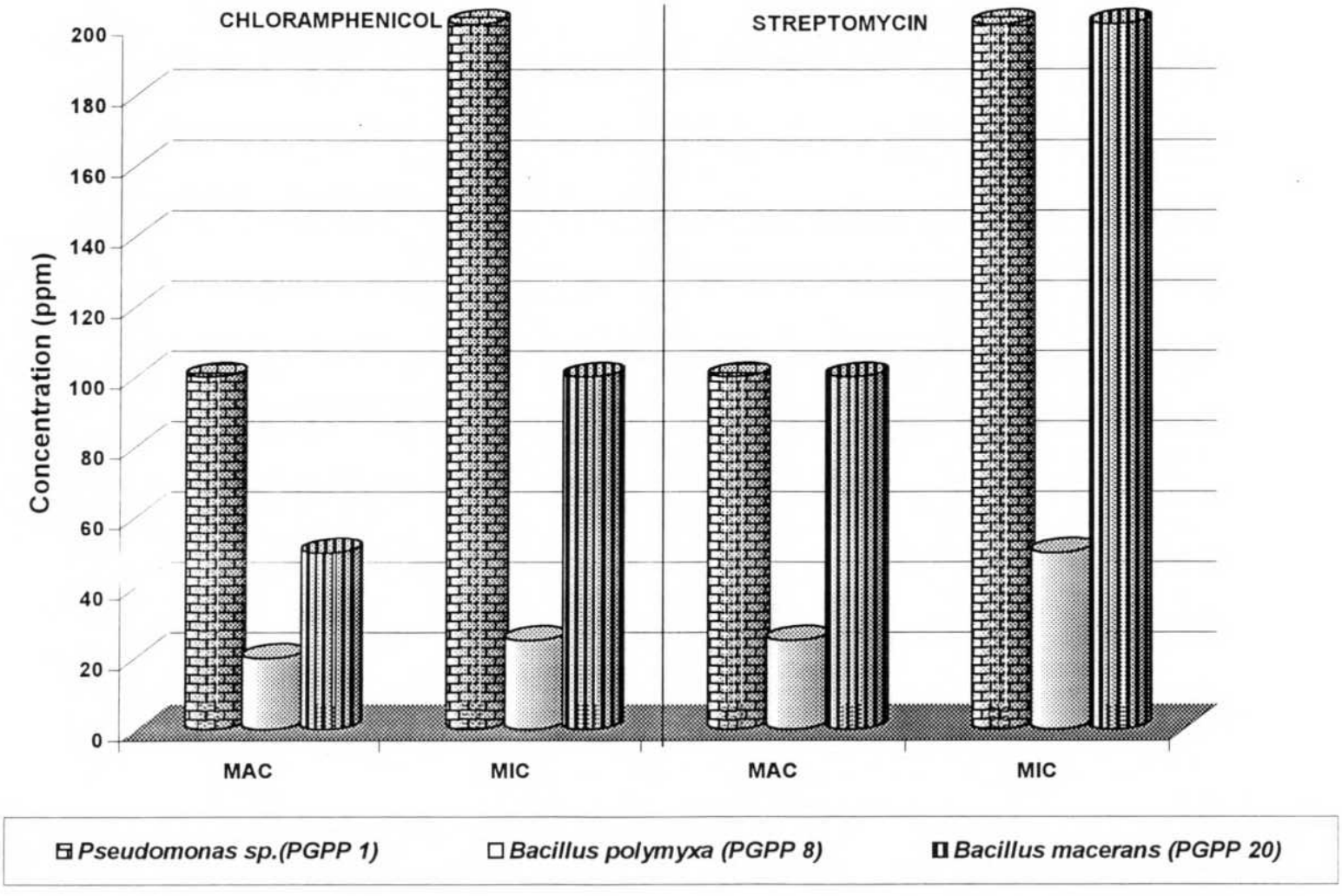
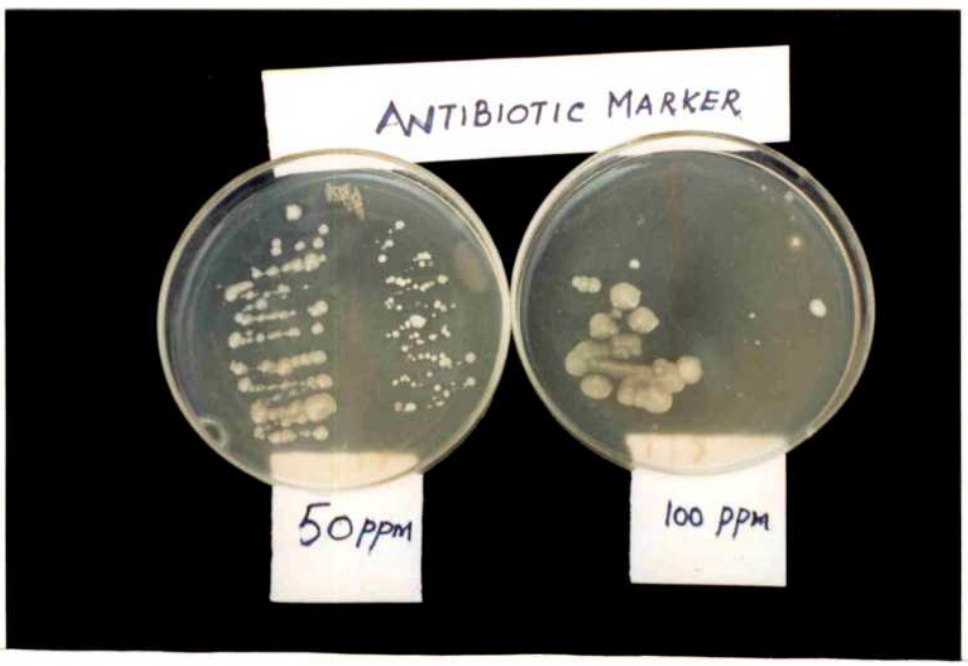


Plate 4. Intrinsic antibiotic resistance (IAR) of plant growth promoting phyllosphere (PGPP) bacteria

a) *Pseudomonas* sp. (PGPP 1) in chloramphenicol



b) *Pseudomonas* sp. (PGPP 1) [1] and *Bacillus polymyxa* (PGPP 8) [2] in streptomycin

growth. Based on their resitance to antibiolity these organism were identified as follows:

Pseudomonas sp.	-->	200 ppm chloramphenicol and 200 ppm streptomycin
Bacillus polymyxa (PGPP 8)	-->	25 ppm chloramphenicol and 50 ppm streptomycin
B. macerans (PGPP 20)	-->	50 ppm chloramphenicol and 200 ppm streptomycin

4.7 COMPATIBILITY OF PGPP BACTERIA WITH THE NITROGEN FIXERS AND PHOSPHATE SOLUBILISERS

The compatibility of PGPP bacteria *viz.*, *Pseudomonas* sp. (PGPP 1), *Bacillus polymyxa* (PGPP 8) and *B.macerans* (PGPP 20) with the authenticated strains of *Rhizobium freidii* (CRS1), *Bradyrhizobium japonicum* (CRS 3), *Azospirillum lipoferum* (AZ 204), *Azotobacter choococcum* (CZR 1) and *Pseudomonas* sp. (PS 2) were tested by cross streak assay (Table 8 and Plate 5). Except *Bacillus mecerans* (PGPP 20) and *Bradyrhizobium japonicum* (CRS 3), all others were compatible with each other.

4.8 EFFECT OF PGPP BACTERIAL INOCULATION ON GROWTH AND YIELD OF RICE (var. CO 43)

A pot culture experiment was conducted (Plate 6) to study the effect of inoculation of PGPP bacteria either alone or in combination with *Azospirillum lipoferum* (AZ 204) on growth and yield of rice (var. CO 43).

Table 8. Compatibility of PGPP bacteria with the nitrogen fixers and phosphate solubilisers.

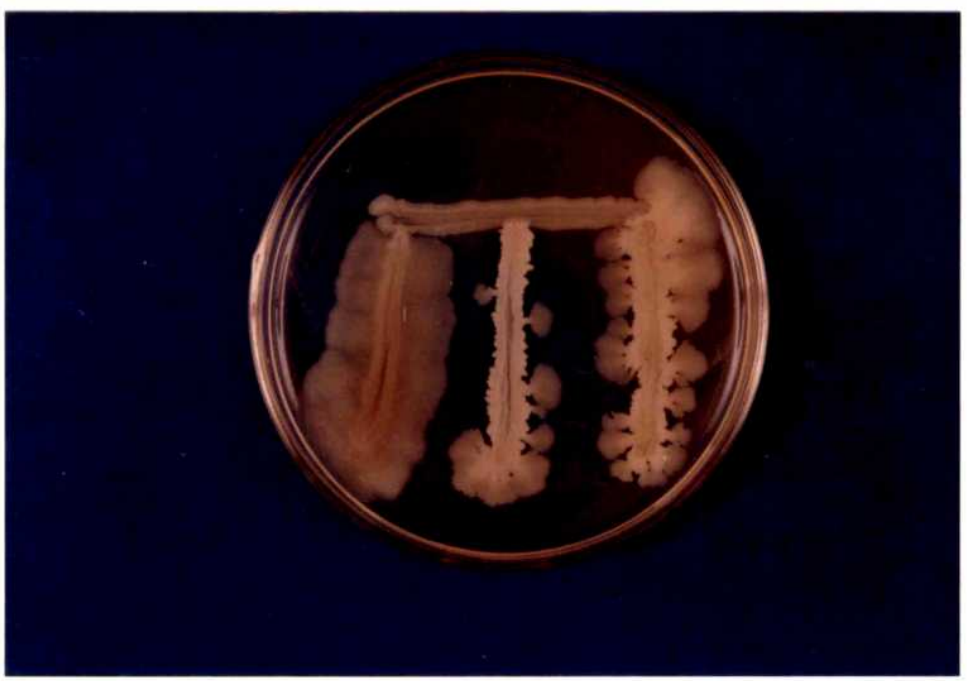
Bacteria	PGPP bacteria		
	Pseudomonas sp. (PGPP 1)	*Bacillus polymyxa* (PGPP 8)	*Bacillus macerans* (PGPP 20)
Rhizobium freidii (CRS 1)	C	C	C
Bradyrhizobium japonicum (CRS 3)	C	C	IC
Azospirillum lipoferum (AZ 204)	C	ND	C
Azotobacter chroococcum (CZR 1)	C	C	C
Pseudomonas sp. (PS 2)	ND	C	C

C -- compatible

IC – incompatible

ND – not determined

Plate 5. Compatibility of plant growth promoting phyllosphere (PGPP) bacteria with *Azotobacter chroococcum* (CZR 1)



A ---> *Azotobacter chroococcum* (CZR 1)

1 ---> *Pseudomonas* sp. (PGPP 1)

2 ---> *Bacillus polymyxa* (PGPP 8)

3 ---> *B. macerans* (PGPP 20)

Bacteria were applied by conventional methods and also through leaf spray. Plant growth and yield parameters like shoot length, root length, tiller production, panicle number and panicle length were recorded besides straw and grain yield. The results are presented in Tables 9 and 10.

4.8.1 Shoot and root length

The shoot length ranged between 40.67 to 56.00 cm at 60 days after sowing (DAS) and 62.33 to 85.50 cm at 120 DAS. Maximum shoot length was recorded by the treatment receiving leaf spray *of Bacillus macerans* (PGPP 20) at 120 DAS (85.50 cm). The root length varied from 26.43 to 39.67 cm at 60 DAS and from 40.63 to 55.57 cm at 120 DAS. The least values in both cases were recorded with uninoculated control plants whereas the highest values were recorded in plants receiving *B.macerans* (PGPP 20) as leaf spray.

4.8.2 Tillers and dry matter production

The number of tillers varied from 3.00 to 4.67 per hill at 60 days after sowing (DAS) and from 2.60 to 5.00 per hill at 120 DAS. The maximum number of tillers per hill (5.00) at 120 DAS was recorded by the treatments receiving leaf spray of *Azospirillum lipoferum* (AZ 204), *Bacillus macerans* (PGPP 20) and also in plants inoculated with both *Azospirillum lipoferum* (AZ 204) and *Bacillus macerans* (PGPP 20) by leaf spray and conventional methods. Statistically there was no significant differences among the treatments for tiller production. The drymatter production ranged between 13.20 and 17.00 g/pl. and among various treatments the highest dry matter

Plate 6. Effect of PGPI' bacterial inoculatiaon on rice (var. CO 43) under pot culture condition

Table 9. Effect of PGPP bacterial inoculation on growth of rice (var. CO 43)

Treatments	Shoot length (cm)		Root length (cm)		Tillers (no./hill)		Dry matter production (g/pl.)
	60 DAS	120 DAS	60 DAS	120 DAS	60 DAS	120 DAS	
Uninoculated control	46.33	77.00	26.43	40.63	4.67	4.00	13.30
CMA							
Azospirillum lipoferum (AZ 204)	42.67	66.67	28.63	42.43	3.67	4.30	13.20
Pseudomonas sp (PGPP 1)	49.67	74.00	31.03	43.93	3.67	4.00	13.40
Bacillus macerans(PGPP 20)	42.83	63.67	30.73	44.70	3.67	4.30	15.13
AZ 204 + PGPP 1	48.67	68.00	31.67	45.23	4.00	3.50	15.10
AZ 204 + PGPP 20	46.17	70.50	31.70	48.37	3.67	3.60	15.03
Leaf spray (LS)							
AZ 204	56.00	83.00	38.07	52.40	4.33	5.00	15.33
PGPP 1	46.33	77.33	36.77	49.83	4.00	4.50	16.33
PGPP 20	49.83	85.50	39.67	55.57	3.67	5.00	17.00
AZ 204 + PGPP 1	49.50	84.25	36.53	50.10	4.00	4.30	16.03
AZ 204 + PGPP 20	42.00	66.17	36.77	52.17	3.67	4.50	14.30
CMA + LS							
AZ 204	49.17	55.00	36.13	50.43	4.33	4.00	15.40
PGPP 1	40.67	79.25	38.40	53.13	3.33	2.60	14.97
PGPP 20	41.17	66.00	37.80	49.47	3.67	3.60	15.00
AZ 204 + PGPP 1	41.67	66.67	36.23	53.10	3.00	4.00	15.20
AZ 204 + PGPP 20	47.67	62.33	36.43	51.00	3.67	5.00	13.77
SEd	5.25	12.40	0.95	1.54	0.95	1.35	0.48
CD	NS	NS	1.93	3.14	NS	NS	0.97

DAS – Days after sowing

CMA [Conventional methods of application] = {Seed treatment + Seedling root dip + Soil application }

production was recorded by foliar application of *Bacillus macerans* (PGPP 20) (17.00 g/pl) followed by *Pseudomonas sp.* (PGPP 1) (16.33 g/pl).

4.8.3 Panicle number and panicle length

The panicle number varied from 2.33 to 5.00 per plant and panicle length ranged between 20.50 and 22.70 cm and maximum being in foliar inoculated plants with *Bacillus macerans* (PGPP 20). However, there was no significant difference among the treatments for both the characters.

4.8.4 Straw yield

Straw yield in various treatments ranged between 14.10 and 18.50 g/pl. Maximum straw yield was recorded in plants inoculated with both *Azospirillum lipoferum* (AZ 204) and *Bacillus macerans* (PGPP 20) though leaf spray and conventional methods of application.

4.8.5 Grain yield

Grain yield varied from 10.30 to 12.07 g/pl. and foliar inoculation of *Bacillus macerans* (PGPP 20) recorded the maximum yield (12.07 g/pl.) followed by leaf spray of *Azospirillum lipoferum* (12.02 g/pl.)[Fig.6].

4.8.6 Effect of PGPP bacterial inoculation on chlorophyll, nitrogen and phosphorus contents of plants

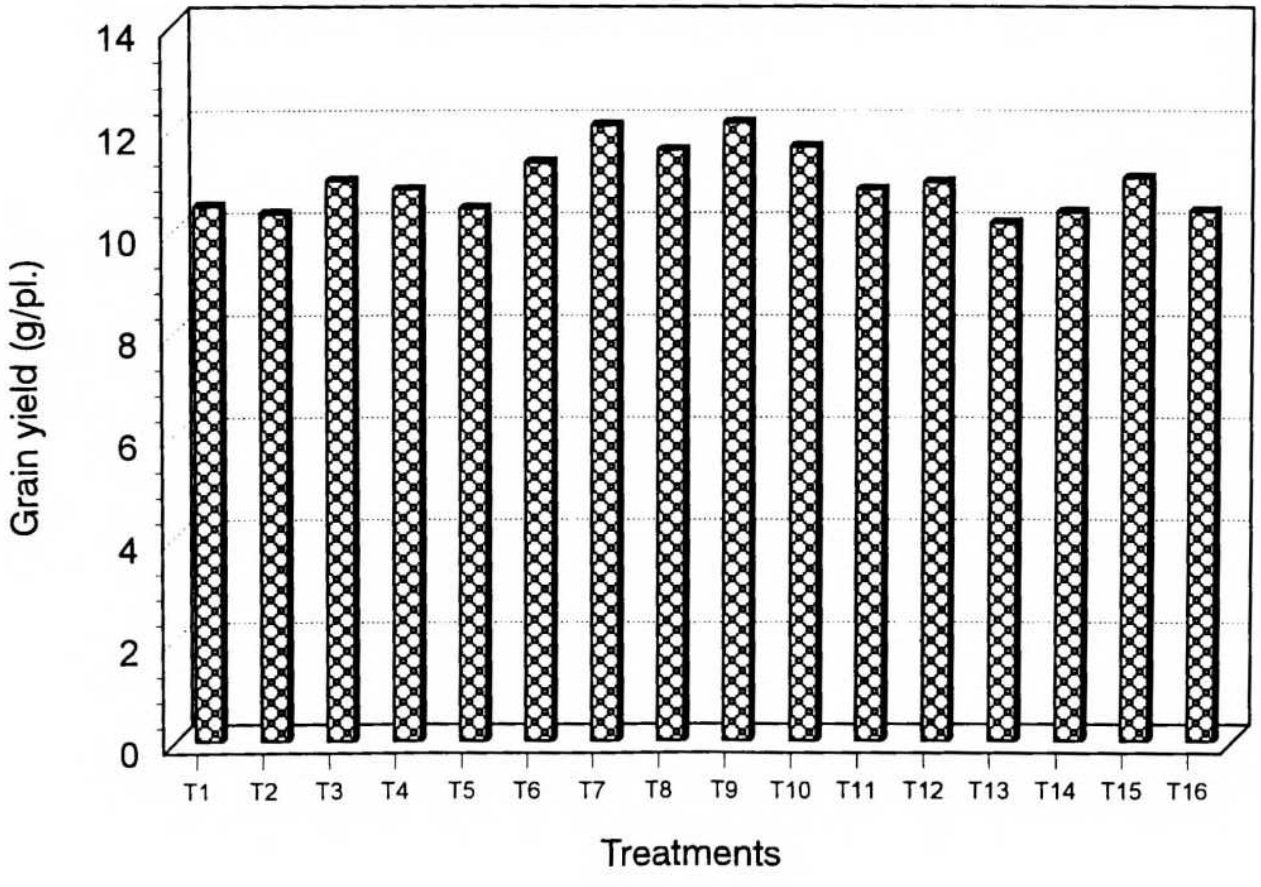
The plant samples from different treatments were analysed for its chlorophyll content during the growth stage and for N and P contents after harvest. The results are presented in Table 11 and Fig.7.

Table 10. Effect of PGPP bacterial inoculation on yield of rice (var. CO 43)

Treatments	Panicles (no./pl.)	Panicle length (cm)	Straw yield (g/pl.)	Grain yield (g/pl.)
Uninoculated control	3.33	20.65	14.50	10.45
CMA				
Azospirillum lipoferum (AZ 204)	4.00	20.50	14.10	10.30
Pseudomonas sp (PGPP 1)	4.00	21.70	15.65	10.94
Bacillus macerans(PGPP 20)	3.66	21.90	16.40	10.77
AZ 204 + PGPP 1	3.66	20.80	17.00	10.40
AZ 204 + PGPP 20	3.33	21.95	16.50	11.30
Leaf spray (LS)				
AZ 204	4.33	22.00	18.00	12.02
PGPP 1	4.66	22.10	17.50	11.54
PGPP 20	5.00	22.70	18.10	12.07
AZ 204 + PGPP 1	4.00	20.80	17.65	11.60
AZ 204 + PGPP 20	4.00	22.00	16.90	10.77
CMA + LS				
AZ 204	3.66	21.00	14.80	10.91
PGPP 1	2.33	20.90	15.73	10.11
PGPP 20	3.66	21.30	18.00	10.33
AZ 204 + PGPP 1	3.66	21.50	18.10	11.00
AZ 204 + PGPP 20	4.66	21.75	18.50	10.35
SEd	0.87	1.20	1.83	0.21
CD	NS	NS	NS	0.42

CMA [Conventional methods of application] = {Seed treatment + Seedling root dip + Soil application }

Fig.6. Effect of PGPP bacterial inoculation on grain yield of rice (var. CO 43)



Treatments

T1=Uninoculated control

Conventional methods of application (CMA)

T2=*Azospirillum lipoferum* (AZ 204)

T3=*Pseudomonas* sp. (PGPP 1)

T4=*Bacillus macerans* (PGPP 20)

T5=AZ 204 + PGPP 1

T6=AZ 204 + PGPP 20

Leaf spray (LS)

T7=AZ 204

T8=PGPP 1

T9=PGPP 20

T10=AZ 204 + PGPP 1

T11=AZ 204 + PGPP 20

CMA + LS

T12=AZ 204

T13=PGPP 1

T14=PGPP 20

T15=AZ 204 + PGPP 1

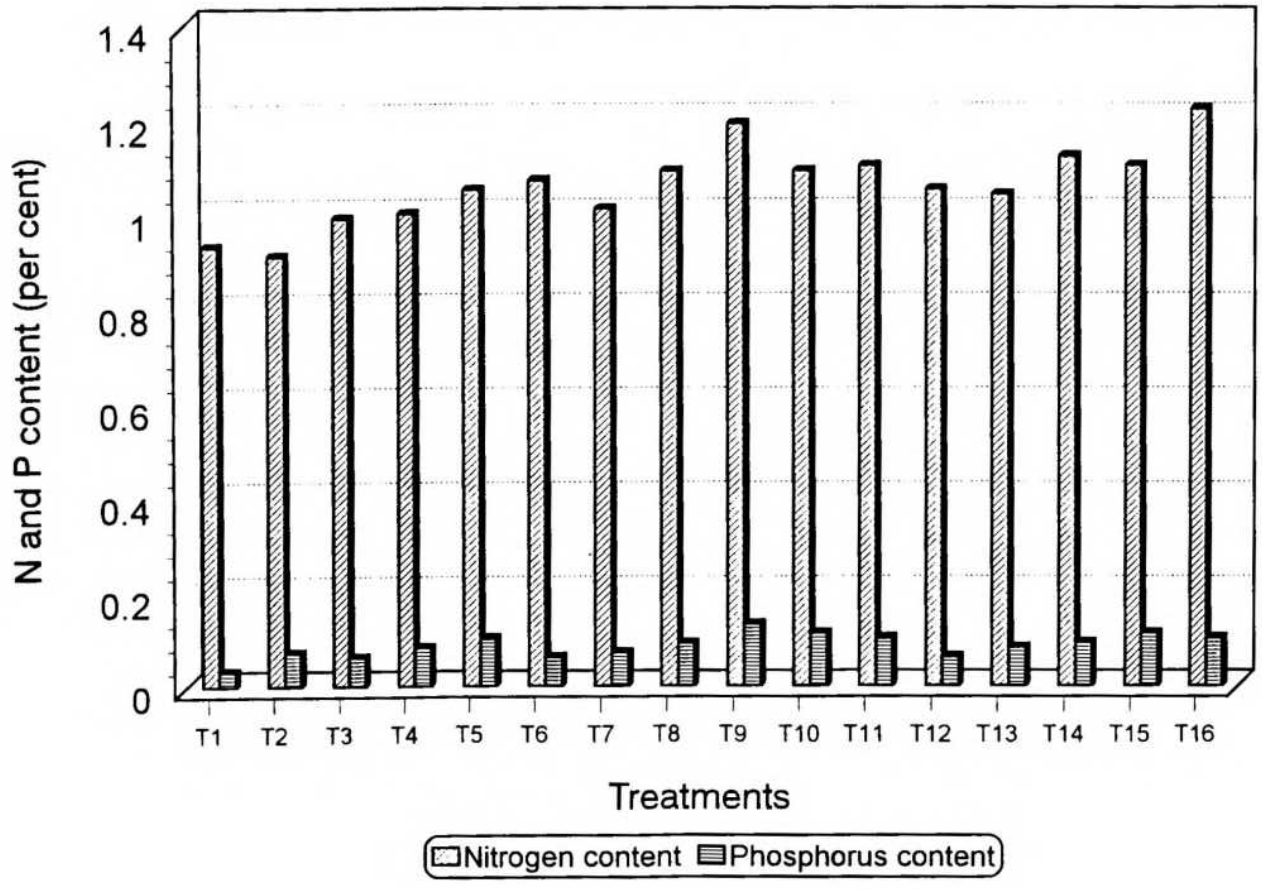
T16= AZ 204 + PGPP 20

Table 11. Effect of PGPP bacterial inoculation on chlorophyll, nitrogen and phosphorus content of rice (var. CO 43)

Treatments	Total chlorophyll content (mg g^{-1} tissue)	Plant nitrogen (%)	Plant phosphorus (%)
Uninoculated control	0.144	0.93	0.03
CMA			
Azospirillum lipoferum (AZ 204)	0.165	0.91	0.07
Pseudomonas sp (PGPP 1)	0.165	0.99	0.06
Bacillus macerans(PGPP 20)	0.314	1.00	0.08
AZ 204 + PGPP 1	0.286	1.05	0.10
AZ 204 + PGPP 20	0.367	1.07	0.06
Leaf spray (LS)			
AZ 204	0.334	1.01	0.07
PGPP 1	0.286	1.09	0.09
PGPP 20	0.443	1.19	0.13
AZ 204 + PGPP 1	0.394	1.09	0.11
AZ 204 + PGPP 20	0.391	1.10	0.10
CMA + LS			
AZ 204	0.371	1.05	0.06
PGPP 1	0.286	1.04	0.08
PGPP 20	0.278	1.12	0.09
AZ 204 + PGPP 1	0.427	1.10	0.11
AZ 204 + PGPP 20	0.334	1.22	0.10
SEd	0.026	0.17	0.04
CD	0.537	0.34	NS

CMA [Conventional methods of application] = {Seed treatment + Seedling root dip + Soil application }

Fig.7. Effect of PGPP bacterial inoculation on N and P content of rice (var. CO 43)



Treatments

T1=Uninoculated control

Conventional methods of application (CMA)

T2=*Azospirillum lipoferum* (AZ 204)

T3=*Pseudomonas* sp. (PGPP 1)

T4=*Bacillus macerans* (PGPP 20)

T5=AZ 204 + PGPP 1

T6=AZ 204 + PGPP 20

Leaf spray (LS)

T7=AZ 204

T8=PGPP 1

T9=PGPP 20

T10=AZ 204 + PGPP 1

T11=AZ 204 + PGPP 20

CMA + LS

T12=AZ 204

T13=PGPP 1

T14=PGPP 20

T15=AZ 204 + PGPP 1

T16= AZ 204 + PGPP 20

4.8.6.1 Total chlorophyll content

There was a significant difference between treatments in increasing leaf chlorophyll content. Single inoculation of *Bacillus macerans* (PGPP 20) by leaf spray alone recorded the maximum chlorophyll content (0.443 mg g-1 tissue) followed by dual inoculation of *Azospirillum lipoferum* (AZ 204) + *Pseudomonas* sp. (PGPP 1) by conventional methods and leaf spray (0.427 mg g-1 tissue). Least chlorophyll content was recorded in uninoculated control plants (0.144 mg g-1 tissue).

4.8.6.2 Plant nitrogen content

There was a significant difference among the treatments in increasing plant N content. Dual inoculation of *Azospirillum lipoferum* (AZ 204) and *Bacillus macerans* (PGPP 20) by conventual methods and leaf spray recorded higher plant N content (1.22 %) closely followed by leaf inoculation of *Bacillus macerans* (1.19%).

4.8.6.3 Plant phosphorus content

Plant P content varied from 0.03 to 0.13 per cent. Maximum P content was recorded in plants sprayed with *Bacillus macerans* (PGPP 20) alone (0.13%). However, differences observed between treatments was statistically not significant.

4.8.7 Effect of PGPP bacterial inoculation on phyllosphere population of rice.

The result of phyllosphere population of *Azospirillum lipoferum* (AZ 204), *Pseudomonas sp.* (PGPP 1) and *Bacillus macerans* (PGPP 20)

enumerated before and after leaf spray is presented in Table 12. The control treatment showed very low population of these bacteria in the phyllosphere.

4.8.7.1 *Azospirillum lipoferum* (AZ 204)

The population of *Azospirillum lipoferum* (AZ 204) was enumerated by MPN method in semisolid malic acid medium at different intervals. In all the treatments higher population was recorded immediately after the leaf spray and declined rapidly before next spray. The maximum final population (4.90×10^5 MPN/cm^2) was recorded in the leaf inoculation of *A.lipoferum* (AZ 204) followed by dual inoculation of *A.lipoferum* (AZ 204) and *Bacillus macerans* (PGPP 20) by conventional and leaf spray methods (4.50×10^5 MPN/cm^2).

4.8.7.2 *Pseudomonas sp.* (PGPP 1)

Pseudomonas sp. (PGPP 1) population was enumerated using chloramphenical (200 ppm) and streptomycin (200 ppm) incorporated medium. The maximum population (3.4×10^5 cfu/cm^2) was recorded in dual inoculation of *Pseudomonas* sp. (PGPP 1) and *Azospirllum lipoferum* (AZ 204) by conventional methods and leaf spray followed by foliar inoculation of *Pseudomonas sp.* (PGPP 1) alone (3.23×10^5 cfu/cm^2).

4.8.7.3 *Bacillus macerans* (PGPP 20)

The *B. macerans* (PGPP 20) population was enumerated by streptomycin (200 ppm) incorporated medium. Maximum population was

Table 12. Population of phyllosphere bacteria on rice inoculated with PGPP bacteria.

Bacteria	Population before and after foliar inoculation (X 10^5 per cm^2)								
	I Spray		II Spray		III Spray		IV Spray		Final count
	BS	AS	BS	AS	BS	AS	BS	AS	
Azospirillum lipoferum (AZ 204)-LS	0.40	5500.00	3.07	6100.00	3.00	5600.00	4.10	6900.00	4.90
AZ 204 +PGPP 1 –LS	1.10	5400.00	2.73	7000.00	2.90	6967.00	2.80	6500.00	3.73
AZ 204+PGPP 20 –LS	0.60	5300.00	2.87	6633.00	3.10	6533.00	3.40	6100.00	3.87
AZ 204 – CMA+LS	0.43	4900.00	3.00	6500.00	2.80	5500.00	3.70	6400.00	4.40
AZ204 +PGPP 1 – CMA+LS	0.30	6100.00	2.70	5967.00	2.70	5767.00	3.90	5733.00	4.40
AZ 204+PGPP 20 –CMA+LS	0.47	6000.00	2.90	6000.00	2.83	6067.00	3.70	5500.00	4.50
Control	0.27	0.37	0.53	0.43	0.37	0.67	0.43	0.37	0.43
Pseudomonas sp. (PGPP 1)-LS	0.30	4567.00	1.90	4900.00	2.50	5100.00	3.07	5300.00	3.23
PGPP 1+AZ 204 –LS	0.40	4033.00	1.97	4500.00	2.37	4967.00	2.57	5500.00	3.00
PGPP 1 – CMA+LS	0.30	4500.00	2.07	4800.00	2.30	4500.00	2.80	5500.00	2.90
PGPP 1 +AZ 204 –CMA+LS	0.20	5500.00	2.50	5200.00	2.60	4700.00	3.10	5167.00	3.40
Control	0.34	0.67	0.43	0.50	0.53	0.37	0.47	0.53	0.43
Bacillus polymyxa (PGPP 8) – LS	0.40	4500.00	2.80	5100.00	3.80	6067.00	3.93	5800.00	3.00
PGPP 8 + AZ 204 – LS	0.33	5800.00	3.10	5900.00	2.90	7100.00	4.10	5967.00	2.50
PGPP 8 – CMA+LS	0.47	4900.00	3.30	6300.00	3.73	6800.00	3.70	5100.00	2.67
PGPP 8 + AZ 204 – CMA+LS	0.30	5100.00	3.20	5700.00	5.17	5933.00	3.50	4900.00	2.73
Control	0.40	0.47	0.33	0.53	0.67	0.53	0.60	0.47	0.53

CMA[conventional methods of application] - { Seed treatment + Seedling root dip+ Soil application }; LS – Leaf spray ;
BS - Before spray ; AS – After spray

observed in *B. macerans* (PGPP 20) incoculated plants by leaf spray alone (3.00×10^5 cfu/cm^2) followed by dual inoculation of *B.macerans* (PGPP 20) and *Azospirillum lipoferum* (AZ 204) by conventional and leaf spray methods (2.73×10^5 cfu/cm^2)

4.9. EFFECT OF PGPP BACTERIAL INOCULATION ON GROWTH AND YIELD OF COTTON (var. LRA 5166)

A pot culture experiment was conducted to study the effect of inoculation of PGPP bacteria *viz.*, *Pseudomnas* sp. (PGPP 1) and *Bacillus macerans* (PGPP 20) either alone or in combinatin with *Azotobacter chroococcum* (CZR 1) *and Azospirillum macerans* (AZ 204) on growth and yield of cotton var. LRA 5166 (Plate 7).

Plant growth and yield parameters like shoot and root length, leaves number, dry matter production, sympodial branches, total number of bolls, boll weight and seed cotton yield were recorded. The results are presented in Tables 13 and 14.

4.9.1 Shoot and root length

The shoot length of cotton plants in various treatments ranged between 21.17 to 27.00 cm at 60 days after sowing (DAS) and 60.66 to 73.34 cm at 120 DAS. The maximum shoot length was recorded by the application of *Azotobacter chroococcum* (CZR 1) by both conventional methods and leaf spray (73.34 cm) at 120 DAS (Table 13). However, satistically no significant

Plate 7. Effect of PGPP bacterial inoculaiton on cotton (var. LRA 5166) under pot culture condition

Table 13. Effect of PGPP bacterial inoculation on growth of cotton (var. LRA 5166)

Treatments	Shoot length (cm)		Root length (cm)		Leaves (no./pl.)		Dry matter production (g/pl.)	
	60 DAS	120 DAS	60 DAS	120 DAS	60 DAS	120 DAS	60 DAS	120 DAS
Uninoculated control	22.67	65.00	18.70	31.80	7.67	28.34	1.90	3.80
CMA								
Azospirillum lipoferum (AZ 204)	21.17	67.30	21.05	37.50	7.67	31.34	2.25	4.76
Azotobacter chroococcum (CZR 1)	24.23	68.30	22.00	39.88	7.67	27.34	3.20	6.97
Pseudomonas sp. (PGPP 1)	23.33	72.00	23.75	43.00	7.67	33.34	3.15	6.52
Bacillus macerans (PGPP 20)	24.83	62.33	22.70	42.50	6.67	21.34	3.22	6.95
Leaf spray (LS)								
AZ 204	25.50	65.00	19.79	44.00	6.00	24.67	2.70	4.93
CZR 1	25.67	60.66	20.75	39.60	8.67	24.34	4.58	9.43
PGPP 1	26.00	70.67	21.08	41.00	7.00	23.67	4.10	8.02
PGPP 20	27.00	69.67	23.77	38.00	7.67	34.67	4.25	8.33
CMA + LS								
AZ 204	21.67	63.34	20.08	32.10	6.33	29.67	3.18	6.19
CZR 1	24.17	73.34	21.85	34.67	5.33	26.67	4.05	8.48
PGPP 1	25.50	72.00	24.64	43.70	7.33	27.34	4.37	8.21
PGPP 20	25.67	70.67	23.37	39.50	7.67	28.67	4.31	8.97
SEd	2.54	7.88	0.46	1.81	1.19	7.77	0.08	0.19
CD	NS	NS	0.94	3.73	NS	15.96	NS	0.38

CMA [Conventional methods of application] = {Seed treatment + Soil application }

DAS - Days after sowing

difference was observed both at 60 and 120 DAS. The root length varied from 18.70 to 24.64 cm at 60 DAS and 31.80 to 44.00 cm at 120 DAS. At 60 DAS, application of *Pseudomonas* sp. (PGPP 1) by conventional methods and leaf spray recorded the maximum root length (24.64 cm) followed by leaf spray of *Bacillus macerans* (PGPP 20) (23.77 cm). At 120 DAS, *Azospirillum lipoferum* (AZ 204) inoculation by leaf spray recorded the maximum root length (44.00) followed by conventional and leaf spray inoculation of *Pseudomonas* sp. (43.70 cm).

4.9.2 Number of leaves and drymatter production

The number of leaves per plant ranged between 5.33 and 8.67 at 60 days after sowing (DAS) and from 21.34 to 34.67 at 120 DAS. At 60 DAS, leaf spray of *Azotobacter chroococcum* (CZR 1) recorded the maximum number of leaves (8.67). At 120 DAS, leaf spray of *Bacillus macerans* (PGPP 20) recorded the maximum number of leaves per plant (34.67) followed by *Pseudomonas* sp. (PGPP 1) application by conventional methods (33.34).

The drymatter production varied from 1.90 to 4.58 g/pl. at 60 DAS and from 3.80 to 9.43 g/pl. at 120 DAS. Both at 60 and 120 DAS, foliar application of *Azotobacter chrooococcum* (CZR 1) produced the maximum dry matter of 4.58 and 9.43 g/pl. respectively followed by the conventional inoculation of *Pseudomonas* sp. (4.37 g/pl) at 60 DAS and by conventional methods and leaf spray inoculation of *Bacillus macerans* (PGPP 20) at 120 DAS (8.97 g/pl.).

4.9.3 Sympodial branches

The number of sympodial branches at 60 days after sowing (DAS) ranged between 1.00 and 4.00 per plant whereas at 120 DAS, it varied from 1.67 to 6.33 per plant. Maximum number of sympodial branches per plant (6.33) was observed in the treatments receiving *Azospirillum lipoferum* (AZ 204) and *Bacillus macerans* (PGPP 20) by both conventional and leaf spray methods.

4.9.4 Number of bolls and boll weight

The total number of bolls per plant varied from 18.00 to 29.33, maximum being in the treatment receiving the inoculation of *Bacillus macerans* (PGPP 20) by both conventional methods and leaf spray followed by leaf spray inculation of *Azospirillum lipoferum* (27.67).

The boll weight ranged between 3.60 and 4.50 g/pl. The highest boll weight (4.50 g/pl.) was recorded by inoculating *B.macerans* (PGPP 20) through conventional methods and leaf spray followed by its leaf spray alone. (4.20 g/pl.).

4.9.5. Seed cotton yield

The seed cotton yield varied from 64.80 to 131.99 g/pl. The highest yield was recorded by the conventional and leaf inoculation of *Bacillus macerans* (PGPP 20) followed by *Azospirilum lipeferum* (AZ 204) foliar

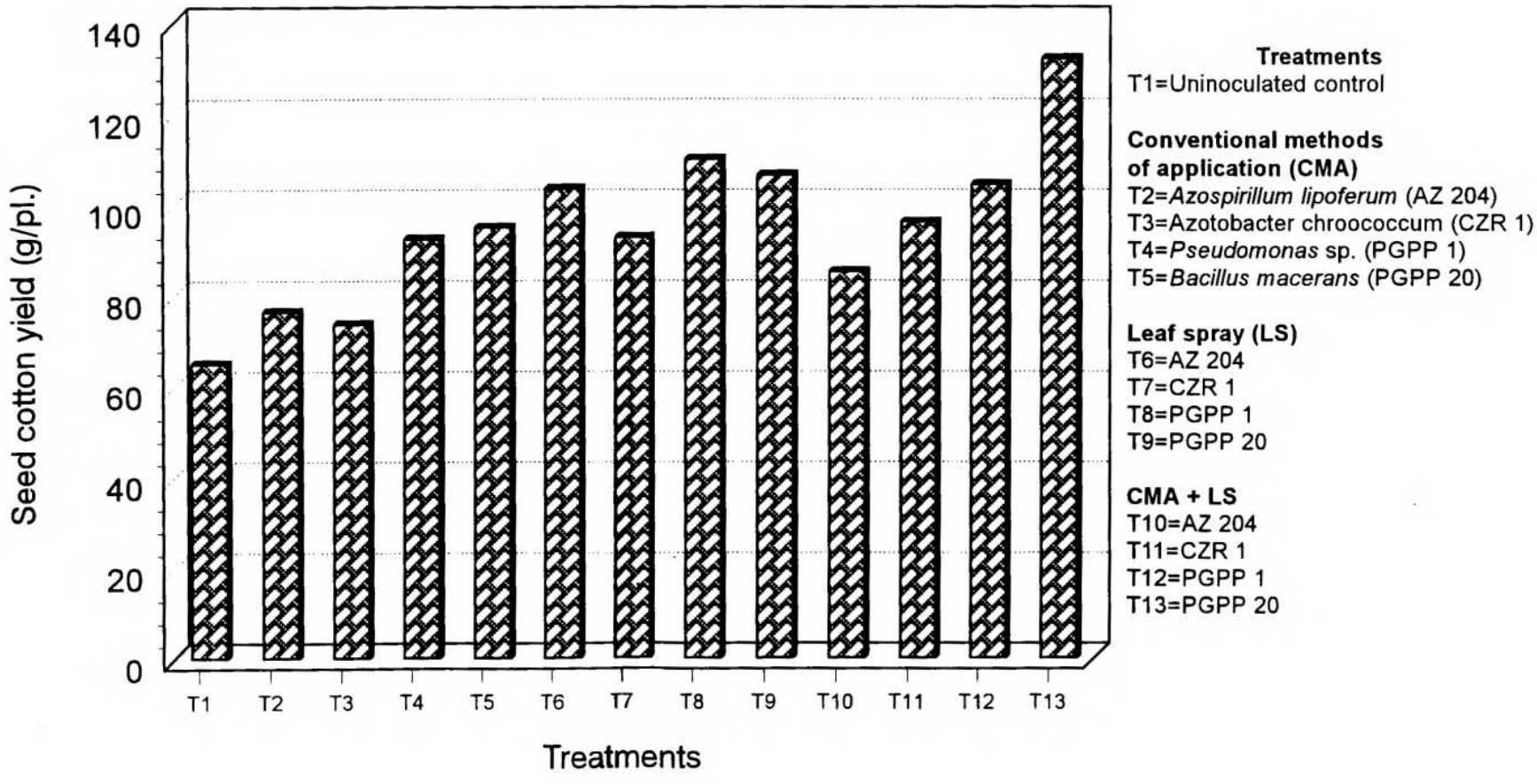
Table 14 . Effect of PGPP bacterial inoculation on yield of cotton (var. LRA 5166)

Treatments	Sympodial brances(no./pl.)		Bolls (no.pl.)	Boll weight g/boll	Seed cotton yield (Kg/ha.)
	60 DAS	120 DAS			
Uninoculated control	2.00	3.67	18.00	3.60	64.80
CMA					
Azospirillum lipoferum (AZ 204)	2.00	4.00	21.00	3.63	76.23
Azotobacter chroococcum (CZR 1)	1.00	1.67	19.00	3.87	73.53
Pseudomonas sp. (PGPP 1)	3.67	3.00	23.67	3.90	92.31
Bacillus macerans (PGPP 20)	2.00	3.67	24.33	3.90	94.89
Leaf spray (LS)					
AZ 204	1.33	2.33	27.67	4.10	113.45
CZR 1	2.67	3.33	23.00	4.03	92.69
PGPP 1	2.67	5.00	27.00	4.07	109.89
PGPP 20	2.33	4.33	25.33	4.20	106.39
CMA + LS					
AZ 204	3.00	6.33	22.00	3.87	85.14
CZR 1	2.00	4.00	24.00	4.00	96.00
PGPP 1	3.00	6.00	25.00	4.17	104.25
PGPP 20	4.00	6.33	29.33	4.50	131.99
SEd	0.90	2.07	1.37	0.12	2.86
CD	NS	NS	2.82	0.25	8.64

CMA [Conventional methods of application] = {Seed treatment + Soil application }

DAS - Days after sowing

Fig.8. Effect of PGPP bacterial inoculation on cotton (var. LRA 5166)



application (113.45 g/pl.). The lowest yield (64.80 g/pl.) was recorded in uninoculated control plants (Fig.8).

4.9.6. Effect PGPP bactrial inoculation on chlorophyll, nitrogen and phosphorus contents of cotton plants.

The plant samples from different treatments were analysed for their chlorophyll content during growth stage and N and P contents after harvest. The results are presented in Table 15 and Fig.9.

4.9.6.1 Total chlorophyll content

The total chlorophyll content ranged between 0.198 and 0.489 mg g^{-1} tissue. The highest chlorophyll content was recorded by the treatment receiving *Bacillus macerans* (PGPP 20) by conventional methods and leaf spray followed by *Azotobacter chroococcum* (AZRI) by same methods (0.411 mg g^{-1} tissue). The least chlorophyll content was recorded in uninoculated control leaves (0.198 mg g^{-1} tissue).

4.9.6.2. Plant nitrogen content

The plant N content varied from 0.90 to 1.39%. The highest value was recorded in plants inoculated with *Bacillus mecerans* (PGPP 20) by foliar conventional methods and leaf spray followed by its inoculation with leaf spray alone (1.3%). The lowest plant nitrogen content was recorded in uninoculated control (0.90%) plants.

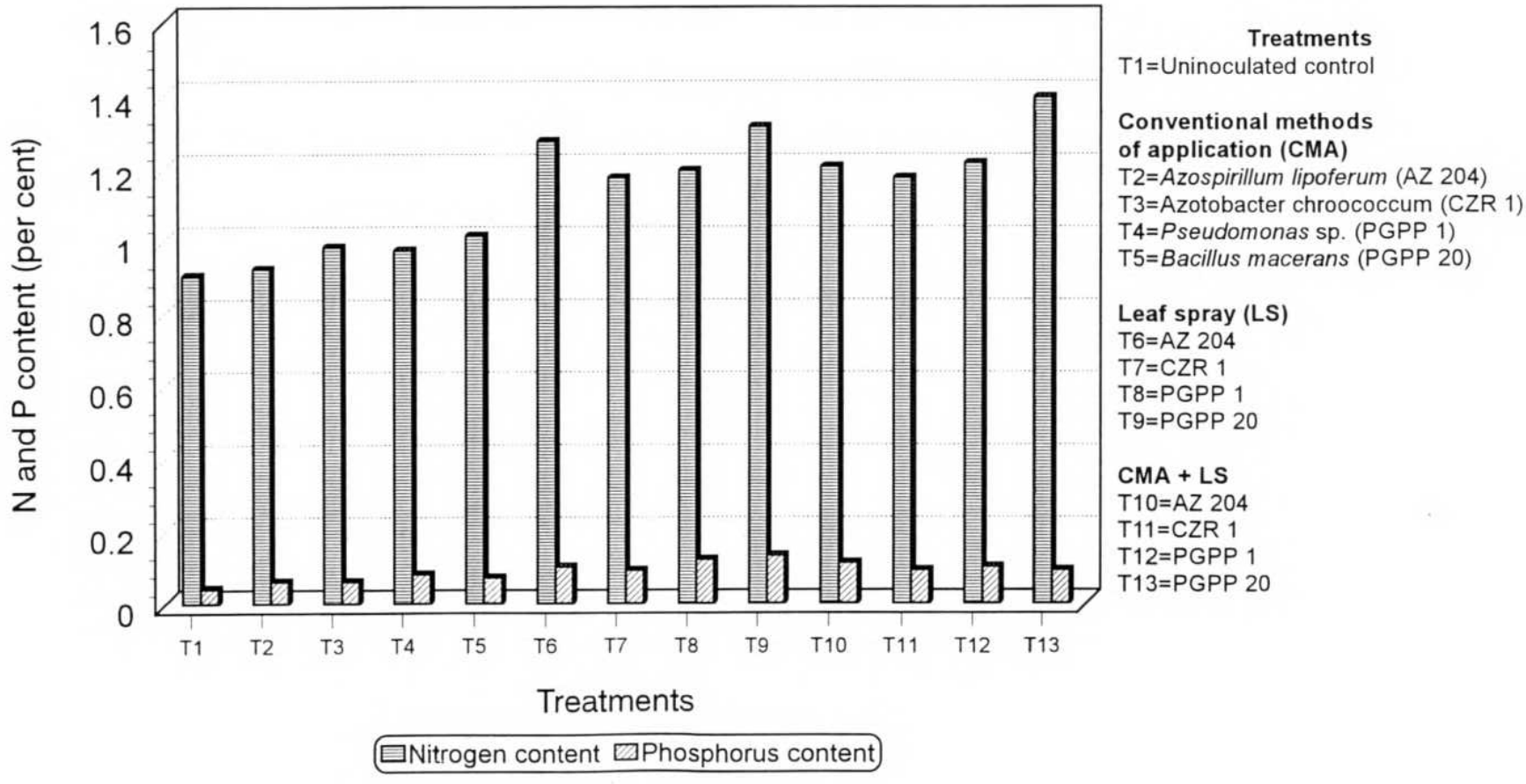
Table 15. Effect of PGPP bacterial inoculation on chlorophyll, nitrogen and phosphorus content of cotton (var. LRA 5166).

Treatments	Total chlorophyll content (mg g^{-1} tissue)	Plant nitrogen content (%)	Plant phosphorus content (%)
Uninoculated control	0.198	0.90	0.04
CMA			
Azospirillum lipoferum (AZ 204)	0.213	0.92	0.06
Azotobacter chroococcum (CZR 1)	0.278	0.98	0.06
Pseudomonas sp. (PGPP 1)	0.305	0.97	0.08
Bacillus macerans (PGPP 20)	0.303	1.01	0.07
Leaf spray (LS)			
AZ 204	0.270	1.27	0.10
CZR 1	0.290	1.17	0.09
PGPP 1	0.297	1.19	0.12
PGPP 20	0.311	1.31	0.13
CMA + LS			
AZ 204	0.357	1.20	0.11
CZR 1	0.411	1.17	0.09
PGPP 1	0.378	1.21	0.10
PGPP 20	0.489	1.39	0.09
SEd	0.018	0.09	0.01
CD	0.036	0.19	0.03

CMA [Conventional methods of application] = {Seed treatment + Soil application }

DAS - Days after sowing

Fig.9. Effect of PGPP bacterial inoculation on N and P content of cotton (var. LRA 5166)



4.9.6.3. Plant phosphorus content

The plant P content varied from 0.04 to 0.14%, maximum being in the plants sprayed with *Bacillus macerans* (PGPP 20) followed by foliar inoculation of *Pseudomonas* sp. (1.12%). The uninoculated control plants recorded the least phosphorous content (0.04%).

4.9.7 Effect of PGPP bacterial inoculation on phyllosphere population of cotton

The phyllosphere population of *Azospirillum lipoferum* (AZ 204), *Azotobacter Chroococcum* (CZRI), *Pseudomonas* sp. (PGPP 1) and *Bacillus macerans* (PGPP 20) on cotton leaves were enumerated before and after leaf spray and the results are presented in Table 16. In all the cases, the population was maximum immediately after foliar spray and declined sharply before the next spray.

4.9.7.1. *Azospirillum lipoferum* (AZ 204)

The higher population (3.17 x 10^5 MPN/cm^2) of *Azospirillum lipoferum* (AZ 204) was recorded in leaf spray inoculation, whereas the inoculation by conventional methods and leaf spray recorded the lower population (3.03 x 10^5 MPN/cm^2) of *A.lipoferum* (AZ 204).

4.9.7.2. *Azotobacter Chroococcum* (CZRI)

Inoculation by both conventional methods and leaf spray recorded higher population (3.30 x 10^5 cfu/cm^2) *of Azotobacter Chroococcum* (CZRI) than the leaf spray inoculation alone (3.10 x 10^5 cfu/cm^2).

Table 16. Population of phyllosphere bacteria on cotton inoculated with PGPP bacteria.

Bacteria	Population before and after foliar inoculation (X 10^5 per cm^2)										
	I Spray		II Spray		III Spray		IV Spray		V Spray		Final count
	BS	AS	BS	AS	BS	AS	BS	AS	BS	AS	
Azospirillum lipoferum (AZ 204) – LS	0.10	2700.00	1.80	4600.00	2.10	4800.00	2.23	5400.00	1.87	5700.00	3.17
AZ 204 – CMA+LS	0.13	2866.00	1.93	4800.00	2.00	5200.00	2.30	5600.00	1.93	5833.0	3.03
Azotobacter chroococcum (CZR 1)- LS	0.20	3633.00	1.40	4100.00	2.90	5366.00	3.10	6200.00	3.23	6600.00	3.10
CZR 1 - CMA+LS	0.17	3400.00	1.63	4300.00	2.63	5400.00	3.03	6100.00	3.17	6300.00	3.30
Pseudomonas sp. (PGPP – 1)-LS	0.13	3300.00	0.47	4333.00	0.30	4100.00	0.70	4400.00	1.23	4800.00	0.97
PGPP 1 - CMA+LS	0.27	4400.00	0.33	4700.00	0.70	4866.00	0.83	5400.00	1.47	5800.00	1.80
Bacillus macerans (PGPP – 20)-LS	0.10	2900.00	1.50	3400.00	1.73	4400.00	1.80	4800.00	2.13	5100.00	2.47
PGPP 20 - CMA+LS	0.23	3300.00	1.43	3900.00	1.87	4800.00	1.97	5900.00	2.30	6200.00	2.83

CMA[conventional methods of application] - { Seed treatment + soil application }; LS – Leaf spray ;

BS - Before spray ; AS – After spray

4.9.7.3 *Pseudomonas* sp. (PGPP 1)

Here also, inoculation by conventional methods and leaf spray recorded the higher population of (1.80 x 10^5 cfu/cm^2) *Psendomonas* sp. (PGPP 1) than the foliar inoculation alone (0.97 x 10^5 cfu/cm^2).

4.9.7.3 *Bacillus macerans* (PGPP 20)

Higher population of *Bacillus macerans* (PGPP 20) was observed (2.83 x 10^5 cfu/cm^2) in plants inoculated by conventional and leaf spray methods than the foliar inoculation with *Bacillus macerans* (PGPP 20) alone (2.47 x 10^5 cfu/cm^2).

4.10 EFFECT OF PGPP BACTERIAL INOCULATION ON GROWTH AND YIELD OF SOYBEAN (var. C0 1)

A field trial was conducted to study the effect of inoculation of PGPP bacteria like *Psendomonas* sp. (PGPP 1) and *Bacillus polymyxa* (PGPP 8) either alone or in combination with *Rhizobium freidii* (CRS 1), phosphate solubilising *Psendomonas* sp. (PS 2) on growth and yield of soybean (Plate 8). Observations were made on plant growth and yield parameters. Shoot and root length, dry matter production, nodule number per plant. nodule dry weight, number of pods per plant and grain yield were recorded and the results are presented in Tables 17 and 18.

4.10.1 Shoot and root length

The shoot length varied from 51.50 to 66.60 cm at 45 days after sowing (DAS), the maximum value being recorded in plants sprayed with

Plate 8. Effect of PGPP bacterial inoculationon soybean (var. CO 1) under field condition

Table 17. Effect of PGPP bacterial inoculation on growth of soybean (var. CO 1)

Treatments	Shoot length (cm)		Root length (cm)		Dry matter production (g/pl.)
	45 DAS	90 DAS	45 DAS	90 DAS	
Uninoculated control	62.20	84.50	11.60	19.90	8.00
CMA					
Bacillus polymyxa (PGPP 8)	63.30	86.10	10.50	21.30	6.60
Pseudomonas sp. (PGPP 1)	58.70	78.70	12.80	19.20	6.30
Pseudomonas sp. (PS 2)	51.50	70.50	12.10	18.80	6.00
Rhizobium freidii (CRS 1)	62.60	79.60	11.40	21.60	8.00
Leaf spray (LS)					
PGPP 8	62.30	77.00	11.70	19.20	8.30
PGPP 1	66.60	82.20	13.50	19.90	7.70
PS 2	62.60	79.20	10.80	20.40	7.30
CRS 1	64.40	84.30	11.80	22.00	8.30
CMA + LS					
CRS 1 + PGPP 8	58.50	77.30	11.80	20.20	8.30
CRS 1 + PGPP 1	62.40	77.80	11.50	23.50	7.30
CRS 1 + PS 2	62.50	81.40	11.10	17.50	7.70
SEd	6.39	15.47	1.95	1.95	3.27
CD	NS	NS	NS	NS	NS

CMA [Conventional methods of application] = {Seed treatment + Soil application }

DAS - Days after sowing

Pseudomonas sp. (PGPP 1). At 90 DAS, the maximum shoot length (86.10 cm) was recorded by conventional methods of application of *Bacillus polymyxa* (PGPP 8). Similarly, the root length varied from 10.50 to 19.50 cm at 45 DAS, the maximum length being recorded by leaf spray of *Pseudomonas* sp. (PGPP 1). At 90 DAS the maximum length (23.50 cm) was recorded by dual inoculation of *Pseudomonas* sp. (PGPP 1) along with *Rhizobium freidii* (CRS 1) by conventional methods and leaf spray application. However, no significant difference was observed between treatments for both shoot and root length.

4.10.2 Dry matter production

The plant dry matter production in various treatments ranged from 6.00 to 8.30 g/pl. Maximum drymatter production was recorded by leaf spray of *Rhizobium freidii* (CRS 1) alone and also by its combination with *Bacillus polymyxa* (PGPP 8) when applied by conventional methods and leaf spray. There was no significant difference among the treatments in increasing the dry matter production.

4.10.3 Nodule number and nodule dry weight

The nodule number in various treatments ranged between 8.20 and 19.30 per plant at 45 days after sowing (DAS) and between 6.10 and 22.00 per plant at 90 DAS (Table 18). The higher nodule number at 90 DAS (22.30 per plant) was recorded by *Rhizobium freidii* (CRS 1) inoculation by conventional methods [Fig.10]. The nodule dry weight varied from 0.077 to 0.206 g per cent at 45 DAS and from 0.060 to 182 g per plant at 90 DAS. The

maximum value at 90 DAS (0.182 g/pl) was recorded by conventional inoculation of *Rhizobium freidii* (CRS 1)

4.10.4 Pod number

The number of pods in various treatment ranged between 43.00 and 62.10 per plant, the maximum (62.10 per plant) being recorded in plants having dual inoculation of *Rhizobium freidii* (CRS 1) and *Bacillus polymyxa* (PGPP 8) by conventional methods and leaf spray [Fig.10].

4.10.5 Grain yield

The grain yield varied from 857.78 to 1394.82 kg/ha [Fig.11]. The highest yield was recorded by the treatment receiving *Bacillus polymyxa* (PGPP 8) by conventional methods followed by dual inoculation of *Rhizobium freidii* (CRS 1) and *Pseudomonas* sp. (PGPP 1) by conventional methods and leaf spray (1315. 18 kg/ha).

4.10.6 Effect of PGPP bacterial inoculation on chlorophyll, nitrogen and phosphorus contents of soybean plants

The plant samples from various treatments were analysed for its total chlorophyll content during growth stage and for N and P contents after harvest. The results are presented in Table 19 and Fig.12.

4.10.6.1 Total chlorophyll content

The total chlorophyll content ranged between 0.145 and 0.398 mg g^{-1} tissue. The highest value was recorded in the leaves inoculated with *Bacillus*

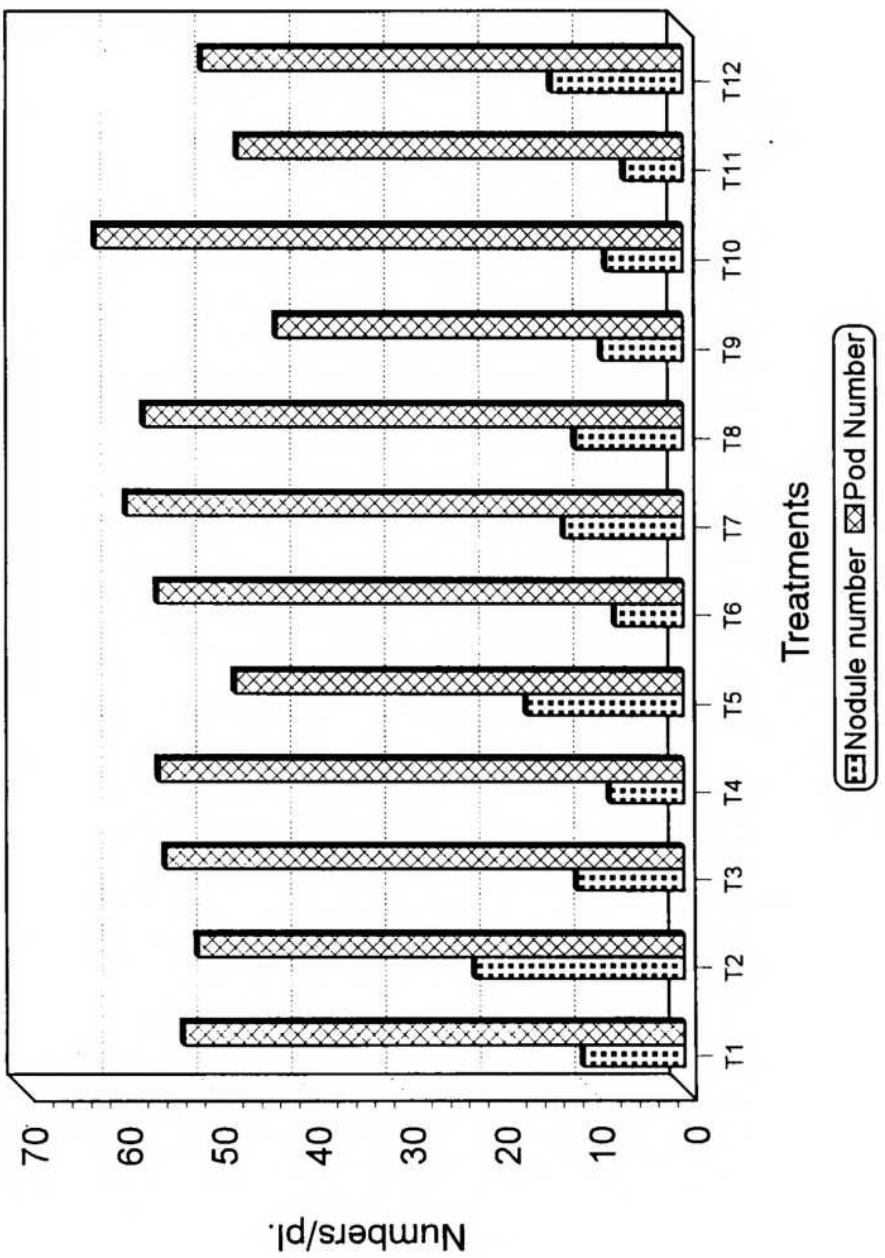
Table 18. Effect of PGPP bacterial inoculation of on yield of soybean (var. CO 1).

Treatments	Nodules (no./pl.)		Nodule dry weight (g/pl.)		Pods (no./pl.)	Grain yield (Kg/ha)
	45 DAS	90 DAS	45 DAS	90 DAS		
Uninoculated control	10.10	10.60	0.082	0.075	52.90	1054.82
CMA						
Bacillus polymyxa (PGPP 8)	8.50	11.20	0.097	0.111	54.80	1394.82
Pseudomonas sp. (PGPP 1)	11.40	7.70	0.097	0.093	55.50	1149.63
Pseudomonas sp. (PS 2)	8.20	16.50	0.077	0.154	47.40	984.44
Rhizobium freidii (CRS 1)	18.20	22.00	0.109	0.182	51.40	1188.15
Leaf spray (LS)						
PGPP 8	12.30	7.10	0.127	0.067	55.60	857.78
PGPP 1	10.80	12.50	0.104	0.123	58.90	1047.04
PS 2	14.70	11.30	0.142	0.120	57.00	900.00
CRS 1	14.70	8.50	0.141	0.119	43.00	1219.26
CMA + LS						
CRS 1 + PGPP 8	13.50	8.00	0.125	0.101	62.10	1273.34
CRS 1 + PGPP 1	19.30	6.10	0.206	0.060	47.10	1315.18
CRS 1 + PS 2	13.90	13.80	0.137	0.109	50.90	1254.07
SEd	4.32	5.34	0.039	0.053	8.59	170.74
CD	NS	NS	NS	NS	NS	354.09

CMA [Conventional methods of application] = {Seed treatment + Soil application }

DAS - Days after sowing

Fig.10. Effect of PGPP bacterial inoculation on nodule and pod number of soybean (var.CO 1)



polymyxa (PGPP 8) through foliar spray alone (3.98 mg g^{-1} tissue) followed by inoculation with conventional methods and leaf spray of *Rhizobium freidii* (CRS 1) plus *B. polymyxa* (PGPP 8) (0.387 mg g^{-1} tissue). The uninoculated control plants recorded the least chlorophyll content (0.145 mg g^{-1} tissue).

4.10.6.2 Plant nitrogen content

Plant nitrogen content varied from 0.81 to 1.31%. Among the treatments, foliar inoculation of *Bacillus polymyxa* (PGPP 8) recorded higher nitrogen content (1.31%) followed by leaf spray (1.27%) of *Pseudomonas* sp. (PS 2). The lowest value (0.81%) was recorded in uninoculated control plants.

4.10.6.3 Plant phosphorus content

The phosphorus content of plants varied from 0.04 to 0.12%, the maximum value being recorded in plants receiving leaf spray of *Bacillus polymyxa* (PGPP 8) followed by same method of inoculation (0.11%) of *Pseudomonas* spp. (PS 2 and PGPP 1). The least phosphorus content (0.04%) was recorded in uninoculated control plants.

4.10.7 Effect of PGPP bacterial inoculation on phyllosphere population of soybean

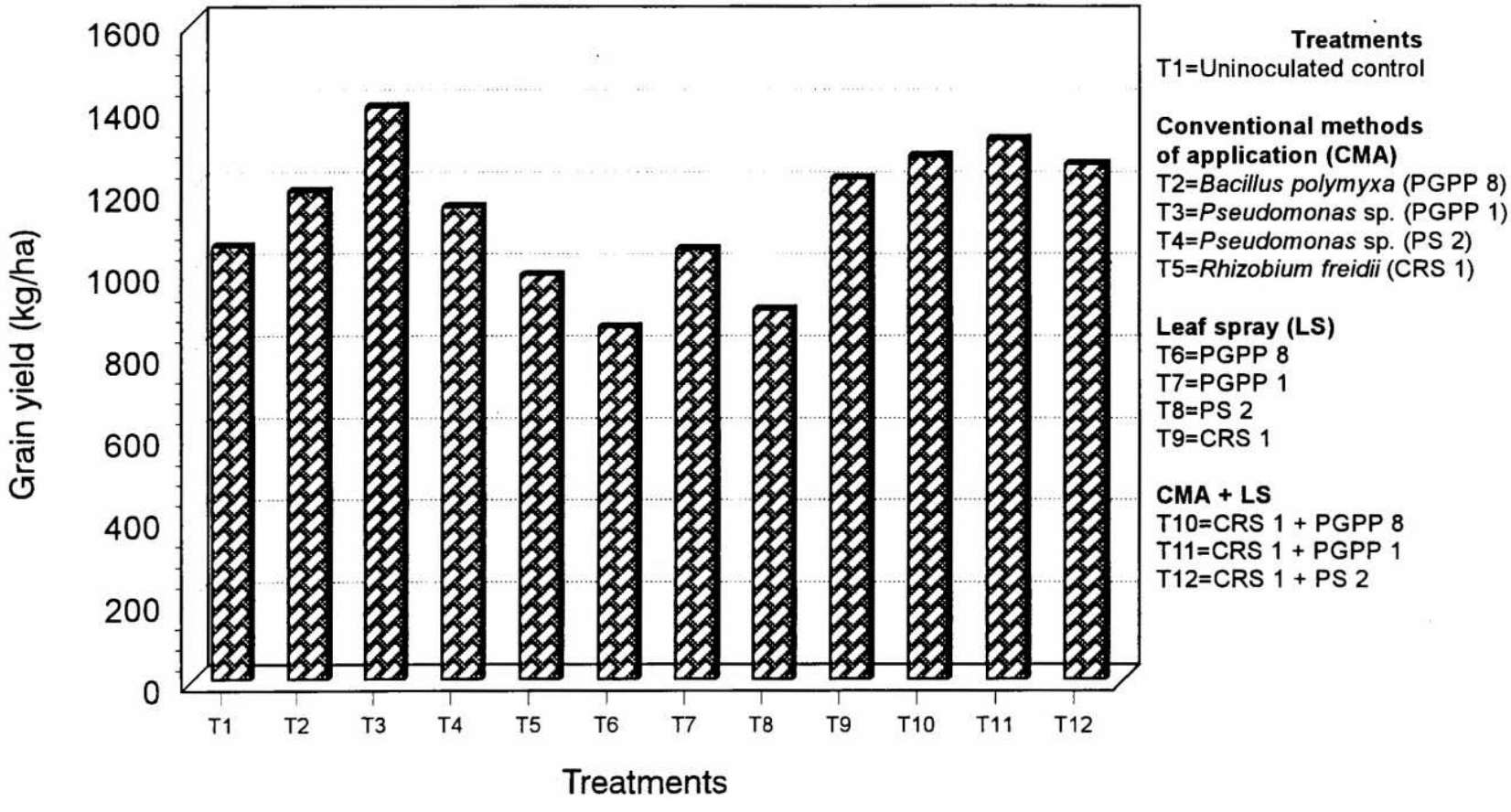
The phyllosphere population of *Bacillus polymyxa* (PGPP 8), *Pseudomonas* sp. (PGPP 8), *Pseudomonas* sp. (PS 2) and *Rhizobium freidii* (CRP 1) were enumerated before and after the leaf spray. The population was

Table 19. Effect of PGPP bacterial inoculation on chlorophyll, nitrogen and phosphorus content of soybean (var. CO 1.)

Treatments	Total chlorophyll content (mg g^{-1} tissue)	Plant nitrogen (%)	Plant phosphorus (%)
Uninoculated control	0.145	0.81	0.04
CMA			
Bacillus polymyxa (PGPP 8)	0.222	0.91	0.08
Pseudomonas sp. (PGPP 1)	0.165	0.87	0.07
Pseudomonas sp. (PS 2)	0.222	1.12	0.10
Rhizobium freidii (CRS 1)	0.165	0.90	0.05
Leaf spray (LS)			
PGPP 8	0.398	1.31	0.12
PGPP 1	0.350	1.27	0.11
PS 2	0.379	1.21	0.11
CRS 1	0.229	1.19	0.10
CMA + LS			
CRS 1 + PGPP 8	0.387	1.08	009
CRS 1+ PGPP 1	0.266	0.90	0.08
CRS 1 + PS 2	0.322	0.97	0.08
SEd	0.017	0.06	0.02
CD	0.035	0.11	0.42

CMA [Conventional methods of application] = {Seed treatment + Soil application }

Fig.11. Effect of PGPP bacterial inoculation on yield of soybean (var.CO 1)



maximum immediately after leaf spray and rapidly declined before next spray. The result is presented in Table 20.

4.10.7.1 *Bacillus polymyxa* (PGPP 8)

The final population of *Bacillus polymyxa* (PGPP 8) was higher in dual inoculated plants with *B. polymyxa* (PGPP 8) and *Rhizobium freidii* (CRS 1) by conventional methods and leaf spray (6.53 x 10^5 cfu/cm^2) than the single inoculation of *B. polymyxa* (PGPP 8) by leaf spray alone (8.37 x 10^5 cfu/cm^2).

4.10.7.2 *Pseudomonas* sp. (PGPP 1)

The final population was higher in dual inoculation of *Pseudomonas* sp. (PGPP 1) and *Rhizobium freidii* (CRS 3) by conventional methods and leaf spray (7.73 x 10^5 cfu/cm^2) than the single inoculation of *Pseudomonas* sp. (PGPP 1) by leaf spray alone (6.10 x 10^5 cfu/cm^2).

4.10.7.3 *Pseudomonas* sp. (PS 2)

The higher population of *Pseudomonas* sp. (PS 2) (1.23 x 10^5 cm^2) was recorded by foliar inoculation of *Pseudomonas* sp. (PS 2) alone than the dual inoculation with *Pseudomonas* sp. (PS 2) and *Rhizobium freidii* (CRS 1) by conventional and leaf spray methods (1.00 x 10^5 cfu/cm^2).

4.10.7.4. *Rhizobium freidii* (CRS 1)

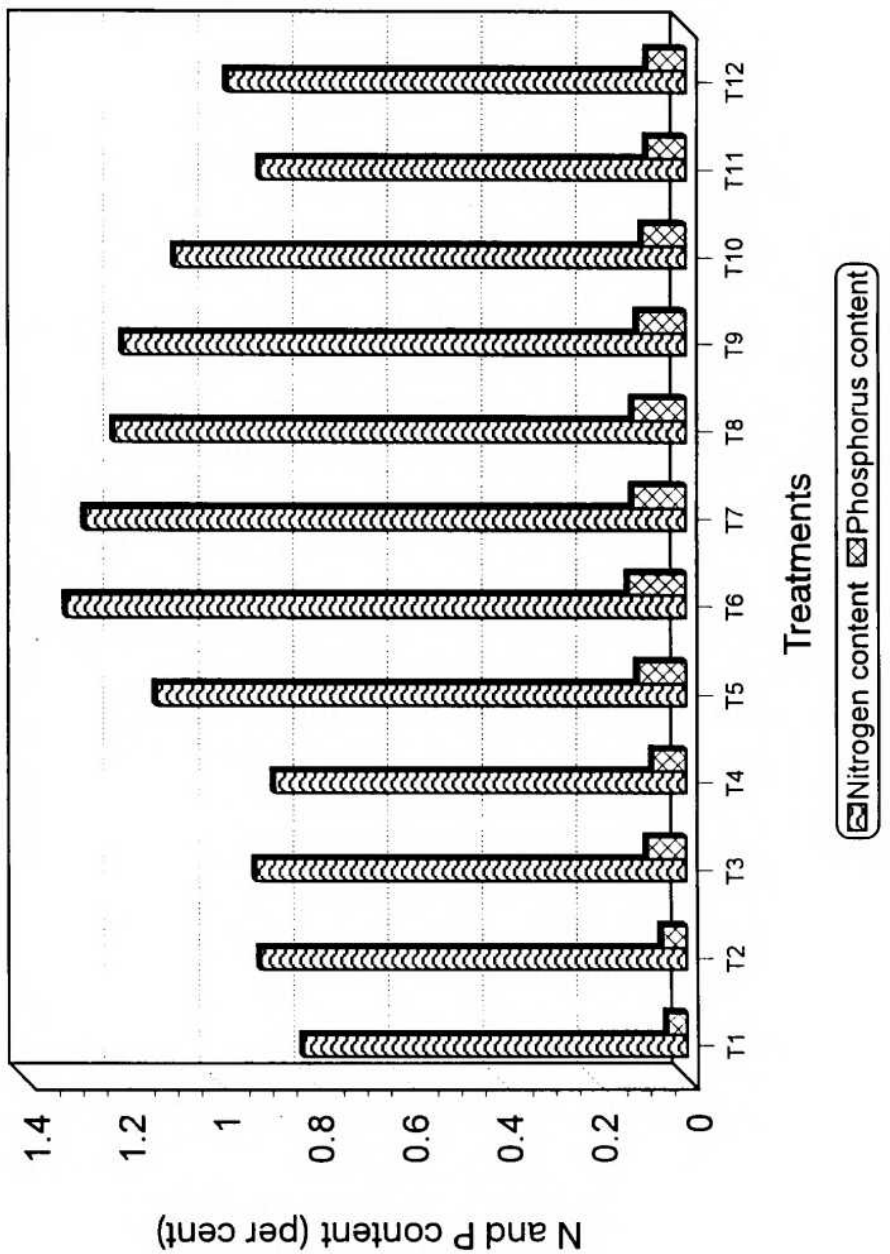
The final population was highest (1.13 x 10^5 cfu/cm^2) in dual inoculated plants with *Bacillus polymyxa* (PGPP) and *Rhizobium freidii* (CRS

Table 20. Population of phyllosphere bacteria on soybean inoculated with PGPP bacteria.

Bacteria	Population before and after foliar inoculation (X 10^5 per cm^2)				
	I Spray		II Spray		Final count
	BS	AS	BS	AS	
Bacillus polymyxa (PGPP 8) – LS	1.43	9233.00	3.13	11300.00	3.37
CRS 3 + PGPP 8 - CMA + LS	1.00	1226.00	6.63	11867.00	6.53
Pseudomonas sp. (PGPP 1)	0.53	1116.00	6.17	11167.00	6.10
CRS 3 + GPP 1 - CMA+ LS	0.27	9267.00	7.70	10467.00	7.73
Pseudomonas sp. (PS 2)	0.13	7867.00	1.40	9500.00	1.23
CRS 3 + PS 2 - CMA + LS	0.10	9200.00	1.33	9633.00	1.00
Rhizobium freidii (CRS 1) – LS	0.17	9400.00	1.03	10633.30	0.50
CRS 3 + PGPP 8 – CMA+LS	0.13	8100.00	1.33	12166.70	1.13
CRS 3 + PGPP 1 – CMA+LS	0.13	8633.00	1.07	9333.00	0.77
CRS 3 + PS 2 – CMA+LS	0.13	7667.00	0.87	8600.00	0.57

CMA [conventional methods of application] - { Seed treatment + Soil application }
LS – Leaf spray
BS – Before spray
AS - After spray

Fig.12. Effect of PGPP bacterial inoculation on N and P contents of soybean (var.CO 1)



1) followed by dual inoculation with *R. freidii* and *Pseudomonas* sp. (0.77 x 10^5 cfu/cm^2) both by conventional and leaf spray methods. Foliar inoculation of rhizobia recorded the least population (0.50 x 10^5 cfu/cm^2).

4.11 ANTAGONISTIC ACTIVITY OF PGPP BACTERIA AGAINST FOLIAR PATHOGENS

The PGPP bacteria *viz.*, *Pseudomonas* sp. (PGPP 1), *Bacillus polymyxa (PGPP 8)* and *B.macerans* (PGPP 20) were tested for antagonistic activity against the foliar pathogens *viz., Xanthomonas oryzae, X. malvacearum, Helminthosperium oryzae, Fusarium orysporum* and *colletotrichum* truncatum. The results on the activity is presented in Table 21.

Pseudomonas sp. (PGPP 1) showed antagonistic activity against *Xanthomonas oryzae* and *Fusarium oxysporum* and was not antagonistic against *X. malvacearum, Helminthosporium oryzae* and *Colletotrichum truncatum. Bacillus polymyxa* (PGPP 8) was antagonistic against *H. oryzae* and *F. oxysporum* and no antagonism was noticed against *X.oryzae, X. malvacearum* and *C.truncatum. Bacillus macerans* (PGPP 20) showed antagonistic activity on *X. oryzae* only and was not antagonistic against other bacterial and fungal pathogens tested.

4.12 EFFECT OF PESTICIDES ON PGPP BACTERIA UNDER *in vitro* CONDITION

Effect of commonly used pesticides like monocrotophos, metasystox, endosulfan, neem oil, dithane, and cypermethrin were tested on the growth of

Table 21. Antagonistic activity of PGPP bacteria against foliar pathogens

Foliar pathogens	Antagonistic activity		
	Pseudomonas sp. (PGPP 1)	*Bacillus polymyxa* (PGPP 8)	*Bacillus macerans* (PGPP 20)
Xanthomonas oryzae	X	NA	X
X. malvacearum	NA	NA	NA
Helminthosporium oryzae	NA	X	ND
Fusarium oxysporum	X	X	NA
Colletotrichum truncatum	NA	NA	NA

X → Antagonism
NA → No antagonism
ND → Not determined

PGPP baceria *viz.*, *Pseudomonas* sp. *Bacillus polymyxa* and *B.macerans*. Growth of the phyllosphere bacteria at recommended dose [R1], double the recommended dose [R2] and half of the recommended dose [R3] of pesticides was observed under *in vitro* condition and the results are presented in Table 22.

The recommended dose of monocrotophos and neem oil had no effect on any of the PGPP bacteria tested, whereas double the recommended dose of monocrotophos inhibited *B.polymyxa* as revealed by inhibition zone (1.90 cm diameter) and that of neem oil inhibited *B.macerans* (1.40 cm).

Metasystox at recommended dose inhibited the growth of *Bacillus polymyxa* (1.40 cm) and *B. macerans* (1.60 cm) but at lower concentration had no effect on these organisms. It had no effect on the growth of *Pseudomonas* sp. at its recommended dose but double the recommended dose inhibited the same organism. Even half of the recommended dose of endosulfan was toxic to *B. macerans* (1.40 cm) and at the recommended level it inhibited both *Pseudomonas* sp. (1.60 cm) and *B. polymyx*a (1.90 cm).

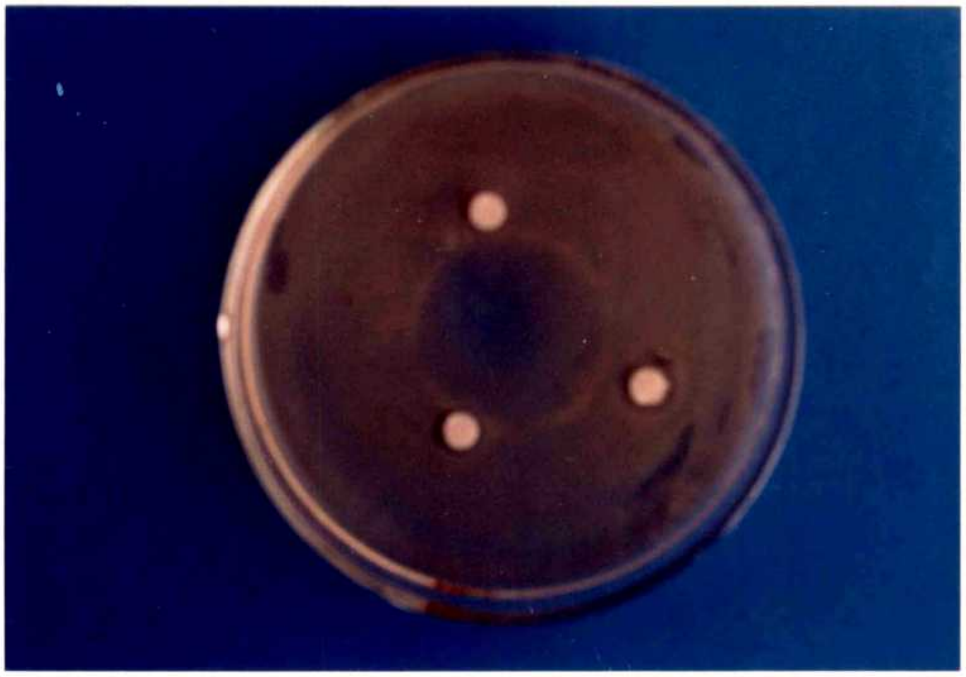
Dithane inhibited the growth of *Pseudomonas* sp. (1.40 cm) and *B. macerans* (1.40 cm) even at the concentration of half of the recommended dose whereas it inhibited *B.polymyxa* (1.70 cm) only at double the recommended dose (Plate 9). At half of the recommended dose, cypermethrin was toxic to both *Pseudomonas* sp. (1.50 cm) and *B.polymyxa* (1.30 cm) but

Table 22. Effect of pesticides on PGPP bacteria under *in vitro* condition

Pesticides	Zone of inhibition (diameter in cm)								
	Pseudomonas sp. (PGPP 1)			*Bacillus polymyxa* (PGPP 8)			*Bacillus macerans* (PGPP 20)		
	R1	R2	R3	R1	R2	R3	R1	R2	R3
Monocrotophos	C	C	C	C	1.90	C	C	C	C
Metasystox	C	1.70	C	1.40	1.90	C	1.60	2.00	C
Endosulfan	1.60	2.10	C	1.90	2.30	C	1.80	2.50	1.40
Dithane	1.70	2.10	1.40	C	1.70	C	1.70	2.10	1.40
Cypermethrin	1.90	2.60	1.50	1.60	1.90	1.30	1.40	1.80	C
Neem oil	C	C	C	C	C	C	C	1.40	C

C - compatible (No zone of inhibition)
R1 - recommended dose
R2 – double the recommended dose
R3 - half of the recommended dose

Plate 9. Effect of dithane on the growth of *Pseudomonas* sp. (PGPP 1)



had not effect on *B. macerans*, while at recommended dose it inhibited the growth (1.40 cm) of *B. macerans* .

4.13 SURVIVAL OF PLANT GROWTH PROMOTING PHYLLOSPHERE (PGPP) BACTERIA IN LIGNITE BASED INOCULANT

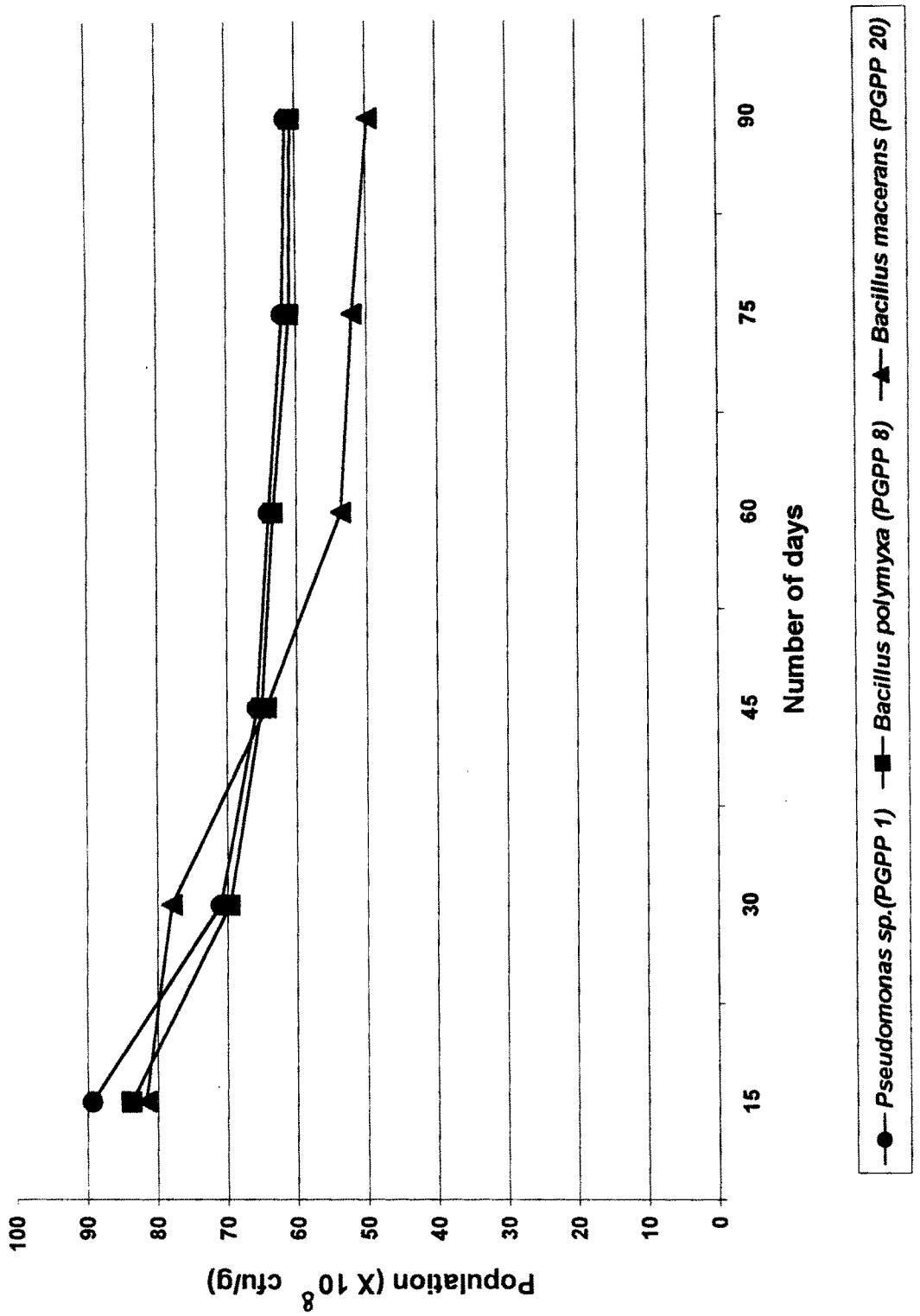
The survival of PGPP bacteria *viz.*, *Pseudomonas* sp. *Bacillus polymyxa* and *B.macerans* in lignite based inoculant material was investigated till 90 days of storage and the results are presented in Table 23 and Fig.13.

The population of PGPP bacteria varied from 81.66 to 89.33 x 10^8/g of carrier material after 15 days of storage. The initial *Pseudomonas* sp. (PGPP 1) population at 15 DAP was (89.33 x 10^8/g) declined and reached a population of 61.33 x 10^8/g at 90 days after storage. Similarly, the population of *Bacillus polymyxa* (PGPP 8) also gradually declined and reached a load of 60.66 x 10^8/g at 90 days after storage. Compared to the other two bacteria the population of *B.macerans* declined rapidly after forty days of storage and showed the least population of 49.66 x 10^8 at 90 days after storage.

Table 23. Survival of PGPP bacteria in lignite based inoculant

Bacteria	Population (X 10^8/g)					
	15 days	30 days	45 days	60 days	75 days	90 days
Pseudomonas sp. (PGPP 1)	89.33	71.00	65.66	64.00	62.00	61.33
Bacillus polymyxa (PGPP 8)	83.73	69.66	65.00	63.33	61.00	60.66
Bacillus macerans (PGPP 20)	81.66	78.00	64.33	53.66	52.00	49.66

Fig.13. Survival of PGPP bacteria in lignite based inoculant



DISCUSSION

5. DISCUSSION

The economic importance of plant pathogenic microorganisms in the phyllosphere of terrestial crop plants intensified our study on the ecology of deleterious leaf surface microorganisms. However, similar importance was not paid for the study of saprophytic and beneficial microorganisms occurring in phyllosphere. Pioneer work of Ruinen (1970) on the occurance of free-living nitrogen fixing bacteria in the phyllosphere and their possible role as providers of organic nitrogen to the plants has triggered the research on phyllosphere nitrogen fixation. Many phyllosphere bacteria also produced variety of plant growth promoting substances on leaf surface (Loper and Schroth, 1986).

In contrast to voluminous work on nitrogen fixation in the rhizosphere, studies on phyllosphere nitrogen fixation is meagre. So, the present study was carried out to understand the role of phyllosphere microorganisms in nitrogen fixation and in the production of plant growth promoting substances.

ENUMERATION AND ISOLATION OF PHYLLOSPHERE MICROORGANISMS

The present study on the occurrence of microorganisms in the phyllosphere of selected agricultural crops *viz.*, rice, wheat, maize, soybean, cowpea, groundnut and cotton revealed the predominance of bacteria over

fungi and actinomycetes. Higher bacterial population than fungi in the phyllosphere of deciduous trees was earlier observed by Warren (1976).

The present study also revealed the domination of gram negative rod shaped bacteria in phyllosphere in contrast to the equal distribution of both gram positive and gram negative bacteria as observed by Billing (1976). Highest total bacterial population (113.30 x $10^4/cm^2$) was recorded in cotton phyllosphere and the presence of large number of trichomes on cotton leaves might have trapped more bacteria as observed by Murty (1985). Type of leaf, wax deposits, silicification and venation on the leaves also influenced microbiol propagules occurring in phyllosphere (Ruinen, 1961).

The population of nitrogen fixing bacteria to total heterotrophs occurring on the leaf surface varied from 8.0 to 50.0 per cent. Cereal leaves harboured more nitrogen fixers than pulses and fibre crops which supported the findings of Sen *et al.* (1985). They observed more nitrogen fixers in rice and wheat phyllosphere.

The number of phyllosphere fungi and actinomycetes were very low when compared to the bacteria and it is in accordance with the findings of Dickinson (1967).

For further studies, 24 bacterial isolates growing on nitrogen free medium with distinct colony characters were selected.

PRELIMINARY SCREENING OF PHYLLOSPHERE BACTERIA FOR ACETYLENE REDUCTION ACTIVITY (ARA)

Among the 24 bacterial isolates, soybean phyllosphere isolate (PGPP 8) showed maximum ARA (495.06 nmol of C_2H_4 produced h^{-1} mg^{-1} of cell protein), however it was lesser than the ARA of root nodulating soybean rhizobia as observed by Natarajan (1998). The rice phyllosphere bacteria (PGPP 1) also reduced considerable quantity of acetylene (429.77 nmol of C_2H_4 produced h^{-1} mg^{-1} of cell protein) but lower than the ARA of the bacteria isolated from rice leaves by Sen Gupta *et al.* (1982).

The groundnut (PGPP 15) and cotton (PGPP 20) phyllosphere isolates recorded 342.93 and 400.77 nmol of C_2H_4 production h^{-1} mg^{-1} of cell protein, respectively. The nitrogen fixation by bacterial isolates of larch phyllosphere as observed by Jones (1976) varied from 7 to 26 µg of C_2H_4 production in 24h.

From the 24 phyllosphere bacterial isolates growing on nitrogen free medium, seven isolates (two each from rice, cotton and groundnut and one from soybean phyllosphere) having higher ARA were selected for further studies.

SCREENING PHYLLOSPHERE BACTERIA FOR THE PRODUCTION OF PLANT GROWTH PROMOTING SUBSTANCES

Indole acetic acid (IAA) production by the epiphytic bacteria were reported by many workers whereas gibberellic acid (GA) was mainly secreted by plant pathogens (Buckley and Pugh, 1971). In the present study, among

the two methods followed for IAA and GA determination, plant bioassay method always gave higher values than the colorimetric method. Similar observation was also made by Kumar and Lonsane (1986).

The Gram positive bacterial isolates, PGPP 8 and PGPP 20 produced more quantity of IAA than that of gram negative bacteria. Similar result of increased IAA production by gram positive *Bacillus polymyxa* was reported by Holl *et al.* (1988).

IAA production in *Phyllobacterium* spp., *Pseudomonas aeruginosa* and *P. fluorescens* were reported by Muller *et al.* (1989), Martens and Frankenberger (1991) and Kalaivani (1998), respectively whereas GA production in *Azospirillum* sp. and *Azotobacter chroococcum* was observed by Tien *et al.* (1979) and Elwan and El-Naggar (1972), respectively.

Based on the production of IAA and GA and also on ARA, three isolates *viz.*, PGPP 1 (rice), PGPP 8 (soybean) and PGPP 20 (cotton) were selected for characterization and futher studies.

CHARACTERIZATION OF PLANT GROWTH PROMOTING PHYLLOSPHERE (PGPP) BATERIA

Studies on morphological, cultural and biochemical characters indicated that the gram negative PGPP 1 isolate is possibly a non fluorescent *Pseudomonas* sp. This short rod shaped, motile bacterium grow well on king's medium B and failed to hydrolyse starch. The various morphological, cultural

and biochemical characters of this organism fitted to the description of non fluorescent *Pseudomonas* (Palleroni, 1984).

The other two gram positive bacterial isolates (PGPP 8 and PGPP 20) produced endospores and hydrolysed the starch. The cultural and biochemical characters of PGPP 8 and PGPP 20 indicated that they belonged to the genus *Bacillus*. Growth in anaerobic agar, absence of gelatin hydrolysis, failure to grow at 50^{0} C and positive Voges - Proskauer test of PGPP 8 bacterium coincided with the characters of *Bacillus polymyxa* (Slepecky and Hemphill, 1991) and hence tentatively identified as *B.polymyxa*. Gelatin liquification and negative Voges - Proskauer test of endospore producing PGPP 20 bacterium fitted to the description of *B.macerans* (Claus and Berkeley, 1986) and so tentatively identified as *B.macerans*.

Occurrence of nitrogen fixing *Pseudomonas* sp. in phyllosphere of trees (Jenni *et al.*, 1989) and nitrogen fixing *Bacillus polymyxa* and *B.macerans* in the phyllosphere of cotton (Oliveira *et al.*, 1993) were observed earlier. They also showed their DNA homology with the nif genes of *Klebsilella pneumonia* by southern hybridization technique.

PHOSPHATE SOLUBILISATION BY PLANT GROWTH PROMOTING PHYLLOSPHERE (PGPP) BACTERIA

The seven phyllosphere bacteria selected based on the acetylene reduction activity were also tested for the solubilisation of hydroxy apatite in

the medium. The gram negative, rod shaped, pigment producing groundnut isolate PGPP 15 only solubilised the insoluble form of phosphate as indicated by the formation of clearing zone. The phyllosphere bacteria, *Pseudomonas* sp. (PGPP 1), *Bacillus polymyxa* (PGPP 8) and *B.macerans* (PGPP 20) which exhibited higher ARA failed to solubilise apatite whereas Arora and Gaur (1978) observed rock phosphate solubilisation in the soil isolates of *Bacillus polymyxa* and *Pseudomonas* sp.

The bacterium PGPP 15 produced higher amount of organic acid (48.55 mg of lactic acid / 50 ml broth) than that of *Bacillus megaterium* var. *phosphaticum* (42.20 mg) but lesser than that of *Pseudomonas* sp. (PS 2) (55.83 mg). This difference in organic acid production will lead to variation in phosphate solubilising ability of phosphobacteria (Cerezine, 1988). The results showed the higher amount of organic acid production and in turn phosphorus release in the culture medium.

The difference observed in the pH change of the medium varied from 5.0 to 5.3 and showed no relation with phosphorus solubilisation which supported the observation of Asea *et al.* (1988).

The secretion of acid phosphatase and inturn mineralisation of organic phosphate was higher in *Pseudomonas* sp. (PS 2) followed by the phyllosphere bacterial isolate PGPP 15 and *Bacillus magaterium* var. *phosphaticum*. The result is in confirmity with the observations of Alexander

(1977) who found *Pseudomonas* sp. as an effective organic phosphate solubiliser besides solubilizing rock phosphate.

INTRINSIC ANTIBIOTIC RESISTANCE (IAR) OF PLANT GROWTH PROMOTING PHYLLOSPHERE (PGPP) BACTERIA

For easy identification of introduced phyllosphere bacteria from native phyllosphere organisms, IAR character was used based on their resistance and susceptibility to various concentrations of chloramphenicol and streptomycin . Lindow (1983) suggested IAR as a reliable marker for identification of the introduced organisms into an environment. Maximum antibiotic concentration (MAC) that allowed the normal growth and minimum inhibitory concentration (MIC) that significantly reduced the growth were evaluated and used for identification (Hopwood, 1970).

Among the three bacterial isolates, *Pseudomonas* sp. (PS 2) showed resistance for both chloramphenicol and streptomycin upto 200 ppm whereas *Bacillus polymyxa* (PGPP 8) tolerated only upto 25 and 50 ppm and *B.macerans* (PGPP 20) upto 100 and 200 ppm of chloramphenicol and streptomycin, respectively. Similar IAR character was used for the identification of introduced *Rhizobium* (Natarajan, 1998) and phyllosphere bacteria (Mussaon *et al.*, 1995). The antibiotic markers (MIC) used in this study for identification of phyllosphere bacteria were as follows.

Pseudomonas sp. (PGPP 1)	--->	200 ppm chloramphenicol and 200 ppm streptomycin
Bacillus polymyxa (PGPP 8)	-->	25 ppm chloramphenicol and 50 ppm streptomycin
B.macerans (PGPP 20)	--->	50 ppm chloramphenicol and 200 ppm streptomycin

COMPATIBILITY OF PGPP BACTERIA WITH NITROGEN FIXERS AND PHOSPHATE SOLUBILIZERS

The compatibility test revealed that all the three PGPP bacteria *viz.*, *Pseudomonas* sp. (PGPP 1), *Bacillus polymyxa* (PGPP 8) and *B.macerans* (PGPP 20) were compatible with nitrogen fixing *Azospirillum lipoferum* (Az 204) and *Azotobacter chroococcum* (CZR 1) and phosphate solubilising *Pseudomonas* sp. (PS 2), and this results confirms the finding of Kalaivani (1998) who observed compatibility of *Azospirillum lipoferum* (AZ 204) with *Pseudononas fluorescens.*

The results showed variation in the compatibility of PGPP bacteria with fast growing *Rhizobium freidii* and slow growing *Bradyrhizobium japonicum* strains. *R.freidii* was compatible with all the three PGPP bacterial isolates whereas *B.japonicum* and phyllosphere bacterium *Bacillus macerans* were incompatible. Natarajan (1998) tested the compatibility of *Bacillus* sp. with fast growing soybean rhizobia and used it as coinoculants with *R.freidii.*

EFFECT OF INOCULATION OF PLANT GROWTH PROMOTING PHYLLOSPHERE (PGPP) BACTERIA ON GROWTH AND YIELD OF RICE

The positive effect of foliar inoculation of *Bacillus macerans* (PGPP 20) in increasing the root length, dry matter production, and grain yield of rice either alone or in combination with *Azospirillum lipoferum* (AZ 204) was in confirmity with the findings of earlier workers. Pati and chandra (1981) also observed more than 70 per cent yield increase in wheat by spraying phyllosphere organisms like *Beijerinckia indica.*

The present study also revealed significant effect of foliar inoculation of *Bacillus macerans* (PGPP 20) and *Azospirillum lipoferum* (AZ 204) on rice yield (12.07 and 12.02 g/pl, respectively) and it supported the findings of earlier workers like Sen *et al.* (1985). They observed 39 to 680 per cent yield increase in rice due to foliar spray of diazotrophs.

However, Sharma and Mukerji (1976) observed no significant effect of foliar inoculation of diazotrophs on shoot length, tiller production, panicle number and straw yield in rice.

Increased chlorophyll, nitrogen and phosphorus contents of the plants due to foliar inoculation of *B.macerans* indicated the availability of more nutrients to the plants by the phyllosphere bacterial activity. The leaf spray of *B.macerans* recorded the highest total chlorophyll (0.443 mg g^{-1} tissue),

plant nitrogen (1.19%) and plant phosphorus content (0.13%). This supported the finding of Ramarethinam *et al.* (1998). They observed higher net photosynthetic rate in chillies due to leaf spray of the diazotroph *B.subtilis.*

The nitrogen fixation and production of plant growth promoting substances by thephyllospherebacterium *Bacillus macerans* (PGPP 20) might have increased the growth and yield of rice by providing the plant with more nutrients and growth factors.

The phyllosphere population estimated before and after leaf spray revealed that the population declined rapidly to the level of thousand times than that of initial population. Since colonization of phyllosphere bacteria depends on host, and environmental factors and vary greatly in time span of less than one minute (Frossard, 1981), continuous monitoring is required to see their establishment. In the present experiment, the sampling interval was more than twenty days and no difference was observed in the survival among the various introduced organisms. So it is suggested that frequent sampling with lesser time interval is required to study the population dynamics of introduced phyllosphere bacteria.

EFFECT OF INOCULATION OF PLANT GROWTH PROMOTING PHYLLOSPHERE (PGPP) BACTERIA ON GROWTH AND YIELD OF COTTON

The present study revealed that leaf spray of phyllosphere bacteria either alone or in combination with conventional methods of application had

beneficial effect on growth and yield of cotton. The leaf spray of *Azospirillum lipoferum* (AZ 204) recorded significant increase in root length (44.00 cm). Maximum number of leaves (34.67/pl.) was observed in the treatment receiving leaf spray of *Bacillus macerans* (PGPP 20). Higher dry matter production (8.97 g/pl.) was also recorded by conventional and foliar inoculation of *Bacillus macerans* (PGPP 20). Similar increased growth due to foliar spray of the diazotroph *Azotobacter* sp. was recorded by Murty (1984a) in wheat, maize and cotton.

The conventional (seed treatment and soil application) and foliar inoculation of *Bacillus macerans* (PGPP 20) increased the yield of cotton and this may be attributed to its nitrogen fixation and secretion of plant growth promoting substances by *B. Macerans*.

Increased chlorophyll content (0.484 mg g^{-1} tissue) and nitrogen content (1.39%) was recorded by the treatment receiving both conventional and foliar inoculation of *Bacillus macerans* (PGPP 20). The same bacteria by leaf spray also recorded the highest phosphorus content (0.13%) in plants. Similar increase in nitrogen content due to foliar spray of diazotrophs was recorded by Wani and Rai (1980) in cotton.

Bacillus macerans (PGPP 20), the phyllosphere isolate of cotton increased the growth and yield of cotton and this may be attributed to its

natural epiphytic fitness, effective nitrogen fixation and to its IAA and GA production (Varvaro and Surico, 1984).

The population of phyllosphere bacteria was enumerated before and after leaf spray. Rapid reduction in baterial population within thirty days was observed in all treatments. Except few cases, all other treatments receiving both conventional and leaf spray inoculations recorded higher population than that of leaf spray alone. The population of foliar inoculated bacteria was higher in the phyllosphere and the results confirmed the findings of Mussaon *et al.*(1995).

EFFECT OF INOCULATION OF PLANT GROWTH PROMOTING PHYLLOSPHERE (PGPP) BACTERIA ON GROWTH AND YIELD OF SOYBEAN

Though the inoculation increased the plant growth parameters, significant difference was not observed except in case of grain yield. Sharma and Mukerji (1976) were also of the view that majority of epiphytic microorganisms are neutral in their effect on the host plants during their growth period. Since the present investigation was in the field, the physical and environmental factors such as temperature, relative humidity, wind speed etc., might have influenced the activity of phyllosphere bacteria in bringing the desired result as reported by Burrage (1976).

Higher nodule number (22.00/pl.) and nodule dry weight (0.182 g/pl.) were recorded by the conventional (seed treatment and soil application) inoculation method of *Rhizobium freidii* (CRS 1). The highest pod number was recorded in plants receiving both *R.freidii* (CRS 1) and *Bacillus macerans* (PGPP 8) by conventional and foliar inoculation. Similar effect due to rhizobial treatment was observed in groundnut (Narendranath, 1995) and in soybean (Natarajan, 1998).

Bacillus macerans (PGPP 8) inoculation caused higher grain yield (1394.82 kg/ha) in soybean and this is in accordance with the result of Sen *et al.* (1985) that inoculation of phyllosphere bacteria increased the yield of soybean by 109 to 182 per cent over control.

The present investigation showed an increase in chlorophyll content (0.398 mg g^{-1} tissue), nitrogen (1.31%) and phosphorus (0.12%) contents of soybean plants due to foliar inoculation of *Bacillus polymyxa* (PGPP 8). This kind of increased nitrogen contribution by phllosphere nitrogen fixing microorganisms was earlier observed by Sadykov and Umarov (1980) in soybean.

The phyllosphere population enumerated before and after leaf spray showed a steady decline within thirty days, i.e., before the subsequent spray. Higher population of phyllosphere bacteria, *Pseudomonas* sp. (PGPP 1) and *Bacillus polymyxa* (PGPP 8) observed in this experiment may be attributed

to their epiphytic fitness and IAA producing ability (Loper and Schroth, 1986).

Since *Bacillus polymyxa* (PGPP 8) was isolated from soybean phyllosphere and also fixed nitrogen with enhanced IAA and GA production, it might have survived better on the leaf and increased the grain yield of soybean by its activity.

ANTAGONISTIC ACTIVITY OF PGPP BACTERIA AGAINST FOLIAR PATHOGENS

The leaf surface harbours variety of microorganisms of saprophytic, parasitic and symbiotic nature. Their association on the leaf surface is exploited for the control of foliar plant pathogenic microorganisms. In the present study, the antagonistic activity of phyllosphere bacteria against pathogens like *Xanthomonas oryzae, X.malvacearum, Helminthosporium oryzae, Fusarium oxysporum and collectotrichum truncatum* reaveled the selective inhibition of pathogens by phyllosphere bacteria.

Pseudomonas sp. (PGPP 1) was antagonistic against *Xanthomonas oryzae* and *Fusarium oxysporum* whereas *Bacillus polymyxa* (PGPP 8) was inhibitory to *Helminthosporum oryzae* and *F.oxysporum.* Similar antagonistic activity of various *Pseudomonas* species against *X.oryzae and Fusarium* sp. were reported by Santhi *et al.* (1987), Rosales *et al.* (1995) and Kalaivani (1998).

None of the PGPP bacteria tested were antagonistic against *Xanthomonas malvacearum* and *Colletotrichum truncatum* and this result differs from the observation of Verma *et al.* (1983). *Bacillus macerans* (PGPP 20) was also selective in antagonistic activity against *X.oryzae* and *X.malvacearum.*

EFFECT OF PESTICIDES ON PGPP BACTERIA UNDER *in vitro* CONDITION

The pesticides used to control different plant diseases accumulate greatly on leaf surface and influence the survival of phyllosphere microorganisms (Crosse, 1967). The *in vitro* studies on the effect of various pesticides at different concentrations on phyllosphere microorganisms revealed that plant products like neem oil had no effect on the growth of PGPP bacteria. Among the organophosphorus compounds, monocrotophos at its recommended dose was safer to use. At the recommended dose, matasystox was not toxic to *Pseudomonas* sp. (PGPP 1) but inhibited the growth of *Bacillus polymyxa* and *B. macerans*.

Endosulfan and cypermethrin inhibited the growth of all PGPP bacteria even at the recommended concentration. Dithane was selective in inhibiting the growth of phylloshere microorganisms. It inhibited the growth of PGPP bacteria *Pseudomonas* sp. and *Bacillus macerans* but not *B. polymyxa.* The inhibitory effect of dithane on the growth of phyllosphere bacterium, *Beijerinckia* sp. (Merilyn and Thomas, 1991) and the toxicity of copper and mercury fungicides on phyllosphere fungi and bacteria (Hislop and Clifford, 1975) were observed earlier.

Though the phyllosphere organisms are generally more tolerant to fungicides than the pathogens as observed by Baker and Cook (1974), it is necessary to identify the compatible non toxic pesticides where beneficial phyllosphere microorganisms are sprayed on the leaf surface. The present study revealed that plant product pesticides are much safer to the phyllosphere microorganisms.

SURVIVAL OF PLANT GROWTH PROMOTING PHYLLOSPHERE (PGPP) BACTERIA IN LIGNITE BASED INOCULANT

Since the carrier based inoculant is convenient to use at field level, PGPP bacterial inoculant was prepared by using lignite as carrier material. The survival of the organisms in the carrier was tested over a period of time.

As lignite supported higher population (10^8cells/g) of all PGPP bacteria even after ninety days of storage, it may be used as an ideal carrier meterial for PGPP bacterial inoculant preparation. Kandasamy and Prasad (1971) recommended the use of lignite as carrier material for rhizobia.

Lignite, alone or with various amendments like soymeal was used by Narendranath (1995) to increase the shelf life of groundnut rhizobial inoculant.

The survival of all the PGPP bacteria in lignite after 90 days of storage is well above the standards prescribed for the minimum population of *Rhizobium* (BIS, 1986) and *Azotobacter* (BIS, 1979) inoculants (10^7 cells/g at expiry date). Hence lignite may be used as a carrier material for preparing the PGPP inoculants also.

SUMMARY

6. SUMMARY

The salient findings of the experiments carried out are given below.

1. The study on the occurrence of phyllosphere microorganisms of selected agricultural crops *viz.*, rice, wheat, maize, soybean, cowpea, groundnut and cotton revealed the domination of bacteria over actinomycetes and fungi. Total heterotrophic bacteria were more on cotton leaves and nitrogen fixers on wheat leaves. The population of nitrogen fixing bacteria on the phyllosphere varied from 8.0 to 50.0 per cent of the total heterotrophic bacteria.

2. The acetylene reduction activity of phyllosphere nitrogen fixing bacteria ranged from 186.56 to 495.06 nmol of C_2H_4 produced h^{-1} mg^{-1} of cell protein, maximum being in *Bacillus polymyxa*(PGPP 8) isolated from soybean phyllophere.

3. Among the seven selected phyllosphere nitrogen fixing bacteria, *Pseudomonas* sp. (PGPP 1), *Bacillus polymyxa* (PGPP 8) and *B.macerans* (PGPP 20) reduced more acetylene and also produced higher amount of indole acetic acid (47.50, 72.50 and 57.50 mg/ml, respectively) and gibberellic acid (4.50, 4.75 and 4.25 mg/ml, respectively).

4. The nitrogen fixing phyllosphere bacteria (PGPP 15) of groundnut leaf also solubilised the insoluble phosphorus under *in vitro* condition by producing more organic acids and acidity in the medium.

5. The phyllosphere bacteria showed variation in their natural resistance to antibiotics like chloramphenicol and streptomycin. The *Pseudomonas* sp. isolated from rice phyllosphere tolerated higher concentration (200 ppm) of chloramphenicol and streptomycin.

6. The cross streak assay revealed that all the PGPP bacteria were compatible with the nitrogen fixers like *Rhizobium freidii, Bradyrhizobium japonicum, Azotobacter chroococuum, Azospirillum lipoferum* and phosphate solubilising *Psendomonas* sp. indicating the possibility of combined inoculation.

7. The foliar inoculation of plant growth promoting phyllosphere (PGPP) bacterium *Bacillus macerans* (PGPP 20) significantly increased the growth and yield of rice under pot culture condition.

8. Inoculation of *Bacillus macerans* (PGPP 20) by leaf spray increased the total chlorophyll content, nitrogen and phosphorus contents of the rice plants.

9. The studies on the phyllosphere population of foliar inoculated bacteria showed an increased population immediately after leaf spray followed by quick decline.

10. Inoculation of *Bacillus macerans* (PGPP 20) by conventional methods (seed treatment and soil application) as well as by leaf spray increased growth and yield of cotton (131.99 g/pl.) over unioculated control (64.80 g/pl).

11. Foliar inoculation of *Bacillus macerans* (PGPP 20) in cotton increased the total chlorophyll (0.489 mg g^{-1} tissue), nitrogen (1.39%) and phosphorus (0.14%) contents of plants.

12. The field trial conducted in soybean revealed the beneficial use of *Bacillus polymyxa* (PGPP 8) in increasing the yield of soybean.

13. *Bacillus polymyxa* (PGPP 8) foliar inoculation increased the total chlorophyll (0.398 mg g^{-1} tissue), nitrogen (1.31%) and phosphorus content (0.12%) of soybean plants over uninoculated control.

14. The phylloshere bacterium *Pseudomonas* sp. (PGPP 1) was found to be antagonistic against the pathogens, *viz.*, *Xanthomanas oryzae* and *Fusarium oxysporum*; where as *Bacillus polymyxa* (PGPP 8) was antagonistic to *Helminthosporium oryzae* and *F.oxysporum*; and *B.macerans* (PGPP 20)

was antagonistic against *Xanthomonas oryzae* indicating the possible role of PGPP bacteria in controlling the plant diseases.

15. The study on the interaction of pesticides with PGPP bacteria indicated that botanic pesticides like neem oil are less harmful to phyllosphere microorganisms. The synthetic pyrethroid, cypermethrin and organophosphorus inhibited the growth of *Pseudomonas* sp. (PGPP 1), *Bacillus polymyxa* (PGPP 8) and *B.macerans* (PGPP 20) under *in vitro* conditions even at lower concentration.

16. The survival study of PGPP inoculants prepared in lignite showed its suitability as an ideal carrier material for inoculant production of PGPP bacteria *viz.*, *Pseudomonas* sp. (PGPP 1), *Bacillus polymyxa* (PGPP 8) and *B.macerans* (PGPP 20). It supported a population of more than 10^8 cells/g even after 90 days of storage.

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APPENDIX

APPENDIX

COMPOSITION OF MEDIA

Kuster's medium (for actinomycetes)

Soluble starch	-	2.0 g
Glycerine	-	3.0 g
Dipotassium hydrogen phosphate	-	0.2 g
Casein	-	1.0 g
Magnesium sulphate	-	0.2 g
Potassium chloride	-	1.0 g
Tap water	-	1000.0 ml
pH	-	7.0
Agar	-	15.0 g

Martin's Rose bengal streptomycin agar medium (for fungi)

Glucose	-	10.0 g
Peptone	-	10.0 g
Dipotassium hydrogen phosphate	-	1.0 g
Magnesium sulphate	-	0.5 g
Rose bengal	-	0.9 g
Tap water	-	1000.0 ml
pH	-	6.5
Agar	-	15.0 g
Streptomycin sulphate	-	0.03 g

Modified Jensen's agar medium (for free living N-fixing bacteria)

Sucrose	-	20.0 g
Dipotassium hydrogen phosphate	-	1.0 g
Magnesium sulphate	-	0.5 g
Sodium molybdate	-	0.001 g
Ferrous sulphate	-	0.01 g
Calcium carbonate	-	2.0 g
Agar	-	18.0 g
Distilled water	-	1000.0 ml
pH	-	7.0 - 7.2

Nitrogen free malicacid medium (for *Azospirillum* sp.)

Malic acid	-	5.0 g
Dipotassium hydrogen phosphate	-	0.5 g
Potassium hydroxide	-	4.0 g
Magnesium sulphate	-	0.1 g
Sodium chloride	-	0.02 g
Calcium chloride	-	0.01 g
Ferrous sulphate	-	0.05 g
Sodium molybdate	-	0.002 g
Manganese sulphate	-	0.01 g
Alcoholic Bromothymol Blue (0.5%)	-	2.0 ml
Agar	-	1.8 g (Semi-Solid)
Distilled water	-	1000.0 ml
pH	-	6.9 - 7.3

Nutrient agar medium (for total heterotrophic bacteria)

Beef extract	-	3.0 g
Bactopeptone	-	5.0 g
Glucose	-	5.0 g
Sodium chloride	-	5.0 g
Agar	-	15.0 g
Distilled water	-	1000.0 ml
pH	-	6.8

Pikovskaya's medium (for phosphobacteria)

Tricalcium phosphate	-	5.0 g
Glucose	-	10.0 g
Ammonium sulphate	-	0.5 g
Sodium chloride	-	0.2 g
Magnesium chloride	-	0.1 g
Potassium chloride	-	0.2 g
Yeast extract	-	0.5 g
Manganese sulphate	-	trace
Ferrous sulphate	-	trace
Agar	-	7.0
Distilled water	-	1000.0 ml
pH	-	7.0

Sperber's hydroxy apatite medium (for phosphobacteria)

Soil extract	-	100.0 ml
Glucose	-	1.0 g
Dipotassium hydrogen phosphate (10%)	-	5.0 ml
Calcium chloride (10%)	-	10.0 ml
pH	-	7.0
Agar	-	15.0 g

(Sterilise separately Dipotassium hydrogen phosphate and calcium chloride; and add to sterilized medium. Adjust the pH to 7.0 with N/10 Sodium hydroxide).

Waksman No. 77 medium (for *Azotobacter* sp.)

Mannitol	-	10.0 g
Dipotassium phosphate	-	0.5 g
Magnesium sulphate	-	0.2 g
Sodium chloride	-	0.2 g
Manganese sulphate	-	trace
Ferric chloride	-	trace
Distilled water	-	1000.0 ml
Agar	-	15.0 g