RESPONSE OF SUNFLOWER (*Helianthus annuus* L.) TO PHOSPHATE SOLUBILIZING BIOINOCULANTS IN CONJUNCTION WITH FORMS AND LEVELS OF PHOSPHORUS

RAVI GADAGI

DEPARTMENT OF AGRICULTURAL MICROBIOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES,
DHARWAD - 580 005.

OCTOBER, 1996.

RESPONSE OF SUNFLOWER (*Helianthus annuus* L.) TO PHOSPHATE SOLUBILIZING BIOINOCULANTS IN CONJUNCTION WITH FORMS AND LEVELS OF PHOSPHORUS

Thesis Submitted to the
University of Agricultural Sciences, Dharwad
in partial fulfillment of the requirements for the

Degree of

Master of Science (Agriculture)

IN

AGRICULTURAL MICROBIOLOGY

By

RAVI GADAGI

DEPARTMENT OF AGRICULTURAL MICROBIOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES,
DHARWAD - 580 005

OCTOBER, 1996.

DEPARTMENT OF AGRICULTURAL MICROBIOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES, DHARWAD.

CERTIFICATE

This is to certify that the thesis entitled **"RESPONSE OF SUNFLOWER (*Helianthus annuus* L.) TO PHOSPHATE SOLUBILIZING BIOINOCULANTS IN CONJUNCTION WITH FORMS AND LEVELS OF PHOSPHORUS"** submitted by ***RAVI GADAGI*** for the degree of **MASTER OF SCIENCE (AGRICULTURE)**in Agricultural Microbiology, to the University of Agricultural Sciences, Dharwad, is a record of research work done by him during the period of his study in this University under my guidance and supervision and the thesis has not previously formed the basis for award of any degree, diploma, associateship, fellowship or other similar titles.

DHARWAD
October, 1996.

(A.R. ALAGAWADI)
Major Advisor

Approved by :
Chairman :

(A.R. ALAGAWADI)

Members :1.

(K.S. JAGADISH)

2.

(V.S. DODDAMANI)

Affectionately Dedicated
to my Father
Late Shri. S. R. Gadagi

ACKNOWLEDGEMENT

It is with immense pleasure that I express my deep sense of gratitude and indebtedness to Dr. A.R. Alagawadi, Associate Professor of Agricultural Microbiology, U.A.S., Dharwad and Chairman of my Advisory Committee for his masterly guidance, constant encouragement, expert advice and constructive criticism throughout the course of study and persual of this manuscript.

I am deeply indebted to Shri. K.S. Jagadish, Assistant Professor, Department of Agricultural Microbiology, Dr. V.S. Doddamani, Soil Physicist, Main Research Station, UAS, Dharwad and Dr. S.M. Hiremath Associate Professor, Department of Agronomy, College of Agriculture, Bijapur who served as members of my advisory committee and for their kind co-operation, valuable assistance and support.

I am highly indebted to Dr. J.H. Kulkarni, Professor and Head of Agricultural Microbiology, for his valuable suggestions and encouragement.

My thanks are due to Dr. M.N. Sreenivasa, Shri, P.U. Krishnaraj and the rest of the staff in the Department of Agricultural Microbiology, for their timely assistance during my research.

The inspiration inculcated in me for an academic career was due mainly to my beloved mother and brothers. I am most beholden to all them. I immensely thank my dear sisters and brother-in-laws for all their affection and keen interest shown in my career.

Thanks is the worst word in friendship. Yet, I shall avail this opportunity to extend by sincere gratitude to Girish Patil, Mahesh Hanchinamani, Arun Sataraddi and Nirmal and all those who have helped directly or indirectly in the preparation of the thesis.

I take this opportunity to acknowledge my sincere gratitude to M/s. Zuari-Agro Chemicals Limited for providing me financial assistance in the form of Junior Fellowship.

Finally, I record my appreciation to Mr. Prakash Agadi for typing the thesis neatly.

Ravi Gadagi

October, 1996
Dharwad.

(RAVI GADAGI)

CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

I. INTRODUCTION

Phosphorus is the `Master key' element in crop production (Pierre, 1938) and is a major plant nutrient required in sufficient amounts for higher crop yields as well as for proper functioning of soil biota. Phosphorus is considered as one of the major limiting factor in successful crop production since the intensive cropping has resulted in widespread deficiency of this nutrient. The situation is becoming more complicated because the natural reserves, as the raw materials for the manufacture of phosphatic fertilizers, are limited and are depleting fast.

Most of the soil phosphorus is not in easily available form, because of its low solubility and its fixation in soil. Hence, it is necessary to apply phosphatic fertilizers to meet the crop requirement. However, the use efficiency of added phosphatic fertilizers ranges from only 15 to 25 per cent, due to fixation of P in the form of iron or aluminium phosphates in acid soils and calcium phosphate in alkaline soils. This native or applied fertilizer phosphorus is a non-renewable resource and therefore a better utilization of phosphorus by plants helps in conserving the limited supply of phosphorus in nature.

In view of the severe shortfall in phosphatic fertilizers and also the cost involved, there is an increasing trend throughout the world, to explore the possibility of utilizing less exploited reserves of rock phosphates. There are large deposits of low grade rock phosphate in India which are unsuitable for manufacture of super phosphate because of low phosphate content and impurities in it. The phosphorus present in rock phosphate is in insoluble form and plants cannot utilize this phosphorus unless it is converted into available form. A number of soil microorganisms are known to solubilize insoluble inorganic phosphates and make it available to plants. The use of phosphate solubilizing microorganisms in making phosphorus available to plants has been studied under different soil conditions throughout the world and they have been recognized as a potential means to convert the unavailable phosphorus into available form.

The first commercial P-solubilizing biofertilizer was prepared in the then USSR under the name `phosphobacterin' incorporating *Bacillus megaterium* var. *phosphaticum*. This was widely used in USSR and other East European countries. Several field studies have also been conducted in India using various P-solubilizing microorganisms like *B. megaterium* var. *phosphaticum*, *B. polymyxa, B. firmus, Pseudomonas striata, Aspergillus awamori, Penicillium digitatum*, etc., as bioinoculants in wheat, rice, potato, bengal gram, cotton and other crops (Gaur, 1990) with an increase in the P-uptake and yield. However, studies on performance of P-solubilizers in sunflower is very limited.

Sunflower (*Helianthus annuus* L.) is an important oilseed crop, ranks third after soybean and groundnut as a source of edible oil in the world. Sunflower is grown over an area of 2.4 m hectars in India with an annual production of 1.8 m tonnes (Anon., 1995). Sunflower though introduced recently, is making headway in

Indian agriculture as an important oilseed crop. However, information on the response of sunflower to inoculation with phosphate solubilizing biofertilizers, specially in combination with different forms of phosphorus and levels of phosphorus is inadequate. The present investigation was therefore undertaken with the following objectives :

1. To study the influence of phosphate solubilizing biofertilizers viz., 'biophos' and *Pseudomonas striata* in combination with different forms and levels of phosphorus on growth, nutrient uptake and yield of sunflower.

2. To study the inoculation and P-fertilizer effect on the rhizosphere microflora of sunflower especially P-solubilizers and free-living N_2 fixers.

3. To study the inoculation and P-fertilizers influence on available P-content of soil at different stages of crop growth.

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

Soil microorganisms play a significant role in making phosphorus available to plants by dephosphorylating phosphorus bearing organic compounds and also by bringing about favourable changes in soil reaction and in the soil micro environment leading to solubilization of insoluble inorganic phosphate sources. The literature on the solubilization of insoluble phosphates by microorganisms and the inoculation effects of phosphate solubilizers on the growth and yield of different crops has been reviewed in this chapter.

Many soil microorganisms can solubilize insoluble phosphates which are largely unavailable to plants. Microbial involvement in the solubilization of insoluble phosphate was first shown by Stalstrom (1903). However, Sackett *et al.* (1908) gave conclusive evidence to show that soil bacteria dissolved rock phosphate, bonemeal, di- and tricalcium phosphates etc. Most of the studies on phosphate solubilization were done first by isolating the microorganisms from soil and studying the solubilization *in-vitro*. The investigations on solubilization of insoluble phosphates under field conditions and its uptake by plants were however started later. A systematic approach to the study was made by Pikovskaya (1948) who isolated soil bacteria capable of dissolving tricalcium phosphate. Various microorganisms capable of dissolving either insoluble phosphorus compounds or fixed form of phosphorus have been isolated from the soil and rhizosphere which include *Bacillus megaterium* var. *phosphaticum, Bacillus megaterium* var *serratia*

(Menkina, 1950), *Achromobacter, Brevibacterium, Flavobacterium, Micromonospora, Sarcina* and *Serratia* (Sperber, 1958), *Pseudomonas striata* (Bardiya and Gaur, 1972), *Bacillus firmus, Streptomyces* sp. (Banik and Dey, 1981), *Aeromonas* sp. (Al-Ghazali *et al.*, 1986). *Penicillium* sp. and *Pseudomonas* sp. (Illmer and Schinner, 1992).

2.1 Solubilization of insoluble phosphate

2.1.1 In pure culture

Pure cultures of several bacteria, fungi, actinomycetes and yeasts inoculated into liquid medium and incubated for varying lengths of time at suitable temperature and pH have been shown to release soluble P from various insoluble P-containing compounds like bonemeal, hydroxy apatite, iron and aluminium phosphate, di and tricalcium phosphate and rock phosphate (Sen and Paul, 1957; Goswami and Sen, 1962; Bardiya and Gaur, 1974; Oriuno *et al.*, 1978; Halder *et al.*, 1990, Illmer and Schinner, 1992; Narsian *et al.*, 1995).

`Phosphobacterin', `posfo-24' and an organism isolated from glands of *Cassia occidentalis* released 4.73, 2.68 and 3.13 mg soluble P_2O_5/100 ml broth respectively from tricalcium phosphate after 15 days of inoculation (Goswami and Sen, 1962). Ostwal and Bhide (1972) studied the solubilization of tricalcium phosphate by 34 pseudomonads isolated from the soils of Maharashtra in liquid medium and found them to solubilize 13-58 per cent tricalcium phosphate. *Pseudomonas putida* among the isolates, showed maximum solubilization of 58 per cent of the added tricalcium phosphate.

Solubilization studies involving insoluble inorganic phosphate compounds by different P-solubilizing organisms in liquid medium indicated better solubilization by fungal cultures than bacteria. *Pseudomonas striata* among bacteria and *Aspergillus awamori* among fungi showed maximum solubilization of the added insoluble P compounds (Arora and Gaur, 1979). However, Gaur and Gaind (1983) found *Aspergillus awamori* to be superior to *Pseudomonas striata* in solubilization of tricalcium phosphate, iron and aluminium phosphates in broth culture. Singh *et al.* (1984) also observed higher solubilization of rock phosphates of different origin by *Aspergillus awamori* than *Pseudomonas striata* in liquid medium.

Forster and Freier (1988) studied P-mobilizing efficiency of 41 isolates of fungi obtained from the fertile arable soil with high phosphorus content. Only four out of 41 isolates tested *in-vitro*, showed a very high dissolution of rock phosphate. The isolates released 1.61 to 3.42 mg of P out of 5.8 mg of insoluble P added in the form of rock phosphate in broth medium.

Even the strains of *Rhizobium* and *Bradyrhizobium* have been found to effectively solubilize rock phosphate and lower the pH of the medium. Among the organisms tested, *Rhizobium leguminosarum* biovar *viceae* BICC 635 was the most effective solubilizer of rock phosphate and the production of 2-ketogluconic acid by this strain was suggested to be the primary cause of rock phosphate solubilization (Halder *et al.*, 1991). Illmer and Schinner (1992) reported high solubilizing ability of a *Penicillium* sp. and a *Pseudomonas* sp. isolated from forest soil to solubilize hydroxy apatite and calcium hydroxy apatite dihydrate. *Aspergillus aculeatus* isolated from rhizosphere of gram, showed highest tricalcium phosphate solubilizing activity in liquid medium after 48 hours of fungal growth in the presence of glucose and ammonium sulphate as the carbon and nitrogen sources (Narsian *et al.*, 1995).

2.1.2 In Soil

The physical and chemical properties of soil determine the nature of environment in which microorganisms are present. These environmental characters in turn affect the composition of the microbial population both qualitatively and quantitatively. Using ^{32}P labelled compounds, several workers have shown that inoculation of pure culture of phosphate solubilizers to soil released more water soluble phosphate from tricalcium phosphate and other mineral phosphates.

P-dissolving ability of some bacterial and fungal strains isolated from forest soil, was examined by Moreau (1959) in soil incubation study. He was of the opinion that, liberation of P from glycerophosphate or tricalcium phosphate was done mainly by bacteria and also by some fungi. The organisms solubilized three to four per cent of the added insoluble phosphorus within ten days of incubation. Bajpai and Sundara Rao (1971) conducted soil incubation studies using ^{32}P labelled super phosphate or apatite with and without farmyard manure and *Bacillus megaterium* or *Bacillus circulans*. They found that inoculation of *Bacillus megaterium* and *Bacillus circulans* to sterilized soil increased the available P_2O_5 of soil. They also observed an increase in solubilization of native soil phosphorus. Ostwal and Bhide (1972) isolated 72 pseudomonads from soils of Maharashtra, and examined 34 efficient cultures for solubilization of tricalcium phosphate in soil. They found them to solubilize 8 to 27 per cent tricalcium phosphate. Among the isolates, *Pseudomonas putida* showed maximum solubilization of 37 per cent of the added tricalcium phosphate in soil.

Five most efficient P-solubilizing strains, isolated from wheat and broad bean, were studied for their P-solubilizing ability in sterile and non-sterile clay loam soils with insoluble inorganic phosphates, RNA, phytin and lecithin as P-sources. Phosphate solubilization was greater in non-sterile soils indicating that inoculation was more effective in the presence of normal soil microflora (Mahmoud *et al.*, 1976). Banik and Dey (1982) studied solubilization of rock phosphate in soil by several phosphate dissolving microorganisms isolated from alluvial soil and reported that *Aspergillus candidus* and *Bacillus firmus* performed better than the other isolates.

Jisha (1993) in a soil incubation study recorded higher available P in soil due to inoculation with *Pseudomonas striata* or *Bacillus polymyxa* in both sterilized and unsterilized soil amended with rock phosphate and cotton stalk. However, *Pseudomonas striata* showed higher solubilization of added rock phosphate than *Bacillus polymyxa.*

2.2 P-solubilizing microorganisms as inoculants and crop response

Since the literature on the response of sunflower crop to inoculation with phosphate solubilizing microorganisms is very limited, the inoculation influence in other crops is also reviewed briefly here under.

2.2.1 Sunflower

The earliest report of increased P-uptake and dry weight of plants through inoculation of phosphate solubilizing organisms was made by Gerretsen

(1948). He found that sunflower, oat, mustard and rape inoculated with pure cultures of rhizosphere bacteria in pots containing sterilized soil amended with poorly soluble phosphate, resulted in increased dry weight of plants as well as P-uptake. Significant increase in N and P uptake and green matter was also observed during the first stage of vegetation in sunflower plants due to soil inoculation with phosphate dissolving microorganisms (Stefan and Boti, 1960).

Investigations carried out by Forster and Freier (1988), on the efficiency of P-mobilizing microorganisms in the rhizosphere of sunflower indicated increased dry weight and phosphorus uptake in sunflower plants. Jones and Sreenivasa (1993) studied the response of sunflower to inoculation with *Pseudomonas striata* and phosphorus application in black clayey soil. The results indicated that inoculation of *Pseudomonas striata* increased the plant height, dry matter content and seed yield over uninoculated treatment. They also recorded increase in the population of P-solubilizers in the rhizosphere of inoculated plants upto 60 days after planting. Use of phosphate solubilizing biofertilizer, produced from *Bacillus megaterium* var. *phosphaticum*, in sunflower, in combination with application of single super phosphate or Mussoorie rock phosphate increased the dry matter yield and phosphorus uptake significantly when compared to uninoculated control (Rachewad *et al.*, 1992).

Ravishankar (1993) in a field trail conducted in vertisol under irrigated condition, recorded significantly higher yield of sunflower due to inoculation with P-solubilizing biofertilizer prepared out of *Pseudomonas striata*.

The higher yields in inoculated treatments were attributed to increased head diameter, total number of seeds and number of filled seeds per head.

2.2.2 Groundnut

Manjaiah (1989) recorded significantly higher pod yield, shelling percentage, test weight and phosphorus uptake in groundnut crop inoculated with *Aspergillus awamori* and applied with Mussoorie rock phosphate and FYM. Similarly, application of Mussoorie rock phosphate plus FYM plus *Pseudomonas striata* increased the phosphorus uptake in field grown groundnut significantly at all the stages of crop growth compared to application of FYM plus Mussoorie rock phosphate without inoculation (Hebbara and Suseeladevi, 1990). Mudalagiriyappa (1993) also recorded significantly higher pod yield and P-uptake in groundnut due to inoculation of *Pseudomonas striata* and *Aspergillus awamori*.

2.2.3 Soybean

Seed inoculation with *Bacillus megaterium* in combination with application of 100 kg P_2O_5 ha^{-1} as rock phosphate has been reported to increase the P-uptake and dry matter yield of soybean (Ahmad and Jha, 1982). Banik and Datta (1988) observed significant increase in the vegetative growth of soybean plants inoculated with *Bacillus firmus* and applied with rock phosphate as P-source. Significantly higher seed yield and P-uptake of field grown soybean due to inoculation with the two phosphate solubilizing biofertilizers viz., `phosphin' and `microphos' has also been reported by Patil (1990).

2.2.3 Other crops

Seed inoculation of maize with *Pseudomonas* sp. significantly increased the dry matter yield and P-uptake when compared to uninoculated control (Kavimandan and Gaur, 1971). Rachewad *et al.* (1991) reported that seed inoculation of maize with *Bacillus polymyxa* and/or application of 75 kg P ha^{-1} in the form of single super phosphate or Mussoorie rock phosphate increased the biomass production, P-content and uptake in plants compared to uninoculated plants. They also found that inoculation along with addition of P was more effective than inoculation alone.

Significant increase in the grain and straw yield as well as phosphorus uptake of wheat due to inoculation with *Bacillus polymyxa* and rock phosphate application has been reported by Gaur and Ostwal (1972). Similar increase in the grain yield of wheat was recorded under field condition when rock phosphate was applied to soil and seeds were inoculated with *Pseudomonas striata*. Further, the response of the crop to bacterial inoculation was equivalent to 50 kg P_2O_5 ha^{-1} as super phosphate (Gaur *et al.*, 1980). Chakraborthy *et al.* (1986) also recorded higher yield of wheat due to application of `phosphobacterin' in combination with Mussoorie rock phosphate.

Asea *et al.* (1988), using ^{32}p isotope dilution method, found that green house grown wheat inoculated with *Penicillium bilaji* was able to obtain 18 per cent of P from sources unavailable to uninoculated plants and the organism was also able to solubilize added rock phosphate. Kucey (1988) observed increased dry matter production and P-uptake in wheat in response to *Penicillium bilaji* inoculation both under green house and field conditions. Field trail conducted by Tiwari *et al.* (1989) indicated that seed inoculation with *Pseudomonas striata* caused a greater impact on wheat crop.

Sharma and Singh (1971) reported higher grain yield and increased N and P-uptake of rice due to inoculation with `phosphobacterin' and bone meal application to sandy loam soil of Karnal. A significant increase in P-uptake and dry weight of pot grown rice plants, due to inoculation with *Bacillus* spp. was recorded by Banik and Dey (1981). Datta *et al.* (1982) also found significant increase in the yield and phosphorus uptake of rice due to inoculation with *Bacillus firmus*. The results of field trails conducted in alluvial soil indicated that even simple inoculation of rice seedlings with *Bacillus polymyxa* increased the grain yield by 5.7 q and 2.7 q ha^{-1} in two successive years. Further, application of rock phosphate @ 60 kg P_2O_5 ha^{-1} plus `microphos' culture was as effective as super phosphate applied at the same rate in enhancing the rice yield (Gaur and Singh, 1982). Kundu and Gaur (1984) reported appreciable increase in the yield and nutrient uptake of rice due to inoculation with P-solubilizing *Pseudomonas striata* and *Aspergillus awamori* with or without the addition of chemical fertilizers.

Inoculation of phosphate solubilizing culture (consisting of *Pseudomonas striata* and *Bacillus polymyxa* mixed in 1 to 1 ratio) alone or in combination with phosphatic fertilizers increased the root cation exchange capacity, available phosphorus content in soil and P-uptake in field grown rice plants (Mohod *et al.*, 1989). Inoculation with the same P-solubilizing culture also increased the number of grains per panicle, grain weight per panicle, 1000 grain weight, grain yield, straw yield and N-uptake of rice (Mohod *et al.*, 1991). Anthoniraj *et al.* (1994) also reported positive response of low land rice under field condition to inoculation with phosphobacteria, although the extent of benefit varied with the P-levels tested. They also noted higher population of phosphobacteria in inoculated plots than in uninoculated plots.

Salih *et al.* (1989) found that, inoculation of sorghum with *Penicillium* and *Aspergillus* isolates in combination with application of triple super phosphate or rock phosphate increased the dry matter yield and P-uptake in plants in calcareous soil. Increase in the grain and straw yield of sorghum under field condition has also been reported by Alagawadi and Gaur (1992) due to inoculation with *P. straita* and *B. Polymyxa.*

Jisha (1993) reported that, the inoculation of *Pseudomonas striata* or *Bacillus polymyxa* showed significant positive influence on growth, yield and nutrient uptake of sorghum and also showed increased available P-content in soil compared to uninoculated control. Further the population of P-solubilizers in the rhizosphere of inoculated plants was higher than in uninoculated plants at 60 days of plant growth.

Use of phosphate solubilizing bacteria viz., *Pseudomonas striata* or *Bacillus polymyxa* and their influence on the nutrient uptake and yield of sorghum was studied by Jisha and Alagawadi (1996), in vertisol amended with cotton stalk and rock phosphate. They observed that inoculation of *Pseudomonas striata* and

Bacillus polymyxa increased the size and weight of earhead, number of spikelets per ear, straw and grain yield as well as nutrient uptake and available P content in soil, significantly over uninoculated control. The population of phosphate solubilizing bacteria was higher in inoculated plants than that of the uninoculated plants.

Alagawadi and Gaur (1988) studied the influence of P-solubilizing *Pseudomonas striata* and *Bacillus polymyxa* with and without added chemical fertilizers on the yield and nutrient uptake of chickpea and reported significant increase in the available P-content of soil, dry matter content, grain yield, and nutrient uptake of plants due to inoculation. They also noted higher population of P-solubilizing bacteria in the rhizosphere of inoculated plants than uninoculated plants. Field trails conducted by Tiwari *et al.* (1989) indicated that seed inoculation with *Bacillus polymyxa* markedly increased the yield of chickpea. Inoculation of P-solubilizing bacteria along with the application of 17.5 kg P ha^{-1} in the form of Mussoorie rock phosphate resulted in increased dry matter in chickpea and was as effective as the application of single super phosphate (Prabhakar and Saraf, 1990). Inoculation with phosphate solubilizing bacteria increased the seed yield of black gram by 9.5 and 22.5 per cent over control in two successive years (Tomar *et al.*, 1993).

2.3 Production of growth promoting substances by phosphate solubilizing microorganisms

Besides solubilizing insoluble phosphates and making it available to plants, the phosphate solubilizing microorganisms are also known to produce growth promoting substances and benefit the plants. Brown (1972), Azcon and Barea (1975) and Barea *et al.* (1976) have reported that phosphate dissolving microorganisms of soil and rhizosphere produce plant growth promoting substances like indole acetic acid, gibberellins, cytokinins, etc.

Out of the 50 phosphate solubilizing bacteria examined by Barea *et al.* (1976), 43 produced IAA, 29 formed gibberellins 45 cultures produced cytokinin like substances. Twenty cultures were able to synthesize all the three types of plant hormones. Sattar and Gaur (1987) also reported production of auxins and gibberellins to a considerable content by phosphate dissolving *Bacillus polymyxa, Bacillus pulvifaciens, P. striata* and *Aspergillus awamori.* However, strains varied in nature and quantity of production of plant growth promoting substances. They suggested that the beneficial effects of these organisms may also be due to the production of plant growth promoting substances, besides phosphate solubilization.

MATERIAL AND METHODS

III. MATERIAL AND METHODS

A field experiment was conducted in deep black soil at the Main Research Station of the University of Agricultural Sciences, Dharwad, under rainfed condition during *kharif* season of 1994-95 to study the effect of P-solubilizing biofertilizers in combination with different forms and levels of phosphorus on growth, phosphorus uptake and yield of sunflower, as well as rhizosphere microflora and available P content in soil. The details of the material used and the experimental techniques adopted during the course of investigation are described in this chapter.

3.1 Location and experimental site

The experiment was conducted in plot number 125 of `E' block of the Main Research Station, University of Agril. Sciences, Dharwad, which is situated at 15°-26' north latitude, 75°-07' east longitude and an altitude of 678 meters above mean sea level.

3.2 Soil characters of experimental site

The soil of the experimental site was deep black clayey in texture. Composite soil sample upto a depth of 30 cm collected from the experimental site before initiating the experiment was analysed for important physical and chemical properties as well as microbial populations by employing standard methods (Table 1).

Table 1. Physical and chemical properties and initial microbial population of the experimental site

Particulars	Value	Method employed
I. Physical properties		
Coarse sand (%)	6.10	Particle size distribution by International pipette method (Piper, 1966)
Fine sand (%)	13.14	
Silt (%)	28.00	
Clay (%)	50.76	
Field capacity (%)	33.00	Field method (Dastane, 1972)
Bulk density (g\cc)	1.29	Core sample method (Dastane, 1972)
II. Chemical properties		
Available N (kg/ha)	196.00	Alkaline permanganate method (Subbaiah and Asija, 1959)
Available P_2O_5 (kg/ha)	14.25	Olsen's method (Jackson, 1967)
Available K_2O (kg/ha)	601.00	Flame photometer (Jackson, 1967)
Organic carbon (%)	0.73	Walkley and Black's wet oxidation method (Jackson, 1967)
pH (1:2.5)	8.10	Beckman's pH meter (Piper, 1966)
III. Initial microbial population		
Bacteria	77.50×10^6	Serial dilution plate count technique
Fungi	23.50×10^3	
Actinomycetes	10.50×10^3	
Free living N_2-fixers	20.00×10^3	
P-solubilizers	15.00×10^3	

3.3 Climatic conditions

The monthly meteorological data for the period from January, 1995 to December, 1995 and the average for the past 44 years (1951-1994) as recorded at meteorological observatory of the Main Research Station, UAS, Dharwad, are furnished in Appendix I.

During 1995, total rainfall received was 790.00 mm which was 11 mm less than the average of 44 years. Out of 790.00 mm rainfall, 299.6 mm was received during the cropping period (4th August to 2nd November). The highest and the lowest mean maximum temperature during crop period was 29.3 and 28.1°C where as, the highest and the lowest mean minimum temperature was 20.70°C and 15.20°C respectively. The relative humidity ranged from 75 per cent in June to 86 per cent in August.

3.4 Previous crop history of experimental site

During the *kharif* season of 1993-94, general maize crop was taken up with all common cultural operations, and in *rabi* season the land was kept fallow.

3.5 Experimental details

The details of the experiment with regard to crop variety, treatment combinations, the design and plot size are furnished below.

3.5.1 Treatments

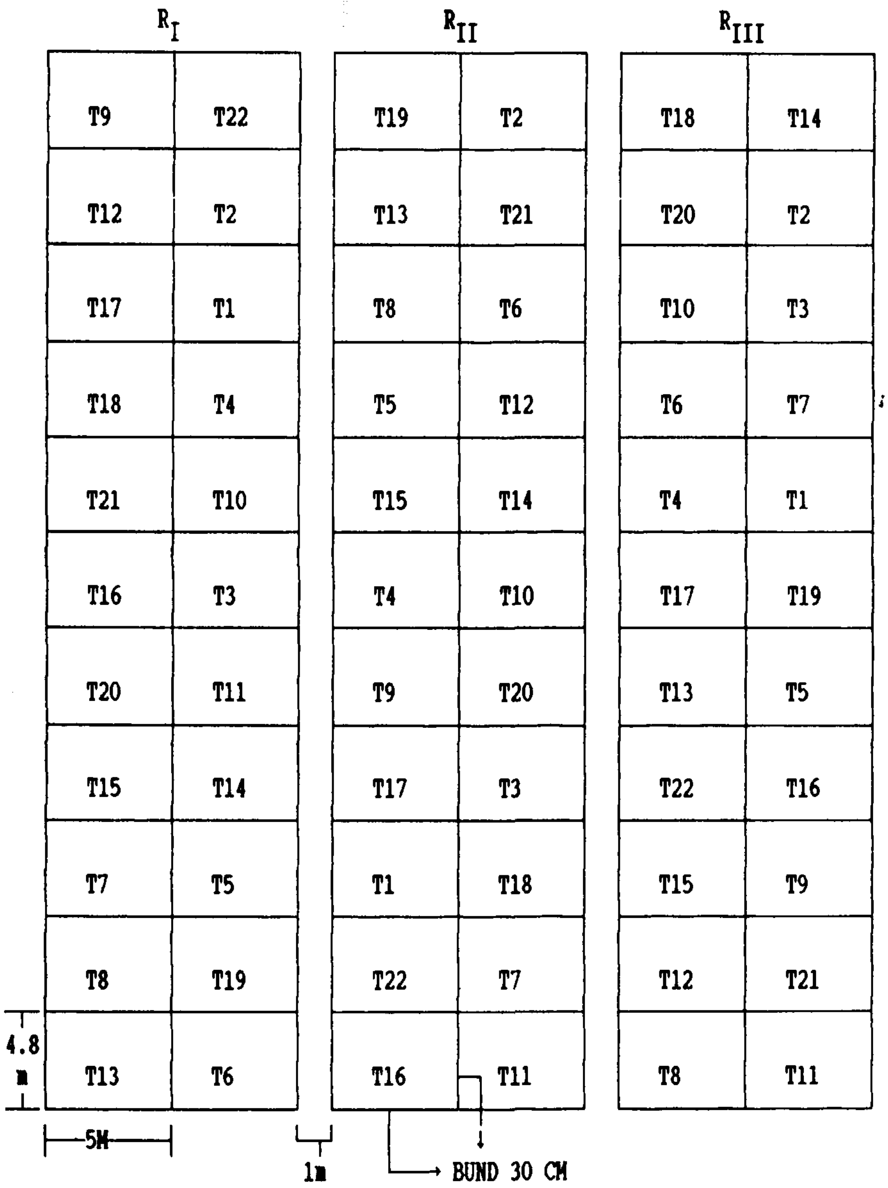
There were 21 treatment combinations involving forms and levels of phosphorus as well as P-solubilizing biofertilizers as given below:

T_1 - No P and uninoculated control (UIC)
T_2 - No P + Biophos
T_3 - No P + *P. striata*
T_4 - SSP at 50% RDP + UIC
T_5 - SSP at 50% RDP + Biophos
T_6 - SSP at 50% RDP + *P. striata*
T_7 - SSP at 75% RDP + UIC
T_8 - SSP at 75% RDP + Biophos
T_9 - SSP at 75% RDP + *P. striata*
T_{10} - SSP at 100% RDP + UIC
T_{11} - SSP at 100% RDP + Biophos
T_{12} - SSP at 100% RDP + *P. striata*
T_{13} - MRP at 50% RDP + UIC
T_{14} - MRP at 50% RDP + Biophos
T_{15} - MRP at 50% RDP + *P. striata*
T_{16} - MRP at 75% RDP + UIC
T_{17} - MRP at 75% RDP + Biophos
T_{18} - MRP at 75% RDP + *P. striata*
T_{19} - MRP at 100% RDP + UIC
T_{20} - MRP at 100% RDP + Biophos
T_{21} - MRP at 100% RDP + *P. striata*

(MRP - Mussoorie rock phosphate;
SSP - Single super phosphate;
RDP - Recommended dose of phosphate)

3.5.2 Design and layout

The experiment was laidout in randomised block design with three replications. The plan of layout is presented in Figure 1. The size of individual plots was 5m x 4.8m.



R_I		R_{II}		R_{III}	
T9	T22	T19	T2	T18	T14
T12	T2	T13	T21	T20	T2
T17	T1	T8	T6	T10	T3
T18	T4	T5	T12	T6	T7
T21	T10	T15	T14	T4	T1
T16	T3	T4	T10	T17	T19
T20	T11	T9	T20	T13	T5
T15	T14	T17	T3	T22	T16
T7	T5	T1	T18	T15	T9
T8	T19	T22	T7	T12	T21
T13	T6	T16	T11	T8	T11

Fig 1. Plan of Layout

3.5.4 Crop variety

Sunflower hybrid KBSH-1, obtained from Senior Farm Superintendent, Main Research Station, UAS, Dharwad, was used in the experiment. The variety has a normal maturity period of 90-95 days with uniform maturity. It is one of the recommended varieties for Karnataka State.

3.6 Biofertilizers

The two phosphate solubilizing biofertilizers used in the study were

1. `Biophos'
2. *Pseudomonas striata*

`Biophos', a commercial carrier based biofertilizer manufactured by M/s Ajay Biotech, Pvt. Ltd. Pune and marketed by M/s Zuari Agrochemicals Ltd., was obtained from the local market. The efficient phosphate solubilizing bacterium, *Pseudomonas striata*, obtained from the Microbiology Division, Indian Agricultural Research Institute, New Delhi, was used as another biofertilizer in the study. The carrier based inoculum of this bacterium was prepared by transferring a loopful of culture from 24 hours old culture slants to 50 ml Pikovskaya's broth (Pikovskaya, 1948). After two days of incubation at 28±2°C, the entire broth culture was transferred to one liter capacity Erlenmeyer flask containing 500 ml Pikovskaya's broth. The flasks were incubated at 28±2°C for three days and then mixed with sterilized lignite in the ratio of 1:2 and cured at room temperature for one week in sealed polythene bags before use. The *P. striata* inoculum had a population of

28×10^8 g^{-1} carrier material where as that in `biophos' was 22×10^8 g^{-1} on the day of inoculation.

3.7.1 Land preparation

The land was ploughed once with tractor drawn iron plough during the month of May, 1995. This was followed by two harrowings to bring the soil to fine tilth. Stubbles and weeds were collected and disposed out of the experimental site. Experimental plots were laidout as per the plan given and each plot was provided with bunds all around to avoid contamination of soil and water from other plots.

3.7.2 Manure and fertilizer application

FYM at the rate of seven tonnes per hectare was uniformly applied to all the plots 15 days prior to sowing. The fertilizer dose recommended for the crop was 35:50:35 kg NPK/ha and all the fertilizers were applied as basal dose at the time of sowing. Nitrogen in the form of urea and potassium in the form of muriate of potash were applied commonly to all the plots. Phosphorus was applied at 0, 50, 75 and 100 per cent of the recommended dose in the form of single super phosphate or Mussoorie rock phosphate as per the treatment schedule.

3.7.3 Seed inoculation and sowing

Seeds were inoculated separately with carrier based inoculum of `biophos' and *Pseudomonas striata* using gum arabic solution as an adhesive. Inoculated seeds were dried in shade for half an hour and were sown immediately.

The seeds were sown on 4th August 1995 at the rate of two seeds per hill by hand dibbling with a spacing of 60 cm between the rows and 20 cm between the plants within each row. Gap filling was taken up after seven days of sowing to ensure uniform plant population. Thinning was done 15 days after sowing to maintain one plant per hill.

3.8 Plant protection and after care

To check the weed growth, inter-cultivation was done at three and six weeks after sowing with the help of a blade hoe and two hand weedings were done at fourth and seventh week after sowing. Three insecticidal sprays consisting of Monocrotophos (0.02%), Endosulfan (0.02%) and Monocrotophos (0.01%) plus Novan (0.05%) respectively at second, fourth and sixth week after sowing, and a fungicidal spray consisting of Mancozeb (0.2%) at eighth week after sowing were given to protect the crop from pests and diseases.

3.9 Harvesting and threshing

The sunflower crop was harvested after 90 days of sowing when the crop attained full maturity as indicated by lemon yellow colour on the backside of head. The heads from each net plot were cut, air dried and hand threshed. The produce was cleaned and seed and straw weight per net plot were recorded after complete drying.

3.10 Observations

Observations on plant growth parameters, nutrient uptake, available P_2O_5 content in soil and rhizosphere microflora were recorded at monthly intervals. Yield and yield parameters were recorded at harvest.

3.10.1 Plant height

The height of five randomly selected plants was measured from the base of the plant to the growing tip and the average height was expressed in centimeters.

3.10.2 Number of green leaves per plant

Total number of fully opened green leaves from the randomly selected five plants were counted and their average was taken as number of green leaves per plant.

3.10.3 Dry matter accumulation

The dry matter accumulation of sunflower plants was recorded at 30 and 60 days after sowing and at harvest by uprooting five plants randomly. From uprooted plants, the root and shoot portions were separated and were separately dried in an oven at 70°C to constant weight. The shoot and root dry weights were recorded and the average weight was expressed in grams per plant.

3.10.4 Days to 50 per cent flowering

The number of days taken for 50 per cent of the plants to flower in each plot was recorded and expressed as days to 50 per cent flowering.

3.10.5 Yield and yield components

a) Diameter of head

The distance between the two diagonally opposite edges of the randomly selected five heads was measured and average diameter was expressed in centimeters.

b) Head weight

The heads of five plants were cut at the neck region of the plants and fresh weight of the heads was recorded and average weight was expressed in grams per head.

c) Number of seeds per head

The air dried heads of five plants were threshed to separate the seeds. The seeds obtained from each head were cleaned and the number of filled seeds were counted. The average number of seeds of five heads was expressed as number of seeds per head.

d) Seed yield per plant

The seeds obtained from each head of the five randomly selected plants were weighed seperately and the mean seed weight of five plants was taken as the seed weight per plant.

e) Thousand seed weight

One thousand seeds were counted from the seed samples drawn from the net plot yield and their weight was recorded and expressed in grams.

f) Seed yield

The air dried heads from each net plot were threshed, cleaned and the weight of the seeds were recorded. Based on the net plot yield, grain yield per hectare was calculated and expressed in quintals.

g) Stalk yield

The air dried stalks of each net plot were weighed and the yield of stalk per hectare was calculated.

3.11 Microbiological analysis

The rhizosphere soil samples collected at 30 and 60 days after sowing and at harvest were analysed for population of free-living N_2-fixers and phosphate solubilizers. The rhizosphere soil samples collected at 60 days after sowing were also analysed for population of total bacteria, fungi and actinomycetes.

The rhizosphere soil samples required for microbiological analysis were collected from the experimental plots by carefully uprooting the plants followed by gentle shaking to remove excess soil. The soil adhering to the roots was carefully collected and used for enumeration of total bacteria, fungi, actinomycetes, free-living N_2-fixers and P-solubilizers by the standard serial dilution plate count technique using soil extract agar (Allen, 1959) for bacteria, Martin's Rose Bengal agar (Martin, 1950) for fungi, Kuster's agar (Kuster and Williams, 1964) for actinomycetes, Norris N-free agar (Norris, 1959) for free-living N_2-fixers and Pikovskaya's agar (Pikovskaya, 1948) for phosphate solubilizers. Plates were incubated at 28 ± 2°C and colony counts were recorded on the respective plates after

3-7 days of incubation. The microbial populations were expressed as number of colony forming units per gram dry weight of soil.

3.12 Chemical analysis

3.12.1 Available phosphorus content in soil

The soil samples collected at 30 and 60 days after sowing and at harvest were air dried, powdered and passed through 2 mm sieve. The available phosphorus content of soil samples was determined by Olsen's method as described by Jackson (1973).

3.12.2 Plant analysis

The plant samples collected at 30 and 60 days of plant growth and the seeds collected at harvest were dried and ground in a Wiley mill and used for the estimation of nitrogen and phosphorus content by the standard procedures. The total nitrogen content in plant samples was estimated following the microkjeldahl method (Jackson, 1973) where as phosphorus content in the plant samples was estimated by the vanadomolybdate phosphoric acid yellow colour method of Jackson (1973). The N and P uptake was worked out by multiplying the N and P percentage with the corresponding dry matter yield and expressed as kg per hectare.

3.13 Statistical analysis of the data

Fisher's method of "analysis of variance" was applied for analysis and interpretation of the data (Panse and Sukhatme, 1967). The level of significance used in "F" test was P=0.05. Critical differences were calculated wherever "F" test was significant.

EXPERIMENTAL RESULTS

IV. EXPERIMENTAL RESULTS

The results of field trial conducted during the *kharif* season of 1995 under rainfed condition, to study the influence of phosphate solubilizing biofertilizers in combination with different forms and levels of phosphorus, on growth, yield and nutrient uptake of sunflower as well as soil available phosphorus and rhizosphere microflora are presented in this chapter.

4.1 Growth parameters

4.1.1 Plant height

The plant height, in general was found to increase markedly in all the inoculation and fertilizer application treatments as compared to control at both 30 and 60 days of crop growth (Table 2). At 30 DAS, inoculation with `biophos' and *Pseudomonas striata* showed significant increase in plant height (38.58 and 37.19 cm, respectively) over uninoculated control (35.35 cm). However, the two biofertilizers were on par with each other. Among the two forms of phosphorus, super phosphate application significantly enhanced the plant height (37.84 cm) over rock phosphate application (36.24 cm). The plant height was also found to increase significantly with increasing levels of phosphorus irrespective of the forms of phosphorus. Maximum plant height, among p-levels, was recorded at 100 per cent RDP (40.29 cm) followed by 75 per cent RDP and 50 per cent RDP (37.94 and 36.07 cm, respectively) and was least in control where no P was applied (33.86 cm). The interaction effects between the forms and levels of phosphorus and/or the inoculations were statistically non-significant.

Table 2. Effect of inoculation with `biophos' and P. striata in combination with different forms and levels of P on plant height (cms) at different growth stages

Forms of P	P-levels (%RDP)	30 DAS				60 DAS			
		UIC	`biophos'	P.striata	Mean	UIC	`biophos'	P.striata	Mean
Super phosphate	0	32.27	34.88	34.44	33.86	192.27	199.10	196.21	195.86
	50	36.66	37.77	37.66	37.36	204.55	210.10	209.66	208.10
	75	36.33	40.11	38.77	38.40	207.99	215.22	214.00	212.40
	100	38.32	43.99	42.99	41.73	213.55	221.11	220.99	218.55
	Mean	33.87	39.16	38.46	37.84	204.59	211.38	210.21	208.73
Rock phosphate	0	32.27	34.88	34.44	33.86	192.27	199.10	196.21	195.86
	50	33.22	38.44	32.66	34.77	196.44	204.51	204.10	201.68
	75	36.10	38.44	37.88	37.47	203.32	213.08	211.88	209.43
	100	37.77	40.10	38.66	38.84	208.66	216.77	215.00	213.48
	Mean	34.84	37.96	35.11	36.24	200.17	208.37	206.80	205.11
Mean		35.35	38.58	37.19	37.04	202.38	209.87	208.51	206.92

Means for levels of phosphorus

	30 DAS				60 DAS			
P-levels	0	50	75	100	0	50	75	100
Mean	33.86	36.07	37.94	40.29	195.86	204.89	210.91	216.01

Sources	SEm±	CD (0.05)	SEm±	CD (0.05)
Forms of P(A)	0.447	1.273	0.358	1.018
Levels of P (B)	0.663	1.801	0.506	1.439
Inoculation (C)	0.548	1.560	0.438	1.246
AxB	0.895	NS	0.715	2.035
AxC	0.775	NS	0.619	NS
BxC	1.096	NS	0.876	NS
AxBxC	1.550	NS	1.239	NS

RDP = Recommended dose of phosphorus, UIC = Uninoculated control, NS = Non-significant,
DAS = Days after sowing

An almost similar trend was also noticed at 60 days of crop growth. `Biophos' inoculation showed maximum plant height (209.87 cm), which was significantly superior over *P. striata* (208.51 cm) as well as uninoculated control (202.38 cm). *P. striata* inoculation also enhanced the plant height significantly over uninoculated control. Once again, application of super phosphate increased the plant height significantly (208.73 cm) over rock phosphate (205.11 cm). Among the phosphorus levels, maximum plant height was recorded at 100 per cent RDP (216.01 cm), followed by 75 per cent RDP (210.91 cm) and 50 per cent RDP (204.89 cm) all of which were significantly superior over control (195.86 cm) but differed significantly among themselves. The interaction effects between the forms and levels of phosphorus were significant at 60 DAS. Super phosphate application at 100 per cent RDP showed maximum plant height (218.55 cm) and was significantly superior over all other similar combinations. The interaction effects between forms of phosphorus and inoculation, inoculation and levels of phosphorus and forms of P x levels P x inoculation were found non-significant.

4.1.2 Number of leaves per plant

Maximum number of leaves per plant among inoculation treatments at 30 DAS (Table 3), was recorded in treatment receiving `biophos' inoculation (18.35 g plant^{-1}) followed by *P. striata* inoculation (18.14 g plant^{-1}) both of which were significantly superior over the uninoculated control (17.55 g plant^{-1}) but were on par with each other. Application of super phosphate, irrespective of inoculation and P-levels, produced significantly higher number of leaves (18.21 g plant^{-1}) than

Table 3. Number of leaves per plant as influenced by P-solubilizers in combination with different forms and levels of P at different growth stages

Forms of P	P-levels (%RDP)	30 DAS				60 DAS			
		UIC	`biophos'	P.striata	Mean	UIC	`biophos'	P.striata	Mean
Super phosphate	0	16.71	17.33	17.10	17.05	13.72	15.11	15.00	14.61
	50	17.99	18.21	18.55	18.25	15.44	19.55	18.44	17.81
	75	17.77	18.88	18.99	18.55	17.33	21.55	20.38	19.75
	100	18.44	19.21	19.33	18.99	19.31	22.88	21.99	21.39
	Mean	17.73	18.41	18.49	18.21	16.45	19.77	18.95	18.39
Rock phosphate	0	16.71	17.33	17.10	17.05	13.72	15.11	15.00	14.61
	50	17.22	18.10	17.88	17.73	15.33	17.66	16.33	16.44
	75	17.66	18.55	17.66	17.96	15.88	19.88	18.10	17.96
	100	17.88	19.22	18.55	18.55	17.22	20.00	21.33	19.59
	Mean	17.37	18.30	17.80	17.82	15.54	18.22	17.69	17.15
Mean		17.55	18.35	18.14	18.01	15.99	18.99	18.32	17.77

Means for levels of phosphorus

	30 DAS				60 DAS			
P-levels	0	50	75	100	0	50	75	100
Mean	17.05	17.99	18.25	18.77	14.61	17.12	18.85	20.49

Sources	SEm±	CD (0.05)	SEm±	CD (0.05)
Forms of P(A)	0.125	0.355	0.251	0.715
Levels of P (B)	0.177	0.503	0.355	1.011
Inoculation (C)	0.153	0.435	0.308	0.875
AxB	0.250	NS	0.502	NS
AxC	0.216	NS	0.435	NS
BxC	0.306	NS	0.615	NS
AxBxC	0.433	NS	0.870	NS

RDP = Recommended dose of phosphorus, UIC = Uninoculated control, NS = Non-significant,
DAS = Days after sowing

application of rock phosphate (17.82 g plant^{-1}). It was also observed that increasing levels of P-application in either form enhanced the number of leaves significantly. Maximum number of leaves, among P-levels, was recorded with 100 per cent RDP (18.77 g plant^{-1}), followed by 75 and 50 per cent RDP (18.25 and 17.99 g plant^{-1}, respectively) and was least in control (17.05 g plant^{-1}) where no P was applied. However, none of the interactions were found to be significant.

A matching trend was also observed at 60 DAS. Among the inoculation treatments, `biophos' showed the highest number of leaves (18.99 g plant^{-1}), followed by *P. striata* (18.32 g plant^{-1}) both of which were significantly superior over the uninoculated control (15.99 g plant^{-1}). However, the two biofertilizers were on par with each other. At 60 DAS also, super phosphate application showed significantly more number of leaves (18.39 g plant^{-1}) than rock phosphate application (17.15 g plant^{-1}). Among the P-levels, maximum number of leaves were recorded at 100 per cent RDP (20.49 g plant^{-1}), followed by 75 and 50 per cent RDP (18.85 and 17.12 g plant^{-1}, respectively) each of which differed significantly among themselves but all were significantly superior over no phosphorus control (14.61 g plant^{-1}). The interaction effects were found to be non-significant at this stage of crop growth also.

4.1.3 Dry matter accumulation and its distribution

4.1.3.1 Root dry weight

In general, all the inoculation and fertilizer treatments showed a marked increase in root dry weight over the respective control treatments (Table 4).

The root dry weight at 30 DAS, among the inoculation treatments, was highest in `biophos' (1.56 g plant^{-1}), followed by *P. striata* (1.52 g plant^{-1}) both of which were on par with each other but significantly superior over uninoculated control (1.32 g plant^{-1}). The root dry weight was significantly higher in treatment receiving P in the form of super phosphate (1.54 g plant^{-1}) than those receiving P in the form of rock phosphate (1.39 g plant^{-1}). The root dry weight was however found to increase significantly with increase in P-levels irrespective of P-forms and inoculations. Among the P-levels, highest root dry weight was observed at 100 per cent RDP (1.72 g plant^{-1}), followed by 75 and 50 per cent RDP (1.54 and 1.41 g plant^{-1}, respectively) and lowest root dry weight was recorded in no phosphorus control (1.19 g plant^{-1}). The interaction effects between the forms and levels of phosphorus were also found to be significant. Super phosphate application at 100 per cent RDP showed highest root dry weight (1.86 g plant^{-1}) and was significantly superior over all other P-form x P-level combinations. The interaction between the levels of P and inoculation also showed significant effect where the highest root dry weight was recorded in the combination of 100 per cent RDP plus `biophos' inoculation (1.87 g plant^{-1}) followed by 100 per cent RDP plus *P. striata* inoculation (1.74 g plant^{-1}) both of which were significantly superior over the all other combinations of P-level x inoculation. However, interaction between the forms of P, levels of P and inoculation was found to be non-significant.

An almost similar trend in root dry weight was also recorded at 60 DAS. Irrespective of the forms and levels of phosphorus, inoculation of `biophos'

Table 4. Root dry weight (g plant^{-1}) at 30 and 60 days of crop growth as influenced by P-solubilizing biofertilizers and different forms and levels of P

Forms of P	P-levels (%RDP)	30 DAS				60 DAS			
		UIC	\`biophos'	P.striata	Mean	UIC	\`biophos'	P.striata	Mean
Super phosphate	0	1.02	1.24	1.30	1.19	2.46	2.73	2.70	2.63
	50	1.34	1.59	1.53	1.48	3.20	3.33	4.10	3.54
	75	1.49	1.75	1.67	1.64	4.50	6.16	5.47	5.38
	100	1.64	2.07	1.87	1.86	5.18	7.40	6.76	6.45
	Mean	1.37	1.66	1.59	1.54	3.83	4.90	4.76	4.50
Rock phosphate	0	1.02	1.24	1.30	1.19	2.46	2.73	2.70	2.63
	50	1.24	1.40	1.36	1.33	2.30	3.33	2.66	2.76
	75	1.35	1.50	1.48	1.44	2.86	3.43	3.30	3.20
	100	1.45	1.68	1.63	1.58	3.03	4.36	5.46	4.28
	Mean	1.26	1.46	1.44	1.39	2.66	3.46	3.53	3.22
Mean		1.32	1.56	1.52	1.46	3.25	4.18	4.14	3.86

Two way table for interaction between inoculation and levels of P

P-levels (%RDP)	30 DAS				60 DAS			
	UIC	\`biophos'	P.striata	Mean	UIC	biophos'	P.striata	Mean
0	1.02	1.24	1.30	1.19	2.46	2.73	2.70	2.63
50	1.29	1.50	1.44	1.41	2.75	3.33	3.38	3.15
75	1.42	1.63	1.58	1.54	3.68	4.80	4.38	4.29
100	1.54	1.87	1.74	1.72	4.11	5.83	5.11	5.37

Sources	SEm±	CD (0.05)	SEm±	CD (0.05)
Forms of P(A)	0.012	0.034	0.063	0.180
Levels of P (B)	0.017	0.049	0.089	0.254
Inoculation (C)	0.015	0.042	0.077	0.220
AxB	0.024	0.069	0.126	0.360
AxC	0.021	NS	0.109	NS
BxC	0.030	0.084	0.155	0.440
AxBxC	0.042	NS	0.219	0.623

RDP = Recommended dose of phosphorus, UIC = Uninoculated control, NS = Non-significant,
DAS = Days after sowing

(4.18 g plant^{-1}) and *P. striata* (4.14 g plant^{-1}) showed almost same root dry weight, but both were significantly superior over the uninoculated control (3.25 g plant^{-1}). Once again application of super phosphate was found to be significantly superior (4.50 g plant^{-1}) over the rock phosphate (3.22 g plant^{-1}). Among P-levels, highest root dry weight was recorded in plants receiving 100 per cent RDP (5.37 g plant^{-1}), followed by 75 and 50 per cent RDP (4.29 and 3.15 g plant^{-1}, respectively) each of which differed significantly among themselves but all were significantly superior over control where no P was applied (2.63 g p^{-1}). The interaction effect between the forms and levels of P was significant. Super phosphate application at 100 per cent RDP showed the highest root dry weight (6.45 g plant^{-1}) which was significantly superior over all other similar combinations. Interaction between inoculation and P-levels also showed significant influence on root dry weight. Treatment receiving 100 per cent RDP plus `biophos' inoculation showed significantly higher root dry weight over the other combinations of inoculation x P-levels. The interaction effect between forms of P, levels of P and inoculations were also found significant. Treatment receiving `biophos' inoculation along with super phosphate at 100 per cent RDP showed the highest root dry weight (7.40 g plant^{-1}) and was significantly superior over all other treatment combinations.

4.1.3.2 Shoot dry weight

The data on shoot dry weight at different growth stages are presented in Table 5. In general, all inoculation and fertilizer treatments were found to be superior over uninoculated and no P control treatment. At 30 DAS, maximum shoot dry weight among inoculation treatments, was recorded with 'biophos' (17.16 g plant^{-1}) which was significantly superior over *P. striata* inoculation (14.82 g plant^{-1}) and uninoculated control (13.82 g plant^{-1}). However, *P. striata* inoculation and uninoculated control were on par with each other. Among the two forms of P, super phosphate application was found to be significantly superior (16.56 g plant^{-1}) over rock phosphate application (14.00 g plant^{-1}). Highest shoot dry weight among P-levels, irrespective of inoculation and forms of phosphorus, was recorded at 100 per cent RDP (19.49 g plant^{-1}), followed by 75 per cent RDP (17.99 g plant^{-1}) which were on par with each other but significantly superior over 50 per cent RDP (12.31 g plant^{-1}) and no P control (11.31 g plant^{-1}). The interaction between the forms of P and levels of P was found to be significant at 30 DAS. Application of super phosphate at 100 per cent RDP showed highest shoot dry weight (22.55 g plant^{-1}), followed by single super phosphate at 75 per cent RDP (20.05 g plant^{-1}) both of which were significantly superior over all other similar combinations. None of the other interactions were found to be significant.

A similar trend in shoot dry weight was also observed at 60 days of crop growth. `Biophos' inoculation increased the shoot dry weight significantly (61.68 g plant^{-1}) over the *P. striata* (58.62 g plant^{-1}) and uninoculated control (52.71 g plant^{-1}). *P. striata* inoculation also showed significantly higher shoot dry weight than uninoculated control. Super phosphate application enhanced the shoot dry weight significantly (60.39 g plant^{-1}) over the rock phosphate application (57.67 g plant^{-1}). Shoot dry weight was significantly influenced by the P-levels. Highest

Table 5. Effect of inoculation with P-solubilizing biofertilizers in combination with different forms and levels of P on shoot dry weight (g $plant^{-1}$) at 30 and 60 days after sowing

Forms of P	P-levels (%RDP)	30 DAS				60 DAS			
		UIC	`biophos'	P.striata	Mean	UIC	`biophos'	P.striata	Mean
Super phosphate	0	9.58	12.36	11.96	11.30	47.30	52.37	50.55	50.07
	50	10.92	14.46	11.64	12.34	49.47	56.17	54.42	53.35
	75	15.46	24.15	20.55	20.05	53.94	69.16	67.35	63.48
	100	23.51	25.38	18.76	22.55	67.75	83.18	72.99	74.64
	Mean	14.86	19.09	15.73	16.56	54.61	65.22	61.33	60.39
Rock phosphate	0	9.58	12.36	11.96	11.30	47.30	52.37	50.55	50.07
	50	11.40	12.45	13.03	12.29	48.52	54.81	52.47	52.64
	75	13.73	16.96	17.06	15.92	51.24	58.52	58.16	59.73
	100	16.46	19.20	13.66	16.44	56.18	66.86	62.46	68.24
	Mean	12.79	15.24	13.92	14.00	50.81	58.14	55.91	57.67
Mean		13.82	17.16	14.82	15.28	52.71	61.68	58.62	57.67

Means for levels of phosphorus (30 DAS)

P level (%RDP)	0	50	75	100
Mean	11.31	12.31	17.99	19.49

Two way table for interaction between inoculation and levels of P (60 DAS)

P-levels (%RDP)	UIC	`biophos'	P.striata	Mean
0	47.34	52.37	50.55	50.07
50	48.99	55.49	53.44	52.64
75	52.59	63.84	62.76	59.73
100	61.96	75.02	67.73	68.24

Sources	SEm±	CD (0.05)	SEm±	CD (0.05)
Forms of P(A)	0.587	1.671	0.326	0.929
Levels of P (B)	0.830	2.363	0.461	1.313
Inoculation (C)	0.719	2.046	0.400	1.137
AxB	1.714	3.341	0.653	1.857
AxC	1.917	NS	0.565	1.609
BxC	1.438	NS	0.799	2.275
AxBxC	2.033	NS	1.130	NS

RDP = Recommended dose of phosphorus, UIC = Uninoculated control, NS = Non-significant,
DAS = Days after sowing

shoot dry weight among P-levels was recorded with 100 per cent RDP (68.24 g plant^{-1}), followed by 75 and 50 per cent RDP (59.73 and 52.64 g plant^{-1}, respectively) and was least in no P control (50.07 g plant^{-1}) all of which differed significantly with each other. The interaction effect between the forms and levels of P was found to be significant. Super phosphate at 100 per cent RDP (74.64 g plant^{-1}), followed by rock phosphate at 100 per cent RDP (68.24 g plant^{-1}) showed significantly higher shoot dry weight over all other P-form x P level combinations. Interaction between inoculations and forms of P was also found to be significant. `Biophos' inoculation along with super phosphate application showed highest shoot dry weight (65.22 g plant^{-1}), followed by *P. striata* plus super phosphate (61.33 g plant^{-1}) both of which were significantly superior over all other similar combinations. Interaction between inoculations and P-levels was also found to have significant effect on shoot dry weight at 60 DAS. `Biophos' inoculation in combination with 100 per cent RDP recorded highest shoot dry weight (75.02 g plant^{-1}), followed by *P. striata* plus 100 per cent RDP (67.73 g plant^{-1}) both of which were significantly superior over all other combinations of P-levels and inoculations. Inoculation with `biophos' or *P. striata* in combination with 75 per cent RDP showed higher shoot dry weight than application of only 100 per cent RDP without inoculation, although the increase was non-significant. The interaction between P-forms, P-levels and inoculation were however non-significant.

4.1.3.3 Total dry matter accumulation

The data presented in Table 6 indicates that, in general, all the treatments receiving inoculation and P fertilizers showed significantly higher total dry matter over the uninoculated and no phosphorus control. At 30 DAS, the maximum total dry matter among the inoculation treatments was recorded in `biophos' (19.07 g plant^{-1}), followed by *P. striata* (17.83 g plant^{-1}) both of which were significantly superior over uninoculated control (13.81 g plant^{-1}). Among the two forms of P, super phosphate application showed significant increase in total dry matter (18.25 g plant^{-1}) over rock phosphate application (15.55 g plant^{-1})). A significant increase in total dry matter content of plants was also recorded with increasing levels of P. Highest total dry matter, among phosphorus levels, was recorded with 100 per cent RDP (22.61 g plant^{-1}) followed by 75 and 50 per cent RDP (18.18 and 13.67 g plant^{-1}, respectively), all of which were significantly superior over no P control but differed significantly among themselves. Interaction effect between forms and levels of P was found to be significant. Super phosphate application at 100 per cent RDP was found to be significantly superior over the all other P-form x P-level combinations. The interaction effect between the forms of P and inoculation was non-significant, but interaction between inoculation and levels of P was significant. Maximum total dry matter as influenced by P-levels x inoculation was recorded in the treatment receiving `biophos' plus 100 per cent RDP (24.96 g plant^{-1}) which was significantly superior over all other combinations except *P. striata* plus 100 per cent RDP. The interaction between forms of P, levels of P and inoculation was found to be non-significant.

A matching trend was also observed at 60 DAS. Significantly higher total dry matter was recorded due to `biophos' (65.93 g plant^{-1}) and *P. striata*

Table 6. Effect of inoculation with P-solubilizing biofertilizers in combination with different forms and levels of P on total dry matter accumulation (g $plant^{-1}$) at 30 and 60 days of crop growth

Forms of P	P-levels (%RDP)	30 DAS				60 DAS			
		UIC	`biophos'	P.striata	Mean	UIC	`biophos'	P.striata	Mean
Super phosphate	0	10.57	13.61	13.27	12.48	49.55	55.31	53.15	52.67
	50	12.26	16.06	14.23	14.18	53.34	59.54	58.52	57.13
	75	13.59	25.35	23.16	20.70	58.45	75.33	73.05	68.94
	100	22.56	28.20	26.21	25.66	72.94	90.59	79.97	81.17
	Mean	14.74	20.80	19.22	18.25	58.57	70.19	66.17	64.98
Rock phosphate	0	10.57	13.61	13.27	12.48	49.55	55.31	53.15	52.67
	50	10.64	14.96	13.86	13.15	50.82	58.15	55.14	54.70
	75	13.18	19.08	18.52	16.92	54.11	61.98	61.46	59.18
	100	17.08	21.71	20.13	19.64	59.22	71.23	67.94	66.13
	Mean	12.86	17.34	16.44	15.55	53.42	61.67	59.42	58.17
Mean		13.80	19.07	17.83	16.96	55.99	65.93	62.80	61.57

Two way table for interaction between inoculation and levels of P

P-levels (%RDP)	30 DAS				60 DAS			
	UIC	`biophos'	P.striata	Mean	UIC	`biophos'	P.striata	Mean
0	10.57	13.61	13.27	12.48	49.55	55.31	53.15	55.67
50	11.45	15.51	14.05	13.67	52.08	58.84	56.83	55.91
75	13.35	22.21	20.83	18.18	56.28	68.65	67.25	64.06
100	19.70	24.96	23.17	22.61	66.08	80.91	73.95	73.65

Sources	SEm±	CD (0.05)	SEm±	CD (0.05)
Forms of P(A)	0.286	0.814	0.326	0.929
Levels of P (B)	0.404	1.151	0.462	1.314
Inoculation (C)	0.350	0.997	0.400	1.138
AxB	0.572	1.628	0.653	1.858
AxC	0.495	NS	0.566	1.609
BxC	0.700	1.994	0.800	2.276
AxBxC	0.991	NS	1.131	3.219

RDP = Recommended dose of phosphorus, UIC = Uninoculated control, NS = Non-significant,
DAS = Days after sowing

(62.80 g plant^{-1}) inoculation compared to uninoculated control (55.99 g plant^{-1}). `Biophos' inoculation was found to be significantly superior over *P. striata* inoculation. Super phosphate application enhanced the total dry matter significantly (64.98 g plant^{-1}) over the rock phosphate application (58.17 g plant^{-1}). Total plant dry matter was found to increase significantly with increase in P levels. Among the P-levels, total dry matter was highest at 100 per cent RDP (73.65 g plant^{-1}), followed by 75 per cent RDP (64.06 g plant^{-1}) both of which were significantly superior over 50 per cent RDP (55.91 g plant^{-1}) and no phosphorus control (55.67 g plant^{-1}). However, 50 per cent RDP and no P control were on par with each other. All the interactions were found to be significant with respect to total plant dry matter content. Among the various treatment combinations, highest total dry matter content of plants at 60 DAS was recorded in the combination of `biophos' plus super phosphate applied at 100 per cent RDP (90.59 g plant^{-1}) followed by *P. striata* plus super phosphate at 100 per cent RDP (79.97 g plant^{-1}) both of which were significantly superior over all other combinations.

4.2 Days to 50 per cent flowering

The data on number of days taken for 50 per cent flowering by the crop as influenced by different treatments are presented in Table 7.

In general, all the inoculation and fertilizer treatments were found to reduce the number of days for 50 per cent flowering. Among the inoculation treatments, `biophos' inoculation showed 50 per cent flowering in 57.37 days which was significantly early compared to *P. striata* inoculation (58.00 days).

Table 7. Days to 50 per cent flowering as influenced by phosphate solubilizers, sources of phosphorus and levels of phosphorus

Inoculation	P-forms at different levels (%RDP)										
	Super phosphate					Rock phosphate					Mean
	0	50	75	100	Mean	0	50	75	100	Mean	
UIC	59.66	59.00	58.00	56.66	58.33	59.66	60.00	59.66	59.00	59.58	58.95
\`biophos'	57.66	58.33	56.33	55.66	57.00	57.60	58.00	58.00	57.33	57.75	57.37
P.striata	58.33	57.66	57.66	56.00	57.41	58.33	59.33	58.33	58.33	58.58	58.00
Mean	58.55	58.33	57.33	56.11	57.58	58.55	59.11	58.66	58.22	58.63	58.11

Means for levels of phosphorus

P-levels (%RDP)	0	50	75	100
Mean	58.55	58.72	58.00	57.16

Sources	SEm±	CD (0.05)
Forms of P(A)	0.103	0.293
Levels of P (B)	0.146	0.414
Inoculation	0.126	0.359
AxB	0.206	0.586
AxC	0.178	NS
BxC	0.252	NS
AxBxC	0.357	NS

RDP = Recommended dose of phosphorus
NS = Non-significant
UIC = Uninoculated control

Uninoculated plants took 58.95 days for 50 per cent flowering which was significantly late than the two inoculation treatments. Among the forms of phosphorus, super phosphate application significantly reduced the days to 50 per cent flowering (57.58 days) compared to application of rock phosphate (58.63 days). With increasing levels of P, specially at higher doses, the days to 50 per cent flowering was reduced significantly. Application of 100 per cent RDP recorded 50 per cent flowering in 57.16 days followed by 75 per cent RDP (58.00 days) both of which were significantly superior over 50 per cent RDP and no P control. The interaction effects between the forms of P and levels of P were found to be significant. Super phosphate at 100 per cent RDP showed significantly less number of days for 50 per cent flowering (56.11 days) compared to all other combinations. All other interactions were however non-significant.

4.3 Yield and yield components

4.3.1 Diameter of head

The diameter of head was found to be significantly more in treatments receiving `biophos' (37.53 cm) and *P. striata* (36.70 cm) inoculation over uninoculated control (33.35 cm) irrespective of the forms and levels of P (Table 8). However, two inoculation treatments were on par with each other. Among the forms of P, application of super phosphate was found to be significantly superior (37.10 cm) over rock phosphate application (34.02 cm). Irrespective of the form of phosphorus used, increasing levels of phosphorus significantly increased the diameter of head. Among the P-levels, maximum diameter of head was recorded at

Table 8. Diameter of head (cms) and head weight (g plant^{-1}) as influenced by phosphate solubilizing biofertilizers, forms and levels of phosphorus

Forms of P	P-levels (%RDP)	Diameter of head (cms)				Head weight (g plant^{-1})			
		UIC	`biophos'	P.striata	Mean	UIC	`biophos'	P.striata	Mean
Super phosphate	0	29.33	35.26	32.33	32.31	45.40	52.13	49.13	48.88
	50	35.26	37.80	37.60	36.88	48.46	73.40	64.06	61.97
	75	36.40	39.20	38.80	38.13	69.66	85.00	78.66	77.77
	100	37.26	42.40	43.60	41.08	75.80	107.13	103.53	95.48
	Mean	34.56	38.66	38.08	37.10	59.83	79.41	73.85	71.03
Rock phosphate	0	29.33	35.26	32.33	32.31	45.40	52.13	49.13	48.88
	50	31.06	34.40	32.13	32.53	49.73	63.40	56.66	56.60
	75	33.40	37.26	38.93	36.53	51.26	76.33	72.00	66.53
	100	34.80	38.73	37.91	37.14	68.33	84.80	76.40	76.51
	Mean	32.14	36.41	35.32	34.02	53.68	69.16	63.55	62.13
Mean		33.35	37.53	36.70	35.86	56.75	74.28	68.70	66.58

Means for levels of phosphorus (Diameter of head)

P level (%RDP)	0	50	75	100
Mean	32.31	34.71	37.33	39.11

Two way table for interaction between inoculation and levels of P (Head weight)

%RDP	UIC	`biophos'	P.striata	Mean
0	45.03	52.13	49.13	48.88
50	49.10	68.40	60.36	59.28
75	60.46	80.66	75.33	72.15
100	72.06	95.96	89.96	86.00

Sources	SEm±	CD (0.05)	SEm±	CD (0.05)
Forms of P(A)	0.478	1.361	0.296	0.841
Levels of P (B)	0.676	1.925	0.418	1.190
Inoculation (C)	0.586	1.667	0.362	1.030
AxB	0.956	2.722	0.591	1.682
AxC	0.828	NS	0.512	1.457
BxC	1.171	NS	0.724	2.060
AxBxC	1.657	NS	1.024	2.914

RDP = Recommended dose of phosphorus, UIC = Uninoculated control, NS = Non-significant

100 per cent RDP (39.11 cm), followed by 75 per cent RDP (37.33 cm) and 50 per cent RDP (34.71 cm) and was least in uninoculated control (32.31 cm). The interaction between forms of P and levels of P was found to be significant. Super phosphate applied at 100 per cent RDP was found to be significantly superior over all other similar treatment combinations. However, the other interactions were found to be non-significant.

4.3.2 Head weight

The head weight (Table 8), among the inoculation treatments, was highest in `biophos' (74.28 g plant$^{-1}$), followed by *P. striata* (68.70 g plant$^{-1}$), both of which were significantly superior over uninoculated control (56.75 g plant$^{-1}$). `Biophos' inoculation showed significantly higher head weight than *P. striata* inoculation. Among the forms of P, super phosphate (71.03 g plant^{-1}) was found to be significantly superior over the rock phosphate (62.13 g plant^{-1}) in increasing the head weight. A significant increase in head weight was recorded with increasing P-levels, irrespective of the forms of P and inoculation treatments. Highest head weight was recorded at 100 per cent RDP (86.00 g plant^{-1}), followed by 75 per cent RDP (72.15 g plant^{-1}) and 50 per cent RDP (59.28 g plant^{-1}) each differing significantly among themselves but all were significantly superior over no P control (48.88 g plant^{-1}). All the interactions were found to be significant with respect to head weight. `Biophos' inoculation in combination with 100 per cent RDP applied in the form of super phosphate recorded the highest head weight (107.13 g plant^{-1}), followed by a similar treatment combination with *P. striata* (103.53 g plant^{-1}) both of which were significantly superior over all other treatment combinations in the experiment.

4.3.3 Number of seeds per head

The data presented in Table 9 indicated that inoculation with `biophos' and *P. striata* significantly increased the number of filled seeds per head (991.00 and 919.00, respectively) compared to uninoculated control (715.00). Among the two biofertilizers, `biophos' was found to be significantly superior over the *P. striata*. Super phosphate, among the P-forms, significantly enhanced the number of filled seeds per head (952.00) as compared to rock phosphate application (798.00). Among the P-levels, highest number of filled seeds were recorded with 100 per cent RDP (1112.00) which was significantly superior over other P-levels. The interaction between forms of P and levels of P was found to be significant. Super phosphate application at 100 per cent RDP recorded highest number of filled seeds (1243.00) and was significantly superior over all other such combinations. Interaction between inoculation and forms of P also showed significant influence on number of filled seeds per head. Super phosphate plus `biophos' inoculation showed highest number of filled seeds and was significantly superior over all other P-form x inoculation combinations. The interaction between inoculation and levels of P was also significant with highest number of filled seeds being recorded in the combination of 100 per cent RDP plus `biophos' (1277). The interaction between inoculation, forms of P and levels of P was also found significant. `Biophos' in combination with super phosphate applied at 100 per cent RDP showed highest number of seeds per head (1429), followed by a similar combination with *P. striata*

Table 9. Number of seeds per head, seed yield per plant (g) and thousand seed weight (g) as influenced by phosphate solubilizers, forms and levels of phosphorus

Forms of P	P-levels (%RDP)	Number of seeds per head				Seed yield per plant (g)				1000 Seed weight (g)			
		UIC	`biophos'	P.striata	Mean	UIC	`biophos'	P.striata	Mean	UIC	`biophos'	P.striata	Mean
Super phosphate	0	556.00	621.00	592.00	590.00	12.03	13.00	12.40	12.47	28.00	29.60	28.90	28.83
	50	789.00	1081.00	989.00	953.00	16.99	18.10	18.25	17.78	33.42	34.61	33.70	33.91
	75	842.00	1195.00	1025.00	1021.00	18.15	21.46	19.44	19.68	33.60	35.22	34.24	34.35
	100	970.00	1429.00	1330.00	1243.00	19.00	25.34	23.68	22.57	33.85	36.37	37.28	35.83
	Mean	789.00	1081.00	984.00	952.00	16.57	19.40	18.44	18.13	32.21	33.95	33.53	33.23
Rock phosphate	0	556.00	621.00	592.00	590.00	12.03	13.00	12.40	12.47	28.00	29.60	28.90	28.83
	50	580.00	864.00	825.00	756.00	12.67	14.14	13.40	13.39	31.20	32.70	32.40	32.10
	75	640.00	991.00	967.00	866.00	13.08	17.26	16.00	15.45	31.75	34.00	33.86	33.20
	100	789.00	1125.00	1033.00	982.00	14.14	19.40	18.34	17.29	32.22	34.50	34.20	33.64
	Mean	641.00	900.00	854.00	798.00	12.98	15.95	15.03	16.38	30.79	32.70	32.34	31.94
Mean		715.00	991.00	919.00	875.00	14.71	17.67	16.74	16.39	31.50	33.32	32.93	32.58

Two way table for interaction between inoculation and levels of P

P-levels (%RDP)	Number of seeds per head				Seed weight per head			
	UIC	`biophos'	P.striata	Mean	UIC	`biophos'	P.striata	Mean
0	556.00	621.00	592.00	589.00	12.03	13.00	12.40	12.47
50	685.00	972.00	907.00	854.00	14.82	16.12	15.82	15.59
75	741.00	1093.00	996.00	943.00	15.61	19.36	17.72	17.56
100	889.00	1277.00	1181.00	1112.00	16.57	22.22	21.01	19.93

Means of thousand seed weight for P-levels

P-levels (%RDP)	0	50	75	100
Mean	28.83	33.00	33.77	37.73

Sources	SEm±	CD (0.05)	SEm±	CD (0.05)	SEm±	CD (0.05)
Forms of P (A)	4.00	11.39	0.141	0.402	0.230	0.654
Levels of P (B)	5.66	16.11	0.200	0.568	0.325	0.924
Inoculation (C)	4.90	13.95	0.173	0.492	0.281	0.800
AxB	8.05	22.78	0.282	0.803	0.459	NS
AxC	6.93	19.73	0.244	NS	0.398	NS
BxC	9.80	27.90	0.346	0.984	0.563	NS
AxBxC	13.86	39.46	0.489	NS	0.796	NS

RDP = Recommended dose of phosphorus, UIC = Uninoculated control, NS = Non-significant,

(1330) both of which were significantly superior over all other treatment combinations.

4.3.4 Seed yield per plant

The data on seed yield per plant (Table 9) showed an almost same trends as that of number of seeds per plant. `Biophos' and *P. striata* inoculation increased the seed yield per plant significantly over uninoculated control (14.71 g). However, seed yield per plant was significantly higher with `biophos' (17.67 g) than with *P. striata* (16.74 g). Irrespective of the P-levels and inoculation, super phosphate application showed significant increase in seed yield per plant (18.13 g) over rock phosphate application (16.38 g). The seed yield per plant was found to increase significantly with increase in P-levels. The interaction between forms of P and levels of P was found to be significant where the highest seed yield per plant was recorded in the combination of 100 per cent RDP applied in the form of super phosphate (22.57 g). The interaction between levels of P and inoculation was also found to be significant. Combination of `biophos' plus 100 per cent RDP recorded the highest seed yield per plant (22.22 g), followed by *P. striata* plus 100 per cent RDP (21.01 g) both of which were significantly superior over all other combinations of P-level and inoculation. The interaction effects between forms of P and inoculation as well as between inoculation, forms of P and levels of P were found to be non-significant.

4.3.5 1000 Seed weight

The data on 1000 seed weight (Table 9) revealed that `biophos' and *P. striata* inoculation recorded significantly higher 1000 seed weight (33.32 and

32.93 g, respectively) than uninoculated control (31.50 g) although the two biofertilizers were on par with each other. Among the P-forms, super phosphate (33.23 g) was significantly superior over rock phosphate (31.94 g) in enhancing the 1000 seed weight. Among P-levels, 100 per cent RDP showed highest 1000 seed weight (37.73 g) which was significantly superior over other P levels. Application of 75 and 50 per cent RDP also significantly enhanced the 1000 seed weight (33.77 and 33.00 g respectively) over no P control (28.33 g) but were on par among themselves. None of the interactions were found to be significant.

4.3.6 Seed yield

The data on seed yield (Table 10) revealed that, in general all the inoculation and P-fertilizer treatments were found to be significantly superior over their respective controls. The treatment receiving `biophos' inoculation recorded the highest seed yield (13.62 q ha^{-1}), followed by *P. striata* (12.70 q ha^{-1}) both of which were significantly superior over the uninoculated control (10.73 q ha^{-1}). However, the two biofertilizers were on par with each other. Among the forms of P, super phosphate showed significantly greater influence on grain yield (14.16 q ha^{-1}) than the rock phosphate (10.57 q ha^{-1}). A significant increase in grain yield was also observed with increasing levels of P. Among the P-levels, maximum seed yield was recorded at 100 per cent RDP (15.40 q ha^{-1}), followed by 75 and 50 per cent RDP (13.62 and 11.50 q ha^{-1}, respectively), all of which were significantly superior over no phosphorus control (8.44 q ha^{-1}). The interaction between forms of P and levels of P was found to be significant where in the super phosphate

Table 10. Seed yield (q ha^{-1}) and stalk yield (q ha^{-1}) as influenced by P solubilizing biofertilizers and forms and levels of phosphorus

Forms of P	P-levels (%RDP)	Seed Yield				Stalk Yield			
		UIC	`biophos'	P.striata	Mean	UIC	`biophos'	P.striata	Mean
Super phosphate	0	7.93	9.00	8.39	8.44	32.10	36.50	35.20	32.20
	50	12.99	14.67	14.25	13.97	34.36	39.03	38.53	37.31
	75	14.15	17.46	15.44	15.68	37.60	45.60	42.80	42.00
	100	15.00	21.04	19.68	18.57	42.43	46.30	45.76	44.83
	Mean	12.52	15.54	14.44	14.16	36.82	41.85	40.82	39.83
Rock phosphate	0	7.93	9.00	8.39	8.44	32.10	36.50	35.20	32.20
	50	8.64	9.13	9.40	9.06	33.23	35.33	35.00	34.52
	75	9.08	13.26	12.00	11.45	32.90	36.50	36.20	35.20
	100	10.14	15.40	14.54	13.36	34.80	36.60	36.03	35.81
	Mean	8.95	11.70	11.08	10.57	33.25	36.23	35.60	35.42
Mean		10.73	13.62	12.70	12.36	35.03	39.04	38.21	37.42

Means of seed yield for levels of phosphorus

P level (%RDP)	0	50	75	100
Mean	8.44	11.50	13.62	15.40

Two way table of stalk yield for interaction between inoculation and levels of P

%RDP	UIC	`biophos'	P.striata	Mean
0	32.20	35.65	35.35	34.40
50	33.79	37.18	36.76	35.91
75	35.85	41.05	39.50	38.60
100	38.61	41.45	40.89	40.31

Sources	SEm±	CD (0.05)	SEm±	CD (0.05)
Forms of P(A)	0.351	0.999	0.145	0.414
Levels of P (B)	0.497	1.413	0.205	0.585
Inoculation (C)	0.430	1.224	0.178	0.506
AxB	0.702	1.999	0.291	0.827
AxC	0.608	NS	0.250	0.716
BxC	0.860	NS	0.356	1.013
AxBxC	1.216	NS	0.503	1.432

RDP = Recommended dose of phosphorus, UIC = Uninoculated control, NS = Non-significant,

applied at 100 per cent RDP showed the highest yield (18.57 g ha^{-1}) and was significantly superior over all other combinations. However, other interactions failed to show any significant effect on seed yield. The results on seed yield also indicated that, application of super phosphate at 75 per cent RDP in combination with `biophos' or *P. striata* produced numerically higher yield than application of only super phosphate at 100 per cent RDP without inoculation. Similarly, application of rock phosphate at 100 per cent RDP in combination with `biophos' or *P. striata* showed almost same yield as that of application of super phosphate at 100 per cent RDP without inoculation.

4.3.7 Stalk yield

The trend in stalk yield of sunflower crop (Table 10) was almost same as that of seed yield. Both `biophos' and *P. striata* showed significantly higher stalk yield than uninoculated control although `biophos' was significantly superior over *P. striata*. Among the forms of P, super phosphate application increased the stalk yield significantly than rock phosphate application. The stalk yield was also found to increase significantly with increasing level of P-application. The interaction between forms of P, levels of P and inoculation was found to be significant. Among the various treatment combinations, `biophos' plus 100 per cent RDP as super phosphate showed the highest stalk yield (46.30 q ha$^{-1}$) closely followed by *P. striata* plus 100 per cent RDP as super phosphate (45.76 q ha$^{-1}$) and `biophos' plus 75 per cent RDP as super phosphate (45.60 q ha^{-1}) which were on par among themselves but significantly superior over rest of the combinations.

4.4 Nutrient uptake

4.4.1 N-uptake

The data on N-uptake by sunflower at different stages of crop growth are presented in Table 11. At 30 days of crop growth, N-uptake in shoot among inoculation treatments, was found to be highest in `biophos' (44.23 kg ha^{-1}) which was significantly higher than *P. striata* (41.02 kg ha^{-1}) and uninoculated control (31.95 kg ha^{-1}). *P. striata* inoculation also showed significantly higher N-uptake than uninoculated control. Among the forms of P, super phosphate application enhanced the N-uptake (43.30 kg ha^{-1}) significantly over rock phosphate application (34.83 kg ha^{-1}). N-uptake was found to increase significantly with increase in P-level. Phosphorus at 100 per cent recommended dose recorded the highest N-uptake (52.28 kg ha^{-1}), followed by 75 and 50 per cent RDP (43.94 and 33.47 kg ha^{-1}, respectively) each of which differed significantly among themselves but significantly superior over no phosphorus control. The interaction between forms of P and levels of P was found to be significant. 100 per cent RDP applied in the form of super phosphate showed maximum N-uptake and was significantly superior over all other combinations. The interaction between levels of P and inoculation was also found to be significant where the highest N-uptake in shoots was recorded at 100 per cent RDP plus `biophos' which was significantly superior over all other treatment combinations. The interaction effects between forms of P and inoculation and forms of P x levels of P x inoculation were found to be non-significant.

An almost similar trend was observed at 60 DAS. Here also `biophos' inoculation enhanced the N-uptake in shoot (102.79 kg/ha) significantly

Table 11. Nitrogen uptake (kg ha^{-1}) in shoots of sunflower at 30 and 60 DAS and in seeds at harvest as influenced by phosphate solubilizing biofertilizers in combination with forms and levels of phosphorus

Forms of P	P-levels (%RDP)	Shoot N-uptake at 30 DAS				Shoot N-uptake at 60 DAS				N-uptake in seeds			
		UIC	`biophos'	P.striata	Mean	UIC	`biophos'	P.striata	Mean	UIC	`biophos'	P.striata	Mean
Super phosphate	0	22.94	28.85	27.91	26.56	80.72	87.05	84.24	84.00	20.81	26.29	23.81	23.64
	50	30.93	44.59	38.11	37.87	83.53	94.00	90.69	89.40	33.41	48.71	45.03	42.38
	75	35.25	58.99	53.40	49.21	89.89	115.26	112.24	105.80	42.60	49.47	44.78	42.61
	100	52.29	65.49	60.86	59.55	112.90	138.63	121.08	124.20	44.16	67.32	61.00	57.49
	Mean	35.35	49.81	45.07	43.30	91.76	108.73	102.06	100.85	35.24	47.95	43.65	42.28
Rock phosphate	0	22.94	28.85	27.91	26.56	80.72	87.05	84.24	84.00	20.81	26.29	23.81	23.64
	50	22.71	33.28	31.24	29.00	80.82	91.22	87.45	86.50	25.07	30.74	29.14	28.32
	75	29.57	43.90	42.57	38.68	85.39	97.48	96.93	93.27	28.50	43.30	40.52	37.44
	100	38.99	49.90	46.15	45.01	93.60	114.43	104.10	103.04	39.54	56.22	52.49	49.42
	Mean	28.55	38.98	36.97	34.83	85.13	96.85	93.17	91.17	28.48	39.14	36.49	34.71
Mean		31.95	44.23	41.02	39.07	88.45	102.79	97.62	96.24	31.86	43.54	40.07	38.49

Two way table for interaction between levels of P and inoculation

P level (%RDP)	Shoot N-uptake at 30 DAS				Shoot N-uptake at 60 DAS				N-uptake in seeds			
	UIC	`biophos'	P.striata	Mean	UIC	`biophos'	P.striata	Mean	UIC	`biophos'	P.striata	Mean
0	22.94	28.85	27.91	26.56	80.72	87.05	84.24	84.00	20.81	26.29	23.81	23.64
50	26.82	38.94	34.67	33.47	82.18	92.61	89.67	87.95	29.24	39.72	37.09	35.35
75	32.41	51.44	47.98	43.94	87.67	106.37	104.58	99.53	35.55	46.38	42.65	41.53
100	45.64	57.69	53.51	52.28	103.25	125.03	112.59	113.62	41.85	61.77	56.75	53.45

Sources	SEm±	CD (0.05)	SEm±	CD (0.05)	SEm±	CD (0.05)
Forms of P (A)	0.746	2.123	0.545	1.551	0.407	1.157
Levels of P (B)	1.055	3.003	0.771	2.194	0.575	1.637
Inoculation (C)	0.914	2.600	0.668	1.900	0.498	1.418
AxB	1.492	4.246	1.090	3.103	0.813	2.315
AxC	1.292	NS	0.944	2.687	0.704	NS
BxC	1.827	5.201	1.335	3.800	0.996	2.835
AxBxC	2.854	NS	1.888	5.374	1.409	4.009

RDP = Recommended dose of phosphorus, UIC = Uninoculated control, NS = Non-significant, DAS = Days after sowing

over *P. striata* (97.62 kg ha^{-1}) and uninoculated control (88.45 kg ha^{-1}). Super phosphate among the forms of P and 100 per cent RDP among levels of P showed maximum N-uptake in shoot. The interaction effects viz., forms of P x levels of P, forms of P x inoculation, levels of P x inoculation and forms of P x levels of P x inoculation were found significant at this stage of crop growth. The treatment combination of `biophos' plus 100 per cent RDP applied in the form of super phosphate showed the highest N-uptake in shoots (138.63 kg ha^{-1}) and was significantly higher than all other treatment combinations.

The N-uptake in seeds was found to be significantly higher in treatment receiving `biophos' inoculation (43.54 kg ha^{-1}) compared to *P. striata* inoculation (40.07 kg ha^{-1}). *P. striata* also showed significantly higher N-uptake than uninoculated control (31.86 kg ha^{-1}). Among the forms of P, super phosphate was found to be significantly superior (42.28 kg ha^{-1}) over rock phosphate (34.71 kg ha^{-1}) in enhancing the N-uptake in seeds. A significant increase in N-uptake in seeds was associated with increasing P-levels irrespective of the forms of P and inoculation. Highest N-uptake was recorded at 100 per cent RDP (53.45 kg ha^{-1}), followed by 75 and 50 per cent RDP (41.53 and 35.35 kg ha^{-1}) and was least in no phosphorus control (23.64 kg ha^{-1}). The interaction between forms of P and levels of P was significant. 100 per cent RDP applied as super phosphate showed highest N-uptake (57.49 kg ha^{-1}) which was significantly superior over all other P-form x P-level combinations. The interaction between levels of P and inoculations led to a significant increase in N-uptake where the combination of 100 per cent RDP plus `biophos' recorded the highest N-uptake (61.77 kg ha$^{-1}$). The interaction between forms of P, levels of P and inoculation was also significant where in the highest N-uptake was recorded in the combination of `biophos' plus 100 per cent RDP applied in the form of super phosphate (67.32 kg ha^{-1}) and was significantly superior over all other treatment combinations.

4.4.2 P-uptake

From the results presented in Table 12, it is clear that there was significant increase in P-uptake due to inoculation and P-application. At 30 DAS, the highest P-uptake in shoot among inoculation treatments was recorded with `biophos' (1.55 kg ha^{-1}) which was significantly higher than that of *P. striata* (1.39 kg ha^{-1}). *P. striata* also showed significantly higher P-uptake than uninoculated control (1.01 kg ha^{-1}). Among the forms of P, super phosphate was found to be significantly superior over rock phosphate in enhancing P-uptake at 30 DAS. The P-uptake in shoot was increased significantly with increasing P levels. Application of 100 per cent RDP showed the highest P-uptake (1.74 kg ha^{-1}), followed by 75 per cent RDP (1.40 kg ha^{-1}) and 50 per cent RDP (1.23 kg ha^{-1}) all of which differed significantly among themselves but superior over no phosphorus control (0.91 kg ha^{-1}). The interaction between forms of P and levels of P was found to be significant. Super phosphate at 100 per cent RDP showed significantly more P-uptake (1.97 kg ha^{-1}) compared to all other P-form x P-level combinations. The interaction between forms of P and inoculation was found to be significant with highest P-uptake being recorded with super phosphate application plus `biophos' inoculation (1.79 kg ha$^{-1}$). The interaction between levels of P and inoculation was also found significant where in maximum P-uptake was recorded at 100 per cent RDP plus `biophos' inoculation (1.92 kg ha^{-1}), followed by 100 per cent RDP plus *P. striata* (1.78 kg ha^{-1}) both of which were on par with each other but significantly superior over all other similar treatment combinations. The interaction between forms of P, levels of P and inoculation was found non-significant.

The trend in shoot P-uptake at 60 DAS was almost same as that observed at 30 DAS. Highest P-uptake was recorded with `biophos' inoculation (12.65 kg ha^{-1}) which was significantly higher than *P. striata* (11.76 kg ha^{-1}). *P. striata* inoculation also showed significantly higher P-uptake than uninoculated control (9.65 kg ha^{-1}). Super phosphate among P-forms and 100 per cent RDP among P-levels showed the highest P-uptake. The interaction between forms of P and levels of P, forms of P x inoculation, levels of P x inoculation and forms of P x levels of P x inoculation were found to be significant. The combination of `biophos' plus 100 per cent RDP in the form of super phosphate showed the highest shoot P at 60 DAS and was significantly superior over all other treatment combinations.

The P-uptake in seeds followed the same trend as that of shoot P-uptake at 30 and 60 DAS. `Biophos' and *P. striata* inoculation recorded significantly higher P-uptake (8.39 and 7.62 kg ha$^{-1}$, respectively) than uninoculated control (5.56 kg ha$^{-1}$), although `biophos' showed significantly higher P-uptake than *P. striata*. Among the P-forms, super phosphate (8.55 kg ha^{-1}) was found to be significantly superior over rock phosphate (5.82 kg ha^{-1}) in enhancing the P-uptak

Table 12. Phosphorus uptake (kg ha^{-1}) in shoots of sunflower at 30 and 60 DAS and in seeds at harvest as influenced by phosphate solubilizing biofertilizers and forms and levels of phosphorus

Forms of P	P-levels (%RDP)	Shoot P-uptake at 30 DAS				Shoot P-uptake at 60 DAS				P-uptake in seeds			
		UIC	`biophos'	P.striata	Mean	UIC	`biophos'	P.striata	Mean	UIC	`biophos'	P.striata	Mean
Super phosphate	0	0.73	1.02	0.99	0.91	7.48	8.26	7.97	7.90	3.40	4.48	4.00	3.96
	50	0.90	2.04	1.50	1.48	10.29	14.50	12.69	12.49	6.10	8.94	8.25	7.76
	75	1.00	1.94	1.94	1.56	11.68	17.27	16.86	15.26	8.06	11.87	10.36	10.10
	100	1.74	2.17	2.02	1.97	15.59	20.57	18.24	18.19	2.29	14.50	13.53	12.38
	Mean	1.09	1.79	1.53	1.48	11.26	15.19	13.93	13.46	6.70	9.95	8.99	8.55
Rock phosphate	0	0.73	1.02	0.99	0.91	7.48	8.25	7.97	7.90	3.40	4.48	4.00	3.96
	50	0.77	1.12	1.07	0.99	7.68	9.11	8.74	8.51	3.91	5.07	5.07	4.68
	75	0.92	1.41	1.41	1.24	8.10	9.74	9.69	9.18	4.63	8.09	6.93	6.55
	100	1.29	1.66	1.54	1.50	8.89	13.36	11.96	11.40	5.67	9.69	8.99	8.11
	Mean	0.93	1.30	1.25	1.16	8.30	10.11	9.56	9.25	4.40	6.83	6.24	5.82
Mean		1.01	1.55	1.39	1.32	9.65	12.65	11.76	11.35	5.56	8.39	7.62	7.19

Two way table for interaction between levels of P and inoculation

P level (%RDP)	Shoot P-uptake at 30 DAS				Shoot P-uptake at 60 DAS				P-uptake in seeds			
	UIC	`biophos'	P.striata	Mean	UIC	`biophos'	P.striata	Mean	UIC	`biophos'	P.striata	Mean
0	0.73	1.02	0.99	0.91	7.48	8.25	7.97	7.90	3.40	4.48	4.00	3.96
50	0.84	1.58	1.28	1.23	8.98	11.81	10.71	10.50	5.00	7.00	6.66	6.22
75	0.96	1.67	1.58	1.40	9.89	13.50	13.26	12.22	6.35	9.98	8.65	8.32
100	1.51	1.92	1.78	1.74	12.24	17.06	15.10	14.80	7.48	12.10	11.17	10.25

Sources	SEm±	CD (0.05)	SEm±	CD (0.05)	SEm±	CD (0.05)
Forms of P(A)	0.029	0.082	0.070	0.200	0.086	0.248
Levels of P (B)	0.041	0.115	0.099	0.283	0.122	0.347
Inoculation (C)	0.035	0.100	0.086	0.245	0.106	0.300
AxB	0.057	0.163	0.141	0.400	0.172	0.491
AxC	0.050	0.141	0.122	0.346	0.149	0.425
BxC	0.070	0.200	0.172	0.490	0.211	0.601
AxBxC	0.099	NS	0.243	0.693	0.299	NS

RDP = Recommended dose of phosphorus, UIC = Uninoculated control, NS = Non-significant, DAS = Days after sowing

in seeds. P-uptake was increased significantly with increasing P-levels and the maximum P-uptake was recorded at 100 per cent RDP (10.25 kg ha^{-1}). The interaction between forms of P and levels of P was significant. 100 per cent RDP applied as super phosphate showed highest P-uptake in seeds (12.38 kg ha^{-1}) which was significantly superior over all other similar treatment combinations. The interaction between forms of P and inoculation was also significant. Maximum P-uptake in seed was recorded in treatment receiving super phosphate plus `biophos' inoculation (9.95 kg ha$^{-1}$) which was significantly superior over all other P-form x inoculation combinations. The interaction between levels of P and inoculation was also significant where in 100 per cent RDP plus `biophos' showed maximum P-uptake (12.10 kg ha^{-1}) in seeds and was significantly superior over all other P-level x inoculation combinations. The interaction between forms of P, levels of P and inoculation was however non-significant.

4.5 Rhizosphere microflora

The population of total bacteria, fungi and actinomycetes in the rhizosphere of sunflower was estimated at 60 days of crop growth whereas the population of P-solubilizers and free-living N_2 fixers was estimated at 30, 60 and 90 days of crop growth. The results are presented in Table 13, 14 and 15.

4.5.1 Bacteria

The data presented in the Table 13 indicated that the forms and levels of P as well as inoculation significantly influenced the bacterial population in the rhizosphere. Among the inoculation treatments, `biophos' inoculation showed the highest bacterial population (86.68 x 10^6 g^{-1}) followed by *P. striata* (86.51 x 10^6 g^{-1}) both of which were significantly superior over the uninoculated control (83.90 x 10^6 g^{-1}). Among the forms of phosphorus, super phosphate application showed significantly higher bacterial population (86.68 10^6 g^{-1}) than that of rock phosphate (84.71 10^6 g^{-1}). The levels of phosphorus, irrespective of the forms of P, also showed a significant influence on the bacterial population in rhizosphere. Among the P-levels, 100 per cent RDP recorded the highest bacterial population (89.37 10^6 g^{-1}) which was however on par with 75 per cent RDP (87.57 10^6) but significantly superior over 50 per cent RDP (85.78 10^6 g^{-1}) and no phosphorus control (80.07 10^6 g^{-1}). Application of phosphorus at 75 per cent RDP and 50 per cent RDP showed significantly higher bacterial population than no P control but were on par among themselves. The interaction effects were found to be non-significant.

4.5.2 Fungi

The population of fungi, among inoculation treatments, was highest in `biophos' inoculation (34.88 10^3 g^{-1}), followed by *P. striata* (33.67 x 10^3 g^{-1}), both of which were significantly superior over the uninoculated control (31.56 x 10^3 g^{-1}) but on par with each other. Among the forms of P, super phosphate application (34.45 x 10^3 g^{-1}) was found to be significantly superior over the rock phosphate application (32.29 x 10^3 g^{-1}) in stimulating rhizosphere fungal population. Maximum population of fungi, among the P-levels was recorded at 100 per cent RDP (35.20 x 10^3 g^{-1}), followed by 75 per cent RDP (34.08 x 10^3 g^{-1}), both of

Table 13. Population of total bacteria, fungi and actinomycetes in sunflower rhizosphere as influenced by P-solubilizers, forms and levels of phosphorus at 60 days of crop growth

Forms of P (%RDP)	P-levels (%RDP)	Bacterial population ($X10^6$ g^{-1})				Fungal population ($X10^3$ g^{-1})				Actinomycete population ($X10^3$ g^{-1})			
		UIC	\`biophos'	P.striata	Mean	UIC	\`biophos'	P.striata	Mean	UIC	\`biophos'	P.striata	Mean
Super phosphate	0	80.23	79.18	80.79	80.07	29.38	33.91	32.17	31.82	34.46	39.55	36.72	36.91
	50	84.75	89.27	87.01	87.01	32.20	35.03	34.46	33.90	37.30	41.24	40.11	39.55
	75	85.88	90.40	89.83	88.70	33.90	36.72	36.16	33.59	39.55	43.50	42.37	41.81
	100	89.27	92.66	90.96	90.96	35.03	38.98	35.51	36.50	42.11	48.59	47.46	46.05
	Mean	85.03	87.87	87.15	86.68	32.62	36.16	34.57	34.45	38.35	43.22	41.66	41.08
Rock phosphate	0	80.23	79.18	80.79	80.07	29.38	33.89	32.20	31.82	34.46	39.55	36.72	36.91
	50	81.36	84.75	87.57	84.56	29.94	31.64	31.07	30.88	35.02	38.42	37.85	37.10
	75	83.62	88.70	87.01	86.44	30.51	33.90	33.33	32.58	36.16	42.37	41.27	39.92
	100	85.88	89.33	88.14	87.78	32.20	35.03	34.46	33.90	40.70	44.07	43.50	42.75
	Mean	82.77	85.49	85.88	84.71	30.51	33.61	32.77	32.29	36.58	41.10	39.83	39.17
Mean		83.90	86.68	86.51	85.70	31.56	34.88	33.67	33.37	37.47	42.16	40.75	40.12

Means for levels of phosphorus

	Bacterial Population				Fungal Population				Actinomycete Population			
P-levels	0	50	75	100	0	50	75	100	0	50	75	100
Mean	80.07	85.78	87.57	89.37	31.82	32.39	34.08	35.20	36.91	38.32	40.86	44.40

Sources	SEm±	CD (0.05)	SEm±	CD (0.05)	SEm±	CD (0.05)
Forms of P(A)	0.453	1.325	0.546	1.597	0.566	1.655
Levels of P (B)	0.640	1.873	0.772	2.258	0.800	2.341
Inoculation (C)	0.555	1.622	0.668	1.956	0.693	2.028
AxB	0.906	NS	1.092	NS	1.132	NS
AxC	0.784	NS	0.945	NS	0.980	NS
BxC	1.109	NS	1.337	NS	1.386	NS
AxBxC	1.568	NS	1.891	NS	1.960	NS

RDP = Recommended dose of phosphorus, UIC = Uninoculated control, NS = Non-significant,

which were significantly superior over the uninoculated control (31.82 x 10^3 g^{-1}). However, fungal population did not differ significantly between the treatments receiving 50 per cent RDP and no phosphorus control as well as between 50 per cent RDP and 75 per cent RDP. The interactions were found to be non-significant.

4.5.3 Actinomycetes

The rhizosphere of plants inoculated with `biophos' harboured the highest actinomycete population (42.16 x 10^3 g^{-1}), followed by *P. striata* (40.75 x 10^3 g^{-1}) both of which were significantly superior over the uninoculated control (37.47 x 10^3 g^{-1}). Super phosphate application stimulated the actinomycete population in the rhizosphere significantly (41.08 x 10^3 g^{-1}) over rock phosphate application (39.17 x 10^3 g^{-1}). Maximum population of actinomycetes among P-levels was recorded at 100 per cent RDP (44.40 x 10^3 g^{-1}), followed by 75 per cent RDP (40.86 x 10^3 g^{-1}) both of which were significantly superior over 50 per cent RDP (38.32 x 10^3 g^{-1}) and no phosphorus control (36.91 x 10^3 g^{-1}). The interactions failed to show significant effect on actinomycetes population.

5.4.4 Free-living nitrogen fixers

The population of free-living N_2 fixers in the rhizosphere recorded at different growth stages of sunflower are presented in Table 14. Among the inoculation treatments, the population of free-living N_2 fixers at 30 DAS was highest in treatment receiving `biophos' (24.01 x 10^3 g^{-1}) which was significantly superior over *P. striata* (21.56 x 10^3 g^{-1}) and uninoculated control (18.48 x 10^3 g^{-1}). However, *P. striata* was significantly superior over uninoculated control.

Application of P in the form of super phosphate stimulated the free-living N_2 fixers (21.95×10^3 g^{-1}) significantly over rock phosphate (21.22×10^3 g^{-1}). Population of free-living N_2 fixers was also increased significantly with increase in P-levels. Highest population was recorded at 100 per cent RDP (25.32×10^3 g^{-1}), followed by 75 per cent RDP (23.67×10^3 g^{-1}) and 50 per cent RDP (20.39×10^3 g^{-1}) and was least in no phosphorus control (17.22×10^3 g^{-1}). The interaction between forms and levels of P was found to be significant. Super phosphate applied at 100 per cent RDP recorded the highest population (26.25×10^3 g^{-1}) which was significantly higher than all other P form and P level combinations. The interaction between inoculation and levels of P was also found to be significant wherein phosphorus at 100 per cent RDP plus `biophos' recorded the highest population ($27.72 \times 10^3$ $g^{-1}$) followed by 75 per cent RDP plus `biophos' (27.55×10^3 g^{-1}) both of which were on par with each other but significantly superior over all other similar combinations. The interaction between inoculation, forms of P and levels of P revealed that super phosphate applied at 100 per cent RDP in combination with `biophos' inoculation showed the highest population of free-living N_2 fixers (28.98×10^3 g^{-1}) and was significantly superior over all other combinations.

Although there was a general increase in the population of free-living N_2 fixers in the rhizosphere at 60 DAS compared to 30 DAS, the trend in population with respect to inoculation, P-forms and P-levels was almost same. `Biophos' inoculation (28.22×10^3 g^{-1}) was significantly superior over *P. striata* inoculation (26.75×10^3 g^{-1}) and both were significantly superior over uninoculated

Table 14. Population of free-living nitrogen fixers (No. x 10^3 g^{-1}) in sunflower rhizosphere as influenced by P-solubilizing biofertilizers and forms and levels of phosphorus at different growth stages

Forms of P	P-levels (%RDP)	30 DAS				60 DAS				At harvest			
		UIC	`biophos'	P.striata	Mean	UIC	`biophos'	P.striata	Mean	UIC	`biophos'	P.striata	Mean
Super phosphate	0	15.12	18.90	17.64	17.22	18.25	24.73	23.16	22.04	19.50	21.50	20.50	20.50
	50	19.53	24.07	22.05	21.88	24.25	28.25	26.55	26.35	21.00	22.00	22.00	21.66
	75	20.15	25.20	22.05	22.46	25.99	31.64	29.94	29.19	21.00	24.50	23.50	23.00
	100	23.31	28.98	26.46	26.25	27.68	32.27	32.20	30.72	22.50	28.00	28.50	26.33
	Mean	19.52	24.28	22.05	21.95	24.04	29.22	27.96	27.07	21.00	24.00	23.62	22.87
Rock phosphate	0	15.12	18.90	17.64	17.22	18.25	24.73	23.16	22.04	19.50	21.50	20.50	20.50
	50	17.64	20.79	18.27	18.90	20.34	25.99	24.23	23.52	19.00	20.00	21.50	20.16
	75	18.84	29.91	25.83	24.87	23.10	27.70	25.99	25.59	20.50	22.50	22.00	21.66
	100	22.18	26.46	24.57	24.40	25.42	30.51	28.25	28.06	22.50	23.50	22.00	22.66
	Mean	18.92	22.57	22.17	21.22	21.77	27.23	25.53	24.84	20.37	21.62	21.50	21.16
Mean		18.48	24.01	21.56	21.34	22.91	28.22	26.75	25.96	20.68	22.81	22.56	22.02

Two way table for interaction between inoculation and levels of P (30 DAS)

%RDP	UIC	`biophos'	P.striata	Mean
0	15.12	18.90	17.64	17.22
50	18.58	22.43	20.16	20.39
75	19.52	27.55	23.94	23.67
100	22.74	27.72	25.51	25.32

Means for levels of phosphorus

	60 DAS				At harvest			
P-levels	0	50	75	100	0	50	75	100
Mean	22.04	24.93	27.39	29.39	20.50	20.91	22.33	24.50

Sources	SEm±	CD (0.05)	SEm±	CD (0.05)	SEm±	CD (0.05)
Forms of P(A)	0.130	0.379	0.371	1.086	0.262	0.766
Levels of P (B)	0.183	0.536	0.525	1.536	0.370	1.083
Inoculation (C)	0.159	0.464	0.455	1.330	0.321	0.938
AxB	0.259	0.758	0.742	NS	0.523	1.531
AxC	0.224	NS	0.643	NS	0.453	NS
BxC	0.317	0.928	0.909	NS	0.641	NS
AxBxC	0.449	1.313	1.286	NS	0.907	NS

RDP = Recommended dose of phosphorus, UIC = Uninoculated control, NS = Non-significant, DAS = Days after sowing

control (22.91 x 10^3 g^{-1}). Among the forms of P, super phosphate (27.07 x 10^3 g^{-1}) was found to be significantly superior over the rock phosphate (24.84 x 10^3 g^{-1}). At this stage of plant growth also, the population of free-living N_2 fixers was increased significantly with increasing P-level and was highest at 100 per cent RDP. However, none of the interactions were found significant at this stage.

There was a general reduction in the population of free-living N_2 fixers in the rhizosphere at harvesting stage as compared to 60 DAS. However, the trend in population as influenced by inoculation, P-forms and P-levels was almost same. `Biophos' and *P. striata* inoculation showed significantly higher population of N_2 fixers over uninoculated control although the two biofertilizers were on par with each other. Super phosphate among the P-forms and 100 per cent RDP among P-levels showed highest population of free-living N_2-fixers in the rhizosphere. The interaction between forms and levels of P was significant at this stage. Super phosphate applied at 100 per cent RDP recorded the highest population and was significantly higher than all other similar treatment combinations. All other interactions were found to be non-significant.

4.5.6 Phosphate solubilizers

The data presented in Table 15 clearly indicated that the population of P-solubilizers in the rhizosphere was significantly influenced by inoculation, forms of phosphorus and levels of phosphorus at different stages of crop growth. At 30 DAS, *P. striata* among inoculation treatments showed the highest population (26.34 x 10^3 g^{-1}) which was however on par with `biophos' inoculation (25.98 x 10^3 g^{-1}) but both were significantly superior over uninoculated control (23.70 x 10^3

g^{-1}). No significant differences in the population of P-solubilizers was noticed due to forms of phosphorus. However, the population of P-solubilizers was found to increase significantly with increase in P-levels. Application of P at 100 per cent RDP recorded the highest population (30.21 x 10^3 g^{-1}), followed by 75 per cent RDP (26.56 x 10^3 g^{-1}) and 50 per cent RDP (24.02 x 10^3 g^{-1}) all of which differed significantly among themselves but were significantly superior over no phosphorus control (20.58 x 10^3 g^{-1}). The interaction between forms and levels of P was found to be significant. Rock phosphate application at 100 per cent RDP recorded the highest population (30.40 x 10^3 g^{-1}), followed by super phosphate applied at 100 per cent RDP (30.03 x 10^3 g^{-1}) both of which were on par with each other, but significantly superior over all other P-form x P-level combinations. The interaction between inoculation and levels of P was found to be significant wherein the combination of *P. striata* plus 100 per cent RDP followed by `biophos' plus 100 per cent RDP recorded maximum population (31.74 x and 30.87 x 10^3 g^{-1}, respectively) both of which were significantly superior over all other similar combinations. The interaction between inoculation, forms of P and levels of P was also found to be significant. The combination of *P. striata* plus 100 per cent RDP as super phosphate or rock phosphate showed maximum population of P-solubilizers.

At 60 days of crop growth, an exactly similar trend in the population of P-solubilizers was noticed as that of 30 DAS, although there was a general increase in the population at 60 DAS. *P. striata* and `biophos' inoculation showed significantly higher population of P-solubilizers over uninoculated control where as

Table 15. Population of phosphate solubilizing microorganisms (No. x 10^3 g^{-1}) in sunflower rhizosphere as influenced by P-solubilizers, and forms and levels of phosphorus at different growth stages

Forms of P	P-levels (%RDP)	30 DAS				60 DAS				At harvest			
		UIC	\`biophos'	P.striata	Mean	UIC	\`biophos'	P.striata	Mean	UIC	\`biophos'	P.striata	Mean
Super phosphate	0	19.53	20.16	22.05	20.58	23.74	28.25	24.99	25.66	17.00	20.00	22.00	19.66
	50	22.05	23.31	22.57	22.64	29.94	35.03	36.72	33.90	21.00	23.50	25.50	23.33
	75	23.31	28.35	29.61	27.09	31.64	33.90	37.85	34.46	22.00	26.00	27.50	25.16
	100	27.09	30.87	32.13	30.03	32.77	39.55	41.24	37.85	25.00	30.50	32.50	29.33
	Mean	22.99	25.67	26.59	25.08	29.52	34.18	35.20	32.97	21.25	25.00	26.87	24.37
Rock phosphate	0	19.53	20.16	22.05	20.58	23.74	28.25	24.99	25.66	17.00	20.00	22.00	19.66
	50	23.94	25.20	27.09	25.41	31.64	36.72	37.48	35.28	25.00	27.00	28.50	26.83
	75	25.20	28.98	23.94	26.04	30.51	40.11	42.37	37.66	26.00	29.50	31.50	29.00
	100	28.98	30.87	31.35	30.40	38.98	42.44	45.76	42.39	28.00	33.00	34.50	31.83
	Mean	24.41	26.30	26.10	25.60	31.21	36.88	37.65	35.25	24.00	27.37	29.12	26.83
Mean		23.70	25.98	26.34	25.34	30.37	35.53	36.42	34.11	42.62	26.18	28.00	25.00

Two way table for interaction between inoculation and levels of P

P level (%RDP)	30 DAS				60 DAS			
	UIC	\`biophos'	P.striata	Mean	UIC	\`biophos'	P.striata	Mean
0	19.53	20.16	22.05	20.58	23.74	28.25	24.99	25.65
50	22.99	24.25	24.83	24.02	30.79	35.87	37.10	34.59
75	24.25	28.66	26.77	26.56	31.07	37.00	40.11	36.06
100	28.03	30.87	31.74	30.21	35.87	40.99	43.50	40.12

Means for levels of phosphorus At harvest

P-levels	0	50	75	100
Mean	19.66	25.08	27.08	30.58

Sources	SEm±	CD (0.05)	SEm±	CD (0.05)	SEm±	CD (0.05)
Forms of P(A)	0.218	NS	0.297	0.868	0.488	1.427
Levels of P (B)	0.308	0.901	0.420	1.228	0.690	2.018
Inoculation (C)	0.267	0.780	0.363	1.063	0.597	1.748
AxB	0.435	1.274	0.593	1.736	0.975	NS
AxC	0.377	NS	0.514	NS	0.845	NS
BxC	0.533	1.560	0.727	2.127	1.195	NS
AxBxC	0.754	2.206	1.028	3.007	1.690	NS

RDP = Recommended dose of phosphorus, UIC = Uninoculated control, NS = Non-significant, DAS = Days after sowing

rock phosphate application was significantly superior over super phosphate application. Among the P-levels, highest population by P solubilizers was recorded in the treatment receiving 100 per cent RDP (40.12 x 10^3 g^{-1}), followed by 75 and 50 per cent RDP (36.06 and 34.59 x 10^3 g^{-1}, respectively) and no phosphorus control (25.65 x 10^3 g^{-1}) all of which differed significantly among themselves. The interaction between forms and levels of P, inoculation and levels of P as well as inoculation x forms of P x levels of P were found to be significant at this stage of crop growth also. *P. striata* inoculation in combination with application of rock phosphate at 100 per cent RDP recorded the highest population of P-solubilizers which was significant over all other treatment combinations.

The trend in population of P-solubilizers at harvest was almost same as that of 30 and 60 days of crop growth but there was a general decrease in the population at harvest as compared to 60 DAS. At harvest, *P. striata* inoculation showed significantly higher population over `biophos' where as `biophos' was significantly superior over uninoculated control. Similarly, rock phosphate among the P-forms and 100 per cent RDP among P-levels showed the highest population of P-solubilizers. However, the interaction effects between P-forms and P-levels, P-forms x inoculation, P-levels x inoculation and P-forms x P-levels x inoculation were non-significant at harvest.

4.6 Available phosphorus content in soil

Available P content in soil as influenced by various treatments at different stages of crop growth are presented in Table 16. At 30 DAS, the highest available phosphorus in soil among the inoculation treatments was recorded in `biophos' (21.92 kg ha$^{-1}$), followed by *P. striata* (20.59 kg ha$^{-1}$), both of which were significantly superior over uninoculated control (18.94 kg ha$^{-1}$). However, `biophos' was significantly superior over *P. striata*. Among the forms of P, super phosphate receiving treatments showed significantly higher available phosphorus in soil (23.86 kg ha^{-1}) than rock phosphate receiving treatments (17.11 kg ha^{-1}). A significant increase in available phosphorus content of soil was associated with increasing P-level. Among the P-levels, maximum soil available phosphorus was recorded in the treatment receiving 100 per cent RDP (24.22 kg ha^{-1}) followed by 75 per cent RDP (21.77 kg ha^{-1}) and 50 per cent RDP (20.20 kg ha^{-1}). Least available P was recorded in no phosphorus control (15.74 kg ha^{-1}). The interaction between forms of P and levels of P was found to be significant where in the highest available phosphorus in soil was recorded in treatment receiving 100 per cent RDP applied as super phosphate (28.89 kg ha^{-1}) which was significantly superior over all other P-form x P-level combinations. The interaction between levels of P and inoculation was also found significant. Treatment combination of 100 per cent RDP plus `biophos' showed the highest available P (26.08 kg ha^{-1}) which was significantly superior over other combinations of P-level x inoculation. Interaction between forms of phosphorus, levels of P and inoculation was also found significant where the maximum available phosphorus in soil was recorded in combination of `biophos' plus 100 per cent RDP as super phosphate (30.68 kg ha^{-1}), followed by *P.striata* plus 100 per cent RDP as super phosphate (29.74 kg ha^{-1}) both of which were on par with each other but significantly superior over all other treatment combinations.

Table 16. Available phosphorus content in soil (kg ha^{-1}) as influenced by phosphate solubilizing biofertilizers, forms and levels of phosphorus at different growth stages

Forms of P	P-levels (%RDP)	30 DAS				60 DAS				At harvest			
		UIC	`biophos'	P.striata	Mean	UIC	`biophos'	P.striata	Mean	UIC	`biophos'	P.striata	Mean
Super phosphate	0	14.01	17.03	16.18	15.74	18.03	18.69	19.69	18.80	18.72	19.89	18.27	18.96
	50	23.79	26.33	23.05	24.39	21.87	22.29	21.80	21.98	18.98	20.98	20.60	20.18
	75	24.13	27.69	27.46	26.42	22.77	26.22	24.11	24.47	18.30	21.69	21.13	20.37
	100	26.26	30.68	29.74	28.89	23.03	28.00	25.21	25.41	19.05	25.05	23.91	22.68
	Mean	22.04	25.43	24.11	23.86	21.42	23.80	22.77	22.66	18.76	21.91	20.97	20.55
Rock phosphate	0	14.01	17.03	16.18	15.74	18.03	18.69	19.69	18.80	18.72	19.89	18.27	18.90
	50	14.65	17.27	16.15	16.02	18.16	19.23	18.33	18.57	18.82	19.60	18.06	18.82
	75	16.61	17.85	16.90	17.12	21.81	22.29	21.80	21.96	19.03	21.47	20.47	20.32
	100	18.10	21.49	19.10	19.56	21.81	23.35	22.51	22.55	21.11	24.64	24.31	23.35
	Mean	15.84	18.41	17.08	17.11	19.95	20.89	20.81	20.55	19.42	21.40	20.27	20.36
Mean		18.94	21.92	20.59	20.48	20.68	22.34	21.79	21.61	19.09	21.65	20.62	20.45

Two way table for interaction between levels of P and inoculation

P level (%)	30 DAS				60 DAS				At harvest			
	UIC	`biophos'	P.striata	Mean	UIC	`biophos'	P.striata	Mean	UIC	`biophos'	P.striata	Mean
0	14.01	17.03	16.18	15.74	18.03	18.69	19.69	18.80	18.72	19.89	18.27	18.96
50	19.22	21.80	19.60	20.20	20.01	20.76	20.06	20.28	18.90	20.29	19.33	19.50
75	20.37	22.77	22.18	21.77	22.29	24.25	23.10	23.12	18.66	21.58	20.80	20.34
100	22.18	26.08	24.42	24.22	22.42	25.67	23.86	23.98	20.03	24.86	24.11	23.01

Sources	SEm±	CD (0.05)	SEm±	CD (0.05)	SEm±	CD (0.05)
Forms of P(A)	0.129	0.377	0.101	0.297	0.112	NS
Levels of P (B)	0.182	0.533	0.143	0.420	0.158	0.463
Inoculation (C)	0.158	0.462	0.124	0.363	0.137	0.401
AxB	0.258	0.754	0.203	0.893	0.224	0.655
AxC	0.223	NS	0.176	0.514	0.194	0.567
BxC	0.316	0.932	0.248	0.727	0.274	0.802
AxBxC	0.446	1.306	0.351	1.028	0.388	NS

RDP = Recommended dose of phosphorus, UIC = Uninoculated control, NS = Non-significant, DAS = Days after sowing

An almost similar trend in soil available phosphorus was noticed at 60 DAS. `Biophos', among the inoculation treatments, showed highest available phosphorus (22.34 kg ha^{-1}) which was significantly superior over *P. striata* inoculation (21.79 kg ha^{-1}). *P. striata* was also significantly superior over uninoculated control. Super phosphate among P-forms and 100 per cent RDP among P-levels showed the highest available phosphorus. The interaction between forms of P and levels of P, forms of P x inoculation, levels of P x inoculation and forms of P x levels of P x inoculation were found to be significant. Among all the treatment combinations, the highest available phosphorus was recorded in the treatment receiving `biophos' plus 100 per cent RDP in the form of super phosphate (28.00 kg ha^{-1}) which was significantly superior over all other combinations.

At harvest, `biophos' among inoculation treatments and 100 per cent RDP among P-levels recorded maximum available P in soil which were significant over the respective other inoculations and P-levels. However, no significant differences were found between the forms of P with respect to available P in soil at this stage. The interaction between forms of P x levels of P, forms of P x inoculation, levels of P x inoculation were found to be significant and the trend in available phosphorus was same as that of previous samplings. However, the interaction between forms of P x levels of P x inoculation was found non-significant at harvest.

DISCUSSION

V. DISCUSSION

The modern agriculture has been heavily dependent on chemical fertilizers to meet the food demands of ever increasing population. The population pressure has also led to fast depletion of fossil fuel resources with concomitant increase in the prices of chemical fertilizers. On the other hand, progressive depletion of major plant nutrients in soil due to intensive cultivation, has necessitated the use of higher doses of chemical fertilizers particularly in tropical soils where the organic matter content is very low. But the continuous use of heavy doses of chemical fertilizers may lead to environmental pollution. Hence, the current trend throughout the world is to explore the possibility of using alternate nutrient sources or increasing the efficiency of chemical fertilizers by supplementing them with organic fertilizers and microbial inoculants.

The supply of nutrients to plants through microorganisms, which play a major role in biological nitrogen fixation, phosphorus solubilization, mineralization and mineral transformations, has gained lot of importance. The biofertilizers containing phosphate solubilizing microorganisms benefit the agricultural production through enhanced nutrient supply, specially the phosphorus. Inoculation with P-solubilizing biofertilizers has shown to increase the P-uptake and yield of several crop plants (Gaur *et al.*, 1980; Anthoniraj *et al.*, 1994; Jisha and Alagawadi, 1996). Further, combined use of chemical fertilizers and biofertilizers has been considered as a workable technology in the recent years (Gaur and Alagawadi, 1987; Alagawadi and Gaur, 1988; Prathibha, 1993). In this context, it was thought appropriate to investigate on the performance of two phosphate solubilizing biofertilizers in conjunction with different forms and levels of phosphorus in an important oilseed crop, sunflower. The results obtained in the investigation are discussed in this chapter.

5.1 Plant growth parameters

The plant growth parameters like height, number of leaves and dry matter yield of sunflower in the present investigation were significantly increased due to inoculation with `biophos' or *P. striata* as compared to uninoculated control. Application of phosphorus in the form of super phosphate or rock phosphate in combination with `biophos' or *P. striata* inoculation further enhanced the plant growth parameters significantly over inoculation alone or phosphorus alone. Increase in the height of several crop plants due to inoculation of phosphate solubilizing microorganisms has been reported by several workers (Barea *et al.*, 1976; Patil, 1990; Jones and Sreenivasa, 1993). Increased cell elongation and multiplication due to enhanced nutrient uptake by plants (Black, 1968) following inoculation of phosphate solubilizing biofertilizers or application of phosphatic fertilizers probably has caused the increased plant height. It can also be attributed to the production of plant growth promoting substances in the vicinity of roots by the inoculated biofertilizers as many P-solubilizers are known to produce IAA, GA and Cytokinin like substances (Sattar and Gaur, 1987), whose role in shoot and root elongation as well as plant growth is well established (Brown, 1972, 1974 and 1975).

The results of increased number of leaves per plant due to inoculation with `biophos' or *P. striata* and their combination with P-fertilizers are in agreement with results of Jones (1992) who also observed increased number of leaves in sunflower plants due to inoculation with *P. striata* and P-fertilizer application.

The investigation also revealed a significant increase in the dry matter production in sunflower plants due to `biophos' and *P. striata* inoculation either alone or in combination with phosphatic fertilizers (Fig. 2). The increased dry matter accumulation due to inoculation and P-fertilizer application can be attributed to increased plant height and number of leaves per plant which are the main contributing factors for plant biomass. Since the available P content in soil was enhanced due to inoculation with biofertilizers (Table 16), there was better uptake of P by the crop (Table 12) which ultimately led to better growth and dry matter accumulation in the present investigation. Similar increase in the dry matter yield of sunflower (Jones and Sreenivasa, 1993) and other crops (Sharma *et al.*, 1983; El-Din and Baber, 1983; Alagawadi and Gaur, 1988; Prabhakar and Saraf, 1990) due to inoculation with P-solubilizers have been reported earlier and were attributed to the production of growth promoting substances in addition to P-solubilization by these organisms.

It was also observed that, among the two biofertilizers, `biophos' was more efficient in enhancing the plant growth and dry matter yield than *P. striata*.

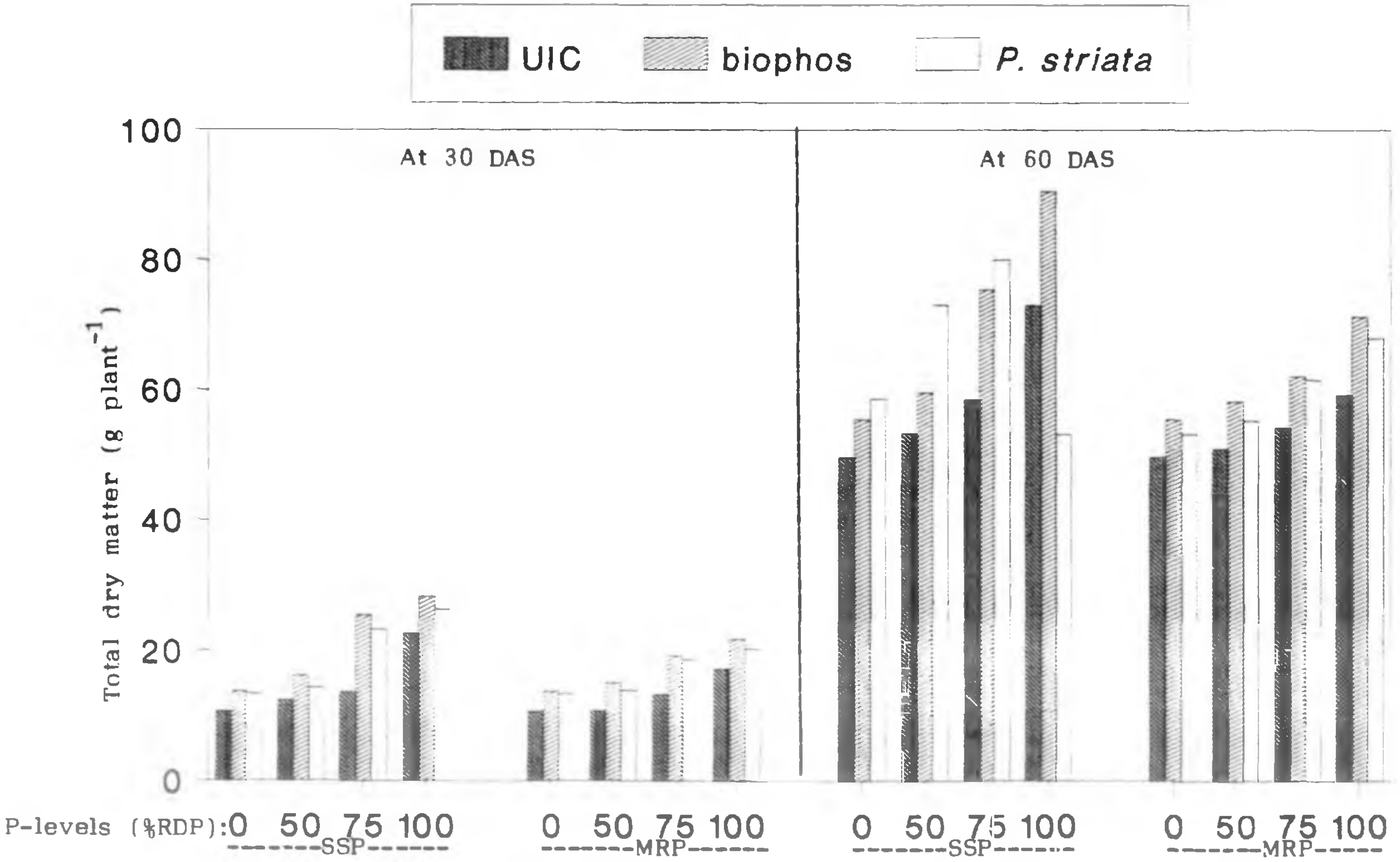


Fig. 2 Effect of inoculation with P-solubilizing biofertilizers in combination with different forms and levels of phosphorus on total dry matter accumulation

(SSP - Single super phosphate MRP - Mussoorie rock phosphate UIC-Uninoculated control)

The observed differences between the two biofertilizers may be ascribed to their differential intrinsic ability to solubilize insoluble phosphorus (Banik and Dey, 1983). The phosphate solubilization is linked to the organic acid production by the organism and the amount of P-solubilized is dependent on the quantity and nature of organic acids produced (Bardiya, 1970). Hence it is probable that the `biophos' contained organism(s) which are more efficient P-solubilizers as evidenced by increased available P-content in soil and P-uptake in plants (Table 12).

5.2 Days to 50 per cent flowering

In addition to improving the plant growth and dry matter yield, `biophos' and *P. striata* inoculation led to early flowering of sunflower crop in the present study (Table 7). Inoculation in combination with application of P-fertilizers, particularly super phosphate has further reduced the days to 50 per cent flowering indicating that the increased phosphorus availability induced the early maturity of crop. This lend support to the long-held view that phosphorus hastens the plant maturity (Nelson and Tisdale, 1968). Similar observations on early flowering of sunflower plants due to inoculation with P-solubilizing biofertilizers have been made earlier (Jones, 1992; Ravishankar, 1993).

5.3 Yield and yield components

Early maturity of the crop was also coupled with significant increase in yield attributing parameters like head size, head weight, number of seeds per head, seed yield per plant and thousand seed weight due to `biophos' and *P. striata* inoculation and P-fertilizer application over respective controls. Inoculation in combination with P-fertilizer application further enhanced the yield attributing parameters significantly over inoculation alone or P-fertilizer alone. The inoculated organisms enhanced the P-availability in the rhizosphere (Table 16) with a concomitant increase in P-uptake by the crop (Table 12) which lead to increased dry matter accumulation and subsequently the yield parameters. Besides increasing the P-availability to the crop, the P-solubilizing organisms are also known to produce growth promoting substances (Sattar and Gaur, 1987) which might also have contributed to the increased yield parameters. Increase in the head size, head weight, seed yield per plant in sunflower (Jones and Sreenivasa, 1993) and other crops like cotton (Prathibha, 1993) and sorghum (Jisha, 1993) due to inoculation with P-solubilizing organisms have been reported earlier which draw support from the results of present investigation.

The results also revealed a significant increase in the seed yield of sunflower (Fig. 3) due to inoculation of `biophos' and *P. striata* as well as P-fertilizers. Seed yield is the manifestation of yield attributing characters like size of the head, weight of the head, number of seeds per head, seed yield per plant and thousand seed weight. Hence, a significant increase in all these characters due to inoculation and/or P-fertilizer application has led to significant increase in seed yield per hectare. The inoculated organisms established well in the rhizosphere as evidenced by their higher population in rhizosphere (Fig. 6) thereby released higher amounts of available phosphorus (Table 16) in the vicinity of root zone to enable the plants to take up more P (Table 12). The increased P-uptake by the crop must have

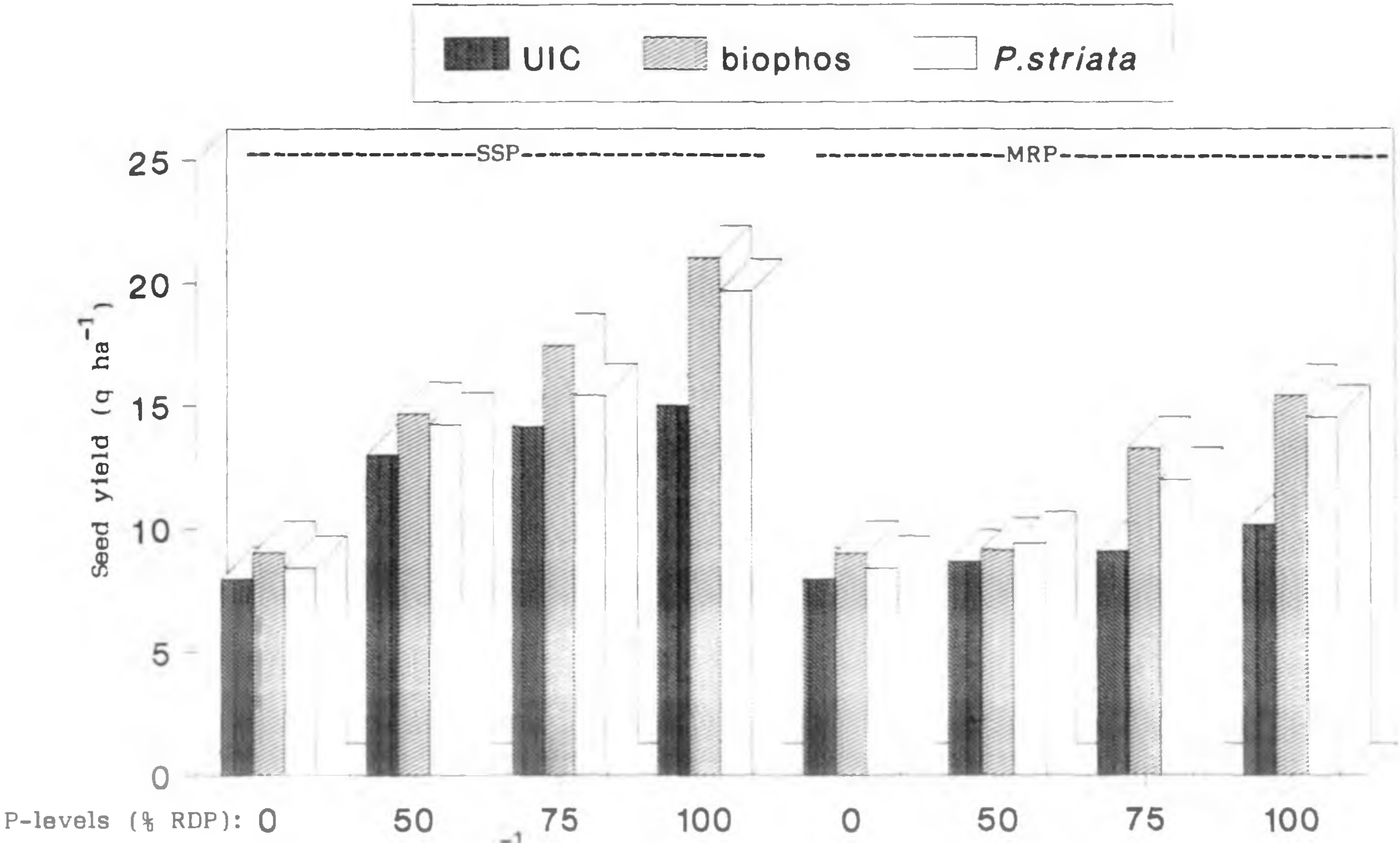


Fig.3 Seed yield (q ha^{-1}) as influenced by phosphate solubilizing biofertilizers in combination with forms and levels of phosphorus

(SSP - Single super phosphate MRP - Mussoorie rock phosphate UIC - Uninoculated control)

led to increased dry matter and seed yield. Similar increase in the yield of sunflower and other crops due to inoculation with P-solubilizers have been reported by several workers (Gaur, 1985; Alagawadi and Gaur, 1988 and 1992; Jones, 1993; Ravishankar, 1993; Prathibha, 1993). The seed yield of sunflower in the inoculation treatments was further enhanced with the application of phosphorus, especially in the form of super phosphate, at 100 per cent recommended dose. This clearly indicates that besides releasing P from rock phosphate, the inoculated organisms increased the use efficiency of super phosphate. This is further confirmed by the fact that `biophos' or *P. striata* inoculation in combination with SSP at 75 per cent RDP or MRP at 100 per cent RDP produced higher or same yield as that of standard practice of applying 100 per cent RDP in the form of super phosphate. These results clearly indicated the possibility of saving 25 per cent P-fertilizer in the form of super phosphate or replacing entire super phosphate with rock phosphate when the crop is inoculated with these two biofertilizers. Similar conclusions were also made earlier by various workers working on P-solubilizing biofertilizers in different crops (Gaur *et al.*, 1980; Shinde and Patil, 1985; Alagawadi and Gaur, 1988 and 1992; Jones and Sreenivasa, 1993).

5.4 Nutrient uptake

The growth and yield of crop plants are determined by the presence of sufficient quantities of nutrients in available form for plant uptake (Babannavar, 1990). The higher yield of sunflower in the present study due to inoculation with P-solubilizers and application of P-fertilizers can be assigned to increased nutrient availability and their uptake by the crop (Table 11 and 12). Inoculation of `biophos' or *P. striata* in combination with P-fertilizer had significant influence on N-uptake by plants (Table 11 and Fig. 4). The increase in N-uptake due to inoculation or P-fertilization can be ascribed to increased P-availability to the crop. Phosphorus is known to increase the root growth and proliferation (Nelson and Tisdale, 1968) thereby creating more absorptive area for uptake of other nutrients specially the N. Mohod *et al.* (1991) also observed significant increase in N-uptake of rice due to inoculation with *P. striata* or *B. polymyxa* which gain support from the results of present investigation. The increased N-uptake by the crop in the present investigation due to inoculation with P-solubilizing biofertilizers may also be attributed to increased population of free-living N_2-fixers (Table 14) as well as other organisms (Table 13) in rhizosphere which in turn probably increased the uptake of nutrients by means of altering the root surface characteristics involved in nutrient uptake (Pacovsky *et al.*, 1985).

The same argument may hold true for the observed increase in P-uptake by sunflower crop due to `biophos' or *P. striata* inoculation and/or application of P-fertilizers (Fig. 5). The inoculated organisms established well in the rhizosphere (Fig. 6), increased the available phosphorus in soil thereby increasing its uptake by the crop. These findings are in line with the results of Kundu and Gaur (1980), Maurya and Sanoria (1982), Rachewad *et al.* (1992), Jisha and Alagawadi (1996) who observed increased P-uptake in potato, chickpea, sunflower and sorghum crops respectively due to inoculation with P-solubilizing organisms.

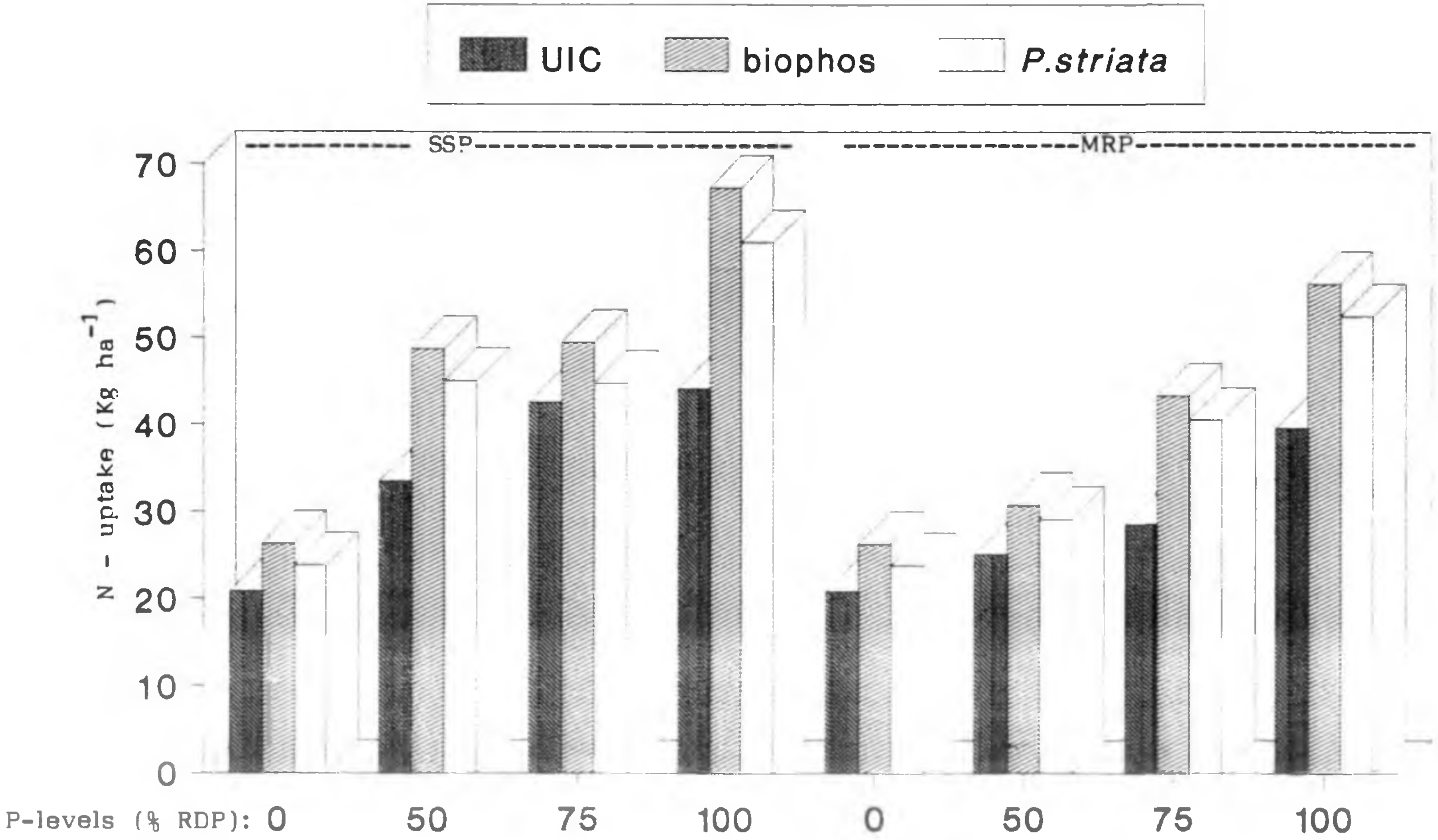


Fig.4 Nitrogen uptake (Kg ha^{-1}) in seeds as influenced by phosphate solubilizing biofertilizers in combination with forms and levels of phosphorus

(SSP - Single super phosphate MRP - Mussoorie rock phosphate UIC - Uninoculated control)

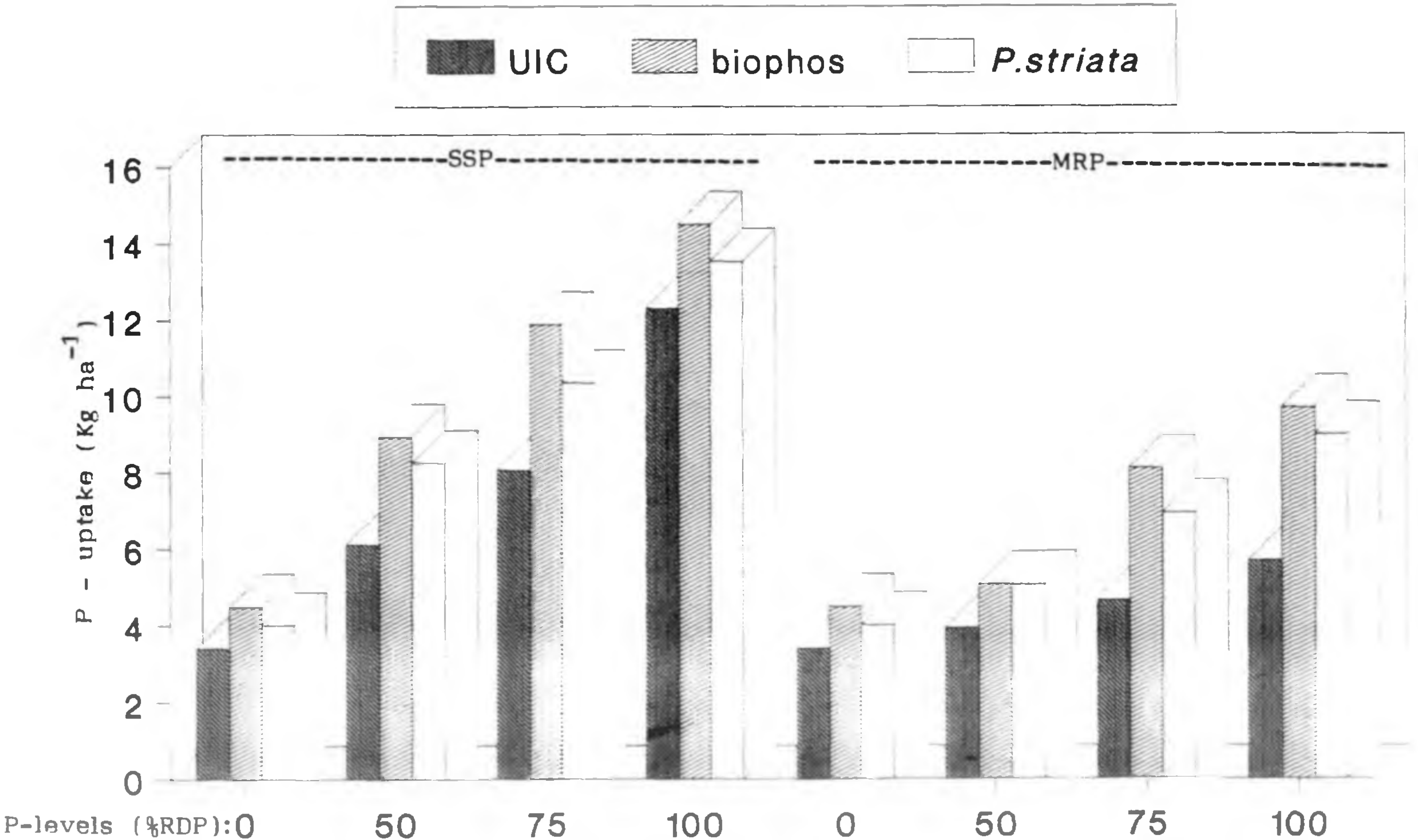


Fig. 5 Phosphorus uptake (Kg ha^{-1}) in seeds as influenced by phosphate solubilizing biofertilizers in combination with forms and levels of phosphorus

(SSP - Single super phosphate MRP - Mussoorie rock phosphate UIC - Uninoculated control)

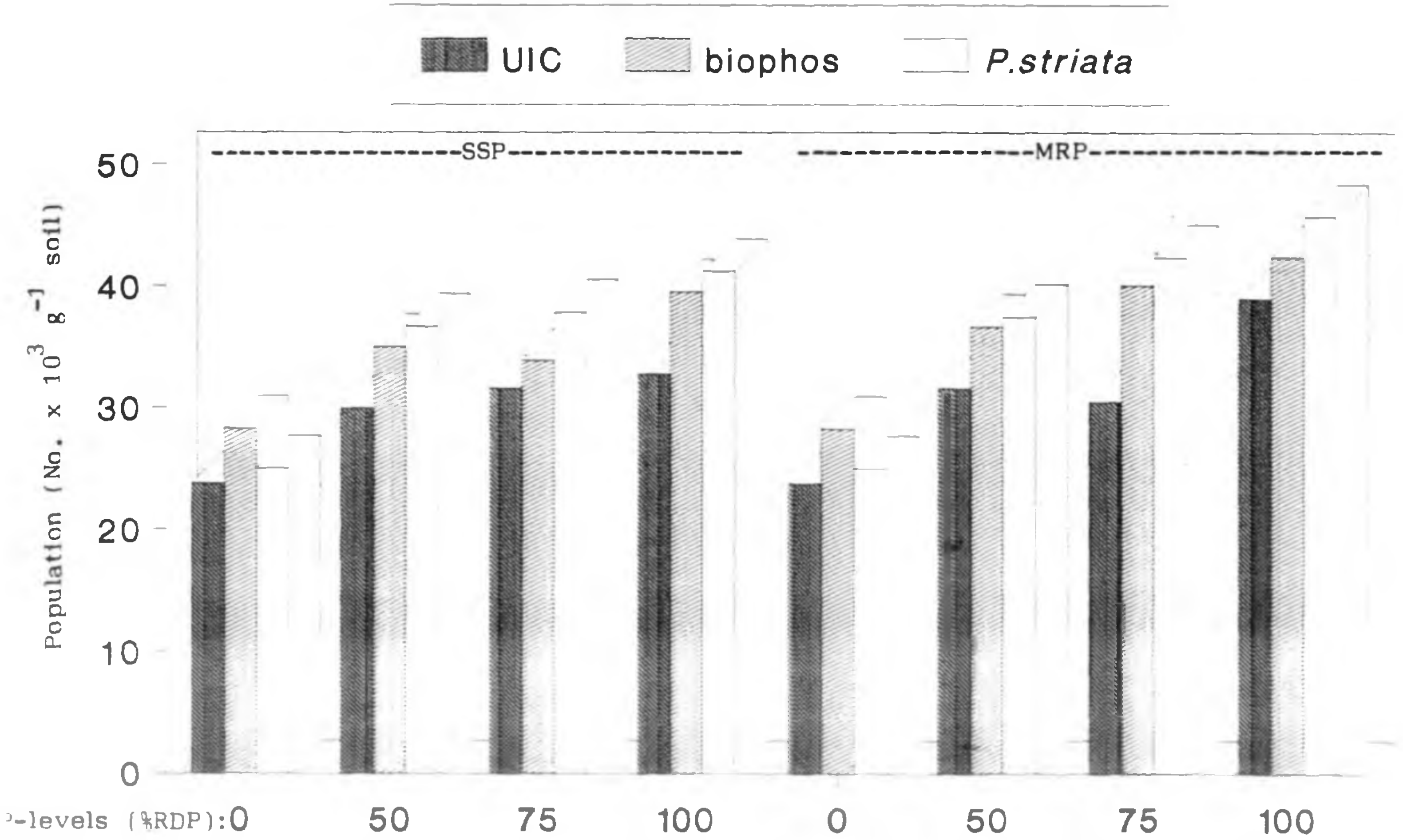


Fig.6 Population of phoshate solubilizing microorganisms in sunflower rhisosphere as influenced by P-solubilizers, forms and levels of phosphorus at 60 DAS

(SSP - Single super phosphate MRP - Mussoorie rock phosphate UIC - Uninoculated control)

5.5 Available P content in soil

In the present study, available P-content in soil was significantly increased due to `biophos' and *P. striata* inoculation in combination with P-fertilizer application (Fig. 7) which are in agreement with results of Asea *et al.* (1988), Salih *et al.* (1989) and Mohod *et al.* (1989). It was also observed that the available P in soil was higher in the initial stages of plant growth where super phosphate was used as P-source but was declined at harvest probably due to increased uptake by the crop which support the findings of Mandal and Khan (1972) and Marwaha *et al.*, (1984). However with rock phosphate application, the available P-content in soil in general was low at initial stages but increased at the later stages of crop growth.

5.6 Rhizosphere microflora

The results obtained in the present investigation also indicated a significant increase in the microbial population in the rhizosphere of sunflower due to inoculation and P-fertilizer application (Tables 13, 14 and 15). This may also be attributed to increased phosphorus availability to the soil biota due to inoculation with P-solubilizers and/or P-fertilizer application. Significant increase in the population of bacteria, fungi, actinomycetes and P-solubilizers due to inoculation with P-solubilizing biofertilizer and P-application has been recorded in sunflower (Jones and Sreenivasa, 1993) and other crops (Prathibha, 1993; Jisha, 1993). Among the various stages of plant growth examined, population of P-solubilizers and free-living N_2-fixers were maximum at 60 DAS and declined at harvest. It is likely that the amount of root exudates were higher at 60 DAS and decreased

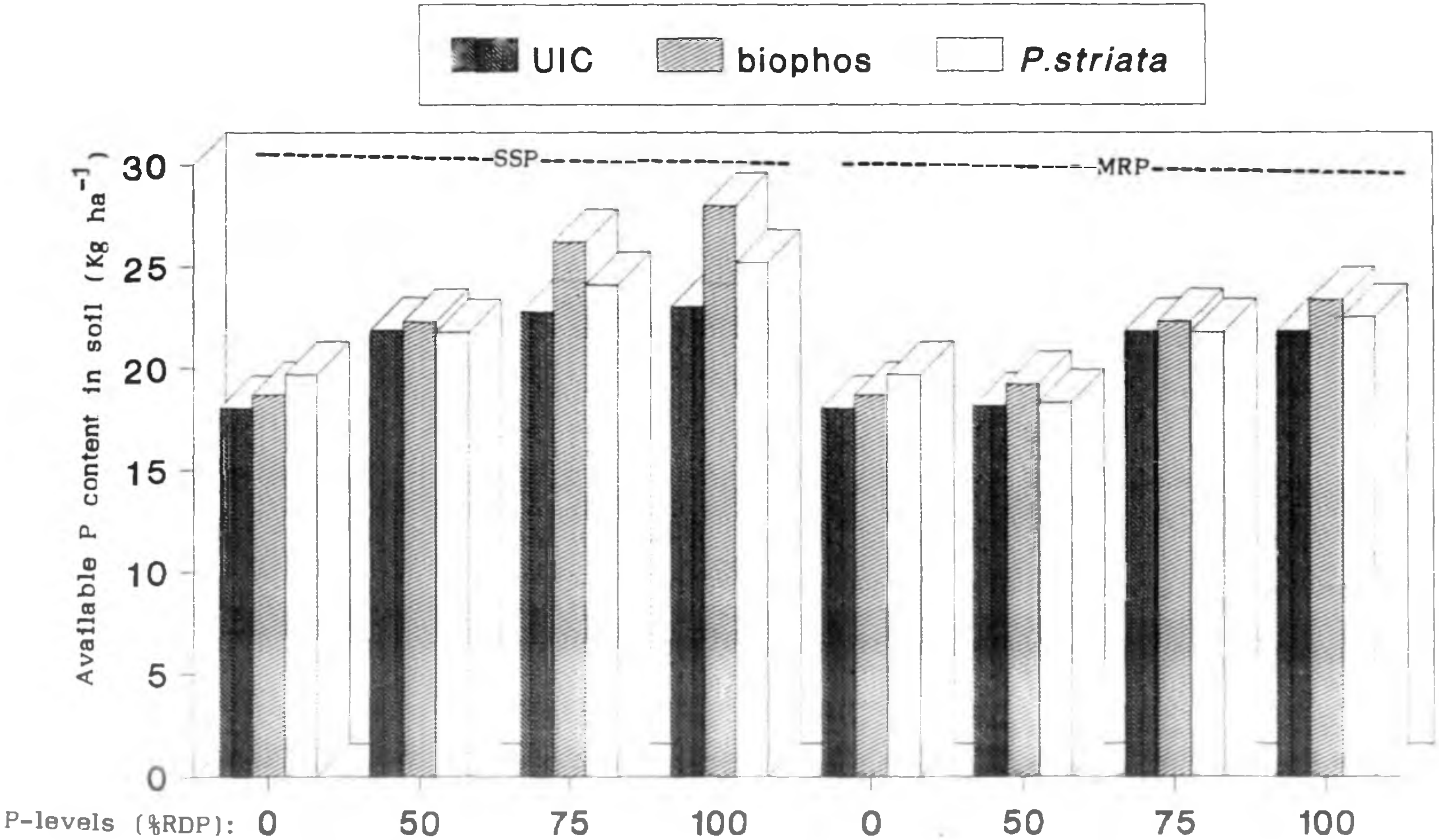


Fig. 7 Available phosphorus content in soil (Kg ha^{-1}) at 60 DAS by phosphate solubilizing biofertilizers in combination with forms and levels of phosphorus

(SSP - Single super phosphate MRP - Mussoorie rock phosphate UIC - Uninoculated control)

thereafter causing a decline in microbial population at harvest. When the plant enters into reproductive phase, most of the energy and photosynthates are directed towards the development of reproductive parts and release less of root exudates. The observed variations in the rhizosphere population of P-solubilizers and N_2-fixers due to plant age agree with the earlier findings of Rovira and Davey (1974) and Neelkanthan and Rangaswami (1965). Rovira (1965) and Peterson (1958) have also opined that the host plant and its growth stages are important in determining the rhizosphere microflora.

From the investigation, it can be concluded that `biophos' and *P. striata* as P-solubilizing biofertilizers can play an important role in the P-nutrition of sunflower. The two biofertilizers performed better in the presence of added P-fertilizers. Inoculation in combination with application of 75 per cent RDP in the form of super phosphate can give same or higher yield than that of the standard practice of applying 100 per cent RDP in the form of super phosphate, or inoculation in combination with 100 per cent RDP in the form of rock phosphate can give same yield as that of 100 per cent RDP in the form of super phosphate indicating the possibility of reducing the input cost on P-fertilizer.

SUMMARY

VI. SUMMARY

The production of chemical fertilizer is based on non-renewable and constantly depleting petroleum based feed stocks. The fast depletion of fossil fuel resources, increasing costs of chemical fertilizers and the environmental pollution caused by them, have called for more attention to use of biofertilizers in combination with chemical fertilizers. Taking into consideration the above factors, a field experiment was conducted at the Main Research Station, UAS, Dharwad on vertisols under rainfed condition during *kharif* season of 1995, to study the performance of P-solubilizing biofertilizers in combination with different forms and levels of P on growth, yield, nutrient uptake of sunflower and available P content in soil as well as rhizosphere microbial population.

The results revealed that `biophos' or *P. striata* inoculation increased the plant height, number of leaves per plant and dry matter yield in sunflower significantly over uninoculated control. Application of phosphorus in addition to inoculation with P-solubilizing biofertilizers further enhanced all the above plant growth parameters significantly over no phosphorus control. Among the forms of phosphorus, super phosphate application had greater impact on plant growth parameters than rock phosphate. Application of phosphorus at 100 per cent recommended dose had maximum influence on all the plant growth parameters compared to other levels of phosphorus. Among the various treatment combinations, `biophos' inoculation in combination with application of 100 per cent

RDP in the form of super phosphate followed by a similar combination of *P. striata* showed the best results.

The inoculation of P-solubilizing biofertilizers also resulted in an early flowering of the sunflower crop. `Biophos', among the two biofertilizers recorded significantly less number of days for 50 per cent flowering than *P. striata*. Super phosphate among the P-forms, and 100 per cent RDP among P-levels significantly reduced the number of days to 50 per cent flowering. Phosphorus applied at 100 per cent RDP in the form of super phosphate in combination with `biophos' inoculation recorded the least number of days to 50 per cent flowering although the results were statistically non-significant.

The yield parameters viz., size of the head, weight of the head, number of filled seeds per head, seed yield per plant and thousand seed weight were increased significantly due to `biophos' or *P. striata* inoculation compared to uninoculated control. All the yield parameters in inoculated treatments were further augmented due to application of phosphorus. Super phosphate was found to be significantly superior over rock phosphate in increasing all the yield parameters. Irrespective of the form in which P was applied, increasing level of P application significantly influenced the yield components. Among the various treatment combinations, `biophos' plus super phosphate applied at 100 per cent RDP followed by a similar combination with *P. striata* performed best in increasing various yield parameters.

The results on seed yield per hectare indicated that `biophos' as well as *P. striata* enhanced the seed yield significantly over uninoculated control. Super phosphate among the P-forms and 100 per cent RDP among P-levels showed maximum seed yield and the interaction between P-form and P-levels had significant influence on seed yield. Although the interaction between inoculation, P-forms and P-levels were non-significant, `biophos' or *P. striata* in combination with super phosphate application at 100 per cent RDP recorded the highest yield. Application of super phosphate at 75 per cent RDP in combination with `biophos' or *P. striata* recorded higher yield than application of super phosphate at 100 per cent RDP. Similarly, rock phosphate application at 100 per cent RDP along with `biophos' or *P. striata* inoculation gave same yield as that of super phosphate applied at 100 per cent RDP. An almost same trend was also noticed with stalk yield.

The N and P uptake by sunflower crop was enhanced due to `biophos' or *P. striata* inoculation which was further augmented significantly in the presence of added phosphatic fertilizers. Once again the combination of `biophos' plus super phosphate applied at 100 per cent RDP followed by *P. striata* with similar combination showed highest N and P-uptake at all the stages of plant growth as well as in seeds.

A significant increase in available phosphorus content in soil was noticed due to inoculation of `biophos' or *P. striata* at all the stages of plant growth. Inoculation with `biophos' or *P. striata* in combination with P-fertilizer application further increased the available phosphorus content in soil. The available P content of soil in the initial stages of crop growth was higher wherever P was applied in the form of super phosphate but in case of rock phosphate, the available P content in soil was low initially but increased at later stages of crop growth in inoculation treatments.

The microbial population in sunflower rhizosphere soil was enhanced due to inoculation of `biophos' or *P. striata*. The highest population of bacteria, fungi, actinomycetes and free-living $N_2$-fixers was seen in the treatment receiving `biophos' inoculation than *P. striata*. However, inoculation of *P. striata* showed higher counts of P-solubilizers than `biophos'. The rhizosphere population increased with increase in the levels of P in both the forms, but super phosphate stimulated the rhizoshpere population to a greater extent than rock phosphate. P-solubilizers and free-living N_2-fixers were increased upto 60 days after sowing and later a decline in their population was noticed.

From the studies it was clear that the inoculated organisms established well in the rhizosphere and enhanced the available P-content in soil (and also other nutrients probably) thereby increasing N and P uptake by plants. Increased nutrient uptake enhanced the growth and yield of sunflower crop.

REFERENCES

VI. REFERENCES

AHMAD, N. AND JHA, K.K., 1982, Effect of phosphate solubilizers on the dry matter yield and phosphorus uptake by soybean. *J. Indian Soc. Soil Sci.*, **30**: 105-106.

ALAGAWADI, A.R. AND GAUR, A.C., 1988, Associative effect of *Rhizobium* and phosphate solubilizing bacteria on yield and nutrient uptake of chickpea. *Pl. Soil*, **105**: 241-243.

ALAGAWADI, A.R. AND GAUR, A.C., 1992, Inoculation of *Azospirillium brasilense* and phosphate solubilizing bacteria on yield of sorghum (*Sorghum bicolor* L. Moench) in dry land. *Trop. Agric.*, **69**: 347-350.

AL-GHAZALI, M.F., KHORSHED, M.S.H. AND AL-AZAWI, K., 1986, Some observations on phosphorus solubilization by aerobic microorganisms isolated from sediments of Al-Khair River, Baghdad, *J. Biol. Sci. Res.* 17: 157-172.

ALLEN, O.N., 1959, The isolation of *Azobacter* sp. In: *Experiments in Soil Microbiology*, Burgess Publishing Company, Minneapolis, Minnesota. pp. 46-47.

ANONYMOUS, 1995, Food and Agriculture Organisation of the United States, Quart. Bullet. of Stat. **8**(1&2): 50.

ANTHONIRAJ, S., GOPALSWAMY, G.AND ABDUL KAREEM, A., 1994, Effect of graded levels of P and phosphobacterial inoculation on rice yield. *Madras Agric. J.*, 81: 457-458.

ARORA, D. AND GAUR, A.C., 1979, Microbial solubilization of different inorganic phosphates. *Indian J. Exptl. Biol.* 17: 1258-1261.

ASEA, P.W.A., KUCEY, R.M.N. AND STEWART, J.W.D., 1988, Inorganic phosphate solubiliztion by two *Penicillium* species in solution culture and soil. *Soil Biol. Biochem.*, 20: 459-464.

AZCON, R. AND BAREA, J.M., 1975, Synthesis of auxins, Gibberellins and cytokinins by *Azotobacter vinelandii* and *A. beijerinckia* related to effects produced on tomato plants. *P. Soil.* **43**: 609-613.

BABANNAVAR, P.B., 1990, Resonse of sunflower (*Helianthus annuus* L.) to varying levels of nitrogen and phosphorus under saline water irrigation. *M.Sc. Thesis*, Univ. Agril. Sci., Dharwad.

BAJAPAI, P.D. AND SUNDARA RAO, W.V.B., 1971, Phosphate solubilizing bacteria, Part-III. Soil inoculation with phosphorus solubilizing bacteria. *Soil Sci. Pl. Nutr.*, 17: 46-53.

BANIK, S. AND DATTA, M., 1988, Effect of inoculation of a phosphate solubilizing phytohormone producing *Bacillus firmus* on the growth and yield of soybean (*Glycine max*) grown in acid soils of Nagaland. *Zbl. Mikrobiol.*, **143**: 139-147.

BANIK, S. AND DEY, B.K., 1981, Phosphate solubilizing microogranisms of a lateritic soil. III. Effect of inoculation of some tricalcium phosphate solubilizing microorganisms on available phosphorus content of rhizosphere soils of rice (*Oryza sativa* L.Cv. IR-20) plants and their uptake of phosphorus. *Zbl. Bakteriol.*, **136**: 493-501.

✓ BANIK, S. AND DEY, B.K., 1982, Available phosphate content of an alluvial soil as influenced by inoculation of some isolated phosphate solubilizing microorganisms. *Pl. Soil*, **69**: 353-356.

BANIK, S. AND DEY, B.K., 1983, Phosphate solubilizing potentiality of the microorganisms capable of utilizing aluminium phosphate as a sole phosphate source. *Zbl. Mikrobiol.*, **138**: 17-23.

BARDIYA, M.C., 1970, Studies on microbial solubilization of rock phosphate. *Ph.D. Thesis.*, P.G. School, I.A.R.I. New Delhi.

BARDIYA, M.C. AND GAUR, A.C., 1972, Rock phosphate dissolution by bacteria. *Indian J. Microbiol.*, **12**: 269-271.

BARDIYA, M.C. AND GAUR, A.C., 1974, Isolation and screening of microorganisms dissolving low grade rock phosphate. *Folia Mikrobiol.* **19**: 388-389.

BAREA, J.M., NAVARRO, E. AND MONTOYA, E., 1976, Production of plant growth regulators by rhizosphere phosphate solubilizing bacteria. *J. Appl. Bacteriol.* 40: 129-134.

BLACK, C.A., 1968, *Soil Plant Relations - Phosphorus Functions in Plants.* John Willey and Sons Publ., P. 626.

BROWN, M.E., 1972, Plant growth substances produced by microorganisms of soil rhizosphere. *J Appl. Bacteriol.*, 35: 443-451.

BROWN, M.E., 1974, Seed and root bacterization, *Ann. Rev. of Phytopathol.*, 12: 311-331.

BROWN, M.E., 1975, Rhizosphere microorganisms opportunists, bandits or benefactors. pp. 21-38. In: *Soil Microbiology*. Ed: Walker, N. Halsted Press, New York.

CHAKRABORTY, T., MAJUMDAR, S.K. AND BHATTACHARJEE, P.K., 1986, Rock phosphate as direct fertilizer for wheat. *Proceedings of National Seminar on Rock phosphate in Agril.*, TNAU. Coimbatore, p. 102.

DASTANE, N.G., 1967, *A Practical Manual for Water Use Research*, Navabharat Prakashan Publication, Poona (India) pp. 5-6.

DATTA, M., BANIK, S. AND GUPTA, R.K., 1982, Studies on the efficacy of a phytoharmone producing p-solubilizing *Bacillus firmus* in augmenting paddy yield in acid soils of Nagaland. *Pl. Soil*, 69: 365-373.

*EL-DIN, S.M.S.B. AND BABER, M.S.M., 1983, Effect of phosphate dissolving bacteria on P-uptake by barley plants grown in a salt affected calcareous soil. *Zeitschrift Fur Pflanzenernahrung und Bodenkunde*, **146**: 545-550.

FORSTER, I. AND FREIER, F., 1988, Contributions to the mobilization of phosphorus by soil microorganisms. 4th comm. investigation on the efficiency of P-mobilizing microorganisms *in vitro* and in the rhizosphere of sunflower and winter wheat. *Zbl. Mikrobiol.*, **143**: 125-138.

GAUR, A.C., 1985, Phosphate solubilizing microorganisms and their role in plant growth and crop yield. *Proc. Soil. Biol. Symp.*, Hisar, India, Feb. 1985, pp. 125-138.

GAUR, A.C., 1990, *Phosphate solubilizing microorganisms as biofertilizers. Omega scientific publishers,* New Delhi. pp. 73-101.

GAUR, A.C. AND ALAGAWADI, A.R., 1987, Interaction of nitrogen fixing and phosphate solubilizing microorganisms on crop productivity. Focal theme (Botany), Indian Sci. Congr. Associ. Symp. pp. 35-46.

GAUR, A.C. AND GAIND, S., 1983, Microbial solubilization of phosphates with particular reference to Iron and Aluminium phosphate. *Sci. Culture.* **49**: 110-112.

GAUR, A.C. AND OSTWAL, K.P., 1972, Influence of phosphate dissolving Bacilli on yield and phosphate uptake of wheat crop. *Indian J. Exp. Biol.*, **10**: 393-394.

GAUR, A.C., OSTWAL, K.P. AND MATHUR, R.S., 1980, Save superphosphate by using phosphate solubilizing cultures. *Kheti*. 32: 23-25.

GAUR, A.C. AND SINGH, R., 1982, Integrated Nutrient System. *Fert. News*, **27**: 87-98.

GERRETSEN, F.C., 1948, The influence of microorganisms on the phosphate uptake by the plant. *Pl. Soil,* **1**: 51-81.

GOSWAMI, K.P. AND SEN, A., 1962, Solubilization of calcium phosphate by three strains of phosphobacteria. *Indian J. Agric. Sci.*, **32**: 96-101.

HALDER, A.K., MISHRA, A.K., BATTACHARYYA, P. AND CHAKRABARTY, P.K., 1990, Solubilizing of rock phosphate by *Rhizobium* and *Bradyrhizobium*. *J. Gen. Appl. Microbiol.* **36**: 81-92.

HALDER, A.K., MISHRA, A.K. AND CHAKRABARTY, P.K., 1991, Solubilizing of inorganic phosphate by *Bradyrhizobium*. *Indian J. Exp. Biol.*, **29**: 28-31.

HEBBARA, M. AND SUSEELA DEVI, L., 1990, Effect of phosphorus solubilizing bacteria (PSB) on phosphorus availability to groundnut from rock phosphate. *Curr. Res.* **19**(4): 56-57.

ILLMER, P. AND SCHINNER, F., 1992, Solubilization of inorganic phosphates by micro organisms isolated from forest soils. *Soil Biol. Biochem.*, 24: 389-395.

JACKSON, M.L., 1967, *Soil Chemical Analysis*, Prentice Hall of India Private Limited, New Delhi, p. 498.

JACKSON, M.L., 1973, *Soil Chemical Analysis*, Prentice Hall of India Pvt. Ltd. New Delhi. pp. 111-203.

JISHA, M.S., 1993, Phosphorus nutrition of sorghum as influenced by phosphate solubilizers in the presence of cellulolytic fungus in soil amended with cotton stalk. M.Sc. (Agri.) Thesis, Univ. Agril. Sci. Dharwad.

JISHA, M.S. AND ALAGAWADI, A.R., 1996, Nutrient uptake and yield of sorghum (*Sorghum bicolor* L. Moench) inoculated with phosphate solubilizing bacteria and cellulolytic fungus in a cotton stalk amended vertisol. *Microbiol. Res.*, **151**: 213-217.

JONES, N.P., 1992, Studies on interactions between VAM fungus and phosphate solubilizing bacteria and their effects on rhizosphere microflora, growth and yield of sunflower. *M.Sc. Thesis.* Univ. Agril. Sci., Dharwad.

JONES, N.P. AND SREENIVASA, M.N., 1993, Effect of inoculation of VA mycorrhiza and/or phosphate solubilizing bacteria on rhizosphere microflora of sunflower II. *Azotobacter* and phosphate solubilizing bacteria. *J. Ecotoxicol. Environ Monit.* 3: 55-58.

KAVIMANDAN, S.K. AND GAUR, A.C., 1971, Effect of seed inoculation with *Pseudomonas* sp. on phosphate uptake and yield of maize. *Curr. Sci.* **40**: 439-440.

KUCEY, R.M.N., 1988, Effect of *Penicillium bilaji* on the solubility and uptake of P and micronutrients from soil by wheat. *Can. J. Soil Sci.*, **68**: 261-270.

KUNDU, B.S. AND GAUR, A.C., 1980, Effect of phosphobacterin on yield uptake of potato crop. *Curr. Sci.*, **49**: 159-160.

KUNDU, B.S. AND GAUR, A.C., 1984, Rice response to inoculation with N_2-fixers and P-solubilizing micro organisms. *Pl. Soil.* **79**: 227-234.

KUSTER, E. AND WILLIAMS, S.T., 1964, Selection of media for isolation of *Streptomyces*. *Nature*. **202**: 926-929.

MAHMOUD, S.A.Z., ABDEL-HAFEZ, A.M., ELSAAY, M. AND HANAFY, E.S., 1976, Efficiency of phosphate mobilizing bacteria on different phosphorus substrates in clay loam soil. *Egyptian J. Soil*, **16**: 9-20.

MANDAL, L.N. AND KHAN, S.K., 1972, Release of phosphorus from insoluble phosphatic materials in acidic low-land rice soils. *J. Indian Soc. Soil Sci.*, **20**: 19-25.

MANJAIAH, K.M., 1989, Investigations on influence of rock phosphate on ground nut in acid soil. M.Sc. (Agri.) Thesis, Univ. Agric. Sci. Dharwad.

MARTIN, J.P., 1950, Use of acid, rose bengal and streptomycin in plate method for estimating soil fungi. *Soil Sci.* **69**: 215-232.

MARWAHA, B.C., KANWAR, B.S. AND TRIPATHI, B.R., 1984, Effectiveness of Mussoorie rock phosphate in an ultisol as measured by changes in some soil properties under field codition. *J. Indian Soc. Soil Sci.,* 32: 381-383.

MAURYA, B.R. AND SANORIA, C.L., 1982, Effictiveness of rhizobial strains with and without co-inoculants and phosphate on bengal gram (*Cicer arietinum*). *J. Agric. Sci.,* **99**: 239-240.

MENKINA, R.A., 1950, Bacteria which mineralizes organic phosphorus compounds. *Mikrobiologiya*, **19**: 308-316.

MOHOD, S.P., GUPTA, D.N. AND CHAVAN, A.S., 1989, Enhancement of phosphate availability and phosphorus uptake in rice by phosphate solubilizing culture. *J. Maharashtra Agric. Univ.,* **14**: 178-181.

MOHOD, S.P., GUPTA, D.N. AND CHAVAN, A.S., 1991, Effects of P solubilizing organisms on yield and N-uptake by rice. *J. Maharashtra Agric. Univ.*, **16**: 229-231.

MOREAU, R., 1959, The biological liberation of soluble phosphates from insoluble phosphorus compound in soil. Compt. *Rend. Akad. Sci.* Paris. **249**: 1804-1806.

MUDALAGIRIYAPPA, 1993, Response of groundnut to sources of phosphate and phosphate solubilizers in vertisols. M.Sc. (Agri.) Thesis. Univ. Agril. Sci. Dharwad.

NARSIAN, V., THAKKAR, J. AND PATEL, H.H., 1995, Mineral phosphate solubilization by *Aspergillus aculeatus*. *Indian J. Exp. Biol.*, **33**: 91-93.

NEELAKANTHAN, S. AND RANGASWAMI, G., 1965, Bacterization of rice and okra seeds with *Azatobacter chroococum* and establishment of the bacterium in the rhizosphere. *Curr. Sci.*, **34**: 157.

NELSON, W.L. AND TISDALE, S.L., 1968, Elements requird in plant nutrition. In: Soil fertility and fertilizer. Ed. The MacMillion, Company, New York, U.S.A. pp. 71-72.

NORRIS, J.R., 1959, The isolation and identification of Azotobacters. *Lab. Pract.* **8**: 239-243.

ORIUNO, A., HERNANSAEZ, A., NOGUERA, J. AND MORALES, V., 1978, Solubilization power of phosphorus by *Asperigellus niger* and *Pseudomonas flouroscens*. *Microbiol.* **30**: 113-120.

OSTWAL, K.P. AND BHIDE, V.P., 1972, Solubilization of tricalcium phosphate by soil *Pseudomonas*. *Indian J. Exp. Biol.* **10**; 153-154.

PACOVSKY, R.S., PAUL, E.A. AND BETHLENFALVAY, G.J., 1985, Influence of soil on the interactions between endomycorrhizae and *Azospirillum* in sorghum. *Soil Biol. Biochem.*, 17: 524-525.

PANSE, V.G. AND SUKHATME, P.V., 1967, *Statistical Methods for Agricultural Workers*, ICAR., New Delhi.

PATIL, S.C., 1990, Studies on the influence of P-solubilizers and sources of phosphate on soybean (*Glycine max* (L) Merril). *M.Sc. (Agri) Thesis*, Univ. Agril. Sci., Dharwad.

PETERSON, E.A., 1958, Observations on fungi associated with plant roots. *Can. J. Microbiol.*, **4**: 257-265.

PIERRE, W.H., 1938, Soil and Man. Year Book of Agriculture, pp. 337-396.

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

PIPER, C.S., 1966, *Soil and Plant Analysis*, Academic Press, New York, pp. 236.

PRABHAKAR, M. AND SARAF, C.S., 1990, Dry matter accumulation and distribution in chickpea as influenced by genotype, P source and irrigation level. *Indian J. Agric. Sci.* **60**: 204-206.

PRATHIBHA, C.K. 1993, Interactions of *Azosphirillum* sp. and *Pseudomans striata* or *Glomus fasciculatum* on growth, yield and nutrient uptake of hybrid cotton. M.Sc. (Agri.) Thesis, Univ. Agril. Sci., Dharwad.

RACHEWAD, S.N., RAUT, R.S., MALEWAR, G.U. AND GANURE, C.K., 1992, Effects of phosphate solubilizing biofertilizer on biomass production and uptake of phosphorus by sunflower. *J. Maharastra Agric. Univ.*, 17: 480-481.

RACHEWAD, S.N. RAUT, R.S., MALEWAR, G.U. AND HANSABADE, A.R., 1991, Effect of phosphate solubilizing biofertiilzer on phosphorus utilization by maize. *Ann. Plant Physiol.* 5; 177-180.

RAVISHANKAR, S.P., 1993, Effect of sources of phosphorus and phosphate solubilizers on irrigted sunflower and its residual effect on succeeding green gram (*Vigna munga*). M.Sc. (Agri.) Thesis, Univ. Agril. Sci. Dharwad.

ROVIRA, A.D., 1965, Interaction between plant roots and soil microorganisms. *Ann. Rev. Microbial.*, **19**: 241-266.

ROVIRA, A.D. AND DAVEY, C.B., 1974, Biology of the rhizosphere. In: *The Plant Roots and Its Environment* Ed. Carso, E.W., University Press. Virginia, U.S.A. pp. 153-204.

SACKETT, W.G., PATTERN, A.J. AND BROWN, C.W., 1908, The solvent action of soil bacteria upon insoluble phosphates of raw bone meal and natural rock phosphate. *Zbl. Bakteriol.*, **28**: 668.

SALIH, H.M., YAHYA, A.I., ABDUAL RAHEM, A.M. ND MUNAM, B.H., 1989, Availability of phosphorus in a calcareous soil treated with rock phosphate or super phosphate as affected by phospate dissolving fungi. *Pl. Soil*, **120**: 181-185.

SATTAR, M.A. AND GAUR, A.C., 1987, Production of auxins and gibberellins by phosphate dissolving micro-organisms. *Zbl. Mikrobiol.*, **142**: 393-395.

SEN, A. AND PAUL, N.B., 1957, Occurrence of phosphobacteria in the glands of *Cassia occidentalis*. *J. Soil Sci.*, **7**: 106-111.

SHARMA, J.P. AND SINGH, M., 1971, Response of rice to phosphate and nitrogenous fertilizers with and without phosphobacteria culture. *Indian J. Agron.*, **16**: 15-18.

SHARMA, S.N., RAY, S.B., PANDEY, S.L. AND PRASAD, R., 1983, Effect of irrigation, pyrites and phosphobacteria on the efficiency of rock phosphate applied to lentils. *J. Agric. Sci.*, **101**: 467-472.

SHINDE, B.N. AND PATIL, A.J., 1985, Use of phosphate solubilizing cultures and rock phosphate on phosphate availability to wheat in black calcareous soil. *Curr. Res. Rep.*, 1: 105-106.

SINGH, H.P., PAREEK, R.P. AND SINGH, T.A., 1984, Solubilizing of rock phosphate by phosphate solubilizers in broth. *Curr. Sci.*, 53: 1212-1213.

SPERBER, J.I., 1958, The incidence of apatite solubilizing organisms in the rhizosphere and soil. *Aust. J. Agric. Res.*, 9: 778-781.

STALSTROM, V.A., 1903, Soil microorganisms in relation to availability of soil phosphorus to plants. *Zbl. Bakteriol.*, 11: 724.

*STEFAN, V. AND BOTI, P., 1960, The influence of bacterial fertilizer. *Cent. Expt. Ingras. Bact. Lucrari.* Stint., 2: 197-205.

SUBBAIAH, B.V. AND ASIJA, G.L., 1959, A rapid procedure for the estimation of available nitrogen in soils. *Curr. Sci.*, 25: 259-260.

TIWARI, V.N., LEHRI, L.K.AND PATHAK, A.N., 1989, Effect of inoculating crops with phosphomicrobes. *Exptl. Agric.*, 25: 47-50.

TOMAR, S.S., PATHAN, .A., GUPTA, K.P. AND KHANDKAR, U.R., 1993, Effect of phosphate solubilizing bacteria at different levels of phosphate on blackgram (*Phaseolus mungo*). *Indian J. Agron.*, 38: 131-133.

---

* - Originals are not seen

APPENDICES

Appendix 1. Monthly meteorological data for the year 1995 and average of 44 years (1950-1994) recorded at the meteorological observatory of the agricultural

Months	Rainfall		Temperature				Mean RH%	
			Mean max.		Mean min			
	1995	1950-94	1995	1950-94	1995	1950-94	1995	1950-94
January	5.20	0.00	27.70	29.17	13.70	14.64	83.00	61.85
February	0.00	0.00	32.60	34.92	16.70	15.63	73.00	55.08
March	0.00	7.0	35.1	35.79	19.10	18.73	68.0	55.97
April	20.60	50.0	36.8	36.16	21.30	21.43	66.0	60.20
May	56.60	90.0	33.8	36.84	21.30	21.47	68.0	67.24
June	143.40	112.0	31.4	29.94	22.10	21.20	75.0	82.13
July	184.40	158.0	27.1	27.06	21.00	20.97	87.0	88.43
August	50.50	102.0	28.1	27.14	20.70	20.65	86.0	86.64
September	121.60	105.0	28.3	28.75	20.60	20.17	82.0	83.31
October	127.50	237.0	29.3	30.24	20.20	19.19	80.0	75.88
November	81.00	34.0	29.1	29.38	15.20	16.26	81.0	67.63
December	0.00	6.0	29.4	29.19	13.50	13.31	77.0	63.70
Mean	790.00	801.00						

Appendix II. Composition of media used

Soil extract agar (Allen, 1959)

Glucose	1.00 g
K_2HPO_4	0.50 g
Soil extract	100 ml
Agar	15.00 g
Distilled water	900 ml
pH	7.0

Martin's rose bengal agar (Martin, 1950)

Dextrose	10.00 g
Peptone	5.00 g
K_2HPO_4	1.00 g
$MgSO_4 . 7H_2O$	0.50 g
Rose bengal	0.03 per cent
Agar	15.00 g
Distilled water	1000 ml
pH	6.0

Note : Just before plating, the medium was mixed with 3ml of 1% streptomycin sulphate solution per litre of media

Kuster's agar (Kuster and Williams, 1964)

Strach	10.00 g
Casein	0.30 g
KNO_3	2.00 g
NaCl	2.00 g
K_2HPO_4	2.00 g
$MgSO_4 . 7H_2O$	0.05 g
$CaCO_3$	0.02 g
Ferrous sulphate	0.01 g
Agar	15.00 g
Distilled water	1000 ml
pH	7.2

Pikovskaya's agar (Pikovskaya, 1948)

Glucose	10.00 g
Tricalcium phosphate	5.00 g
Yeast extract	1.00 g
$MgSO_4 \cdot 7H_2O$	0.25 g
$(NH_4)_2 SO_4$	0.25 g
NaCl	0.10 g
KCl	0.20 g
$MnSO_4$	traces
Ferrous sulphate	traces
Distilled water	1000 ml
Agar	15.00 g
pH	7.0

Norris N-free agar (Norris, 1959)

Glucose	10.00 g
K_2HPO_4	1.00 g
$MgSO_4 \cdot 7H_2O$	0.20 g
$CaCO_3$	1.00 g
NaCl	0.20 g
Ferrous sulphate	0.10 g
Sodium molybdate	0.005 g
Distilled water	1000 ml
Agar	15.00 g
pH	7.1