

**INFLUENCE OF PLANT GROWTH PROMOTING
RHIZOBACTERIA ON THE POPULATION DYNAMICS OF THE
RHIZOSPHERE MICROFLORA, GROWTH AND DEVELOPMENT
IN COTTON (*Gossypium hirsutum*)**

*Thesis submitted in part fulfillment of the requirements for the degree of
Doctor of Philosophy (Agriculture) in the Agricultural Microbiology
to the Tamil Nadu Agricultural University, Coimbatore 641 003*

By

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CERTIFICATE

This is to certify that this thesis entitled "INFLUENCE OF PLANT GROWTH PROMOTING RHIZOBACTERIA ON THE POPULATION DYNAMICS OF THE RHIZOSPHERE MICROFLORA, GROWTH AND DEVELOPMENT IN COTTON (*Gossypium hirsutum*)" submitted in part fulfillment of the requirements for the award of the degree of **DOCTOR OF PHILOSOPHY (AGRICULTURE)** in **AGRICULTURAL MICROBIOLOGY** to the Tamil Nadu Agricultural University, Coimbatore is a *bonafide* record of research work carried out by **Miss K. SUGUNA RANI** 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 or prizes and that the work has not been published in part or full in any scientific popular journal or magazine.

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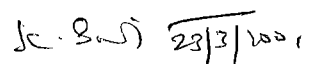
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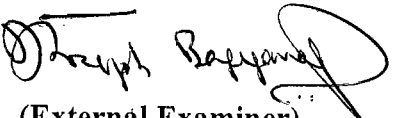
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ABSTRACT

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INFLUENCE OF PLANT GROWTH PROMOTING RHIZOBACTERIA ON THE POPULATION DYNAMICS OF RHIZOSPHERE MICROFLORA, GROWTH AND DEVELOPMENT IN COTTON (*Gossypium hirsutum*)

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Forty five isolates of *Azospirillum*, thirty seven isolates of phosphobacteria and twenty five isolates of *Pseudomonas* were obtained from Cotton rhizosphere samples collected at different locations of Tamil Nadu. The rhizobacterial isolates were screened for their efficiency under *in vitro* condition. Screening of *Azospirillum* isolates for nitrogen fixing ability revealed that the efficiency of nitrogen fixation varied from 6.26 to 45.83 mg of N g⁻¹ of malate. Phosphobacterial isolates solubilized, 3.25 to 18.56 mg 'P' from 100 mg⁻¹ TCP. The presence of certain groups of rhizobacteria exhibiting antagonistic activity in and around the root zone might be of value in suppressing pathogens capable of causing damage to seedling emergence. The *Pseudomonas* isolates exhibited antagonistic activity against *R. solani* with an inhibition zone of 6 mm to 15 mm.

The efficient rhizobacterial isolates were characterized. Among the 12 isolates, seven of the *Azospirillum* isolates were identified as *A. brasilense* and five as *A. lipoferum*.

Among the 11 phosphobacterial isolates seven isolates were identified as *Bacillus* spp. and four as *Pseudomonas* spp. Eight *Pseudomonas* isolates were identified as *P. fluorescens*.

Production of IAA by rhizobacterial isolates increased due to addition of tryptophan to the medium. Among the *Azospirillum* isolates Azo 11 recorded maximum amount of $93.2 \mu\text{g } 100 \text{ ml}^{-1}$, followed by phosphobacteria (PB 9) and *Pseudomonas* (Pfa 2). The GA production was highest in *Pseudomonas* (Pfa 2) followed by *Azospirillum* (Azo 11) and phosphobacteria (PB 9). The rhizobacterial isolates produced phenolate type of 2,3-dihydroxy benzoic acid (2,3-DHBA) siderophore at the rate of $8.11 \mu \text{ moles ml}^{-1}$ in *Pseudomonas* (Pfa 2) followed by phosphobacteria (PB 9) and *Azospirillum* (Azo 11). The poly- β hydroxy butyrate (PHB) accumulation of *Azospirillum* (Azo 11) increased with age of isolate @ $1.870 \mu\text{g g}^{-1}$ of dry wt of the cell. The hydrogen cyanide (HCN) production of *Pseudomonas* varied from 2.42 to $9.56 \mu\text{g ml}^{-1}$ and Pfa 2 isolate recorded the higher amount.

Intrinsic antibiotic resistance (IAR) of Azo 11 of *Azospirillum* isolate varied from 250, 20, 150 and 200 ppm; the phosphobacterial isolate PB 9, recorded 300, 15, 250 and 200 ppm of streptomycin, chloramphenicol, ampicillin and kanamycin respectively. Similarly, the *Pseudomonas* isolates Pfa 2 recorded the maximum resistance to all the antibiotics tested. Growth performance of Azo 11, PB 9 and Pfa 2 under different carbon sources indicated that malate, glucose and glycerol supported the growth respectively. The phosphobacterial isolate PB 9 exhibited varying degree of solubilization of different type of rock phosphates. Among the rock phosphates, Mussorie rock phosphate solubilized faster compared to Udaipur and Gafsa rock phosphates.

Germination per cent and seedling vigour index of Cotton seedlings improved by the inoculation of *Azospirillum* (Azo 11), phosphobacteria (PB 9) and *Pseudomonas* (Pfa 2). The rhizoplane, rhizosphere colonization and adsorption of cells to the Cotton roots under hydroponics condition was higher due to the inoculation of Azo 11, PB 9 and

Pfa 2. The nutrient contents viz., total sugars, reducing sugars and amino nitrogen of the root exudates were also higher due to the inoculation of these rhizobacterial isolates. These rhizobacterial isolates were also compatible under different carrier materials. Lignite enhanced the survival upto 180 days than peat. Logistic population dynamics model of rhizosphere microflora was developed to study the influence of combined inoculation of rhizobacterial isolate with graded levels of N and P. The growth rate of total diazotroph, was comparatively higher than bacteria and actinomycetes. A declining growth rate was observed for fungal population in the rhizosphere of Cotton due to the combined inoculation of the rhizobacterial isolates. In general the total diazotroph growth rate was higher at 75 per cent N and P when compared with that of bacteria and actinomycetes. The logistic population dynamics model is useful in understanding the growth rate and population dynamics of the rhizosphere in cotton, which in turn might throw some light in designing the time of application of antagonistic microorganisms.

The survival of rhizobacterial isolates was higher due to the combined inoculation than individual inoculation at graded levels of N and P. The growth parameters like shoot and root length, number and length of lateral roots, and root volume, the yield parameters viz., monopodial and sympodial branches, number of bolls and boll weight were enhanced due to the combined inoculation of *Azospirillum* (Azo 11) + phosphobacteria (PB 9) + *Pseudomonas* (Pfa 2) with graded levels of N and P. The macro and micronutrients uptake was higher due to the combined inoculation of these rhizobacterial isolates. The seed cotton yield (20.53 Q ha^{-1}) and dry matter (4453 kg ha^{-1}) production increased due to the combined inoculation of *Azospirillum* (Azo 11) + phosphobacteria (PB 9) + *Pseudomonas* (Pfa 2) at recommended level (100%) and also on par with reduced level of N and P (75%). This study provides evidence that there is a possibility of saving of 25 per cent of N and P with combined inoculation of *Azospirillum* (Azo 11) + phosphobacteria (PB 9) + *Pseudomonas* (Pfa 2) to obtain the desired yield and to improve the fertility status of the soil.

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INTRODUCTION

CHAPTER I

INTRODUCTION

Cotton (*Gossypium* sp.) the king of fibre of the globe is grown in 68 countries in about 33.5 million hectares with an estimated production of 20 million tonnes. Being a premier crop of commerce in our country, it accounts for nearly thirty per cent of total foreign exchange earnings. India has the distinction of having the largest cotton area (8.8 to 9.1 m ha) in the world and accounts for 15 per cent of world cotton lint production (3 m tonnes). India have nearly tripled cotton productivity since 1950's, still the production (320 kg lint ha⁻¹) remains low compared to the world average (560 kg lint ha⁻¹). In Tamil Nadu, 2.22 lakh ha area is under cotton cultivation with the productivity of 421 kg ha⁻¹.

Cotton (*Gossypium* sp.) needs adequate fertilizer for obtaining the desired yield. There are plentiful opportunities available to raise productivity of cotton *i.e.* the use of biofertilizer and biocontrol agents like nitrogen fixers, phosphate solubilizers and *Pseudomonas fluorescens*.

Agricultural soils often contain inadequate and ineffective beneficial microflora, such as diazotrophs and phosphate solubilizing organisms. A major barrier to the successful agronomic use of bacterial inoculum is the need to establish population densities of the introduced bacterium in the root environment, an environment characterized by intense microbial competition for nutrients (Weller, 1988). The rhizobacteria belong to several genera of soil bacteria which are able to colonize roots *viz.*, *Acetobacter*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Bradyrhizobium*, *Serratia* and *Xanthomonas* (Kloepper *et al.*, 1989) plays a vital role in the crop improvement.

The proliferation of rhizobacteria in host plant rhizosphere helps in the fixation of atmospheric nitrogen and supplies it to plants. They also synthesize siderophores that can solubilize and sequester iron from soil and provide it to plant cells. They synthesize different phytohormones that can act to enhance various stages of plant growth. The phosphate solubilizing rhizobacteria possess mechanisms for the solubilization of

minerals such as phosphorus that then become more readily available for plant growth and *Pseudomonas* helps in the reduction of plant diseases and nematode infection (Brown, 1974 and Glick, 1995).

The compatible existence of these nitrogen fixing, phosphate solubilizing and disease suppressing rhizobacteria in the rhizosphere could result in enhanced biological activities, which in turn helps in better plant growth and crop yield. Radhakrishnan (1996) reported that dual inoculation of *Azospirillum* and phosphobacteria resulted in enhanced plant height, root biomass and kapas yield in Cotton.

Inoculation of seeds and soil with beneficial microorganisms that will proliferate in the rhizosphere is essential to enhance plant growth. In large scale inoculant production, selection of suitable / elite strains is essential. Normally elite strains are multiplied and mixed in suitable organic carriers for easy application. These organic carriers should have the ability to carry the selected microorganisms in viable condition. Normally, Peat is commonly used as a carrier material. Due to the non-availability of peat, in large scale and at present little information is available for dual / combined application in the carrier material, hence attempts have to be made to select a suitable carrier for dual inoculation.

The introduction of efficient growth promoting rhizobacteria in the rhizosphere of crops increases the availability of plant nutrients by bringing changes in soil microenvironment. The ecology of rhizosphere microflora and their effects on plant growth has gained much interest in recent past. Plant root exudates determine the nature and extent of rhizosphere microflora (Brown, 1975). Hence, the factors affecting the root growth and metabolism will result in qualitative and quantitative changes in rhizosphere microflora and in turn affect plant growth (Rovira, 1969). The information on the effect of inoculants on general rhizosphere microflora is scanty.

To obtain the desirable yield in Cotton, there is a need for the development of efficient isolates, which save the use of nitrogen and phosphorus and control the plant diseases in addition to yield increase.

With these in view, the research programme has been framed with the following objectives.

- Isolation and characterization of suitable rhizobacterial strains for cotton from different locations of Tamil Nadu.
- Screening of rhizobacterial isolates under *in vitro* conditions.
- Studies on the production of growth promoting substances and intrinsic antibiotic resistance of rhizobacterial isolates.
- Studies on efficacy of root colonization by rhizobacterial isolates under *in vitro* conditions.
- Selection of carrier material for dual inoculum production.
- Studies on combined inoculum on the growth and development of cotton var. MCU 9 with graded levels of N and P under field conditions.
- Development of logistic microbial model to study the effect of rhizobacterial inoculation on the rhizosphere microflora of cotton.

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Cotton is an important commercial crop and India export nearly one third of its cotton as lint, yarn and fabrics which accounts for nearly one third of total export earnings. However, compared to the rapid progress since 1990's there are a severe jolt and set back to cotton production in 1997-98. The production is estimated at 148 lakh bales and the average yield declined to 288 kg ha⁻¹ from 343 kg ha⁻¹. The significant loss in production and productivity is mainly due to certain unseasonal rains, improper nutrient management and pest and diseases. In general agricultural soils often contain inadequate and ineffective beneficial microflora, such as diazotroph and phosphate solubilizing organisms. To increase the productivity of cotton, application of elite rhizobacterial strains through proper nutrient management is essential.

The challenge of agrobiological studies is to characterize rhizosphere microbial relationship and to optimize all components so that they function coordinately in the system. The results available suggest that plant growth can be optimized to obtain maximum yields using specific combinations of selected microorganisms that function in a synergistic form and this biological potential may be used in sustainable agriculture and crop management studies (Anonymous, 1999).

The management strategy will involve the selection of suitable microbial isolates in combination with beneficial rhizobacteria producing biologically active compounds or favouring nutrient cycling (Ames *et al.*, 1987 and Hoflich *et al.*, 1992) and the selection may be made for compatibility and combined efficiency regarding a particular soil-plants and environmental system (Azcon *et al.*, 1991).

In this view, plant growth promoting rhizobacteria from cotton rhizosphere has been studied for the present research programme.

2.1. RHIZOSPHERE

Hiltner (1904) first introduced the term rhizosphere to designate the zone of soil in which microflora is subjected to the influence of plant roots. The region of soil immediately surrounding the roots of higher plant together with the root surface constitutes

rhizosphere (Stanier *et al.*, 1977). Rhizosphere is a site rich with nutrients to support microbial activity and interactions among soil microorganisms. Characteristics that might enhance the establishment and survival of introduced microbes include high growth rate, resistance to adverse conditions, production of substances that aid in added production of antibiotics (Mazzola *et al.*, 1992).

2.2. PLANT GROWTH PROMOTING MICROORGANISMS IN THE RHIZOSPHERE

Beneficial free-living soil bacteria are usually referred to as plant growth promoting rhizobacteria (or) PGPR (Kloepper *et al.*, 1989) or by one group of workers in China, as yield increasing bacteria or YIB (Tang, 1994). Bacteria which are mainly considered as PGPR include the genera of *Acetobacter*, *Azotobacter*, *Azospirillum*, *Bacillus* and *Pseudomonas* (Brown, 1974; Elmerich, 1984; Kloepper *et al.*, 1988; Bashan and Levany, 1990 and Tang, 1994).

2.3. ECOLOGY OF PGPR

2.3.1. *Azospirillum*

Beijerinck in 1922 first isolated the bacterium from N₂ poor sandy soil of the Netherlands and originally named as *Spirillum lipoferum* (Beijerinck, 1925). The bacterium was able to fix atmospheric nitrogen in enrichment culture. The importance of these microorganisms was realized when Dobereiner and Day (1976) isolated it from the roots of *Digitaria decumbens* cv *transvala*, which exhibited nitrogenase activity. In this host, bacteria are in close proximity to the vascular tissue of the plant (endophytic) and have easy-access to the photosynthates. The fixed nitrogen inturn is supplied directly to the plant (endophytic diozotrophs). They also reported that *S. lipoferum* as a very common root and soil inhabitant in the tropics.

Five species have been described to date in the genus *Azospirillum*. Both *A. brasilense* and *A. lipoferum* are the best studied among the azospirilla and a large variety of morphological and physiological features differentiate both species (Tarrand *et al.*, 1978). Later *A. amazonense*, an acid tolerant species was isolated from the grasses and palm trees in Brazil (Magalhaes *et al.*, 1983). *A. halopraeferens* had only been isolated from the root surface of *Leptochloa fusca* in Pakistan (Reinhold *et al.*, 1987). Kabir *et al.* (1995) characterized and identified four *Azospirillum* spp at molecular level using different oligonucleotide probes.

Azospirillum irakense, a species with pectin hydrolyzing ability was found only in the rhizosphere of rice in the region of Diwaniyah in Iraq (Khammas *et al.*, 1989). Direct sequencing of PCR amplified 16S rDNA gene distinguished *A. irakense* and *A. lipoferum* (Bally *et al.*, 1992). All species except *A. irakense* could be identified with an *Azospirillum* specific probe, based on the sequence of the 23S rRNA (Kirchhof and Hartmann, 1992).

Plethora and papers documented the wide spread geographic distribution of *Azospirillum* spp. Azospirilla are abundant particularly in tropical soils. It appeared to be able to grow in temperate regions and cold climate spodosols of Finland (Haahtela *et al.*, 1981). The occurrence of *Azospirillum* in the rhizosphere varied from 1 to 10 per cent to the total rhizosphere population (Okon, 1985). The rhizosphere contains 100 times more azospirilla than the non-rhizosphere soils (De Coninck *et al.*, 1988).

Mascarua-Esparca *et al.* (1988) reported the occurrence of *Azospirillum* in the roots of giant cactaceous plants. Its occurrence in the spermosphere of graminaceous plants (Rao and Venkateswarlu, 1988) was also reported. Seeds of graminaceous plants were found as carriers of azospirilla. Sundaram *et al.* (1988) isolated *Azospirillum* from the rhizosphere of turf grass. The roots of poaceae of Indian tropical forests also found to harbour *Azospirillum* (Gajendiran and Mahadevan, 1989).

Azospirilla have been isolated from a wide variety of plants from all over the world from tropical, temperate, cold climates, desert plants, water-flooded conditions and also from salt affected soils (Michiels *et al.*, 1989a). Sumner (1990) reported the occurrence of *Azospirillum* sp in the rhizosphere of various field grown crops such as rice, wheat, maize, sorghum and pearl millet of different locations.

Fages and Lux (1991) isolated *Azospirillum* sp. from the roots of sunflower. Gamo and Ahn (1991) reported the presence of *Azospirillum* in the roots of non-graminaceous plants in Japan. Bashan (1991) reported the air borne phase of azospirilla and speculated it as a mode of dispersal. The occurrence of *Azospirillum* spp. in the rhizosphere of columnar cardon cactus, *Pachycereus pringlei* (Puente and Bashan, 1993) and Mediterranean herbaceous swards (Zaady *et al.*, 1994) has also been reported (Table 1).

Table 1. Isolation of *Azospirillum* from different plant species and families

Plant species	Scientific Name	References
Family : Graminae		
Maize	<i>Zea mays</i>	Van Bulow and Dobereiner (1975)
Wheat	<i>Triticum aestivum</i>	Neyra <i>et al.</i> (1977)
Rice	<i>Oryza sativa</i>	Rao <i>et al.</i> (1978)
Sorghum	<i>Sorghum bicolor</i>	Veena (1999)
Pearl millet	<i>Pennisetum typhoides</i>	Wani <i>et al.</i> (1988a, 1988b)
Sugarcane	<i>Saccharum officinarum</i>	Quintero and Garza (1978), Hegazi <i>et al.</i> (1979)
Digitaria and other forage grasses	<i>Digitaria decumbans</i>	Neves <i>et al.</i> (1976), Day <i>et al.</i> (1975)
Barley	<i>Hardium vulgare</i>	Reynders and Vlassak (1978)
Rye	<i>Secale cerelae</i>	
Oats	<i>Avena sativa</i>	
Temperate grasses		Glatzle and Martin (1981)
Blue panic grass	<i>Panicum maximum</i> ,	Dobereiner <i>et al.</i> (1976), Silva de and Dobereiner (1977)
Green panic mutants	<i>Bracharia mutica</i>	
Para grass	<i>Pennisetum purpureum</i>	
Bamboo	<i>Bambus vulgaris</i> <i>Dendrocalamus strictus</i>	Mahesh Kumar (1997)
Family : Euphorbiaceae		
Peepal tree	<i>Ficus religious</i>	Laxmi Kumari <i>et al.</i> (1976)
Rubber plant	<i>Euphorbia antisyphilitica</i> <i>E. caudifolia</i>	Rao and Venkateswarlu (1982)
Family : Cactaceae		
Cactus plant	<i>Opuntia vulgaris</i> <i>O. microdays</i> <i>O. cylindrica</i> <i>O. coccinellifera</i> <i>O. dellini</i> <i>O. ficus-indica</i>	Rao and Venkateswarlu (1982)

(Contd...)

Table 1 (Contd...)

Plant species	Scientific Name	References
Family : Fabaceae		
	<i>Crotalaria burhia</i>	Rao and Venkateswarlu (1982)
Family : Boraginaceae		
Small Shrubs	<i>Saricostoma pauciflorum</i> <i>Heliotropicum zeylanicum</i>	Rao and Venkateswarlu (1982)
Family : Amaranthaceae		
Amaranthus (Weed)	<i>Amaranthus</i> sp.	Govindan and Purushothaman (1985)
Amaranthus	<i>Amaranthus spinosa</i>	Laxmi Kumari <i>et al.</i> (1976)
Family : Portulacaceae		
Table rose	<i>Portulacca</i> spp.	Laxmi Kumari <i>et al.</i> (1976)
Aquatic plant	<i>Potamogeton filiformis</i>	Sylvester-Bradley (1976)
Family : Malvaceae		
Cotton	<i>Gossypium</i> spp.	Bashan <i>et al.</i> (1989) Prathibha (1993)
Family : Leguminaceae		
Soybean	<i>Glycine max</i> L.	Reynders and Vlassak (1978) Bashan <i>et al.</i> (1989)
Family : Cruciferaeaceae		
Radish Cabbage	<i>Raphanus sativus</i> <i>Brassica oleracea</i>	Reynders and Vlassak (1978)
Family : Compositae		
Sunflower	<i>Helianthus annuus</i> L.	Kumar <i>et al.</i> (1995)
Family : Solanaceae		
Tomato	<i>Lycopersicon esculentum</i>	Reynders and Vlassak (1978)
Potato	<i>Solanum tuberosum</i>	Reynders and Vlassak (1978)
Capsicum	<i>Capsicum</i> spp.	Reynders and Vlassak (1978)
Family : Convolvulaceae		
Sweet potato Tapioca	<i>Ipomoea batatas</i> <i>Manihot esculenta</i>	Dobereiner (1988)

2.3.2. Phosphobacteria

Pikovskaya (1948) made a pioneering attempt in isolating "phosphorite" an organism capable of actively solubilizing tricalcium phosphate (TCP) and coined the name "Bacterium P". Several strains of phosphate solubilizing microorganisms (PSM) which liberated inorganic form of 'P' from the organic compounds like lecithin and phytin were isolated (Menkina, 1951; Szember, 1960 and Greaves *et al.*, 1965). Bacteria capable of solubilizing tricalcium phosphate were isolated from the glands of *Cassia occidentalis* by Sen and Paul (1958).

Gerretsen (1948); Sperber (1957); Louw and Welbey (1958); Katznelson and Bose (1959) designed enrichment technique and special media for the isolation of phosphate dissolving microorganisms from soils and from rhizosphere. Solubilization of insoluble phosphate by Gram positive and Gram negative rods, cocci shaped bacteria and several fungi including yeast were reported by Ahmad and Jha (1968). Phosphate solubilizing bacterial and fungal isolates like *Bacillus* sp. *Aspergillus flavus*, *A. carbonoum*, *A. fumigatus* and *A. wentii* were obtained from Mussorie rock phosphate, Jha Markotra and Maton rock phosphate deposits (Gaur *et al.*, 1973). Besides inorganic phosphate solubilization, they were also found to solubilize organic phosphates.

Kalugasalam (1981) compared the phosphate solubilization efficiency of *Pseudomonas* sp. with *Aspergillus niger*, isolated from the rhizosphere of cowpea and Bengal gram. Bacterial isolate was found to be more efficient in phosphorus solubilization than fungi. Ramamourty (1982) attributed more phosphorus solubilization to the presence of *Bacillus* sp. in the rhizosphere of pearl millet. Gaur and Gaind (1983) suggested that the isolation based on rock phosphate solubilization was an appropriate approach because most of the rock phosphate solubilizers possessed the capability to dissolve different forms of insoluble phosphates also.

Molla *et al.* (1984) isolated several species of *Bacillus*, *Proteus*, *Serratia*, *Pseudomonas*, *Micrococcus* and *Streptomyces* from the rhizosphere of rye grass and wheat. Thomas (1985) noted higher population of P-solubilizing fungi in the laterite, alluvial and clayey soils than in the sandy soils of coconut plantations. Four *Bacillus* species *viz.*,

B. cereus, *B. megaterium*, *B. polymyxa* and *B. subtilis* with potential for solubilizing inorganic phosphates were isolated from the rhizosphere of *Aristida* by Charul Joshi and Pandya (1991). Illmer and Schinner (1992) isolated two phosphate solubilizing microorganisms, *Penicillium* sp. and *Pseudomonas* sp. from the forest soils of Austria, which solubilize high amount of inorganic phosphates.

Narula *et al.* (1995) isolated six phosphate solubilizing isolates of *Azotobacter chroococcum* from the rhizosphere soils of oilseed crops, which solubilized TCP and RP at 37 and 42°C respectively. By "rock phosphate enrichment technique" twenty isolates of *Azospirillum* showing P-solubilizing property were obtained by Tamilvendan (1995). However, these isolates were found inferior to PSB *Bacillus megaterium* var *phosphaticum* in P-solubilization. An interesting observation was made by Mishra *et al.* (1996) regarding the solubilization of insoluble phosphates by two bacterial isolates. *Rhizobium phaseoli* a symbiotic N₂ fixing bacteria isolated from the nodules of French bean was found to be more efficient in phosphate solubilization than *Pseudomonas*.

2.3.3. *Pseudomonas fluorescens*

Old and Nicholson (1975) reported internal populations of bacteria in a study using electron microscopy. Bacteria were found to occur in root cortical tissues and in pits of the endodermis. Bacteria belonging to 19 genera and 46 species were recovered from seeds and ovules, where in *Pseudomonas fluorescens* was recovered from 93 per cent of the seeds tested (Mundt and Hinkle, 1976).

Howell and Stipanovic (1979) isolated fluorescent pseudomonads from the rhizosphere of cotton. Scher and Baker (1982) isolated fluorescent *Pseudomonas* from using the fungus *Fusarium* sp. as bait. Geels and Schippers (1983a) isolated fluorescent pseudomonads from potato tubers using modified King's medium B, supplemented with cycloheximide, antimycin and chloramphenicol.

The rhizosphere bacterial populations estimated on entire root systems of young green house and field grown plants showed total aerobic bacterial populations, total fluorescent bacterial population and populations of inoculated plant growth promoting

strains of *Pseudomonas putida* generally approximated a log normal distribution (Loper *et al.*, 1984). *Pseudomonas putida* was isolated from soybean roots by Kloepper *et al.* (1985).

New media was formulated that provide a high degree of selectivity and detection of fluorescent *Pseudomonas*. The media was based on a detergent, sodium lauryl sarcosine and antibiotic trimethoprim (Gould *et al.*, 1985). Mew and Rosales (1986) isolated fluorescent bacteria from rhizosphere of rice plants. Misaghi (1990) isolated *P. fluorescens* and *P. putida* from the rhizosphere and rhizoplane of cotton, tomato, cucumber and alfalfa.

Weststeijn (1990) obtained *Pseudomonas* isolate from rhizosphere by using King's medium B supplemented with cycloheximide (100 ppm), chloramphenicol (12.5 ppm), antimycin (50 ppm) and β -hydroxy quinoline (200 ppm). Vandenhove *et al.* (1991) isolated *P. fluorescens* from the rhizosphere of winter wheat. Seong *et al.* (1991) used M9 solid medium for the recovery of *P. fluorescens* strain ANP 15 from barley roots. Isolates from the rhizosphere of soybean were found to be predominantly *Bacillus* sp, *Corynebacteria*, *Pseudomonas cepacia* and *Pseudomonas gladioli* (Kloepper *et al.*, 1992).

Gamiel and Katan (1993) using modified King's medium B incorporated with cycloheximide (100 ppm), ampicillin (50 ppm), chloramphenicol (125 ppm) and pentachlorobenzene (5 ppm) obtained strains of *P. putida* from tomato roots. Glick *et al.* (1995) demonstrated a novel procedure for rapid isolation of plant growth promoting pseudomonads by using 1-aminocyclopropane-1-carboxylate (ACC) as a sole source of nitrogen. The *Pseudomonas* strain designated as RRLJ 134 was isolated from the top soil (pH 5, sandy loam) of a tea plantation in the Dooars region (Dileep Kumar and Bezbaruah, 1996).

2.4. NITROGEN FIXATION BY *Azospirillum*

Azospirillum strains fix atmospheric nitrogen efficiently as free-living bacteria or in association with plants and participate in the nitrogen cycle (Heulin *et al.*, 1987). N₂ fixation was the first major mechanism of action suggested for the enhancement of plant growth by *Azospirillum*. Incorporation of atmospheric nitrogen into the host plant by

Azospirillum was evaluated mainly by the indirect assay *i.e.* acetylene reduction (Van Berkum and Bohlool, 1980). Nitrogen fixation by *Azospirillum* has now been confirmed by several workers too, using not only conventional microkjeldhal assay, but also the more definite method of isotopic enrichment involving $^{15}\text{N}_2$ (Laxmikumari *et al.*, 1976; Okon *et al.*, 1976b; Barbar *et al.*, 1978 and Scott and Scott, 1978).

In the past decade, there have been further reports of substantial increases in total N accumulated in field grown crops following inoculation with *Azospirillum* (Bashan and Levanony, 1990). In most experiments, increases have been less than 20 per cent (Bouton *et al.*, 1979; Albrecht *et al.*, 1981 and Reynders and Vlassak, 1982). However, many reports also showed increase of 20-60 per cent (Bouton *et al.*, 1979; Tayler *et al.*, 1979; Kapulnik *et al.*, 1981a; Okon *et al.*, 1981) and highly extreme values 60-128 per cent (Hegazi *et al.*, 1981; Kapulnik *et al.*, 1981b; Subba Rao, 1981 and Baldani *et al.*, 1983) in N uptake than in uninoculated control.

Lima *et al.* (1987) demonstrated that upto 50 per cent of the N content of crops such as sugarcane, *Panicum maximum* and *Paspalum notatum* could be supplied by associated nitrogen fixers mainly *Azospirillum*. Mallik *et al.* (1987) showed that using *A. brasilense* DSM 1891 and ^{15}N , the percentage of N in Kallar grass derived from atmospheric nitrogen were between 30 and 60 per cent. Inoculation of wheat and maize with *Azospirillum* spp. (Rennie and Thomas, 1987 and Kucey, 1988) indicated that upto 18 per cent of the plant nitrogen was derived from nitrogen fixation.

Several workers have examined the nitrogen fixing efficiency of *Azospirillum* species, Dobereiner and Day (1976) reported *Azospirillum* to fix as much as 150 mg of N g^{-1} of malate utilized. Okon *et al.* (1977) reported 20-24 mg N fixed g^{-1} of carbon source utilized whereas Nelson and Knowles (1978) observed 4.7 to 28.0 mg N g^{-1} carbon sources. Dobereiner and Boddey (1980) reported that the efficiency of nitrogen fixation increased with increasing age of culture reaching values of 98 mg g^{-1} carbon sources and 49 mg g^{-1} of glucose for *Azospirillum brasilense* and *Azospirillum lipoferum* respectively in the early stationary phase. Purushothaman *et al.* (1988) reported that the nitrogen fixing potential of *Azospirillum* sp. varied between 1.6 to 23.96 mg g^{-1} of carbon source. Prathibha (1993) observed 2.4 to 18.23 mg g^{-1} of carbon source utilized by *Azospirillum* strains from cotton

genotypes. Mahesh Kumar (1997) isolated six *Azospirillum* strains from the roots of bamboo plants and found them to fix 15.6 to 22.4 mg g⁻¹ carbon sources utilized. *Azospirillum lipoferum* and *Azospirillum brasilense* showed nitrogen fixation in the range of 7.54 to 24.53 mg of nitrogen g⁻¹ malic acid after seven days at 28°C under static conditions (Tamilvendan and Purushothaman, 1996).

Variation in the nitrogenase activity was observed between sites, seasons and growth stages of the forage grasses (Day *et al.*, 1975). Organic matter, pH and soil redox potential governs nitrogen-fixing ability of *Azospirillum*. *Azospirillum brasilense* contributed 12.6 per cent of nitrogen in maize plants (Rennie, 1980). *Azospirillum* strains isolated from rice roots showed consistent varietal variation with respect to nitrogen fixing ability (Nayak *et al.*, 1981).

Okon *et al.* (1983), using ¹⁵N-labelled N₂ and AR assay, demonstrated that *A. brasilense* in the rhizosphere of *Setaria italica* fixed N₂ but only 5 per cent of the N₂ fixed was incorporated into the plant. Wheat plants derived only minor amounts of nitrogen from *Azospirillum* if exogenous carbohydrate were not present in the rhizosphere (Lethbridge and Davidson, 1983). According to Hartman *et al.* (1988) different species of *Azospirillum* varied in utilization of amino acids for growth and nitrogen fixation. Further, amino acid concentration of 10 mM inhibited nitrogen fixation in most of the *A. brasilense* strains. Factors for the contribution of nitrogen by *Azospirillum* to plant and the growth conditions necessary for nitrogen fixation were reported to be highly variable and erratic (Bashan and Levanony, 1990). Boddey and Dobereiner (1995) concluded that, nitrogen fixation by *Azospirillum* was restricted to rhizosphere or rhizoplane of host plant.

Okon *et al.* (1988) suggested that *Azospirillum* supplied less than 1 kg ha⁻¹ season⁻¹ in most cases. Nevertheless, all wild type *Azospirillum* strains were found to fix N₂ efficiently either as free-living bacteria or in association with plants (Hurek *et al.*, 1988; and Heulin *et al.*, 1989). By using the ¹⁵N dilution technique the effect of inoculation of wheat with *Azospirillum* was calculated as 0.067 mg N₂ fixed plant⁻¹ (Christiansen-Weniger and Van Veen, 1991a). In association with rice (Var BAS 370), *Azospirillum lipoferum* N₂ contributed about 66 per cent of the total N₂ in plants as was demonstrated with ¹⁵N isotopic studies (Mallik *et al.*, 1997).

2.5. PRODUCTION OF PHYTOHORMONES AND OTHER GROWTH PROMOTING SUBSTANCES

Many root colonizing bacteria are known to produce vitamins and growth hormones which often leads to morphological changes in the plants (Brown, 1972; Prikryl *et al.*, 1985 and Derylo and Skorupska, 1993).

2.5.1. *Azospirillum*

Many authors (Table 2) provided the evidence for the production of various plant hormones by *Azospirillum* in the culture.

Table 2. Production of plant growth hormone by plant associated rhizosphere bacteria

Plant growth hormone	References
Indole acetic acid (IAA)	Tien <i>et al.</i> (1979); Hartmann <i>et al.</i> (1983a,b); De Francesco <i>et al.</i> (1985); Horemans <i>et al.</i> (1986); Harari <i>et al.</i> (1988); Zimmer and Bothe (1988); Omay <i>et al.</i> (1993) and Baca <i>et al.</i> (1994)
Indole lactic acid	Tien <i>et al.</i> (1979)
Indole - 3- ethanol	Crozier <i>et al.</i> (1988)
Indole - 3 - methanol	Crozier <i>et al.</i> (1988)
Indole - 3 - butyric acid (IBA)	Fallik <i>et al.</i> (1989)
Gibberellins	Tien <i>et al.</i> (1979) and Bottini <i>et al.</i> (1989)
Absciscic acid	Omay <i>et al.</i> (1993)
Cytokinins like substances	Tien <i>et al.</i> (1979) and Whallon <i>et al.</i> (1985)

Azospirillum brasilense produces extremely high amounts of IAA and was mostly superior to strains of *Azospirillum lipoferum* (Horemans *et al.*, 1986; Mascarua Esparca *et al.*, 1988 and Martin *et al.*, 1989). Cultures of *A. brasilense* and *A. lipoferum* excreted high amounts of IAA in logarithmic phase (Zimmer and Bothe, 1988). *Azospirillum* converts tryptophan to indole acetic acid (Reynders and Vlassak, 1979). IAA, gibberellins and cytokinins were excreted by *A. brasilense* (Tien *et al.*, 1979). They stated that root exudates of plants contained tryptophan which probably acted as precursor for the synthesis of IAA.

The plant growth promoting influence of *Azospirillum* has been extensively examined (Michiels *et al.*, 1989a and Sumner, 1990). Tryptophan stimulated the IAA production and the concentration of IAA was low during logarithmic growth phase and increased rapidly with the beginning of stationary phase and showed a significant increase in the IAA due to the presence of tryptophan (Baca *et al.*, 1994).

An *Azospirillum* strain and a mutant, which over produced IAA in culture strongly affected plant root morphology (Jain and Patriquin, 1985; Kolb and Martin, 1985). However, mutants that failed to produce IAA in culture had no effect on root morphology (Barbieri *et al.*, 1986 and 1990). Inoculation with *Azospirillum* improved the hormonal balance of a hormonal defective mutant of wheat (Inbal and Feldman, 1992). The phytohormone synthesized by *Azospirillum* influenced the root hair development, respiration rate, metabolism and root proliferation which inturn resulted in better mineral uptake in the inoculated plant (Bar and Okon, 1993).

The gibberellins A₁, A₃ and 150-A₃ were detected in *A. lipoferum* sp 33 cultures in concentrations of 20 to 40 µg ml⁻¹ by combined gas chromatography and mass spectrometry (Bottini *et al.*, 1989).

2.5.2. *Pseudomonas*

Plant growth promoting substances *viz.*, Indole Acetic Acid (IAA) and Gibberellins (GAs) were isolated from specific strains of rhizosphere bacteria. There were shown to stimulate growth on radish and were given the epithet PGPR (Kloepper and Schroth, 1978.). Specific strains of *Pseudomonas fluorescens* have been used as seed inoculant to promote growth and increased yield of crops.

Nita (1964) reported the production of 'gibberellinic' substances by *Pseudomonas fluorescens*. Katznelson and Cole (1965) reported that *P. fluorescens* produced 1-14 µg l⁻¹ of gibberellic acid. They also reported that plant could derive maximum benefit from microbial synthesis of gibberellins and related substances only at the root surface. Eklund (1970) obtained effect on cucumber growth very similar to those of tomato after treating seeds with *Pseudomonas fluorescens*. IAA produced by bacteria may constitute a mechanism for

growth promotion by bacterial inocula (Brown, 1974). Many examples of plant growth stimulation by fluorescent pseudomonads have been reported. (Gardner *et al.*, 1984; Bashan, 1986b; Weller and Cook, 1986 and Van Peer and Schippers, 1988).

Rhizosphere colonizing fluorescent pseudomonads significantly increased growth and yield of certain crops (Suslow and Schroth, 1982a). Growth promotion is evidenced by increase in seedling emergence, vigour, seedling weight, root system development and yield (Kloepper *et al.*, 1985). A specific group of PGPR was seen to increase emergence of seedlings in cold field soil and was termed "Emergence Promoting Rhizobacteria" (EPR) (Kloepper *et al.*, 1986).

Dowling and O'Gara (1994) reported that many strains of pseudomonads produce IAA. Although IAA has not been directly implicated as a metabolite in disease control, it is bioactive and stimulates root elongation by stimulating the growth of root hairs. Sridar (1996) isolated and characterized ten isolates of *P. fluorescens* from the rhizosphere of cotton and soybean. Among those isolates, Pfc-2 produced 3.8 μg and 0.9 μg IAA ml^{-1} culture medium in the presence and absence of tryptophan respectively.

2.6. PRODUCTION OF SIDEROPHORE

Siderophore are iron chelating compounds secreted by bacteria on or around the roots that affect the growth of the plants. PGPR produce siderophores, which sequester iron in the root zone making it unavailable to certain rhizoplane microorganisms. They act differently depending on the nature of organism. (Table 3).

2.6.1. *Azospirillum*

Iron salts have been reported to be essential for N_2 fixation (Carnahan and Castle, 1958). Several azospirilla are endowed with a very efficient Fe-acquisition system. Saxena *et al.* (1986) reported that under iron limiting conditions *Azospirillum lipoferum* D₂ excrete the phenolate siderophores 2, 3 and 2, 5 - dihydroxy benzoic acid (DHBA). They conjugated with lysine and leucine as well salicylic acid. The yield of 2,3-DHBA was comparable to that observed in cowpea *Rhizobium* RA-1, which produced 2,3-DHBA (Modi *et al.*, 1985). Iron uptake was enhanced in the presence of siderophores.

Table 3 . Production of siderophores by plant associated rhizosphere bacteria

Siderophore	Producing bacteria	References
a. Pyoverdinin	<i>Pseudomonas</i> sp. <i>P. fluorescens</i> <i>P. aeruginosa</i> <i>P. putida</i> <i>P. syringae</i>	Buyer <i>et al.</i> (1995) Demange <i>et al.</i> (1986) Barbhaiya and Rao (1989) Bar-Ness <i>et al.</i> (1991) Cody and Gross (1987)
b. Catechols		
i. Agrobactin	<i>Agrobacterium tumefaciens</i>	Ong <i>et al.</i> (1979)
ii. Chrysobactin	<i>Erwinia chrysanthami</i>	Persmark <i>et al.</i> (1989)
iii. Enterobactin	<i>Enterobacteriaceae</i>	Pollack and Neilands (1970)
iv. 2,3-Dihydroxy benzoic acid	<i>Azotobacter vinelandii</i>	Page and Hayer (1984)
v. Azotochelin	<i>A. vinelandii</i>	Corbin and Bulen (1969)
c. Hydroxamates		
i. Cepabactin	<i>P. cepacia</i>	Meyer <i>et al.</i> (1989)
ii. Hydroxmate K	<i>Rhizobium leguminosarum</i>	Carson <i>et al.</i> (1992)
iii. Frankobactin	<i>Frankia</i> sp.	Aronson and Boyer (1994)
d. Catechol and Hydroxamate type	<i>Azotobacter chroococcum</i> <i>Azospirillum lipoferum</i> D ₂ <i>Pseudomonas</i> sp.	Fekete <i>et al.</i> (1989) Saxena <i>et al.</i> (1986) De Weger <i>et al.</i> (1986)

In *Azospirillum brasilense* RG, 2,3-DHBA conjugated with ornithine and serine and spirillobactins was characterized (Bachhawat and Ghosh, 1987). Siderophore known was known to increase the rhizosphere competency of *Azospirillum* (Michiels *et al.*, 1989a).

Sridar and Balasubramanian (1996) studied the siderophore production of different species of *Azospirillum* with ammonium under aerobic conditions. *Azospirillum* spp. produced salicylate type of siderophores ranged from 1.28 to 3.20 mg l⁻¹. *A. lipoferum* produced higher quantity of siderophores than other species.

2.6.2. *Pseudomonas* sp.

Soil *Pseudomonas* generally produce fluorescent, yellow green, water soluble siderophores with both a hydroxamate and phenolate group. Siderophores synthesized by fluorescent *Pseudomonas* is called pyoverdine (Meyer and Abdallah, 1978). Kloepper *et al.* (1980a) reported that PGPR produces extra cellular siderophores which efficiently complexes environmental iron, making it less available to certain native microflora. They named the siderophore as pseudobactin. Scher and Baker (1982) found that the rhizosphere bacteria which promoted plant growth showed high specificity, high affinity iron uptake systems exerting a strong influence on the microbial environment in the rhizosphere that can be quite beneficial for the plants.

Siderophore production by fluorescent pseudomonads is influenced by a great variety of factors viz., concentration of iron (Kloepper *et al.*, 1980b), nature and concentration of carbon and nitrogen sources, level of phosphates (Barbhaiya and Rao, 1985); pH and light (Greppin and Gouda, 1965); degree of aeration (Lenhoff, 1963); presence of trace elements such as magnesium (Georgin and Poe, 1931), zinc (Chakrabarty and Roy, 1964) and molybdenum (Lenhoff *et al.*, 1956) and temperature (Weisbeck *et al.*, 1986).

Specific strains of *Pseudomonas* produce siderophores which enhanced the plant growth and acted as biocontrol agent (Leong, 1986). With respect to siderophore mediated competition for iron (III) between different *Pseudomonas* strains in plant rhizosphere, the ability of exploiting heterologous siderophores has been postulated to confer a competitive advantage (Loper, 1988).

Dileep Kumar and Dube (1993) found a fluorescent *Pseudomonas*, isolated from tomato rhizoplane, produced siderophore in succinate medium deficient in iron. They found that iron suppressed siderophore production. Dileep Kumar and Bezbaruah (1996) reported the presence of hydroxamate siderophores in the crude extract of *Pseudomonas* strain isolated from rhizosphere of tea.

2.7. PHOSPHATE SOLUBILISATION BY PLANT GROWTH PROMOTING RHIZOBACTERIA

Chandrasekharan (1969) reported high degree of inorganic phosphate solubilization by rhizosphere bacterial isolates compared to non-rhizosphere bacterial isolates. Alexander (1977) observed soil phosphate solubilization by the microorganisms like *Pseudomonas*, *Bacillus*, *Aspergillus* and *Streptomyces*. Kalugasalam (1981) compared the phosphate solubilization among various *Pseudomonas* sp.

Molla *et al.* (1984) isolated several species of phosphate solubilizing *Bacillus*, *Micrococcus*, *Proteus*, *Pseudomonas*, *Serratia* and *Streptomyces* from the rhizosphere of rye grass and wheat. PGPR stimulated the plant growth by facilitating the uptake of minerals by the plant especially phosphate (Kloepper *et al.*, 1988).

Two phosphate solubilizing microorganisms *Penicillium* sp. and *Pseudomonas* sp. isolated from forest soils solubilized high amounts of inorganic phosphates (Illmer and Schinner, 1992). By rock phosphate enrichment technique twenty isolates of *Azospirillum* showing P-solubilizing property were obtained by Tamilvendan (1995). Anjani *et al.* (1996) isolated a growth promotory fluorescent pseudomonad also having the characteristics of phosphate solubilization and siderophore production. Mishra *et al.* (1996) reported higher solubilization of phosphate by *Rhizobium phaseoli* than *Pseudomonas* sp. Vassilev *et al.* (1997) studied the rock phosphate solubilization by immobilized cells of *Enterobacter* sp in fermentation and soil conditions.

Growth of phosphate solubilizing microorganisms in liquid medium containing insoluble phosphates results in the pH reduction of the medium (Sethi and Subba Rao, 1968; Gaur, 1972 and Venkateswarlu *et al.*, 1984). The production of organic acids by microorganisms seems to be the main cause for the solubilization of inorganic phosphates.

Pareek and Gaur (1973) correlated the release of phosphate from tricalcium phosphate and rock phosphate to organic acids produced by the phosphate solubilizing microorganisms. The aliphatic acids were more effective in solubilization of phosphate than phenolic acids. Barthakur (1978) detected organic acids in culture solutions, but unable to correlate the 'P' solubilization with the quantity of organic acids produced.

Many microbes in the soil are able to solubilize the unavailable forms of calcium bound phosphate by excreting organic acids which either directly dissolve rock phosphate or chelate calcium ions to bring phosphorus into solution (Kucey, 1983). Masky and Msanya (1985) observed the increased amount of soluble phosphorus with increasing concentration of organic acids. They also stated that in calcareous soil the citric acid had strong 'P' solubilizing ability than the other organic acids. Kapoor *et al.* (1989) showed that the solubilization of inorganic phosphate depends upon the type of organic acids produced by phosphate solubilizing microorganisms, which chelate the phosphate ions. Tri and dicarboxylic acids were more effective compared to monocarboxylic and aromatic acids in chelation.

Illmer and Schinner (1992) observed no correlation in pH reduction and P solubilization in culture medium of *Pseudomonas* sp. indicating that acid production is not the only reason for 'P' release in the medium. It was suggested by David and Peter (1994) that the complexing action of the citrate anion and dissolution properties of the protons released from citric acid as the reasons responsible for P solubilization. The organic acids is of little consequence in alkali soils, but in low pH soils it may be involved with micronutrient uptake or as a potential Al detoxification mechanism.

Illmer and Schinner (1995) examined the phosphorus solubilization mechanisms of *Penicillium aurantiogriseum* and *Pseudomonas* sp. (PI 18/89) and reported that no direct contact between microorganisms and calcium phosphate were necessary for effective solubilization of phosphorus and questioned the widely accepted theory that phosphorus solubilization was due to the release of organic acids. They suggested the release of protons accompanying respiration or NH_4^+ assimilation is the possible reasons for P solubilization in the absence of acid production.

Soil organic matter made up of plant and animal residues contain large quantities of organic phosphorus, which cannot be utilized by plants without their microbial conversion to inorganic form, which is mainly accomplished by enzymatic action.

The general name "phosphatase" had been used to describe a broad group of enzymes that catalyse the hydrolysis of both esters and anhydrides of phosphoric acids. Phosphatases are classified into acid and alkaline phosphatases (Eivazi and Tabatabai, 1977 and Gould *et al.*, 1979). Acid and neutral pH influenced the production of acid phosphatase, where as the alkaline phosphatase was produced in alkaline pH (Dick and Tabatabai, 1984).

Both Gram positive and negative bacteria secreted acid phosphatase into the medium, which mineralized the organo phosphate compounds (Spiers and McGill, 1979). Organisms like *B. subtilis*, *B. cereus*, *B. megaterium* and *P. aeruginosa* produced large quantity of phosphatase in the culture medium, which mineralized organic phosphorus (Kelly *et al.*, 1984; Nakas *et al.*, 1987 and Strom and Lory, 1987).

Lazzaroni *et al.* (1985) observed the production of the enzyme phosphatase by phosphate solubilizing bacteria and it was maximum between 12 and 15 hr of incubation. In the cultural medium, pH 6.0 was favoured for more acid phosphatase secretion (Boquet *et al.*, 1987), where as pH 7.3 for alkaline phosphatase (Magnouloux and Portalier, 1988). Juma and Tabatabai (1988) reported that the phosphatase activity in sterile root materials of corn and soybean was optimum at pH 4-5 indicating that these roots contain acid phosphatase activity.

Acid phosphatase also promotes the hydrolysis of the synthetic organo phosphatic substrates like P-nitrophenyl phosphate (Pradel and Boquet, 1988). *Bacillus* isolate (TV-4) from wheat rhizosphere was more effective in mineralizing P-nitrophenyl phosphate than the other isolates and the secretion of acid phosphatase was higher than alkaline phosphatase (Ravichandran, 1989). Chhonkar (1994) observed that crop characteristics had positive bearing on the accumulation of enzymes causing hydrolytic cleavage of phosphate monoesters at the root soil interface.

Mohammed (1984) reported significant release of 'P' from organic compounds through the action of phosphatase produced by *Rhizobium leguminosarum*. Organic 'P' source like P-nitrophenyl phosphate could be mineralized by isolates of *Azospirillum lipoferum*, but the efficiency of mineralization was lesser than the phosphate solubilizing bacteria *B. megaterium* var *phosphaticum* (Tamilvendan, 1995).

2.8. *Pseudomonas* AS A POTENTIAL BIOCONTROL AGENT

Rhizobacteria having an antagonistic interactions with various soil borne plant pathogens are being exploited and considered to be potentially effective as biocontrol agent due to their ability to colonize the rhizosphere easily and quickly along with production of metabolites beneficial for plant growth, in addition to the production of pathogen suppressive bio-compounds. Fluorescent *Pseudomonas* appears frequently among isolates from plant rhizosphere (Rouatt and Katznelson, 1961) and often comprises an important group of the bacterial population (Katznelson *et al.*, 1969).

In recent years there has been much success in obtaining biological control of plant pathogens using bacterization techniques (Cook and Rovira, 1976). A general screening of the rhizobacteria for antibiotic activity showed that they were inhibitory to a wide variety of microorganisms.

PGPR exhibited antibiotic activity towards to native microflora (Burr *et al.*, 1978). Kloepper and Schroth (1978) observed fluorescent pseudomonads group as potent rhizobacteria for controlling most of plant pathogens.

Fluorescent *Pseudomonas* also protects plants against other seed and root diseases (Howell and Stipanovic, 1979). The fluorescent *Pseudomonas* was noted for production of metabolites inhibitory to life process of bacteria and fungi. They identified different metabolites *viz.*, pterines, pyroles, indoles, pyocompounds, peptides and phenazines produced by fluorescent pseudomonads which are involved in the suppression of plant pathogens (Leisinger and Margraff, 1979).

For example pyrolnitron and pyolutecorin, produced by *Pseudomonas fluorescens* (Strain pf-5) are inhibitory to *Rhizoctonia solani* and *Pythium ultimum* respectively (Howell and Stipanovic, 1979). The antagonistic activity is based, among other

things on their capacity to colonize the surface of plant roots in an aggressive manner. Where by other microorganisms including phytopathogenic fungi can be suppressed. Besides, iron binding chelating agents called siderophores, are formed which are at least partially involved in the antagonistic activity. On the basis of their high affinity to Fe^{3+} ions, these chelating agents cause a deficiency of iron for pathogens to be inhibited (Kloepper *et al.*, 1980a and b).

Thus, a root or seed treatment of sugarbeet and radish with fluorescent *Pseudomonas* led to a reduced severity of take all on the roots (Suslow and Schroth, 1982a and b). Good results were obtained in the suppression of soil borne pathogens and sometimes yield increase by treatment with fluorescent *Pseudomonas*.

Savithri and Gnanamanickam (1987) reported that the seed treatment of peanut with *P. fluorescens* resulted in 59 per cent increased yield besides the reduction in stem rot severity caused by *Rhizoctonia solani*.

Weller (1988) observed antibiotic production in several beneficial pseudomonads which are antagonistic to plant pathogens. Lee and Kobayashi (1988) isolated *Pseudomonas*, which inhibited the *Rhizoctonia solani* of sugar beet. Dube and Podile (1989) reported that a fluorescent *Pseudomonas* protected peanut seedlings from *R. solani* and *S. rolfsii*. The disease incidence by *S. rolfsii* was reduced by 72 per cent and *R. solani* by 58 per cent when this *Pseudomonas* strain was used as a seed inoculant.

One of the mechanisms involved in disease suppression is the production of antifungal metabolites (Weller, 1988). However production of specific metabolites by microorganisms is dependent on both chemical and physical factors in the environment. Weller (1988) also reported that strain PFM 2 of *P. fluorescens* produced the antibiotic 2,4, diacetyl-phoroglucinol (DPAG) which was responsible for the inhibition of *Septoria tritici* under *in vitro* conditions.

Genetic alternation of *Pseudomonas fluorescens* strains to increase their antibiosis phenomenon render them deleterious to the plant (Maurhofer *et al.*, 1992). Production of antibiotic is the primary mechanism of suppression by this strain (Ownley *et al.*, 1992).

Dowling and O'Gara (1994) viewed that various metabolites produced by *Pseudomonas* involved in the biocontrol of plant diseases. Thara and Gnanamanickam (1994) observed *in vitro* inhibition of rice sheath blight pathogen *Rhizoctonia solani* by *P. fluorescens*.

Prevalence of 2,4-diacetylphloroglucinol production among strains of *Pseudomonas fluorescens* that suppress plant disease was observed (Nowak-Thompson *et al.*, 1994). Liu *et al.* (1995) reported that induction of systemic resistance in cucumber against *Fusarium* wilt by PGPR. Rhizobacteria from the local soil, having antifungal activity could be used as seed inoculants to partially suppress the disease in chick pea (Khot *et al.*, 1996). Food poisoning bacteria and moulds are controlled by *Pseudomonas* sp. by the production of siderophores (Laine *et al.*, 1996). *Pseudomonas* strain RRLJ-134 isolated from tea plantations were found to inhibit fungal pathogens and to enhance plant growth in tea and in some crop plants which are grown in vacant land in tea plantations (Dileep Kumar and Bezbaruah, 1996).

2.9. HYDROGEN CYANIDE PRODUCTION BY *P. fluorescens*

The term 'cyanide' will be loosely referred to both the cyanide anion (CN) and undissociated hydrogen cyanide (HCN). Cyanide formation (cyanogenesis) by bacteria was noted before world war (Clawson and Young, 1913).

Cyanide is a secondary metabolite of several microorganisms. It can be produced from glycine and from cyanogenic glycosides (Knowles, 1976) both of which have been demonstrated in root exudates (Rovira *et al.*, 1974; Curl and Trulove, 1986). The rate of HCN production by *Pseudomonas* depends on several environmental factors (Knowles, 1976 and Castric, 1975). Freeman *et al.* (1975) reported the production of HCN by *Pseudomonas fluorescens*.

The production of HCN by fluorescent *Pseudomonas* impart to the induction of plant resistance to certain pathogens, PGPR also increases plant growth indirectly by production of HCN which have been implicated in the reduction of plant pathogens (Ahl *et al.*, 1986). The role of siderophores in potato yield increase by *Pseudomonas* spp. in short potato rotations has been suggested due to HCN production (Bakker *et al.*, 1987).

Naturally occurring fluorescent *Pseudomonas* were seen to suppress black root rot of tobacco due to production of hydrogen cyanide (Voisard *et al.*, 1989). The cyanide producing isolates and growth-promoting isolates WCS-358 and WCS-374 respectively are fluorescent *Pseudomonas* isolated from the rhizosphere of potato, their ecological niches are likely to overlap, as will the actual locations where they are active.

2.10. INTRINSIC ANTIBIOTIC RESISTANCE (IAR)

Difficulties in ecological studies in the field arose because of the lack of convenient techniques, for selecting particular strains from amidst the large number of bacteria in the soil and differentiating between strains of bacteria. One apparent solution to this problem is the use of antibiotic resistance markers, either as intrinsic antibiotic resistance typing (Josey *et al.*, 1979; Rafique-Uddin *et al.*, 1984 and Meade and O'Gara, 1985) or by introducing one or more spontaneous mutations with variations in its antibiotic resistance pattern (Mc Loughlin *et al.*, 1984).

The genetic diversity of soil bacterium has been demonstrated using the patterns of intrinsic antibiotic resistance (Somasegaran and Hoben, 1994). They have been used extensively to mark the strains in genetics and molecular biology experiments (Krishnaraj, 1996). The IAR is due to the presence of genes, which are responsible for synthesis of enzyme systems, present both on the main chromosome and plasmid (Hayes and Wolf, 1990), that detoxify the antibiotics and produce proteins that inhibit the cellular transfer of the antibiotic.

2.10.1. *Azospirillum*

Within the species of *Azospirillum*, differences have been found between strains isolated from comparable habitats or even from the same habitat (Balandreau, 1986). Dobereiner and Baldani (1979) found diversity among *Azospirillum* strains with respect to their resistance to streptomycin, tetracycline, gentamycin, erythromycin and chloramphenicol.

Franché and Elmerich (1981) found that the wild type strains of *A. lipoferum* and *A. brasilense* were sensitive to streptomycin, rifampicin, nalidixic acid cephaloridine and Cefalotin. While, Magalhaes *et al.* (1983) observed that all the *A. amazonense* strains were resistant to penicillin, but relatively tolerant to chloramphenicol and erythromycin.

About 11 per cent of the total *Azospirillum* isolates showed the resistance to 50 ppm streptomycin and only five per cent of the total *Azospirillum* isolates recorded resistance to 50 ppm chloromphenioal (Govindan, 1993).

2.10.2. Phosphobacteria

Phosphate solubilizing *Bacillus* sp. isolated from the rhizosphere of pearl millet could not grow even at 100 ppm of streptomycin indicating the lower tolerance level of *Bacillus* sp. to the particular antibiotic (Ramamourty, 1982). Spontaneous mutants of *Pseudomonas* sp. NT 15 isolated from the rhizosphere of winter wheat were resistant to rifampicin than wild strain (Bolton *et al.*, 1990).

Belimov *et al.* (1995) suggested the gradient plate technique for detecting spontaneous resistant mutants of phosphate solubilizing organism *Agrobacterium radiobacter* (rifampicin 40 $\mu\text{g ml}^{-1}$ and streptomycin 500 $\mu\text{g ml}^{-1}$). Ramamourty (1982) developed mutant colonies of *Bacillus* sp. using nitrosoguanidine (NTG) which produced clear phosphate solubilization zone with simultaneous resistance to streptomycin (500 ppm).

Ravichandran (1989) induced mutation by employing NTG in *Bacillus* sp. which resulted in the mutants resistant to streptomycin (100 $\mu\text{g ml}^{-1}$), ampicillin (100 $\mu\text{g ml}^{-1}$), tetracycline (100 $\mu\text{g ml}^{-1}$) and chloramphenicol (25 $\mu\text{g ml}^{-1}$). The mutants possessed the capacity to solubilize more of 'P' over a long period of time than parent, indicating some possible change in nucleotide sequence.

2.10.3. *Pseudomonas* isolates

Weller and Cook (1983) obtained single and double antibiotic marker strains of *Pseudomonas fluorescens* for rifampicin and nalidixic acid. To investigate the migration of *Pseudomonas* from potato and seed tubers to roots in soil and their persistence on the tuber, antibiotic resistant antagonists were obtained by transferring colonies of the antagonist to increasing concentration of nalidixic acid. Geels and Shippers (1983a) developed rifampicin resistant mutant of fluorescent pseudomonads isolated from maize, soybean, sorghum and wheat. *P. fluorescens* and *P. putida* used as inoculum in the rhizosphere was identified by testing their resistance to antibiotic rifampicin (100 $\mu\text{g ml}^{-1}$). These strains showed resistance to rifampicin consistently for over 50 generations (Loper *et al.*, 1985).

Kloepper *et al.* (1985) isolated *P. putida* from water and sugar beet root. Sixty four strains resistant to 100 $\mu\text{g ml}^{-1}$ rifampicin were selected. Gutterson *et al.* (1986) developed resistance for *P. fluorescens* against kanamycin, chloramphenicol, streptomycin, ampicillin and tetracycline at 50, 34, 100, 7 and 12.5 $\mu\text{g ml}^{-1}$ respectively. James and Gutterson (1986) reported that strain HV 379 was resistant to nalidixic acid and spectinomycin. Mutant strains of *P. fluorescens* and *P. putida* were selected for resistance to rifampicin (100 $\mu\text{g ml}^{-1}$) and nalidixic acid (100 $\mu\text{g ml}^{-1}$) and the antibiotic resistant strains were checked for *in vitro* inhibition for *Erwinia caratovora* (Xu and Gross, 1986).

Bakker *et al.* (1987) developed Tn5 transposon mutants, which carried resistance marker against kanamycin and streptomycin, and they were distinguished from wild type isolate by isolation on a medium containing the antibiotics. Ganesan and Gnanamanickam (1987) inoculated peanut plants with cells of strain of *P. fluorescens* resistant to streptomycin (pf. st-R) (100 $\mu\text{g/ml}$) for biological control of *S. rolfisii*. Loper (1988) grew *Pseudomonas* spp. routinely in nutrient agar antibiotic supplemented medium at the levels ($\mu\text{g/ml}$), rifampicin 100, kanamycin 50 and cycloheximide 150. Thomashow and Weller (1988) isolated *P. fluorescens* from wheat using rifampicin 100 μg ; cycloheximide 100 μg ; nalidixic acid 100 μg , tetracycline 25 μg ; ampicillin 100 μg , chloramphenicol 30 μg and kanamycin 100 μg .

Weststeijn (1990) obtained *Pseudomonas* isolates from rhizosphere soil of tulip on king's medium B, supplemented with cycloheximide (100 $\mu\text{g ml}^{-1}$), chloramphenicol (12.5 $\mu\text{g ml}^{-1}$), antimycin (50 $\mu\text{g ml}^{-1}$) and 8-hydroxyquinoline (0.2 $\mu\text{g ml}^{-1}$).

Mishagi (1990) developed rifampicin and nalidixic acid resistant mutants of *P. fluorescens* and *P. putida* by supplementing 100 $\mu\text{g ml}^{-1}$ of the above antibiotics. Seong *et al.* (1991) recovered strain AVP 1 of *P. aeruginosa* with kanamycin (200 $\mu\text{g ml}^{-1}$) and cycloheximide (200 $\mu\text{g ml}^{-1}$) supplemented medium. Vandenhove *et al.* (1991) tested survival ability of *P. fluorescens* a neomycin resistant Tn 5 mutant developed on King's B medium supplemented with 200 μg of neomycin. Saxena *et al.* (1998) studied the inter and intra specific transfer of antibiotic resistance from fluorescent pseudomonads.

2.11. CARRIER BASED MICROBIAL INOCULANT PRODUCTION

Voluminous literatures are available in the selection of carriers for inoculant preparation especially with legume inoculant. Among the large list of carrier peat and lignite are extensively used for inoculant preparation. The geo-organic chemistry of lignite and peat revealed remarkable differences in the macromolecular structures of their fibrillic components. Certain undetermined characteristics of coal suggested that it could be used to produce good quality inoculants (Dube *et al.*, 1975).

Tilak and Subba Rao (1978) established that lignite, pressmud and farm yard manure (FYM) after suitable amendment with charcoal or coconut shell powder could substitute peat under Indian conditions. Kloepper and Schroth (1978) have developed powdered formulations of PGPR for use on potato by mixing bacteria with xanthan gum and talc. Twenty per cent xanthan gum preparation supported the highest population of PGPR.

Suslow (1980) suggested the incorporation of methyl cellulose in a powder formulation to coat PGPR on sugar beat seeds for better survival of rhizobacteria. Kulkarni *et al.* (1980) reported a wide variation in the composition of peat within the same location. Besides low grade quality and limited availability, high charges involved in transporting it from the source prohibit the extensive use of peat for commercial manufacture of legume inoculants in India (Rewari and Subba Rao, 1981).

Kloepper and Schroth (1981) reported that the dry powder inoculum of fluorescent *Pseudomonas* in talc powder with Xanthan gum was effective for seed tuber treatment of potato. Kundu and Gaur (1981) studied the suitability of peat, FYM, soil and cowdung cake powder for preparation of inoculants of efficient phosphate solubilizing bacteria and fungi and reported that although soil could serve as a good carrier for spore formers like *B. polymyxa* and *A. awamori* for pure and mixed inoculant preparations of PSM, peat is the best solid carrier.

Jauhri and Philip (1982) reported the pressmud with charcoal (13:1) was a superior inoculant base. The annual production of pressmud is estimated to be 5.45 m tonnes in India. Geels and Schippers (1983b) formulated the suspensions of *Pseudomonas* cells by mixing cell suspensions in 0.2 per cent protease peptone and used 2 per cent carboxy methyl cellulose in distilled water for potato tuber treatment.

Wood charcoal and soil mixture was found to be a good carrier material for the survival and maintenance of *Pseudomonas striata*, *Bacillus polymyxa* and *Aspergillus awamori* (Gaur and Gaiind, 1984). An inverse relationship existed between the moisture content of the carrier and shelf life. Weller and Cook (1986) reported that fluorescent *Pseudomonas* suspended in water containing 0.1M magnesium sulphate when used as seed treatment increased the growth of wheat.

Favilli *et al.* (1987) observed high viability of cells (10^9 /g) in peat based inoculants upto 90 days of storage. A granular peat formulation of *P. fluorescens* showed promise for control of seedling pathogens of cotton (Weller, 1988). Gaiind and Gaur (1990) studied the suitability of carriers like charcoal soil mixture, paddy straw compost and wheat bran for preparing phosphate solubilizing bacteria and fungal inoculants. The charcoal - soil mixture was found to be the best carrier for phosphate solubilizing bacteria and wheat bran for phosphate solubilizing fungi.

Govindarajan (1996) studied the growth and survival of *Azospirillum lipoferum* in peat, coirpith and peat and coirpith mixture. Peat supported higher proliferation of the inoculated organism than other carriers. Vermicompost as an alternative carrier to lignite was suggested in the inoculant preparation of phosphobacterium, *Azospirillum* and *Acetobacter diazotrophicus* (Muthukumarasamy *et al.*, 1996). Pressmud supported higher population of phosphobacteria, *Rhizobium*, *Azospirillum*, *Azotobacter* than peat, lignite and composted coir pith (Rajannan *et al.*, 1996). Vidyasekaran *et al.* (1997) suggested the use of talc based powder formulation of *P. fluorescens* for the effective control of pigeonpea (*Cajanus cajan*) wilt caused by *Fusarium oxysporum* fsp *udam*

2.12. ESTABLISHMENT OF PGPR IN PLANT ROOTS AND SOIL

2.12.1. *Azospirillum*

Attachment of azospirilla to roots, root hairs, or cells in suspension cultures has been reported. Umali-Garcia *et al.* (1980) found that *A. brasilense* strains were attached in significantly higher numbers to roots of pearl millet compared to other soil bacteria.

Root colonization of *Azospirillum* starts generally from a chemotactic attraction of the bacteria towards the roots. Azospirilla showed characteristic behaviour towards specific sugar, organic acids and amino acids. Chemotaxis was shown to be strain specific and

attuned to the composition of the host root exudate. Strains isolated from C₄ plants such as kallar grass, sorghum and maize showed strong attraction to maize, while *Azospirillum* isolates of wheat responded strongly to wheat (Reinhold *et al.*, 1985).

The chemotaxis of *Azospirillum lipoferum* Sp 596 and *A. brasilense* Sp 7 and Sp Cd to aromatic substrates like benzoate, protocatechuate, catechol and 4-hydroxybenzoate was demonstrated (Lopez-de-Victoria and Lovell, 1993) found that benzoate was the strongest chemoattractant tested. The requirement of active plant metabolism for attachment of *A. brasilense* Cd to maize roots was indicated (Gafny *et al.*, 1986). The same strain however was attached to live and killed wheat roots in equal numbers (Bashan *et al.*, 1986) indicating the presence of different mechanisms of attachment. Active bacterial metabolism seems to be a general requirement, since heat-killed azospirilla lost their attachment capability (Eyers *et al.*, 1988).

The binding of *A. brasilense* to wheat roots was pH and cation dependent. *A. brasilense* Cd cells that had been treated with either protease or Na EDTA showed significantly decreased adsorption to root hairs (Bashan and Levanony, 1989a). Twenty five fold reduction of *A. brasilense* Sp 7, adsorption in the presence of 25 mM EDTA was reported (Michiels *et al.*, 1989a).

The specific mechanism by which *Azospirillum* attaches itself to the roots remains unclear. It was speculated that the different cellular components of the bacteria like outer membrane polysaccharides, fimbriae, flagella and from the plant, mucilages, polysaccharides and lectins were likely to be involved (Del Gallo and Fendrik, 1994).

Azospirillum can colonize roots externally and internally. They are capable of internal colonization, *Azospirillum* preferentially colonized the elongating and root hair zones (Bashan and Levanony, 1989b). In external colonization, *Azospirillum* preferentially colonized the elongating and root hair zones (Bashan and Levanony, 1989b; Vande Broek *et al.*, 1993). The presence of *Azospirillum* in the rhizosphere and its colonization of wheat were localized with fluorescently labeled rRNA-targeted oligonucleotide probes (Assmus *et al.*, 1995).

2.12.2. *Pseudomonas fluorescens*

Root colonization is the microbial capacity to multiply and keep pace with growing root in the field soil. Ideally, a seed applied inoculant should spread from its site of application to give long term protection of root system. The ability to be agglutinated by root surface components is reported to contribute to rhizosphere competence of bacteria (Anderson *et al.*, 1988) and is associated with the apparent host preferences of individual strains (Van Peer *et al.*, 1990).

A major barrier to the successful agronomic use of bacterial inocula is the need to establish high population density of the introduced bacterium in the root environment, an environment characterized by intense microbial competition for nutrients.

Burr *et al.* (1978) reported that when potato seed pieces were treated with PGPR strains, the bacteria could be detected on root upto two months after planting. Kloepper *et al.* (1980b) studied root colonization by using double marked, rifampicin and nalidixic acid resistant mutants of PGPR. Population declined in older roots. They also detected the mutant strains on plants outside experiment plots in neighbouring rows, indicating the aggressive nature of the strains and their ability to move great distances on roots. Suslow and Schroth (1982a) demonstrated PGPR strains, which showed the ability to move from treated seed and to colonize developing roots in field soil at population upto 3.7×10^4 cfu/cm root. Root colonization varied with soil type and moisture. Ahmad and Baker (1987) used the term "rhizosphere competence" to describe the ability of biocontrol agents to grow and function in the rhizosphere. Bahme and Schroth (1987) reported that colonization of *Pseudomonas fluorescens* in potato rhizosphere was 10 fold greater in a sandy loam soil. Van Peer *et al.* (1990) proposed agglutinin to be important in the initial phase of adherence of bacteria to the root surface of tomato. Al-Achi *et al.* (1991) reported that *Pseudomonas putida* did not prevent the growth of *P. fluorescens* on roots. Srivastava *et al.* (1999) examined the root colonization of wheat in nonsterile soil employing two promising growth promoting strains (GRP₃ and PRS₉) of fluorescent pseudomonad.

2.13. CROP RESPONSE TO PLANT GROWTH PROMOTING RHIZOBACTERIAL INOCULATION

A large number of rhizosphere bacteria have potential for use as inoculants in crops and the plants inoculated with these bacteria have shown increased grain yield and plant biomass accumulation (Bashan and Levanony, 1990 and Glick, 1995).

2.13.1. Crop response to *Azospirillum* inoculation

During the past twenty years numerous reports of inoculation effect of *Azospirillum* on all kinds of crop plants have been published (Bashan and Levanony, 1990; Sumner, 1990 and Fages, 1994) (Table 4).

The effect of *Azospirillum* inoculation on the total yield increases of field grown plants generally ranged from 10 to 30 per cent (Rao *et al.*, 1983 and Watanabe and Lin, 1984). A few reports indicated extremely higher values, 50-270 per cent over uninoculated control (Bashan and Levanony, 1990). Even moderate yield increases (upto 20 per cent) attributed to inoculation with *Azospirillum* are considered commercially valuable to moderate agriculture, if obtained consistently.

Fayez and Daw (1987) studied the response of the cotton plant to inoculation with six strains *Azospirillum brasilense* under subtropical conditions in Egypt. Okon (1985) and Wani (1990) evaluated the worldwide success of *Azospirillum* inoculation and concluded that positive effects on yield were obtained in approximately 65 per cent of all field experiments. About 70 to 75 per cent of all pot experiment in cotton and several vegetables resulted in yield increase (Bashan *et al.*, 1989).

The inoculation studies in the field using *Azospirillum* have shown increased crop yields in cereals in several countries. In temperate regions, inoculation of wheat caused significant yield increase in the range of 1.5 to 3.0 t ha⁻¹ (Cabellero-Mellodo *et al.*, 1992). Statistically significant yield increases were obtained upto 60 to 70 per cent in the field trials of USSR, Israel and India. Experiments with sorghum showed that inoculation with *Azospirillum* increased the grain yield and drymatter marginally by 2 to 10 per cent over the uninoculated control (Okon and Labandera-Gonzalez, 1994). Fulchieri and Frioni (1994) observed that maize (*Zea mays*) inoculated with *Azospirillum* has enhanced dry weight of seeds by 59 per cent and also the yield, which was similar to 60 kg urea ha⁻¹.

Table 4. Effect of *Azospirillum* inoculation on the plant biomass, nitrogen uptake, grain yield etc.

Organism	Crop	Agronomic Importance (Over UIC)	References
<i>Azospirillum brasilense</i> sp7	Maize	18% Dry matter and 18% total nitrogen	Rennie (1980)
<i>Azospirillum brasilense</i> , 2 cd, cd1, cd3, sp7	Maize	7 to 27% total dry matter and nitrogen 13 to 37%	Nur <i>et al.</i> (1980)
<i>Azospirillum brasilense</i> , 2cd, cd1, cd3, sp7	<i>Setaria italica</i>	17 to 79% dry matter, 50 to 128% nitrogen	Nur <i>et al.</i> (1980)
<i>Azospirillum</i> sp. SP 107	Wheat	16% dry matter and 312% total nitrogen	Dobereiner and Baldani (1981)
<i>Azospirillum lipoferum</i> SP 242	Maize	36% total dry matter, 37% total nitrogen	Dobereiner and Baldani (1981)
<i>Azospirillum brasilense</i> , SP 107	Sorghum	53% grain yield, 5.82 to 19.6 kg ha ⁻¹	Pal and Mallik (1981)
<i>Azospirillum</i> sp.	Clover, Leucern and chickpea	Yield	Tilak <i>et al.</i> (1981)
<i>Azospirillum</i> sp.	Maize and sorghum	Dry matter yield	Tilak <i>et al.</i> (1982)
<i>Azospirillum</i> sp.	Rice	Grain and straw yield significantly increased	Rao <i>et al.</i> (1983)
<i>Azospirillum</i> sp.	Rice	Increased grain yield	Watanabe and Lin (1984)
<i>Azospirillum</i> sp.	Sweet potato	Increased Root and foliage dry weight	Hill <i>et al.</i> (1983)
<i>Azospirillum brasilense</i>	Sorghum	25% increase dry matter and Nitrogen content	Paczkovsky <i>et al.</i> (1985)

(Contd...)

Table 4. (Contd...)

Organism	Crop	Agronomic Importance (Over UIC)	References
<i>Azospirillum</i> spp.	Sorghum Wheat Bajra and cotton	3 to 23% grain yield, 1 to 16% grain yield, 2 to 76% grain yield, 12 to 16% cotton yield were increased	Shah and Joshi (1986)
<i>Azospirillum brasilense</i>	Pearl millet Sorghum	10 to 17% yield pearl millet, 7 to 31% yield sorghum	Subba Rao (1986)
<i>Azospirillum</i> sp.	<i>Rhododendran nilagiricens</i> and <i>Syzygium arnattianum</i>	Increased seedling height	Rangarajan <i>et al.</i> (1987)
<i>Azospirillum</i> sp.	Wheat, Maize, Barley and Rice	25% increased grain yield (7 years consecutive study)	Favilli <i>et al.</i> (1987)
<i>Azospirillum</i> sp.	Mulberry	Improved rooting	Santhanakrishnan and Obliswami (1988)
<i>Azospirillum lipoferum</i>	Pearl millet	11% grain yield	Wani <i>et al.</i> (1988b)
<i>Azospirillum brasilense</i>	Tomato, cotton, egg plant and pepper	30% tomato yield, 16% cotton yield, 23% egg plant yield and 18% pepper yield were increased	Bashan <i>et al.</i> (1989)
<i>Azospirillum</i> sp.	Rice	Total biomass and grain yield increased	Balasubramaniam and Kumar (1989)

(Contd...)

Table 4. (Contd...)

Organism	Crop	Agronomic Importance (Over UIC)	References
<i>Azospirillum</i> sp.	Rice	Inoculation along with 75 kg N/ha gave 17.5 per cent yield increase over respective control	Gopalswamy <i>et al.</i> (1989)
<i>Azospirillum</i> sp.	Onion	Inoculation alone gave an increased yield (19.1 – 20.5 t/ha) than control (15.9 t/ha) but was significantly less due to application of N and P at 150 kg/ha level (24.2 t/ha)	Gurubatham <i>et al.</i> (1989)
<i>Azospirillum</i> sp.	Okra	16.4 – 45.4 % yield increase	Parvatham and Vijayan (1929)
<i>Azospirillum</i> sp.	Finger millet	16.4 – 45.4% yield increase	Pradhan (1990)
<i>Azospirillum</i> sp.	Sugarcane	9.5 – 10.4% cane yield	Mishra and Naidu (1990)
<i>Azospirillum</i> sp.	Barley	19.4 – 60.8% yield increase	Saxena <i>et al.</i> (1990)
<i>Azospirillum</i> sp.	Tomato	3.14 – 20.8% yield increase saving 50 per cent N	Subbiah (1990)
<i>Azospirillum</i> sp.	Pearl millet	16 – 19% yield increase and net saving 20 kg N/ha	Jadhav <i>et al.</i> (1991)
<i>Azospirillum</i> sp.	Sugarcane	34.5% yield increase	Bangar <i>et al.</i> (1992)
<i>Azospirillum</i> sp.	Sunflower	Highest mean seed yield, higher capitulum diameter	Ram <i>et al.</i> (1992)

(Contd...)

Table 4. (Contd...)

Organism	Crop	Agronomic Importance (Over UIC)	References
<i>Azospirillum brasilense</i>	Sorghum	Leaf area, dry matter and grain yield	Sarig <i>et al.</i> (1990)
<i>Azospirillum brasilense</i>	Coffee and Tea seedlings	Improved root, shoot length and dry weight	Merina (1991)
<i>Azospirillum</i> sp.	Rice	25% yield increased (Two consecutive years)	Sharma <i>et al.</i> (1992)
<i>Azospirillum brasilense</i>	<i>Casurina cunlghanina</i>	More nutrient uptake and plant growth	Subba Rao (1993)
<i>Azospirillum</i> sp.	Sugarcane	7% cane yield, 75 kg N saving ha ⁻¹	Durrai and Manickam (1991)
<i>Azospirillum</i> sp.	Sugarcane	9% cane yield and 25 % fertilizer saving	Shinde <i>et al.</i> (1991)
<i>Azospirillum lipoferum</i> and <i>A. brasilense</i> strains	<i>Narcissus tazetta</i>	Improved plant growth, flower yield and bulb productivity	El-Naggar and Mohmaud (1994)
<i>Azospirillum</i> sp.	Tomato	Significant yield increased	Bashan <i>et al.</i> (1995)
<i>Azospirillum</i> sp.	Wet land rice	Shoot growth, straw yield and N-uptake	Goviandan (1993)
<i>Azospirillum</i> sp.	Neem	Dry weight and nutrient uptake significantly highest	Karthikeyan (1994)
<i>Azospirillum</i> sp.	Grasses	Dry matter significantly maximum	Bashan and Dubrovsky (1996)
<i>Azospirillum</i> sp.	Maize	Grain yield	Salomone and Doberienner (1996)

Contd...

Table 4. (Contd...)

Organism	Crop	Agronomic Importance (Over UIC)	References
<i>Azospirillum</i> sp.	<i>Setaria italica</i> and Maize	Shoot length, shoot dry weight	Fallik and Okon (1996)
<i>Azospirillum brasilense</i>	Chrysanthemum	Plant growth and flower yield	Hemavathi (1997)
<i>Azospirillum</i> sp.	Sorghum bicolor	10 kg ha ⁻¹ grain yield sorghum	Mastiholi and Itnal (1997)
<i>Azospirillum</i> sp.	Bamboo	Growth of seedling	Mahesh Kumar <i>et al.</i> (1998)
<i>Azospirillum</i> ACD-15 and ACD 20	Sorghum	Grain yield	Alagawadi and Krishanraj (1998)
<i>Azospirillum</i> sp.	Rabi sorghum	Increased plant weight, grain and fodder yield and 25% N fertilizers savings	Tippannavar and Alagawadi (1998)
<i>Azospirillum</i> sp.	Chrysanthemum	Increased plant growth parameters and flower yield	Gadagi <i>et al.</i> (1999)
<i>Azospirillum</i> sp.	Marigold and China aster	Increased plant growth parameters and flower yield	Gadagi <i>et al.</i> (1999)

In India, the results of multi location trials of *Azospirillum* with sorghum, pearl millet and finger millet indicated that with sorghum under different agroclimatic conditions, the average increase in yield following inoculation was about 19 per cent. In finger millet *A. brasilense* caused an average of 15 per cent increase in yield over non-inoculated controls. The application of 10 to 30 kg N ha⁻¹ was found to be suitable for maximum benefit of bacterial inoculation in pearl millet (Rao, 1986; Wani, 1990).

Subba Rao *et al.* (1986) reported that an increase in grain yield in sorghum (CSH6) due to inoculation over the non-inoculated control (no nitrogen) was equivalent to that obtained by the application of 15 to 20 kg N ha⁻¹. The isolates of *Azospirillum* differed in their interaction with sorghum genotypes (Wani, 1986). Inoculation with efficient strains of *Azospirillum* enhanced the yield of CSH5 and CO 24 sorghum while K-tall and USH1 showed negligible response (Purushothaman and Oblisami, 1986).

2.13.2. Crop response to *Pseudomonas* inoculation

Application of *P. fluorescens* to seed resulted in yield increases with radish (Kloepper and Schroth, 1978) and 11-33 per cent in potatoes (Burr *et al.*, 1978). Kloepper and Schroth (1981) observed the enhancement of plant growth and increased stolon lengths of potato plants treated with PGPR. Suslow and Schroth (1982a) reported 13 per cent yield increase of sugarbeets due to seed application of *P. fluorescens*.

Two fold increase in shoot dry weight and three fold increase in root dry weight was observed due to the inoculation of the seed pieces and stem cuttings of potato with rhizobacteria (Howie and Echandi, 1984). *Pseudomonas fluorescens* treatment increased the plant vigour, height and grain yield of wheat (Weller and Cook, 1986). Mew and Rosales (1986) reported increased height of rice plants treated with *P. fluorescens* than the control plants.

De Frietas and Germida (1990a&b) observed the enhanced growth of wheat plants inoculated with rhizobacteria. *P. fluorescens*, *P. aeruginosa* and *P. putida*. Howie and Echandi (1984) reported that treatments of potato seed pieces and stem cuttings with strains of PGPR increased shoot dry weight from 1.23 to 2 fold and root dry weight from 1.27 to 2.78 fold. Weller and Cook (1986) reported that *P. fluorescens* treatment increased number of heads and grain yield of wheat, Mew and Rosales (1986) reported that rice plants treated

with *P. fluorescens* were taller than control. Dileep kumar and Dube (1991) reported significant increase in the emergence of tomato seedlings when *P. fluorescens* was applied to seeds. The seedlings showed a consistent and greater root length shoot height, fresh and dry weights than control.

Hofte *et al.* (1991) reported that plant growth promoting strains of *Pseudomonas aeruginosa* TMSK 2 and *P. fluorescens* ANP-15 significantly increased the germination of maize seeds by 60-300 per cent. Several strains of *P. fluorescens* when applied to seeds increased the plant growth of rice and cotton by 27 and 40 per cent respectively (Sakthivel *et al.*, 1986 and Lin *et al.*, 1992). Growth promotion of winter wheat by treating with strains of *Pseudomonas* spp. led to the increase in grain yield by 46-75 per cent in green house experiment and 11 per cent in field trials (de Frietas and Germida, 1990a).

Dileep kumar and Dube (1992) reported *P. fluorescens* increased the yield of chickpea and soybean by 23.6 per cent and 36.6 per cent respectively. Gnanamanickam and Mew (1992) reported increase in the grain yields of rice due to seed treatment with *P. fluorescens*. Tosi and Zazzerini (1994) recorded increase in the length of safflower seedlings by seed treatment with *P. fluorescens* strain 14.

2.13.3. Crop response to dual inoculation of *Azospirillum* and phosphate solubilizers and *Pseudomonas*

Watanabe and Lin (1984) recorded that dual inoculation of *Azospirillum lipoferum* and *Pseudomonas* sp. in wetland rice promoted early tillering and reproductive growth as well as increased ARA of roots.

Alagwadi and Gaur (1988b, 1992) observed that the combined inoculation of *A. brasilense* and *Bacillus polymyxa* or *Pseudomonas striata* had significant increase in the grain, dry matter yield, N and P uptake of sorghum, over single inoculation. The rhizosphere population of *Azospirillum* and phosphate solubilizing were also higher in the respective inoculated treatments. Prabhakara and Ravi (1991) recommended the application of *A. brasilense* SP 7 and *Pseudomonas* sp. for getting higher dry matter, nitrogen and phosphorus content in *Zea mays* plants.

Prabhakara and Ravi (1991) recommended the application of *A. brasilense* Sp 7 and *Pseudomonas* sp for higher dry matter, nitrogen and phosphorus content in *Zea mays* plants. They also noticed possible replacement of entire super phosphate with rock phosphate due to inoculation. Radhakrishnan (1996) reported that dual inoculation of *Azospirillum* and phosphobacteria resulted in higher root biomass and more bolls in cotton. Inoculation with phosphobacteria enhanced the plant height and kapas yield.

Alagwadi and Gaur (1992) reported that the combined inoculation of *A. brasilense* and *Pseudomonas striata* had significant increase in the grain, dry matter yield, N and P uptake of sorghum, over single inoculation. The rhizosphere population of *Azospirillum* and phosphate solubilizing bacteria were also higher in the inoculated treatments. Muthamizhan (1994) found that *P. fluorescens* did not inhibit *Azospirillum lipoferum* under *in vitro* conditions. Vijaya Nirmala and Sundaram (1996) stated that dual inoculation of phosphobacterium and *Azospirillum* with 75 per cent recommended dose of NPK was superior to uninoculated control in increasing the yield of cumbu variety UCC 5.

2.14. NUTRIENT, MINERAL UPTAKE AND PLANT WATER RELATIONSHIP

In addition to increasing root characteristics, plant inoculation with *Azospirillum* affected many other yield determinants. These changes were directly attributed to positive bacterial effects on mineral uptake by plant.

2.14.1. *Azospirillum*

Enhancement in uptake of NO_3^- , NH_4^+ , PO_4^{2+} , K^+ , Rb^+ and Fe^{2+} by *Azospirillum* was proposed to cause an increase in foliar dry matter and accumulation of minerals in stems and leaves. During the plant reproductive period, these minerals could have been translocated to the panicles and spikes and ultimately resulted in a high yield (Barton *et al.*, 1986; Jain and Patriquin, 1984; Kapulnik *et al.*, 1985; Morgenstern and Okon, 1987; Murty and Ladha, 1988; Lin *et al.*, 1983; Sarig *et al.*, 1988 and Wani, 1990).

Increased mineral uptake by plants due to *Azospirillum* inoculation, has been suggested to be due to a general increase in root volume and not to any specific enhancement of the normal ion uptake mechanism (Morgenstern and Okon, 1987; Murty and Ladha, 1988). It has been further suggested that *Azospirillum* inoculation may

promote the availability of ions in the soils by helping the plant scavenge limiting nutrients (Lin *et al.*, 1983). This may also explain the accumulation of nitrogen compounds in the plant without any apparent nitrogen fixation. Inoculation of maize with *Azospirillum brasilense* sp. increased the uptake of nitrate, potassium and phosphorus into excised root segments by 30 to 50 per cent over the control (Okon and Kapulnik, 1986).

In addition to improved mineral uptake *Azospirillum* inoculation improved water status of sorghum plants under stress. Inoculated plants exhibited tolerance to water stress with more water in their foliage, higher leaf water potential and lower canopy temperature than uninoculated plants. The increased yield in sorghum in inoculated plants was attributed primarily to improved utilization of soil moisture (Sarig *et al.*, 1988). Although increased mineral uptake by inoculated plants has been suggested to be due to the general increase in the volume of root system, there are evidences that not all the strains could share this ability (Bashan *et al.*, 1990).

Inoculation with *Azospirillum brasilense* Cd significantly increased the proton extrusion from the intact wheat root. Stress treatments such as starvation, high temperature of 45°C, anaerobiosis or high antibiotic level on bacterial cells prior to inoculation decreased the effect of the bacteria on proton efflux (Bashan, 1990).

2.14.2. Phosphobacteria

Beneficial influence of artificial inoculation with phosphate solubilizing organisms had been reported for different crops under diverse agroclimatic conditions. Gerretsen (1948) was the first to report the increased yield accompanied with high phosphorus uptake in oat plants inoculated with soil containing phosphate solubilizing microorganisms as compared to uninoculated control. Similar findings were also observed by Pikovskaya (1948) and Dorosinsky and Lazarev (1949).

Sundara Rao (1965) reported that 9 out of 27 trials conducted using PSM significantly increased the crop yields and the increase over control ranged between 10 and 20 per cent. Sharma and Singh (1971) concluded that phosphobacterin culture enhanced the efficiency of plant uptake of phosphoric fertilizers, particularly super phosphate in maize.

Gaur and Ostwal (1972); Kundu and Gaur (1980) observed positive effect of inoculation of PSM such as *Aspergillus awamori*, *Bacillus polymyxa* and *Pseudomonas striata* on the yield and phosphorus uptake of soybean, wheat and rice crops, supplied with rock phosphate. Among the organisms, *P. striata* gave maximum yield of 17.86 q ha⁻¹ followed by *B. polymyxa* and *A. awamori* in soybean. Similar response was also observed in wheat and rice due to phosphobacteria inoculation.

Inoculation of *P. striata* with rock phosphate gave significantly higher yield in soybean than the application of 80 kg P₂O₅ as super phosphate alone (Gaur, 1979). Inoculation of *P. striata* increased the grain and straw yield and also 'P' uptake in rice (Kundu and Gaur, 1984). Banik and Dey (1985) found that the inoculation of native phosphate solubilizing bacteria *Bacillus firmus* (Ac B₆) had positive effect on rice crop by early tillering, enhanced plant growth and higher dry weight besides 'P' uptake. Gaur (1985) reported that inoculation of chickpea seed with *B. polymyxa*, *A. awamori* and *P. striata* along with rock phosphate (60 kg ha⁻¹) gave higher grain yield of 7.4, 7.0 and 5.4 q ha⁻¹ respectively, compared to 1.9 q ha⁻¹ in uninoculated control.

Banik and Datta (1988) reported that the inoculation of *Bacillus firmus* (NLIM 2636) with soybean seeds had little effect on grain yield but increased the available 'P' content of the rhizosphere soil. Mohod *et al.* (1989) stated that the use of P-solubilizing culture either alone or in combination with phosphate fertilizer increased the root cation exchange capacity (CEC), available phosphorus in soil and P-uptake in rice plant. Seed inoculation of *P. striata* significantly increased the yield of pea by 25.3 per cent and 22 per cent respectively over uninoculated control in two season field experiments conducted at IARI, New Delhi (Gaur, 1990).

Pharande and Patil (1991) reported good response in jowar due to the inoculation of *Bacillus megaterium* and *Pseudomonas aeruginosa*. Application of compost along with phosphate solubilizing bacteria enhanced the grain and dry matter yields of pearl millet (Rasal and Patil, 1991) whereas Veer *et al.* (1991) could not get any positive response by inoculating seeds with 'P' solubilizing cultures like *A. awamori* and *Emercella nodulance* in chickpea.

2.15. MATHEMATICAL MODELS FOR ASSESSING POPULATION DYNAMICS

The analytical approach to quantitative microbial ecology involves estimation of constants or parameters in equations chosen to represent processes such as substrate depletion, growth, or surface colonization. There are several models available viz., exponential; Monod equation (Powel, 1967, Robinson, 1985) to describe microbial growth in the soil, in a system such as cotton rhizosphere soil. The simplest microbial model states that, the change in the growth of the microbial population (dp/dt) with respect to time is directly proportional to the size of the population. Thus, dp/dt is directly proportional to the size of the population. Thus, $dp/dt = \beta P$ where β is the population growth, the resulting model is exponential population growth model. If the value of β is negative, the size of the population decline exponentially, such a model may reasonably fit, a variety of microbial process (Brand and Pinnock, 1981) and Pinnock *et al.*, (1978) used exponential models to predict the persistence of *B. thuringiensis* spores on foliage and to relate these population levels to the mortality of insects feeding on the foliage.

Another, commonly used growth model is Monod equation which expresses, growth rate as a function of substrate concentration (Robinson, 1985). The other model, such as Blackman bilinear (Blackman, 1905) and Powell diffusional model (Powell, 1967), may better describe growth for some organisms. We know that bacterial population size in a non replenished medium usually approaches to the maximum limit (or) carrying capacity (P_M) at which the specific growth rate approaches to zero (Lambrect *et al.*, 1988). The logistic growth model has drawbacks when compared to general model of population growth, since, it predicted asymptotic stable density is probably almost never achieved by natural population (Krebs, 1985). Kundsén and Hudler (1987) incorporated logistic growth into their simulation model for *P. fluorescens* on pine foliage and remarked on the unrealistic limitations imposed by the invariant carrying capacity (asymptote) of the logistic model.

Hence in the present study, the logistic growth model found to be suitable for studying the microbial growth dynamics in the rhizosphere of cotton. The modeling in soil microorganisms will provide a clear picture about the growth of bacteria, fungi and algae and their interactions in the soils and its influence in the crop yield. Systems analysis and simulation models have been used by the biological scientist for effective nutrient management (Kailasam, 2000).

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

3.1. SURVEY FOR THE COLLECTION OF SAMPLES

Survey was conducted to isolate efficient strains of *Azospirillum*, phosphobacteria and *Pseudomonas* spp. from cotton growing belts of Tamil Nadu viz., Coimbatore, Madurai, Ramanathapuram and Tirunelveli districts (Fig. 1). Soil and root samples from different cotton varieties at different growth stages of crop were collected as detailed in Table 6. The samples were placed in polythene bags and transported to the laboratory.

3.2. ISOLATION OF RHIZOBACTERIAL ISOLATES

The rhizosphere soil samples were used to isolate plant growth promoting rhizobacteria viz., *Azospirillum*, phosphobacteria and *Pseudomonas*.

3.2.1. *Azospirillum*

The root samples were rinsed thrice in sterile distilled water and cut into 2-3 cm size. The root bits were surface sterilized by 80 per cent ethanol and in 0.1 per cent mercuric chloride. Subsequently roots were washed in sterile distilled water and in 25 mM phosphate buffer, followed by three more washings in sterile distilled water. The root samples were then placed in test tubes containing 10 ml of N-free semisolid bromothymol blue agar medium (NFb) (Dobereiner and Day, 1975) and incubated at room temperature for 24 to 72 h.

For the isolation of *Azospirillum* from the rhizosphere soil samples, the soil suspensions were prepared and diluted sufficiently with sterile distilled water. A quantity of 0.1 ml of the suspensions was added to the tubes containing 10 ml of sterile NFb medium. The cultures were incubated at room temperature for 24 to 72 h.

3.2.1.1. Identification of *Azospirillum* isolates

After inoculation, the tubes containing NFb medium was observed for the development of white sub-surface pellicles. A loopful of the pellicle was streaked on Congo red plates (Caceres, 1982) and incubated at room temperature for 72 h. Individual colonies were then streaked on potato infusion agar plates (Baldani and Dobereiner, 1980) and incubated at room temperature for seven days. The purified individual colonies formed were transferred to yeast extract glucose agar slants and maintained as stock cultures.

Fig.1 SURVEY LOCATION

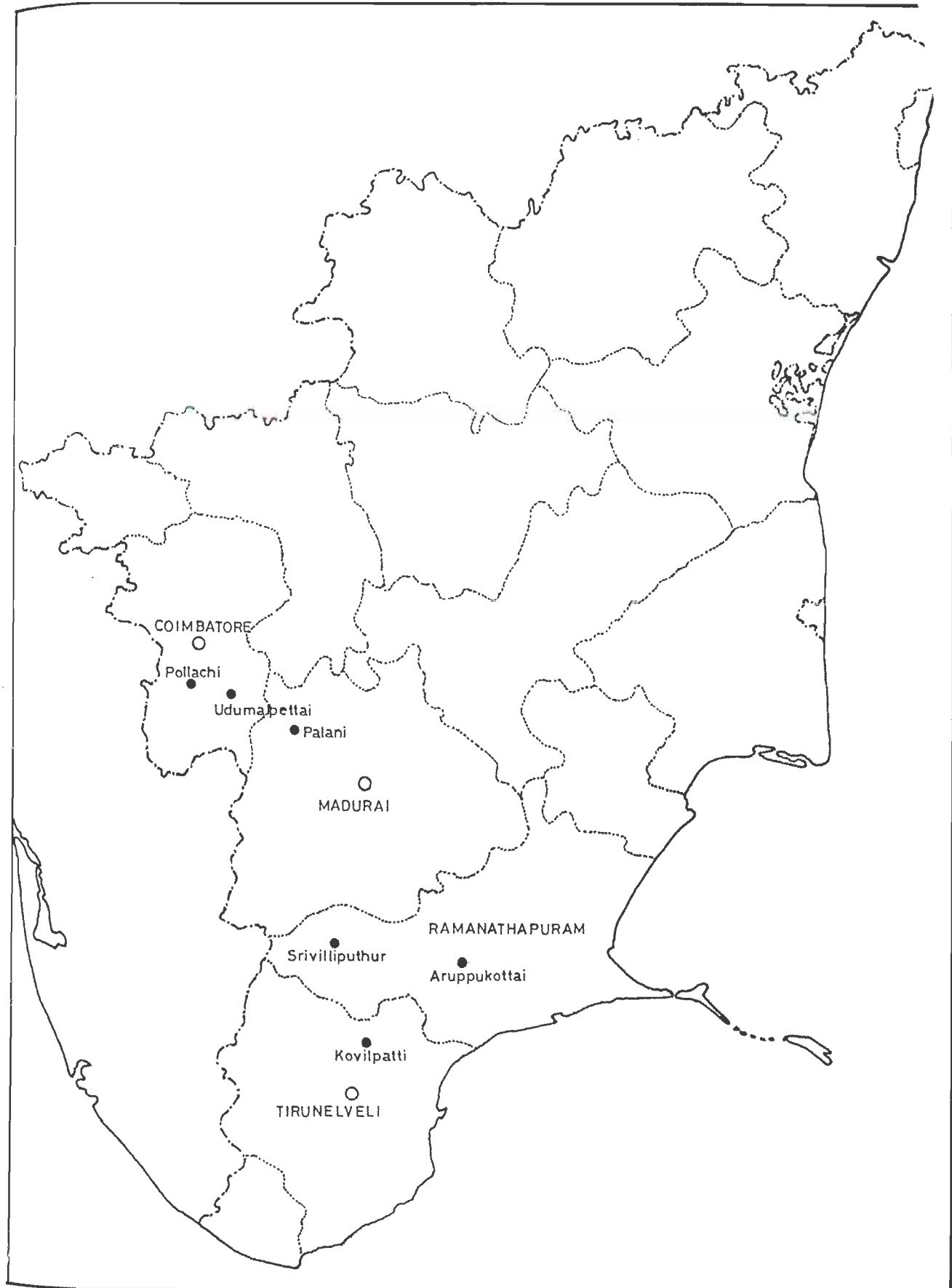


Table 6. Survey locations, Cotton varieties and number of rhizobacterial isolates taken for the study

Location	Soil type	Stage of the crop	Variety	Number of isolates			
				<i>Azospirillum</i>		Phosphobacteria	<i>Pseudomonas</i>
				Root	Rhizosphere soil		
Ramanathapuram Dt.							
Aruppukottai	Black soil	Flowering	MCU 10	2	1	3	2
Kovilangulam	Black soil	Flowering	SVPR 2	2	1	2	2
Palavanatham	Black soil	Flowering	LRA 5166	1	2	3	1
Coimbatore Dt.							
Thondamuthur	Black soil	Vegetative	MCU 5	2	2	2	2
Pollachi	Black soil	Square formation	MCU 9	3	1	2	3
Udumalaipad	Black soil	Square formation	MCU 7	1	1	2	2
Madurai Dt.							
Palani	Black soil	Square formation	MCU 10	1	2	2	2
Madurai	Black soil	Square formation	MCU 5	1	1	2	1
Neikarapatti	Black soil	Square formation	LRA 5166	1	1	3	1
Trunelveli Dt.							
Kovilpatty	Black soil	Flowering	LRA 5166	2	1	2	1
Kalugasalapuram	Black soil	Vegetative	KC 2	2	2	3	1
Keelaeral	Black soil	Vegetative	K 11	1	1	3	1
Sankaralingapuram	Black soil	Flowering	K 10	1	1	2	1
Ramanathapuram Dt.							
Srivilliputhur	Black soil	Flowering	TSH 288	1	1	2	2
Rajapalayam	Black soil	Flowering	SVPR 1	1	2	2	1
Vembakottai	Black soil	Square formation	MCU 10	1	-	-	-
Watrap	Black soil	Square formation	SVPR 2	1	1	2	1
Total number of isolates				24	21	37	24

Microscopic examination was done using the wet mounts of the 24 h old cultures the shape, motility and presence of poly- β -hydroxy butyrate granules. Gram staining was carried out as per Hucker's modifications (Rangaswami, 1975).

3.2.2. Phosphobacteria

Bacteria that solubilize unavailable form of phosphate are termed as phosphate solubilizing bacteria (PSB) or phosphobacteria (PB). Phosphate solubilizing bacteria were isolated from the rhizosphere soil of different cotton cultivars from different locations of Tamil Nadu by dilution plate technique using Sperber's hydroxy apatite medium (Sperber, 1958). Colonies obtained after 48 h of incubation at room temperatures were purified and maintained in Pikovskaya's slants for further studies.

3.2.3. *Pseudomonas*

Pseudomonas isolates were obtained from soil samples collected from the rhizosphere of different cotton varieties from different locations of Tamil Nadu by dilution plate technique, using King's B agar medium (King *et al.*, 1954) following serial dilution technique. Colonies obtained after 48 h of incubation at room temperature were purified and transferred to King's B agar slants and maintained at 4°C for further studies.

3.2.4. Preparation of inoculum

Pure stock cultures of the rhizobacteria grown in respective broth for 48 h were washed clean by centrifugation with normal saline (0.8 per cent NaCl). The cells were resuspended in sterile distilled water and adjusted to a final concentration of 4×10^8 cells mL^{-1} was used as inoculum for the various studies.

3.2.5. Standard strains used

The standard strain of *Azospirillum brasilense* Sp7 (ATCC 29145), *Azospirillum lipoferum* (Az204) and *Bacillus megaterium* var *phosphaticum* were obtained from the culture collection centre of the Department of Agricultural Microbiology, Centre for advanced studies in Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore 3 and the standard strain *Pseudomonas fluorescens* (Pfl) was obtained from the culture collection centre of the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore 3 used for further studies.

3.3. SCREENING RHIZOBACTERIAL ISOLATES UNDER *in vitro* CONDITIONS

3.3.1. *Azospirillum*

The forty five isolates were screened for their efficiency in nitrogen fixation and nitrogenase activity based on indirect method of acetylene reduction activity under *in vitro* conditions.

3.3.1.1. *In vitro* nitrogen fixation by *Azospirillum* isolates

The quantity of nitrogen fixed *in vitro* by the *Azospirillum* isolates was estimated by the method of Humphries (1956). Semisolid nitrogen free malic acid medium was prepared and supplemented with L-glutamic acid at the rate of 100 mg lit⁻¹ and dispensed in 100 ml quantities in 250 ml Erlen Meyer flasks. The medium was inoculated with one ml of standard inoculum and incubated at 32°C for seven days. After seven days of incubation, the culture was homogenized in a waring blender, five ml was withdrawn and digested with 5 ml of concentrated sulphuric acid and five g of digestion catalyst (K₂SO₄ and CuSO₄ in 10:1 ratio) in Tecator digestion system until the contents became clear. After sufficient cooling, the aliquot was transferred to microkjeldahl distillation unit. Ten ml of 40 per cent sodium hydroxide was added and steam distilled. Ammonia evolved was collected over two per cent boric acid (20 ml) containing two drops of double indicator (20 ml) (83.3 mg bromocresol green + 16.6 mg methyl red indicator dissolved in 10 ml of 95 per cent ethanol), and back titrated against N/50 H₂SO₄ (1 ml of N/50 H₂SO₄ = 0.00028 g of N) and using the titre value, the nitrogen fixed was calculated and expressed as mg g⁻¹ of malate.

$$\text{Total nitrogen content (\%)} = \text{Titre value} \times 0.00028 \times \frac{100}{10} \times \frac{1}{0.5}$$

3.3.1.2. Acetylene reduction activity of the *Azospirillum* isolates

The acetylene reduction activity of the *Azospirillum* isolates was determined by using the procedure followed by Bergersen (1980). Semisolid nitrogen free malic acid medium was prepared and dispensed in 40-ml quantities in 130 ml glass bottles and sterilized. The bottles were inoculated with one ml of standard inoculum and incubated at 32°C under static conditions. After four days of growth, cotton plugs were replaced with sterilized suba seal rubber stoppers. The air inside the bottles was replaced with nitrogen by flushing with nitrogen through a syringe. Using an air tight syringe, pure acetylene (C₂H₂) gas was

injected at the rate of one per cent of the remaining gas phase, after with drawing same volume of nitrogen without disturbing the pellicles and incubated for 12 h at room temperature. At the end of the incubation period one ml of the gas sample was withdrawn from the bottle and injected into a gas chromatograph (with FID system and 80-100 poropak column). The column temperature was maintained at 85°C, injection temperature at 100°C and ionization temperature at 125°C. The flow rate of carrier gas used was nitrogen was 30 ml sec⁻¹ whereas hydrogen and oxygen were set at flow rate of 30 ml sec⁻¹ and 60 ml sec⁻¹ respectively. The area of the ethylene peak was recorded for each culture. The acetylene reduction activity was calculated using the formula.

$$\text{Acetylene reduction activity} = \frac{\text{Area count} \times \text{Gas volume of the flask} \times 0.0006}{\text{Volume of gas sample injected into GC} \times \text{hrs of incubation} \times \text{mg of protein in the sample}}$$

Acetylene reduction activity was expressed as n moles of C₂H₄ produced hr⁻¹ mg⁻¹ cell protein.

3.3.1.3. Estimation of cell protein

The protein content of the cells was estimated by the method described by Lowry *et al.* (1951). The cultures used for the estimation of ARA were homogenized in an all glass Braun homogenizer and one ml of the homogenized broth was taken in a boiling tube and four ml of 0.1 N sodium hydroxide was added. The contents of tube were boiled over a water bath for 10 min, till the solution became clear. This clear solution was used for estimation of protein.

Reagents

Alkaline copper solution

Reagent A: Two per cent sodium carbonate in 0.1 N sodium hydroxide solution.

Reagent B: Copper sulphate 0.5 per cent in one per cent sodium tartarate.

A quantity of 50 ml of the reagent 'A' was mixed one ml of 'B' at the time of use.

Procedure

One ml of the sample was taken in a test tube and the volume was made upto 4.5 ml with distilled water. To each tube, five ml of the alkaline copper solution was added, mixed

well and allowed to stand for 10 minutes. Then 0.5 ml of Folin-ciocalteu reagent (1 N), mixed well and the intensity of blue colour developed was read in a 2000Z double beam spectrophotometer at 720 nm against appropriate blank. The protein content was calculated by referring to a standard curve prepared with bovine serum albumin (BSA).

3.3.2. Phosphobacteria

The efficiency of thirty seven phosphobacterial isolates were determined by acid phosphatase activity, amount of soluble phosphorus released and estimating the titrable acidity of the medium with concomitant changes in pH

3.3.2.1. Medium and culture

To 50 ml of Pikovskaya's broth, 100 mg of tricalcium phosphate (TCP) was added. The flasks were sterilized at 15 psi for 20 min and inoculated with desired cultures and incubated at 30°C in a psychrotherm at 100 rpm.

3.3.2.2. Assay of acid phosphatase

The acid phosphatase activity was determined based on the liberation of p-nitrophenol from p-nitrophenyl phosphate by colorimetric method as described by Morton (1955).

The phosphate solubilizing bacterial strains were grown in Pikovskaya's broth. The pH of the medium was adjusted to 5.2 and incubated at 30±2°C for seven h in an environmental shaker. Ten ml quantity of the broth was centrifuged at 5000 rpm for 15 min. The supernatant was discarded and the pellets of phosphobacteria were suspended in five ml of sterile distilled water. The cells served as enzyme source.

3.3.2.3. Reagents

Acetate buffer for acid phosphatase (pH 5.2)

Solution A : 0.2 M solution of acetic acid
(11.55 ml of acetic acid in 1000 ml of distilled water)

Solution B : 0.2 M solution of sodium acetate
(27.2g of sodium acetate dissolved in 100 ml of distilled water)

A quantity of 25.5 ml of solution A and 24.5 ml of solution B was mixed and diluted to 100 ml with distilled water.

Substrate solution

A quantity of 100 mg of p-nitrophenyl phosphate was dissolved in 100 ml of distilled water at the time of use.

Reaction mixture

A quantity of 10 ml of the acetate buffer, one ml of the substrate solution and two ml of phosphobacterium cell suspension were taken in a cleanly washed oven dried Erlenmeyer flask and incubated at 30°C for 24 h. After incubation, 10 ml of the assay mixture was withdrawn, centrifuged at 5000 rpm for 15 minutes and the supernatant was used for the estimation of released phenol.

Development of colour

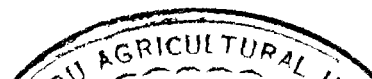
One ml of the supernatant was transferred into a clean test tube to which one ml of 1 N folin ciocalteau reagent was added followed by two ml of 20 per cent sodium carbonate. The contents were mixed thoroughly and immersed in a boiling water bath for one minute, cooled and the volume was made up to 10 ml with distilled water. The intensity of the blue colour was read in a colorimeter at 600 nm. The acid phosphatase activity was calculated by referring to a standard graph prepared with p-nitrophenol. Enzyme activity was expressed as n moles of p-nitrophenol released per ml of the culture supernatant.

3.3.2.4. Determination of soluble phosphorus

The phosphate solubilizing bacteria were grown in 50 ml Pikovoskaya's broth containing 100 mg TCP and the amount of soluble 'P' released was estimated on 15th and 30th day after inoculation (DAI). The culture medium was centrifuged at 10000 rpm for 10 min and the clear supernatant was used for soluble 'P' estimation by the method described by Olsen *et al.* (1954).

Reagents**Reagent A**

A quantity of 12.0 g of ammonium molybdate was dissolved in 250 ml of distilled water. Separately 0.294 g of antimony potassium tartarate was dissolved in 100 ml of distilled water. Both the solutions were added to 100 ml of 5N sulphuric acid. This solution was mixed thoroughly and the volume was made upto two litre with distilled water.



Reagent B

A quantity of 1.056 g of ascorbic acid was dissolved in 200 ml of reagent A.

Procedure

One ml of 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 and the volume was made up to 25 ml with distilled water. The intensity of blue colour developed was read at 660 nm in 2000Z double beam spectrophotometer using appropriate reagent blank. The standard graph was prepared with potassium dihydrogen orthophosphate (KH_2PO_4). The quantity of 'P' solubilized was determined with the standard graph.

3.3.2.5. Estimation of titrable acidity in the culture filtrate

The phosphobacteria grown in Pikovskaya's medium were centrifuged at 10000 rpm for 10 minutes to remove the cells and traces of phosphate inoculation. 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 0.01N NaOH consumed per 5.0 ml of culture filtrate.

3.3.2.6. Determination of pH

Changes in the pH of the culture medium due to PSB were measured using a digital pH meter on 15th and 30th days after incubation (DAI).

3.3.3. *Pseudomonas*

Twenty four *Pseudomonas* isolates were isolated from the rhizosphere of cotton and screened for their antagonistic activity against *Rhizoctonia solani* under *in vitro* condition.

3.3.3.1. Antagonistic activity against *Rhizoctonia solani*

The antagonistic activity of *Pseudomonas* against *R. solani* was carried out based on dual culture technique described by Muthusamy (1972). Potato dextrose agar (PDA) medium was prepared and one end of the Petri plate was streaked with loop of *Pseudomonas* isolate while in the opposite side a disc of *R. solani* was placed and incubated at room temperature. Growth of *R. solani* in the dual culture plates was compared with growth in the control (*R. solani*) plate.

3.4. CHARACTERIZATION OF COTTON RHIZOBACTERIAL ISOLATES

3.4.1. *Azospirillum*

3.4.1.1. Growth on glucose peptone broth

The glucose peptone broth was prepared and dispensed in five ml quantities in test tubes and sterilized. The tubes were inoculated with 0.1 ml of 24 h cultures of *Azospirillum* isolates and incubated at room temperature. The relative growth of the isolates was determined after 48 h of incubation.

3.4.1.2. Production of acid from glucose

Production of acid from glucose by the *Azospirillum* isolates was carried out by using the method described by Krieg and Tarrand (1978). The pH of the medium was adjusted to 7.0 with 1N potassium hydroxide. The medium was dispensed in five ml quantities in test tubes and sterilized. The tubes were inoculated with 0.1 ml of 48 h isolates of *Azospirillum* and incubated for 96 h at room temperature. The change in the colour of the broth to yellow indicated the acid production.

3.4.1.3. Utilization of different carbon sources

The ability of *Azospirillum* isolates to utilize different carbon sources was tested in nitrogen free malic acid medium. The medium was prepared without bromothymol blue and the carbon source. The various carbon sources viz., malate, glucose, mannitol, sucrose and α -keto glutaric acid were filter sterilized and incorporated separately at one per cent level. After sterilization the medium was inoculated with 0.1 ml 48 h isolates of *Azospirillum*. The tubes were incubated at room temperature for two to three days and observed for growth.

3.4.1.4. Biotin requirement

The nitrogen free malate medium was used for assessing the requirement for biotin. Two sets of media were prepared with and without biotin ($100 \mu\text{g ml}^{-1}$). The cultures were grown for 48 h in glucose peptone broth, centrifuged at 8000 rpm for 15 min. and washed twice with sterile distilled water and resuspended in sterile distilled water to a uniform density. A quantity of 0.1ml of this suspension was used as inoculum. For each set, a control was maintained without inoculation. The tubes were incubated for 48 h at room temperature and the growth the isolates was observed.

3.4.1.5. Nitrate Reductase (NR) activity

The nitrate reductase activity of the *Azospirillum* isolates was tested based on the method described by Yordy and Ruoff (1981). *Azospirillum* isolates were grown in 10 ml of malate broth supplemented with 10mM of sodium nitrate, and incubated at room temperature for five days in a shaker. The broth was centrifuged and the supernatant was collected. To 10 ml of the supernatant 0.3 ml of one per cent sulphanilamide in 1.5 N hydrochloric acid and 0.2 ml of 0.002 per cent N (1-naphtyl)-ethylene diamine dihydrochloride were added. The appearance of pink colour indicated the presence of NR activity.

3.4.1.6. Nitrite Reductase (NiR) activity

The nitrite reductase activity of the *Azospirillum* isolates was tested based on the method described by Yordy and Ruoff (1981). *Azospirillum* isolates were grown in 10 ml of malate broth supplemented with 10mM of sodium nitrite. This was incubated at room temperature for five days in shake culture conditions. The broth was centrifuged and the supernatant was collected. To one ml of the supernatant 0.3 ml of one per cent sulphanilamide in 1.5 N hydrochloric acid and 0.2 ml of 0.002 per cent N (1-naphtyl) ethylene diamine dihydrochloride were added. The disappearance of pink colour indicated the presence of NiR activity.

3.4.2. Phosphobacteria

The eleven phosphobacterial isolates were characterized by subjecting to the following tests described by Seeley *et al.* (1995).

The morphological test *viz.*, shape of the bacterial cell and Gram reaction was carried out as per Hucker's modifications (Rangaswami, 1975). Presence of spores was detected through spore staining and the motility was observed in the microscope by wet mount preparation.

On the agar plates the colony characteristics *viz.*, form, margin and elevation of the colony were observed.

The physiological tests *viz.*, catalase test, Voges - Proskauer test, citrate utilization, acid production, H₂S production, gelatin hydrolysis and starch hydrolysis were performed as per the method described by Aneja (1993).

Based on the above characters, the organisms were identified by referring to bacterial identification and classification suggested by Slepecky and Hemphill (1991).

3.4.3. *Pseudomonas fluorescens*

Following tests were performed for characterizing the isolates (Cappucino and Sharma, 1983).

The gram staining was carried out as per the Hucker's modifications (Rangaswamy, 1975), and the shape of the cells and motility was observed through the microscope. The isolates were checked for fluorescent pigment production under UV light.

The isolates streaked on agar slants, incubated at 4°C were checked for their growth. The isolates streaked on the nutrient agar medium supplemented with five per cent sucrose were observed for levan formation.

The physiological tests *viz.*, starch hydrolysis, gelatin hydrolysis, acid and gas production, litmus milk reduction and nitrate reduction were carried out by following the method described by Aneja (1993).

3.5. PRODUCTION OF GROWTH HORMONE BY THE COTTON RHIZOBACTERIAL ISOLATES

The growth hormones like indole acetic acid (IAA) and gibberellic acid (GA) production by the rhizobacteria were estimated.

3.5.1. Estimation of Indole acetic acid (IAA) production by rhizobacterial isolates

The IAA production by rhizobacterial isolates under *in vitro* conditions was determined by following the method of Tien *et al.* (1979). Nitrogen free malate broth was prepared with out bromothymol blue indicator for *Azospirillum* isolates, Nutrient broth, and King 's B broth were prepared for phosphobacteria and *Pseudomonas* isolates and dispensed in 100 ml quantities in 250 ml Erlenmeyer flasks and sterilized. Freshly prepared, filter-sterilized solution of L-tryptophan was added to the one set of flasks to a final concentration of 100 mg l⁻¹, while another set was prepared without L-tryptophan. One ml of standard inoculum of rhizobacterial isolates was added into each flask and incubated at 37°C for seven days in dark.

After seven days of incubation, the culture was centrifuged at 6000 rpm to remove the bacterial cells. The supernatant was brought to pH 2.8 with 1N HCl. Fifteen ml of the acidified supernatant was taken in 100 ml conical flasks and to it equal volume of diethyl ether was added and incubated in dark for four h. IAA extraction was done at 4°C in a separating funnel using diethyl ether. The organic phase was discarded and the solvent phase was pooled and evaporated to dryness. To the dried material two ml of methanol was added, pooled and the IAA present in the methanol extract was determined using the method of Gordon and Paleg (1957).

To 0.5 ml of the methanol extract, 1.5 ml of distilled water and four ml Salper's reagent (1.0 ml of 0.5 M FeCl_3 in 50 ml of 35 per cent perchloric acid) was added and incubated in dark for one hour. The intensity of pink colour developed was read at 535 nm in 2000Z double beam spectrophotometer. From a standard curve prepared with known concentrations of IAA, the quantity of IAA in the culture filtrate was determined and expressed as $\mu\text{g } 100 \text{ ml}^{-1}$ of culture.

3.5.2. Estimation of Gibberillic acid (GA) production by rhizobacterial isolates

The gibberellic acid production by rhizobacterial isolates under *in vitro* conditions was determined by following method of Borrow *et al.* (1955).

Reagents

(a) Zinc acetate solution

A quantity of 21.9 g of zinc acetate was dissolved in 80 ml of distilled water, one ml of glacial acetic acid was added and the volume was made upto 100 ml with distilled water.

(b) Potassium ferrocyanide solution

A quantity of 10.6 g of potassium ferrocyanide was dissolved in 100 ml of distilled water.

Estimation of GA

Nitrogen free malate broth was prepared without bromothymol blue indicator nutrient broth, and King 's B broth were prepared for phosphobacteria and *Pseudomonas* isolates dispensed in 100 ml quantities in 250 ml. Erlenmeyer flasks and sterilized. One ml of standard inoculum of rhizobacterial isolates was added into each flask and incubated at 37°C for seven days.

After seven days of incubation, the culture was centrifuged at 8000 g for 10 minutes to remove the bacterial cells. Fifteen ml of the culture filtrate was pipetted out separately to the test tube and two ml of zinc acetate solution was added. After two minutes, two ml of potassium ferrocyanide solution was added and centrifuged at 8000 g for 10 min. Five ml of supernatant was added to five ml of 30 per cent hydrochloric acid and the mixture was incubated at 20°C for 75 min. The blank was prepared with five per cent hydrochloric acid. The absorbance was measured at 254 nm in a spectrophotometer. From the standard graph using standard gibberellic acid solution the amount of GA produced by the culture was calculated and expressed as $\mu\text{g ml}^{-1}$ broth.

3.6. ESTIMATION OF SIDEROPHORE PRODUCTION BY RHIZOBACTERIAL ISOLATES

Siderophore production by the rhizobacterial isolates was estimated by the method described by Reeves *et al.* (1983)

Nitrogen free malate broth was prepared without bromothymol blue indicator nutrient broth, and King 's B broth were prepared for *Azospirillum*, phosphobacteria and *Pseudomonas* isolates respectively and dispensed in 100 ml quantities in 250 ml Erlenmeyer flasks and sterilized. One ml of standard inoculum of rhizobacterial isolates was added into each flask and incubated at 37°C for seven days. After seven days of incubation, the broth culture was centrifuged at 10,000 rpm for 20 min. The supernatant was divided into three equal parts for estimation of catechol type, hydroxymate and salicylate type siderophore.

Twenty ml of culture supernatant was taken and the pH was adjusted to 2.0 with dilute HCl. To 20 ml of supernatant, 20 ml of ethyl acetate was added and extraction was done twice. Hathway reagent (One ml of 0.1M ferric chloride and 1 ml of 0.1N HCl was added to 100 ml of distilled water and to this 1 ml of 0.1M potassium ferricyanide was added) was prepared. Five ml of the assay solutions was added with five ml of Hathway reagent and absorbance was determined at 560 nm with sodium salicylate as standard for the estimation of salicylate type of siderophore.

To measure the catechol type siderophore, five ml of the assay solution was added with five ml of Hathway reagent and absorbance was determined at 700 nm with 2,3 DHBA as a standard. One μ mol of 2,3- dihydroxy benzoic acid (2,3 DHBA) gave on absorbance of 0.75. From the absorbance value of the sample, the quantum of siderophore was calculated and expressed as μ moles per ml of culture medium.

Hydroxamate type of siderophore was measured in the culture supernatants of rhizobacterial isolates by the method of Gibson and Magrath (1969). To 0.5 ml of culture supernatant, 0.5 ml of 6M H_2SO_4 was added and the mixture was autoclaved in a glass tube. One μ l of sulphanilic acid (1% w/v) in 30 per cent acetic acid and 0.5 μ l 1.3 per cent iodine in 30 per cent acetic acid was added. The excess of iodine was destroyed by the addition of one ml of 2 per cent (w/v) Na_3AsO_4 solution. A solution of one ml of α -naphthylamine (0.3% in 30% acetic acid) was added and the total volume was made upto 10 ml with distilled water and absorbance was determined at 460 nm with hydroxylamine hydrochloride as a standard.

3.7. DETERMINATION OF POLY- β -HYDROXYBUTYRATE (PHB) CONTENT OF THE *Azospirillum* ISOLATES

The poly - β - hydroxybutyrate content of the *Azospirillum* isolates was estimated by following the method described by Zevenhuizen (1981). The *Azospirillum* cultures were grown in 100 ml of yeast extract glucose broth under shake culture till the cultures reached the log phase. The cells were harvested by centrifugation at 8300 G for 20 min at 4°C and the pellets were suspended in sterile distilled water. The cell suspension was used for PHB determination.

A quantity of one ml of cell suspension was added to one ml of 2N HCl. The mixture was digested in loosely stoppered tubes at 100°C for two h over a water bath, cooled and extracted with chloroform by shaking two times with 2.5 ml of chloroform. The samples containing lipids were transferred to test tubes and the chloroform was evaporated in a boiling water bath. Then five ml of H_2SO_4 (96%) was added and the samples were heated at 100°C for 10 min over a water bath. The solution is cooled and

after thorough mixing, PHB were recorded at 235 nm against H₂SO₄ blank in a 2000Z double beam spectrophotometer. The amount of PHB was calculated from the extinction coefficient, already determined with pure samples of PHB.

$$E_{235} = 0.35 \text{ per } 10 \mu\text{g PHB.}$$

3.8. HYDROGEN CYANIDE (HCN) PRODUCTION BY *Pseudomonas*

The hydrogen cyanide content in the *Pseudomonas* isolates was estimated by following the method described by Lork (1948). *Pseudomonas* isolates were grown on King's B medium supplemented with glycine 4.4 g l⁻¹ to screen for cyanide production. A piece of filter paper impregnated with picric acid solution (2.5 g picric acid and 12.5 g sodium carbonate in one litre of water) was placed in the lid of each Petri dish. The Petri dishes were sealed with parafilm. Thereafter, the Petri plates were inverted and incubated for 2-4 days. Discolouration of the filter paper to orange brown after incubation indicated microbial production of cyanide.

To ascertain if discolouration was actually due to cyanide production, 25 ml of King's B broth supplemented with glycine 4.4 g l⁻¹ was taken in 500 ml conical flask and inoculated with *Pseudomonas* isolates. Then the filter paper was cut into 10-12 cm long and 0.5 cm wide, and saturated with alkaline picrate solution. The saturated filter paper was placed in the hanging position with the help of a cork stopper inside the conical flask. The flasks were incubated at room temperature for 2-4 days. The sodium picrate present in the filter paper is reduced to reddish compound in proportion to the amount of hydrocyanic acid evolved. The colour was eluted by placing the paper in a clean test tube containing 10 ml of distilled water and compared with standards at 625 nm. The standard curve was prepared with KCN and the hydrogen cyanide content of the sample was expressed as $\mu\text{g ml}^{-1}$ of culture.

3.9. INTRINSIC ANTIBIOTIC RESISTANCE BY COTTON RHIZOBACTERIAL ISOLATES

The N-free malate, Nutrient and King's B broth were prepared in 50 ml quantity 100 ml Erlenmeyer flasks. The broth was inoculated with 72 h old cultures of different isolates of *Azospirillum*, phosphobacteria and *Pseudomonas* previously grown at 30°C for 24 h.

At the end of the incubation period, the cultures were centrifuged at 8000 g for 10 min and the cell pellets were washed twice with physiological saline by centrifugation. The cell pellet was dissolved in physiological saline were used for intrinsic antibiotic resistance test. The respective medium was prepared for different rhizobacterial isolates, and different antibiotics *viz.*, streptomycin, chloramphenicol, ampicillin and kanamycin with various concentration (Table 6) was filter sterilized and added to the medium at luke warm temperature before plating. The Petri plates were carefully spotted with the different bacterial isolates and incubated at 30°C for 2-3 days. The growth was qualitatively assessed by comparing with growth on plates without antibiotics.

Table 6. Antibiotics and their concentrations used for intrinsic antibiotic resistance test

S.No.	Antibiotic	Concentration (ppm)
1.	Streptomycin	25, 50, 75, 100, 125, 150, 200, 250, 300, 400, 500
2.	Chloramphenicol	5, 10, 15, 20, 25, 30, 40, 50, 75, 100
3.	Ampicillin	25, 50, 75, 100, 125, 150, 200, 250, 300, 400, 500
4.	Kanamycin	25, 50, 75, 100, 125, 150, 200, 250, 300, 400, 500

3.10. GROWTH PERFORMANCE OF COTTON RHIZOBACTERIAL ISOLATES UNDER DIFFERENT CARBON SOURCES

The Okon's broth without any carbon source was prepared in 100 ml quantities in 250 ml Erlenmeyer flasks. The carbon sources *viz.*, malic acid, succinic acid, glucose and sucrose were filter sterilized and added separately at 0.5 per cent levels. The broth was inoculated with 0.1 ml of cell suspension (0.6 OD at 560 nm) of 24 h old cultures of *Azospirillum* strains and then growth at room temperature with gentle agitation (100 min⁻¹) to maintain aerobic conditions. The optical density was read at 3 h intervals in a 2000Z double beam spectrophotometer at 560 nm using appropriate blank. Growth curve was drawn by plotting the OD values and time on semi logarithmic paper described by Umbreit (1976).

Similarly for the phosphobacterial isolates glucose, xylose, mannitol and sucrose were added and for the *Pseudomonas*, glycerol, glucose, mannitol and sucrose were used.

3.11. ESTIMATION OF PHOSPHATE RELEASE FROM DIFFERENT ROCK PHOSPHATE BY SELECTED ISOLATES OF PHOSPHOBACTERIA

Experiment was conducted to study the efficiency of the phosphobacterial isolates in solubilizing three types of rock phosphate under cultural conditions. The source and 'P' content of rock phosphate are given in the Table 7.

Table 7. Different types of rock phosphates used for the study

Name of rock phosphate	Source	P content (% Total P_2O_5)
Mussorie	India	23.12
Gafsa	Tunisia	36.00
Udaipur	India	27.68

The phosphobacterial isolates were grown in Pikovskaya's broth and tricalcium phosphate ($100 \text{ mg } 50 \text{ ml}^{-1}$) was replaced with different types of rock phosphates viz., Mussorie, Gafsa and Udaipur rock phosphate.. The flasks containing the cultures were incubated at 30°C in an environmental shaker at 100 rpm. The final pH of the culture broth, available phosphorus released from rock phosphates and acidity production (titrable acidity) were determined on 15 and 30 DAI as detailed in section 3.3.2.5, 3.3.2.6 and 3.3.2.7

3.12. TESTING VIABILITY AND SEEDLING VIGOUR OF COTTON (MCU 9) UNDER STERILE GROWTH POUCHES INOCULATED WITH RHIZOBACTERIAL ISOLATES

Selective rhizobacterial isolates were used to study the effect of seedling growth in cotton using sterile growth pouch method (Plate 1-2). The treated seeds of cotton were allowed to germinate at room temperature $28^\circ\text{C} \pm 1$ and at 95% relative humidity in the germination chamber. Then shoot length, root length and germination percentage was recorded after 10 days, vigour index was calculated by the procedures suggested by Abdul-Baki and Andersen (1973). The vigour index was worked out using the formula

$$\text{Vigour index} = \text{Germination per cent} \times \text{Plant height}$$

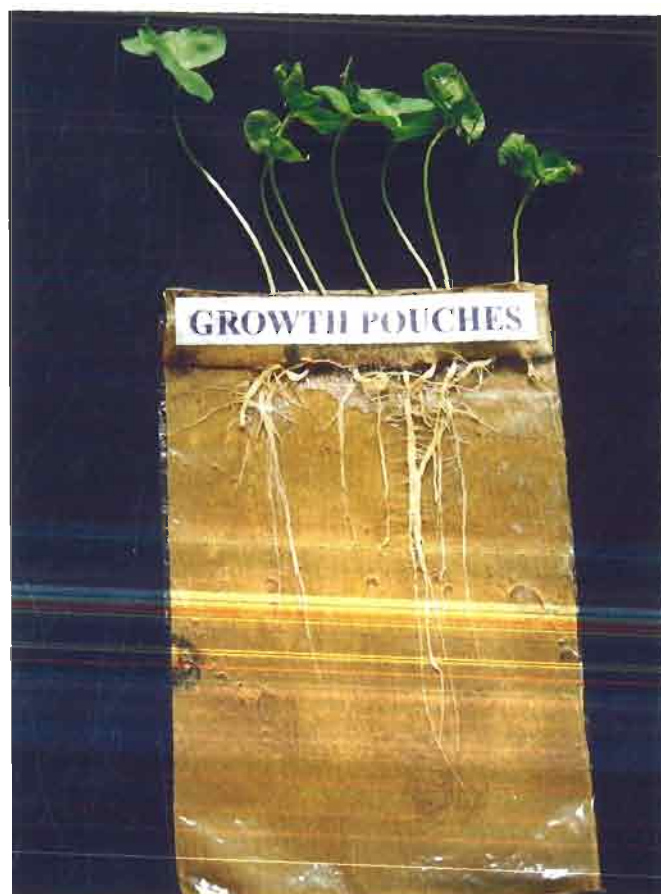


Plate 1. Sterile growth pouches used for germination studies



Plate 2. Studies on the effect of rhizobacterial isolates on the germination of cotton seeds

3.13. STUDIES ON THE ESTABLISHMENT OF RHIZOBACTERIAL ISOLATES IN PLANT UNDER HYDROPONICS CULTURE

3.13.1. Plant cultivation under hydroponics condition

Seeds of cotton (var. MCU9) were surface sterilized with ethanol (80 per cent) and one per cent sodium hypochlorite for five min and thoroughly washed in sterile distilled water. The seeds were pre-germinated on sterile soft agar (0.6 per cent) plates for 12 h in the dark at 30°C. Germinating seeds at the rate of two per tube were transferred to 30 x 3.5 cm (l x b) test tubes containing 60 ml of Hoagland's nutrient solution. The tubes containing nutrient solution were cotton plugged and sterilized earlier. The seeds placed inside the tubes were supported on wire screens just above the nutrient solution, so that the elongating roots grew into the solutions. The tubes were maintained at room temperature in the growth chamber (Plate 3).

3.13.2. Bacterial inoculation

The cell suspension (cfu 6×10^8 cells ml⁻¹) of efficient isolates were used for the bacterization of seeds (10 ml 100 seeds⁻¹). To each tube one ml of inoculum was added to the hydroponics culture one week after the transfer of the seeds.

3.13.3. Rhizoplane and endorhizosphere colonization of Cotton roots (MCU 9) by the rhizobacterial isolates

The root colonization of the rhizobacterial isolates was studied by following the method described by Van Peers and Schippers (1988). A random sample of root pieces of five cm size from each tube was used to determine the number of rhizoplane isolates. Root pieces in glass tubes containing five ml of sterile phosphate buffer (0.2M, pH 7.0) and 2.5 g of three mm diameter glass beads were shaken vigorously for one min. The phosphate buffer containing bacterial cells were serially diluted and plated on yeast extract glucose agar medium for the counting of the rhizoplane isolates on 5th, 10th and 15th DAI.

Colonization of the endorhizosphere was estimated after external disinfection of root pieces. Root pieces of five cm size were dipped in 10 per cent H₂O₂ for 15 sec followed by several washings with sterile 0.01 M MgSO₄ and dried in sterile filter paper. Root pieces were then macerated with a pestle and mortar in one ml 0.1 M MgSO₄.

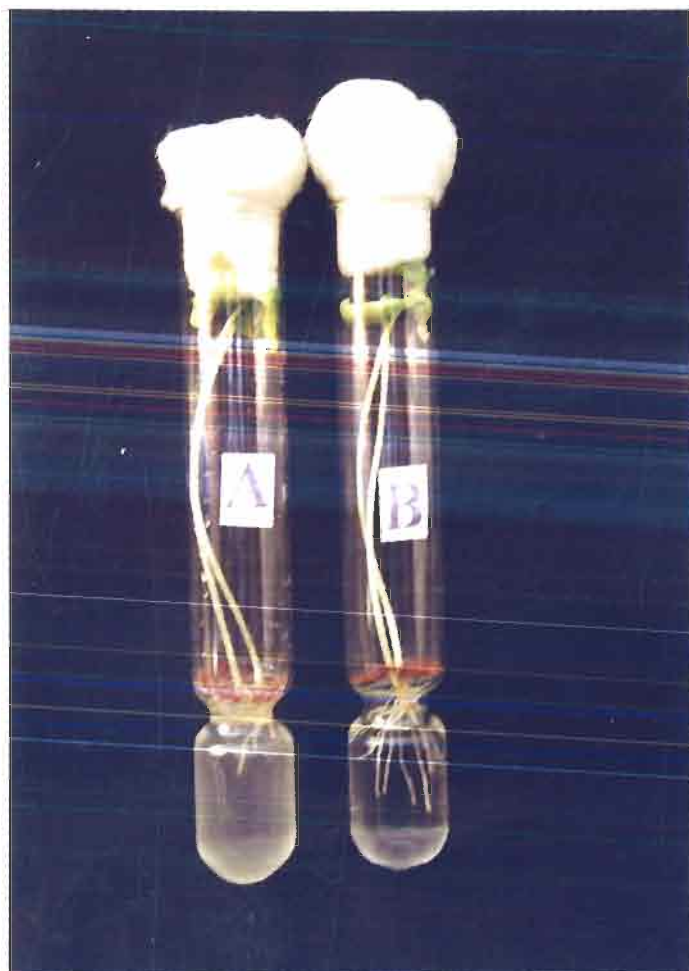


Plate 3. Hydroponics culture of Cotton inoculated with rhizobacterial isolates

There after, four ml of 0.1 M MgSO_4 was added and roots were homogenized on ice with tissue homogeniser. The homogenate was then diluted and plated on yeast extract glucose agar plates on 5th, 10th and 15th DAI.

3.13.4. Estimation of nutrient content of root exudate of cotton seedling (MCU 9) with rhizobacterial isolates under hydroponics condition

3.13.4.1. Estimation of total sugars in the root exudate of cotton seedlings

The nutrient solutions (root exudate) were analysed for total sugar content on 15 and 30 days of plant age by the procedure described by Wale Waja and Smith (1963).

The root exudates were hydrolysed with 2N HCl, cooled and neutralized with sodium carbonate. The suspension was centrifuged at 8000 g for 10 min and the clear supernatant was taken for analysis.

One ml of the supernatant was added to one ml of five per cent phenol solution. Five ml of 96 per cent sulphuric acid was added and then shaken well. After 10 min the tubes were kept in a water bath at 30°C for the development of green colour and read at 490 nm in a 2000Z double beam spectrophotometer. The total sugars present in the sample were calculated using the standard graph of glucose and expressed as μg of glucose mg^{-1} fresh weight of roots.

3.13.4.2. Estimation of reducing sugars in the root exudate of cotton seedlings

The reducing sugar content in the root exudates was analysed on 15th and 30th DAI by the method described by Nelson (1944).

Nelson's reagent A

A quantity of 2.5 g of anhydrous sodium carbonate, 2 g sodium bicarbonate, 2.5 g potassium sodium tartarate and 20 g anhydrous sodium sulphate were mixed in 80 ml distilled water and the volume made up to 100 ml.

Reagent B

A quantity of 15 g copper sulphate was dissolved in 80 ml of distilled water. To this, two drops of conc. sulphuric acid was added and the volume made up to 100 ml. The solutions A and b were mixed at the rate of 1: 24 before use.

Arsenomolybdate reagent

A quantity of 2.5 g of ammonium molybdate was dissolved in 45 ml distilled water. To this 2.1 ml of conc. sulphuric acid was added and stirred well.

In another beaker, 2.5 ml of distilled water was taken and 0.3 g of sodium arsenate was dissolved. The above two solutions were mixed and the volume made up to 50 ml.

Procedure

An aliquot of two ml of root exudate was taken in a test tube and one ml of Nelson's reagent was added. The contents, were boiled in a water bath for 10 minutes. After cooling, one ml of arsenomolybdate reagent was added, shaken well and the volume made up to 25 ml with distilled water. The intensity of the blue colour developed was read at 600 nm in a 2000Z double beam spectrophotometer. Using a standard graph of glucose, the reducing sugars are calculated and expressed as μg of glucose mg^{-1} of fresh weight of roots.

3.13.4.3. Estimation of amino nitrogen in the root exudate of cotton seedlings

Nutrient solutions containing root exudates were analyzed for amino nitrogen content on 15 and 30 DAI by the method developed by Moore and Stein (1948)

Reagents: Citric acid buffer 0.2 M, pH 5.0

A quantity of 21 g of citric acid was dissolved in 200 ml of 1N sodium hydroxide in a 500 ml volumetric flask and made up to 500 ml with glass distilled water.

Ninhydrin reagent

In 500 ml of citrate buffer at pH 5.0, 800 mg of hydrated stannous chloride was dissolved. In 500 ml of methyl cellosolve (E.Merk, Germany) (Ethyleneglycol monomethyl ether) 20 g of recrystallised ninhydrin (BDH-Enhland) was dissolved. The two solutions were mixed at the time of use.

Diluent solution

Equal quantities of glass distilled water and n-propanol were mixed.

Procedure

One ml of the root exudate was taken in a test tube, a drop of methyl red indicator was added and neutralized with 0.1 N sodium hydroxide. One ml of ninhydrin reagent was added, mixed thoroughly and boiled over a water bath 5 min. Glass marbles were placed on the top of the tubes to avoid evaporation. After cooling, 5 ml of diluent solution was added to each tube and mixed well. Blanks were run with distilled water in the place of the root exudate. The absorbance of the purple colour developed was read against the blank at 570 nm in a 2000Z double beam spectrophotometer. The quantity of amino nitrogen present in the sample was calculated based on a standard graph prepared with the amino acid glycine and expressed as μg glycine mg^{-1} fresh weight of the root.

3.14. ADSORPTION OF CELLS OF RHIZOBACTERIAL ISOLATES ON THE ROOTS OF COTTON SEEDLINGS (MCU 9)

The adsorption of cells of rhizobacterial isolates on the roots of the cotton seedlings was estimated by the method developed by Zamudio and Bastarrachea (1994).

Surface sterilized cottonseeds were grown for one week in hydroponics culture under aseptic conditions as described earlier. Test tubes of size 30 x 3.5 cm (lxb) were used for the study. There was no prior inoculation. After one week of growth, the seedlings were transferred to flasks containing 25 ml sterile Hogland's solution containing 6×10^8 rhizobacterial cells ml^{-1} . The flasks were kept in an incubator shaker maintained at 30°C. After 30 min and 60 min shaking at 60 rev min^{-1} , three seedlings from each tube were removed and washed in Hogland's solution for 15 min at 300 rev min^{-1} . The root pieces were then crushed in a tissue grinder containing one ml of Hogland's solution. The root suspension and the washings were diluted and plated on respective agar plates and the number of adsorbed rhizobacterial cells were determined.

3.15. STUDIES ON THE COMPATABILITY OF RHIZOBACTERIAL ISOLATES UNDER *in vitro* CONDITION

3.15.1. Studies on compatibility of rhizobacterial isolates by cross streak assay

A nutrient agar medium was prepared and sterilized. The medium was poured to sterile Petri plates and allowed to solidify. After solidification, the *Azospirillum* was

streaked as a straight line. The *P. fluorescens* and phosphobacteria were streaked perpendicular to the first streak and incubated at room temperature for 2-3 days and growth of the organisms was observed and compatibility was assessed.

3.15.2. Studies on the compatibility of rhizobacterial isolates under different carriers

3.15.2.1. Physico-chemical properties of carrier

The carrier materials peat and lignite were collected from the following sources.

Carrier material	Source
Peat	Ootacamund, Nilgiris Dt, Tamil Nadu
Lignite	Neyveli Lignite Corporation Neyveli, Tamil Nadu

Carrier materials were dried in shade, powdered, sieved through 200 micro sieve and analyzed for physical and chemical properties.

Water holding capacity, bulk density and particle density was analyzed as per the method described by Dakshina Murty and Gupta (1967). Chemical properties viz., pH (Piper, 1966), total nitrogen (Humphries, 1956) and organic carbon content (Walkley and Black, 1934) were analyzed as per the standard procedure.

Azospirillum (Azo 11), phosphobacteria (PB 9) and *Pseudomonas* (Pfa 2) were grown in malic acid broth nutrient broth and King's B broth respectively. When the population reached 10^9 cfu ml⁻¹ of broth was added to 100g of sterile, CaCO₃ amended peat and lignite.

Survival of the rhizobacterial isolates in peat and lignite was enumerated at periodic intervals viz., initial, 15, 30, 60, 90, 120, 150 and 180 days after inoculation. Standard dilution plate technique was adopted using selective media and the population is expressed as No. of cfu g⁻¹ of carrier. The experiment is replicated thrice with the following treatments.

Treatments

- T₁ - *Azospirillum* (Azo 11) alone (40 ml 100 g⁻¹ of carrier)
- T₂ - Phosphobacteria (PB 9) alone (40 ml 100 g⁻¹ of carrier)
- T₃ - *Pseudomonas* (Pfa 2) alone (40 ml 100 g⁻¹ of carrier)

- T₄ - 20 ml of *Azospirillum* (Azo 11) + 20 ml of Phosphobacteria (PB 9)
(100 g⁻¹ of carrier)
- T₅ - 20 ml of *Azospirillum* (Azo 11) + 20 ml of *Pseudomonas* (Pfa 2)
(100 g⁻¹ of carrier)
- T₆ - 20 ml of *Pseudomonas* (Pfa 2) + 20 ml of Phosphobacteria (PB 9)
(100 g⁻¹ of carrier)

3.16. EFFECT OF COMBINED INOCULATION OF *Azospirillum* WITH PHOSPHOBACTERIA AND *Pseudomonas* ON THE DYNAMICS OF RHIZOSPHERE MICROFLORA, GROWTH AND DEVELOPMENT OF COTTON (VAR. MCU 9)

A field experiment was conducted at Tamil Nadu Agricultural University, Coimbatore of Tamil Nadu during August to February, 1999-2000 to study the effect of combined inoculation of rhizobacterial isolates on the dynamics of rhizosphere microflora, growth and development of cotton (var. MCU 9).

The soil characteristics with respect to physical and chemical properties are given in Table 8.

The microbial isolates viz., *Azospirillum* (Azo 11), phosphobacteria (PB 9), and *Pseudomonas* (Pfa 2) were taken for the study. The rhizobacterial isolates were grown in their respective media. After sufficient growth, the individual culture was mixed thoroughly with sterile lignite (10⁹ cells g⁻¹ of lignite) and sealed in polythene bags. The cottonseed (var. MCU9) were seed - bacterized with individual and combined rhizobacterial isolates, *Azospirillum* (Azo 11) and phosphobacteria (PB 9) @ 20g kg⁻¹ of seed and *Pseudomonas* (Pfa 2) @ 4 g Kg⁻¹ of seed. After seed bacterization with respective lignite based inoculum, the inoculant treated seeds were dried under shade and sown. Soil application of the rhizobacterial isolates was applied @ 10 packets of *Azospirillum* (Azo11) and phosphobacteria (PB 9) and @ 2.5 kg ha⁻¹ for *Pseudomonas* (Pfa 2).

Table 8. Physical and chemical properties of the experimental field

Properties	Field soil
Physical properties	
Soil type	Vertisol
Coarse sand (%)	15.3
Fine sand (%)	13.4
Silt (%)	23.7
Clay (%)	37.6
Chemical properties	
Available N (kg ha ⁻¹)	210 (Low)
Available P(kg ha ⁻¹)	18 (Medium)
Available K (kg ha ⁻¹)	607 (High)
pH	7.6
EC (dSm ⁻¹)	0.4
Organic carbon (%)	0.3

The experiment was laid in factorial randomized block design as shown in Fig 2.

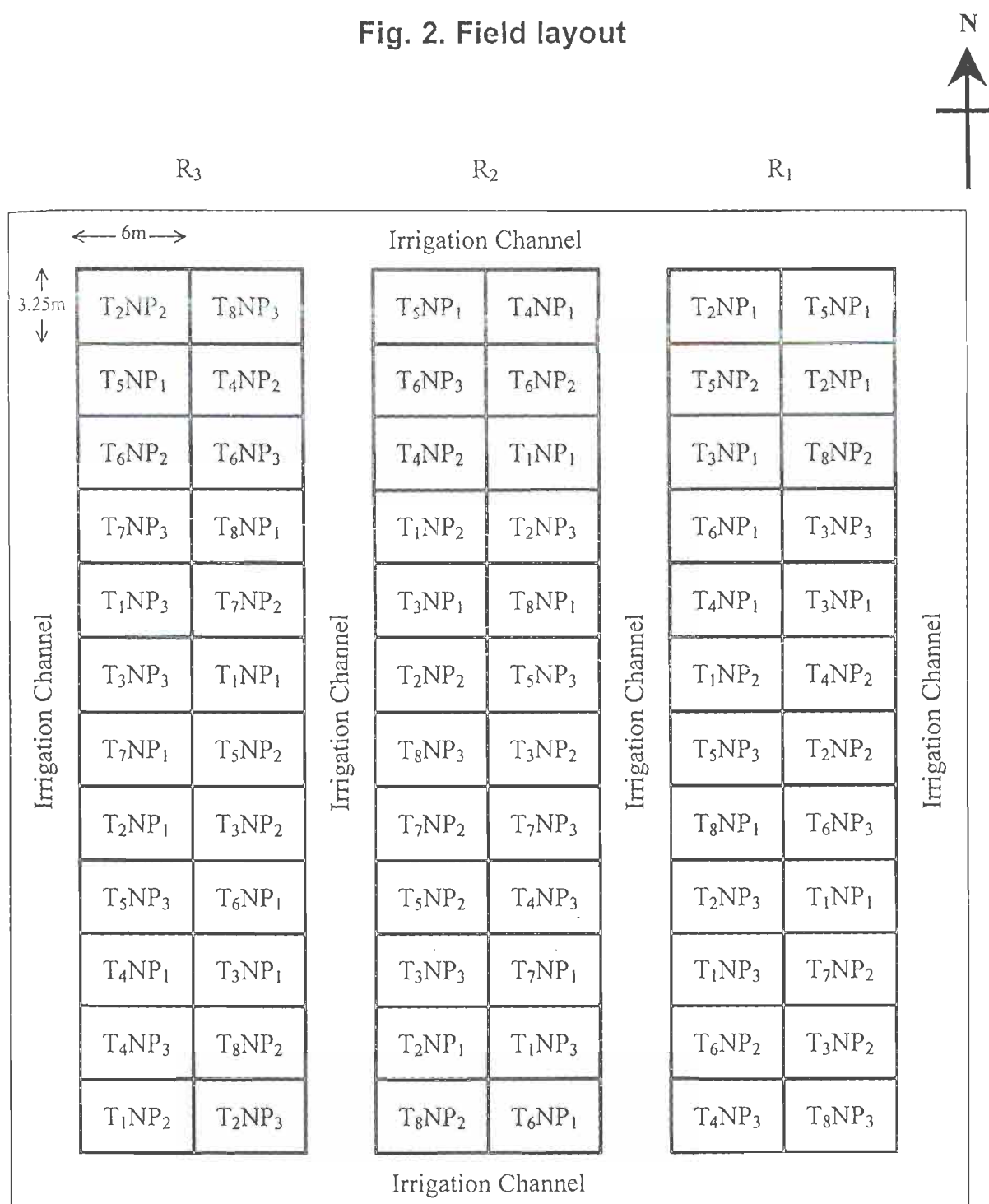
With following treatments

- T₁ - *Azospirillum* (Azo 11) alone
- T₂ - Phosphobacteria (PB 9) alone
- T₃ - *Pseudomonas* (Pfa 2) alone
- T₄ - *Azospirillum* (Azo 11) + Phosphobacteria (PB 9)
- T₅ - *Azospirillum* (Azo 11) + *Pseudomonas* (Pfa 2)
- T₆ - Phosphobacteria (PB 9) + *Pseudomonas* (Pfa 2)
- T₇ - *Azospirillum* (Azo 11) + Phosphobacteria (PB 9) + *Pseudomonas* (Pfa 2)
- T₈ - Uninoculated control

Fertilizer level

- F₁ - 50% N and P
- F₂ - 75% N and P
- F₃ - 100% N and P

Fig. 2. Field layout



Recommended level of fertilizers - 80:40:40 of NPK

Spacing	:	75-x 30 cm
Rows / Treatment	:	5
Area / plot	:	20 m ²
Date of sowing	:	23.8.99
Replication	:	Three

The meteorological data during the crop growth period was given in the annexure.

3.16.1. Population dynamics of total diazotrophs, bacteria, fungi and actinomycetes in the cotton rhizosphere due to the combined inoculation of rhizobacterial inoculants

The population of total diazotrophs, bacteria, fungi and actinomycetes in the cotton rhizosphere was studied by using Watanabe and Barraquo medium, nutrient agar medium, rose-bengal agar and Kenknight's medium respectively at different time intervals viz., 0, 25, 45, 90, 120 and 180th DAS. The growth rate of rhizosphere microflora at various time intervals was estimated by logistic growth modelling

3.16.1.1. Logistic microbial population dynamics growth model development

Microbial population is a function of time in the rhizosphere soil in the cotton crop i.e. $P(t) = f(t)$, where $P(t)$ microbial population at time t . We make the following suitable assumptions on the system (rhizosphere of cotton) the changes in the microbial population, $\Delta P(t)$ is directly proportional to change in time (Δt).

$$\text{i.e. } \Delta P(t) \propto \Delta t \quad \dots 1$$

The second assumption is the change in the microbial population is also directly proportional to the population at that specific time.

$$\text{i.e. } \Delta P(t) \propto P(t) \quad \dots 2$$

The third assumption in the non-replenished medium, the microbial population approaches to maximum capacity at the time of harvest (P_M), at which time, the growth rate approaches to zero.

$$\text{i.e. } \Delta P(t) \propto P_M - P(t) \quad \dots 3$$

Combining all the three assumptions that is the change in the microbial growth is directly proportional to change in time (Δt), the population at time $[P(t)]$, the carrying capacity $[P_M - P(t)]$

$$\text{i.e. } \Delta P(t) \propto \Delta t P(t) [P_M - P(t)]$$

$$\Delta P(t) = r \Delta t P(t) [P_M - P(t)]$$

'r' is the proportionality constant

$$\frac{\Delta P(t)}{\Delta t} = r P(t) [P_M - P(t)]$$

Considering the time interval as small as possible that is limit $\Delta t \rightarrow 0$, here time interval is considered in days.

$$\frac{dP(t)}{dt} = r [P_M - P(t)] P(t)$$

$$\frac{dP(t)}{P(t) [P_M - P(t)]} = r dt$$

$$\left[\frac{1}{P(t)} + \frac{1}{[P_M - P(t)]} \right] dP(t) = P_M r dt$$

Integrating on both sides

$$\log P(t) - \log [P_M - P(t)] = P_M r t + c$$

Where, c is integral constant

$$\log \left[\frac{P(t)}{P_M - P(t)} \right] = P_M r t + c$$

$$\frac{P(t)}{P_M - P(t)} = e^{P_M r t + c}$$

Simplifying

$$P(t) = \frac{P_M}{1 + \alpha e^{-\beta t}} \quad \dots 4$$

where,

$$\beta = P_M r$$

$$\alpha = e^c$$

$$\text{Growth rate} = - \frac{[P(t)]^2}{P_M} \alpha \beta e^{\beta t} \quad \text{.....5}$$

The logistic growth model equation (4 & 5) is used to study the microbial population growth in the present study. The model is estimated to explain the population dynamics at various nutrient levels with different microbial inoculation.

3.16.2. Survival of lignite based microbial inoculants in the cotton rhizosphere

The survival of lignite based microbial inoculants viz., *Azospirillum* (Azo 11) phosphobacteria (PB 9) and *Pseudomonas* (Pfa 2) was studied at different time intervals viz., 0, 7, 25, 45, 90, 120 and 180th DAI by using respective medium.

3.16.3. Biometrics observation

Five plants at random from net area of each plot were selected, tagged and the growth and yield characters were studied as detailed below.

3.16.3.1. Plant height

The height of the plant was measured from the cotyledonary node to the base of the last fully opened leaf. The height was measured at four stages viz., 25th (seedling), 45th (square formation), 90th (boll development) and 120th (first boll bursting) days after sowing and during harvesting (180th day). Root length, root volume (by water displacement method and expressed as w/v), number and length of lateral roots and length of lateral roots of the five plants at random in each plot was measured at four stages.

3.16.3.2. Number of monopodia and sympodia per plant

The vegetative monopodia and reproductive sympodia arising from axillary buds and extra axillary buds respectively were counted on 120th day.

3.16.3.3. Number of bolls per plant

The number of bolls retained by the plant was recorded on 120th day.

3.16.3.4. Boll weight

The weight of seed cotton from ten matured and fully opened bolls of 5 plants selected at random was recorded. The individual boll weight was worked out and expressed in g boll⁻¹.

3.16.3.5. Dry matter production

The samples for estimating dry matter production, five plants on 120th day were pulled out at random at ground level from the reserved sample rows of each plot. The samples were initially air dried and latter on dried in an oven till they become moisture free. Their weights were recorded on moisture free basis and computed as kg ha⁻¹.

3.16.3.6. Methods of nutrient analysis in plant samples

Plant samples collected treatment wise was washed in running tap water and then with 0.1 N HCl followed by double distilled water. The samples were then oven dried at 60-70°C. Samples were powdered in Wiley Mill. One g of powdered plant sample was digested with 12 ml of triple acid extract HNO₃: H₂SO₄: HClO₄ = 9:2:1 v/v), kept for over night and digested till the solution slowly becomes colourless. The volume of the extract was made upto 100 ml with double distilled water and analyzed for macro (N, P, K), secondary (Ca, Mg) and micro nutrients (Cu, Fe, Mn, Zn).

3.16.3.6.1. Estimation of total nitrogen

The nitrogen content of the plant samples were analyzed following Micro-kjeldahl method (Humphries, 1956).

3.16.3.6.2. Estimation of phosphorus

The phosphorus content in the plant material was estimated by the method of Olsen *et al.* (1954) as described earlier.

3.16.3.6.3. Estimation of potassium (Jackson, 1973)

The potassium content in the plant sample was estimated by following the method of Jackson (1973).

3.16.3.6.4. Estimation of calcium and Magnesium

The content of calcium and magnesium in the sample was analyzed following versenate titration method of Jackson (1973).

3.16.3.6.5. Assay of micronutrients

The micronutrients, copper, iron, zinc and manganese were estimated using Atomic Absorption spectrophotometer (AAS) (Jackson, 1973).

The triple acid extract was fed into Varian Tectron atomic absorption spectra (Model 20 BQ) using respective cathode lamps and read under the following wave lengths and slit indices (AAS) (Jackson, 1973).

Elements	Wavelength (nm)	Slit width (nm)
Cu	324.7	0.5
Fe	372.0	0.2
Mn	403.1	0.2
Zn	213.9	1.0

3.16.4. Estimation of soil nutrient content

3.16.4.1. Estimation of soil available nitrogen

The available nitrogen content of the soil sample was estimated by following the alkaline permanganate method of Subbiah and Asija (1956).

3.16.4.2. Estimation of available phosphorus

The available phosphorus content of the soil sample was estimated by following the method of Olsen's *et al* (1954).

3.16.4.3. Estimation of available potassium

The available potassium content of the sample was estimated by following the Neutral ammonium acetate method of Stanford and English (1949).

3.17. STATISTICAL ANALYSIS

Statistical analysis of the experiments are done based on the methods given by Panse and Sukhatme (1985).

RESULTS

CHAPTER IV

RESULTS

4.1. ISOLATION AND CHARACTERIZATION OF RHIZOBACTERIA

4.1.1. *Azospirillum*

Forty five *Azospirillum* isolates were isolated from the roots and rhizosphere soil of different varieties of Cotton cultivated in Tamil Nadu. All the isolates formed white dense, undulating pellicle, 1-3 cm below the surface of Nfb semisolid medium and the colour of the medium changed from yellowish green to blue. Growth of these isolates on Congo red plates showed scarlet colour colonies. These isolates were purified by streak plate method in Nfb medium and single dry wrinkled colonies were selected and used for the study of morphological character (Plate 4).

All these isolates were gram negative and microscopic examination of 18-24 hr old cultures showed motile, slender, curved and short rods with active spiral movement with refractive granules (Table 9).

4.1.2. *Phosphobacteria*

Thirty seven isolates were isolated from the rhizosphere soils of different varieties of Cotton. The isolates which are capable of solubilizing hydroxy apatite in the media, as indicated by the clear zone around the colony growth in Petri plates were selected, purified and used for further studies (Plate 5).

4.1.3. *Pseudomonas* sp

Twenty five Cotton rhizobacterial isolates grown on King's B medium supplemented with ampicillin 100 ppm, chloramphenicol 50 ppm and cycloheximide 10 ppm were isolated, purified and subjected to various morphological and biochemical tests for identification (Plate 6).

4.2. SCREENING OF RHIZOBACTERIAL ISOLATES UNDER *in vitro* CONDITIONS

4.2.1. Screening of *Azospirillum* isolates

The *Azospirillum* isolates were screened for their nitrogen fixing properties under *in vitro* condition.

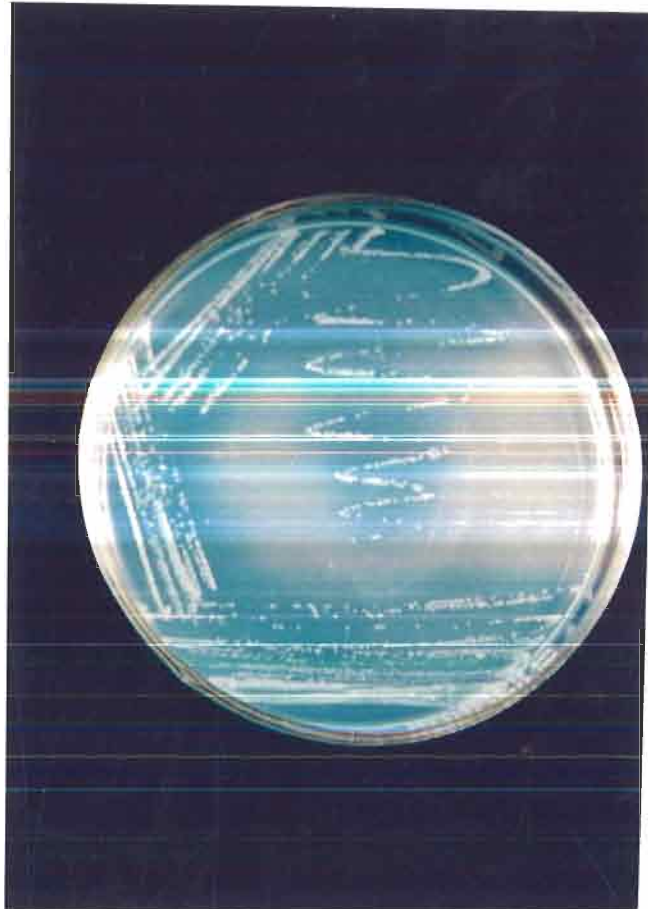


Plate 4. Growth of *Azospirillum* on Nfb agar

Table 9. General characteristics of *Azospirillum* isolates

Isolates	Subsurface pellicle	Change of colour of medium to blue	Scarlet colour formation in Congo red medium	Motility	Gram reaction	Isolates	Subsurface pellicle	Change of colour of medium to blue	Scarlet colour formation in Congo red medium	Motility	Gram reaction
A ₁ R ₁ *	+	+	+	+	-	P ₃ R ₁	+	+	+	+	-
A ₁ R ₂	+	+	+	+	-	P ₃ S ₁	+	+	+	+	-
A ₁ S ₁ **	+	+	+	+	-	K ₁ R ₁	+	+	+	+	-
A ₂ R ₁	+	+	+	+	-	K ₁ R ₂	+	+	+	+	-
A ₂ R ₂	+	+	+	+	-	K ₁ S ₁	+	+	+	+	-
A ₂ S ₁	+	+	+	+	-	K ₂ R ₁	+	+	+	+	-
A ₃ R ₁	+	+	+	+	-	K ₂ R ₂	+	+	+	+	-
A ₃ R ₂	+	+	+	+	-	K ₂ S ₁	+	+	+	+	-
C ₁ R ₁	+	+	+	+	-	K ₂ S ₂	+	+	+	+	-
C ₁ R ₂	+	+	+	+	-	K ₃ R ₁	+	+	+	+	-
C ₁ S ₁	+	+	+	+	-	K ₃ S ₁	+	+	+	+	-
C ₁ S ₂	+	+	+	+	-	K ₄ R ₁	+	+	+	+	-
C ₂ R ₁	+	+	+	+	-	K ₄ S ₁	+	+	+	+	-
C ₂ R ₂	+	+	+	+	-	S ₁ R ₁	+	+	+	+	-
C ₂ R ₃	+	+	+	+	-	S ₁ S ₁	+	+	+	+	-
C ₂ R ₁	+	+	+	+	-	S ₂ R ₁	+	+	+	+	-
C ₃ R ₁	+	+	+	+	-	S ₂ S ₁	+	+	+	+	-
C ₃ S ₁	+	+	+	+	-	S ₂ S ₂	+	+	+	+	-
P ₁ R ₁	+	+	+	+	-	S ₃ R ₁	+	+	+	+	-
P ₁ S ₁	+	+	+	+	-	S ₄ R ₁	+	+	+	+	-
P ₁ S ₂	+	+	+	+	-	S ₄ S ₁	+	+	+	+	-
P ₂ R ₁	+	+	+	+	-	Az 204***	+	+	+	+	-
P ₂ S ₁	+	+	+	+	-	Sp 7***	+	+	+	+	-

R* - Root S** - Rhizosphere soil *** Standard strain

Plate 5. Growth of phosphobacteria on hydroxy apatite medium

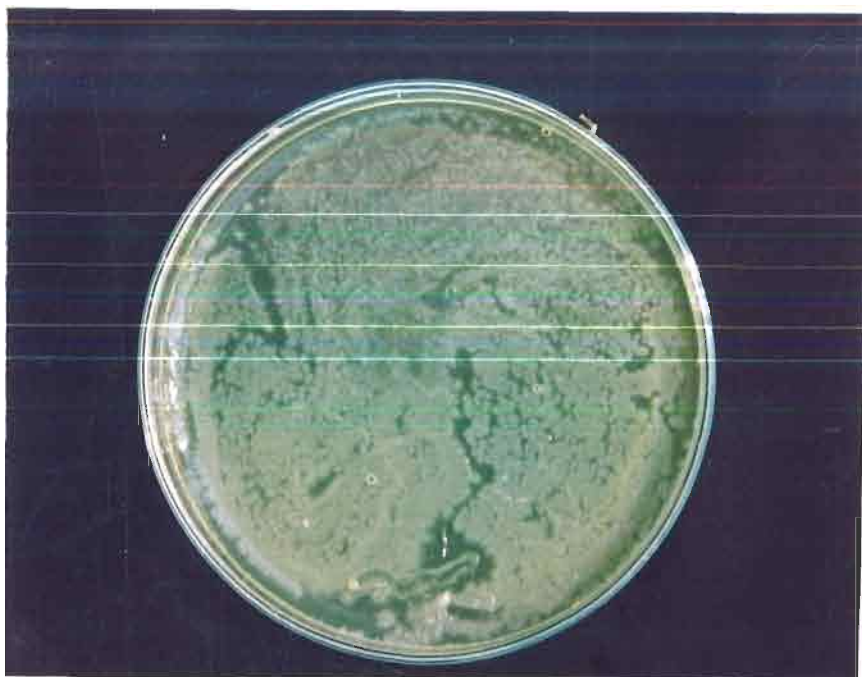


Plate 6. Growth of *Pseudomonas* on King's B agar

4.2.1.1. Nitrogen fixation under *in vitro* conditions

Nitrogen fixation among the isolates varied widely between 5.24 to 45.83 mg of N g⁻¹ of malate and the results is presented in Table 10 and Fig 3. Among the isolates 30 per cent of the isolates fixed more than 20 mg of N g⁻¹ malate. The isolates, S₂S₂ and C₂R₁ recorded a maximum of 45.83 and 42.40 mg N g⁻¹ of malate when compared to standard strain *viz.*, Az204 and Sp7 which recorded of 32.62 and 35.41 mg N g⁻¹ of malate respectively.

4.2.1.2. Nitrogenase activity

The nitrogenase activity of the 45 isolates and the standard strain, Az204 and Sp7 were estimated and the result is presented in Table 10 Fig 4. Among the isolates S₂S₂, C₂R₁ and K₂S₂ recorded significantly higher nitrogenase activity than the standard strains Az204 and Sp7. About 30 per cent of the isolates showed more than 200 n moles of C₂H₄ produced hr⁻¹ mg⁻¹ of cell protein.

4.2.1.3. Selection of efficient isolates

Based on the *in vitro* nitrogen fixation and nitrogenase activity, the 12 *Azospirillum* isolates *viz.*, A₁S₁, A₂S₁, C₁R₂, C₂R₁, C₃S₁, P₃R₁, K₁S₁, K₂S₂, K₄R₁, S₁S₁, S₂S₂ and S₄R₁ were selected and renamed as Azo 1, Azo 2, Azo 3, Azo 4, Azo 5, Azo 6, Azo 7, Azo 8, Azo 9, Azo 10, Azo 11 and Azo 12 respectively and used for further analysis.

4.2.2. Screening of Phosphobacteria isolates

Thirty seven phosphobacterial isolates were screened based on the acid phosphatase activity and the amount of 'P' released from TCP in Pikovskaya's broth. The change in the pH and the titrable acidity of the culture medium due to the growth of phosphobacterial isolates are presented in Table 11 and Plate 7.

4.2.2.1. Acid phosphatase activity

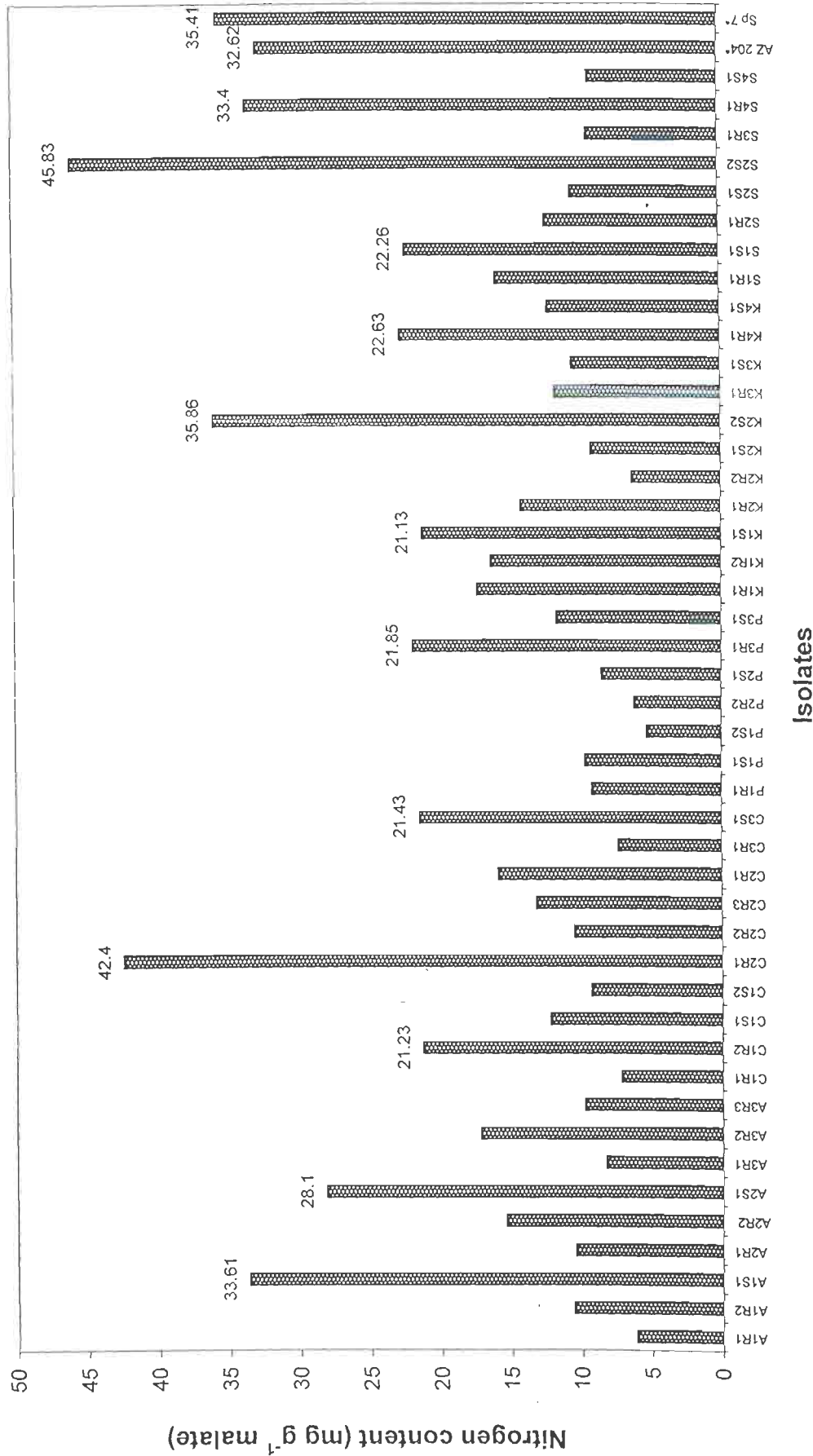
The phosphatase activity among the isolates ranged from 14.12 to 80.35 n moles of p-nitrophenol released min⁻¹ mg⁻¹ of cell protein (Fig 5). The maximum phosphatase activity was recorded by K₃₃ followed by P₃₂, S₂₁ and the standard strain whereas C₁₂ recorded the lowest phosphatase activity. About 38 per cent of the isolates released more than 30 n moles of p-nitrophenol min⁻¹ mg⁻¹ of cell protein.

Table 10. *In vitro* nitrogen fixation and nitrogenase activity of *Azospirillum* isolates

Isolate	Nitrogen content (mg g ⁻¹ of malate)	Nitrogenase activity (n moles of C ₂ H ₄ produced per hr ⁻¹ mg ⁻¹ of cell protein)	Isolate	Nitrogen content (mg g ⁻¹ of malate)	Nitrogenase activity (n moles of C ₂ H ₄ produced per hr ⁻¹ mg ⁻¹ of cell protein)
A ₁ R ₁	6.12	74.48	P ₃ R ₁	21.85	245.96
A ₁ R ₂	10.56	127.62	P ₃ S ₁	11.62	102.37
A ₁ S ₁	33.61	277.18	K ₁ R ₁	17.24	176.23
A ₂ R ₁	10.42	155.71	K ₁ R ₂	16.26	157.52
A ₂ R ₂	15.34	162.83	K ₁ S ₁	21.13	282.46
A ₂ S ₁	28.10	259.85	K ₂ R ₁	14.12	156.73
A ₃ R ₁	8.24	65.12	K ₂ R ₂	6.26	35.25
A ₃ R ₁	17.16	178.32	K ₂ S ₁	9.12	85.32
A ₃ R ₂	9.76	72.63	K ₂ S ₂	35.86	414.52
C ₁ R ₁	7.12	62.15	K ₃ R ₁	11.62	108.16
C ₁ R ₂	21.23	314.10	K ₃ S ₁	10.43	92.83
C ₁ S ₁	12.16	155.32	K ₄ R ₁	22.63	298.76
C ₁ S ₂	9.24	78.52	K ₄ S ₁	12.12	123.65
C ₂ R ₁	42.40	489.22	S ₁ R ₁	15.80	105.26
C ₂ R ₂	10.46	98.65	S ₁ S ₁	22.26	296.69
C ₂ R ₃	13.12	127.32	S ₂ R ₁	12.26	135.12
C ₂ R ₁	15.82	162.73	S ₂ S ₁	10.42	99.65
C ₃ R ₁	7.32	53.21	S ₂ S ₂	45.83	555.32
C ₃ S ₁	21.43	257.70	S ₃ R ₁	9.25	88.32
P ₁ R ₁	9.12	88.35	S ₄ R ₁	33.40	306.11
P ₁ S ₁	9.62	91.60	S ₄ S ₁	9.16	83.16
P ₁ S ₂	5.24	48.35	Az204*	32.62	368.84
P ₂ R ₂	6.12	52.81	Sp 7*	35.41	334.50
P ₂ S ₁	8.42	70.15	CD (P=0.05)	4.421	6.189

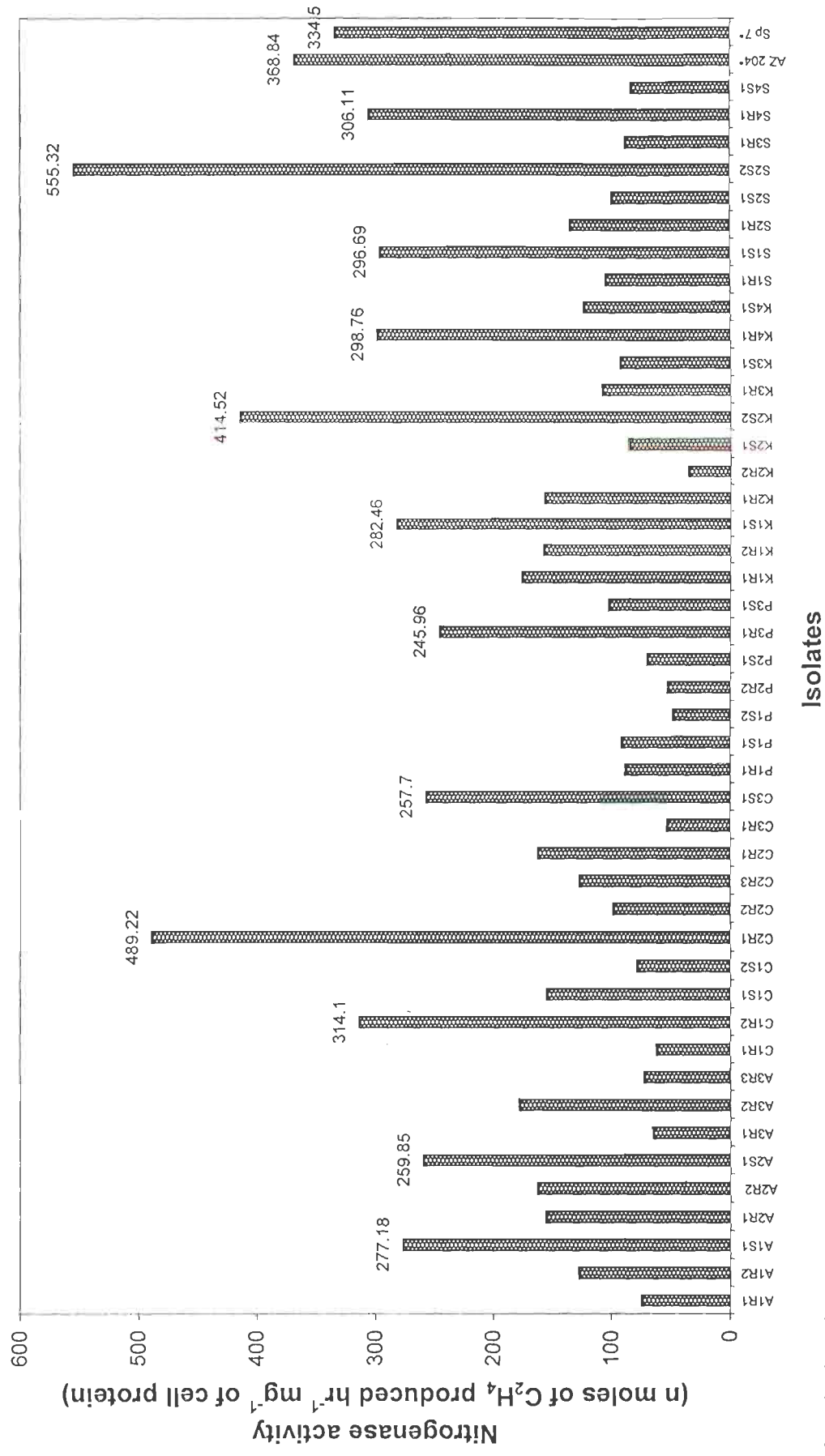
* Standard strain

Fig. 3. *In vitro* nitrogen fixation of *Azospirillum* isolates



* Standard strain

Fig. 4. Nitrogenase activity of *Azospirillum* isolates



* Standard strain

Table 11. Acid phosphatase activity, tricalcium phosphate solubilization, titrable acidity and pH of culture supernatant of phosphobacterial isolates

Isolate	Acid phosphatase activity (n moles of p-nitrophenol released min ⁻¹ mg ⁻¹ of cell protein)	Phosphorus solubilized from 100 mg TCP in Pikovskaya's broth (mg)		Titrable acidity of culture supernatant (ml of 0.01 N NaOH consumed)		pH of the culture medium	
		15 DAI*	30 DAI	15 DAI	30 DAI	15 DAI	30 DAI
A ₁₁	28.65	8.22	10.15	3.5	6.2	6.7	6.5
A ₁₂	32.16	9.25	12.35	4.6	6.7	6.7	6.6
A ₁₃	55.72	15.12	22.26	7.5	9.6	5.7	5.3
A ₂₁	22.15	7.26	9.52	3.2	5.9	7.0	6.8
A ₂₂	21.65	6.52	8.56	2.8	5.2	7.0	6.8
A ₃₁	25.14	8.16	9.98	3.4	6.0	6.7	6.6
A ₃₂	38.32	9.55	13.35	4.8	7.0	6.7	6.6
A ₃₃	60.11	14.63	19.98	7.3	9.8	6.0	5.5
C ₁₁	21.63	8.12	9.85	3.3	5.9	6.7	6.6
C ₁₂	14.42	4.23	6.36	2.1	3.5	7.0	6.0
C ₂₁	46.70	14.71	20.08	6.8	8.4	6.3	6.1
C ₂₂	16.35	3.25	6.55	2.2	3.8	7.0	7.0
C ₃₁	44.56	15.08	21.15	6.4	8.1	6.0	6.0
C ₃₂	18.35	6.16	8.15	2.5	5.0	7.0	7.0
P ₁₁	60.80	16.11	24.51	8.1	10.1	5.1	4.7
P ₁₂	28.52	8.32	12.35	4.5	6.8	6.8	6.6
P ₂₁	33.15	9.57	14.15	5.1	7.6	6.5	6.5
P ₂₂	16.72	4.15	7.83	1.8	4.9	6.7	6.6
P ₃₁	20.22	6.86	9.15	3.0	5.8	6.8	6.5
P ₃₂	78.46	18.28	29.55	8.4	10.3	5.1	4.6
P ₃₃	28.17	9.26	13.35	4.7	7.2	7.0	6.8
K ₁₁	30.63	10.37	13.98	4.9	7.3	6.8	6.5
K ₁₂	20.45	8.25	9.45	3.2	5.1	7.0	6.8
K ₂₁	58.33	15.89	22.20	7.0	9.4	5.8	5.5
K ₂₂	15.33	4.15	7.36	1.5	4.6	6.8	6.5
K ₂₃	51.25	14.32	22.95	7.3	9.1	5.4	5.6
K ₃₁	17.81	3.66	7.15	1.1	4.2	6.7	6.6
K ₃₂	16.55	7.15	10.15	3.1	6.1	6.8	6.7
K ₃₃	80.35	18.56	31.10	8.8	10.7	4.9	4.5
K ₄₁	23.65	8.96	10.35	3.3	6.2	7.0	6.8
K ₄₂	28.32	9.55	12.61	4.7	7.0	6.7	6.5
S ₁₁	60.73	16.52	25.60	7.2	7.2	5.9	5.6
S ₁₂	31.56	9.83	12.35	4.3	4.3	6.8	6.6
S ₂₁	65.52	18.36	30.25	8.5	9.5	5.1	4.7
S ₂₂	35.35	10.15	14.13	5.0	5.0	6.3	6.0
S ₄₁	28.52	9.65	10.64	3.6	3.6	6.7	6.2
S ₄₂	22.13	8.52	11.35	3.8	3.8	6.8	6.8
<i>Bacillus megaterium</i> ** (Bm)	63.71	18.25	28.60	8.0	8.8	5.0	4.7
Uninoculated control	-	0.58	0.58	0.8	0.8	7.0	7.0

* DAI - Days after inoculation ** Standard strain

	CD (P=0.05)	CD (P=0.05)	CD (P=0.05)
Days	1.298	0.408	0.398
Treatment	3.309	0.589	0.549
Days x Treatment	4.680	1.075	1.978

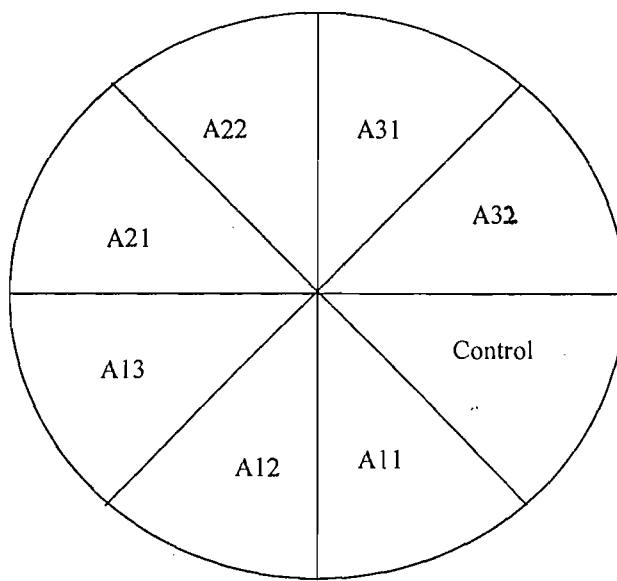
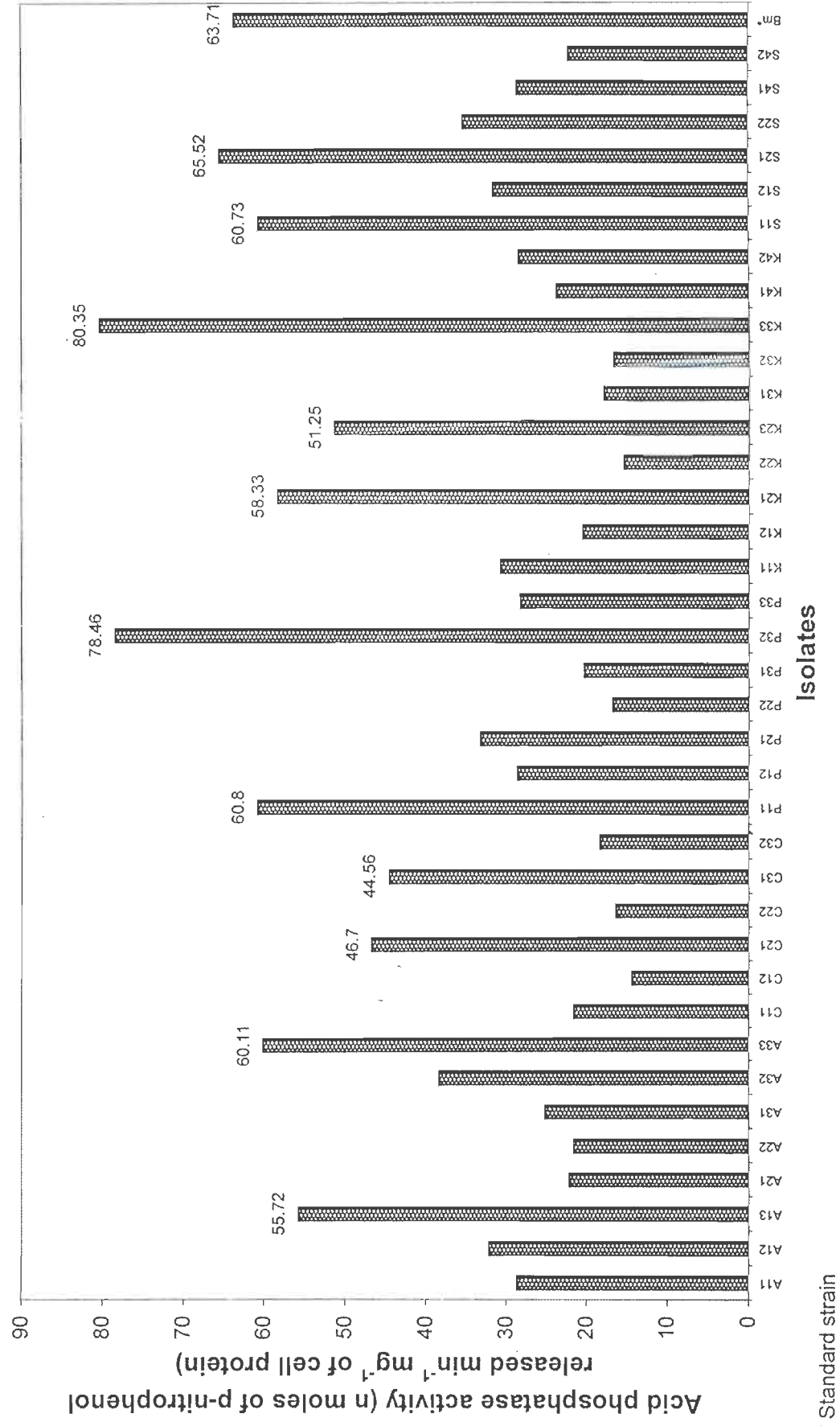


Plate 7. Tricalcium phosphate (TCP) solubilization by phosphobacterial isolates



Plate 7. Tricalcium phosphate (TCP) solubilization by phosphobacterial isolates

Fig. 5. Acid phosphatase activity of phosphobacterial isolates



4.2.2.2. Solubilization of tricalcium phosphate (TCP)

The amount of 'P' solubilized from TCP by various bacterial isolates was found to be higher on 30th day when compared 15th day. About 30 per cent of the isolates showed solubilization above 20 mg 'P' 100 mg TCP. The amount of P released in the broth from 100 mg⁻¹ TCP varied from 3.25 to 18.56 mg on 15 days after inoculation (DAI) and 6.55 to 31.1 mg 'P' from 100 mg⁻¹ TCP on 30 DAI respectively. The K₃₃ isolate recorded a maximum solubilization of 18.56 mg 100 mg⁻¹ of TCP on 15th DAI and 31.10 mg of 'P' 100 mg⁻¹ TCP on 30 DAI followed by P₃₂ isolate and S₂₁ isolate whereas standard strain *Bacillus megaterium* recorded 18.25 and 28.60 mg of 'P' 100 mg⁻¹ TCP (Fig 6).

4.2.2.3. Estimation of titrable acidity

The amount of acid production in the culture medium due to bacterial growth was estimated by titrating against standard alkali (0.01N NaOH). The titre value for 5.0 ml of culture supernatant varied from 1.1 to 8.8 ml of 0.01 N NaOH during 15 DAI and 3.5 to 10.7 ml of 0.01N NaOH on 30 DAI respectively. About, 43 per cent of phosphobacterial isolates recorded more than 7 ml of 0.01N NaOH consumption. The titrable acidity was higher, in K₃₃ followed by P₃₂, P₁₁ and standard strain *Bacillus megaterium* (Fig 7) on 30 DAI.

4.2.2.4. pH reduction

The growth of phosphobacterial isolates decreased the pH of the culture medium. But there is no significant change in the pH of the broth between 15 and 30 DAI. The pH reduction varied from 4.9 to 7.0 in the culture supernatant on 15 DAI whereas on 30 DAI the pH was 4.5 to 7.0 as shown in Fig 8. Among the isolate K₃₃ isolate recorded maximum solubilization and acid phosphatase activity, which leads to reduction in pH from 7 to 4.9 on 15 DAI and 7 to 4.5 on 30 DAI.

4.2.2.5. Selection of efficient isolates

Based on the phosphatase activity and solubilization of tricalcium phosphate 11 isolates viz., A₁₃, A₃₃, C₂₁, C₃₁, P₁₁, P₃₂, K₂₁, K₂₃, K₃₃, S₁₁ and S₂₁ were selected and renamed as PB 1, PB 2, PB 3, PB 4, PB 5, PB 6, PB 7, PB 8, PB 9, PB 10 and PB 11 respectively. These 11 isolates were taken for further studies.

Fig. 6. Solubilization of tricalcium phosphate (TCP) by phosphobacterial isolates

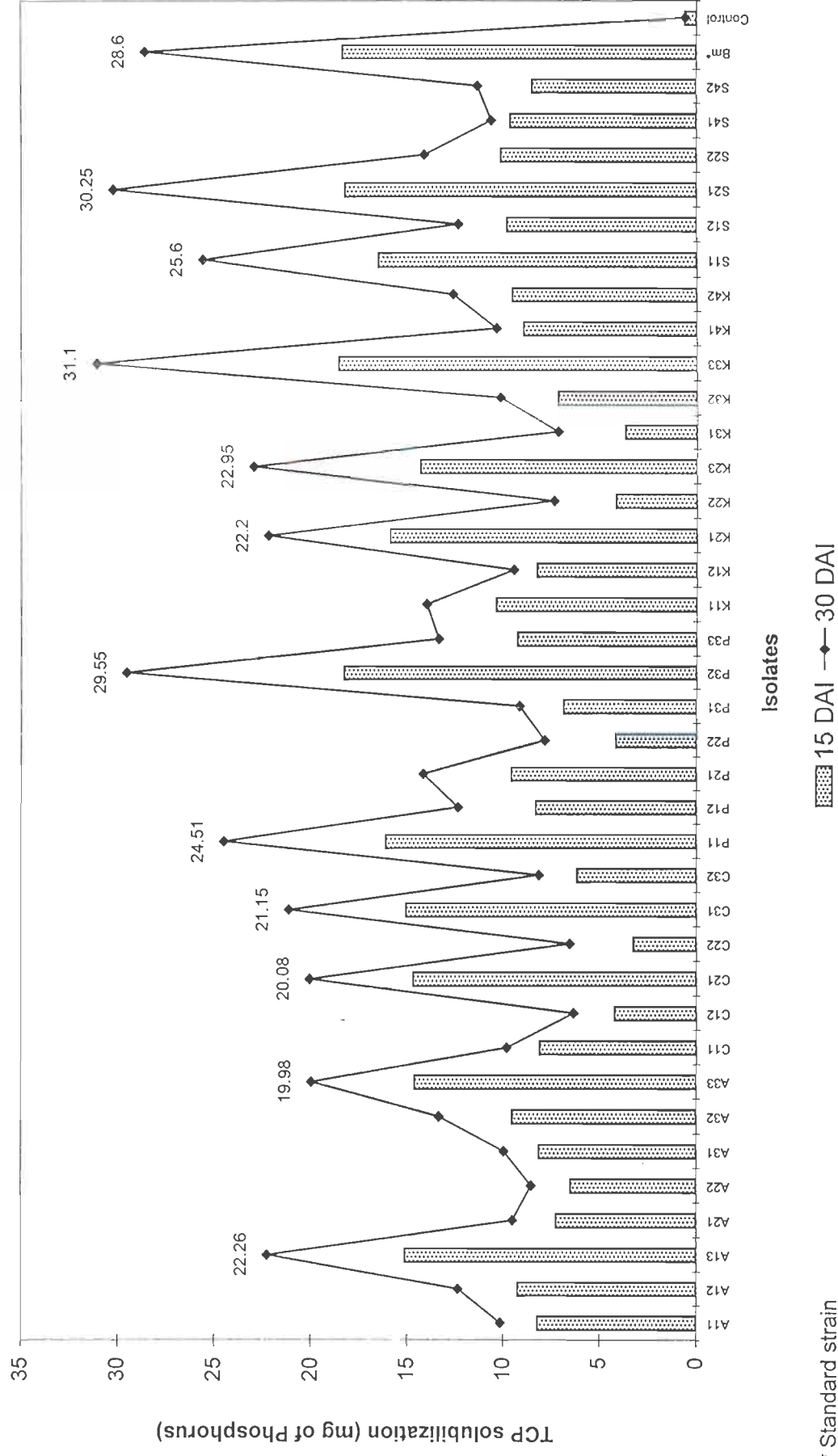
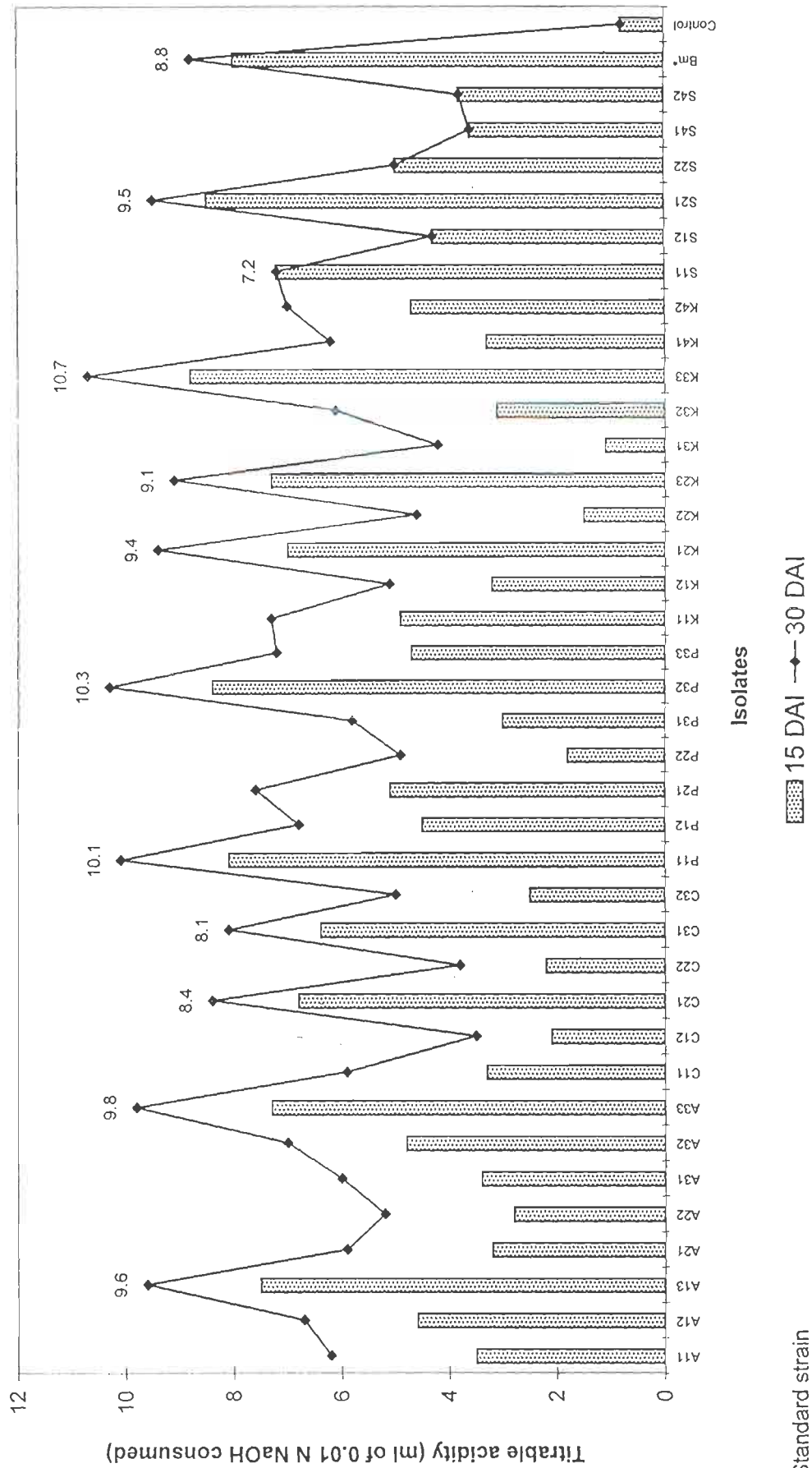


Fig. 7. Titrable acidity produced in the culture supernatant of phosphobacterial isolates



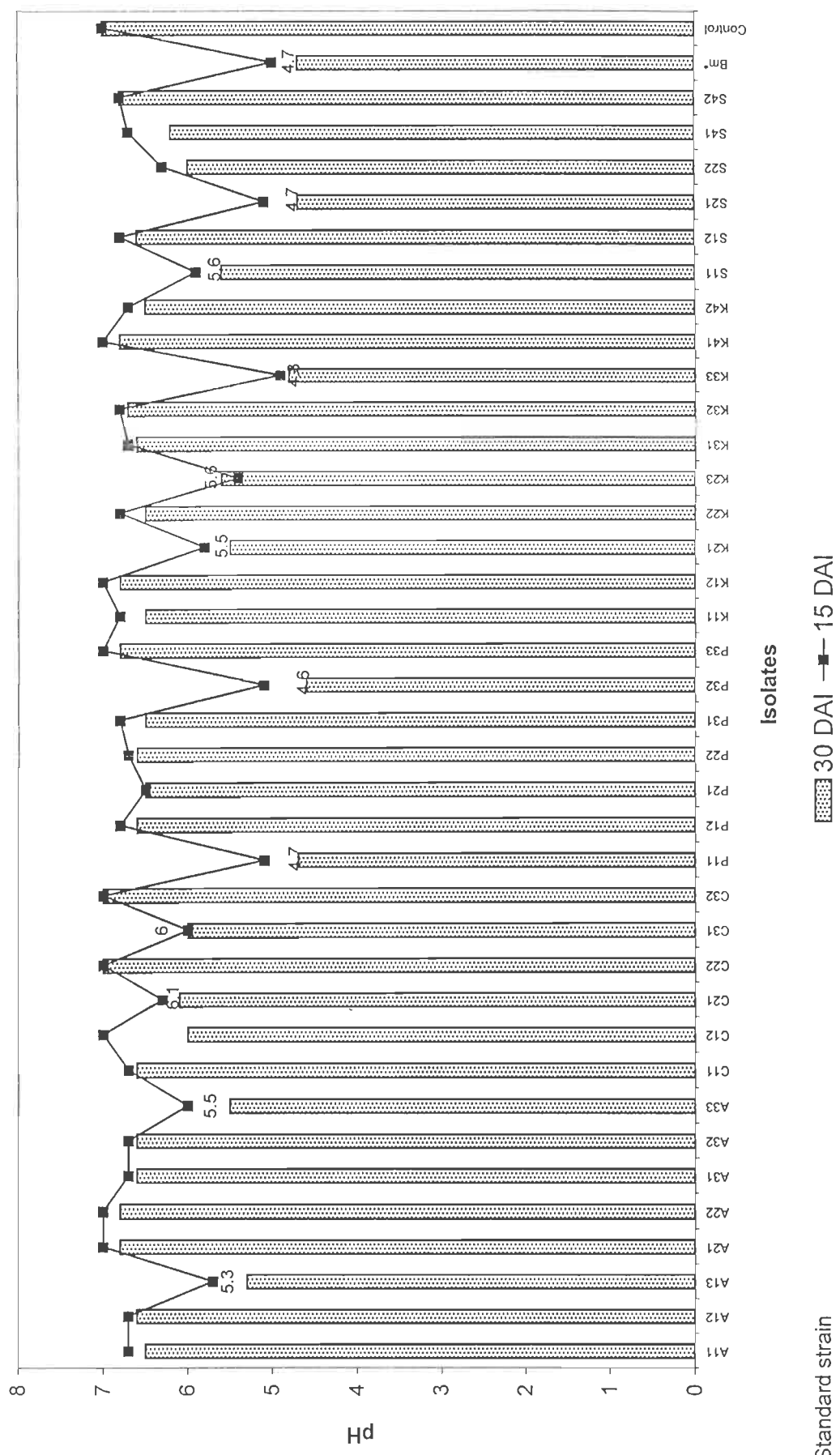
* Standard strain

15 DAI 30 DAI

Isolates

Titration acidity (ml of 0.01 N NaOH consumed)

Fig. 8. pH of the culture medium of phosphobacterial isolates



4.2.3. Screening of *Pseudomonas* isolates

Twenty four *Pseudomonas* isolates were primarily screened for their antagonistic activity against *Rhizoctonia solani*.

4.2.3.1. Antagonistic activity against *R. solani*

The antagonistic activity was measured in terms of inhibition zone, which varied widely from 6 mm to 15 mm. Only 33 per cent of the isolates showed the antagonistic activity against *R. solani*. Among the isolates, A₂₂ isolate showed maximum inhibition zone of 15 mm followed by K₄₁ (12 mm), standard strain *Pseudomonas fluorescens* Pf 1 recorded an inhibition zone of 12 mm, whereas A₁₁ and C₂₂ recorded minimum inhibition zone of 6 mm (Table 12 and Plate 8).

4.2.3.2. Selection of efficient isolates

Based on the antagonistic activity against *R. solani* under *in vitro* condition, eight *Pseudomonas* isolates viz., A₁₁, A₂₂, C₂₂, P₂₁, K₂₁, K₄₁, S₁₂ and S₄₁ were selected and renamed as Pfa 1, Pfa 2, Pfc 3, Pfp 4, Pfk 5, Pfk 6, Pfs 7 and Pfs 8 and used for further studies.

4.3. CHARACTERIZATION OF COTTON RHIZOBACTERIAL ISOLATES

4.3.1. *Azospirillum* isolates

The isolates were characterized for the species level based on various biochemical tests and the results are presented in Table 13.

Among the 12 isolates, five isolates viz., Azo 1, Azo 2, Azo 3, Azo 7, Azo 9 produced acid from glucose and exhibited good growth with glucose, succinate, lactate, mannitol and α -ketoglutaric acid as carbon sources. These isolates had strong requirement of biotin for their growth. These isolates showed more nitrate and less nitrite reductase activity. Biotin requirement showed that, biotin was essential for the growth of acid producers whereas vice versa for the growth of non-acid producer.

The other seven isolates viz., Azo 4, Azo 5, Azo 6, Azo 8, Azo 10, Azo 11 and Azo 12 were poor or unable to produce acid from glucose. These isolates did not grow well on glucose, mannitol and α -ketoglutaric acid as carbon sources. These isolates showed poor nitrate and nitrite reductase activity.

Table 12. Antagonistic activity of *Pseudomonas* isolates against *R. solani*

Isolates	Antagonistic activity of <i>Pseudomonas</i>	
	Growth of <i>R. solani</i> (mm)	Inhibition zone (mm)
A ₁₁	66	6
A ₁₂	90	-
A ₂₁	90	-
A ₂₂	50	15
A ₃₁	90	-
C ₁₁	90	-
C ₁₂	90	-
C ₂₁	90	-
C ₂₂	65	6
C ₂₃	90	-
C ₃₁	90	-
C ₃₂	90	-
P ₁₁	90	-
P ₁₂	90	-
P ₂₁	56	10
P ₃₁	90	-
K ₁₁	90	-
K ₂₁	58	8
K ₃₁	90	-
K ₄₁	52	12
S ₁₁	90	-
S ₁₂	60	8
S ₂₁	90	-
S ₄₁	60	8
Pf1 *	52	12
Control	90	00

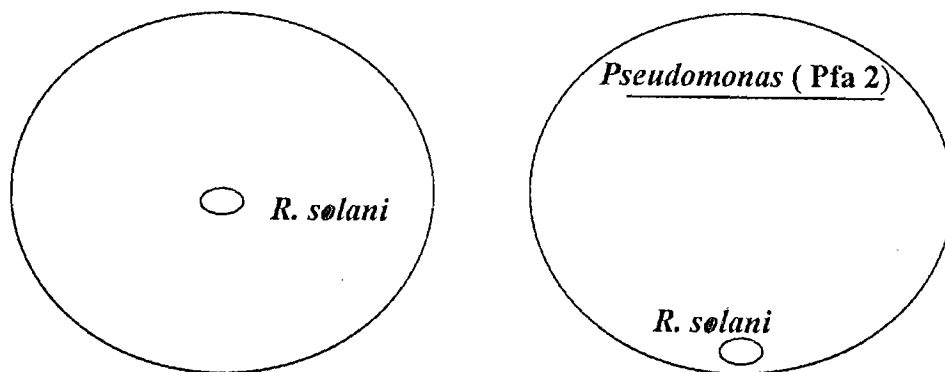


Plate 8. Antagonistic activity of *Pseudomonas* isolates against *R.solani*

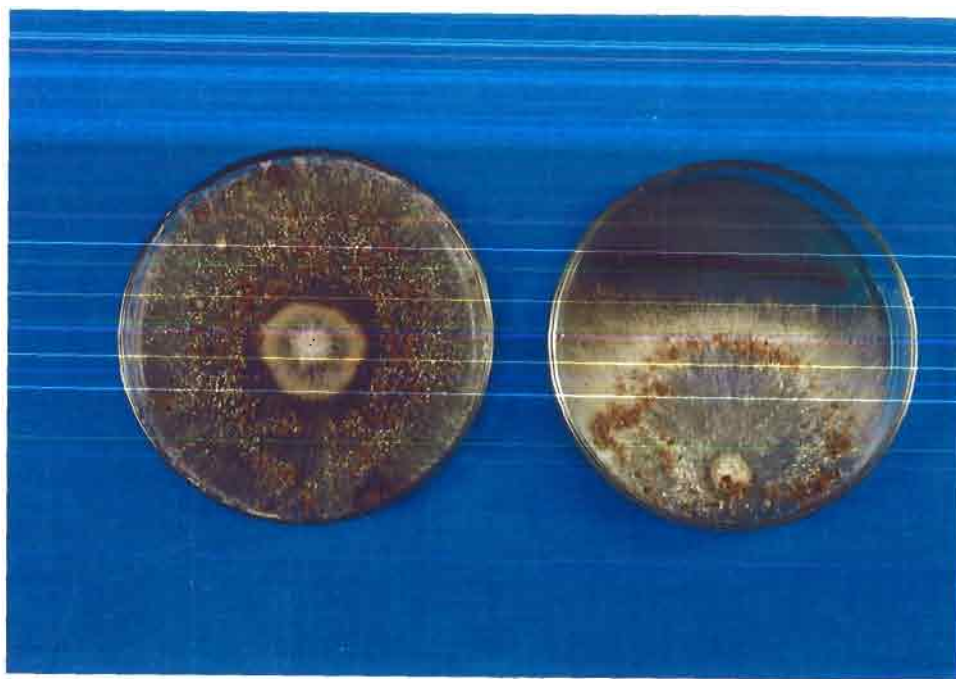


Plate 8. Antagonistic activity of *Pseudomonas* isolates against *R. solani*

Table 13. Biochemical characterization of *Azospirillum* isolates

Isolates	Growth in glucose peptone broth	Acid production	Utilization of carbon source						Biotin requirements	Nitrate reductase activity	Nitrite reductase activity	Tentative identification
			Glucose	Pyruvate	Succinate	Lactose	Mannitol	α -keto glutarate				
Azo 1	++	+++	+++	+	++	++	+++	+++	++	+++	+	<i>A. lipoferum</i>
Azo 2	+++	+++	+++	+	+++	+++	++	++	++	++	-	<i>A. lipoferum</i>
Azo 3	+++	+++	+++	+	+++	+++	+++	++	+++	+++	++	<i>A. lipoferum</i>
Azo 4	+	-	+	++	+++	+++	++	+++	+	+	+	<i>A. brasilense</i>
Azo 5	-	-	+	+++	+++	+++	++	+++	-	++	+++	<i>A. brasilense</i>
Azo 6	+	-	+	+++	+++	+++	++	+++	-	+	++	<i>A. brasilense</i>
Azo 7	+++	+++	+++	+	++	++	+++	++	+++	+++	+	<i>A. lipoferum</i>
Azo 8	+	+	+	+++	+++	+++	++	++	+	++	++	<i>A. brasilense</i>
Azo 9	+++	+++	+++	+	++	++	+++	+++	+++	++	+	<i>A. lipoferum</i>
Azo 10	-	-	+	++	+++	+++	+	+	+	+	+	<i>A. brasilense</i>
Azo 11	+	-	+	++	+++	++	+	+	+	+++	+	<i>A. brasilense</i>
Azo 12	+	+	+	+++	+++	++	++	++	-	+	-	<i>A. brasilense</i>
Az204*	+++	+++	+++	+	++	++	+	++	+++	+++	+	<i>A. lipoferum</i>
Sp7*	+	+	+	+++	+++	++	+	+++	-	+	+	<i>A. brasilense</i>

* Standard strain (-) Negative (+) Poor (++) Moderate (+++) Good

4.3.2. Phosphobacteria isolates

The results of morphological, cultural and biochemical characters exhibited by the 11 phosphobacterial isolates are presented in Table 14. The morphological characters varied widely among the isolates. About 54.5 per cent of isolates were short rods (6), 27.3 per cent were long rods (3), followed by 18.2 per cent of coccus (2). Seven isolates viz., PB 1, PB 2, PB 3, PB 6, PB 7, PB 9 and PB 10 were gram positive and spore staining observed the presence of spores. The four isolates viz., PB 4, PB 5, PB 8 and PB 11 were gram negative and there was no sporulation observed. Under microscopic observations, only two isolates viz., PB 2 and PB 7 were non motile and the remaining cultures showed motility.

Colony characters of these isolates on agar plates varied as circular, entire and irregular to lobate either flat or raised colonies with wrinkled (or) smooth surfaces. Colony sizes ranged from 0.1 to 0.6 cm size with different colours *i.e* white, dull white, creamy white, light pink and dull brown.

Among the 11 isolates, only PB 1 responded negative for gelatin hydrolysis and PB 4, PB 5, PB 8, PB 11 showed negative response for starch hydrolysis, acid and gas production. All the 11 isolates showed positive catalase activity. While PB 1, PB 3 and PB 9 isolates responded positively for Voges-Proskauer test. The H₂S production was observed in the isolates viz., PB 2, PB 3, PB 6, PB 7 and PB 10. All isolates were unable to grow at 45°C and only PB 4, PB 5, PB 8 and PB 11 were able to grow at 4°C.

4.3.3. *Pseudomonas* isolates

Morphological, colony growth characters and biochemical characters exhibited by the isolates from Cotton rhizosphere are presented in Table 15. The isolates were compared with the authenticated, *Pseudomonas fluorescens* (Pf 1) strain obtained from the culture collection centre of Department of Plant Pathology, TNAU, Coimbatore.

Microscopic examination of the isolates, showed that the isolates are short rods like standard strain *Pseudomonas fluorescens* (Pf1). These isolates are capable of growing at 4°C and showed active motility.

Table 14. Characterization of phosphobacterial isolates

Characters	PB 1	PB 2	PB 3	PB 4	PB 5	PB 6	PB 7	PB 8	PB 9	PB 10	PB 11	<i>Bacillus megaterium</i> *
Morphological characters												
Shape	Long rod	Short rod	Coccus	Long rod	Short rod	Short rod	Short rod	Long rod	Short rod	Coccus	Short rod	Short rod
Gram staining	Positive	Positive	Positive	Negative	Negative	Positive	Positive	Negative	Negative	Positive	Negative	Positive
Spore staining	Positive	Positive	Positive	Negative	Negative	Positive	Positive	Negative	Positive	Positive	Negative	Positive
Motility	Motile	Non motile	Motile	Motile	Motile	Motile	non Motile	Motile	Motile	Motile	Motile	Motile
Colony characters												
Form of the colony	Irregular	Irregular	Circular	Circular	Circular	Circular	Irregular	Circular	Circular	Irregular	Circular	Circular
Margin	Lobate	Lobate	Entire	Entire	Entire	Entire	Lobate	Entire	Entire	Lobate	Entire	Entire
Elevation	Flat	Flat	Raised	Raised	Raised	Flat	Raised	Raised	Raised	Flat	Raised	Raised
Surface	Wrinkled	Wrinkled	Smooth and glistening	Smooth	Smooth	Wrinkled	Smooth	Smooth	Smooth and glistening	Wrinkles	Smooth	Smooth and glistening
Size	Small (0.2)	Large (0.6)	Small (0.1)	Small (0.2)	Small (0.2)	Small (0.2)	Small (0.3)	Small (0.2)	Small (0.3)	Small (0.2)	Small (0.2)	Small (0.3)
Colour	White	Light pink	Dull white	Dull brown	Dull brown	Creamy white	White	Dull brown	Cream white	Dull white	Dull brown	Dull white
Physiological tests												
Gelatin hydrolysis	Negative	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Starch hydrolysis	Positive	Positive	Positive	Negative	Negative	Positive	Positive	Negative	Positive	Positive	Negative	Positive
Acid and gas production	Positive	Positive	Positive	Negative	Negative	Positive	Positive	Negative	Positive	Positive	Negative	Positive
Catalase activity	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Voges-Proskauer	Positive	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Positive
H ₂ S production	Negative	Positive	Positive	Negative	Negative	Positive	Positive	Negative	Negative	Positive	Negative	Negative
Growth at 45°C	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Growth at 4°C	Negative	Negative	Negative	Positive	Positive	Negative	Negative	Positive	Negative	Negative	Positive	Negative
Tentative identification	<i>Bacillus</i> sp.	<i>Bacillus</i> sp.	<i>Bacillus</i> sp.	<i>Pseudomonas</i> sp.	<i>Pseudomonas</i> sp.	<i>Bacillus</i> sp.	<i>Bacillus</i> sp.	<i>Pseudomonas</i> sp.	<i>Bacillus</i> sp.	<i>Bacillus</i> sp.	<i>Pseudomonas</i> sp.	--

* Standard strain

Table 15. Characterization of *Pseudomonas* isolates

Isolates	Fluorescent pigment production	Gram stain reaction	Growth at 4°C	Levan formation	Motility	Catalase activity	Starch hydrolysis	Gelatin liquefaction	Nitrate reduction	Acid production	Gas production	Litmus milk reaction	Tentative identification
Pfa 1	+	G -ve rod	+	+	+	+	-	+	+	-	-	Alkaline	<i>P. fluorescens</i>
Pfa 2	+	G -ve rod	+	+	+	+	-	+	+	-	-	Alkaline	<i>P. fluorescens</i>
Pfc 3	+	G -ve rod	+	+	+	+	-	+	+	-	-	Alkaline	<i>P. fluorescens</i>
Pfp 4	+	G -ve rod	+	+	+	+	-	+	+	-	-	Alkaline	<i>P. fluorescens</i>
Pfk 5	+	G -ve rod	+	+	+	+	-	+	+	-	-	Alkaline	<i>P. fluorescens</i>
Pfk 6	+	G -ve rod	+	+	+	+	-	+	+	-	-	Alkaline	<i>P. fluorescens</i>
Pfs 7	+	G -ve rod	+	+	+	+	-	+	+	-	-	Alkaline	<i>P. fluorescens</i>
Pfs 8	+	G -ve rod	+	+	+	+	-	+	+	-	-	Alkaline	<i>P. fluorescens</i>
Pfl *	+	G -ve rod	+	+	+	+	-	+	+	-	-	Alkaline	<i>P. fluorescens</i>

*Standard strain



On agar plates circular, raised and non-wrinkled colonies with entire margin with fluorescent green pigments was observed at the initial stages of growth. After 4-5 days of incubation brown colour pigmentation was observed. In nutrient agar medium supplemented with 5 per cent sucrose, levan formation was observed by convex, mucoid, white colonies after three to five days of incubation.

The isolates from Cotton rhizosphere showed similarity with the standard strain Pfl, both in morphological and in biochemical characters.

4.4. GROWTH HORMONE PRODUCTION BY THE COTTON RHIZOBACTERIAL ISOLATES

The growth hormones like, Indole acetic acid (IAA) and gibberellic acid (GA) production was observed in all the rhizobacterial isolates and the results are presented in Table 16.

4.4.1. Indole acetic acid production

In general, IAA produced by the rhizobacterial isolates in presence and absence of precursor tryptophan varied significantly as shown in Fig 9. Among the *Azospirillum* isolates, Azo 11 recorded maximum amount of IAA ($93.2 \mu\text{g } 100 \text{ ml}^{-1}$) followed by Azo 4 ($78.6 \mu\text{g } 100 \text{ ml}^{-1}$) in the presence of tryptophan and in the absence of tryptophan, 23.0 and $18.7 \mu\text{g } 100 \text{ ml}^{-1}$ were recorded respectively. The IAA production by the standard strains viz., Az204 and Sp7 was 58.6 and $60.1 \mu\text{g } 100 \text{ ml}^{-1}$ in the presence of tryptophan whereas in the absence of tryptophan, it was 18.6 and $20.30 \mu\text{g } 100 \text{ ml}^{-1}$ respectively.

Among the 11 phosphobacterial isolates, the PB 9 isolate recorded higher amount of IAA ($37.8 \mu\text{g } 100 \text{ ml}^{-1}$) in the presence of tryptophan and in the absence of tryptophan, $10.3 \mu\text{g } 100 \text{ ml}^{-1}$ of IAA which is followed by PB 6 and PB 11 isolates. The standard strain *B. megaterium* produced 31.2 and $9.6 \mu\text{g } 100 \text{ ml}^{-1}$ of IAA in the presence and in the absence of precursor tryptophan.

Among the eight *Pseudomonas* isolates, Pfa 2 recorded 87.3 and $25.0 \mu\text{g } 100 \text{ ml}^{-1}$ of IAA in the presence and in the absence of precursor, which is, followed by Pfk 6 and Pfp 4 respectively. The standard strain produced 70.2 and $19.6 \mu\text{g } 100 \text{ ml}^{-1}$ of IAA in the presence and absence of tryptophan respectively.

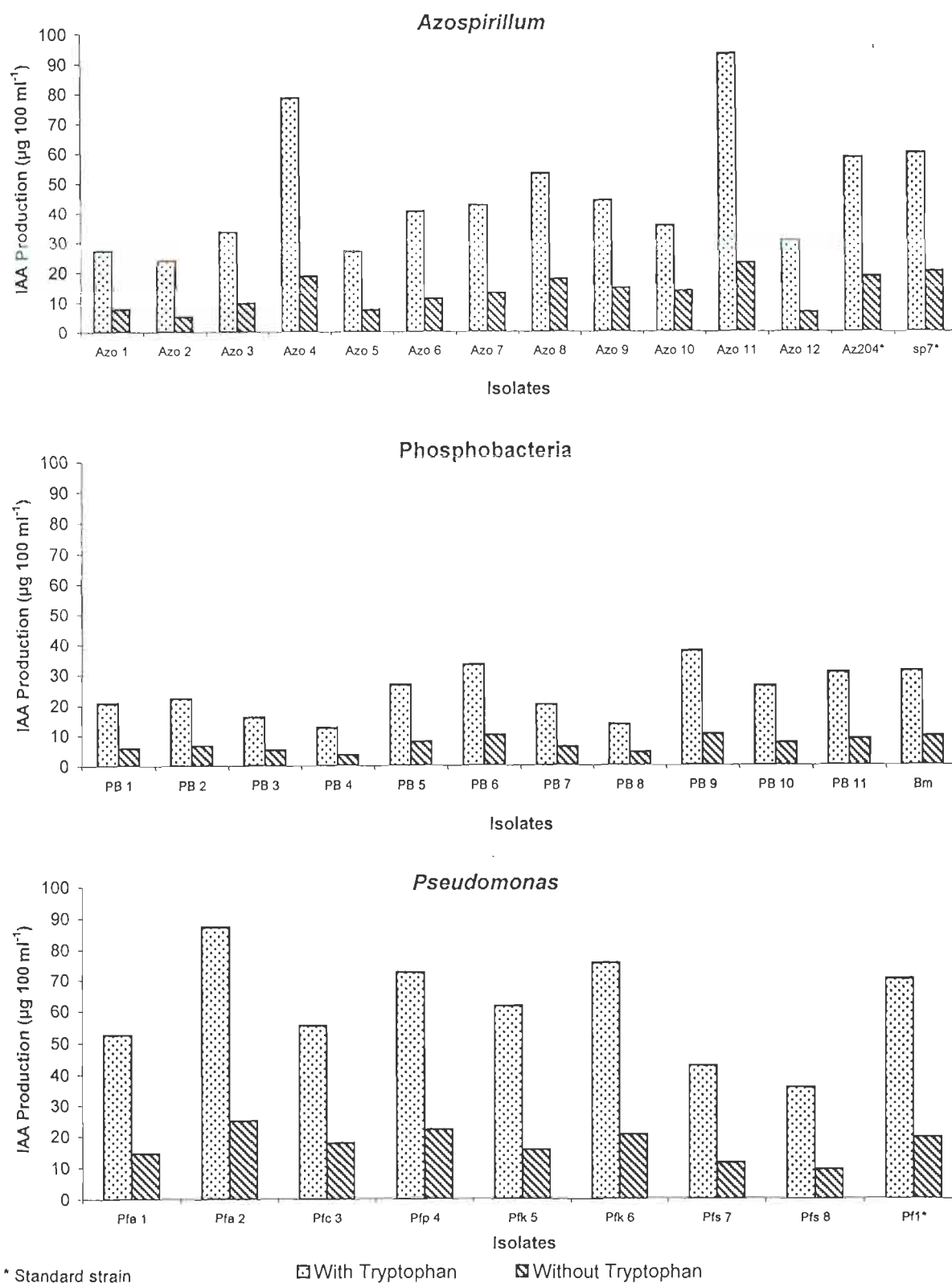
Table 16. Indole acetic acid (IAA) and gibberellic acid (GA) production by Cotton rhizobacterial isolates

Isolates	IAA ($\mu\text{g } 100 \text{ ml}^{-1}$)		GA ($\mu\text{g ml}^{-1}$)
	With tryptophan	Without tryptophan	
<i>Azospirillum</i>			
Azo 1	27.2	7.8	4.87
Azo 2	23.8	5.3	4.38
Azo 3	3.5	9.7	4.10
Azo 4	78.6	18.7	5.12
Azo 5	27.0	7.5	4.64
Azo 6	40.5	11.2	4.13
Azo 7	42.6	13.1	4.10
Azo 8	53.2	17.7	5.40
Azo 9	44.1	14.7	4.26
Azo 10	35.6	13.8	3.33
Azo 11	93.2	23.0	6.41
Azo 12	30.5	6.7	3.85
Az204*	58.6	18.6	4.75
Sp7*	60.1	20.3	4.92
<i>Phosphobacteria</i>			
PB 1	20.8	5.8	2.66
PB 2	22.4	6.6	1.25
PB 3	16.1	5.2	2.40
PB 4	12.6	3.5	3.32
PB 5	26.8	7.9	2.61
PB 6	33.3	10.1	3.65
PB 7	20.4	6.1	2.10
PB 8	13.6	4.3	1.65
PB 9	37.8	10.3	4.30
PB 10	26.4	7.4	3.25
PB 11	30.7	8.8	3.57
<i>B.megaterium</i> *	31.2	9.6	3.46
<i>Pseudomonas</i>			
Pfa 1	52.6	14.6	4.15
Pfa 2	87.3	25.0	6.64
Pfc 3	55.4	17.8	4.05
Pfp 4	72.7	22.2	4.51
Pfk 5	61.6	15.7	3.43
Pfk 6	75.6	20.6	5.92
Pfs 7	42.8	11.5	3.87
Pfs 8	35.7	9.39	4.25
Pfl*	70.2	19.6	5.66

* Standard Strain

IAA	CD (P=0.05)	<i>Azospirillum</i>	<i>Phosphobacteria</i>	<i>Pseudomonas</i>
Isolates		0.676	0.919	2.131
Tryptophan		1.788	2.251	4.521
Isolates x Tryptophan		2.529	3.184	6.394
GA	CD (P=0.05)	0.696	0.694	0.849

Fig. 9. Indole acetic acid (IAA) production by Cotton rhizobacterial isolates



In general, in all the three different genera of rhizobacteria viz., *Azospirillum*, phosphobacteria and *Pseudomonas* the presence of tryptophan triggers the IAA production. In the absence of tryptophan also, *Azospirillum* (Azo 11), *Pseudomonas* (Pfa 2) and Phosphobacteria (PB 9) recorded maximum amount of IAA, when compared to other rhizobacterial isolates. The *Azospirillum* isolate (Azo 11) recorded maximum amount followed by *Pseudomonas* (Pfa 2) and phosphobacteria (PB 9).

4.4.2. Gibberellic acid production

The GA production by the rhizobacterial isolates is shown in Fig 10. The GA production in *Azospirillum* isolates ranged from 3.33 to 6.41 $\mu\text{g ml}^{-1}$ respectively. The isolate Azo 11 produced significantly higher amount of 6.41 $\mu\text{g ml}^{-1}$ followed by Azo 8 which recorded 5.40 $\mu\text{g ml}^{-1}$. The isolate Azo 10 recorded the lowest amount of GA (3.33 $\mu\text{g ml}^{-1}$). While the standard strain Az204 and Sp7 recorded GA of 4.75 and 4.92 $\mu\text{g ml}^{-1}$ respectively.

The GA production by phosphobacterial isolates varied from 1.25 to 4.30 $\mu\text{g ml}^{-1}$. The maximum GA production was recorded by PB 9 (4.30 $\mu\text{g ml}^{-1}$) followed by PB 6 (3.65 $\mu\text{g ml}^{-1}$) and PB 11 (3.57 $\mu\text{g ml}^{-1}$). The standard strain *B. megaterium* recorded 3.46 $\mu\text{g ml}^{-1}$, whereas the minimum level of GA was produced by PB 2 (1.25 $\mu\text{g ml}^{-1}$).

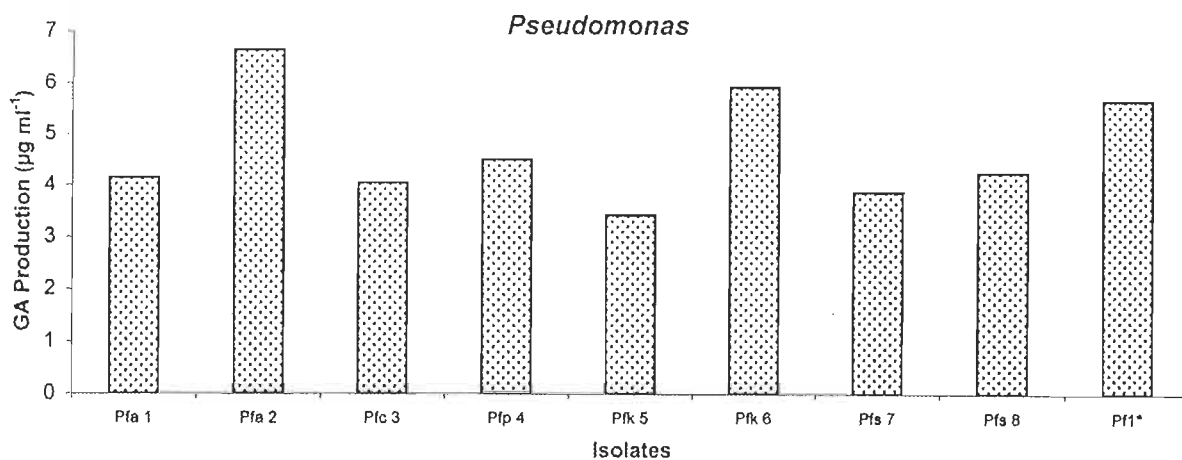
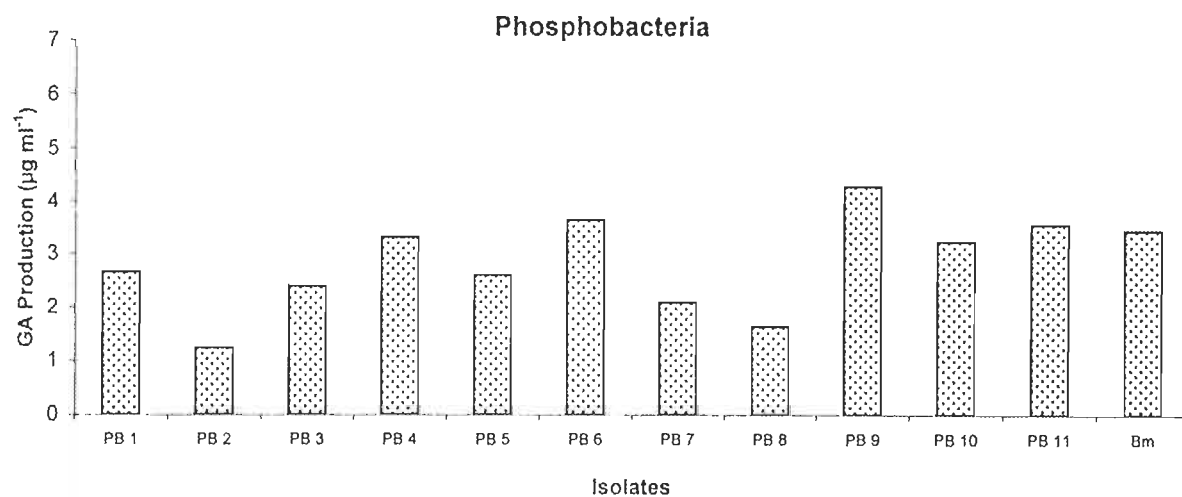
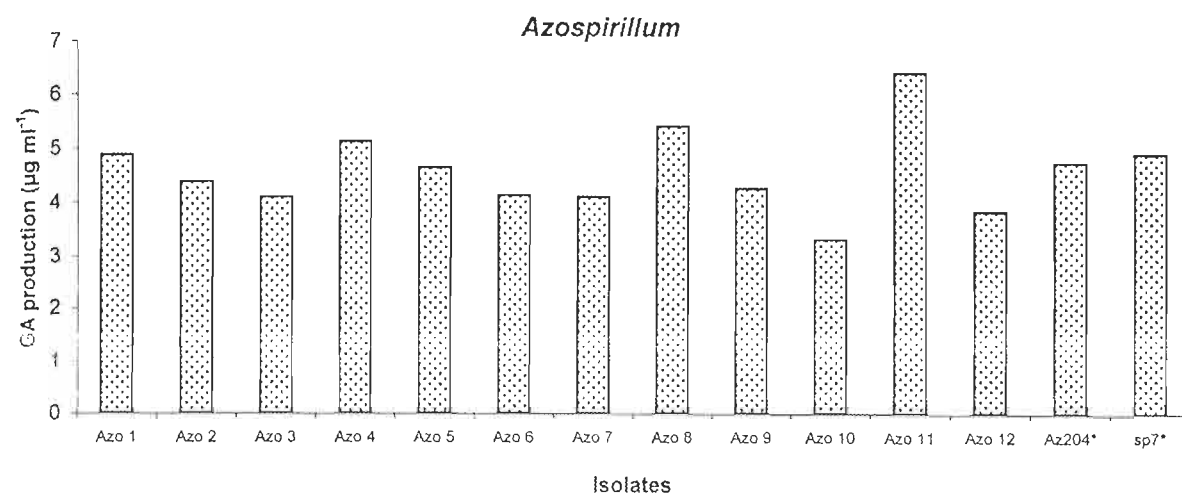
The GA production in *Pseudomonas* isolates varied from 3.43 to 6.64 $\mu\text{g ml}^{-1}$. The higher GA production was recorded in Pfa 2 (6.64 $\mu\text{g ml}^{-1}$) followed by Pfk 6 (5.92 $\mu\text{g ml}^{-1}$) and Pfp 4 (4.51 $\mu\text{g ml}^{-1}$). The standard strain, Pfl recorded GA production of 5.66 $\mu\text{g ml}^{-1}$.

In general, among the three genera higher GA production was recorded by *Pseudomonas* isolate (Pfa 2) followed by *Azospirillum* (Azo 11) and phosphobacterial isolate (PB 9).

4.5. SIDEROPHORE PRODUCTION BY COTTON RHIZOBACTERIAL ISOLATES

The development of wine red colour and the absence of orange colour due to the addition of 0.1 M FeCl_3 to the spent medium of isolates indicated the absence of hydroxamate type of siderophore. Absorbance at 560 nm indicates the absence of salicylate type siderophore production. All the strains produced only the phenolate type of siderophore, which was colorimetrically estimated by measuring the OD value at 700 nm using 2,3-DHBA as a standard, which is, presented in the Table 17 and Fig 11.

Fig. 10. Gibberellic acid (GA) production by Cotton rhizobacterial isolates



* Standard strain

Table 17. Siderophore production by Cotton rhizobacterial isolates

Isolates	Siderophore content (2,3-DHBA) (μ moles ml ⁻¹)
<i>Azospirillum</i>	
Azo 1	1.40
Azo 2	1.62
Azo 3	2.36
Azo 4	3.58
Azo 5	2.12
Azo 6	1.60
Azo 7	2.19
Azo 8	3.54
Azo 9	1.46
Azo 10	1.71
Azo 11	3.86
Azo 12	1.86
Az204*	3.62
Sp7*	3.56
<i>Phosphobacteria</i>	
PB 1	3.21
PB 2	3.73
PB 3	3.42
PB 4	3.32
PB 5	2.76
PB 6	4.81
PB 7	3.65
PB 8	3.00
PB 9	5.82
PB 10	3.86
PB 11	5.57
<i>B.megaterium</i> (Bm)*	5.11
<i>Pseudomonas</i>	
Pfa 1	3.56
Pfa 2	8.41
Pfc 3	3.10
Pfp 4	6.12
Pfk 5	4.25
Pfk 6	6.66
Pfs 7	3.71
Pfs 8	3.56
Pfl*	6.64

CD (P=0.05)

Azospirillum

0.785

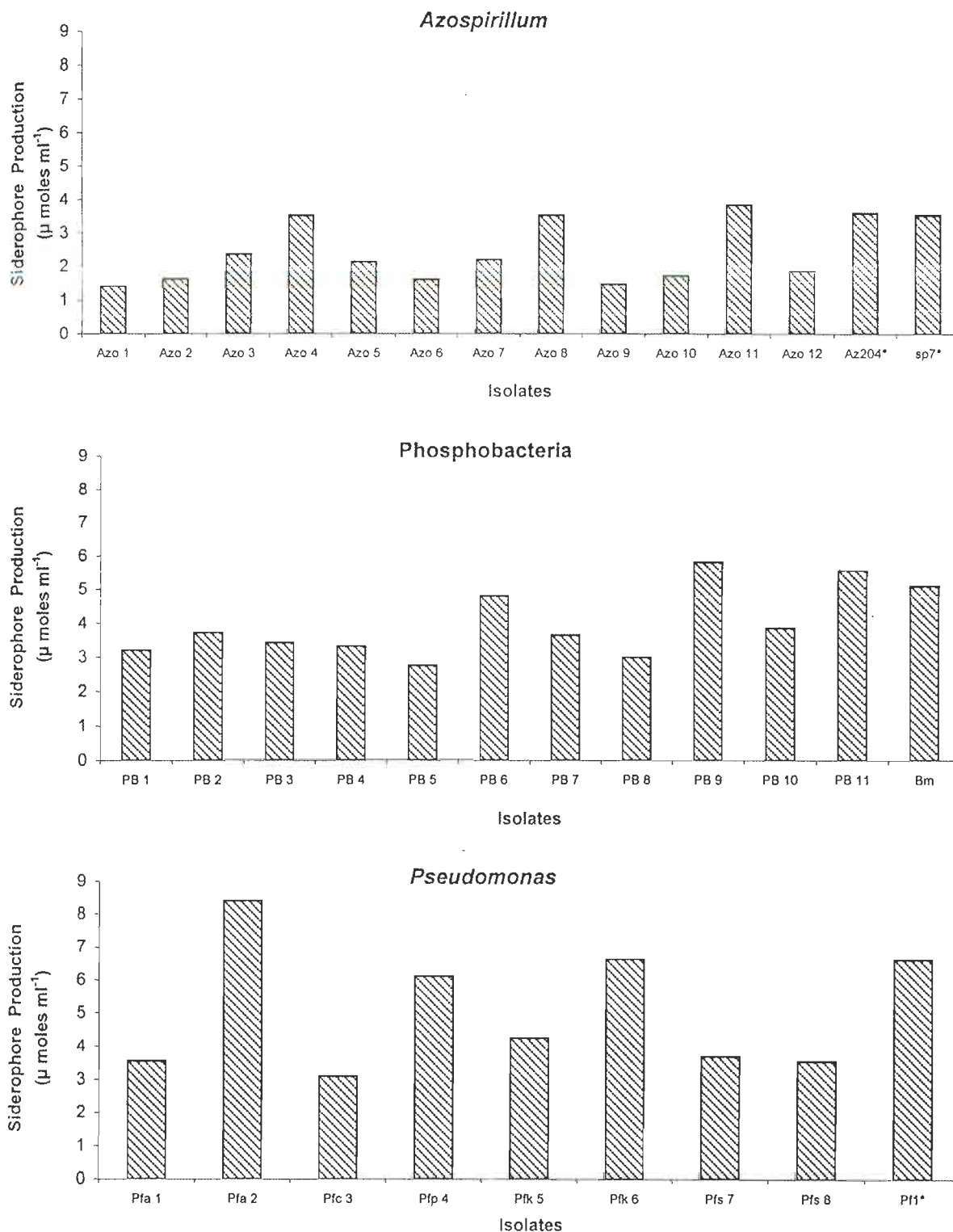
Phosphobacteria

0.642

Pseudomonas

0.832

Fig. 11. Siderophore (2,3-DHBA) production by Cotton rhizobacterial isolates



* Standard strain

Among the *Azospirillum* isolates the siderophore production ranged between 1.40 to 3.86 $\mu\text{moles ml}^{-1}$. The isolate Azo 11 produced 3.86 $\mu\text{ moles ml}^{-1}$ followed by Azo 4 (3.58 $\mu\text{ moles ml}^{-1}$) and Azo 8 (3.54 $\mu\text{ moles ml}^{-1}$). The standard strains, Az204 and Sp 7 produced 3.62 and 3.56 $\mu\text{ moles ml}^{-1}$ respectively. Whereas siderophore production was least in Azo 1 isolate which recorded 1.40 $\mu\text{ moles ml}^{-1}$.

Among the phosphobacterial isolates, the PB 9 isolate recorded maximum siderophore production of 5.82 $\mu\text{ moles ml}^{-1}$, whereas lowest siderophore production of 2.76 $\mu\text{ moles ml}^{-1}$ was recorded by PB 9 isolate. The isolates PB 11 recorded 5.57 $\mu\text{ moles ml}^{-1}$ of siderophore followed by PB 6 (4.81 $\mu\text{ moles ml}^{-1}$) whereas 5.11 $\mu\text{ moles ml}^{-1}$ was produced by the standard strain *B. megaterium* respectively.

The *Pseudomonas* isolate, Pfa 2 isolate recorded higher amount of 8.41 $\mu\text{moles ml}^{-1}$ siderophore followed by Pfk 6 (6.66 $\mu\text{ moles ml}^{-1}$) and Pfp 4 (6.12 $\mu\text{ moles ml}^{-1}$), while Pfc 3 recorded lower amount of 3.10 $\mu\text{ moles ml}^{-1}$ whereas the standard strain (Pfl) produced 6.64 $\mu\text{ moles ml}^{-1}$ of siderophore.

In general, among the different genera *Pseudomonas* (Pfa 2) produced significantly higher siderophore than *Azospirillum* (Azo 11) and phosphobacteria (PB 11).

4.6. Poly β -hydroxy butyrate (PHB) content in the *Azospirillum* isolates

The PHB content of the *Azospirillum* isolates grown in nutrient rich medium is presented in Table 18 and Fig 12. In general PHB accumulation increases with the age of the isolates. The PHB content was higher on 10 DAI when compared 5 DAI in all the isolates. Among the isolates, Azo 11 isolate showed maximum PHB content of 1.021 and 1.870 mg g^{-1} of dry wt of the cell followed by Azo 4 (0.802 and 0.911) and Azo 8 (0.751 and 0.893) respectively during 5 DAI and 10 DAI. In standard strain viz., Az204 and Sp7, the PHB content was 0.510 and 0.827 mg g^{-1} of dry wt of the cell and 0.664 and 0.891 mg g^{-1} of dry wt of the cell on 5 DAI and 10 DAI respectively. The PHB content was significantly higher in Azo 11 isolate than the standard strains.

Table 18 . Poly β -hydroxy butyrate (PHB) content in the *Azospirillum* isolates

Isolates	PHB content (mg g ⁻¹ dry weight of cells)	
	5 th DAI	10 th DAI
Azo 1	0.352	0.523
Azo 2	0.281	0.351
Azo 3	0.514	0.862
Azo 4	0.802	0.911
Azo 5	0.341	0.540
Azo 6	0.422	0.572
Azo 7	0.408	0.612
Azo 8	0.751	0.893
Azo 9	0.631	0.670
Azo 10	0.532	0.684
Azo 11	1.021	1.870
Azo 12	0.422	0.617
Az204*	0.510	0.827
Sp7*	0.664	0.891

*Standard strain

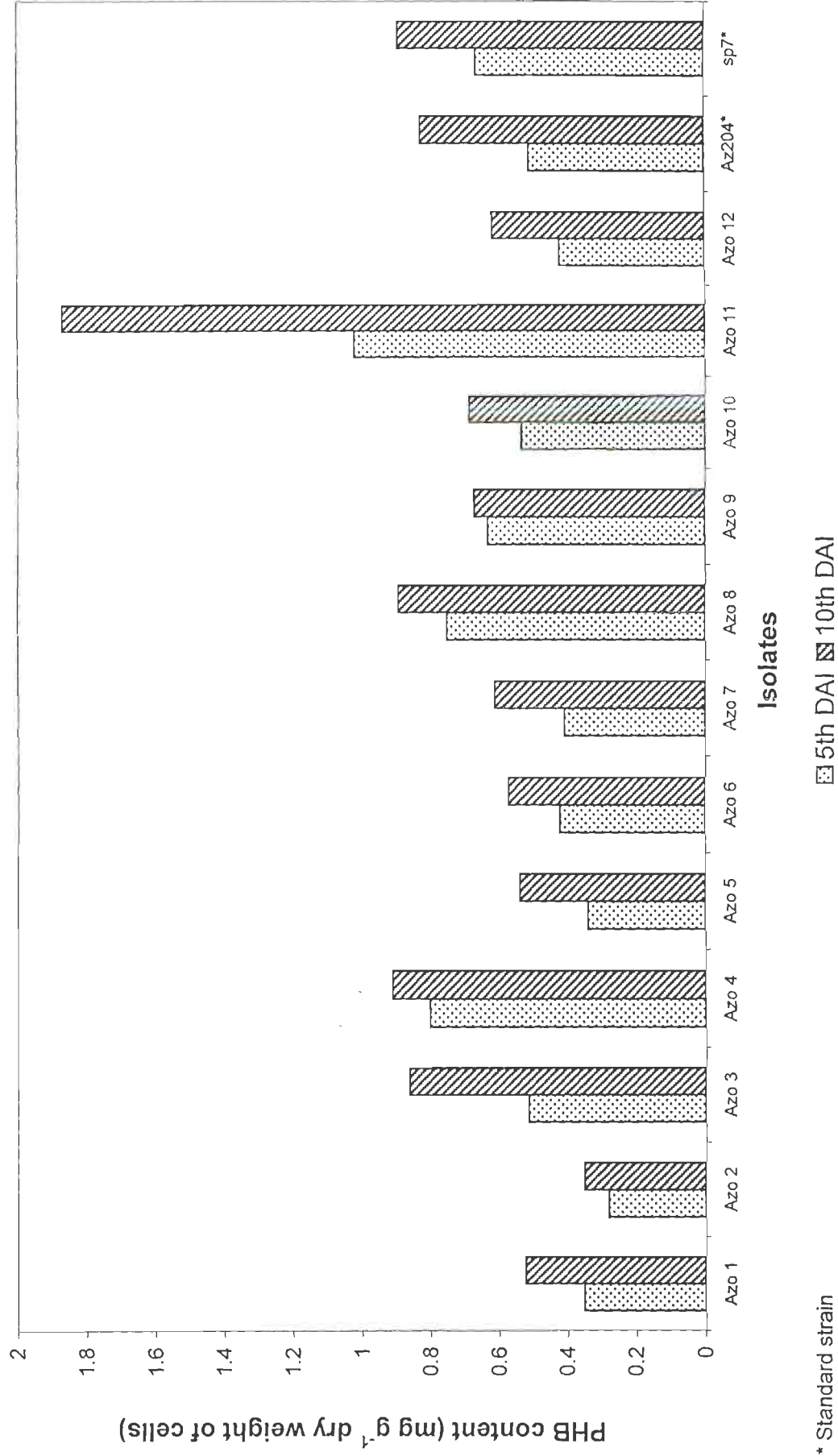
CD (p = 0.05)

Isolates 0.039

Days 0.105

Isolates x Days 0.148

Fig. 12. Poly β -hydroxy butyrate (PHB) content in the *Azospirillum* isolates



4.7. Hydrogen cyanide (HCN) production by *Pseudomonas* isolates

Productions of HCN by *Pseudomonas* isolates are presented in Table 19 and Plate 9. The amount of HCN production varied from 2.42 to 9.56 $\mu\text{g ml}^{-1}$. Among all the eight isolates, Pfa 2 isolate (9.56 $\mu\text{g ml}^{-1}$) recorded significantly higher HCN followed by Pfk 6 (7.23 $\mu\text{g ml}^{-1}$) as shown in Fig 13. The isolate Pfs 7 (2.42 $\mu\text{g ml}^{-1}$) recorded lowest HCN content whereas in the standard strain *Pseudomonas fluorescens* Pfl, recorded the HCN content of 7.15 $\mu\text{g ml}^{-1}$.

4.8. Intrinsic antibiotic resistance (IAR) by Cotton rhizobacterial isolates

The intrinsic antibiotic resistance (IAR) of Cotton rhizobacterial isolates to various antibiotics like streptomycin, chloramphenicol, ampicillin and kanamycin are shown in Table 20. All the isolates showed different level of resistance to various antibiotics.

IAR for streptomycin was tested upto 500 ppm for all the rhizobacterial isolates. Among the *Azospirillum* isolate, Azo 11 showed maximum tolerance of 250 ppm. Similarly in phosphobacteria, PB 9 isolate recorded 300 ppm and in *Pseudomonas* Pfa 2 isolate showed 300 ppm of resistance which is significantly higher than the standard strains for all the isolates.

The IAR of rhizobacterial isolates for chloramphenicol was tested upto 100 ppm level. Among the rhizobacterial isolates, Azo 11, PB 9 and Pfa 2 isolate of *Azospirillum*, phosphobacteria and *Pseudomonas* showed maximum resistance of 20, 15 and 75 ppm of chloramphenicol respectively which was higher than the standard strains.

IAR for ampicillin was tested upto 500 ppm. The antibiotic concentration of 150, 250 and 300 ppm of ampicillin was allowed the growth of Azo 11, PB 9 and Pfa 2 isolate of *Azospirillum*, phosphobacteria and *Pseudomonas*.

The IAR for kanamycin was tested upto 500 ppm. The kanamycin resistance of rhizobacterial isolates was 200 ppm for Azo 11 and PB 9 isolates of *Azospirillum* and phosphobacteria and 250 ppm for Pfa 2 isolate of *Pseudomonas* respectively.

In general the isolates showing higher level of resistance to more than one antibiotic can be used for the development of double antibiotic marker.

Table 19. Hydrogen cyanide (HCN) production by *Pseudomonas* isolates

Isolates	Hydrogen cyanide production ($\mu\text{g ml}^{-1}$)
Pfa 1	4.12
Pfa 2	9.56
Pfc 3	3.16
Pfp 4	6.65
Pfk 5	4.18
Pfk 6	7.23
Pfs 7	2.42
Pfs 8	5.65
Pfl *	7.15

* Standard strain

CD (p= 0.05)

0.719

Plate 9. Hydrogen cyanide production (HCN) by *Pseudomonas* (Pfa 2) isolates

A- *Pseudomonas* (Pfa 2)

B- Control

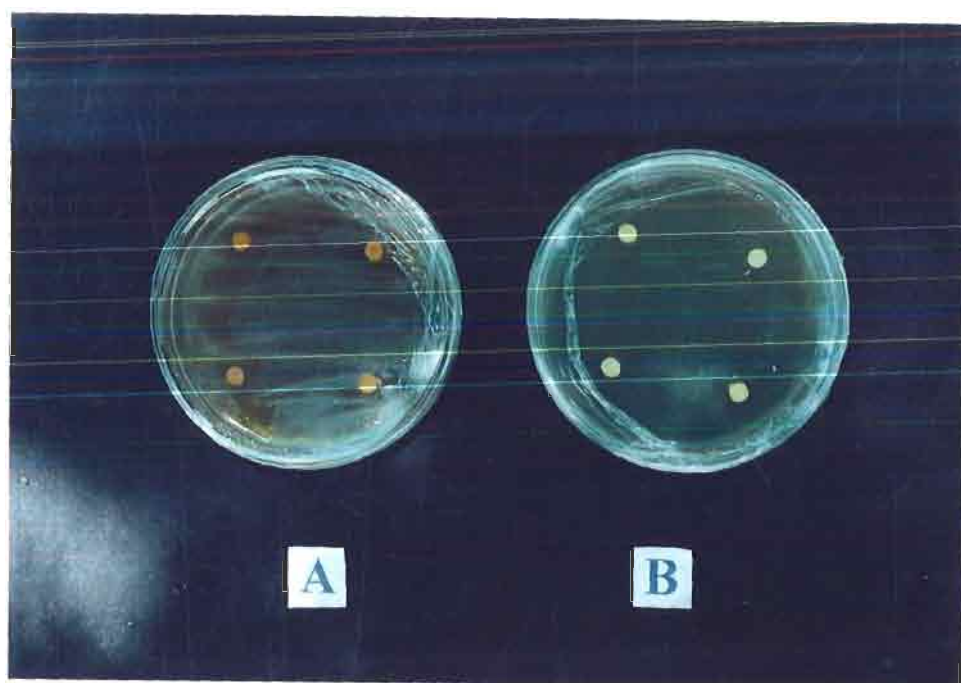
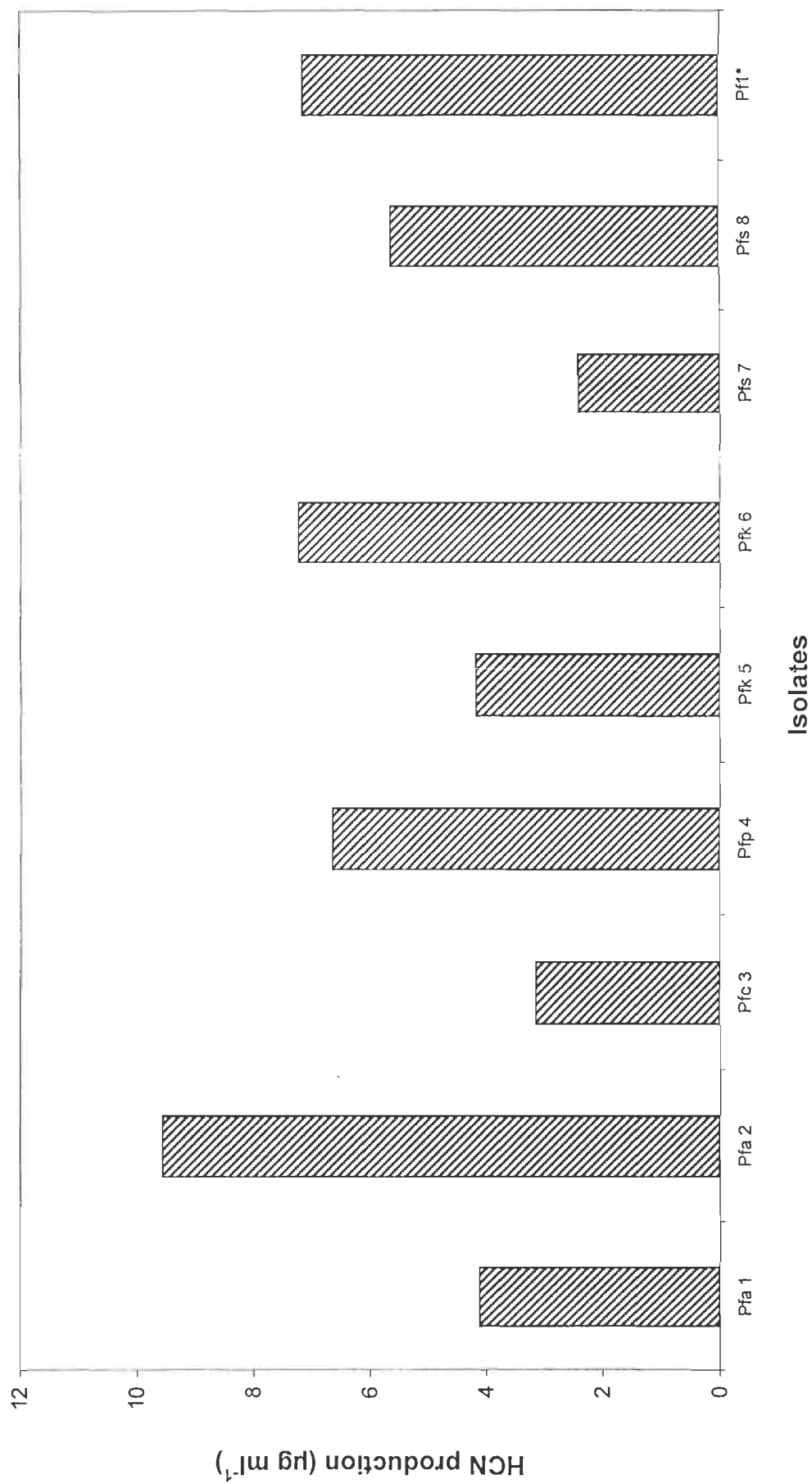


Plate 9. Hydrogen cyanide production (HCN) by *Pseudomonas* (Pfa 2) isolates

Fig. 13. Hydrogen cyanide (HCN) production by *Pseudomonas* isolates

* Standard strain

Table 20. Intrinsic antibiotic resistance of Cotton rhizobacterial isolates

Isolates	Antibiotic concentration** (ppm)			
	Streptomycin	Chloramphenicol	Ampicillin	Kanamycin
<i>Azospirillum</i>				
Azo 1	50	10	25	125
Azo 2	75	15	75	100
Azo 3	75	5	100	125
Azo 4	200	10	100	125
Azo 5	100	5	25	100
Azo 6	100	5	50	50
Azo 7	75	5	100	50
Azo 8	175	10	125	125
Azo 9	50	5	125	100
Azo 10	125	5	100	50
Azo 11	250	20	150	200
Azo 12	100	10	100	125
Az204*	150	15	150	125
Sp7*	100	10	100	150
<i>Phosphobacteria</i>				
PB 1	100	5	50	25
PB 2	150	5	150	50
PB 3	75	5	150	100
PB 4	50	10	150	125
PB 5	125	5	50	50
PB 6	200	10	200	150
PB 7	150	5	50	75
PB 8	200	5	100	100
PB 9	300	15	250	200
PB 10	50	5	50	25
PB 11	150	10	100	100
<i>B. megaterium</i> (Bm)*	250	10	150	200
<i>Pseudomonas</i>				
Pfa 1	150	50	150	50
Pfa 2	300	75	300	250
Pfc 3	100	50	100	100
Pfp 4	200	50	250	50
Pfk 5	150	50	150	100
Pfk 6	250	50	200	150
Pfs 7	150	50	250	200
Pfs 8	200	50	100	50
Pfl*	250	50	250	200

* Standard strain

** Maximum antibiotic concentration that allow the normal growth

4.9. SELECTION OF EFFICIENT RHIZOBACTERIAL ISOLATES

Based on the phytohormone production, siderophore production, PHB content and intrinsic antibiotic resistance (IAR) under *in vitro* conditions, three isolates of *Azospirillum* viz., Azo 11, Azo 4 and Azo 8 were selected. Among the phosphobacterial isolates based on the performance of phytohormone production, siderophore production and IAR, three isolates viz., PB 9, PB 6 and PB 11 were selected. Similarly three *Pseudomonas* isolates, viz., Pfa 2, Pfp 4 and Pfk 6 were selected based on HCN production in addition to the above said parameters.

These selected isolates were tested for their efficiency on the growth under different carbon sources, colonization and adsorption to Cotton seedlings.

4.10. GROWTH PERFORMANCE OF COTTON RHIZOBACTERIAL ISOLATES UNDER DIFFERENT CARBON SOURCES

4.10.1. *Azospirillum*

The growth of the *Azospirillum* isolates under different carbon sources like, malate, succinate, glucose and sucrose were presented in the Fig. 14. Among the isolates Azo 11 exhibited better growth followed by standard strain Sp7, Azo 4 and Azo 8 in malic acid. Similar trend was observed for other carbon sources preferentially in the order of malate, succinate, glucose and sucrose. All the cultures attained the stationary phase within 72-96 hrs of incubation.

4.10.2. *Phosphobacteria*

The growth performance of the phosphobacterial isolates under carbon sources like, glucose, xylose, sucrose and mannitol are shown in Fig.15. The isolates PB 9 showed maximum growth in all the carbon sources. Among isolates, PB 6 isolate recorded the lowest growth in all the carbon sources. In general, all the carbon sources supported the growth of the organisms in the order of glucose followed by xylose mannitol and sucrose. All the cultures attained stationary phase with in 48 hrs of incubation.

4.10.3. *Pseudomonas*

The growth performance of the *Pseudomonas* isolates under various carbon sources like glycerol, glucose, sucrose and mannitol were shown in Fig. 16. All the isolates utilized

Fig. 14. Growth performance of *Azospirillum* isolates under different carbon sources

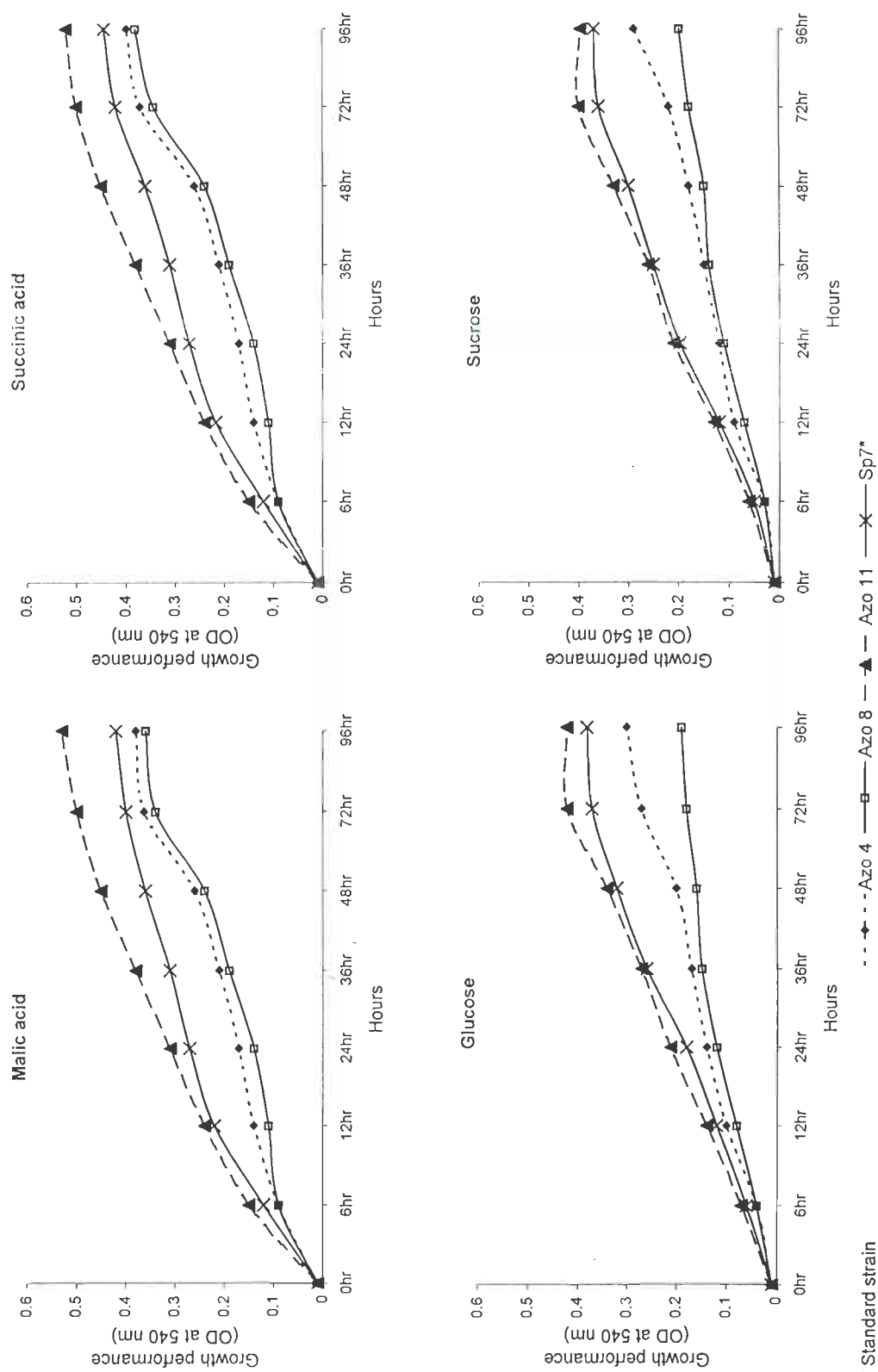


Fig. 15. Growth performance of phosphobacterial isolates under different carbon sources

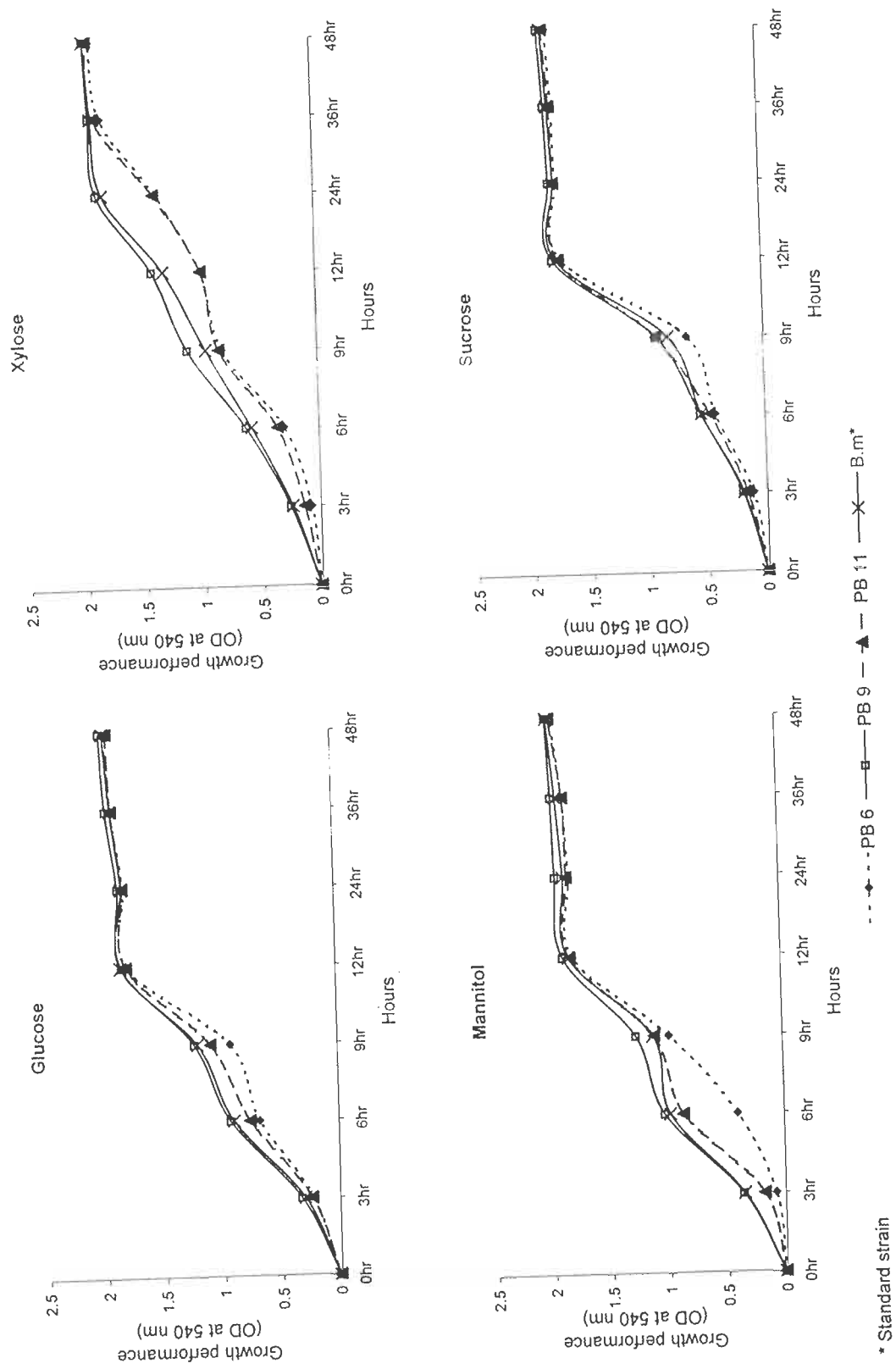
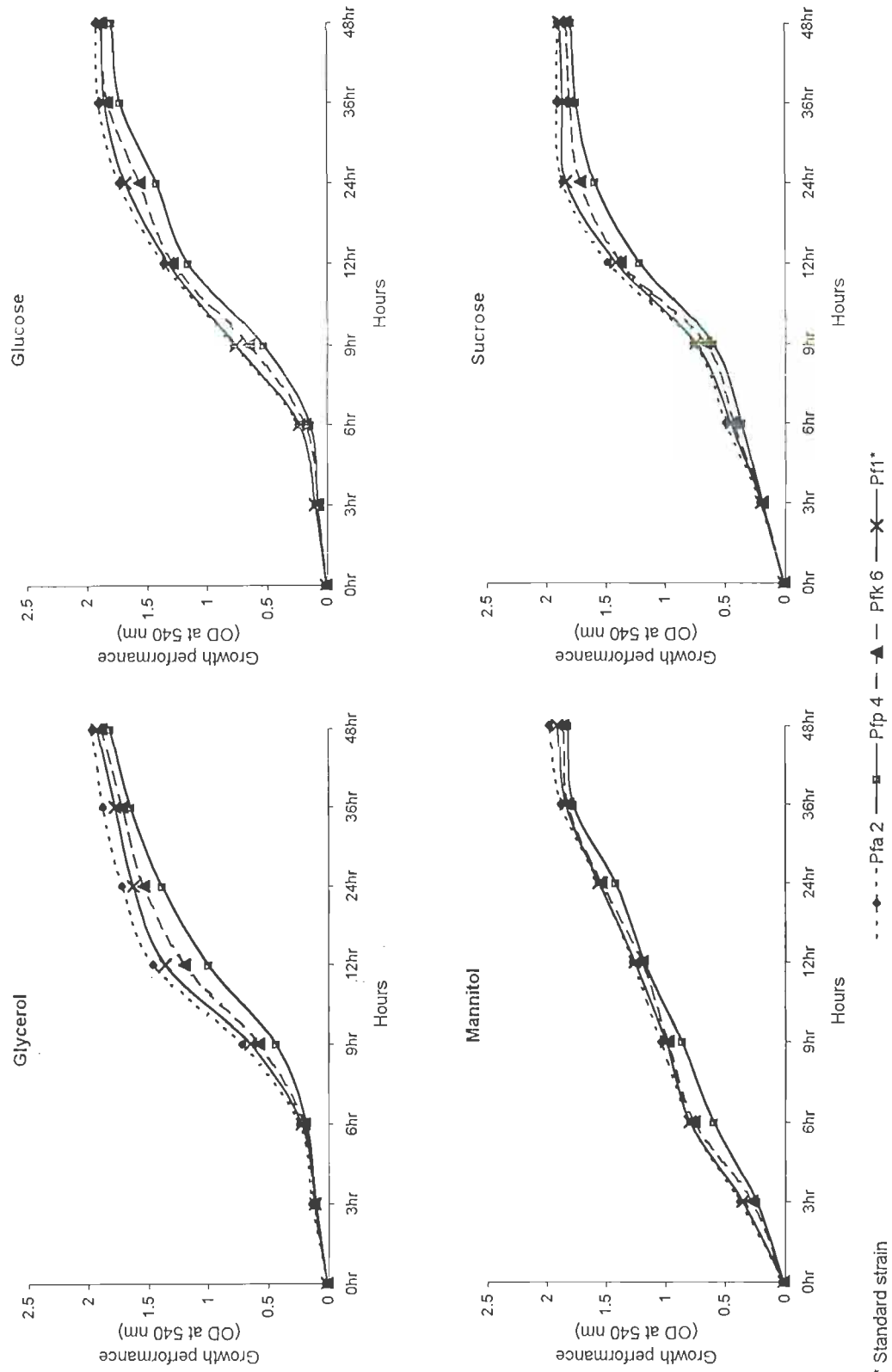


Fig. 16. Growth performance of *Pseudomonas* isolates under different carbon sources



glycerol followed by sucrose, glucose and mannitol. Among the isolates, Pfa 2 isolate recorded maximum growth and Pfp 4 isolate recorded the least growth in all carbon sources. Stationary phase was observed with in 48 hrs of incubation.

4.11. SOLUBILIZATION EFFICIENCY OF PHOSPHOBACTERIAL ISOLATES ON DIFFERENT TYPES OF ROCK PHOSPHATES

Efficiency of selected phosphobacterial isolates in releasing phosphorus from different sources of rock phosphates (RP) viz., Mussorie, Gafsa and Udaipur rock phosphate. The amount of 'P' released, titrable acidity, and reduction in pH of the culture filtrate due to bacterial growth were estimated.

4.11.1. Solubilization of different types of rock phosphate

The result is presented in Table 21 and Fig. 17. The phosphorus solubilization by PB 9 isolate was found to be significantly higher than other isolates in all the three types of rock phosphates viz., Mussorie, Gafsa and Udaipur rock phosphate. It solubilized a mean of 7.30 mg of 'P' 100 mg⁻¹ of different rock phosphates followed by mean of 6.95 mg of 100 mg⁻¹ in standard strain *B. megaterium*.

Among the different rock phosphate sources, solubilization was higher in Mussorie rock phosphate (5.74 mg) followed by Udaipur rock phosphate (5.23 mg) and Gafsa rock phosphate (4.58 mg). In general, the 'P' release was found to be higher on 30 DAI than 15 DAI. The solubilization of Mussorie rock phosphate was higher (5.03 & 6.44 mg) followed by Udaipur rock phosphate, (4.45 & 6.00 mg) and Gafsa rock phosphate (4.02 & 5.13 mg) on 15 and 30 DAI respectively.

Specific interactions between selected phosphobacterial isolates and source of rock phosphate was also noticed in 'P' solubilization. The PB 9 isolate solubilized mean of 7.60, 6.62 and 7.68 mg 100 mg⁻¹ of Mussorie, Gafsa and Udaipur rock phosphate respectively which was significantly higher than the solubilization of standard strain *B. megaterium* (7.29, 6.08 & 7.46 mg respectively).

4.11.2. Titrable acidity

Acid production in the culture media incorporated with different sources of rock phosphate due to growth of phosphobacteria is presented in Table 22.

Table 21. Solubilization of different types of rock phosphate by phosphobacterial isolates

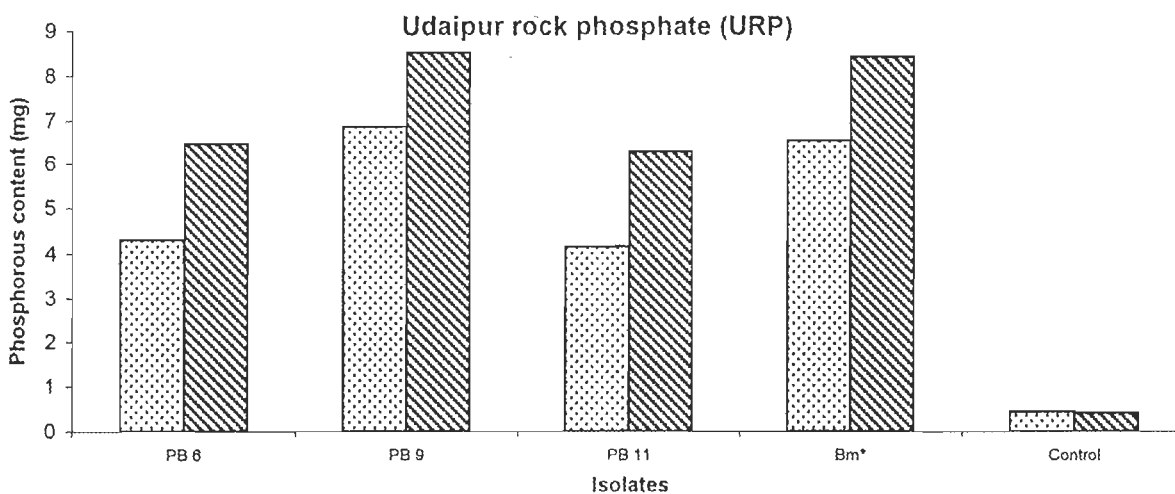
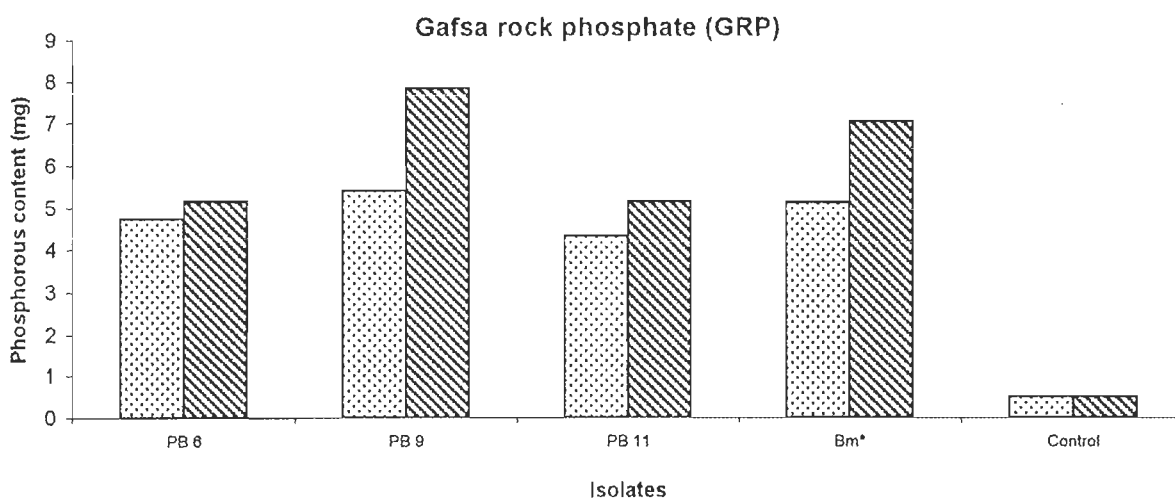
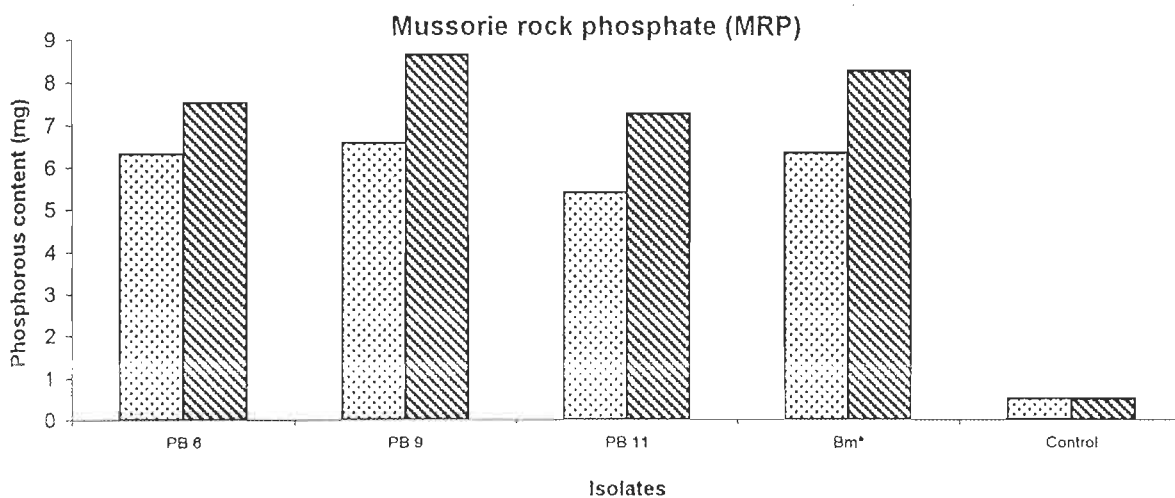
Isolates	Phosphorus solubilized from 100 mg of rock phosphate in Pikovskaya's broth (mg)										Mean
	Mussorie rock phosphate(MRP)			Gafsa rock phosphate (GRP)			Udaipur rock phosphate (URP)				
	15 DAI	30 DAI	Mean	15 DAI	30 DAI	Mean	15 DAI	30 DAI	Mean		
PB 6	6.30	7.52	6.91	4.73	5.15	4.94	4.30	6.45	5.38	5.74	
PB 9	6.57	8.63	7.60	5.41	7.82	6.62	6.85	8.50	7.68	7.30	
PB 11	5.41	7.25	6.33	4.33	5.15	4.74	4.15	6.26	5.20	5.43	
<i>Bacillus megaterium</i> *	6.33	8.27	7.29	5.12	7.03	6.08	6.51	8.41	7.46	6.94	
Uninoculated control	0.52	0.51	0.52	0.51	0.51	0.51	0.43	0.40	0.42	0.48	
Mean	5.03	6.44	5.74	4.02	5.13	4.58	4.45	6.00	5.23	5.18	

* Standard strain; DAI - Days after inoculation

CD (P=0.05)

Phosphate 1.214
Days 0.992
Treatment 1.568
Phosphate x Days 1.718
Days x Treatment 2.217
Phosphate x Treatment 2.715
Phosphate x Days x Treatment 3.840

Fig. 17. Solubilization of different types of rock phosphate by phosphobacterial isolates



* Standard strain

□ 15 DAI

▨ 30 DAI

Table 22. Effect of phosphobacterial inoculation on the titrable acidity of the culture supernatant incorporated with different types of rock phosphate

Isolates	Titrable acidity of the culture supernatant (ml of 0.01N NaOH consumed)										Mean
	Mussorie rock phosphate(MRP)			Gafsa rock phosphate (GRP)			Udaipur rock phosphate (URP)				
	15 DAI	30 DAI	Mean	15 DAI	30 DAI	Mean	15 DAI	30 DAI	Mean		
PB 6	8.5	9.7	9.10	7.4	9.2	8.30	8.7	9.3	9.00	8.80	
PB 9	9.6	10.2	9.90	8.6	10.5	9.55	9.6	10.1	9.85	9.77	
PB 11	8.2	8.6	8.40	7.1	8.3	7.65	8.2	8.5	8.35	8.13	
<i>Bacillus megaterium</i> *	9.4	10.0	9.70	8.8	10.1	9.45	9.5	10.0	9.75	9.63	
Uninoculated control	1.2	1.2	1.20	0.9	0.9	0.90	0.8	0.8	0.80	0.93	
Mean	7.38	7.94	7.66	6.54	7.8	7.17	7.36	7.74	7.55	7.45	

* Standard strain; DAI - Days after inoculation

CD (P=0.05)

Phosphate
Days
Treatment
Phosphate x Days
Days x Treatments
Phosphate x Treatments
Phosphate x Days x Treatments

1.276
1.041
1.647
1.804
2.329
2.852
4.034

The titrable acidity of media inoculated with phosphobacteria was significantly higher over uninoculated control. The maximum titrable acidity was observed due to inoculation of PB 9 isolate which consumed mean of 9.77 ml of 0.01N NaOH. In general, the acid production was higher on 30 DAI when compared to 15 DAI. Among the isolates, PB 9 recorded a titrable acidity of 9.9, 9.55 and 9.85 ml of 0.1 N NaOH followed by standard strain *B. megaterium* of 9.7, 9.45 and 9.75 ml of 0.01N NaOH in Mussorie, Gafsa and Udaipur rock phosphate respectively. There was no significant effect on the titrable acidity, due to the inoculation of different phosphobacterial isolates. The titrable acidity among the phosphobacterial isolates not varied significantly (Fig 18).

Among the rock phosphates, the titrable acidity was higher in the media incorporated with Mussorie rock phosphate (7.66 ml) followed by Udaipur rock phosphate (7.55 ml) and Gafsa rock phosphate (7.17 ml).

4.11.3. pH reduction

In growth medium, containing rockphosphate, the pH changes due to phosphabacterial growth and the results are presented in Table 23 and Fig 19.

Growth of phosphobacterial isolates reduced the pH of the medium significantly than uninoculated control. The higher pH reduction was noticed due to the inoculation of PB 9 isolate and the final pH was reduced to mean of 5.18 on 30 DAI in Mussorie rock phosphate, 5.2 in Gafsa rock phosphate and 5.0 in Udaipur rock phosphate. The phosphobacterial isolate PB 6 recorded the lowest reduction in pH. The reduction of pH was maximum in media incorporated with Mussorie rock phosphate (5.54) followed by Udaipur rock phosphate (5.68) and least pH reduction of 5.78 was observed in Gafsa rock phosphate. The pH reduction was higher on 30 DAI when compared to 15 DAI.

The PB 9 isolate, reduced the pH of media incorporated with Mussorie rock phosphate in the range of 5.05, followed by Udaipur rock phosphate (5.25) and Gafsa rock phosphate (5.30). Due to inoculation of standard strain *B. megaterium*, the pH was 5.10, 5.30 and 5.40 in Mussorie, Udaipur and Gafsa rock phosphates respectively.

Fig. 18. Effect of phosphobacterial inoculation on the titrable acidity of the culture supernatant incorporated with different types of rock phosphate

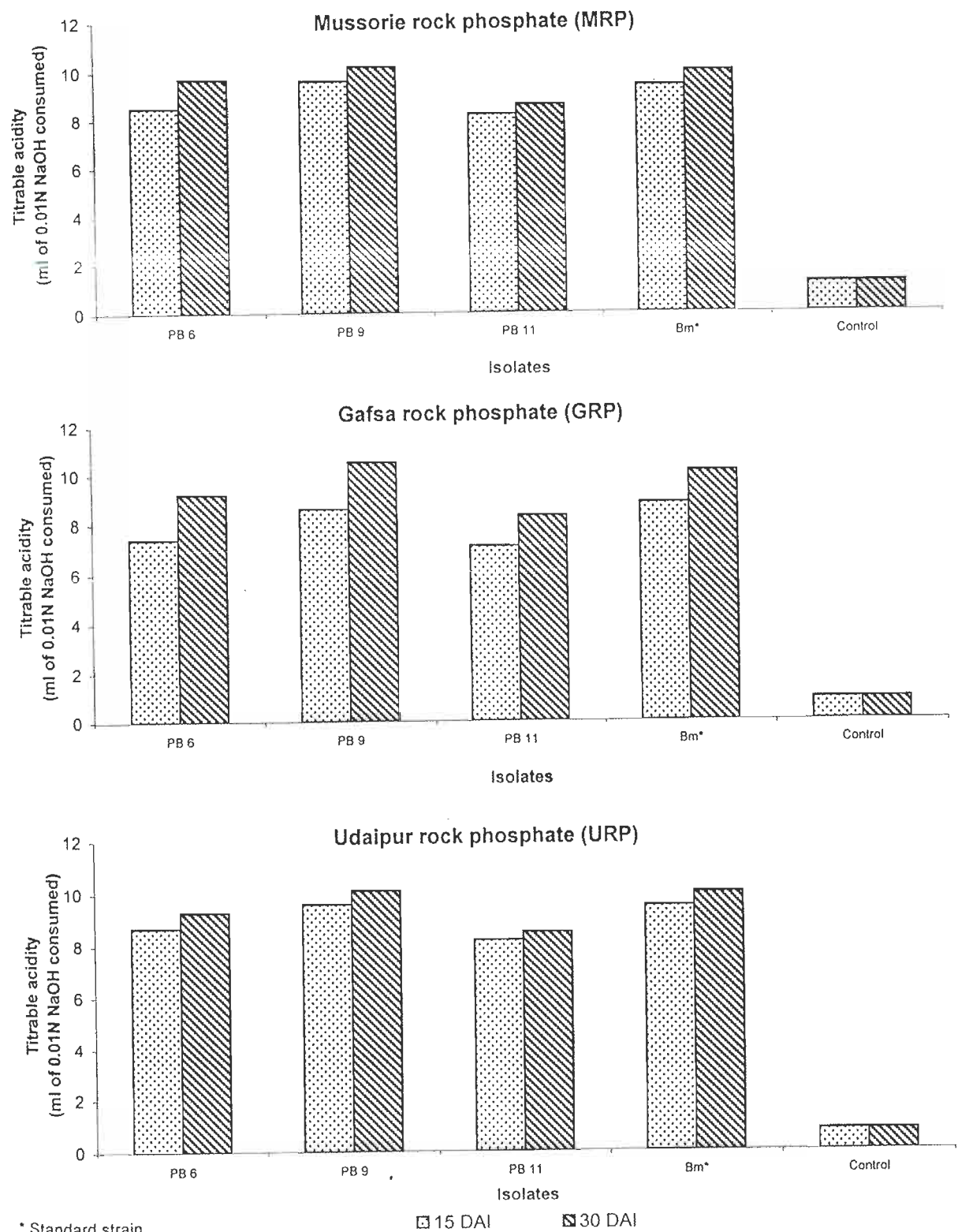


Table 23. Effect of phosphobacterial inoculation on the pH of the culture supernatant incorporated with different types of rock phosphate

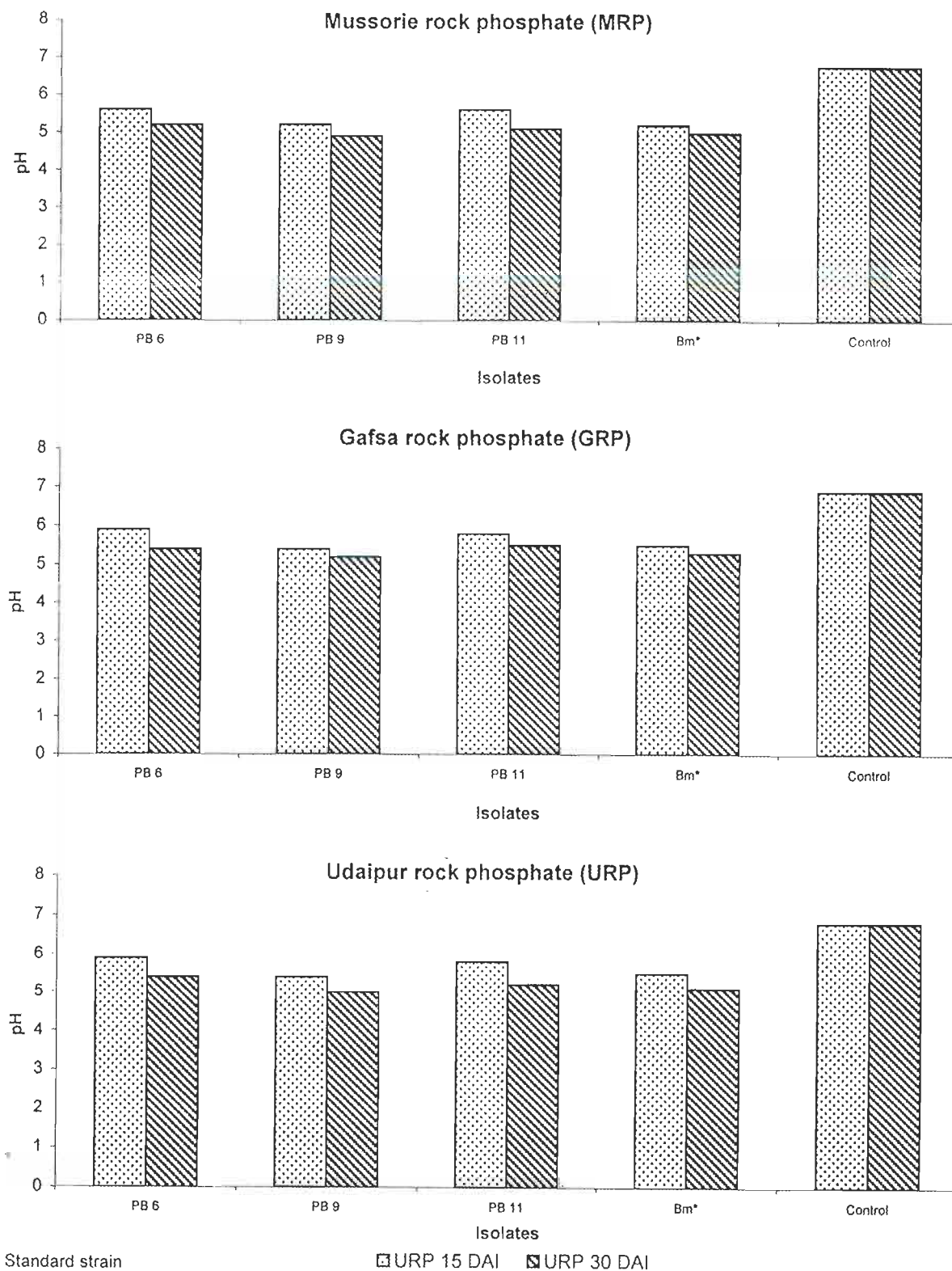
Isolates	pH of the culture medium										Mean
	Mussoorie rock phosphate(MRP)			Gafsa rock phosphate (GRP)			Udaipur rock phosphate (URP)				
	15 DAI	30 DAI	Mean	15 DAI	30 DAI	Mean	15 DAI	30 DAI	Mean		
PB 6	5.6	5.2	5.40	5.9	5.4	5.65	5.9	5.4	5.65	5.57	
PB 9	5.2	4.9	5.05	5.4	5.2	5.30	5.4	5.0	5.20	5.18	
PB 11	5.6	5.1	5.35	5.8	5.5	5.65	5.8	5.2	5.50	5.45	
<i>Bacillus megaterium</i> *	5.2	5.0	5.10	5.5	5.3	5.40	5.5	5.1	5.30	5.27	
Uninoculated control	6.8	6.8	6.80	6.9	6.9	6.90	6.8	6.8	6.80	6.86	
Mean	5.68	5.4	5.54	5.9	5.66	5.78	5.88	5.48	5.68	5.67	

* Standard strain; DAI - Days after inoculation

CD (P=0.05)

Phosphate	0.670
Days	0.547
Treatment	0.865
Phosphate x Days	0.948
Days x Treatment	1.224
Phosphate x Treatment	1.499
Phosphate x Days x Treatment	2.119

Fig. 19. Effect of phosphobacterial inoculation on the pH of the culture supernatant incorporated with different types of rock phosphate



4.12. GERMINATION PERCENTAGE AND SEEDLING VIGOUR INDEX OF COTTON SEEDLING (MCU9) UNDER STERILE GROWTH POUCHES INOCULATED WITH RHIZOBACTERIAL ISOLATES

The germination percentage and seedling vigour index of Cotton (MCU 9) due to the inoculation of rhizobacterial isolates under sterile growth pouch condition is presented in Table 24 and Fig. 20.

4.12.1. Germination percentage

The germination per cent of seeds inoculated with rhizobacterial isolates was higher than uninoculated control. The *Azospirillum* isolate, Azo 11 recorded germination per cent of 82.5 whereas inoculation of Azo 8 recorded the lowest germination per cent of 60. The standard strain Sp7 recorded germination per cent of 75, which is less than that germination per cent of *Azospirillum* isolate Azo 11.

Among the phosphobacterial isolates, PB 9 increased the germination of Cotton seeds with 77.5 per cent followed by standard strain *B. megaterium* (72.5%) and PB 11 (60%) while PB 6 showed least germination per cent of 55.

The *Pseudomonas* isolates recorded germination per cent of 80 to 55. Among them Pfa 2 recorded 80 per cent germination followed by standard strain Pfl (75%), Pfp 4 (60%) and Pfk 6 (55%).

In general, among the three genera of rhizobacterial isolates, Azo 11 of *Azospirillum* showed higher germination percentage followed by *Pseudomonas* isolate Pfa 2 and PB 9 isolate of phosphobacteria.

4.12.2. Seedling vigour index

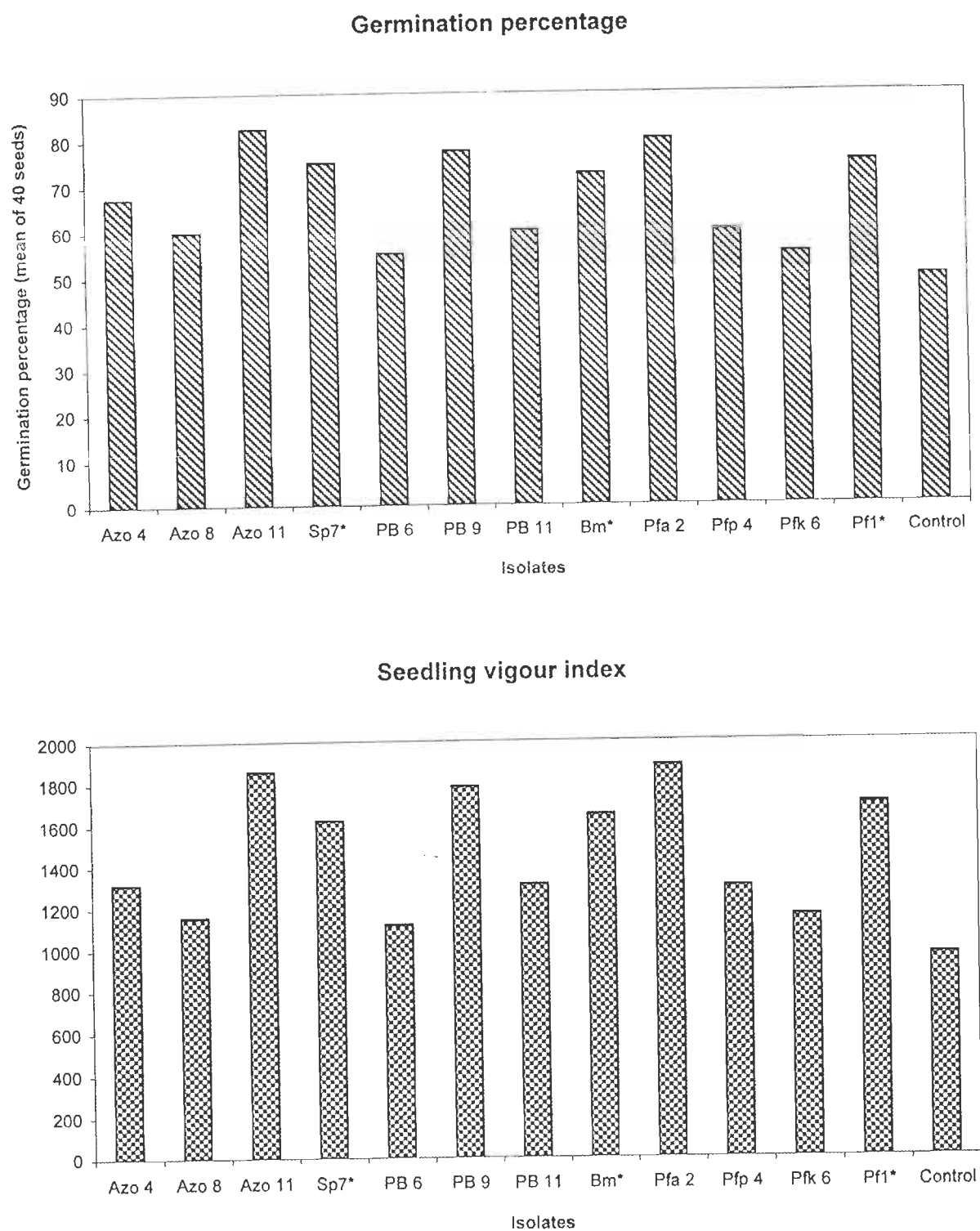
The seedling vigour index was significantly enhanced due to the inoculation of rhizobacterial isolates. Among the *Azospirillum* isolates, Azo 11 showed maximum vigour index followed by standard strain Sp7, Azo 4 and Azo 8. The phosphobacterial isolate PB 9 recorded higher vigour index followed by standard strain *B. megaterium*, PB 11 and PB 6. The *Pseudomonas* isolate Pfa 2 enhanced the vigour index greatly followed by standard strain Pfl, Pfp 4 and Pfk 6. In general among the rhizobacterial isolates, Azo 11 of *Azospirillum* highly enhanced the seedling vigour index followed by Pfa 2 of *Pseudomonas* and PB 9 of phosphobacteria (Table 24 and Fig. 20).

Table 24 Germination percentage and seedling vigour index of Cotton seedling (MCU9) under sterile growth pouches inoculated with rhizobacterial isolates

Isolates	germination percentage (mean of 40 seeds)	Shoot length (cm)	Root length (cm)	Seedling vigour index
<i>Azospirillum</i>				
Azo 4	67.5	8.5	9.0	1316.25
Azo 8	60.0	9.6	9.7	1158.00
Azo 11	82.5	12.0	10.5	1856.25
Sp7*	75.0	12.0	9.6	1620.00
<i>Phosphobacteria</i>				
PB 6	55.0	10.2	10.1	1116.50
PB 9	77.5	13.2	11.2	1782.50
PB 11	60.0	12.0	9.8	1308.00
<i>B. megaterium</i> *	72.5	11.8	9.5	1645.75
<i>Pseudomonas</i>				
Pfa 2	80.0	12.2	11.3	1880.00
Pfp 4	60.0	11.8	9.8	1296.00
Pfk 6	55.0	11.8	9.2	1155.00
Pfl*	75.0	12.0	10.6	1695.00
Uninoculated control	50.0	10.8	8.5	965.00

*Standard strain

Fig. 20. Germination percentage and seedling vigour index of cotton seedlings (MCU9) under sterile growth pouches inoculated with rhizobacterial isolates



* Standard strain

4.13. RHIZOSPHERE COLONIZATION OF RHIZOBACTERIAL ISOLATES ON COTTON SEEDLINGS (MCU9) UNDER HYDROPONICS CONDITION

4.13.1. Rhizoplane colonization of rhizobacterial isolates on Cotton seedlings (MCU 9) under hydroponics condition

The rhizoplane colonization of rhizobacterial isolates under hydroponics condition is presented in the Table 25 and Fig. 21. The rhizoplane population was higher during 30 DAI in all the rhizobacterial isolates. The rhizoplane population of Azo 11 isolate of *Azospirillum* varied from 32.24, 82.78 and 104.13 cfu $\times 10^6$ g⁻¹ of fresh wt of roots on 5, 15, and 30 DAI respectively whereas the rhizoplane population of standard strain Sp7 was 24.66, 75.33 and 98.22 cfu $\times 10^6$ g⁻¹ of fresh wt of roots. Among the *Azospirillum* isolates, Azo 8 recorded the minimum colonization of 15.35 cfu g⁻¹ of fresh wt of root on 5th DAI and similar trend was observed on 15 and 30 DAI.

Colonization of phosphobacteria on rhizoplane of Cotton varied from 22.40, 87.12 and 113.28 cfu $\times 10^6$ g⁻¹ of fresh wt of roots due to the inoculation of PB 9 isolate. The standard strain recorded 21.33, 84.67 and 96.00 cfu $\times 10^6$ g⁻¹ of fresh wt of roots on 5, 15 and 30 DAI respectively, followed by PB 6 and PB 11.

Among the *Pseudomonas* isolates, Pfa 2 colonized to an extend of 24.4, 88.12, and 123.13 cfu $\times 10^6$ g⁻¹ fresh wt of root followed by standard strain Pfl which recorded 23.66, 86.43 and 120.12 cfu $\times 10^6$ g⁻¹ fresh wt of root on 5, 15 and 30 DAI respectively. The Pfp 4 isolate recorded the minimum colonization on 5th day and similar trend was recorded upto 30 DAI.

In general, among the three genera of rhizobacterial isolate colonization pattern varied significantly. *Pseudomonas* isolate (Pfa 2) recorded maximum rhizoplane colonization followed by phosphobacteria (PB 9) and *Azospirillum* (Azo 11) isolate.

4.13.2. Endorhizosphere colonization of rhizobacterial isolates on Cotton seedlings (MCU9) under hydroponics condition

Endorhizosphere colonization of rhizobacterial isolates in Cotton seedlings (MCU9) is presented in Table 26 and Fig. 22. In general, the endorhizosphere colonization increased with increase in the age of the crop.

Table 25. Rhizoplane colonization of rhizobacterial isolates on Cotton seedlings (MCU9) under hydroponics condition

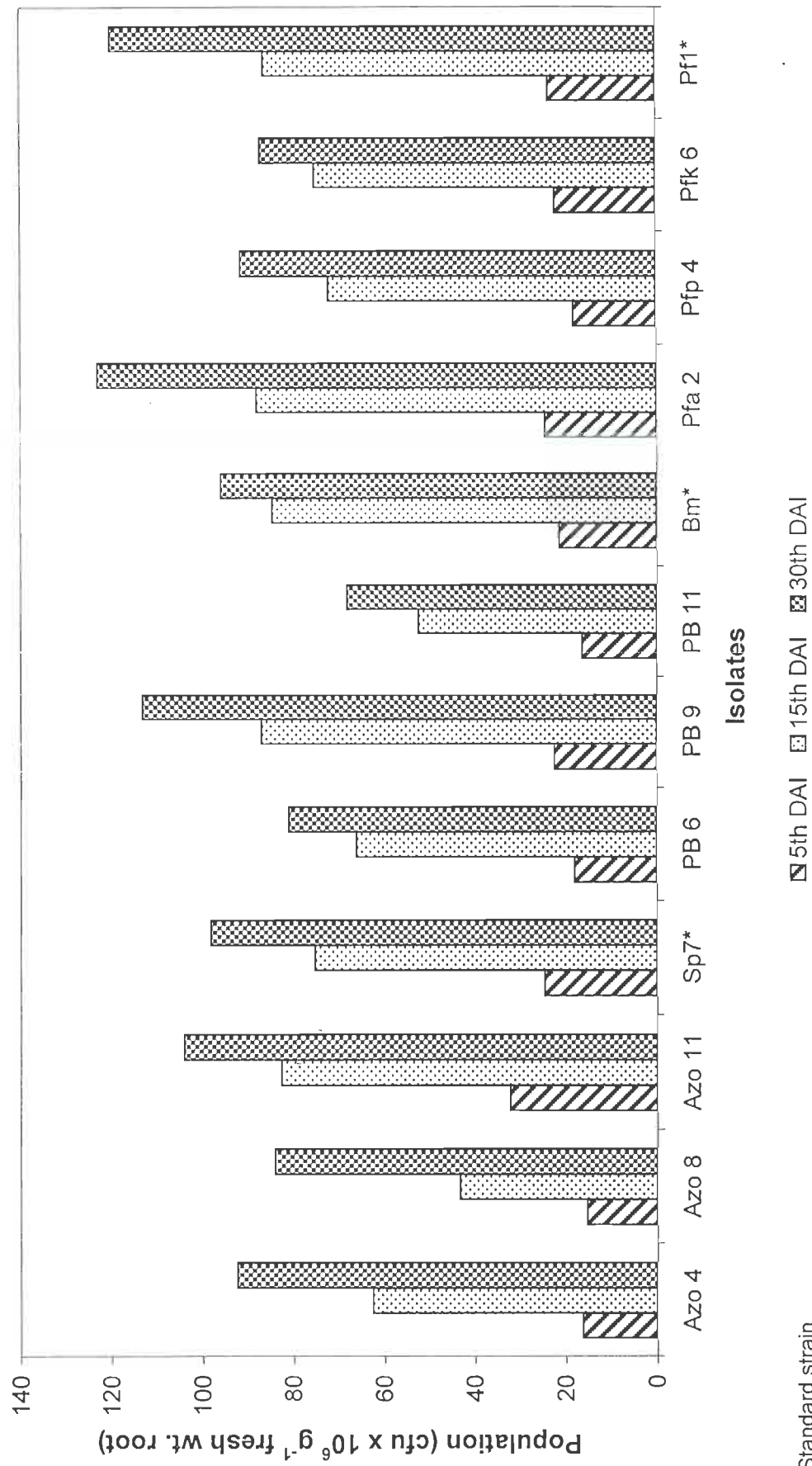
Isolates	Population (cfu x 10 ⁶ g ⁻¹ fresh wt. of root)		
	Days after inoculation (DAI)		
	5	15	30
<i>Azospirillum</i>			
Azo 4	16.30	62.60	92.42
Azo 8	15.35	43.33	84.13
Azo 11	32.24	82.78	104.13
Sp7*	24.66	75.33	98.22
<i>Phosphobacteria</i>			
PB 6	18.00	66.16	81.14
PB 9	22.40	87.12	113.28
PB 11	16.28	52.40	68.17
<i>B. megaterium</i> *	21.33	84.67	96.00
<i>Pseudomonas</i>			
Pfa 2	24.4	88.12	123.13
Pfp 4	18.14	72.30	91.66
Pfk 6	22.17	75.33	87.24
Pfl*	23.66	86.43	120.12

* Standard strain

CD (p=0.05)

Days	1.749
Isolates	3.498
Days x Isolates	6.059

Fig. 21. Rhizoplane colonization of rhizobacterial isolates on Cotton seedlings (MCU 9) under hydroponics condition



* Standard strain

Table 26. Endorhizosphere colonization of rhizobacterial isolates on Cotton seedlings (MCU9) under hydroponics condition

Isolates	Population (cfu x 10 ⁶ g ⁻¹ fresh wt. of root)		
	Days after inoculation (DAI)		
	5	15	30
<i>Azospirillum</i>			
Azo 4	1.16	7.81	15.26
Azo 8	0.96	6.33	13.34
Azo 11	2.52	18.05	30.13
Sp 7*	2.33	16.68	28.12
Phosphobacteria			
PB 6	1.63	5.12	16.00
PB 9	2.23	8.92	22.80
PB 11	1.52	5.33	12.63
<i>B. megaterium</i> *	2.16	8.62	22.31
<i>Pseudomonas</i>			
Pfa 2	3.33	12.12	28.16
Pfp 4	2.12	8.27	21.33
Pfk 6	1.65	8.10	15.20
Pfl *	3.10	11.03	27.24

* Standard strain

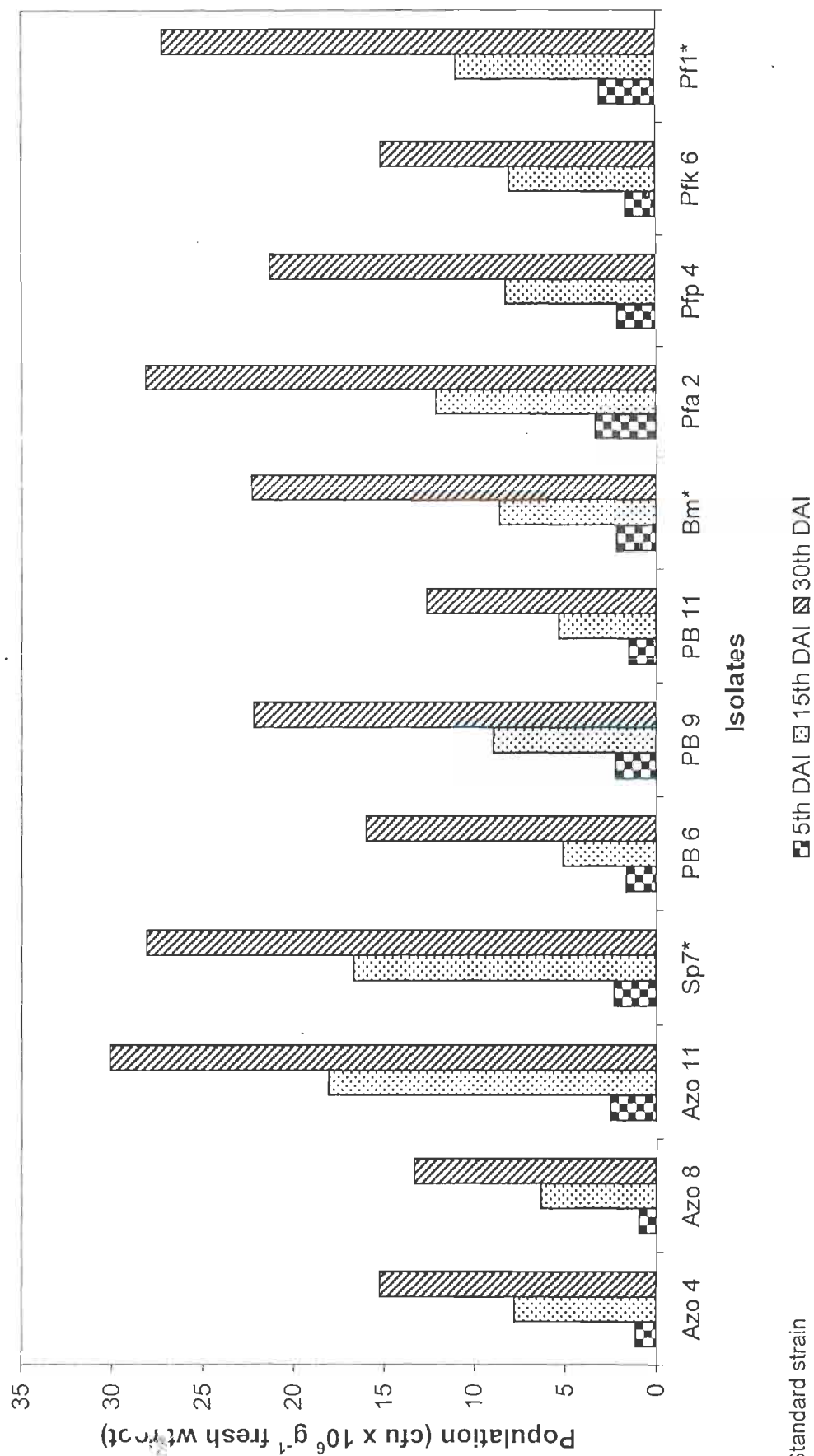
CD (p=0.05)

Days 1.324

Isolates 2.649

Days x Isolates 4.589

Fig. 22. Endorhizosphere colonization of rhizobacterial isolates in Cotton seedlings (MCU9) under hydroponics condition



The endorhizosphere population varied from 2.52, 18.05 and 30.13 cfu $\times 10^6$ g⁻¹ fresh wt of root due to the inoculation of *Azospirillum* (Azo 11) followed by Sp7 with the colonization of 2.33, 16.68 and 28.12 cfu $\times 10^6$ g⁻¹ fresh wt of root on 5, 15 and 30 DAI respectively. The least colonization was observed by Azo 4 followed by Azo 8 on 5th DAI and similar trend was observed upto 30th DAI.

Among the phosphobacterial isolates, PB 9 showed maximum endorhizosphere colonization of 2.23, 8.92, 22.80 cfu $\times 10^6$ g⁻¹ fresh wt of root followed by standard strain *B. megaterium* which recorded 2.16, 8.62 and 22.31 cfu $\times 10^6$ g⁻¹ fresh wt of roots on 5, 15 and 30 DAI. Among the phosphobacterial isolate PB 11 recorded the minimum colonization of 1.52 cfu g⁻¹ of fresh wt of root and on 5th DAI and on 30 DAI the colonization was 12.63 cfu g⁻¹ of fresh wt of root.

The *Pseudomonas* isolates, Pfa 2 colonized a greater extent with a population of 3.33, 12.12 and 28.16 cfu $\times 10^6$ g⁻¹ fresh wt of root followed by standard strain Pfl with 3.10 and 11.03 and 27.24 cfu $\times 10^6$ g⁻¹ fresh wt of root during 5, 15 and 30 DAI respectively.

In general, the endorhizosphere colonization was found to be higher with the inoculation of *Azospirillum* (Azo 11) followed by *Pseudomonas* (Pfa 2) and phosphobacteria (PB 9) isolate.

4.13.3. Nutrient content of root exudate of Cotton seedling (MCU9) under hydroponics condition inoculated with rhizobacterial isolates

The total sugars, reducing sugars and amino nitrogen content of root exudate of Cotton seedlings (MCU9) under hydroponics condition inoculated with rhizobacterial isolates were presented in the Table 27.

The total sugar content in the root exudates due to inoculation of *Azospirillum* ranged from 72.2 to 114.4 mg g⁻¹ fresh wt of root and 171.6 to 193.4 mg g⁻¹ fresh wt of root on 15th and 30th DAI. Inoculation of phosphobacterial isolate showed the total sugar content from 81.6 to 104.3 mg g⁻¹ fresh wt of root whereas due to the inoculation of *Pseudomonas* isolates, the total sugar content varied from 92.7 to 126.5 mg g⁻¹ of root fresh wt on 15th. In general, the amount of total sugars increased with the age of plants.

Table 27. Total sugars, reducing sugars and amino nitrogen content of root exudate of Cotton seedling (MCU 9) under hydroponics condition inoculated with rhizobacterial isolates

Isolates	Total sugar (mg g ⁻¹ fresh wt of roots)		Reducing sugar (mg g ⁻¹ fresh wt of roots)		Amino nitrogen (mg g ⁻¹ fresh wt of roots)	
	15 th DAI	30 th DAI	15 th DAI	30 th DAI	15 th DAI	30 th DAI
<i>Azospirillum</i>						
Azo 4	98.4	185.7	22.1	35.2	20.3	29.1
Azo 8	72.2	171.6	18.3	28.6	18.5	28.6
Azo 11	114.4	193.4	30.6	47.1	28.1	36.4
Sp7*	108.1	186.2	25.5	42.8	24.4	35.8
<i>Phosphobacteria</i>						
PB 6	86.7	173.3	20.3	36.3	19.3	28.6
PB 9	104.3	192.5	29.1	51.6	26.7	32.3
PB 11	81.6	171.2	19.7	34.1	18.7	26.7
<i>B. megaterium</i> *	97.6	187.6	23.4	45.8	26.2	30.1
<i>Pseudomonas</i>						
Pfa 2	126.5	212.5	36.8	57.6	29.3	34.6
Pfp 4	92.7	196.1	21.3	48.8	18.2	25.7
Pfk 6	98.3	181.3	24.4	41.3	22.3	28.1
Pfl *	114.2	206.7	36.6	52.7	28.4	33.4
Control	53.4	102.7	17.1	21.4	12.6	18.4

* Standard strain; DAI - Days after inoculation

CD (P=0.05)

Days 1.288
Isolates 3.284
Days x Isolates 4.644

CD (P=0.05)

1.74
4.44
6.28

CD (P=0.05)

0.947
2.415
3.415

Among the *Azospirillum*, isolates, Azo 11 inoculation increased the total sugar content followed by standard strain Sp7, Azo 4 and Azo 8 on 15th and 30th DAI. Among the phosphobacterial isolates, inoculations of PB 9 isolate enhanced the total sugar content of root exudate followed by standard strain (*B. megaterium*), PB 6 and PB 11. The inoculation of *Pseudomonas* isolate Pfa 2 registered 126.3 and 212.5 mg g⁻¹ fresh wt of root of total sugar followed by standard strain Pfl, Pfk 6 showed least amount of 98.3 and 181.3 mg g⁻¹ fresh wt of root of total sugars on 15th and 30th DAI respectively.

Among the different rhizobacterial isolates the *Pseudomonas* (Pfa 2) isolate followed by phosphobacteria (PB 9) and *Azospirillum* isolate (Azo 11) improved the total sugar content of Cotton seedlings (Fig. 23).

Similar trend was observed in the case of reducing sugar content in the root exudates inoculated with rhizobacterial isolates (Fig. 24).

Amino nitrogen content showed similar trend, but *Azospirillum* isolate (Azo 11) inoculation recorded higher amino nitrogen content in the root exudates of Cotton seedlings followed by inoculation of *Pseudomonas* (Pfa 2) isolates and phosphobacterial isolate (PB 9) (Fig. 25).

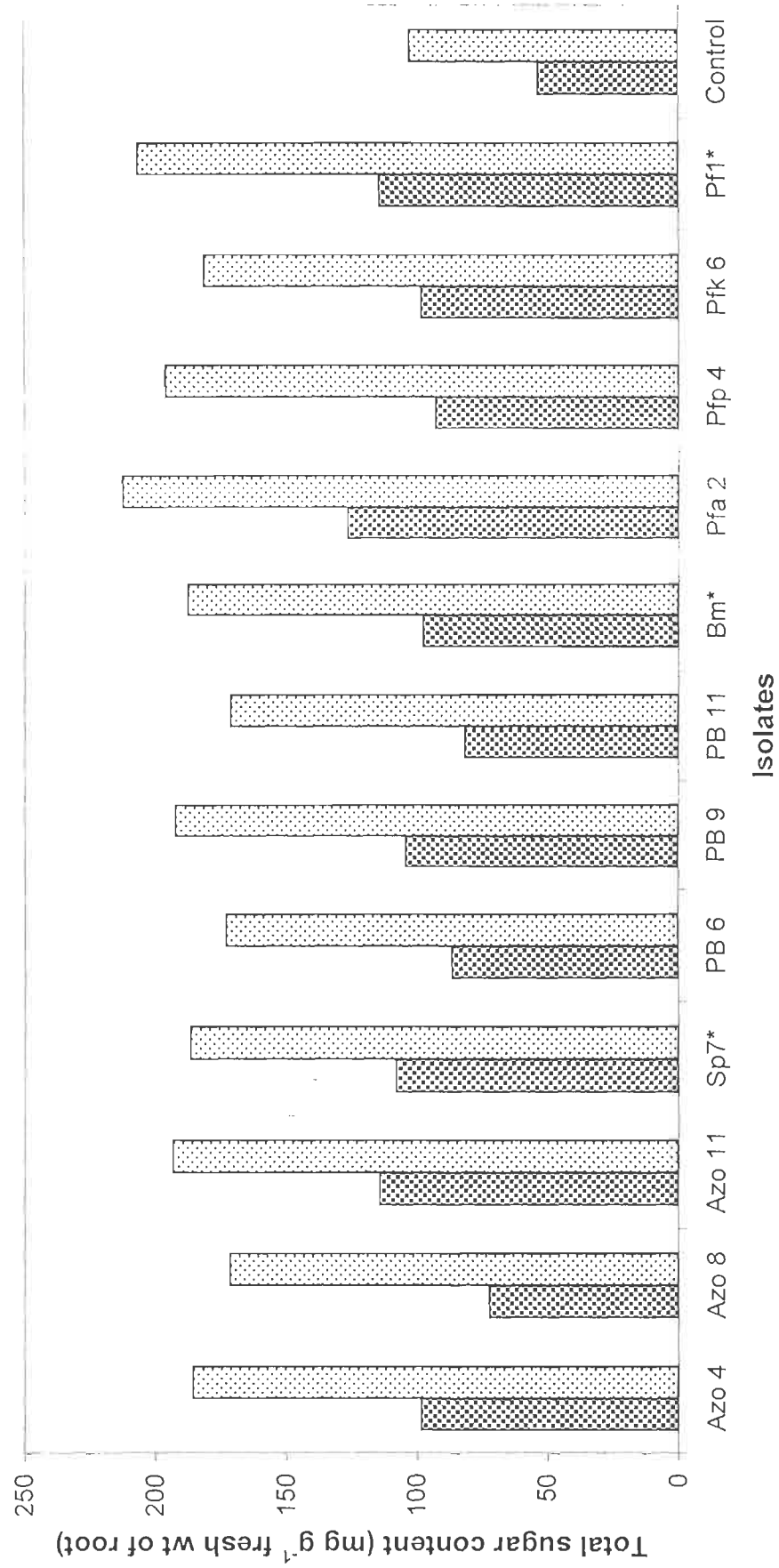
4.14. ADSORPTION OF CELLS OF RHIZOBACTERIAL ISOLATES ON THE ROOTS OF COTTON SEEDLINGS (MCU9)

The adsorption of rhizobacterial isolates on the roots of Cotton seedling (MCU9) is presented in Table 28 and Fig. 26.

The adsorption percentage ranged from 17.43 to 26.22 and 21.02 to 30.90 for *Azospirillum* isolates on 30 min and 60 min of incubation respectively. The phosphobacterial isolates recorded adsorption percentage of 26.30 to 30.20 and 33.04 to 42.26 on 30 and 60 min of incubation respectively. Similarly, the *Pseudomonas* isolates showed 35.39 to 47.48 percentage of adsorption respectively for 30 and 60 min of incubation.

Among the *Azospirillum* isolates, Azo 11 showed higher rate of adsorption with 146.24 and 184.43 cfu 10⁶ g⁻¹ fresh wt of roots during 30 and 60 min of incubation which was followed by Sp7, Azo 4 and Azo 8. The phosphobacterial isolate PB 9 recorded

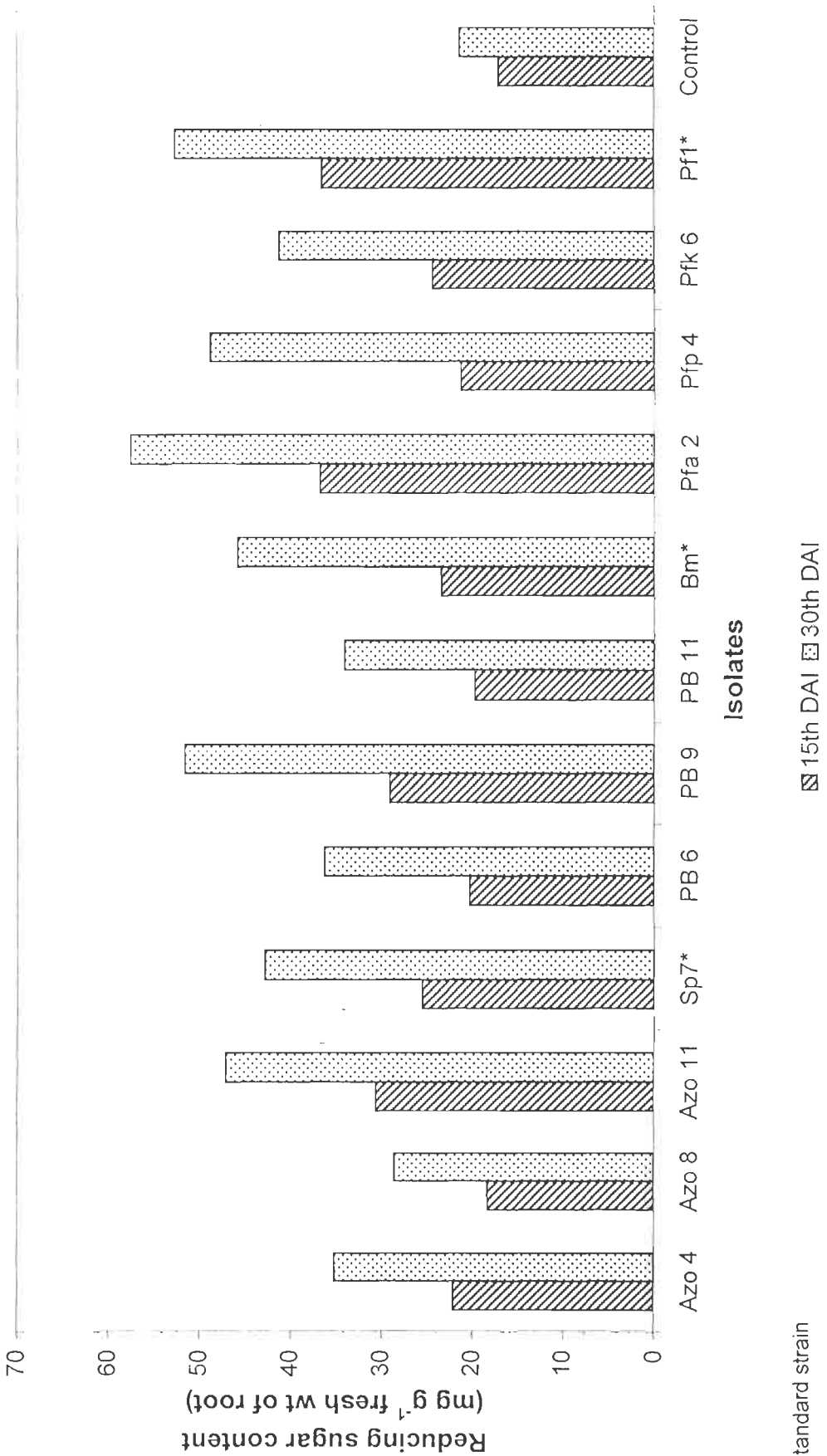
Fig. 23. Total sugar content in root exudate of Cotton seedlings (MCU9) under hydroponics condition inoculated with rhizobacterial isolates



* Standard strain

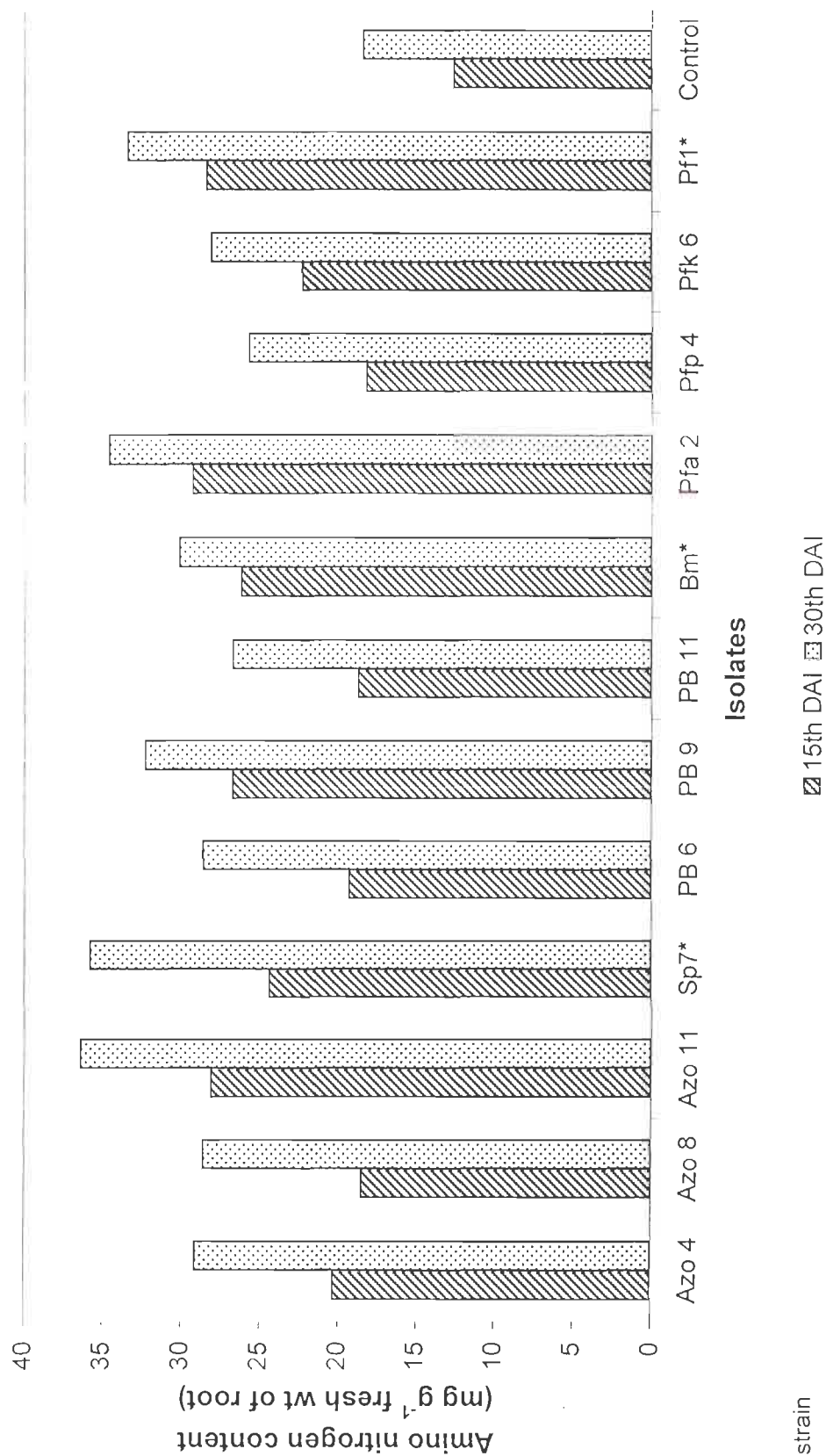
[dotted] 15th DAI [checkered] 30th DAI

Fig. 24. Reducing sugar content in root exudate of Cotton seedlings (MCU9) under hydroponics condition inoculated with rhizobacterial isolates



* Standard strain

Fig. 25. Amino nitrogen content in root exudate of Cotton seedlings (MCU9) under hydroponic condition inoculated with rhizobacterial isolates



* Standard strain

Table 28. Adsorption of cells of rhizobacterial isolates on the root of Cotton seedlings (MCU9) under *in vitro* condition

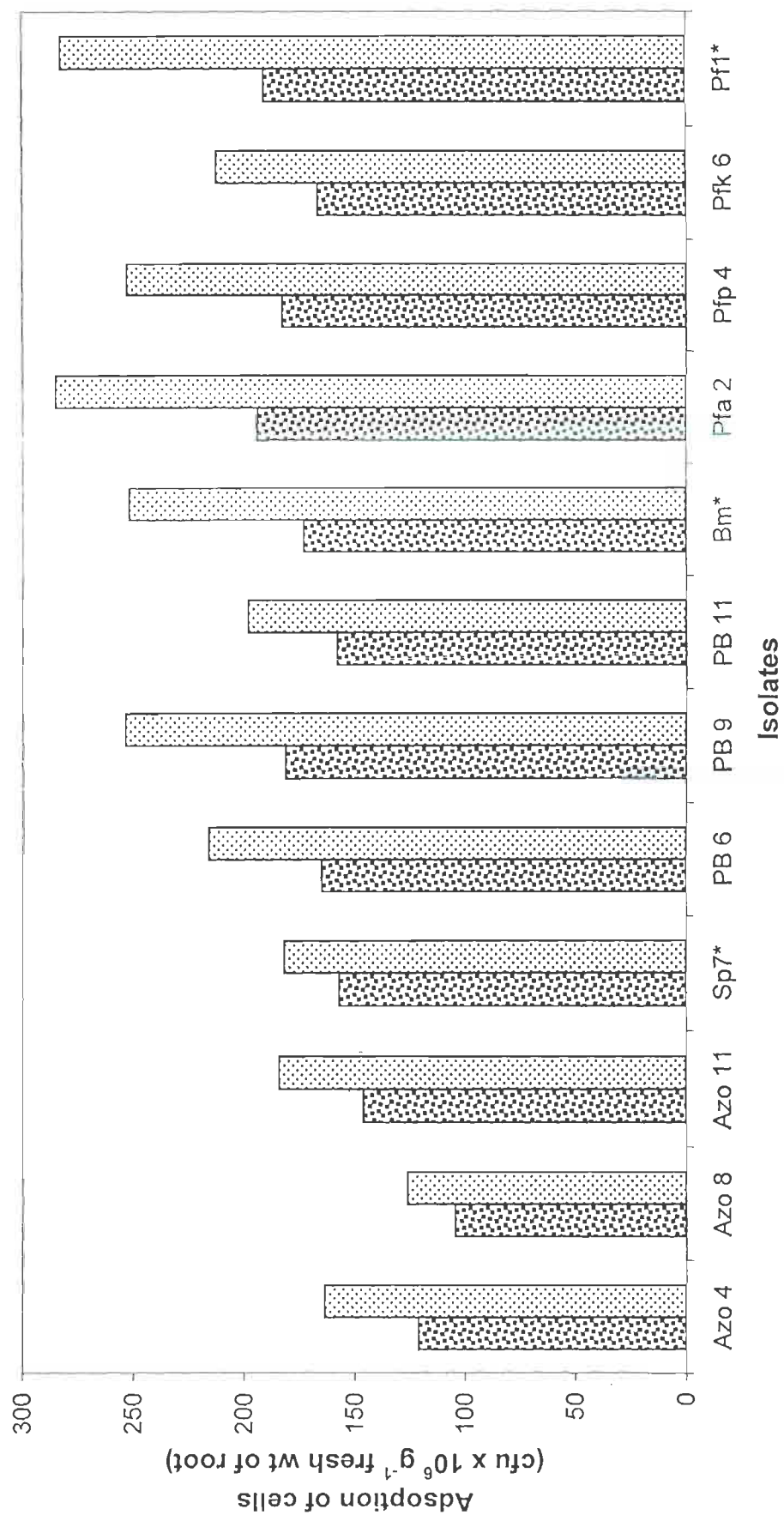
Isolates	30 min		60 min	
	No. of adsorbed cells cfu x 10 ⁶ g ⁻¹ fresh wt. of root	Adsorption %	No. of adsorbed cells cfu x 10 ⁶ g ⁻¹ fresh wt. of root	Adsorption %
<i>Azospirillum</i>				
Azo 4	121.3	20.21	163.64	27.27
Azo 8	104.62	17.43	126.14	21.02
Azo 11	146.24	24.37	184.43	30.90
Sp 7*	157.33	26.22	182.05	30.34
Phosphobacteria				
PB 6	165.16	27.52	216.04	36.00
PB 9	181.24	30.20	253.61	42.26
PB 11	157.80	26.30	198.25	33.04
<i>B. megaterium</i> (Bm)*	173.16	28.86	251.82	41.97
<i>Pseudomonas</i>				
Pfa 2	193.89	32.31	284.92	47.48
Pfp 4	182.63	30.43	252.83	42.13
Pfk 6	166.71	27.78	212.35	35.39
Pfl*	191.06	31.84	282.63	47.10

* Standard strain

No. of adsorbed cells expressed in No. x 10⁶g⁻¹ fresh wt. of root

Initial population of PGPR 6 x 10⁸ No. of cells ml⁻¹

Fig. 26. Adsorption of cells of rhizobacterial isolates on the roots of Cotton seedling (MCU9) under *in vitro* condition



* Standard strain

maximum adsorption of 181.24 and 253.61 cfu $\times 10^6$ g⁻¹ fresh wt of root followed by *B. megaterium*, PB 6 and PB 11. Among the *Pseudomonas* isolates, Pfa 2 recorded higher adsorption rate with 193.89 and 284.92 cfu $\times 10^6$ g⁻¹ fresh wt of roots respectively during 30 and 60 min of incubation followed by standard strain Pf 1, Pfp 4 and Pfk 6.

The adsorption rate was higher in *Pseudomonas* (Pfa 2) isolate followed by phosphobacteria (PB 9) and *Azospirillum* (Azo 11). In general, the rate of adsorption was higher during 60 min of incubation of Cotton roots for all the isolates.

4.15. SELECTION OF EFFICIENT ISOLATES

Based on the performance of rhizobacterial isolates on the growth and development of Cotton in increasing seedling vigour index, rhizosphere colonization and adsorption under *in vitro* conditions, *Azospirillum* (Azo 11), phosphobacteria (PB 9) and *Pseudomonas* (Pfa 2) were selected. These isolates were used for their compatibility studies under *in vitro* conditions.

4.16. STUDIES ON THE COMPATABILITY OF RHIZOBACTERIAL ISOLATES UNDER *in vitro* CONDITIONS

4.16.1. Compatibility of *Azospirillum* (Azo 11) with phosphobacteria (PB 9) and *Pseudomonas* (Pfa 2) by cross streak assay

The compatibility of *Azospirillum* (Azo 11) with phosphobacteria (PB 11) and *Pseudomonas* (Pfa 2) indicated no inhibition zone in the cross streak assay. The compatibility of *Azospirillum* (Azo 11) with phosphobacteria (PB 9) and *Pseudomonas* (Pfa 2) suggested the feasibility of using the three organisms together as microbial inoculants (Plate 10) for increasing the growth and development of Cotton.

4.16.2. Co-inoculation of *Azospirillum* (Azo 11) with phosphobacteria (PB 9) and *Pseudomonas* (Pfa 2) in carrier material

4.16.2.1. Physio-chemical properties of carrier material

Physical properties like bulk density, particle density and water holding capacity were estimated and presented in Table 29.

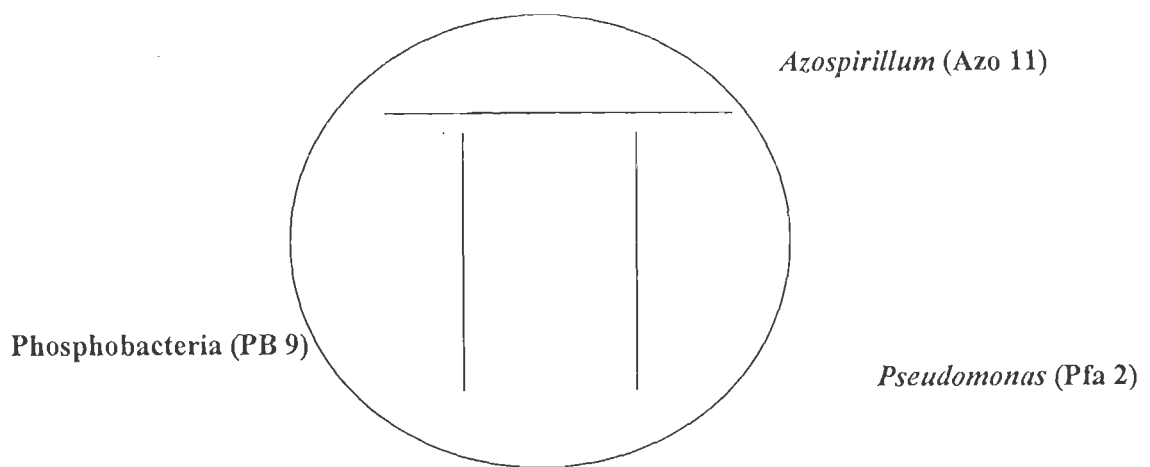


Plate 10. Compatibility of rhizobacterial isolates.

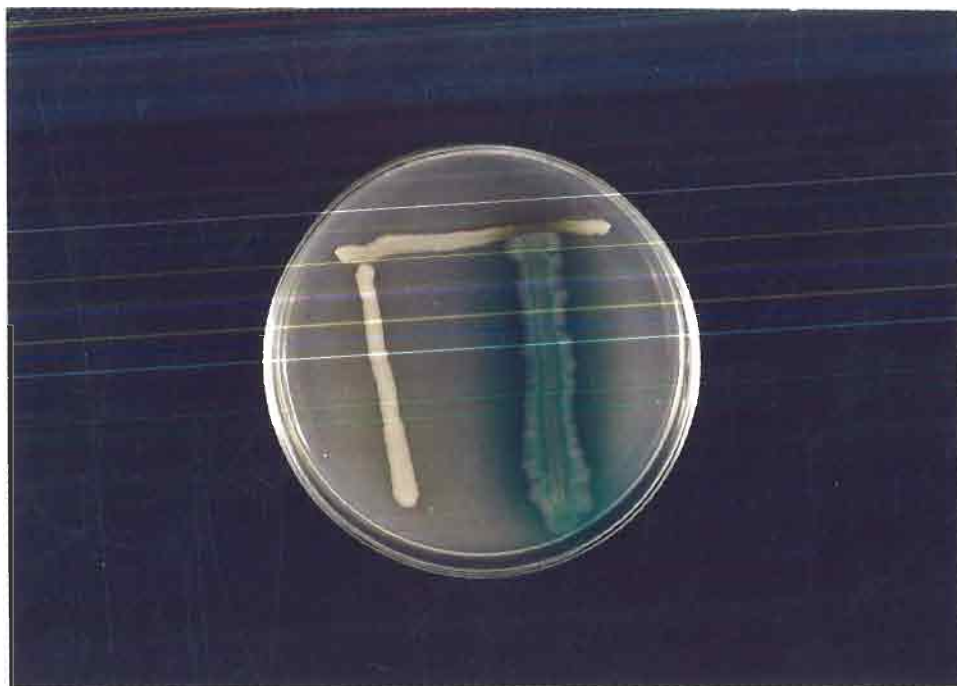


Plate 10. Compatibility of rhizobacterial isolates.

Table 29. Physico-chemical properties of carrier material

Carrier	Bulk density (g cm ⁻³)	Particle density (g cm ⁻³)	Water holding capacity (%)	pH	Carbon content (%)	Total nitrogen (%)	Total phosphorus (%)
Peat	1.01	2.08	1.28	4.8	25.10	0.72	0.52
Lignite	1.03	1.58	1.86	5.6	43.94	0.28	0.17

The bulk density was maximum (1.03 g cm^{-3}) in lignite followed by peat (1.01 g cm^{-3}). The particle density was high, 2.08 g cm^{-3} in peat when compared to lignite which recorded 1.58 g cm^{-3} . The water holding capacity (WHC) varied widely. In lignite the WHC was 86 per cent whereas in peat the WHC was 128 per cent. Studies on the chemical properties like pH showed that peat was more acidic, having a pH of 4.8 whereas in lignite pH was 5.6. Among the two carriers, lignite had maximum organic carbon content (43.94%) and peat soil recorded the least amount (25.10%) of carbon. The nitrogen content of peat was (0.72%) higher than lignite (0.28%). Peat recorded maximum phosphorus content (0.52%) followed by lignite (0.27%).

4.16.2.2. Survival of rhizobacterial isolates in different carrier materials

The survival and compatibility of rhizobacterial isolates *Azospirillum* (Azo 11), phosphobacteria (PB 9) and *Pseudomonas* (Pfa 2) was assessed as individual (single) and combined inoculation in the carrier materials upto 180 days and the results are presented in Fig. 27.

In general among the two carrier materials, lignite recorded higher population of rhizobacterial isolates than peat. Irrespective of the carrier material, the population of rhizobacterial isolates increased upto 30 days of storage and remained constant upto 90 days. The population of combined inoculation was on par with individual inoculum, which indicates the compatibility of all the rhizobacterial isolates. Particularly among the rhizobacterial isolates *Pseudomonas* (Pfa 2) population was higher than phosphobacteria (PB 9) and *Azospirillum* (Azo 11).

4.16.2.2.1. Compatibility of *Azospirillum* with phosphobacteria and *Pseudomonas* in different carrier material

The compatibility of *Azospirillum* (Azo 11) with phosphobacteria (PB 9) and *Pseudomonas* (Pfa 2) was studied upto 180 days in carrier material lignite and the results showed that *Azospirillum* (Azo 11) registered maximum population of $65 \times 10^9 \text{ cfu g}^{-1}$ of lignite with *Pseudomonas* (Pfa 2) on 30 DAI. Whereas with phosphobacteria (PB 9) combination *Azospirillum* (Azo 11) recorded $43 \text{ cfu} \times 10^9$ of carrier in peat soil. The population present in the combined inoculation of the carriers was on par with individual inoculation of the *Azospirillum* (Azo 11) (Table 30a).

Fig. 27. Compatibility of rhizobacterial isolates under different carrier materials

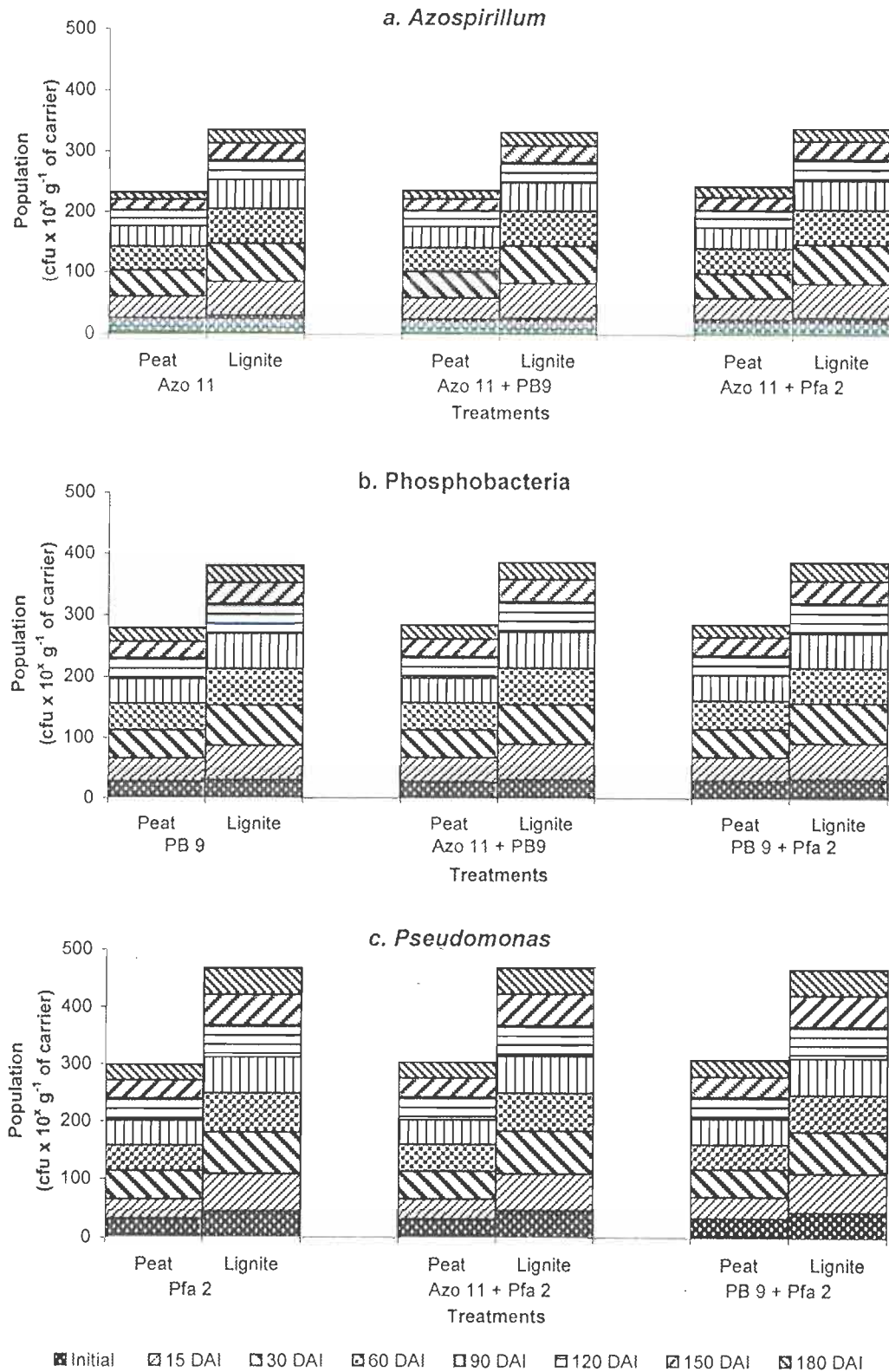


Table 30a. Compatibility of *Azospirillum* with phosphobacteria and *Pseudomonas* in different carrier material

Treatments	Population (cfu g ⁻¹ of carrier)															
	Peat								Lignite							
	Initial x 10 ⁹	15 DAI x 10 ⁹	30 DAI x 10 ⁹	60 DAI x 10 ⁸	90 DAI x 10 ⁸	120 DAI x 10 ⁷	150 DAI x 10 ⁷	180 DAI x 10 ⁷	Initial x 10 ⁹	15 DAI x 10 ⁹	30 DAI x 10 ⁹	60 DAI x 10 ⁸	90 DAI x 10 ⁸	120 DAI x 10 ⁷	150 DAI x 10 ⁷	180 DAI x 10 ⁷
Azo 11 alone	25 (1.4)	35 (1.6)	42 (1.6)	40 (1.6)	34 (1.5)	26 (1.4)	18 (1.3)	12 (1.0)	29 (1.5)	55 (1.8)	62 (1.8)	58 (1.8)	48 (1.7)	32 (1.5)	28 (1.5)	22 (1.4)
PB 9 alone	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)
Pfa 2 alone	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)
Azo 11+PB 9	24 (1.4)	34 (1.5)	43 (1.6)	40 (1.6)	35 (1.5)	26 (1.4)	20 (1.3)	14 (1.1)	26 (1.4)	56 (1.8)	63 (1.8)	58 (1.8)	47 (1.7)	33 (1.5)	28 (1.4)	22 (1.3)
Azo 11+Pfa 2	25 (1.4)	34 (1.5)	40 (1.7)	42 (1.6)	35 (1.5)	28 (1.4)	22 (1.3)	18 (1.2)	28 (1.5)	55 (1.5)	65 (1.8)	58 (1.8)	48 (1.7)	35 (1.5)	30 (1.5)	21 (1.3)
PB 9+Pfa 2	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)

(Figures in parentheses are log₁₀ transformed values)

DAI - Days after inoculation

CD (P=0.05)

Days 0.0225

Carrier 0.0112

Treatment 0.0195

Days x Carriers 0.0318

Carriers x Treatment 0.2760

Days x Treatments 0.0552

Days x Carriers x Treatments 0.0780

4.16.2.2.2. Compatibility of phosphobacteria (PB 9) with *Azospirillum* (Azo 11) and *Pseudomonas* (Pfa 2) in different carrier

The combined inoculation of Phosphobacteria (PB 9) and *Pseudomonas* (Pfa 2) recorded maximum population of 66 and 47×10^9 cfu g⁻¹ of lignite and peat respectively. The population recorded in individual inoculation was on par with combination of phosphobacteria (PB 9) with *Azospirillum* (Azo 11) and *Pseudomonas* (Pfa 2) on 30 DAI (Table 30b).

4.16.2.2.3. Compatibility of *Pseudomonas* (Pfa 2) with *Azospirillum* (Azo 11) and phosphobacteria (PB 9) in different carriers

The individual inoculation of *Pseudomonas* (Pfa 2) recorded the population of 72 and 49×10^9 cfu g⁻¹ of lignite and peat respectively which is on par with other combination of *Azospirillum* (Azo 11) and phosphobacteria (PB 9) (Table 30c).

4.17. EFFECT OF RHIZOBACTERIAL INOCULATION ON THE RHIZOSPHERE MICROFLORA OF COTTON VAR MCU9 UNDER *in vivo* CONDITION

A field experiment was conducted to study the effect of inoculation of rhizobacterial isolates viz., *Azospirillum* (Azo 11), phosphobacteria (PB 9) and *Pseudomonas* (Pfa 2) alone and in combination on rhizosphere microflora of Cotton (MCU9) with graded levels of N and P. The influence of rhizobacterial isolates on the population dynamics of rhizosphere microflora was evaluated by mathematical modeling.

4.17.1. Modeling microbial population dynamics in the rhizosphere of Cotton inoculated with rhizobacterial isolates

The interactions of microbial growth in the rhizosphere of Cotton inoculated with rhizobacterial isolates are represented by means of set of equations, *i.e.* mathematical model. These models provide complete scenario of the relationship between an environment and its microbial inhabitants and the role of these microorganisms shaping in soils. The solutions to these models are the solutions to the microorganic systems. The valid inference drawn from these microbial models of the rhizosphere of Cotton are presented in the Table 31-38 and Fig. 28-31. The inferences are drawn from cumulative increase in microbial population over control and in general total diazotrophs, bacteria and actinomycetes showed positive growth rate whereas negative growth rate was observed in the case of fungi.

Table 30b. Compatibility of phosphobacteria with *Azospirillum* and *Pseudomonas* in different carrier material

Treatments	Population (cfu g ⁻¹) of carrier															
	Peat								Lignite							
	Initial x 10 ⁹	15 DAI x 10 ⁹	30 DAI x 10 ⁹	60 DAI x 10 ⁸	90 DAI x 10 ⁸	120 DAI x 10 ⁷	150 DAI x 10 ⁷	180 DAI x 10 ⁷	Initial x 10 ⁹	15 DAI x 10 ⁹	30 DAI x 10 ⁹	60 DAI x 10 ⁸	90 DAI x 10 ⁸	120 DAI x 10 ⁷	150 DAI x 10 ⁷	180 DAI x 10 ⁷
Azo 11 alone	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)
PB 9 alone	27 (1.4)	39 (1.6)	46 (1.7)	44 (1.6)	40 (1.6)	34 (1.5)	27 (1.4)	22 (1.3)	29 (1.5)	58 (1.8)	66 (1.8)	60 (1.7)	57 (1.8)	48 (1.7)	34 (1.5)	28 (1.4)
Pfa 2 alone	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)
Azo 11+PB 9	27 (1.4)	41 (1.6)	45 (1.7)	45 (1.7)	40 (1.6)	35 (1.5)	29 (1.5)	22 (1.3)	30 (1.5)	60 (1.8)	65 (1.8)	60 (1.7)	58 (1.8)	49 (1.7)	36 (1.6)	28 (1.5)
Azo 11+Pfa 2	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)
PB 9+Pfa 2	29 (1.5)	40 (1.6)	47 (1.7)	46 (1.7)	42 (1.6)	32 (1.5)	30 (1.5)	20 (1.3)	32 (1.5)	60 (1.8)	66 (1.8)	58 (1.6)	56 (1.6)	49 (1.6)	36 (1.6)	30 (1.5)

(Figures in parentheses are log₁₀ transformed values)DAI - Days after inoculation
CD (P=0.05)

Days 0.0209

Carrier 0.0104

Treatment 0.0182

Days x Carriers 0.0296

Carriers x Treatment 0.0256

Days x Treatments 0.0513

Days x Carriers x Treatments 0.0726

Table 30c. Compatibility of *Pseudomonas* with *Azospirillum* and phosphobacteria in different carrier material

Population (cfu g ⁻¹) of carrier																
Treatments	Peat								Lignite							
	Initial x 10 ⁹	15 DAI x 10 ⁹	30 DAI x 10 ⁹	60 DAI x 10 ⁸	90 DAI x 10 ⁸	120 DAI x 10 ⁷	150 DAI x 10 ⁷	180 DAI x 10 ⁷	Initial x 10 ⁹	15 DAI x 10 ⁹	30 DAI x 10 ⁹	60 DAI x 10 ⁸	90 DAI x 10 ⁸	120 DAI x 10 ⁷	150 DAI x 10 ⁷	180 DAI x 10 ⁷
Azo 11 alone	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)
PB 9 alone	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)
Pfa 2 alone	30 (1.5)	35 (1.5)	49 (1.7)	45 (1.8)	42 (1.6)	38 (1.6)	32 (1.5)	28 (1.5)	42 (1.6)	67 (1.8)	22 (1.9)	68 (1.8)	63 (1.8)	56 (1.8)	53 (1.7)	46 (1.7)
Azo 11+PB 9	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)
Azo 11+Pfa 2	30 (1.5)	36 (1.6)	48 (1.7)	47 (1.8)	42 (1.6)	39 (1.6)	34 (1.5)	28 (1.4)	44 (1.6)	66 (1.8)	74 (1.9)	66 (1.8)	64 (1.8)	54 (1.7)	53 (1.7)	45 (1.7)
PB 9+Pfa 2	34 (1.5)	36 (1.6)	49 (1.7)	43 (1.6)	44 (1.6)	38 (1.6)	36 (1.5)	30 (1.6)	44 (1.6)	67 (1.8)	72 (1.9)	64 (1.8)	64 (1.8)	56 (1.7)	54 (1.7)	45 (1.7)

(Figures in parentheses are log₁₀ transformed values)

DAI - Days after inoculation

CD (P=0.05)

Days

0.0159

Carrier

0.0079

Treatment

0.0137

Days x Carriers

0.0225

Carriers x Treatment

0.0194

Days x Treatments

0.0389

Days x Carriers x Treatments

0.0551

4.17.1.1. Estimated logistic microbial population model in the rhizosphere of Cotton (MCU9) due to the combined inoculation of rhizobacterial isolates with graded levels of N and P

The results of the estimated logistic microbial population due to inoculation of rhizobacterial isolates in the rhizosphere of Cotton (MUC9) at graded levels of N and P are presented in the Tables 31-34.

The carrying capacity (P_M) is ranged from 65 to 400, 85 to 360, and 10 to 60 cfu g^{-1} of rhizosphere soil in the case of total diazotrophs ($\times 10^6$), bacteria ($\times 10^6$), and actinomycetes ($\times 10^3$) respectively. In the case of fungi the carrying capacity was negative with 12 to 65 $\text{cfu} \times 10^4 \text{ g}^{-1}$ of rhizosphere soil which showed the declined growth of fungi due to the inoculation of rhizobacteria.

The carrying capacity of total diazotrophs varied from 65 to 300, 90 to 400 and 90 to 300 $\text{cfu g}^{-1} \times 10^6$ of rhizosphere soil at 50, 75, 100 per cent N and P levels respectively. The bacterial carrying capacity ranged from 85 to 230, 110 to 260 and 125 to 360 $\text{cfu g}^{-1} \times 10^6$ of rhizosphere soil at 50, 75, 100 per cent N and P levels respectively. The actinomycetes carrying capacity in the rhizosphere soil varied from 12 to 40, 10 to 32 and 23 to 60 $\text{cfu g}^{-1} \times 10^3$ of rhizosphere soil at 50, 75, 100 per cent N and P levels respectively. The negative carrying capacity of fungi in the rhizosphere soil ranged from 12 to 55, 25 to 60 and 25 to 55 $\text{cfu g}^{-1} \times 10^4$ of rhizosphere soil at 50, 75, 100 per cent N and P levels respectively.

The parameter α value ranged from 3.9585 to 18.4716, 3.9851 to 31.3391, 2.8933 to 20.8878 and 3.9647 to 19.1938 and the parameter β value varied from -0.0145 to -0.0268, -0.0205 to -0.0327, -0.0103 to 0.0266, -0.0150 to -0.0268 for total diazotrophs, bacteria, fungi and actinomycetes respectively.

The correlation coefficient (r) value varied from 0.87 to 0.99 for the various microbial populations in the rhizosphere of Cotton, which indicates the suitability of the model for assessing the population dynamics of the microbes in the rhizosphere of Cotton.

Table 31. Estimated logistic total diazotrophic population dynamics model in the rhizosphere of Cotton (MCU9) due to the combined inoculation of rhizobacterial isolates with graded levels of N and P

Treatments	100% N & P				75% N & P				50% N & P			
	P _M	α	β	r'	P _M	α	β	r'	P _M	α	β	r'
Azo II alone	130	9.6812	-0.0256	0.95	175	8.6509	-0.0204	0.98	85	4.2334	-0.0147	0.96
PB 9 alone	100	10.4416	-0.0258	0.97	130	6.0087	-0.0192	0.97	90	4.1465	-0.0145	0.97
Pfa 2 alone	90	17.6618	-0.0244	0.91	90	9.1083	-0.0202	0.97	65	3.9587	-0.0164	0.95
Azo II + PB 9	275	6.8703	-0.0239	0.96	360	9.7238	-0.0228	0.98	275	6.7364	-0.0212	0.98
Azo II + Pfa 2	210	7.9490	-0.0219	0.98	310	10.3118	-0.0221	0.98	215	7.0452	-0.0209	0.98
PB 9 + Pfa 2	175	18.4716	-0.0268	0.93	270	10.1869	-0.0220	0.98	130	4.4012	-0.0199	0.96
Azo II + PB 9 + Pfa 2	300	9.0757	-0.0265	0.98	400	11.7675	-0.0294	0.99	300	6.3763	-0.0203	0.98

Table 32. Estimated logistic bacterial population dynamics model in the rhizosphere of Cotton (MCU9) due to the combined inoculation of rhizobacterial isolates with graded levels of N and P

Treatments	100% N & P				75% N & P				50% N & P			
	P _M	α	β	r	P _M	α	β	r	P _M	α	β	r
Azo II alone	250	4.3604	-0.0206	0.98	200	4.7415	-0.0223	0.97	120	4.5680	-0.0221	0.95
PB 9 alone	125	4.3516	-0.0194	0.96	140	3.9851	-0.0222	0.93	85	16.7436	-0.0289	0.92
Pfa 2 alone	175	4.5130	-0.0205	0.95	180	4.6803	-0.0206	0.94	100	6.6941	-0.0228	0.96
Azo II + PB 9	260	12.8717	-0.0297	0.98	180	10.1228	-0.0278	0.95	170	10.7460	-0.0253	0.94
Azo II + Pfa 2	275	21.3340	-0.0299	0.93	120	31.3391	-0.0327	0.90	100	19.4871	-0.0312	0.95
PB 9 + Pfa 2	125	18.8803	-0.0276	0.95	110	12.2538	-0.0305	0.90	175	8.3824	-0.0258	0.98
Azo II + PB 9 + Pfa 2	360	14.0699	-0.0280	0.98	260	12.4738	-0.0310	0.98	230	8.2209	-0.0246	0.98

Table 33. Estimated logistic negative* fungal population dynamics model in the rhizosphere of Cotton (MCU9) due to the combined inoculation of rhizobacterial isolates with graded levels of N and P

Treatments	100% N & P				75% N & P				50% N & P			
	P _M	α	β	r'	P _M	α	β	r'	P _M	α	β	r'
Azo II alone	25	7.4013	-0.0196	0.97	25	9.9758	-0.0138	0.94	12	2.8933	-0.0103	0.87
PB 9 alone	35	7.8583	-0.0189	0.98	35	6.3839	-0.0157	0.96	22	3.3661	-0.0134	0.90
Pfa 2 alone	40	6.5642	-0.0190	0.97	35	6.8005	-0.0201	0.97	32	5.3679	-0.0187	0.94
Azo II + PB 9	35	7.3361	-0.0208	0.98	30	16.4374	-0.0245	0.96	25	7.8491	-0.0203	0.95
Azo II + Pfa 2	55	20.8878	-0.0266	0.90	40	4.8271	-0.0193	0.95	37	8.9494	-0.0225	0.97
PB 9 + Pfa 2	60	8.4428	-0.0223	0.97	45	8.4638	-0.0231	0.96	45	6.9059	-0.0203	0.97
Azo II + PB 9 + Pfa 2	65	9.6922	-0.0247	0.95	55	9.4010	-0.0232	0.98	60	6.8940	-0.0229	0.95

* Declined growth rate

Table 34. Estimated logistic actinomycetes population dynamics model in the rhizosphere of Cotton (MCU9) due to the combined inoculation of rhizobacterial isolates with graded levels of N and P

Treatments	100% N & P				75% N & P				50% N & P			
	P _M	α	β	r'	P _M	α	β	r'	P _M	α	β	r'
Azo II alone	30	11.1135	-0.0256	0.93	18	11.7815	-0.0192	0.97	12	7.7542	-0.0145	0.89
PB 9 alone	30	9.4903	-0.0239	0.91	15	9.2848	-0.0135	0.94	32	6.9479	-0.0208	0.93
Pfa 2 alone	23	8.8915	-0.0208	0.91	12	7.8062	-0.0172	0.97	10	5.4081	-0.0160	0.93
Azo II + PB 9	30	15.7568	-0.0273	0.96	30	19.1938	-0.0268	0.96	10	5.9437	-0.0136	0.95
Azo II + Pfa 2	40	10.8896	-0.0267	0.97	25	9.9946	-0.0198	0.98	22	3.9647	-0.0150	0.91
PB 9 + Pfa 2	35	13.6442	-0.0268	0.93	25	19.1421	-0.0222	0.98	20	4.7887	-0.0157	0.87
Azo II + PB 9 + Pfa 2	60	9.2499	-0.0220	0.97	40	21.3318	-0.0274	0.96	30	5.2461	-0.0199	0.93

4.17.1.2. Estimated growth rate of total diazotrophic population in the rhizosphere of Cotton (MCU 9) due to the combined inoculation of rhizobacterial isolates with graded levels of N and P

The results are presented in Table 35 and Fig. 28. In general the growth rate of diazotrophs were high during 90 DAI and decreasing gradually, irrespective of all the nutrient levels and various rhizobacterial inoculants level which indicates that the microbial activity was found to be high in the rhizosphere of Cotton during 90 DAI.

Among the various nutrient levels tested, the growth rate was found to be high at reduced nutrient level (75% N & P) followed by recommended level (100% N & P) and 50 per cent N and P.

When comparing the growth rate of total diazotroph in individual and combined inoculation of rhizobacterial isolates it was higher in the case of combined inoculation at various graded nutrient levels.

Among the individual inoculation, the growth rate of total diazotroph was found to be higher due to the inoculation of *Azospirillum* (Azo 11) with 75 per cent N and P (0.8739) followed by 100 per cent N and P (0.8317) at 90 DAI. Interestingly, at 50 per cent N and P level the growth rate of total diazotrophs increased with phosphobacteria (PB 9) inoculation (0.3251) at 90 DAI.

In the case of combined inoculation of *Azospirillum* (Azo 11) + phosphobacteria (PB 9) + *Pseudomonas* (Pfa 2) showed a greater growth rate of total diazotrophs than other dual inoculation and individual inoculation of rhizobacterial isolates.

Among dual inoculation of rhizobacterial isolates, the growth rate of total diazotrophs was found to be higher at *Azospirillum* (Azo 11) + phosphobacteria, PB 9 (2.0267) followed by *Azospirillum* (Azo 11) + *Pseudomonas*, Pfa 2 (1.6629) and phosphobacteria (PB 9) + *Pseudomonas*, Pfa 2 (1.4479) at 75 per cent N and P.

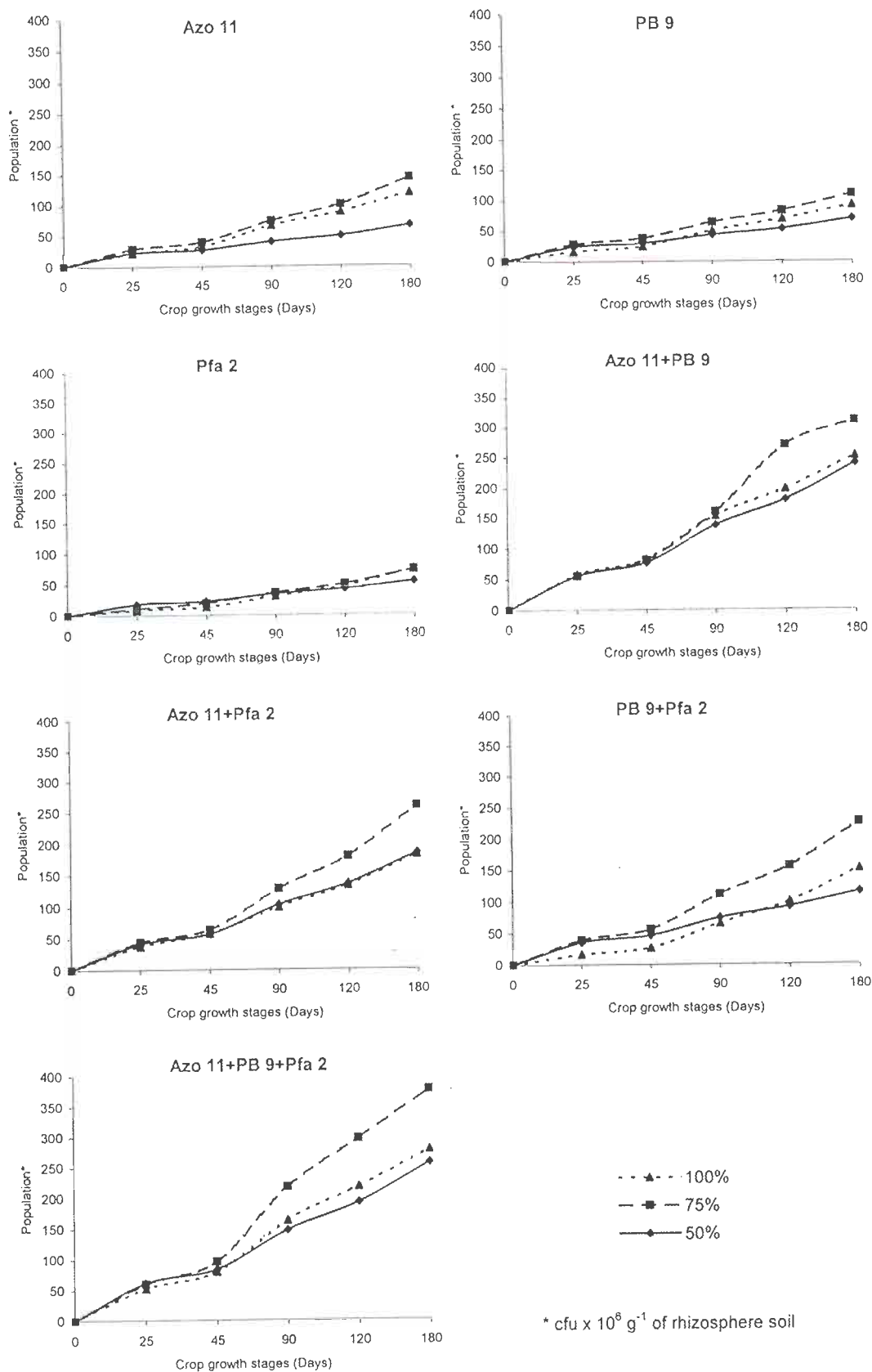
In general the lower growth rate of total diazotrophs was obtained by the individual inoculation of *Pseudomonas*, Pfa 2 at various nutrient levels tested.

Table 35. Estimated growth rate of total diazotrophic population in the rhizosphere of Cotton (MCU9) due to the combined inoculation of rhizobacterial isolates with graded levels of N and P

Treatments	100% N & P					75% N & P					50% N & P				
	25 DAI	45 DAI	90 DAI	120 DAI	180 DAI	25 DAI	45 DAI	90 DAI	120 DAI	180 DAI	25 DAI	45 DAI	90 DAI	120 DAI	180 DAI
Azo II alone	0.4558	0.6178	0.8317	0.7114	0.2672	0.4832	0.6215	0.8739	0.8698	0.5276	0.2369	0.2691	0.3112	0.3024	0.2219
PB 9 alone	0.3367	0.4627	0.6449	0.5621	0.2140	0.4169	0.5065	0.6233	0.5850	0.3344	0.2494	0.2823	0.3251	0.3181	0.2336
Pfa 2 alone	0.1886	0.2740	0.4936	0.5502	0.3213	0.2367	0.3059	0.4490	0.4375	0.2838	0.2128	0.2411	0.2658	0.2444	0.1513
Azo II + PB 9	1.0873	1.3777	1.6227	0.7534	0.5118	1.0686	1.4219	2.0267	1.9465	0.9782	0.9377	1.1706	1.4575	0.1295	0.6557
Azo II + Pfa 2	0.6748	0.8670	1.1467	1.0656	0.5325	0.8454	1.1274	1.6699	1.6629	0.9226	0.7002	0.8786	1.1721	1.0409	0.5432
PB 9 + Pfa 2	0.4057	0.6082	1.1465	1.1010	0.5277	0.7381	0.9819	1.4479	1.4426	0.8089	0.5122	0.5941	0.6315	0.5302	0.2514
Azo II + PB 9 + Pfa 2	1.3840	1.8643	2.3659	1.8978	0.6330	1.5038	2.1565	2.9162	2.2445	0.6206	0.9985	1.2306	1.5222	1.3999	0.7406

DAI - Days after inoculation

Fig. 28. Logistic population of total diazotroph in the rhizosphere of Cotton (MCU9) due to the combined inoculation of rhizobacterial isolates with graded level of N and P



4.17.1.3. Estimated growth rate of bacterial population in the rhizosphere of Cotton (MCU 9) due to the combined inoculation of rhizobacterial isolates with graded levels of N and P

The results are presented in Table 36 and Fig. 29. The growth rate of bacteria was invariably higher during 90 DAI and declined at all the parameters tested.

Among the various nutrient levels tested, 100 per cent N and P favoured higher growth rate of bacteria with various rhizobacterial inoculations at 90 DAI. Surprisingly, the bacterial growth rate was higher at 75 per cent N and P level inoculated with phosphobacteria (PB 9) and *Pseudomonas* (Pfa 2) alone at 90 DAI.

When comparing the growth rate of bacteria among individual and combined inoculation of rhizobacterial isolates, the higher growth rate was obtained with combined inoculation at 90 DAI.

The individual inoculation of *Azospirillum* (Azo 11) followed by *Pseudomonas* (Pfa 2) gave higher growth rate of bacteria at 75 per cent N and P (1.0602 and 0.9004) followed by 100 per cent N and P (1.2418 and 0.8717) respectively. Interestingly at 50 per cent N and P level, the bacterial growth rate was higher with *Azospirillum*, Azo 11 (0.6277) followed by phosphobacteria (PB 9) (0.6069) inoculation during 90 DAI.

The combined inoculation of *Azospirillum* (Azo 11) + phosphobacteria (PB 9) + *Pseudomonas* (Pfa 2) increased the growth rate of bacteria to 2.0853, 2.0122 and 1.4104 with 100 per cent N and P followed by 75 per cent N and P and 50 per cent N and P at 90 DAI.

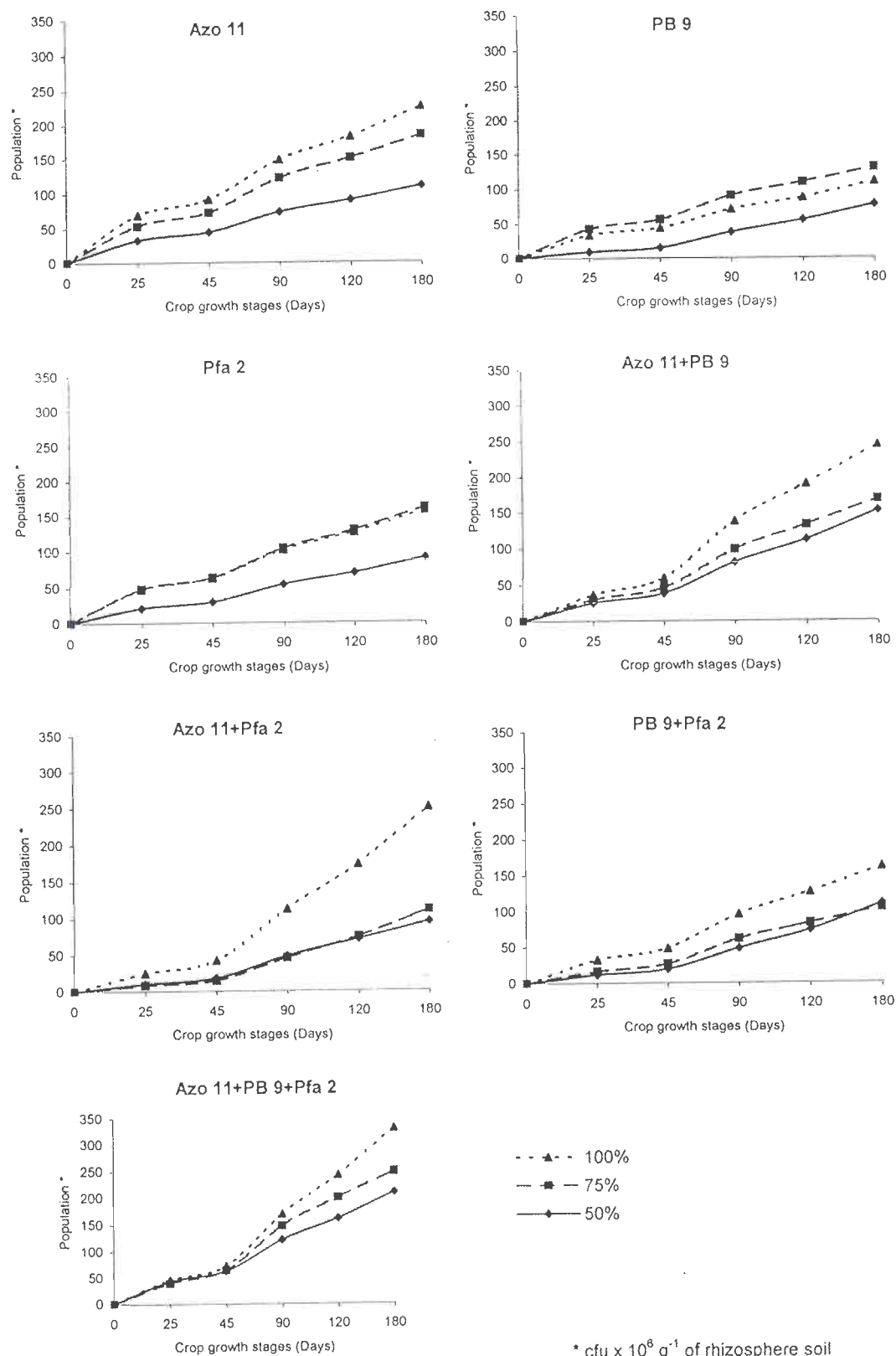
When comparing the growth rate of bacteria due to dual inoculation of rhizobacterial isolates, it was higher at 100 per cent N and P followed by 75 and 50 per cent N and P. In the case of 100 per cent N and P, *Azospirillum* (Azo 11) + *Pseudomonas*, Pfa 2 (1.9871) followed by *Azospirillum* (Azo 11) + phosphobacteria, PB 9 (1.9238) and phosphobacteria (PB 9) + *Pseudomonas*, Pfa 2 (0.8330), in 75 per cent N and P level, *Azospirillum* (Azo 11) + phosphobacteria, PB 9 (1.2401) followed by *Azospirillum* (Azo 11) + *Pseudomonas*, Pfa 2 (0.9267) and phosphobacteria (PB 9) + *Pseudomonas*, Pfa 2 (0.8262) and in 50 per cent N and P level the phosphobacteria (PB 9) + *Pseudomonas*, Pfa 2

Table 36. Estimated growth rate of bacterial population in the rhizosphere of Cotton (MCU9) due to the combined inoculation of rhizobacterial isolates with graded levels of N and P

Treat- ments	100% N & P					75% N & P					50% N & P				
	25 DAI	45 DAI	90 DAI	120 DAI	180 DAI	25 DAI	45 DAI	90 DAI	120 DAI	180 DAI	25 DAI	45 DAI	90 DAI	120 DAI	180 DAI
Azo II alone	1.0322	1.1962	1.2418	1.0128	0.4494	0.8773	1.0339	1.0602	0.8274	0.3240	0.5294	0.6194	0.6277	0.4886	0.1924
PB 9 alone	0.4799	0.5520	0.5949	0.5071	0.2504	0.6577	0.7491	0.7078	0.5286	0.1977	0.2395	0.3623	0.6069	0.5535	0.1898
Pfa 2 alone	0.7071	0.8244	0.8717	0.7205	0.3263	0.7194	0.8442	0.9004	0.7527	0.3425	0.3768	0.4734	0.5667	0.4812	0.2042
Azo II + PB 9	0.9315	1.3600	1.9238	1.5119	0.4206	0.6902	0.9545	1.2401	0.9741	0.2980	0.5455	0.7502	1.0727	0.9657	0.3926
Azo II + Pfa 2	0.6738	1.0629	1.9871	1.9189	0.6690	0.2466	0.4204	0.9267	0.9217	0.2891	0.2824	0.4460	0.7749	0.6738	0.1929
PB 9 + Pfa 2	0.2980	0.4517	0.8330	0.8195	0.3540	0.4251	0.6180	0.8262	0.6115	0.1537	0.6814	0.9019	1.1180	0.8999	0.3117
Azo II + PB 9 + Pfa 2	0.8901	1.3106	2.0853	0.0920	0.6664	1.0637	1.5632	2.0122	1.3923	0.3068	0.8483	1.1125	1.4104	1.1891	0.4604

DAI - Days after inoculation

Fig. 29. Logistic population of bacteria in the rhizosphere of Cotton (MCU9) due to the combined inoculation of rhizobacterial isolates with graded levels of N and P



(1.1180) followed by *Azospirillum* (Azo 11) + phosphobacteria, PB 9 (1.0727) and *Azospirillum* + *Pseudomonas* (0.7749) showed higher and lower growth rates during 90 DAI respectively.

4.17.1.4. Estimated negative growth rate of fungal population in the rhizosphere of Cotton (MCU9) due to the combined inoculation of rhizobacterial isolates with graded levels of N and P

The results are presented in Table 37 Fig. 30. The growth rate of fungus was negative at various parameters tested. The highest growth rate was obtained during 90 DAI irrespective of various nutrient levels and rhizobacterial inoculation.

The negative growth rate was maximum at 100 per cent N and P followed by 75 and 50 per cent N and P. Interestingly, at 50 per cent N and P the negative growth rate was higher than 75 per cent N and P in inoculation of phosphobacteria (PB 9) and *Pseudomonas* (Pfa 2) on 90 DAI.

Among, the individual and combined inoculation, the higher negative growth rate was obtained due to combined inoculation than individual inoculation.

The higher negative growth rate was observed due to inoculation of *Pseudomonas*, Pfa 2 (0.1886, 0.1753 and 0.1496) followed by phosphobacteria, PB 9 (0.1601, 0.1373 and 0.0736) and *Azospirillum*, Azo 11 (0.1207, 0.0819 and 0.0307) individually at 100, 75 and 50 per cent N and P respectively at 90 DAI.

When comparing growth rate of fungi in combined inoculation of rhizobacterial isolates, *Azospirillum* (Azo 11) + Phosphobacteria (PB 9) + *Pseudomonas* (Pfa 2) showed (0.4011, 0.3420 & 0.3171) higher growth rate at 100, 75 and 50 per cent N and P respectively than other dual inoculation of rhizobacterial isolates.

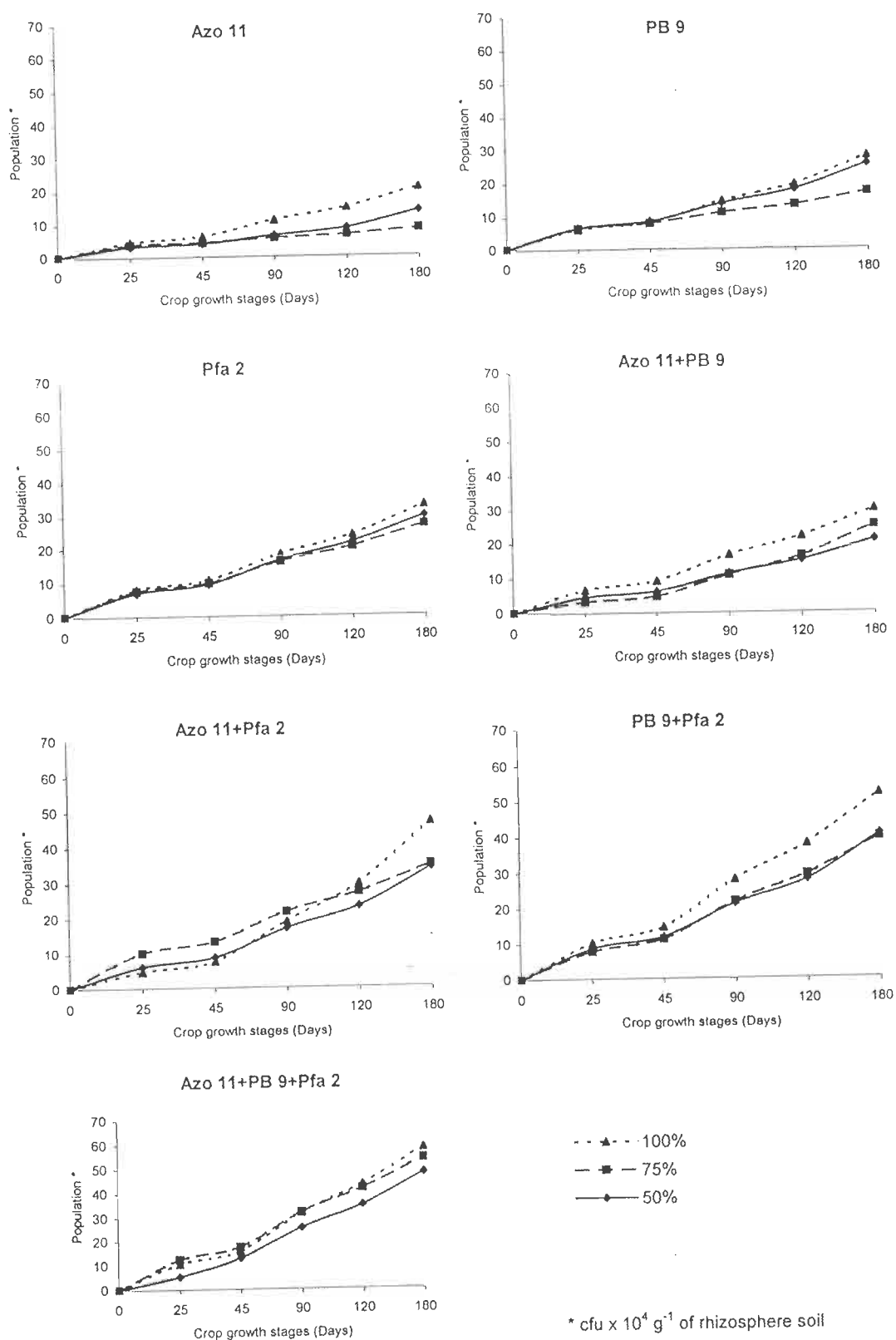
Among the dual inoculation of rhizobacterial isolates, phosphobacteria (PB 9) + *Pseudomonas* (Pfa 2) followed by *Azospirillum* (Azo 11) + *Pseudomonas* (Pfa 2) and *Azospirillum* (Azo 11) + phosphobacteria (PB 9) showed higher negative growth rate of 0.3331, 0.3301 and 0.1813 at 100 per cent N and P, 0.2277, 0.2066, 0.1251 at 50 per cent N and P and 0.2900, 0.1917 and 0.1424 at 75 per cent N and P respectively.

Table 37. Estimated negative* growth rate of fungal population in the rhizosphere of Cotton (MCU9) due to the combined inoculation of rhizobacterial isolates with graded levels of N and P

Treat- ments	100% N & P					75% N & P					50% N & P				
	25 DAI	45 DAI	90 DAI	120 DAI	180 DAI	25 DAI	45 DAI	90 DAI	120 DAI	180 DAI	25 DAI	45 DAI	90 DAI	120 DAI	180 DAI
Azo II alone	0.0725	0.2196	0.1207	0.1188	0.0718	0.0445	0.0556	0.0948	0.0819	0.0917	0.0263	0.0282	0.0307	0.0306	0.0265
PB 9 alone	0.0931	0.1169	0.1636	0.1601	0.1087	0.0839	0.1005	0.1373	0.1309	0.1094	0.0611	0.0672	0.0736	0.0709	0.0524
Pfa 2 alone	0.1201	0.1475	0.1886	0.1826	0.1106	0.1106	0.1375	0.1753	0.1655	0.0918	0.1057	0.1260	0.1496	0.1382	0.0789
Azo II + PB 9	0.1104	0.1393	0.1813	0.1709	0.0917	0.066	0.0961	0.1828	0.1684	0.1020	0.0731	0.0928	0.1251	0.1225	0.0712
Azo II + Pfa 2	0.1139	0.1727	0.3636	0.3301	0.1846	0.1452	0.1708	0.1917	0.1687	0.0873	0.1141	0.1497	0.2066	0.1952	0.0971
PB 9 + Pfa 2	0.1900	0.2469	0.3331	0.3110	0.1536	0.1493	0.1951	0.2900	0.2352	0.1073	0.1427	0.1780	0.2277	0.2144	0.1175
Azo II + PB 9 + Pfa 2	0.2164	0.2917	0.4011	0.3568	0.1515	0.1711	0.2273	0.3171	0.2965	0.1406	0.2235	0.2823	0.3420	0.2919	0.1242

*Declined growth rate ; DAI - Days after inoculation

Fig. 30. Logistic negative population of fungi in the rhizosphere of Cotton (MCU9) due to the combined inoculation of rhizobacterial isolates with graded levels of N and P



4.17.1.5. Estimated growth rate of actinomycetes population in the rhizosphere of Cotton (MCU 9) due to the combined inoculation of rhizobacterial isolates with graded levels of N and P

The results are presented in the Table 38 and Fig. 31. In general the growth rate of actinomycetes was higher during 90 DAI at 50, 75 and 100 per cent N and P level with different rhizobacterial inoculation.

In general, the growth rate was higher at 100 per cent N and P followed by 75 and 50 per cent N and P at various rhizobacterial inoculants tested. The combined inoculation of rhizobacterial isolates enhanced the growth rate of actinomycetes than individual inoculation at various nutrients levels tested.

Among the individual inoculation of rhizobacterial isolates, *Azospirillum*, Azo 11 (0.1914) followed by phosphobacteria (PB 9) (0.1788) and *Pseudomonas*, Pfa 2 (0.1167) at 100 per cent N and P during 90 DAI enhanced the growth of actinomycetes. At 75 and 50 N and P, with inoculation of *Azospirillum*, Azo 11 (0.0858 & 0.0424) followed by *Pseudomonas*, Pfa 2 (0.0515, 0.0395) at 90 DAI showed higher growth rate of actinomycetes respectively. While phosphobacterial inoculation (0.0501, 0.0385) recorded lower growth rate of actinomycetes at 75 and 50 per cent N and P on 90 DAI and *Pseudomonas*, Pfa 2 (0.1167) during 90 DAI at 100 per cent N and P level respectively.

The dual inoculation of *Azospirillum* (Azo 11) + *Pseudomonas*, Pfa 2 (0.2669) followed by phosphobacteria (PB 9) + *Pseudomonas*, Pfa 2 (0.2404) and *Azospirillum* (Azo 11) + phosphobacteria PB 9, (0.2002) at 100 per cent N and P during 90 DAI recorded higher and lower growth rates respectively.

The growth rate of actinomycetes at 75 per cent N and P on 90 DAI with the inoculation of *Azospirillum* (Azo 11) + phosphobacteria PB 9 (0.1976) recorded maximum followed by phosphobacteria (PB 9) + *Pseudomonas* Pfa 2, (0.1359) and *Azospirillum* (Azo 11) + *Pseudomonas* Pfa 2, (0.1235) whereas at 50 per cent N and P level recorded the minimum growth rate.

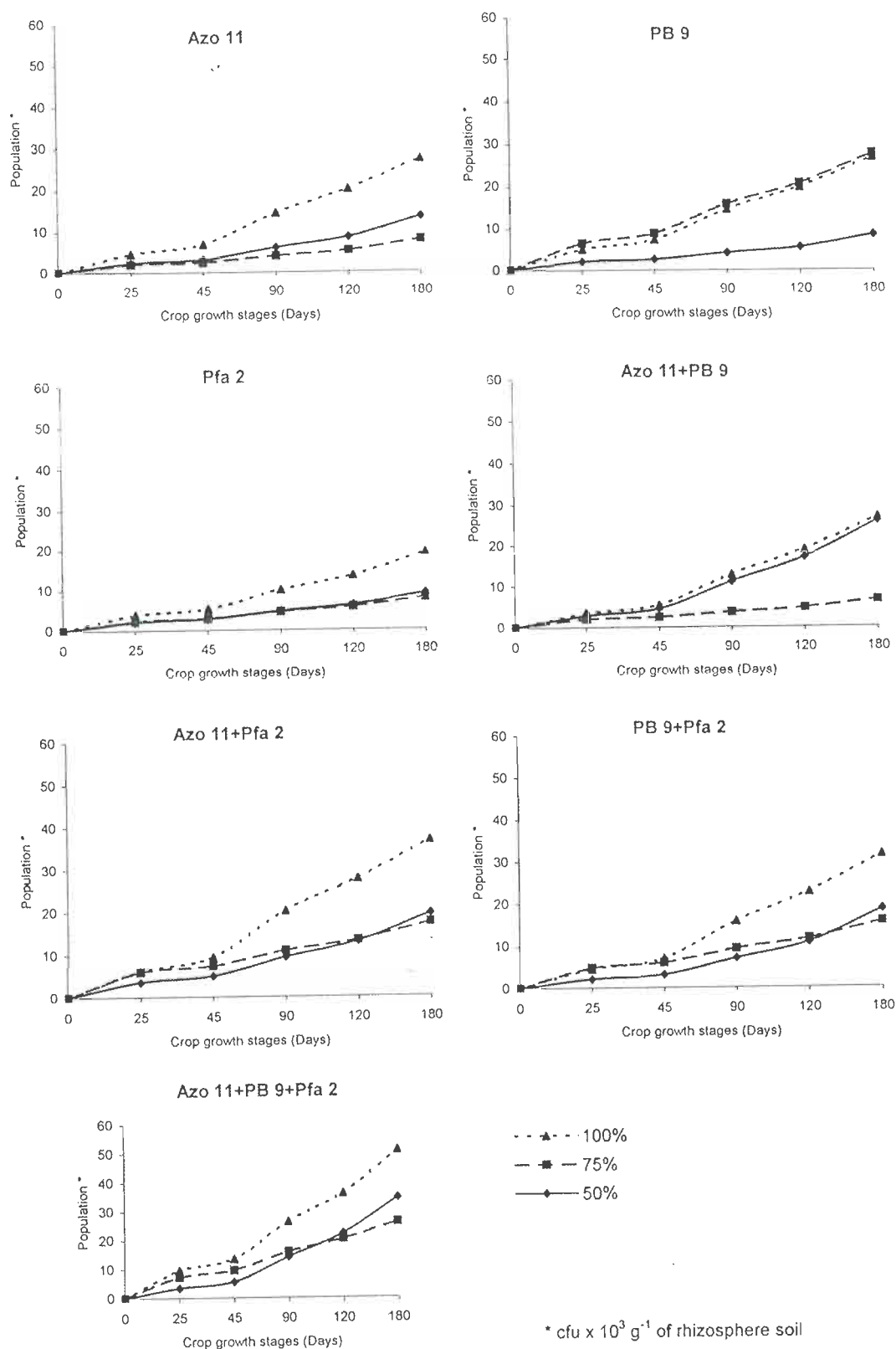
The combined inoculation of *Azospirillum* (Azo 11) + phosphobacteria (PB 9) + *Pseudomonas* (Pfa 2) recorded highest growth rate than other dual inoculation of rhizobacterial isolates. The growth rate was 0.3251, 0.2704 and 0.1485 at 100, 75 and

Table 38. Estimated growth rate of actinomycetes population in the rhizosphere of Cotton (MCU9) due to the combined inoculation of rhizobacterial isolates with graded levels of N and P

Treatments	100% N & P					75% N & P					50% N & P				
	25 DAI	45 DAI	90 DAI	120 DAI	180 DAI	25 DAI	45 DAI	90 DAI	120 DAI	180 DAI	25 DAI	45 DAI	90 DAI	120 DAI	180 DAI
Azo II alone	0.0956	0.1324	0.1914	0.1723	0.0689	0.0366	0.0482	0.0756	0.0858	0.0682	0.0229	0.0276	0.0424	0.0380	0.0402
PB 9 alone	0.0967	0.1292	0.1788	0.1631	0.0723	0.0230	0.0279	0.0131	0.0501	0.0462	0.0261	0.0326	0.0385	0.0150	0.0201
Pfa 2 alone	0.0639	0.0828	0.1167	0.1167	0.0687	0.0283	0.0351	0.0484	0.0515	0.0398	0.0272	0.0320	0.0395	0.0395	0.0285
Azo II + PB 9	0.0811	0.0119	0.2002	0.1915	0.0761	0.0674	0.1015	0.1869	0.1976	0.0930	0.0210	0.02450	0.03380	0.03148	0.0304
Azo II + Pfa 2	0.1375	0.1913	0.2669	0.2270	0.0802	0.0601	0.0782	0.1158	0.1235	0.0848	0.0648	0.0731	0.0824	0.0789	0.0548
PB 9 + Pfa 2	0.1074	0.1559	0.2404	0.2142	0.0764	0.0424	0.0603	0.1114	0.1359	0.1068	0.0566	0.0656	0.0780	0.0765	0.0540
Azo II + PB 9 + Pfa 2	0.1754	0.2304	0.3251	0.3161	0.1682	0.0853	0.1308	0.2704	0.2511	0.1266	0.1084	0.1295	0.1485	0.1309	0.0663

DAI - Days after inoculation

Fig. 31. Logistic population of actinomycete in the rhizosphere of Cotton (MCU9) due to the combined inoculation of rhizobacterial isolates with graded levels of N and P



50 per cent N and P during 90 DAI respectively. In general, the growth rate of total diazotrophs was higher followed by bacteria and actinomycetes whereas fungi recorded negative growth rate at various parameters tested. Combined inoculation of rhizobacterial isolates improved the growth rate of total diazotrophs, bacteria and actinomycetes than individual inoculation and decreased the growth rate of fungi.

The growth rate of 2.0673, 2.0853 and 0.3251 was obtained for total diazotrophs, bacteria and actinomycetes at 75 per cent N and P level during 90 DAI with the combined inoculation of *Azospirillum* (Azo 11) + phosphobacteria (PB 9) + *Pseudomonas* (Pfa 2) respectively and negative growth rate of 0.4011 for fungi was recorded with the above treatments.

4.17.2. Effect of rhizobacterial inoculation of *Azospirillum* (Azo 11), phosphobacteria (PB 9) and *Pseudomonas* (Pfa 2) on the growth, nutrient content and yield of Cotton var. MCU9

The survival of inoculated rhizobacterial isolates was tested at intervals as mentioned in the materials and methods. Plant growth and yield parameters like shoot and root length, root volume, number and length of lateral roots, monopodial and symbodial branches, total number of bolls, boll weight, dry matter production and seed Cotton yield were recorded. Available N, P, K level of soil after harvest was also estimated (Plate 11).

4.17.2.1. Survival of *Azospirillum* (Azo 11) isolates in Cotton rhizosphere (MCU9) with combined inoculation of rhizobacterial isolates at graded levels of N and P

The results have revealed that *Azospirillum* isolate (Azo 11) registered the maximum population of 79.4, 87.3, 64.3 x 10⁸ cfu g⁻¹ of rhizosphere soil on 90 DAI at 100, 75, 50 per cent of N and P respectively in combined inoculation of *Azospirillum* (Azo 11) + phosphobacteria (PB 9) + *Pseudomonas* (Pfa 2). The *Azospirillum* (Azo 11) population was found to be maximum on 90 DAI in single and combined inoculation. The population was found to decline from 120th DAI onwards (Table 39). Similar trend was also observed due to dual inoculation of phosphobacteria (PB 9) and *Pseudomonas* (Pfa 2) alone. The population of *Azospirillum* (Azo 11) increased due to the combination of phosphobacteria (PB 9) when compared to *Pseudomonas* (Pfa 2). At reduced level of N and P (75%)



Plate 11. Effect of combined inoculation of rhizobacterial isolates on Cotton (MCU9)

Table 39. Survival of *Azospirillum* isolate in the Cotton rhizosphere (MCU9) with combined inoculation of rhizobacterial isolates at graded levels of N and P

Treatment	Population (cfu g ⁻¹ of rhizosphere soil)																				
	Initial x 10 ⁸			7 DAI x 10 ⁸			25 th DAI x 10 ⁸			45 th DAI x 10 ⁸			90 th DAI x 10 ⁸			120 th DAI x 10 ⁷			180 th DAI x 10 ⁶		
	100% N&P	75% N&P	50% N&P	100% N&P	75% N&P	50% N&P	100% N&P	75% N&P	50% N&P	100% N&P	75% N&P	50% N&P	100% N&P	75% N&P	50% N&P	100% N&P	75% N&P	50% N&P	100% N&P	75% N&P	50% N&P
Azo 11 alone	2.3 (0.4)	1.2 (0.2)	2.3 (0.3)	16.0 (1.2)	18.3 (1.3)	17.0 (1.2)	24.6 (1.4)	28.7 (1.6)	21.3 (1.5)	43.3 (1.3)	44.6 (1.7)	34.6 (1.5)	51.6 (1.7)	62.3 (1.8)	42.3 (1.6)	35.0 (1.5)	54.2 (1.7)	32.3 (1.5)	26.6 (1.4)	30.0 (1.5)	28.0 (1.5)
Azo 11 + PB 9	1.3 (0.3)	1.3 (0.3)	3.3 (0.5)	22.0 (1.3)	26.3 (1.4)	18.3 (1.3)	28.3 (1.5)	32.6 (1.7)	24.6 (1.5)	53.2 (1.4)	63.7 (1.8)	42.6 (1.6)	66.3 (1.8)	78.7 (1.9)	56.6 (1.8)	58.3 (1.8)	64.2 (1.8)	42.0 (1.6)	33.4 (1.5)	36.7 (1.6)	36.6 (1.6)
Azo 11 + Pfa 2	0 (0.3)	1.2 (0.2)	2.0 (0.4)	25.1 (1.4)	28.1 (1.5)	21.6 (1.3)	34.2 (1.5)	46.1 (1.8)	28.6 (1.7)	57.7 (1.5)	74.1 (1.9)	54.3 (1.7)	65.3 (1.8)	65.4 (1.8)	62.0 (1.8)	54.1 (1.7)	51.1 (1.7)	56.6 (1.7)	41.6 (1.6)	47.3 (1.7)	47.0 (1.7)
Azo 11 + PB 9 + Pfa 2	3 (0.5)	0 (-0.3)	1.2 (0.2)	26.7 (1.4)	32.4 (1.5)	23.6 (1.4)	38.5 (1.6)	43.3 (1.8)	33.4 (1.6)	62.3 (1.5)	76.3 (1.9)	56.1 (1.8)	79.4 (1.9)	87.3 (1.9)	64.3 (1.8)	63.2 (1.8)	65.7 (1.8)	62.3 (1.8)	52.5 (1.7)	35.8 (1.6)	38.1 (1.6)
Uninoculated control	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)

(Figures in parentheses are log₁₀ transformed values) ; DAI - Days after inoculation

CD (P=0.05)

Days 0.0283

N & P 0.0185

Treatment 0.239

Days x N & P 0.0490

N & P x Treatments 0.0414

Days x Treatments 0.0632

Days x N & P x Treatments 0.1096

Azospirillum (Azo 11) population was high when compared to recommended level (100% N & P) (Fig 32). In general combined inoculation increased the population of *Azospirillum* (Azo 11) than individual inoculation.

4.17.2.2. Survival of phosphobacterial isolate (PB 9) in Cotton rhizosphere with combined inoculation of rhizobacterial isolates at graded levels of N and P

Combined inoculation of *Azospirillum* (Azo 11), phosphobacteria (PB 9) and *Pseudomonas* (Pfa 2) under graded levels of N and P increased the phosphobacterial population, 87.6, 112.3, 76.3 $\times 10^8$ cfu $\times 10^8$ g⁻¹ of rhizosphere soil on 90 DAI. In general population of phosphobacteria was high at reduced level (75% N & P) on 90 DAI. Decrease in the population of phosphobacteria was noticed after 90 DAI. The maximum population was recorded at 100 per cent N and P level due to combined inoculation of *Azospirillum* (Azo 11) and *Pseudomonas* (Pfa 2) (Table 40 and Fig. 33).

4.17.2.3. Survival of *Pseudomonas* (Pfa 2) isolate in Cotton rhizosphere (MCU9) with combined inoculation of rhizobacterial isolates at graded levels of N and P

The combined inoculation of *Azospirillum* (Azo 11), Phosphobacteria (PB 9) and *Pseudomonas* (Pfa 2) increased *Pseudomonas* (Pfa 2) population upto 144.6 cfu $\times 10^8$ g⁻¹ of rhizosphere soil on 90 DAI at 75 per cent of N and P. At recommended level of N and P (100%) the *Pseudomonas* (Pfa 2) population was 98.6 $\times 10^8$ cfu g⁻¹ rhizosphere soil on 90 DAI due to combined inoculation of phosphobacteria (Pfa 2) and *Azospirillum* (Azo 11) (Table 41).

The population of *Pseudomonas* tends to decrease after 90 DAI, at all levels of N and P tested. *Azospirillum* (Azo 11) and phosphobacteria (PB 9) found to increase the *Pseudomonas* (Pfa 2) population at 75 and 50 per cent N and P levels whereas at 100 per cent N and P the population of *Pseudomonas* (Pfa 2) was less. In general, combined inoculation of rhizobacterial isolates increased the population of *Pseudomonas* (Pfa 2) than individual inoculation (Fig. 34).

4.17.3. Effect of combined rhizobacterial inoculation on growth parameters of Cotton (MCU9) with graded levels of N and P

4.17.3.1. Shoot length

The combined application of rhizobacterial isolates, *Azospirillum* (Azo 11), phosphobacteria (PB 9) and *Pseudomonas* (Pfa 2) increased the shoot length at all levels of N and P when compared to uninoculated control. Among the single inoculation,

Fig. 32. Survival of *Azospirillum* in Cotton rhizosphere (MCU9) with combined rhizobacterial inoculation under graded levels of N and P

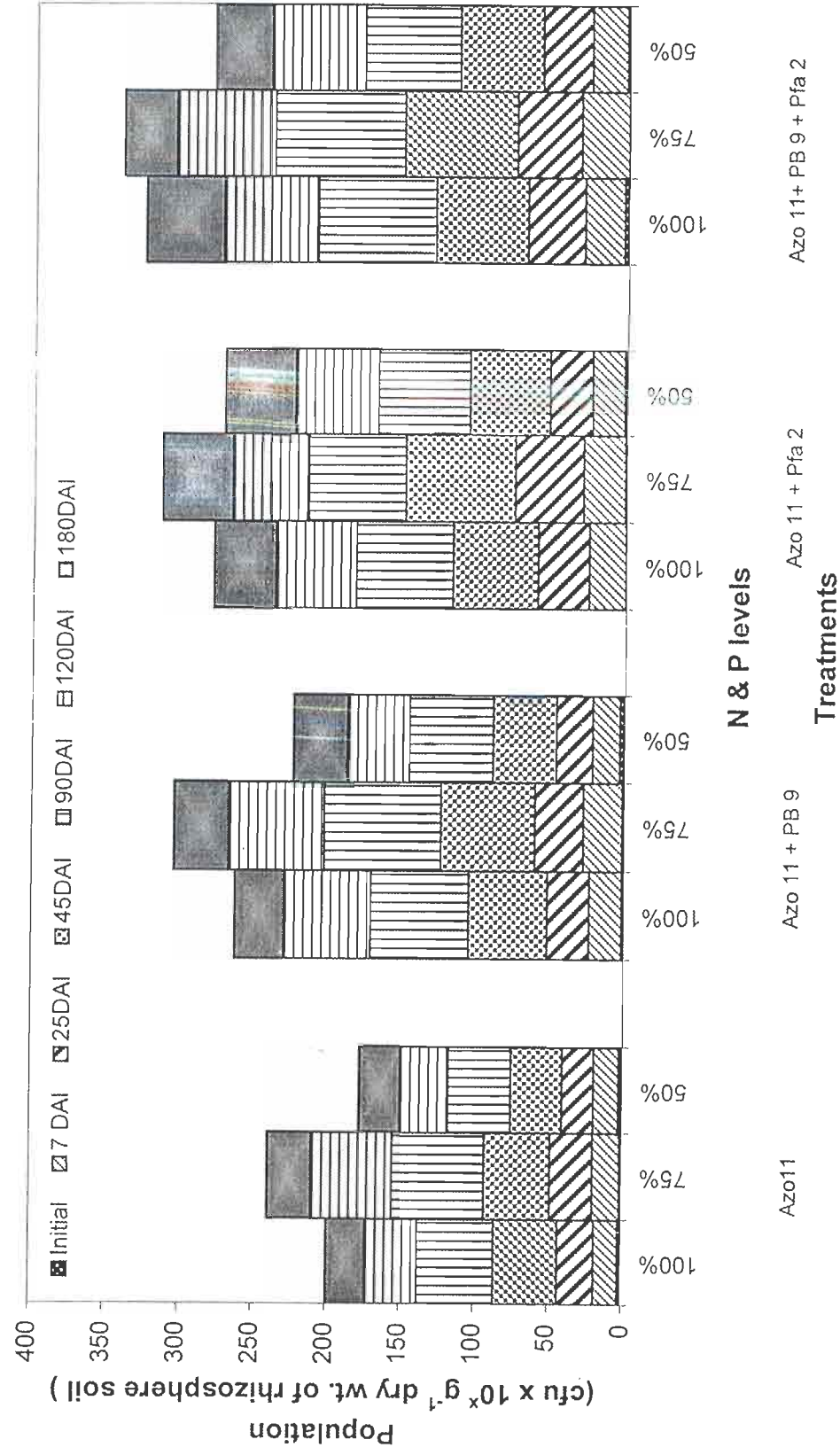


Table 40. Survival of phosphobacterial isolate in the Cotton rhizosphere (MCU9) with combined inoculation of rhizobacterial isolates at graded levels of N and P

Population (cfu g ⁻¹ of rhizosphere soil)																					
Treatment	Initial x 10 ⁸			7 DAI x 10 ⁸			25 th DAI x 10 ⁸			45 th DAI x 10 ⁸			90 th DAI x 10 ⁸			120 th DAI x 10 ⁷			180 th DAI x 10 ⁶		
	100% N&P	75% N&P	50% N&P	100% N&P	75% N&P	50% N&P	100% N&P	75% N&P	50% N&P	100% N&P	75% N&P	50% N&P	100% N&P	75% N&P	50% N&P	100% N&P	75% N&P	50% N&P	100% N&P	75% N&P	50% N&P
Azo 11 alone	1.8 (0.4)	2.5 (0.5)	2.0 (0.3)	28.3 (1.5)	32.2 (1.7)	24.6 (1.5)	45.6 (1.4)	56.1 (1.8)	35.0 (1.5)	65.7 (1.8)	73.8 (1.9)	52.6 (1.7)	51.3 (1.7)	58.3 (1.8)	48.0 (1.7)	46.3 (1.7)	36.2 (1.6)	30.3 (1.5)			
Azo 11 + PB 9	1.3 (0.3)	1.5 (-0.3)	1.5 (1.5)	44.3 (1.8)	48.7 (1.7)	35.0 (1.5)	65.6 (1.9)	72.6 (1.8)	58.7 (1.9)	73.5 (1.9)	82.6 (1.9)	65.0 (1.8)	63.5 (1.8)	78.5 (1.9)	54.0 (1.7)	43.6 (1.6)	48.1 (1.7)	43.3 (1.6)			
Azo 11 + Pfa 2	2.7 (0.5)	0 (-0.3)	0 (-0.3)	52.1 (1.7)	54.4 (1.9)	46.7 (1.7)	72.3 (1.7)	87.3 (1.9)	65.0 (1.8)	78.6 (1.9)	108.2 (2.0)	71.0 (1.9)	66.2 (1.8)	83.3 (1.9)	59.3 (1.8)	48.3 (1.7)	45.6 (1.7)	56.6 (1.8)			
Azo 11 + PB 9 + Pfa 2	1.4 (0.3)	1.8 (0.3)	0 (0.3)	56.3 (1.8)	63.4 (1.9)	48.6 (1.8)	83.0 (1.7)	92.7 (2.3)	32.3 (1.8)	87.6 (1.9)	112.3 (2.1)	76.3 (1.9)	71.2 (1.9)	72.1 (1.9)	54.6 (1.7)	66.5 (1.8)	42.7 (1.6)	43.3 (1.6)			
Uninoculated control	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)

(Figures in parentheses are log₁₀ transformed values)

DAI - Days after inoculation

CD (P=0.05)

Days

0.0215

N & P

0.0141

Treatment

0.0182

Days x N & P

0.0374

N & P x Treatments

0.0316

Days x Treatments

0.0482

Days x N & P x Treatments

0.0836

Fig. 33. Survival of phosphobacteria in Cotton rhizosphere (MCU9) with combined rhizobacterial inoculation under graded levels of N and P

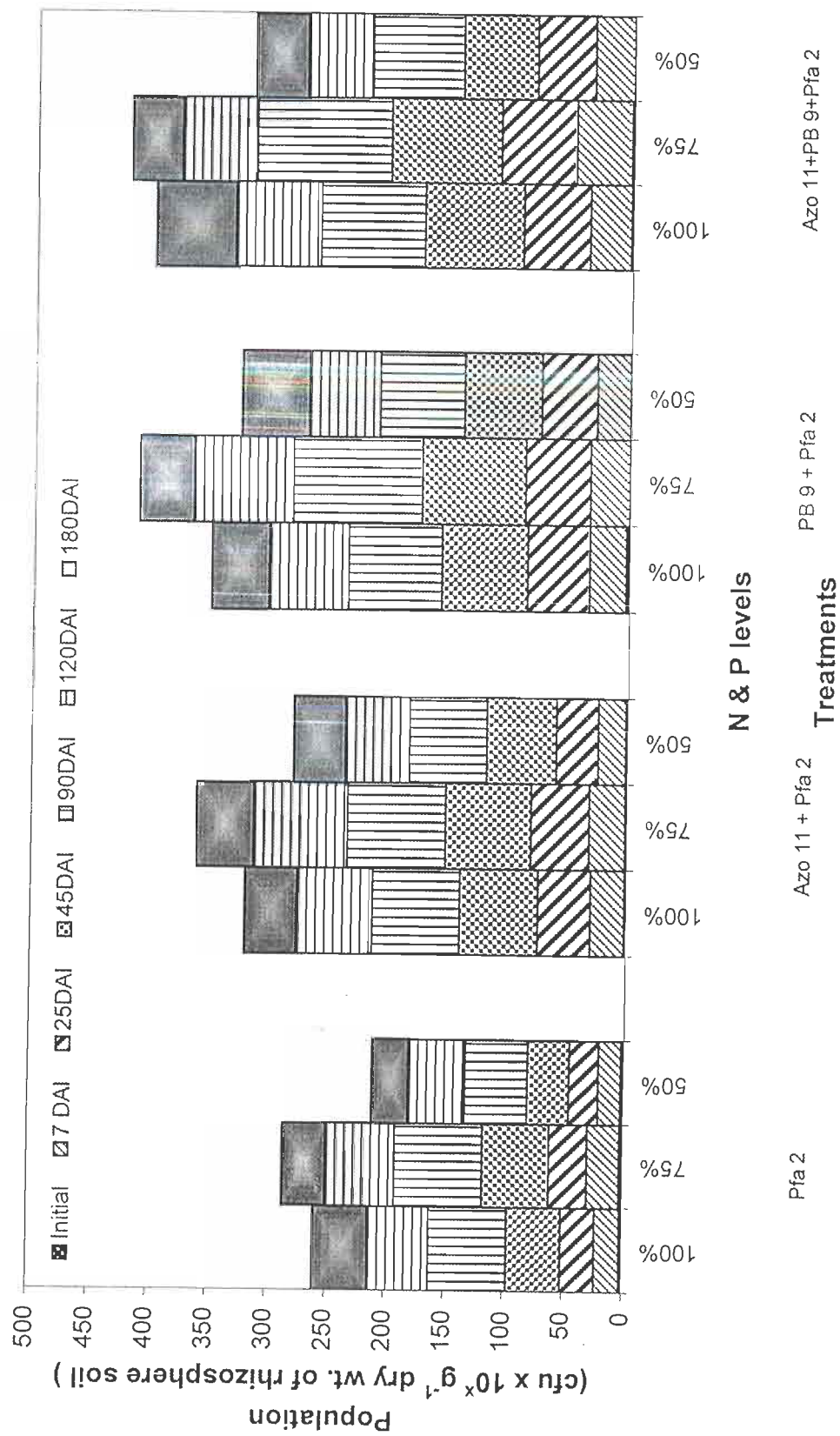


Table 41. Survival of *Pseudomonas* isolate in the Cotton rhizosphere (MCU9) with combined inoculation of rhizobacterial isolates at graded levels of N and P

Treatment	Population (cfu g ⁻¹ of rhizosphere soil)																				
	Initial x 10 ⁸			7 DAI x 10 ⁸			25 th DAI x 10 ⁸			45 th DAI x 10 ⁸			90 th DAI x 10 ⁸			120 th DAI x 10 ⁷			180 th DAI x 10 ⁶		
	100% N&P	75% N&P	50% N&P	100% N&P	75% N&P	50% N&P	100% N&P	75% N&P	50% N&P	100% N&P	75% N&P	50% N&P	100% N&P	75% N&P	50% N&P	100% N&P	75% N&P	50% N&P	100% N&P	75% N&P	50% N&P
Azo 11 alone	2.0 (0.4)	2.6 (0.5)	4.5 (0.3)	28.2 (1.5)	33.0 (1.5)	22.3 (1.4)	44.3 (1.6)	51.6 (1.8)	34.3 (1.7)	61.3 (1.5)	74.3 (1.9)	46.5 (1.7)	73.6 (1.9)	86.4 (1.9)	65.3 (1.8)	67.1 (1.8)	62.1 (1.8)	56.6 (1.8)	49.3 (1.7)	45.8 (1.7)	47.6 (1.7)
Azo 11 + PB 9	3.0 (0.5)	3.0 (0.5)	0 (-0.3)	41.3 (1.6)	32.4 (1.5)	30.3 (1.5)	46.2 (1.7)	52.6 (1.9)	48.0 (1.7)	76.4 (1.7)	85.4 (1.9)	65.3 (1.8)	78.1 (1.9)	92.8 (2.0)	76.3 (1.9)	58.4 (1.8)	78.2 (1.9)	61.3 (1.8)	33.7 (1.5)	56.3 (1.8)	53.3 (1.7)
Azo 11 + Pfa 2	0 (-0.3)	2.0 (0.4)	1.5 (0.3)	43.4 (1.6)	46.1 (1.7)	37.0 (1.6)	63.7 (1.8)	62.3 (1.9)	56.0 (1.8)	84.5 (1.8)	97.6 (2.1)	68.0 (1.8)	81.6 (1.9)	124.4 (2.3)	78.0 (1.9)	73.5 (1.9)	77.1 (2.0)	63.6 (1.8)	39.1 (1.6)	48.2 (1.7)	46.5 (1.7)
Azo 11 + PB 9 + Pfa 2	2.0 (0.4)	1.5 (0.3)	0 (-0.3)	44.3 (1.7)	48.1 (1.7)	40.1 (1.6)	68.1 (1.8)	68.6 (2.0)	62.3 (1.8)	93.6 (1.8)	101.1 (2.0)	78.1 (1.9)	98.6 (2.0)	144.6 (2.2)	85.3 (1.9)	86.6 (1.9)	78.6 (1.9)	53.2 (1.7)	71.7 (1.9)	40.2 (1.6)	38.7 (1.6)
Uninoculated control	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)	0 (-0.3)

(Figures in parentheses are log₁₀ transformed values) ; DAI - Days after inoculation

CD (P=0.05)

Days

N & P

Treatment

Days x N & P

N & P x Treatments

Days x Treatments

Days x N & P x Treatments

0.02371

0.0155

0.0200

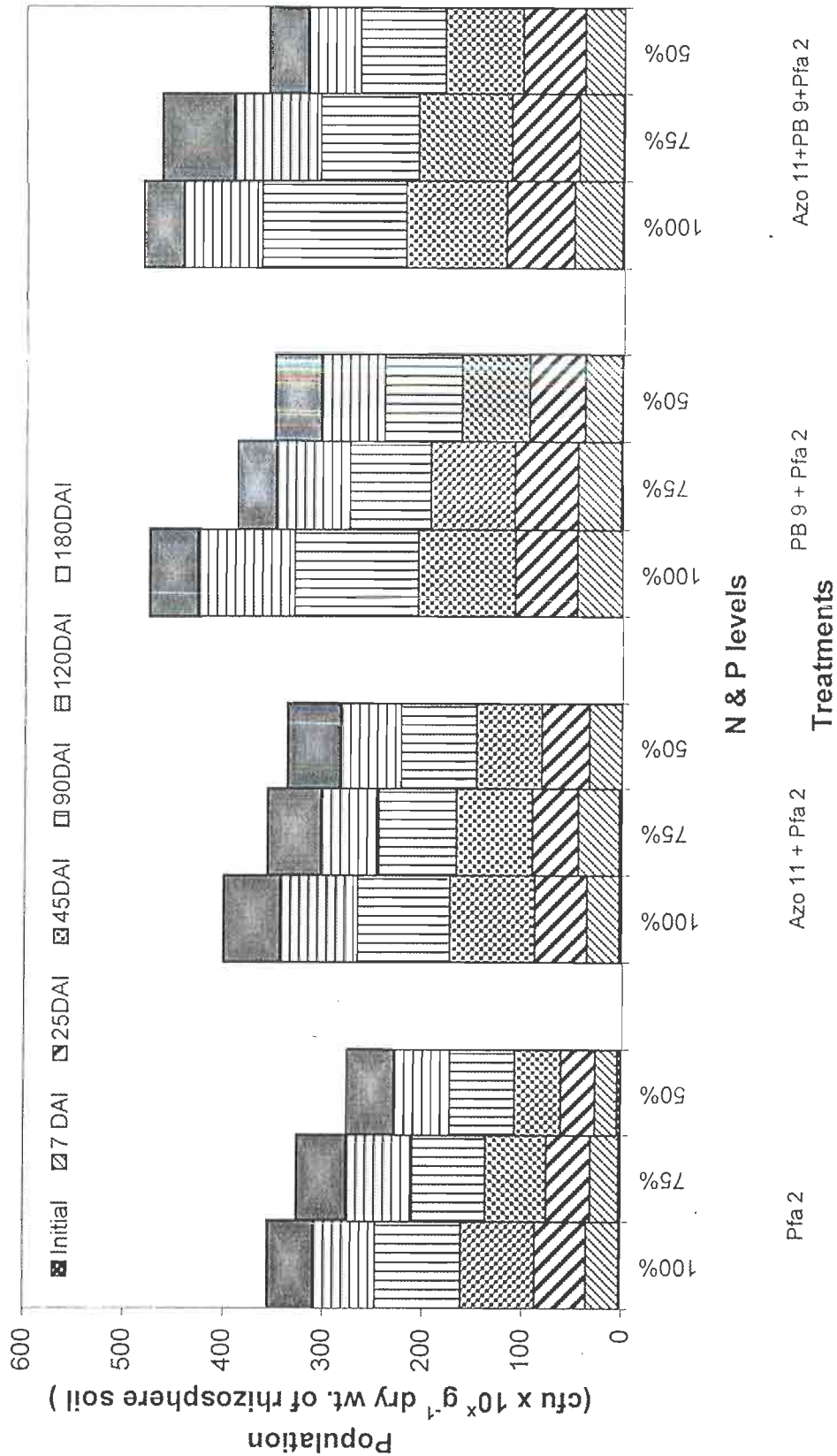
0.0410

0.0347

0.0630

0.0918

Fig. 34. Survival of *Pseudomonas* in Cotton rhizosphere (MCU9) with combined rhizobacterial inoculation under graded levels of N and P



Azospirillum (Azo 11) recorded maximum shoot length of 73 cm on 90th day whereas inoculation of phosphobacteria (PB 9) and *Pseudomonas* (Pfa 2) recorded 72.22 and 72.90 cm at 50 per cent N and P. Similar trend was observed on 25, 45, 120, 180 DAI in 75 and 100 per cent N and P (Table 42).

Inoculation of *Azospirillum* (Azo 11) with *Pseudomonas* (Pfa 2) or combination with phosphobacteria (PB 9) showed the maximum growth when compared to combination of phosphobacteria (PB 9) and *Pseudomonas* (Pfa 2) (Fig. 35).

In general, the shoot length increased with the age of the crop. There was no significant difference in shoot length due to combined inoculation of *Azospirillum* (Azo 11), phosphobacteria (PB 9) and *Pseudomonas* (Pfa 2) at 75 and 100 per cent N and P levels.

4.17.3.2. Root length

The root length of Cotton plants increased with the age of the crop. The single individual inoculation of *Azospirillum* (Azo 11) increased the root length to a maximum of 14.32 cm on 25 DAI when compared to *Pseudomonas* (Pfa 2) and phosphobacteria (PB 9) at reduced level of N and P (50%). Similar trend was observed in 45, 90, 120, 180 DAI in 75 and 100 per cent N and P (Table 43). But it is significantly higher than uninoculated control.

Among the dual inoculation, maximum root length was observed due to the inoculation of *Azospirillum* (Azo 11) and phosphobacteria (PB 9) followed by phosphobacteria (PB 9) and *Pseudomonas* (Pfa 2) at all the time intervals and N and P levels (Fig. 36).

The combined inoculation of *Azospirillum* (Azo 11) + phosphobacteria (PB 9) + *Pseudomonas* (Pfa 2) enhanced the shoot length at recommended level of N and P (100%) but it was on par with the reduced level of N and P (75%). In general the root length increased with the age of the crop.

4.17.3.3. Number of lateral roots

The results are presented in Table 44 and Fig. 37. The number of laterals increased to 26.1 cm at 180 DAI with the combined inoculation of *Azospirillum* (Azo 11) + phosphobacteria (PB 9) and *Pseudomonas* (Pfa 2) at recommended level of N and P. However, it is on par with 75 per cent N and P with the same treatment. Among the dual inoculation, *Azospirillum* (Azo 11) and phosphobacteria (PB 9) increased the number

Table 42. Effect of combined rhizobacterial inoculation on the shoot length of Cotton (MCU9) with graded levels of N and P

Treatments	Shoot length (cm)														
	100% N&P					75% N&P					50% N&P				
	25 DAI	45 DAI	90 DAI	120 DAI	180 DAI	25 DAI	45 DAI	90 DAI	120 DAI	180 DAI	25 DAI	45 DAI	90 DAI	120 DAI	180 DAI
Azo 11 alone	16.20	31.60	85.80	117.72	127.32	16.00	31.25	80.66	116.41	125.51	14.79	30.70	73.0	113.74	121.81
PB 9 alone	16.14	31.50	83.90	117.31	126.75	15.96	31.18	79.25	116.05	124.97	14.74	30.65	72.22	113.43	121.33
Pfa 2 alone	16.18	31.55	84.55	117.54	127.16	15.98	31.20	79.90	116.33	125.43	14.75	30.69	72.90	113.51	121.52
Azo 11 + PB 9	16.60	32.18	93.60	120.33	130.90	16.40	31.90	89.70	119.01	129.11	15.01	30.95	76.20	115.08	123.60
Azo 11 + Pfa 2	16.58	32.08	91.70	119.90	130.33	16.35	31.80	87.80	118.63	128.52	14.95	31.01	77.80	114.75	123.13
PB 9 + Pfa 2	16.59	32.15	92.33	120.11	130.71	16.37	31.85	88.44	118.92	128.90	14.97	30.90	76.00	114.82	123.46
Azo 11 + PB 9 +Pfa 2	16.75	32.45	97.60	121.50	132.53	16.70	32.32	95.70	121.22	132.11	16.02	31.30	81.90	116.41	125.55
Uninoculated control	15.02	31.03	77.90	115.11	123.74	14.80	30.74	74.00	113.80	121.90	14.73	30.63	72.10	112.52	118.01

CD (p=0.05)

DAI - Days after inoculation

N & P

Days

Treatment

N & P x days

Days x Treatments

N & P x Treatments

N & P x Treatments x days

0.1811

0.2338

0.2958

0.4051

0.6615

0.5124

1.1458

Fig. 35. Effect of combined rhizobacterial inoculation on the shoot length of Cotton (MCU9) with graded levels of N and P

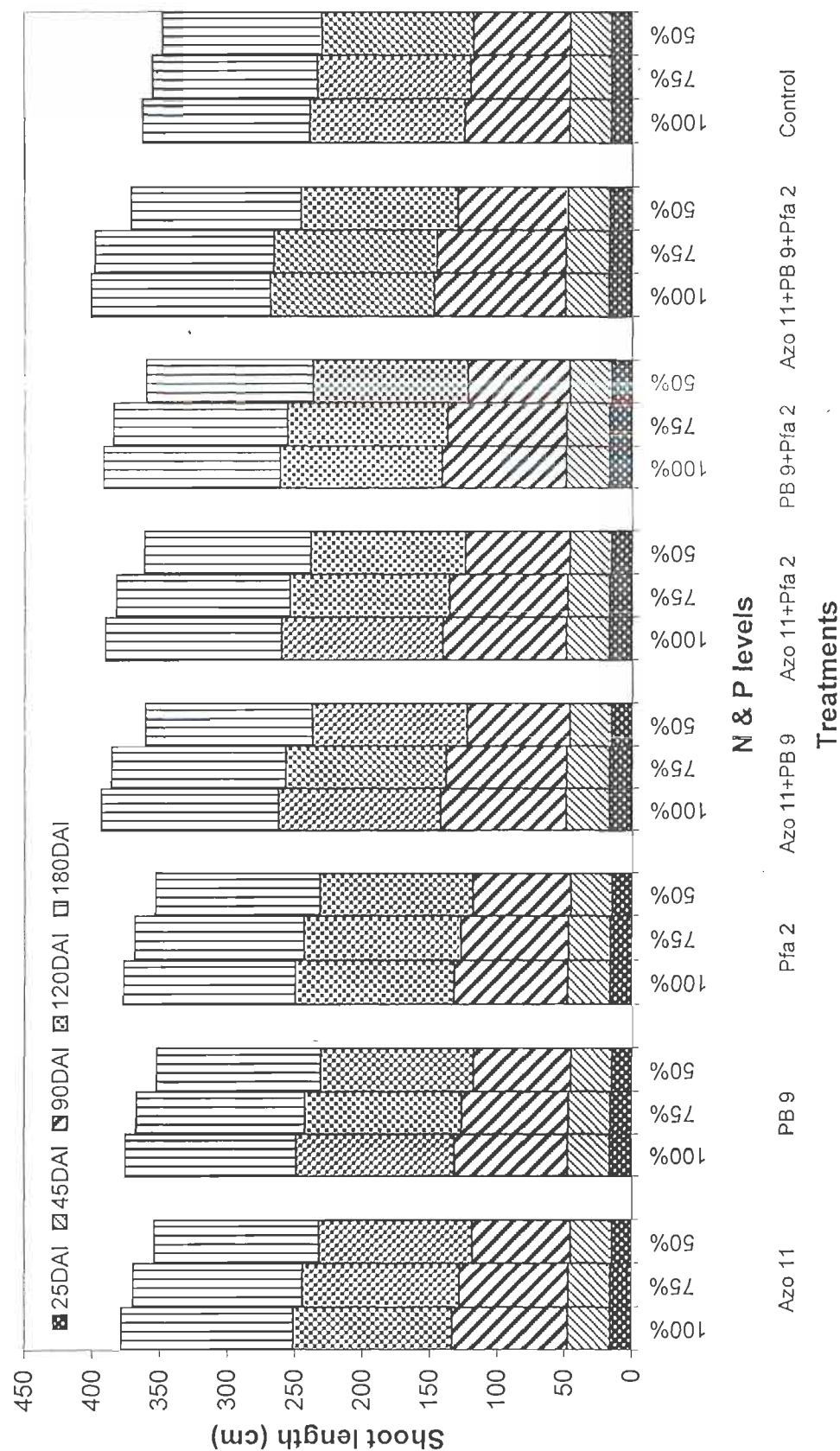


Table 43 . Effect of combined rhizobacterial inoculation on the root length of Cotton (MCU9) with graded levels of N and P

Treatments	Root length (cm)														
	100% N&P					75% N&P					50% N&P				
	25 DAI	45 DAI	90 DAI	120 DAI	180 DAI	25 DAI	45 DAI	90 DAI	120 DAI	180 DAI	25 DAI	45 DAI	90 DAI	120 DAI	180 DAI
Azo 11 alone	14.32	22.20	38.30	43.95	47.72	13.30	21.00	37.12	42.75	46.55	12.82	20.42	36.52	42.13	45.96
PB 9 alone	14.23	22.12	38.24	43.87	47.63	13.22	20.84	36.99	42.55	46.48	12.65	20.23	36.38	41.99	45.78
Pfa 2 alone	14.30	22.18	38.27	43.92	47.65	12.30	19.81	35.94	41.56	45.32	12.76	20.36	36.46	42.08	45.83
Azo 11 + PB 9	13.82	21.60	37.73	43.37	47.13	13.28	20.95	37.08	42.62	46.52	11.80	19.21	35.31	40.93	44.73
Azo 11 + Pfa 2	13.71	21.53	37.68	43.26	46.98	12.18	19.65	35.77	41.40	45.16	11.64	19.03	35.17	40.83	44.58
PB 9 + Pfa 2	13.76	21.58	37.71	43.33	47.08	12.25	19.76	35.81	41.47	45.28	11.72	19.15	35.29	40.85	44.66
Azo 11 + PB 9 +Pfa 2	14.64	22.61	38.74	44.37	48.16	14.12	22.01	38.14	43.79	47.58	13.64	21.41	37.55	43.18	46.96
Uninoculated control	13.74	20.80	36.96	42.57	46.37	11.24	18.57	34.66	40.26	43.98	11.31	18.62	34.72	40.33	44.14

CD (p=0.05)

N & P 0.1302

Days 0.1680

Treatment 0.2126

N & P x days 0.2911

Days x Treatments 0.4754

N & P x Treatments 0.3682

N & P x Treatments x days 0.8234

DAI - Days after inoculation

Fig. 36. Effect of combined rhizobacterial inoculation on the root length of Cotton (MCU9) with graded levels of N and P

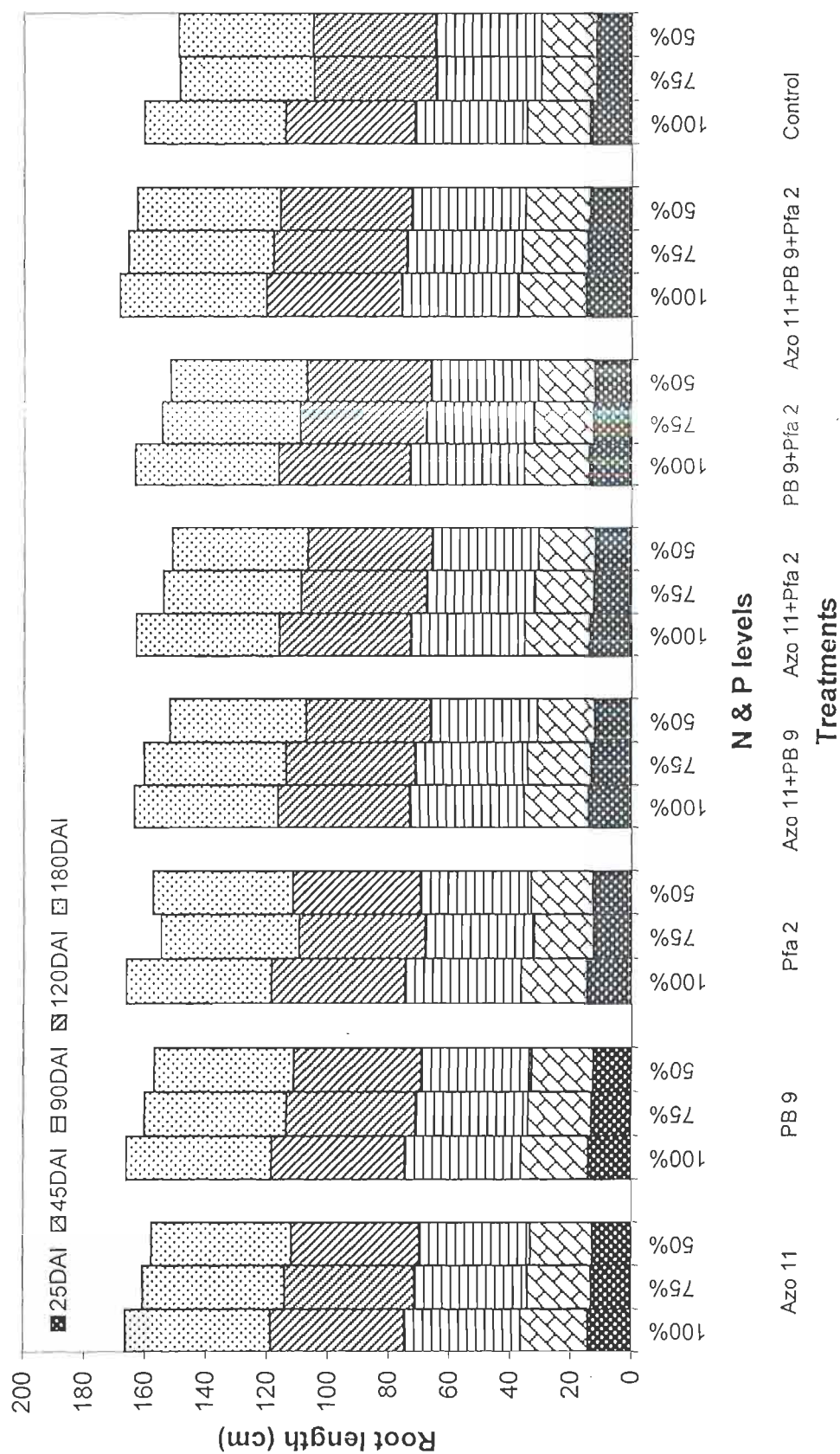


Table 44. Effect of combined rhizobacterial inoculation on the number of lateral roots of Cotton (MCU9) with graded levels of N and P

Treatments	No. of lateral roots														
	100% N&P					75% N&P					50% N&P				
	25 DAI	45 DAI	90 DAI	120 DAI	180 DAI	25 DAI	45 DAI	90 DAI	120 DAI	180 DAI	25 DAI	45 DAI	90 DAI	120 DAI	180 DAI
Azo 11	12.2	14.8	20.0	22.1	22.3	10.9	13.5	18.6	20.9	21.0	8.6	11.2	16.3	18.4	18.5
PB 9	11.9	14.5	19.7	21.8	22.1	10.7	13.3	18.5	20.6	20.8	8.5	10.9	16.1	18.2	18.4
PFA 2	12.0	14.7	19.8	21.9	22.2	10.9	13.3	18.5	20.8	20.9	8.5	10.9	16.3	18.4	18.4
Azo 11 + PB 9	14.5	17.2	22.1	24.3	24.7	13.4	16.1	21.2	23.1	23.5	9.8	12.4	17.5	19.7	19.8
Azo 11 + PFA 2	14.3	16.9	21.5	24.0	24.4	13.1	17.7	20.9	22.9	23.2	9.6	12.1	17.3	19.4	19.6
PB 9 + PFA 2	14.5	17.0	21.8	24.1	24.5	13.3	17.8	21.1	23.0	23.4	9.7	12.2	17.4	19.6	19.8
Azo11 + PB9 +PFA2	15.7	18.3	23.4	25.6	26.1	15.4	18.3	23.4	25.2	25.8	11.0	13.6	18.8	20.9	21.1
Uninoculated control	9.8	12.4	17.6	19.7	19.9	8.7	11.2	16.4	18.5	18.7	8.0	10.1	15.0	16.4	17.5

CD (p=0.05)

DAI - Days after inoculation

N & P

0.1277

Days

0.1648

Treatment

0.2085

N & P x days

0.2855

Days x Treatments

0.4663

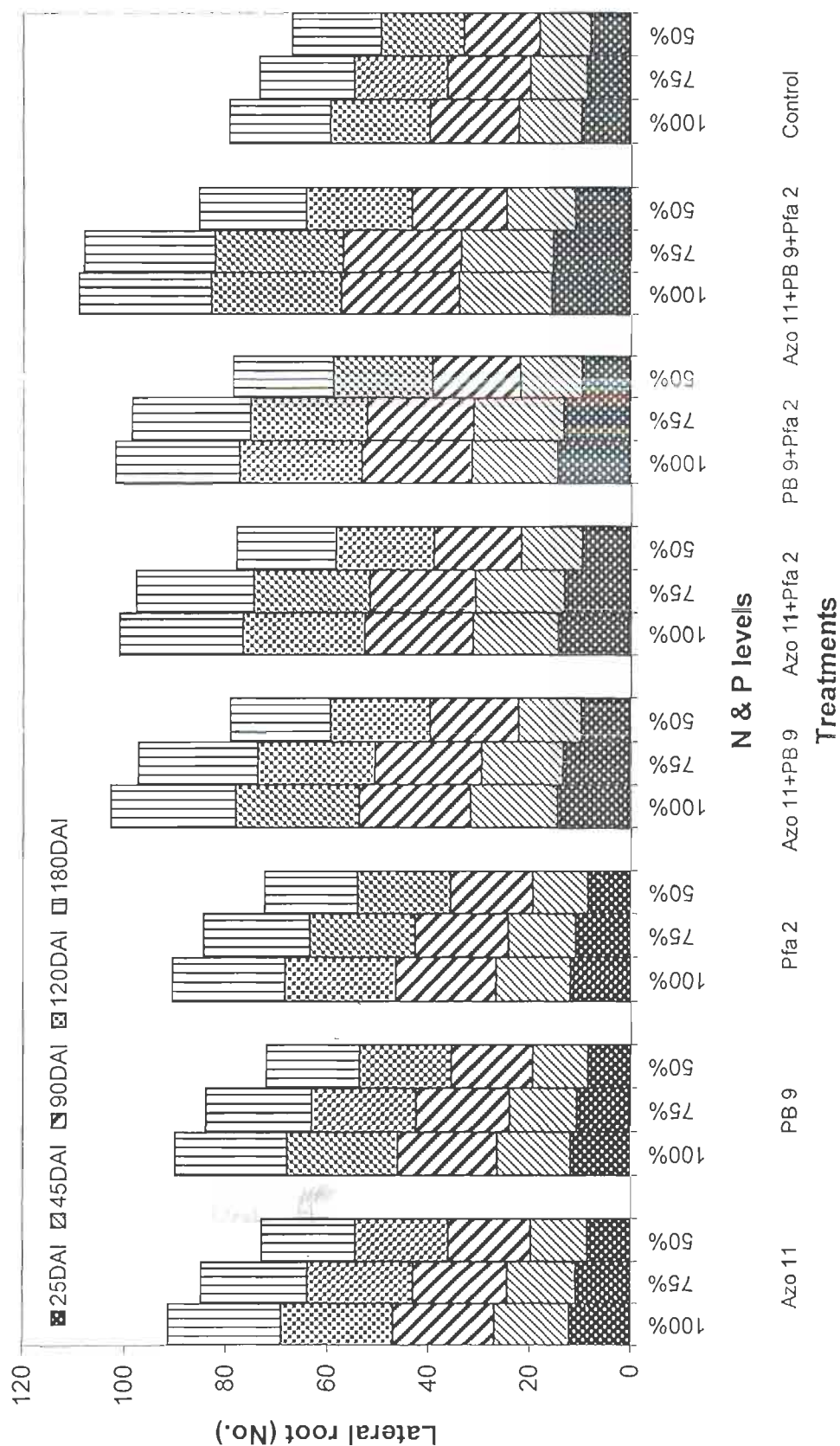
N & P x Treatments

0.3612

N & P x Treatments x days

0.8077

Fig. 37. Effect of combined rhizobacterial inoculation on the number of lateral roots of Cotton (MCU9) with graded levels of N and P



of lateral roots followed by phosphobacteria (PB 9) + *Pseudomonas* (Pfa 2) and *Azospirillum* (Azo 11) + *Pseudomonas* (Pfa 2) at recommended level of N and P (100%). The individual inoculation of *Azospirillum* (Azo 11) increased the number of lateral roots irrespective of N and P applied, which was followed by *Pseudomonas* (Pfa 2) and phosphobacteria (PB 9). In general, combined inoculation of rhizobacterial isolates increased the number of laterals than individual inoculation irrespective of the nutrient levels.

4.17.3.4. Length of lateral roots

In general, the length of lateral roots increased gradually with age of the crop in all the nutrient levels and inoculation levels, which is presented in the Table 45 and Fig. 38. Among the nutrient levels tested, recommended level (100% N & P) with the rhizobacterial inoculation of *Azospirillum* (Azo 11) + phosphobacteria (PB 9) and *Pseudomonas* (Pfa 2) enhanced the length of lateral roots as 54.2 cm during 180 DAI. Among the dual inoculation *Azospirillum* (Azo 11) + phosphobacteria (PB 9) inoculation showed, increased lateral root length followed by phosphobacteria (PB 9) + *Pseudomonas* (Pfa 2) and *Azospirillum* (Azo 11) + *Pseudomonas* (Pfa 2) at all the levels of N and P. Among the individual inoculation, *Azospirillum* (Azo 11) recorded higher lateral root length followed by *Pseudomonas* (Pfa 2) and phosphobacteria (PB 9) which is significantly higher than uninoculated control. Similar trend was observed during 25, 45, 90, 120 DAI.

4.17.3.5. Root volume

The results are presented in Table 46 and Fig. 39. The combined inoculation of *Azospirillum* (Azo 11) + phosphobacteria (PB 9) + *Pseudomonas* (Pfa 2) at 100 per cent N and P increased the root volume at the rate of 7.5 ml of water displacement (W/V) on 25 DAI which is on par with similar inoculation at 75 per cent N and P. Similar trend was noticed in 45, 90, 120 and 180 DAI. Among the dual inoculation, *Azospirillum* (Azo 11) + phosphobacteria (PB 9) increased the root volume followed by phosphobacteria (PB 9) + *Pseudomonas* (Pfa 2) and *Azospirillum* (Azo 11) + *Pseudomonas* (Pfa 2). The individual inoculation of *Azospirillum* (Azo 11) recorded higher volume than *Pseudomonas* (Pfa 2) and phosphobacteria (PB 9) which is significantly higher than uninoculated control.

Table 45. Effect of combined rhizobacterial inoculation on the length of lateral roots in Cotton (MCU9) with graded levels of N and P

Treatments	Length of laterals roots (cm)														
	100% N&P					75% N&P					50% N&P				
	25 DAI	45 DAI	90 DAI	120 DAI	180 DAI	25 DAI	45 DAI	90 DAI	120 DAI	180 DAI	25 DAI	45 DAI	90 DAI	120 DAI	180 DAI
Azo 11 alone	9.6	18.6	42.5	48.8	51.5	8.6	17.7	41.4	47.5	50.8	6.6	15.5	39.1	45.7	48.5
PB 9 alone	9.4	18.4	42.2	48.5	51.3	8.4	17.5	41.1	47.3	50.6	6.3	15.4	38.9	45.4	48.4
Pfa 2 alone	9.6	18.6	42.3	48.6	51.3	8.6	17.5	41.2	47.5	50.8	6.5	15.5	39.1	45.6	48.4
Azo 11 + PB 9	11.6	20.7	44.9	51.2	53.3	10.5	19.7	43.7	49.7	52.4	7.4	16.6	40.3	46.6	49.6
Azo 11 + Pfa 2	11.4	20.4	44.6	50.5	53.0	10.2	19.4	43.4	49.5	52.1	7.3	16.4	40.3	46.4	49.4
PB 9 + Pfa 2	11.5	20.5	44.8	51.1	53.2	10.5	19.6	43.6	49.5	52.3	7.3	16.4	40.1	46.4	49.6
Azo 11 + PB 9 +Pfa 2	12.5	21.3	45.8	52.3	54.2	12.3	21.2	45.6	52.0	53.9	8.6	17.7	41.4	47.6	50.9
Uninoculated control	7.4	16.6	40.3	46.6	49.7	6.6	15.7	39.2	45.7	48.6	5.4	14.3	38.0	44.5	47.3

CD (p=0.05)

DAI - Days after inoculation

N & P

Days

Treatment

N & P x days

Days x Treatments

N & P x Treatments

N & P x Treatments x days

0.5412

0.6987

0.8838

1.2103

1.9764

1.5309

3.4232

Fig. 38. Effect of combined rhizobacterial inoculation on the length of lateral roots of Cotton (MCU9) with graded levels of N and P

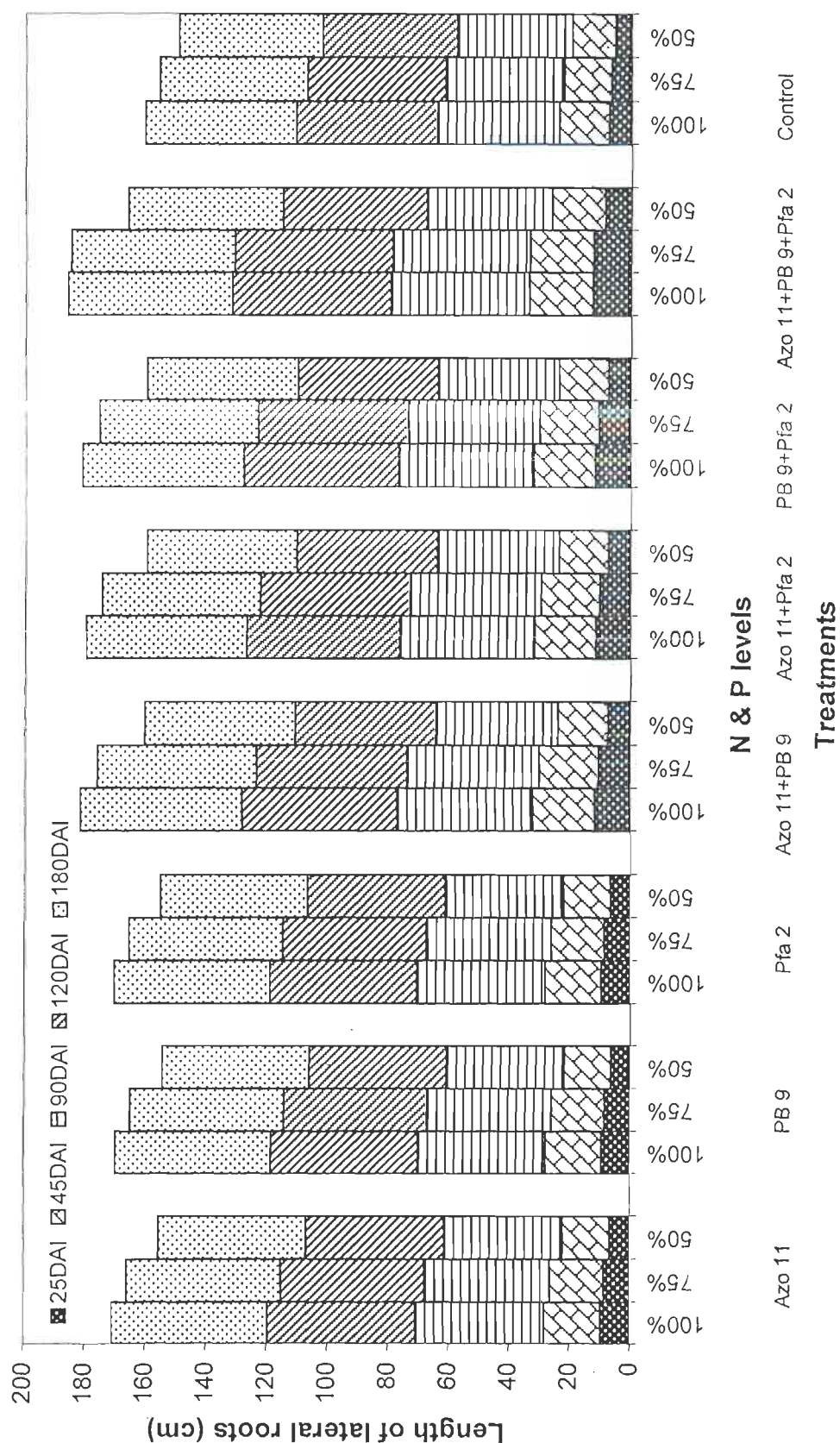


Table 46. Effect of combined rhizobacterial inoculation on the root volume of Cotton (MCU9) with graded levels of N and P

Treatments	Root volume (ml) (w/v)														
	100% N&P						75% N&P						50% N&P		
	25 DAI	45 DAI	90 DAI	120 DAI	180 DAI	25 DAI	45 DAI	90 DAI	120 DAI	180 DAI	25 DAI	45 DAI	90 DAI	120 DAI	180 DAI
Azo 11 alone	5.1	19.0	46.2	58.6	72.4	4.7	18.1	45.3	57.4	71.4	3.0	16.4	43.2	55.6	68.7
PB 9 alone	5.2	18.7	45.9	58.2	72.3	4.4	17.9	45.0	57.1	71.0	2.9	16.1	42.9	55.3	68.5
Pfa 2 alone	5.1	18.9	46.1	58.5	72.3	4.5	17.9	45.2	57.3	71.3	2.9	16.2	43.1	55.5	68.6
Azo 11 + PB 9	6.7	20.5	47.8	61.0	74.8	5.9	19.7	46.9	59.8	73.5	3.9	17.3	44.1	56.4	70.1
Azo 11 + Pfa 2	6.4	20.3	47.5	60.7	74.4	5.6	19.4	46.8	59.4	73.3	3.6	16.9	43.8	56.2	69.8
PB 9 + Pfa 2	6.6	20.5	47.7	60.9	74.5	5.8	19.6	46.9	59.7	73.5	3.7	17.0	43.9	56.3	69.9
Azo 11 + PB 9 +Pfa 2	7.5	21.3	48.7	62.1	76.4	7.2	21.2	48.4	61.8	75.9	4.7	18.2	45.3	57.5	71.4
Uninoculated control	3.9	17.4	44.4	56.4	70.2	3.1	16.4	43.2	55.6	68.7	2.3	14.6	40.1	53.7	67.4

CD (p=0.05) DAI - Days after inoculation

N & P 0.1276

Days 0.1648

Treatment 0.2085

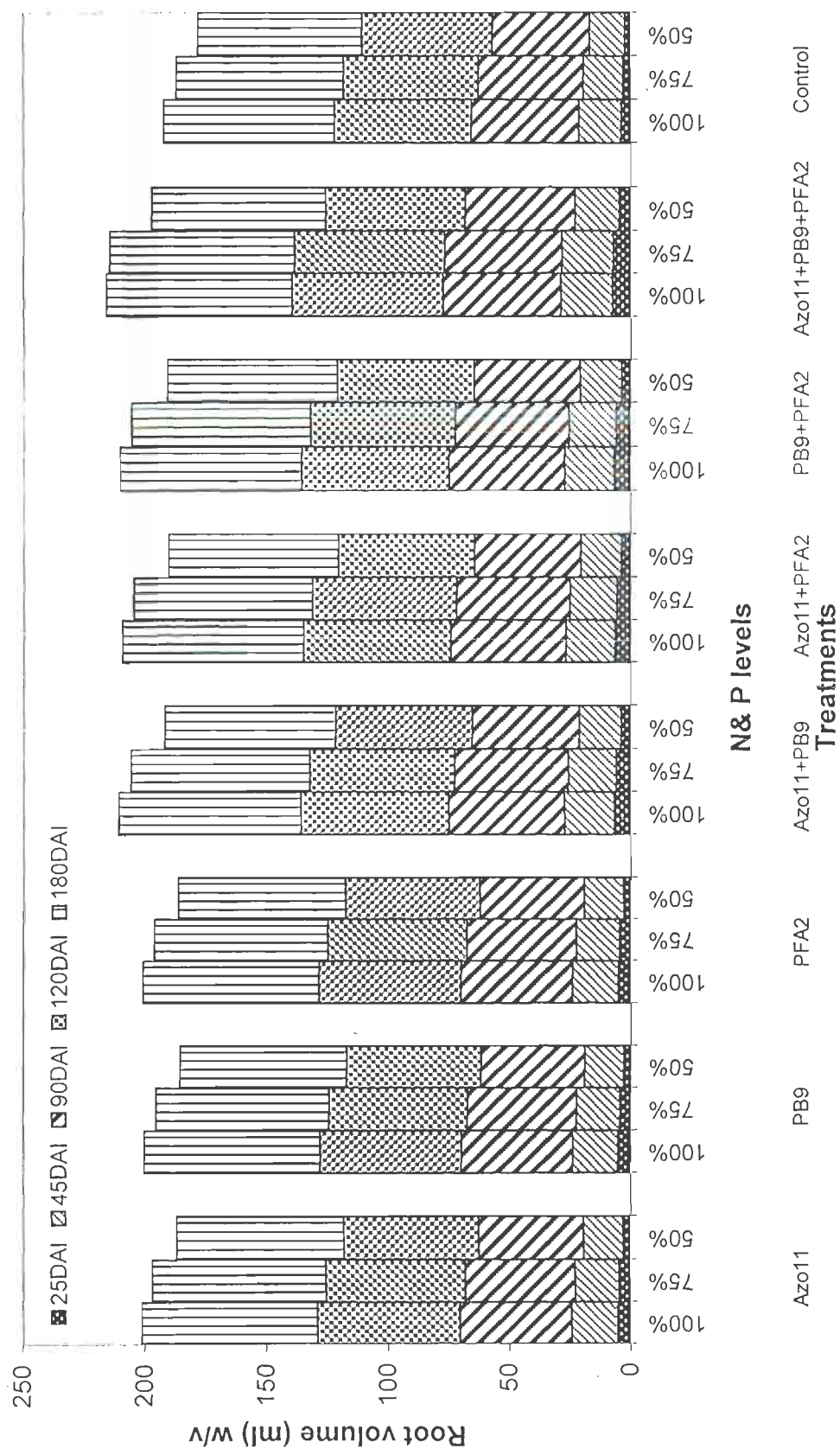
N & P x days 0.2855

Days x Treatments 0.4662

N & P x Treatments 0.3611

N & P x Treatments x days 0.8076

Fig. 39. Effect of combined rhizobacterial inoculation on the root volume of Cotton (MCU9) with graded levels of N and P



4.17.4. Effect of combined inoculation on the yield parameters of Cotton (MCU9) with graded levels of N and P

The yield parameters like monopodial and symbodial branches, number of bolls and boll weight was estimated and presented in the Table 47 and Fig. 40.

The number of monopodial and symbodial branches was increased due to inoculation of *Azospirillum* (Azo 11) + phosphobacteria (PB 9) + *Pseudomonas* (Pfa 2) at recommended nutrient level (100% N & P) which is on par with reduced level of nutrients (75% N & P). The dual inoculation of *Azospirillum* (Azo 11) + phosphobacteria (PB 9) enhanced the number of branches followed by *Azospirillum* (Azo 11) + *Pseudomonas* (Pfa 2) and phosphobacteria (PB 9) + *Pseudomonas* (Pfa 2). Among the individual inoculation, *Azospirillum* (Azo 11) increased the branch numbers whereas less numbers were observed with the inoculation of phosphobacteria (PB 9) at recommended level of nutrients (100% N & P) and it was on par with reduced level of nutrients (75% N & P).

In the case of number of bolls and boll weight a similar trend was observed. The combined inoculation of *Azospirillum* (Azo 11) + phosphobacteria (PB 9) + *Pseudomonas* (Pfa 2) increased the boll number and weight at recommended level (100% N & P) of nutrients and showed similar results at reduced level of nutrients (75% N & P). The dual inoculation of *Azospirillum* (Azo 11) + phosphobacteria (PB 9) increased the boll number and weight followed by phosphobacteria (PB 9) + *Pseudomonas* and *Azospirillum* (Azo 11) + *Pseudomonas* (Pfa 2). In general, combined inoculation of rhizobacterial isolates enhanced the yield parameters than individual inoculation.

4.17.5. Effect of combined rhizobacterial inoculation on the plant uptake of major (N, P, K, Ca and Mg) and micronutrients (Cu, Zn, Mn, Fe) with graded levels of N and P

The macronutrients like N, P, K Ca and Mg uptake of plants were estimated and presented in the Table 48 and Fig. 41.

The nutrient uptake was found to be high in the plants inoculated with *Azospirillum* (Azo 11) + phosphobacteria (PB 9) + *Pseudomonas* (Pfa 2) at recommended level of N and P (100%). The results obtained at reduced level (75%) were on par with the recommended

Table 47. Effect of combined rhizobacterial inoculation on the yield parameters of Cotton (MCU9) with graded levels of N and P

Treatments	Monopodial branches (no pl ⁻¹)			Symbolical branches (no pl ⁻¹)			Bolls (no pl ⁻¹)			Boll weight (g ⁻¹ boll)		
	100% N&P	75% N&P	50% N&P	100% N&P	75% N&P	50% N&P	100% N&P	75% N&P	50% N&P	100% N&P	75% N&P	50% N&P
Azo 11 alone	2.10	1.92	1.61	19.25	18.10	16.20	17.63	16.60	15.27	3.85	3.75	3.25
PB 9 alone	2.04	1.88	1.58	18.80	17.80	15.92	17.24	16.27	14.52	3.80	3.61	3.21
Pfa 2 alone	2.07	1.90	1.59	19.02	17.93	16.03	17.42	16.47	14.70	3.83	3.68	3.26
Azo 11 + PB 9	2.40	2.25	1.74	21.30	20.25	16.94	19.84	18.54	15.54	3.99	3.95	3.39
Azo 11 + Pfa 2	2.39	2.20	1.75	20.90	19.80	17.10	19.56	18.22	15.63	3.94	3.85	3.46
PB 9 + Pfa 2	2.37	2.22	1.73	20.87	19.93	16.80	19.63	18.31	15.30	3.95	3.86	3.31
Azo 11 + PB 9 + Pfa 2	2.56	2.50	1.94	22.30	21.85	18.25	20.82	20.50	16.70	4.33	4.25	3.79
Uninoculated control	1.78	1.63	1.57	17.25	16.24	15.75	15.70	15.29	14.29	3.58	3.28	3.24

CD (P=0.05)

CD (P=0.05)

CD (P=0.05)

CD (P=0.05)

N & P	0.152	0.296	0.192	0.198
Treatments	0.249	0.485	0.381	0.380
N & P x Treatments	0.431	0.839	0.730	0.765

Fig. 40. Effect of combined rhizobacterial inoculation on the yield parameters of Cotton (MCU9) with graded levels of N and P

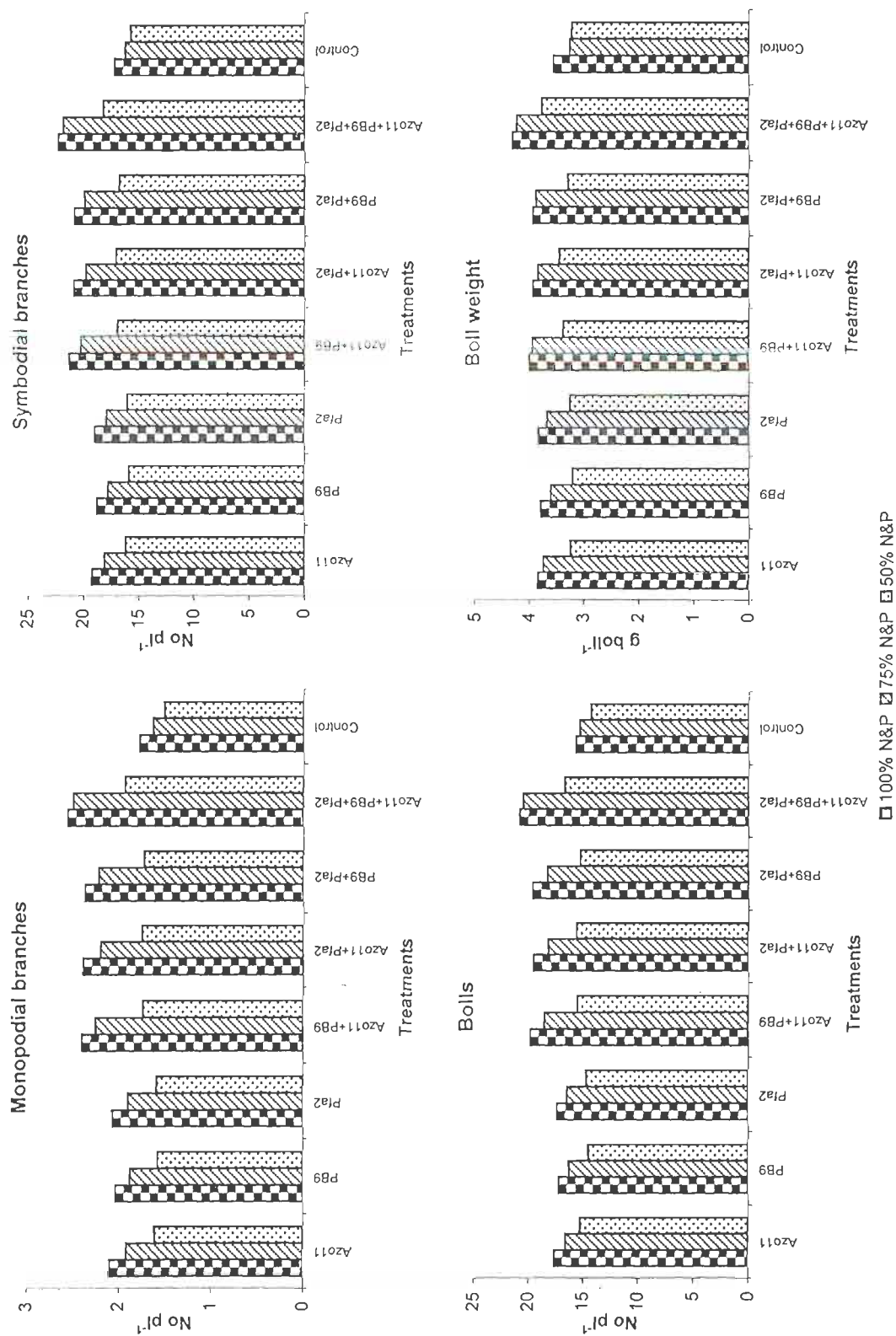


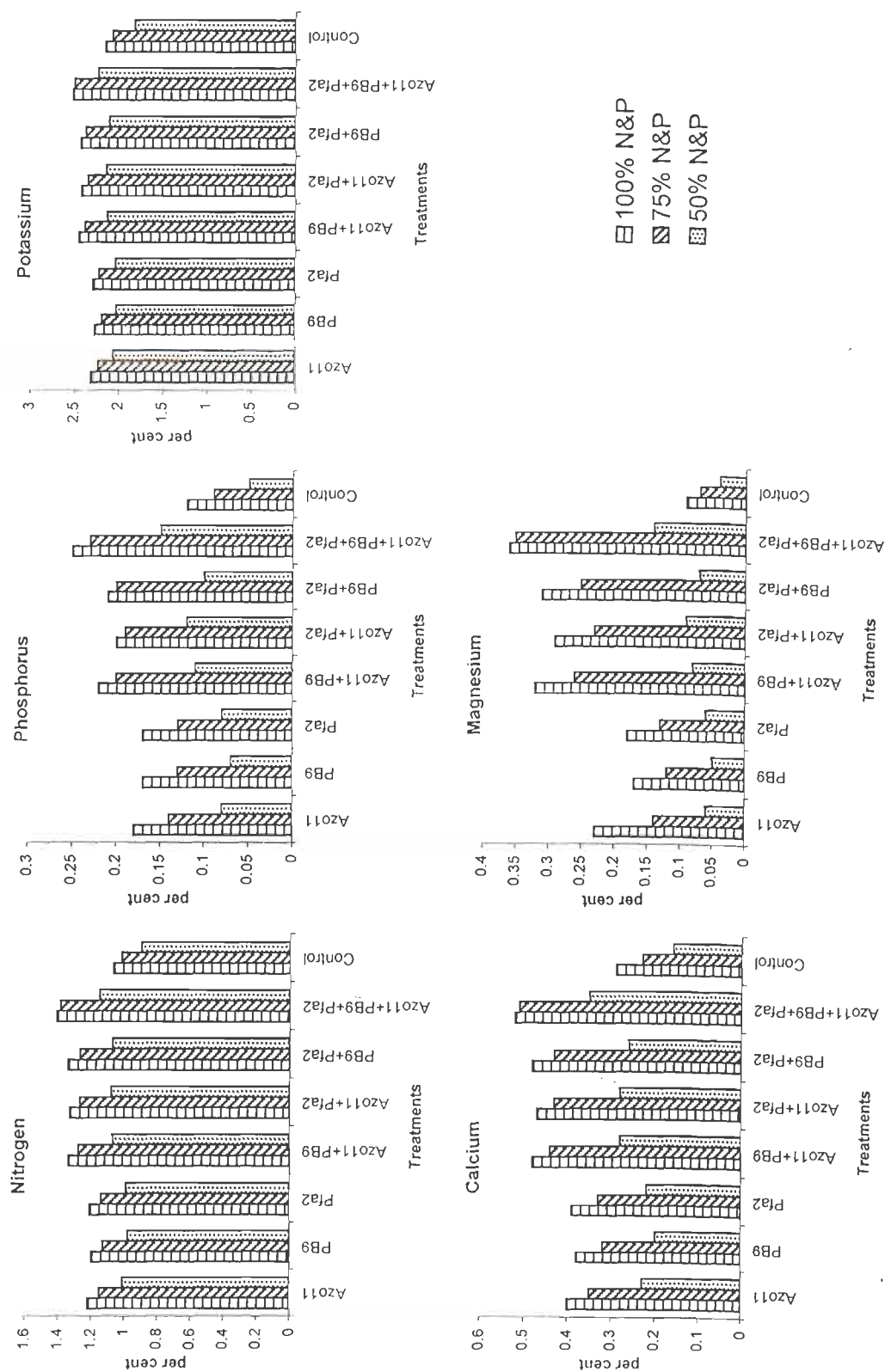
Table 48. Effect of combined rhizobacterial inoculation on the macronutrients content of Cotton (MCU 9) with graded levels of N and P

Treatments	Nitrogen (%)			Phosphorus (%)			Potassium (%)			Calcium (%)			Magnesium (%)		
	100% N&P	75% N&P	50% N&P	100% N&P	75% N&P	50% N&P	100% N&P	75% N&P	50% N&P	100% N&P	75% N&P	50% N&P	100% N&P	75% N&P	50% N&P
Azo 11 alone	1.22	1.15	1.01	0.18	0.14	0.08	2.31	2.23	2.06	0.40	0.35	0.23	0.23	0.14	0.06
PB 9 alone	1.20	1.13	0.98	0.17	0.13	0.07	2.27	2.19	2.03	0.38	0.32	0.17	0.17	0.12	0.05
Pfa 2 alone	1.21	1.14	0.99	0.17	0.13	0.08	2.29	2.22	2.04	0.39	0.33	0.18	0.18	0.13	0.06
Azo 11 + PB 9	1.34	1.28	1.07	0.22	0.20	0.11	2.45	2.38	2.13	0.48	0.44	0.32	0.32	0.26	0.08
Azo 11 + Pfa 2	1.33	1.27	1.08	0.20	0.19	0.12	2.42	2.35	2.14	0.47	0.43	0.29	0.29	0.23	0.09
PB 9 + Pfa 2	1.34	1.27	1.07	0.21	0.20	0.10	2.43	2.37	2.11	0.48	0.43	0.31	0.31	0.25	0.07
Azo 11 + PB 9 +Pfa 2	1.41	1.39	1.15	0.25	0.23	0.15	2.52	2.50	2.23	0.52	0.51	0.36	0.36	0.35	0.14
Uninoculated control	1.07	1.02	0.90	0.12	0.09	0.05	2.15	2.07	1.82	0.29	0.23	0.09	0.09	0.07	0.04

CD (P=0.05) CD (P=0.05) CD (P=0.05) CD (P=0.05)

N & P 0.237 0.009 0.459 0.023 0.010
Treatments 0.388 0.014 0.749 0.037 0.016
N & P x Treatments 0.671 0.025 1.298 0.065 0.028

Fig. 41. Effect of combined rhizobacterial inoculation on the macronutrient content of Cotton (MCU9) with graded levels of N and P



level. Significant difference was not observed among the individual and dual inoculation of isolates at same nutrient levels and are on par with recommended (100%) and reduced level (75%) of N and P.

The micronutrient uptake by Cotton plants inoculated with different rhizobacterial isolates is presented in Table 49 and Fig. 42.

Inoculation of *Azospirillum* (Azo 11) + phosphobacteria (PB 9) + *Pseudomonas* (Pfa 2) enhanced the plant uptake at recommended level of N and P (100%) and on par with reduced level of N and P (75%).

Among the dual inoculation of *Azospirillum* (Azo 11) + phosphobacteria (PB 9) increased the plant uptake of micronutrients followed by *Azospirillum* (Azo 11) + *Pseudomonas* (Pfa 2) and phosphobacteria (PB 9) + *Pseudomonas* (Pfa 2). When comparing the individual inoculation, *Azospirillum* (Azo 11) showed higher plant uptake followed by *Pseudomonas* (Pfa 2) and phosphobacteria (PB 9) at recommended level of (N & P) which is on par with reduced level of N and P (75%).

4.17.6. Effect of rhizobacterial inoculation on the yield and drymatter production of Cotton (MCU9) with graded levels of N and P

The results are presented in Table 50 and Fig. 43. Combined inoculation of *Azospirillum* (Azo 11) + phosphobacteria (PB 9) + *Pseudomonas* (Pfa 2) increased the seed cotton yield of 20.53 Q ha⁻¹ with recommended level of nutrients (100% N & P) but at reduced level of N and P (75%) showed 20.33 Q ha⁻¹ yield which is on par with above.

The dual inoculation of *Azospirillum* (Azo 11) + phosphobacteria (PB 9) increased the yield at recommended level followed by phosphobacteria (PB 9) + *Pseudomonas* (Pfa 2). The individual inoculation of *Azospirillum* (Azo 11) increased the seed cotton yield followed by *Pseudomonas* (Pfa 2) and phosphobacteria (PB 9) which is significantly higher than uninoculated control at recommended level of N and P.

In general, combined inoculation of rhizobacterial isolates enhanced the seed cotton yield than individual inoculation. The yield obtained with combined inoculation at recommended level and reduced level (75% N & P) was on par which indicated the saving of 25 per cent N and P nutrients for field application.

Table 49 . Effect of combined rhizobacterial inoculation on the micronutrients content of Cotton (MCU9) with graded levels of N and P

Treatments	Cu (ppm)			Zn (ppm)			Mn (ppm)			Fe (ppm)		
	100% N&P	75% N&P	50% N&P	100% N&P	75% N&P	50% N&P	100% N&P	75% N&P	50% N&P	100% N&P	75% N&P	50% N&P
Azo 11 alone	20.9	19.2	15.6	54.5	52.4	36.7	73.5	67.8	55.4	602.1	538.6	416.7
PB 9 alone	19.8	18.5	13.8	53.0	50.1	30.6	70.1	64.5	51.4	576.5	514.1	393.5
Pfa 2 alone	20.1	18.7	14.6	53.1	50.8	34.5	72.3	67.6	53.6	598.3	526.6	402.1
Azo 11 + PB 9	24.4	23.2	17.2	60.4	57.5	49.0	81.3	78.6	60.3	726.2	665.1	463.3
Azo 11 + Pfa 2	23.5	21.1	18.1	58.2	55.0	49.5	79.1	74.3	62.1	694.7	638.5	475.6
PB 9 + Pfa 2	23.7	23.0	16.3	59.7	56.7	48.5	80.7	76.6	59.2	702.4	642.7	455.1
Azo 11 + PB 9 +Pfa 2	26.3	25.0	19.4	64.6	63.7	53.0	84.2	83.7	68.5	762.0	758.3	540.5
Uninoculated control	18.4	16.3	12.0	49.9	47.0	25.6	62.3	57.1	45.4	482.4	425.0	322.4

CD (P=0.05)

CD (P=0.05)

CD (P=0.05)

CD (P=0.05)

N & P
Treatments
N & P x Treatments

0.905
1.478
2.560

0.924
1.510
2.614

0.918
1.499
2.595

7.003
11.436
19.807

Fig. 42. Effect of combined rhizobacterial inoculation on the micronutrient content of Cotton (MCU9) with graded levels of N and P

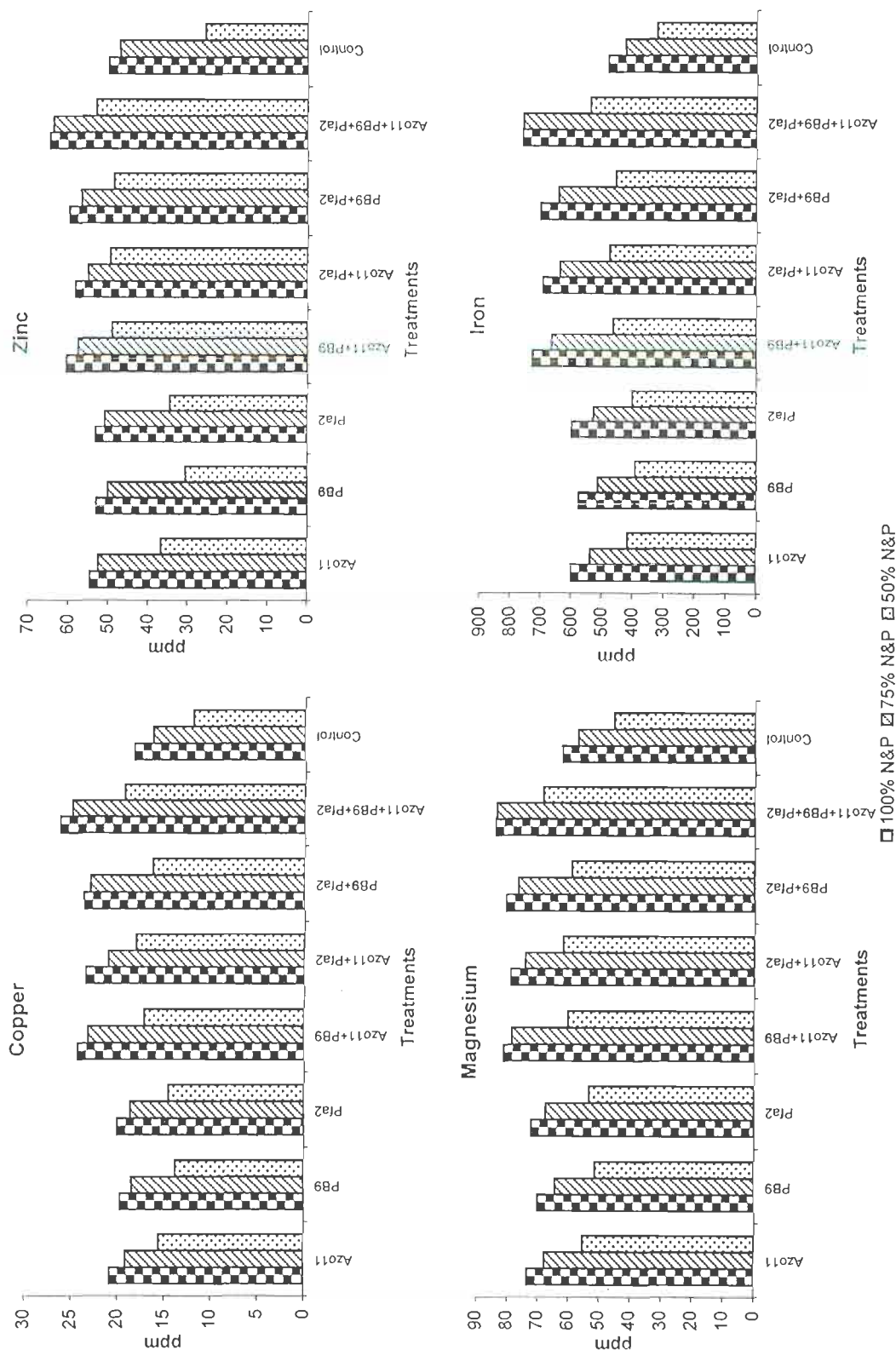


Table 50. Effect of combined rhizobacterial inoculation on the seed cotton yield and dry matter production of Cotton (MCU9) with graded levels of N and P

Treatments	Seed cotton yield (Q ha ⁻¹)			Dry matter production (kg ha ⁻¹)		
	100% N&P	75% N&P	50% N&P	100% N&P	75% N&P	50% N&P
Azo 11 alone	17.23	15.90	12.62	3866	3644	3228
PB 9 alone	16.88	15.63	12.35	3806	3596	3176
Pfa 2 alone	17.06	15.70	12.43	3842	3621	3193
Azo 11 + PB 9	19.36	18.35	14.23	4284	4068	3395
Azo 11 + Pfa 2	19.21	18.25	13.92	4253	4016	3427
PB 9 + Pfa 2	19.25	18.21	14.12	4226	4035	3386
Azo 11 + PB 9 + Pfa 2	20.53	20.33	16.05	4453	4436	3656
Uninoculated control	16.61	12.51	10.61	3446	3236	3026

CD (P=0.05)

CD (P=0.05)

N & P

0.329

0.292

Treatments

0.537

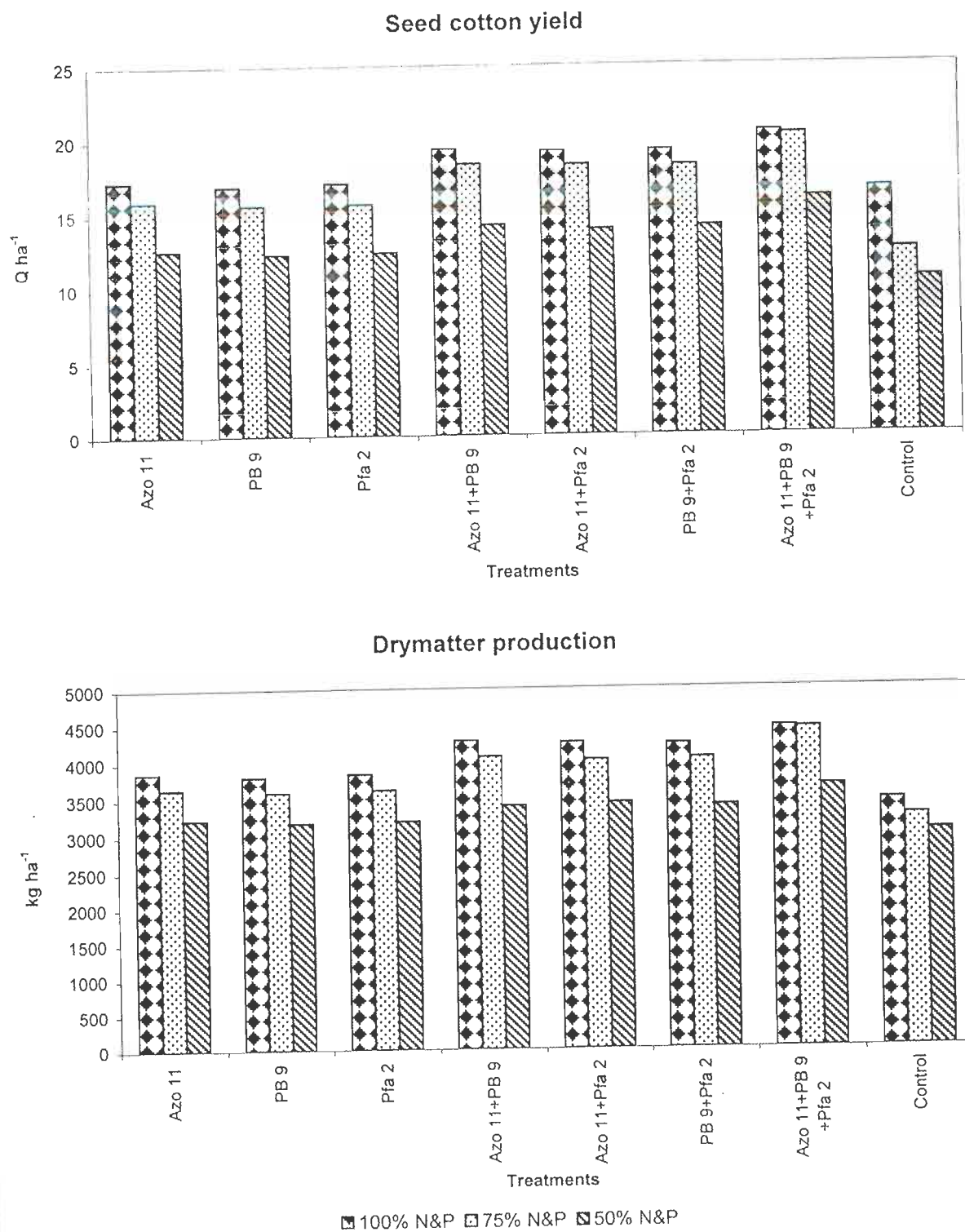
0.477

N & P x Treatments

0.931

0.827

Fig. 43. Effect of combined rhizobacterial inoculation on the seed cotton yield and drymatter production of Cotton (MCU9) with graded levels of N and P



The drymatter production was high with inoculation of *Azospirillum* (Azo 11) + *Pseudomonas* (Pfa 2) at recommended level of N and P (100%) and it is on par with reduced level of N and P (75%) but significantly higher other treatments. Among the dual inoculation, the drymatter production was higher with inoculation *Azospirillum* (Azo 11) + phosphobacteria (PB 9) followed by *Azospirillum* (Azo 11) + *Pseudomonas* (Pfa 2) and phosphobacteria (PB 9) + *Pseudomonas* (Pfa 2) at recommended level of N and P which is significantly higher than reduced level of N and P. The individual inoculation of *Azospirillum* (Azo 11) increased dry matter content followed by *Pseudomonas* (Pfa 2) and phosphobacteria (PB 9) which is comparatively higher than uninoculated control at recommended level of N and P alone. In general, combined inoculation improved dry matter production and seed cotton yield of Cotton (MCU9).

4.17.7 Available nutrient content of soil during harvest of Cotton (MCU9)

The available nutrient content of soil during harvest of crop is presented in Table 51. In general available potassium content was high, nitrogen content was medium and total available phosphorus content was low in soil during harvest. The available nutrient content in the soil increased due to the combined inoculation than dual inoculation and individual inoculation at recommend level of N and P. But the nutrient content at reduced level (75% N & P) was on par with the above treatment.

Table 51. Available nutrient content of soil during harvest of Cotton (MUC 9)

Treatments	Soil Nitrogen (kg ha ⁻¹)			Soil Phosphorus (kg ha ⁻¹)			Soil Potassium (kg ha ⁻¹)		
	100% N&P	75% N&P	50% N&P	100% N&P	75% N&P	50% N&P	100% N&P	75% N&P	50% N&P
Azo 11 alone	236.6	23.5	227.3	21.0	20.0	18.5	625.0	622.0	618.0
PB 9 alone	235.1	232.1	226.1	20.6	19.8	18.2	625.0	621.7	617.5
Pfa 2 alone	236.2	232.7	227.0	20.9	19.8	18.3	624.1	621.9	617.9
Azo 11 + PB 9	241.1	238.1	230.5	22.2	21.4	19.1	628.3	626.1	620.3
Azo 11 + Pfa 2	242.6	239.9	229.1	22.6	21.8	19.0	629.4	627.2	619.5
PB 9 + Pfa 2	240.7	238.1	229.2	22.5	21.4	19.0	629.0	626.1	619.8
Azo 11 + PB 9 + Pfa 2	245.6	244.1	233.6	23.4	23.1	20.1	631.6	630.8	622.5
Uninoculated control	230.6	227.6	222.3	19.5	18.6	17.5	620.5	618.2	615.2

CD (P=0.05)

CD (P=0.05)

CD (P=0.05)

N & P

Treatments

N & P x Treatments

0.634

1.021

2.305

0.526

0.882

1.325

0.612

0.981

1.521

DISCUSSION

CHAPTER V

DISCUSSION

Cotton, king of fibres is cultivated mostly under black soil, vertisol in Tamil Nadu. The nature of soil is often clayey with an alkaline pH and most of the nutrients are lost by fixation often leads to symptoms of nutrient deficiency during different stages of crop growth. Also, the crop suffers much due to invasion of root rot pathogens during rainy season. Hence, there is a need for selection of suitable rhizobacterial strains, which can fix atmospheric nitrogen, solubilize phosphorus and offer protection against root rot disease.

Repeated attempts have been made to inoculate the soil with microorganisms of desired traits in order to improve native soil fertility and plant growth. The challenge of agrobiological studies is to characterize rhizosphere microflora; microbial relationship and to optimise all components, so that they function coordinately in the system.

5.1. ISOLATION AND CHARACTERIZATION OF COTTON RHIZOBACTERIAL ISOLATES

In the present study, 45 *Azospirillum* isolates, 37 phosphobacterial isolates and 24 *Pseudomonas* isolates were isolated from the roots and rhizosphere soil of different cotton varieties cultivated in cotton growing zones of Tamil Nadu.

Fertility of the soil depends not only on the physical and chemical properties of the soil but also depends on the quantitative and qualitative nature of microorganism specifically in rhizosphere of crop plants.

The *Azospirillum*, a common soil inhabitant is found to be in association with different plants viz., cereals, legumes and horticultural crops cultivated in tropical, subtropical and temperate regions (Dobereiner *et al.*, 1976; Tayler *et al.*, 1979; Bally *et al.*, 1983; Hill *et al.*, 1983; Sundaram *et al.*, 1988; Horemaans *et al.*, 1986; Bashan *et al.*, 1989; Hemavathi, 1997, John Kennedy, 1998 and Thiruchenthilvasan, 1999). These *Azospirillum* isolates are capable of forming sub-surface pellicle (Okon *et al.*, 1977) and also species of *Bacillus* and *Herbaspirillum* are known to form pellicle in 'N' free semisolid malate medium (Kreig and Dobereiner, 1984). Differentiation of *Bacillus* and *Herbaspirillum*

from *Azospirillum* was done based on the colony morphology on the Congo red medium given by Rodriguez-Caceres (1982). Small pink colonies later turning to scarlet were selected and streaked on nitrogen free bromothymol blue (Nfb) agar, *Azospirillum* which formed small dense and mucoid colonies were used for further studies. The morphological characteristics of these isolates on comparison with the standard cultures of *Azospirillum brasilense* (Sp7) and *Azospirillum lipoferum* (Az204) showed similarity and were in accordance with the description of *Azospirillum* sp given by Krieg and Dobereiner (1984) and Tarrand *et al.* (1978).

Microscopic examinations of these *Azospirillum* isolates revealed that they were Gram negative and vibroid showing spirillar movement. These characters are in accordance with the species of the genus *Azospirillum* given in section 2 of Bergey's Manual of Systematic Bacteriology vol I, described by Kreig and Dobereiner (1984) and Tarrand *et al.* (1978).

Based on their utilization of biotin, different sources of carbon and production of acid in glucose peptone medium, the isolates were categorized into two groups. Group I, which used biotin for their growth and utilized glucose as sole carbon source but not sucrose and produced acid in glucose peptone medium. The characteristics of group I isolates were similar to the reference culture Az204 and also to the type culture of *Azospirillum* i.e *Azospirillum lipoferum* (Tarrand *et al.*, 1978). Thus group I isolates were identified as *Azospirillum lipoferum* which contributed 41.66 percent of the 12 isolates selected.

Group II isolates could not utilize both glucose and sucrose but used only malate for growth and did not produce acid in glucose peptone medium. These characters agreed with that of the type species described for *Azospirillum brasilense* by Tarrand *et al.* (1978) and contributed about 58.33 of the total 12 isolates.

In all the *Azospirillum* isolates PHB granules were present. Few of the isolates of *Azospirillum* were poor in denitrification. In a study conducted by Neyra *et al.* (1977) half of *A. brasilense* and *A. lipoferum* strains did not reduce NO_3 to N_2 gas. Boddy and Dobereiner (1981) also found higher percentage of nir^- strains (nitrate reduction negative) in plant roots and soil.

Occurrence of more phosphate solubilizing bacteria in the rhizosphere compared to non-rhizosphere has been observed in wheat and maize (Katznelsen and Bose, 1959), oats (Louw and Welbey, 1958).

In the present study, 34 phosphate solubilizing bacteria were isolated from the rhizosphere soil of cotton and screened for their efficiency under *in vitro* condition. Based on the phosphate solubilizing ability 11 phosphobacterial isolates were selected.

Based on the morphological and biochemical characters these phosphobacterial isolates were identified as *Bacillus* (63.64%) and *Pseudomonas* (36.36%). Phosphobacteria belonging to genus *Bacillus* viz., *B. megaterium*, *B. subtilis*, *B. brevis*, *B. polymyxa*, *B. pumilis* and *B. pumilis* were isolated from the rhizosphere of berseem and green gram (Paul and Sundara Rao, 1971), maize (Ghazvintzaheh *et al.*, 1980), rice (Sattar and Gaur, 1987), pearl millet (Ramamourty, 1982) and wheat (Ravichandran, 1989). The *Pseudomonas* a dominant phosphate solubilizing bacteria found in the rhizosphere of soybean, mungbean and chickpea (Sindu *et al.*, 1997).

Pseudomonas, a soil bacterium capable of increasing the crop growth by the production of plant growth promoting substances and also act as a potent biocontrol agent against many root rot pathogens (Rao *et al.*, 1999). Howell and Stipanovic (1979) isolated fluorescent *Pseudomonas* from the rhizosphere of cotton.

In the present investigation the *Pseudomonas* isolates obtained from the rhizosphere of cotton were characterized based on the morphological and biochemical activities. All the isolates were found to be Gram negative and reacted positively for fluorescent pigment production, growth at 4°C and levan formation on King's B medium supplemented with 5 per cent sucrose. These isolates also exhibited positively for motility, catalase activity, and gelatin hydrolysis and nitrate reduction. The isolates showed negative for starch hydrolysis, acid and gas production. The litmus milk reaction was alkaline. Based on the results obtained and on comparison with standard strain of *Pseudomonas fluorescens* (Pf1), these rhizobacterial isolates were found to be *Pseudomonas fluorescens*. These characteristic features were found to be in accordance with Stanier *et al.* (1966); Palleroni (1977) and Rao *et al.* (1999).

5.2. SCREENING RHIZOBACTERIAL ISOLATES FOR EFFICIENCY

The crop management strategy involves, selection of suitable rhizobacterial isolates in combination with beneficial diazotrophs producing biologically active compounds or favouring nutrient cycling and the selection may be made for compatibility and combined efficiency regarding a particular soil plants and environmental system (Azcon *et al.*, 1991).

The nitrogen fixing capacity of *Azospirillum* isolates was studied by estimating total nitrogen fixed and by assay of nitrogenase activity under *in vitro* conditions. The nitrogen fixing ability of *Azospirillum* isolates varied from 6.12 to 45.83 mg N g⁻¹ malate. Such variations in the nitrogen fixing abilities among the *Azospirillum* strains were observed by several workers (Day *et al.*, 1975; Venkateswarlu and Rao, 1983; Zafar *et al.*, 1986; Balasubramanian and Prabhu, 1994; Agarwal and Chaudhary, 1995; Maheshkumar, 1997; John kennady, 1998; Thiruchenthilvasan, 1999 and Anand *et al.*, 1999). The quantity of nitrogen fixed by the *Azospirillum* isolates was indeed low as compared to 83.3 mg N g⁻¹ of carbon source (Pedrosa *et al.*, 1981) and 115 mg N g⁻¹ of carbon source (Dobereiner *et al.*, 1972).

Incorporation of atmospheric nitrogen into the host plant by *Azospirillum* was evaluated mainly by indirect method of acetylene reduction activity (Van Berkum and Bohlool, 1980). In the present study, the acetylene reduction activity of *Azospirillum* isolates ranged from 35.25 to 555.32 n moles of C₂H₄ mg⁻¹ cell protein h⁻¹. Isolates that had nitrogenase activity showed higher N₂ fixing capacity. Fayeze and Daw (1987) observed highest C₂H₄ reduction activities on cotton roots with inoculation of *Azospirillum* strain S₆₃₁ and SpBr 14 (means 216.85 and 209.5 n moles C₂H₄ g⁻¹ root h⁻¹ respectively) while strain M4 gave the lowest activity (means of 100.8 n moles C₂H₄ g⁻¹ root h⁻¹). Similar variations in the nitrogenase activity were observed by Day *et al.* (1975), Kavimandan *et al.* (1978).

The variation in nitrogenase activity depends upon many factors such as source of carbon; pH of the medium, incubation temperature and dissolved oxygen levels at site of nitrogen fixation (Day and Dobereiner, 1976; Okon *et al.*, 1976b and Nelson and Knowles, 1978). However, populations of efficient strains in soil and their access to the plant roots may be a limiting factor for associative N₂ fixation. This clearly explains the need for isolation, screening and inoculation to augment the population of efficient and competing strains in the root zone of cotton crop.

The phosphobacterial isolates were screened based on the ability to release soluble 'P' into the culture medium as suggested by several workers (Moreau, 1959; Bardiya and Gaur, 1974; Banik and Dey, 1981; Ramamourty, 1982; Illmer and Schinner, 1992 and Dave and Patel, 1999).

In the present study solubilization of phosphorus by phosphobacterial isolates varied from 3.25 to 18.56 mg P from 100 mg TCP in Pikovskaya's broth on 15 DAI. Similar trend was observed on 30 DAI. Many species of *Bacillus* were good phosphate solubilizers (Mohammed, 1984 and Thomas and Shantaram, 1986). Hence attempts had been made to select elite strains of phosphobacteria based on the solubilization of fixed phosphorus and nearly 63.6 per cent of isolates were identified as *Bacillus*.

Phosphate solubilizing bacteria in liquid medium reduces the pH of the medium due to the release of organic acids, which plays a major role in phosphorus solubilization (Swaby and Sperber, 1958; Dave and Patel, 1999). However, in the present study no such correlation between the degree of 'P' solubilization and pH reduction was observed. Asea *et al.* (1988) and Thomas (1985) observed high phosphate solubilization without detectable pH change. Since no correlation was made between solubilized 'P' on one hand and the amount of organic acids produced by phosphobacteria on the other hand in the media, doubt was expressed about the role of organic acids in 'P' solubilization (Barthakur, 1978 and Banik and Dey, 1982). Under such condition formation of chelates with Ca^{2+} (Duff *et al.*, 1963 and Kucey, 1983) and proton excretion accompanying NH_4 assimilation (Illmer and Schinner, 1995) were suggested as the possible mechanism of 'P' solubilization.

In the present study, the phosphobacterial isolates release soluble 'P' from organic phosphorus compounds like p-nitrophenyl phosphate (PNPP) besides solubilizing inorganic 'P'. Alexander (1977) observed similar results that *Pseudomonas* and *Bacillus* were extremely effective in mineralizing organic phosphates besides solubilising rock phosphate or tricalcium phosphate. In the present study, the mineralization of organic phosphate (PNPP) ranged from 14.42 to 80.35 n moles of p-nitrophenol released $\text{min}^{-1} \text{mg}^{-1}$ of cell protein. Ravichandran (1989) observed that the *Bacillus* isolate TV-4 from the wheat

rhizosphere mineralized maximum amount of PNPP coupled with high TCP solubilization. Laxmipriya (1997) observed that mineralisation was highest in PNPP with *P. striata* followed by *B. megaterium* var *phosphaticum* and *Bacillus* (SB).

The ability of phosphate solubilizing bacteria to solubilize rock phosphate (RP) is considered as one of the important criteria. Many phosphobacterial isolates, which solubilize tricalcium phosphate, do not release 'P' from rock phosphate. The quantity of the phosphorus released varies with the source and nature of phosphates. Tricalcium phosphate was more easily solubilised when compared to poor solubilization in rock phosphate. In the present study, the phosphobacterial isolates were tested for 'P' release from three types of powdered rock phosphates viz., Mussorie (India), Gafsa (Tunisia) and Udaipur (India). The results revealed lower solubilization of 'P' in various rock phosphates compared to tricalcium phosphate, which was found to be in accordance with the findings of Arora and Gaur (1979).

The quantity of rock phosphate solubilization depends on the organisms involved besides the nature and source of rock phosphate (Dave and Patel, 1999). The phosphobacterial isolate PB 9 was found to be more effective in solubilizing the rock phosphate followed by *Bacillus megaterium* var *phosphaticum*. Similar to solubilization of TCP, there was no concomitant reduction in pH with the amount of 'P' released from rock phosphate.

Various isolates of phosphobacteria viz., PB 9, PB 6 and *Bacillus megaterium* var *phosphaticum* used in this study were able to solubilize 4-5 per cent of rock phosphate within 15 days indicating the possibility of using low grade rock phosphates like Mussorie and Udaipur phosphates directly as 'P' fertilizer along with phosphobacteria in the place of costly phosphoric fertilizers.

Greater microbial solubilization was observed with Mussorie rock phosphate followed by Udaipur and Gafsa rock phosphate. Mehta *et al.* (1979) found greater microbial solubilization with high-grade rock phosphate. In the present study high grade Gafsa rock phosphate with 36 per cent total phosphate was poorly solubilized. Probably the other mineral fractions in RP could influence the microbial solubilization.

The fluorescent pseudomonads are capable of arresting mycelial growth of *R. solani* which cause root rot disease of cotton. Frindlender *et al.* (1993) also observed the antagonistic activity of *P. fluorescens* to *R. solani*. Inhibition of mycelial growth of *R. solani* by *P. fluorescens* has been demonstrated by Hebber *et al.* (1991). Hagedorn *et al.* (1993) observed that improved seedling stand in cotton as a result of *Pseudomonas* spp. application which was due to the suppression of root rot pathogens in cotton.

The potential of fluorescent pseudomonads in inhibiting the pathogens under *in vitro* condition, displacing the pathogen at the root surface and offering protection to the roots of crop plants like cotton, potato, tobacco, wheat and rice has been reported (Kloepper, 1983; Mew and Rosales, 1986; Chen and Yin, 1992 and Sridar, 1996). The deleterious effect of fluorescent *Pseudomonas* inhibiting the mycelial growth and sclerotial viability of *R. solani* was observed by Mew and Rosales (1986) and Kalaivani (1998).

Many rhizobacteria are capable of producing HCN, which plays a major role in the biological control of the pathogens (Schippers, 1988). Nearly 50 per cent of the pseudomonads isolated from potato and wheat rhizosphere produced HCN, although the rate of release differs widely (Bakker and Schippers, 1987).

In the present study all the eight isolates and standard strain of *P. fluorescens* Pfl inhibited the mycelial growth of *R. solani* and also produce HCN under *in vitro* condition. The production of HCN varied from 3.16 to 9.56 $\mu\text{g ml}^{-1}$ which played a major role in disease controlling mechanisms (Bakker *et al.*, 1987).

Poly - β butyrate (PHB) is considered as an energy storage material, which is essential at the times of, stresses (Okon *et al.*, 1976a and Lamm and Neyra, 1981). The synthesis and further degradation of PHB in *A. brasilense* under starvation could be a mechanism, which favoured the establishment and survival of *Azospirillum* in the rhizosphere (Tal *et al.*, 1990). In the present study *Azospirillum* (Azo 11) accumulated larger amounts of PHB on 10 DAI. In contrast Sp 7 and Az 204 exhibited very less content of PHB on 10 DAI. The PHB accumulation by *Azospirillum* strains also found to favour the survival of *Azospirillum* under high temperature.

5.3. PRODUCTION OF PHYTOHORMONE AND PLANT GROWTH PROMOTING SUBSTANCES BY PLANT GROWTH PROMOTING RHIZOBACTERIA

Root colonizing rhizobacteria are known to produce vitamins and growth hormones (auxins, cytokinins *etc.*) which often leads to morphological changes in the plants. In this context, rhizobacterial isolates were screened for their efficiency in synthesizing IAA, GA and also siderophore production by *Pseudomonas* isolates.

Production of IAA by rhizosphere microorganisms is well known (Roberts and Roberts, 1939). Many soil bacteria able to produce IAA in culture medium (Kloepper *et al.*, 1989). Production of plant growth hormones by *Azospirillum* in pure cultures has been documented by several workers (Tjepkema and Burris, 1976; Gaskins *et al.*, 1977; Hubbell *et al.*, 1979; Gaskins and Hubbell, 1979; Tien *et al.*, 1979; Hartmann *et al.*, 1983a; Bar and Okon, 1993; Fulchieri *et al.*, 1993; Rademacher, 1994 and Chitrakumari and Purushothaman, 1995). Production of IAA in the presence of precursor compounds like tryptophan available in the root exudates was also reported (Scott, 1972 and Brown, 1972). Prisen *et al.* (1993) postulated a number of biochemical pathways for IAA biosynthesis like indole acetamide pathway, tryptophan dependent pathway and tryptophan independent pathway in *Azospirillum*. The conversion of tryptophan to IAA by an enzyme, indole-2-acetamide hydrolyze with an intermediate compound, indole-3-acetamide in *Azospirillum* was observed by Bar and Okon (1993).

In the present study, higher production of IAA by *Azospirillum* isolates was observed due to addition of tryptophan in the medium. The increased synthesis of tryptophan often leads to the tryptophan mediated pathways for increased IAA biosynthesis (Scott, 1972; Reynders and Vlassak, 1979; Bar and Okon, 1993; Sridhar, 1996 and Thiruchenthilvasan, 1999).

In the present study, IAA produced by *Azospirillum* isolates ranged from 13.5 (Azo 3) to 93.2 (Azo 11) $\mu\text{g } 100 \text{ ml}^{-1}$. Among the phosphobacterial isolates PB 9 produced maximum amount of 37.8 $\mu\text{g } 100 \text{ ml}^{-1}$ and PB 3 produced least amount of 16.1 $\mu\text{g ml}^{-1}$ in the presence of precursor. In *Pseudomonas* isolates, IAA produced varied from 35.7 (Pfs 8) to 87.3 (Pfa 2) $\mu\text{g } 100 \text{ ml}^{-1}$ due to addition of tryptophan. Fallick *et al.* (1989) observed 32 to 40 $\mu\text{g ml}^{-1}$ IAA productions by *Azospirillum* strains. Hartmann *et al.* (1983a) observed

that certain mutants of *A. brasilense* excreted higher quantities of IAA in the culture medium upto $16 \mu\text{g ml}^{-1}$, 30 times more than the wild type. Dubeikousky *et al.* (1993) and Dowling and O'Gara (1994) have observed IAA production by *Pseudomonas* strains. Ruberchik *et al.* (1958) suggested that enhanced activity of *Azotobacter* in the rhizosphere of wheat, barley, rye and oat are due to the production of biologically active compounds produced by *Bacillus megaterium* var *phosphaticum*. All the rhizobacterial isolates produced varied quantity of IAA and cytokinins, which is in accordance with Hussian and Vancura (1970) and Brown (1972). They observed the auxin production by many root colonising bacteria including *Pseudomonas* sp, which is in conformity with the present investigation.

Besides auxin production, a higher quantity of GA synthesis was observed in rhizobacterial isolates. Janzen *et al.* (1992) observed GA production of *A. brasilense* in chemically defined medium and co-cultured with barley straw and the fungus *Trichoderma harzianum*. Nita (1964) and Eklund (1970) observed gibberellic acid production by *Pseudomonas fluorescens*. Veena (1999) reported that *Azospirillum* strains produced 16.20 to $40.21 \mu\text{g IAA}$ and 0.56 to $1.84 \mu\text{g GA } 25 \text{ ml}^{-1}$. The present study supported the findings of Tien *et al.* (1979); Hartmann *et al.* (1983b); Barbieri *et al.* (1986); Fallik *et al.* (1989); Sridar (1996) and Thiruchenthilvasan (1999). These plant growth-promoting substances must have been implicated with the early vigour, good crop growth and increased yield of cotton in the present investigation.

The production of antimicrobial activity of siderophores of *Azospirillum* against several plant pathogens have been reported (Suslow, 1982b and Saxena *et al.*, 1986). Specific strains of the *Pseudomonas fluorescens-pūtida* group were found to secrete yellow-green fluorescent pigments displaying siderophore activity (Kloepper *et al.*, 1980a and 1980b) and the siderophore produced by PGPR strains showed to enhance the plant growth.

In the present study, the *Azospirillum* isolates (Azo 11) secreted higher quantities of 2,3 DHBA siderophore in the medium. Similarly, phosphobacterial isolate (PB 9) and *Pseudomonas* isolate (Pfa 2) secreted siderophores in the culture medium. Similar production of siderophores by rhizobacterial isolates was observed by several workers (Saxena *et al.*, 1986; Bachhawat and Ghosh, 1987; Dileepkumar and Dube, 1993; Sridar, 1996; Vijila, 1998 and Thiruchenthilvasan, 1999).

5.4. INTRINSIC ANTIBIOTIC RESISTANCE (IAR) OF PLANT GROWTH PROMOTING RHIZOBACTERIA

The introduced rhizobacteria frequently compete with the existing population in the soil. Serological typing and antibiotic marker techniques are generally used to distinguish the inoculant from indigenous strains and their survival in soil. Generally drug resistant markers were used as a tool to study the population dynamics of introduced bacteria in the rhizosphere of plants (Danzo *et al.*, 1973 and Brockwell *et al.*, 1977).

The IAR of rhizobacterial isolates were examined at different concentrations of streptomycin, chloramphenicol, ampicillin and kanamycin. All the rhizobacterial isolates showed varied resistance to different antibiotics.

In the present study, the *Azospirillum* (Azo 11) showed resistance upto 250 ppm of streptomycin, 200 ppm of kanamycin and ampicillin and 20 ppm of chloramphenicol. Whereas the phosphobacterial isolates (PB 9) exhibited resistance upto 300 ppm of streptomycin, 15 ppm of chloramphenicol, 250 ppm of ampicillin and 200 ppm of kanamycin. The *Pseudomonas* (Pfa 2) showed upto 300 ppm of streptomycin and ampicillin, 75 ppm of chloramphenicol and 250 ppm of kanamycin.

The results clearly indicated the wide variations among the rhizobacterial isolates of cotton. Balandreau (1986) reported the diversity with respect of IAR among *Azospirillum*, isolated from different habitat or the same habitat. Diversity of *Azospirillum* with respect to IAR also reported by several workers (Dobereiner and Baldani, 1979; Baldani and Dobereiner, 1980; Frenche and Elmerich, 1981; Singh and Wonzel, 1982 and Magalhaes *et al.*, 1983). Sridar (1996) developed *P. fluorescens* strain for 100 ppm of rifampicin and nalidixic acid. However, Gutterson *et al.* (1986) and Saxena *et al.* (1998) used other antibiotic viz., kanamycin, chloramphenicol, tetracycline, ampicillin and streptomycin for developing resistance.

The difference in their resistance is due to the presence or absence of excretory enzyme system that detoxify the antibiotics or due to difference in the membrane permeability (Hayes and Wolf, 1990), mediated by plasmids or transposable elements (Shaw *et al.*, 1992).

5.5. SEED BACTERIZATION OF RHIZOBACTERIA AND SEEDLING EMERGENCE

Seed bacterization with plant growth promoting rhizobacteria has been reported to enhance plant growth under controlled and field conditions. High rhizobacterial concentration in roots has been considered as a key point for successful plant-microbe interaction and seed inoculation before germination could enhance the vigour of seedlings (Kloepper and Schroth, 1981). Girija *et al.* (1994) and Bashan (1998) observed that the seed inoculation with *Azospirillum* did not effect the germination of seeds. Govindan and Purushothaman (1984) and Purushothaman *et al.* (1987) have reported the positive effect of *Azospirillum* inoculation on vigour index. Dileep kumar and Bezbaruah (1996) observed the seedlings germinated from *Pseudomonas* treated seeds showed increased shoot height and rooting in tea. In the present study, the results revealed higher percentage of seed germination and emergence with rhizobacteiral inoculation than uninoculated control. Stimulation of germination due to seed bacterization can be attributed safely by the production of growth promoting substances by these rhizobacterial isolates as shown by Tien *et al.* (1979); Chanway and Holl, (1993); Fulchieri *et al.* (1993) and Girija *et al.* (1994).

5.6. ROOT COLONIZATION BY RHIZOBACTERIA ON COTTON

Selection of specific combinations of suitable microorganisms to function in a synergistic form, and selection should be made for compatibility and combined efficiency regarding a particular soil plant and environmental system (Azcon *et al.*, 1991).

Migration of beneficial rhizosphere bacteria towards their respective host plants provides an important ecological advantage. Also, species of rhizobacteria with poor survival in soil should reach the root environment for their survival (Rovira, 1969). The ability of beneficial bacteria such as *Azospirillum* and *Pseudomonas* to attain a significant population is a prerequisite for their effects on plant growth (Suslow, 1982a).

Root colonization by beneficial bacteria (active/passive attachment to the root surface of inoculated bacteria) is fundamental for increased plant productivity (Bashan and Levanyon, 1990 and Parke, 1991). The pattern of initial adsorption of bacterial cells to different parts of the root was affected by differences in plant species and bacterial strain (Jain and Patriquin, 1984).

Nitrogen-fixing bacteria of genus *Azospirillum* are known to colonize the rhizosphere of economically important crops to exert beneficial effects on plant growth under certain environmental conditions (Okon and Labandera-Gonzalez, 1994 and Okon and Vanderleydon, 1997). Colonization patterns were shown to differ as a function of time and restricted to the sites of lateral root emergence and root hair zones (Vande Broek *et al.*, 1998). *Azospirillum* attachment is primarily dependent on active bacterial metabolism (Okon and Kapulnik, 1986).

In the present study, the rate of adsorption was higher during 60 min. of incubation. Among the three genera of rhizobacteria, *Pseudomonas* (Pfa 2) exhibited better adsorption followed by phosphobacteria (PB 9) and *Azospirillum* (Azo 11). Bashan (1986b) observed that *Pseudomonas* were more motile than *Azospirillum*. Adsorption of *A. brasilense* strain on corn (*Zea mays*) roots increased rapidly for 90 min. and reached a maximum after 45 hours at a pH of 6.1 (Okon and Kapulnik, 1986).

Azospirillum sp. can colonize the root surface as well as penetrate the root and colonize intercellular spaces of the interior roots (Baldani *et al.*, 1986). In the present study, *Azospirillum* (Azo 11), phosphobacteria (PB 9) and *Pseudomonas* (Pfa 2) efficiently proliferated in the rhizoplane and endorhizosphere of sorghum roots under hydroponics conditions. Small aggregates of bacteria were formed on the root surface, and larger population within the cortex due to inoculation of *A. brasilense* in wheat (Bashan and Levanony, 1988).

Rhizobacteria exhibit chemotaxis towards several attractants, including sugars, amino acids, organic acids (Okon *et al.*, 1980; Barak *et al.*, 1982; Reinhold *et al.*, 1985; Zhulin and Armitage, 1993 and Vande Broek *et al.*, 1998) and aromatic compounds (Lopez-De-Victoria *et al.*, 1993) as well as towards root exudates (Heinrich and Hess, 1985) and root mucilage (Mandimba *et al.*, 1986). Amino acids and vitamins were found in the root exudates of cotton (Sulochana, 1962). In the present study, total sugar, reducing and aminonitrogen present in root exudate served as suitable nutritional sources for proliferation of microorganisms in the rhizosphere.

5.7. SURVIVAL OF RHIZOBACTERIAL INOCULANTS IN THE PEAT AND LIGNITE BASED INOCULANTS

Rhizobacterial cells are commonly incorporated into various types of inoculant carriers for easy application (Fages, 1992).

In the present investigation, a mixed inoculum of *Azospirillum* (Azo 11) with phosphobacteria (PB 9) and *Pseudomonas* (Pfa 2) as well as phosphobacteria (PB 9) and *Pseudomonas* (Pfa 2) were prepared using peat and lignite as a carrier. In mixed inoculum, *Pseudomonas* did not inhibit *Azospirillum* (or) phosphobacteria. This may be due to the antifungal nature of the antibiotic produced by *Pseudomonas* and hence co-inoculants were not suppressed as observed by Sridar (1996) supported that the present investigation. The survival of rhizobacterial isolates as individual and mixed inoculation in lignite supported the survival of isolates than peat. Kalaivani (1998) indicated that *Pseudomonas fluorescens* and *Bradyrhizobium* could multiply and survive satisfactorily in lignite and is in agreement with the findings of Paczkowski and Berryhill (1979) and Crawford and Berryhill (1983). Hence lignite can be readily used as an ideal carrier for the large-scale production of PGPR inoculants.

Similar studies were conducted by Azcon *et al.* (1978) and Meyer *et al.* (1986a) in which a multicomponent inoculum for legumes consisting of *Rhizobium*, mycorrhizae and free-living bacterium was tested. The authors demonstrated the greatest plant response to multicomponent inoculum due to synergistic action of the organisms with each other. The combined inoculum consisting of rhizobia and rhizobacteria have the potential for enhancing legume development (Kloepper *et al.*, 1989); which is similar to the findings of present investigation. The effective use of dual or multiple inoculants will depend on matching the microorganisms of interest with the environment that favour its activities (Saxena and Tilak, 1994).

5.8. MATHEMATICAL MODELLING OF POPULATION DYNAMICS OF RHIZOSPHERE MICROFLORA OF COTTON DUE TO INOCULATION OF RHIZOBACTERIAL ISOLATES

The modeling approach leads into a logical framework in which a potential control agent can be subjected to simulated conditions. Bacterial population growth was modeled as a temperature and nutrient dependent logistic increase in the presence of free water and

an exponential decline in the absence of free water (Loper *et al.*, 1984). Kundsén and Spurr (1985) suggested the use of an 'expert system' approach which couples a population model with a data base containing information on attributes of bacterial strains, formulations and weather data. Kundsén *et al.* (1988) described a simulation model to predict dynamics of survival and conjugative plasmid transfer of genetically engineered donor and non-recombinant recipient strains of *Pseudomonas cepacia* in simple rhizosphere and phyllosphere microcosms.

Prathibha *et al.* (1994) found increase in population of total bacteria, fungi, actinomycetes and free-living N₂ fixers in the rhizosphere of cotton. In the present investigation, logistic model was developed to study the population dynamics of rhizosphere microflora of cotton due to the application of rhizobacterial inoculants with graded levels of N and P. Combined inoculation of rhizobacterial isolates improved the growth rate of total diazotrophs, bacteria and actinomycetes than individual inoculation and decreased the growth rate of fungi. Microbial growth rate was high in the rhizosphere and rhizoplane. Fungi tend to be relatively less important in the rhizosphere probably because of the dense packing of the minimum void zone around the root, which effectively ensures that there are insufficient large channels for some fungi to approach the root (Killham, 1994). Seong *et al.* (1992) observed that in the presence of *Pseudomonas* TNSK 2, the fungal population numbers decreased to about 50 per cent in the control soil and no significant difference in total numbers of bacteria. Most investigation suggested that these rhizobacterial strains alter rhizosphere microflora and reduce deleterious fungal species, thus aiding plant growth (Katznelson and Cole, 1965; Brown, 1972 and Kloepper *et al.*, 1991).

Generally the size of the rhizosphere population per unit weight of root increases with the growth and age of roots. The population growth model accurately predicts the population growth on a time scale. The total bacterial population numbers reached a peak after 21 days and decreased again 35 days after germination (Seong *et al.*, 1992). In the present study, the growth rate of rhizosphere microflora was observed high upto 90 DAI and found to decline afterwards. Rapid proliferation of microbes and on the root surface however will continue only until the carbon supply from the root becomes limiting. In plant solution culture, the growth rate of bacteria in the rhizosphere decreased rapidly with time (Barber and Lynch, 1977).

The present study shows the usefulness of similar growth models in predicting the population growth of rhizosphere microflora to rhizobacterial inoculants with various nutrient levels. Among the various nutrient levels, total diazotrophic population found to decline at 100 per cent N and P. The observation suggested that higher doses of chemical fertilizers are inhibitory to total diazotrophs as observed by Prathibha *et al.* (1994). Azaizeh *et al.* (1996) observed that soil type, N- source and thiamine application stimulate the growth of not only diazotrophic bacteria, but also of total bacteria, fungi and actinomycetes present in the soil.

Different plant species (Lemanceau *et al.*, 1995; Wiehe and Hoflich, 1995 and Wiehe *et al.*, 1996) as well genotypes within a species differentially influence quantitative and qualitative composition of microbial populations in the rhizosphere. Higher estimates of rhizosphere microflora observed in this experiments might be due to higher availability of nutrients through root exudates (Brown, 1975). The amount of root exudation might have decreased after flowering stage, reducing the availability of nutrients to this microflora resulting in decline in their population. It is usual that photosynthates and energy are diverted towards reproductive parts after flowering (Prathibha *et al.*, 1994).

5.9. SURVIVAL OF RHIZOBACTERIAL ISOLATES IN THE RHIZOSPHERE OF COTTON

The establishment of introduced rhizobacterial isolates in rhizosphere is essential to obtain desired effect on the growth and yield of crop plants. The increase in rhizobacterial population was found to be from 7 DAI to 90 DAI indicating the growth rate was found to be decreasing with the aging of the crop. The decline in the rate of growth may be due to the reduction in the quantum of available root exudates as the crop grow old or in the quality of the root exudates.

In inoculated roots taken from the field, *Azospirillum* has been found to compose 1-10 per cent of the total bacterial population of the rhizosphere (Okon, 1985). Relatively little information has been published on the establishment and multiplication of *Azospirillum* especially under field conditions. Following inoculation, many strains survive in the rhizosphere, but invariably populations stabilize at low levels

(Sumner, 1990). Several workers reported the variation in the survival of the *Azospirillum* cells in soils (Vandenhove *et al.*, 1993 and Bashan *et al.*, 1995). In the present study also survival of *Azospirillum* was less when compared to other rhizobacterial isolates.

A large number of phosphate solubilizing (PS) microorganisms has been found in the rhizosphere of plants but in fact, this constitutes only a small percentage of the total microbial population. It is widely accepted that an additional quantity of phosphate solubilizing microorganisms has to be introduced into soils in order to increase the availability of phosphorus from insoluble sources (Vassilev *et al.*, 1997).

Rao *et al.* (1999) stated that the population of fluorescent pseudomonads on lentil roots varied with the strain and ranged from 10^4 to 10^6 cfu g⁻¹ of root fresh weight. Kloepper *et al.* (1980a) found fluorescent pseudomonads population at several locations in sandy loam soil between 1.9×10^2 to 9.6×10^5 cfu g⁻¹ roots two weeks after plant emergence. Burr *et al.* (1978) obtained high degree of success in establishing *P. fluorescens* in soil. de Freitas and Germida (1992) also found increase in *P. fluorescens* populations as plants grow old which is similar to the findings of the present investigation.

5.10. EFFECT OF COMBINED INOCULATION OF RHIZOBACTERIAL ISOLATES ON THE GROWTH NUTRIENT UPTAKE AND YIELD OF COTTON UNDER GRADED LEVELS OF N AND P IN FIELD CONDITION

The objective of studying tripartite inoculation of *Azospirillum*, phosphobacteria and *Pseudomonas* has focused upon obtaining an additive effect on plant growth by selection of suitable microorganisms which function in a synergistic form and this biological potential may be used in sustainable agriculture and crop management strategy (Azcon-Aguilar and Barea, 1992). Information on interrelationship among the three-rhizosphere organisms and their effect on growth of cotton is scanty.

As the root length increased, the height of the plant also increased, proportionately and the maximum plant height was obtained when the root depth was the maximum in cotton (MCU 5) (Sivanappan and Saifudeen, 1977). *Pseudomonas* strains promoted wheat growth in terms of root, shoot length and weight (Srivastava *et al.*, 1999). The inoculated PGPR strains usually have been found to increase the root length and root biomass

(Hong *et al.*, 1991) and this better developed root system may increase the mineral uptake in plants. Bashan *et al.* (1990) observed that the enhanced growth of wheat and soybean due to increase in mineral uptake by *Azospirillum* species.

In the present study, shoot and root length, number and length of lateral root and root volume were increased due to the combined inoculation of the rhizobacterial inoculation than individual inoculation, at recommended level of N and P which favoured the growth of cotton. However, the growth rate of cotton was found to be on par with the reduced level of N and P (75%). Okon and Kapulnik (1986) demonstrated that *Azospirillum* inoculation enhanced the root elongation, root branching, increased root and leaf area and root dry weight in crops. These effects found to be vary with inoculum concentration and environmental conditions (Okon and Kapulnik, 1986). de Frietas and Germida (1990b) showed that inoculation of *P. aeruginosa*, *P. cepacia*, *P. putida*, *P. fluorescens* strains on winter wheat increased the plant height, root and shoot biomass and number of tillers under growth chamber conditions. In general, production of growth promoting substances like gibberellins and auxins by the rhizobacteria enhanced proliferation of root system which inturn enhanced mineral uptake (N, P and K) and consequently increased dry matter accumulation (Okon, 1985 and Sattar and Gaur, 1987).

Increase in the height of crop plants due to inoculation of *Azospirillum* has been reported by several workers (Rangarajan *et al.*, 1987; Merina, 1991, Subba Rao, 1993; El Naggat and Mohamoud, 1994 and Hemavathi, 1997). Increased cell elongation and multiplication due to enhanced nutrient uptake by plants (Black, 1968) following inoculation of *Azospirillum* as well as application of nitrogen fertilizer probably has caused the increased plant height. Plant growth promoting substance *viz.*, IAA and GA play an important role in root elongation and shoot growth (Tien *et al.*, 1979).

Plants receiving dual inoculation of *A. lipoferum* and phosphobacteria recorded maximum N and P content of plants than the individual inoculation (Laxmipriya, 1997). It is reasonable to think that the combined inoculation might have enhanced the 'P' and 'N' uptake by the plant due to increased availability in the rhizosphere by the activity of the inoculated bacteria.

Positive interaction of *Azospirillum* with phosphobacteria and *Pseudomonas* has been reported in many cereals and vegetable crops. Inoculation of *A. lipoferum* along with phosphate solubilizing bacteria *Bacillus* (SB1) and recorded 17.5 and 13.9 per cent yield increase over control in rice (Laxmipriya, 1997). Combined inoculation particularly stimulated the population of *A. lipoferum* as well as the population of phosphate solubilizing bacteria. The increased activity of rhizobacteria in the rhizosphere might have influenced the nutrient uptake, biomass and grain yield than the single inoculation. Similar results have been reported in pearl millet (Ramamourty, 1982); Barley (Belimov *et al.*, 1995). Belimov *et al.*, (1995) supported the above view through isotope studies that combined inoculation of nitrogen fixing and phosphate solubilizing organisms enhanced the absorption of P and N in barley plants.

It has been suggested that factors such as production of plant growth promoting substances and increased rate of mineral uptake by roots (Umali Garcia *et al.*, 1980) contribute to yield enhancement.

Inoculation of associative symbiotic diazotrophic species viz., *Azospirillum* increased the dry matter production, grain yield and nitrogen input in most cereals and vegetable crops (Smith *et al.*, 1984 and Kapulnik *et al.*, 1985). The effect of *Azospirillum* inoculation on the total yield increase in field grown plants ranged from 10-30 percent (Kapulnik *et al.*, 1981a and Okon and Labandra-Gonzalez, 1994). The highest yield increase was obtained when the level of N fertilization was sub-optimal for maximum yield (Bashan and Levannony, 1990). Therefore *Azospirillum* inoculation was considered as a substitute for partial nitrogen. Kolb and Martin (1985) Bashan *et al.* (1989b) showed that yield was increased by inoculation under high levels of N fertilization and that N can influence the number of bacteria in the rhizosphere.

Yield increase following inoculation occurred even at high levels of N fertilization (Kapulnik *et al.*, 1983 and Reynders and Vlassak, 1982). The present study showed that the combined inoculation had synergistic effect in increasing the growth of cotton at low level of N and P.

Prasad and Prasad (1994) observed significant increase in the number of bolls/plant, boll weight and seed cotton yield due to inoculation of *Azotobacter/Azospirillum*. In the present study the combined inoculation of rhizobacterial inoculation increased the seed cotton yield (20.53 Q ha^{-1}) than control (16.61 Q ha^{-1}) at recommended level of N and P. However the yield (20.33 Q ha^{-1}) is on par with reduced level of N and P (75%). The results revealed that there is a saving of 25 per cent N and P fertilizer with combined rhizobacterial inoculation.

Prathibha *et al.* (1994) showed higher uptake of N and P in plants inoculated with *Azospirillum* and *P. striata* at 50 per cent N and 100 percent P as rock phosphate which in turn resulted in highest plant biomass production. FYM carrier based *Azospirillum* culture increased the boll number, N uptake and seed cotton yield. Also *Azospirillum* seed treatment along with the application of 50 per cent of recommended N level increased seed cotton yield on par with the recommended N level (Ravinder Raju *et al.*, 1998). Fayez and Daw (1987) observed increased plant dry weight and nitrogen uptake of cotton due to *Azospirillum* inoculation.

SUMMARY

CHAPTER VI

SUMMARY

Plant growth promoting rhizobacteria viz., *Azospirillum*, phosphobacteria and *Pseudomonas* were isolated from soil and roots of different varieties of cotton at various locations of Tamil Nadu. The rhizobacterial isolates were screened primarily based on nitrogen fixation by *Azospirillum*, phosphorus solubilization by phosphobacteria and antagonistic activity by *Pseudomonas*. Morphological and physiological characteristics of these isolates were studied. These rhizobacterial isolates were examined for their phytohormone production, siderophore production and poly- β -hydroxy butyrate (PHB) accumulation in *Azospirillum*, rock phosphate solubilization by phosphobacteria and hydrogen cyanide (HCN) production by *Pseudomonas* under *in vitro* condition. The efficient isolates were selected based on their growth performance under various carbon sources and Cotton (MUC9) seeds were bacterized with these isolates to study the promotion of seed germination, root colonization and adsorption on roots under *in vitro* condition. The nutrient contents of the root exudates inoculated with rhizobacterial isolates were measured. The selected efficient isolates were examined for their compatibility using different carrier materials for combined inoculum production. A field experiment was carried out to study the influence of rhizobacterial inoculation on the rhizosphere microflora and growth and development of Cotton var MCU 9 with graded levels of N and P. A mathematical model was developed to study the population dynamics of rhizosphere microflora due to inoculation of different rhizobacteria with graded levels of N and P.

A brief summary of the results obtained are given below:

- 1) Forty five isolates of *Azospirillum*, 37 isolates of phosphobacteria and 24 *Pseudomonas* isolates were obtained from root and soil samples collected from four districts of Tamil Nadu.
- 2) The forty five *Azospirillum* isolates were screened for their efficiency in nitrogen fixation. About 30 percent of isolates fixed more than 20 mg of N g⁻¹ malate and showed more than 200 n moles of C₂H₄ produced hr⁻¹ mg⁻¹ of cell protein of nitrogenase activity of which 12 isolates were selected.

- 3) Thirty seven phosphobacterial isolates were screened for their acid phosphatase activity and the amount of 'P' released from tricalcium phosphate (TCP) in Pikovskaya's broth, the change in pH and the titrable acidity. About 38 percent of the isolates released more than 30 n moles of p-nitrophenol $\text{min}^{-1} \text{mg}^{-1}$ of cell protein and 30 per cent isolates showed solubilization of above 20 mg 'P' from 100 mg^{-1} TCP. About 43 per cent of isolates recorded more than seven ml of 0.01N NaOH consumption and pH was reduced to 4.5 from 7.0 and 11 isolates were finally selected.
- 4) Twenty four *Pseudomonas* isolates were screened for their antagonistic activity against *R solani* and 33 per cent of isolates showed antagonism of which eight isolates were selected.
- 5) Characterization of rhizobacterial isolates based on morphological and biochemical characters was carried out. Among the 12 *Azospirillum* isolates, 58.3 per cent isolates were *A. brasilense* and the remaining were found to be *A. lipoferum*. Similarly, among the 11 phosphobacterial isolates seven isolates were indentified and assigned to the genus *Bacillus* and the remaining four to *Pseudomonas*. The *Pseudomonas* isolates selected were characterized upto species level and found to be *P. fluorescens*.
- 6) Among the three different rhizobacteria, *Azospirillum* isolate (Azo 11) recorded maximum amount of 93.2 $\mu\text{g } 100^{-1}$ of IAA in the presence of precursor tryptophan followed by *Pseudomonas* (Pfa 2) @ 87.3 $\mu\text{g } 100 \text{ ml}^{-1}$ and phosphobacteria (PB 9) @ 37.8 $\mu\text{g } 100 \text{ ml}^{-1}$. The GA production was highest (6.64 $\mu\text{g ml}^{-1}$) in *Pseudomonas* (Pfa 2) followed by (6.4 $\mu\text{g ml}^{-1}$) *Azospirillum* (Azo 11) and (4.3 $\mu\text{g ml}^{-1}$) phosphobacteria (PB 9).
- 7) The rhizobacterial isolates produced phenolate type of siderophore like 2,3-dihydroxy benzoic acid (DHBA). Among the rhizobacterial isolates, the *Pseudomonas* isolate (Pfa 2) recorded 8.11 $\mu\text{ moles ml}^{-1}$ followed by 5.82 $\mu\text{ moles ml}^{-1}$ of phosphobacteria (PB 9) and 3.86 $\mu\text{ moles ml}^{-1}$ by *Azospirillum* (Azo 11).
- 8) The poly- β -hydroxy butyrate (PHB) accumulation increased with the age of isolates. Among the *Azospirillum* isolates Azo 11 isolate recorded 1.870 mg g^{-1} of dry wt of the cell.

- 9) The hydrogen cyanide (HCN) production of *Pseudomonas* isolates varied from 2.42 to 9.56 $\mu\text{g ml}^{-1}$ and Pfa2 recorded the higher amount.
- 10) Intrinsic antibiotic resistance by rhizobacterial isolates was tested for streptomycin, chloramphenicol, ampicillin and kanamycin. Among the *Azospirillum* isolates Azo 11 showed 250, 20, 150 and 200 ppm, the phosphobacterial isolates, PB 9 registered 300, 15, 250 and 200 ppm and Pfa 2 isolate of *Pseudomonas* recorded 300, 75, 300 and 250 ppm of resistance respectively in the order of antibiotics as mentioned above.
- 11) Growth performance of cotton rhizobacterial isolates under different carbon sources was studied. Among the rhizobacterial isolates, Azo 11 of *Azospirillum*, PB 9 of phosphobacteria and Pfa 2 of *Pseudomonas* exhibited better growth in malate glucose and glycerol respectively.
- 12) The phosphobacterial isolate PB 9 recorded a mean of 7.3 mg of rock phosphate from 100 mg in Pikovskaya's broth with the titrable acidity of 9.77 ml of 0.01N NaOH consumption. It was found to reduce the pH from 7 to 5.18. Among the rock phosphates, Mussorie rock phosphate solubilized faster than Udaipur and Gafsa rock phosphate.
- 13) Among the rhizobacterial isolates, *Azospirillum* (Azo 11), showed a highest germination per cent of 82.5 followed by *Pseudomonas* (Pfa 2) with 80 and phosphobacteria (PB 9) with 77.5 of cotton seeds than the other rhizobacterial isolates. The seedling vigour index was 1880.0, 1856.25 and 1782.5 for *Pseudomonas* (Pfa 2), *Azospirillum* (Azo 11) and phosphobacteria (PB 9) respectively which is significantly higher than uninoculated control (965.0).
- 14) The rhizoplane and endorhizosphere colonization of rhizobacterial isolates on the Cotton seedling under hydroponics condition was studied. *Azospirillum* (Azo 11), phosphobacteria (PB 9) and *Pseudomonas* (Pfa 2) colonized significantly in the rhizoplane and endorhizosphere than the other rhizobacterial isolates and there was rapid population build up during 15 days after inoculation (DAI) when compared to 5 DAI.

- 15) The total sugar, reducing sugars and amino nitrogen content of root exudates inoculated with rhizobacterial isolates were studied and the content of the above nutrients of root exudate were higher with the inoculation of *Azospirillum* (Azo 11), phosphobacteria (PB 9) and *Pseudomonas* (Pfa 2) isolates.
- 16) The adsorption per cent of *Azospirillum* (Azo 11) was 24.37 and 30.90, phosphobacteria (PB 9) showed 30.20 and 42.26 and *Pseudomonas* (Pfa 2) registered 32.31 and 47.48 at 30 and 60 min. after incubation, respectively. The rate of adsorption increased with the increase in the incubation period.
- 17) Compatibility test through cross streak method showed no inhibition zone which suggested that rhizobacterial isolates were compatible among themselves.
- 18) Co-inoculation of rhizobacterial isolates in different carriers was studied. Among the two carriers, lignite supported the survival of rhizobacterial isolates better than peat. The population of combined inoculation is on par with individual inoculation thus indicating compatibility of the rhizobacterial isolates.
- 19) Logistic population dynamics model was developed to study the influence of combined and individual inoculation of rhizobacterial isolates on the rhizosphere microflora of Cotton at graded levels of N and P. The growth rate of total diazotrophs (2.0673) was higher followed by bacteria (2.0853) and actinomycetes (0.3251) due to the combined inoculation of *Azospirillum* (Azo 11), phosphobacteria (PB 9) and *Pseudomonas* (Pfa 2) on 90 days after sowing, whereas negative growth of fungi (0.4011) indicated the declining population in the rhizosphere. Among the N and P levels, the diazotrophic growth rate decreased at recommended levels of N and P and found to be higher at reduced level of N and P (75%). However, the bacteria and actinomycetes population was found to be higher at recommended level of N and P.
- 20) The survival of rhizobacterial isolates was higher due to the combined than individual inoculation at graded levels of N and P. Among the rhizobacterial isolates, the survival rate of *Pseudomonas* (Pfa 2) was higher followed by phosphobacteria (PB 9) and *Azospirillum* (Azo 11). The reduced level of N and P (75% & 50%) supported the

survival of *Azospirillum* (Azo 11) isolate than recommended level (100% N & P), whereas phosphobacteria (PB 9) and *Pseudomonas* (Pfa 2) recorded highest survival rate in reduced level of N and P (75%) which is on par with recommended level of N and P (100%).

- 21) The growth parameters viz., shoot and root length, number and length of lateral roots and root volume were increased with combined inoculation of *Azospirillum* (Azo 11), phosphobacteria (PB 9) and *Pseudomonas* (Pfa 2) at recommended level of N and P (100%) which is on par with reduced level of N and P (75%).
- 22) The yield parameters like monopodial and symbodial branches, no. of bolls and boll weight were also enhanced due to the combined inoculation of rhizobacterial isolates at recommended level of N and P (100%) which is on par with reduced level of N and P (75%).
- 23) The macro and micro nutrients uptake were higher due to the combined inoculation of rhizobacterial isolates with recommended level of N and P (100%); Similar results with reduced level of N and P (75%) were also recorded.
- 24) The increase in yield (20.53 Q ha⁻¹) and dry matter production (4453 kg ha⁻¹) of cotton due to the combined inoculation of rhizobacterial isolates at recommend level of N and P (100%) was found to be on par with reduced level of N and P (75%) indicating a saving of 25 per cent of N and P with rhizobacterial inoculation.
- 25) The combined inoculation of rhizobacterial isolates with graded levels of N and P improved the fertility status of the soil as noticed by the increment in the soil available N, P and K levels after harvest.

In the present study the mathematical model proved that the increased growth rate of total diazotroph, bacteria and actinomycetes and decreased growth rate of fungi in rhizosphere was due to inoculation of rhizobacterial isolates. Increased growth rate of total diazotroph population implies higher nitrogen fixation in the soil which in turn encourage farmers to reduce the quantum of nitrogenous fertilizer application. Reduction in the growth rate of fungi in the rhizosphere indicate disease free cropping and thereby

minimizing the yield loss due to the soil borne pathogens. Farmers are benefited due to the application of PGPR in two ways: 1. Saving of 25 per cent inorganic fertilizer which in turn help in reducing the cost of cultivation. 2. In addition it will also help in increasing the yield. Moreover, this will also help in improving the fertility status of soil due to PGPR inoculation.

The future thrust

Evolving a common medium which will promote the growth of rhizobacterial isolates for combined inoculum production is worthwhile since it will reduce the cost of production.

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*Originals not found.

ANNEXURE

ANNEXURE

Composition of N-free Semisolid Malate Medium (Dobereiner and Day, 1975)

Malic acid	:	5.0 g
Dipotassium hydrogen orthophosphate	:	0.5 g
Magnesium sulphate	:	0.2 g
Sodium chloride	:	0.1 g
Calcium chloride	:	2.0 g
Fe-EDTA (1.64 per cent w/v aqueous)	:	4.0 ml
Trace element solution	:	2.0 ml
Alcoholic solution of Bromothymol blue (0.5 per cent)	:	2.0 ml
Vitamin solution	:	1.0 ml
Potassium hydroxide	:	4.0 g
Agar	:	1.75 g
Distilled water	:	1000 ml
pH	:	6.8

Trace element solution

Sodium molybdate	:	200 mg
Manganous sulphate	:	235 mg
Boric acid	:	280 mg
Copper sulphate	:	8.0 mg
Zinc sulphate	:	24.0 mg
Distilled water	:	200 ml

Vitamin solution

Biotin (Sigma, USA)	:	10.0 mg
Pyridoxin (Sigma, USA)	:	20.0 mg
Distilled Water	:	1000 ml

Composition of Yeast Extract Glucose Agar medium (Allen, 1953)

Glucose	:	5.0 g
Yeast extract	:	3.0 g
Peptone	:	5.0 g
Agar	:	15.0 g
Distilled water	:	1000 ml
pH	:	6.7 to 7.8

Composition of Congo Red Medium (Caceres, 1982)

Malic Acid	:	5.0 g
Dipotassium hydrogen orthophosphate	:	0.5 g
Magnesium sulphate	:	0.2 g
Sodium chloride	:	0.1 g
Ferric chloride	:	0.015 g
Potassium hydroxide	:	4.8 g
Agar	:	20.0 g
Distilled water	:	1000 ml

pH adjusted to 7.0 with 0.1 N KOH

Composition of Glucose peptone broth

Glucose	:	5.0 g
Peptone	:	3.0 g
Distilled water	:	1000 ml

pH of the broth was adjusted to 7.0 to 7.1

Production of acid from Glucose (Krieg and Tarrand, 1978)

Glucose	:	10.0 g
Peptone	:	2.0 g
Ammonium sulphate	:	1.0 g
Magnesium sulphate	:	1.0 g
Ferric chloride	:	0.002 g
Manganous sulphate	:	0.002 g
Alcoholic solution of bromothymol blue	:	2.0 ml
Distilled water	:	1000 ml

pH of the medium was adjusted to 7.0 to 7.1

Composition of Hogland's Nutrient solution (g l⁻¹)

(Hogland and Snyder, 1933)

Dipotassium hydrogen

Orthophosphate : 0.136

Potassium nitrate : 1.02

Calcium nitrate : 0.492

Magnesium sulphate : 0.49

Trace element solution : 1 ml

pH : 6.2

Trace element solution (g l⁻¹)

Boric acid : 2.86

Manganous chloride : 1.81

Zinc sulphate : 0.22

Copper sulphate : 0.08

Sodium molybdate : 0.11

Media for Determining Phosphate Solubilization

Composition of Sperber's hydroxy apatite medium (Sperber, 1958)

Soil extract : 100 ml *

Glucose : 1.0 g

Agar : 2.0 g

(*Autoclave soil suspensions, filter and use clear filtrate)

Sterilize in 100 ml lots, cool and add 5 ml of 10 % K₂HPO₄ and 10 ml of 10% CaCl₂ to each flash. Adjust pH to 1.0 with sterile N/10 NaOH. Pour plates immediately and allow to solidify.

Composition of Pikovskaya's medium (Pikovskaya, 1948)

Tricalcium phosphate : 5.0 g

Glucose : 10.0g

Ammonium sulphate : 0.5 g

Sodium chloride : 0.2 g

Magnesium sulphate : 0.1 g

Potassium chloride : 0.2 g

Yeast extract : 0.5 g

Manganese sulphate	:	trace
Ferrous sulphate	:	trace
Agar	:	15.0 g
pH	:	7.0

Composition of King's medium B (King *et al.*, 1954)

Glycerol	:	10 ml
Protease peptone	:	20 g
Dipotassium hydrogen orthophosphate	:	1.5 g
Magnesium chloride	:	1.5 g
Ampicillin	:	100 ppm
Chloramphenicol	:	50 ppm
Cycloheximide	:	10 ppm
Distilled water	:	1000 ml

Composition of Potato Dextrose Agar (PDA)

Potato (peeled)	:	200.0 g
Dextrose	:	20.0 g
Agar	:	15.0 g
Distilled water	:	1000 ml

Peel off the skin of potatoes, cut into small pieces and boil (200 g) in 500 ml of water, till they are easily penetrated by a glass rod. Filter through cloth. Add dextrose to the filtrate. Dissolve agar in water and bring up to the required volume by the addition of water. Autoclave at 15 lb pressure for 15 minutes.

Composition of Nutrient Glucose agar medium (for bacteria) (Anonymous, 1974)

Glucose	:	5.0 g
Peptone	:	5.0 g
Beef extract	:	3.0 g
Sodium chloride	:	5.0 g
Agar	:	15-20 g
pH	:	7.0

Composition of Martin's rose bengal medium (for isolation of fungi)

(Anonymous, 1974)

Glucose	:	10.0 g
Peptone	:	5.0 g
Potassium dihydrogen		
Orthophosphate	:	1.0 g
Magnesium sulphate	:	0.5 g
Rose Bengal	:	33 mg
Streptomycin sulphate	:	3 ml
(10 % solution)		
Agar	:	15.0-20.0 g
Distilled water	:	1000 ml

Composition of Ken Knight medium (for actinomycetes) (Anonymous, 1974)

Dipotassium hydrogen

orthophosphate	:	1.0 g
Sodium nitrate	:	0.1 g
Potassium chloride	:	0.1 g
Magnesium sulphate	:	0.1 g
Cellulose	:	10.0 g
Distilled water	:	1000 ml
pH	:	7.0-7.2

Composition of Watanabe and Baraqqia medium (for total diazotroph)

(Watanabe and Baraqqia, 1979)

Glucose	-	0.5 g
Malic acid	-	0.5 g
Yeast extract	-	0.1g
Dipotassium hydrogen orthophosphate	-	0.5g
Ferrous sulphate	-	0.4 g
Sodium molybdate	-	trace
Calcium chloride	-	0.2 g
Boric acid	-	0.5 g
Zinc sulphate	-	0.1 g
Manganous chloride	-	0.1g
Distilled water	-	1000 ml
pH	-	7.0
N-free agar	-	15.0g

Monthly weather data during the crop period 1999-2000

Month and year	Rainfall (mm)	Rainy days	Temperature		RH (%)		Sunshine (Hrs.)	EVP (mm)	Solar Radiation Cal/cm ² /day
			Max.	Min.	Morning	Evening			
Aug'99	21.9	2	31.8	21.6	83	51	5.9	5.7	354.9
Sep'99	28.0	3	32.7	22.2	85	45	7.9	5.9	440.0
Oct'99	306.1	16	30.0	22.1	91	66	5.2	2.9	325.6
Nov'99	104.2	6	29.2	19.9	91	59	6.9	3.1	389.0
Dec'99	23.2	3	27.8	19.2	90	58	6.0	3.0	382.9
Jan'99	23.2	-	30.7	19.5	89	46	7.1	3.9	417.6
Feb'99	36.9	4	31.6	19.3	90	48	6.7	4.1	392.9

