STUDIES ON BACTERIA SOLUBILIZING BOTH POTASSIUM AND PHOSPHORUS AND THEIR EFFECT ON MAIZE (Zea mays L.)

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INTRODUCTION

Plant nutrition is the study of the chemical elements that are necessary for plant growth. There are several principle that apply to plant nutrition some of elements is directly involved in plant metabolism. Plant requires specific elements for growth and reproduction.

Soil is a dynamic natural body on the earth crust. There are several mineral available in soil, but most important mineral elements are nitrogen (N), phosphorus (P) and potassium (K). Potassium is the third most important plant nutrient. According to Ghosh and Hasan (1980), soil test result for Potassium fertility status among India's agricultural soil categorized accordingly, 21 per cent low, 51 per cent medium, 28 per cent high. Thus in India, 72 per cent cultivated area representing 266 districts were deficient in potassium fertilization.

Microorganisms play a key role in the P and K cycle. There are considerable population of P or K-solubilizing bacteria in soil and plant rhizosphere (Sperberg, 1957). Different bacterial species such as *Pseudomonas, Agrobacterium, Bacillus, Rhizobium* and *Flavobacterium,* had been tested for their ability to solubilize inorganic phosphate compounds such as tri-calcium phosphate, hydroxylapatite and rock phosphate (Goldstein, 1986). Silicate bacteria were found to resolve potassium, silicon and aluminium from insoluble mineral P-solubilizing bacteria and exert beneficial effect upon plant growth (Aleksandrov *et al*., 1967).

Use of plant growth promoting rhizobacteria (PGPR) including phosphate and potassium solubilizing bacteria (PSB and KSB) as biofertilizer was suggested to sustainable solution to improve plant nutrition and production (Vessey, 2003).

Phosphorus and potassium are the major essential macronutrients for biological growth and development of crop. However, the concentrations of soluble P and K in soil are usually very low and the large proportion of P and K in soil are insoluble rocks, minerals and other deposits (Goldstein, 1994). In spite of that, these sources constitute the biggest reservoirs of P and K in soil because, under appropriate conditions, they can be solubilize and become available for plants.

A wide range of bacteria namely *Pseudomonas, Burkholderia, Acidothiobacillus ferrooxidans, Bacillus mucilaginosus, B. edaphicus, B. circulans* and *Paenibacillus* sp. had been reported to release potassium in accessible form potassium-bearing minerals in soils (Sheng, 2006 and Lin *et al*., 2002). These potassium solubilizing bacteria (KSB) were found to dissolve potassium, silicon and aluminum from insoluble K-bearing minerals such as micas, illite and orthoclases, by excreting organic acids which either directly dissolved rock K or chelated silicon ions to bring K into the solution (Aleksandrov *et al*., 1967; Ullaman *et al*., 1996 and Bennett *et al*., 1998).

Inoculation with potassium solubilizing bacteria had been reported to exert beneficial effects on growth of cotton and rape (Sheng, 2006), pepper and cucumber (Han *et al*., 2006), sorghum (Badar *et al.,* 2006), wheat (Sheng and He*,* 2006) and Sudan grass (Basak and Biswas*,* 2009 and Basak and Biswas, 2010). Similarly, inoculation of maize and wheat plants with *Bacillus mucilaginosus, Azotobacter chroococcum* and *Rhizobium* resulted in significant higher mobilization of potassium from waste mica, which in turn acted as a source of potassium for plant growth (Singh *et al*., 2010).

Potassium is an essential macronutrient that plays an important role in the growth and development of plants. In the soil, K exists mainly in four different pools, that is mineral, non-exchangeable, exchangeable, and soluble K. The concentration of soluble K in soils is usually very low (1 to 2% of total). However, the major portion of K is in the rocks and the minerals (98%) in insoluble forms (Goldstein, 1994). For optimum crop production, soil solution and exchangeable K need to be replenished continually with K releasing by non-exchangeable K through weathering of K resources such as micas and feldspars (Sparks and Huang, 1985) or by addition of K fertilizers. Fortunately, certain soil microorganisms are able to solubilize unaviable form of K-bearing mineals, such as micas, illite and orthoclases. This solubilization could be attributed to excreting organic acids which either directly dissolves rock K or chelate silicon ions to bring K into solution (Groudev, 1987; Friedrich *et al*., 1991; Ullman, *et al.,* 1996 and Bennet *et al.,* 1998). Therefore, the application of K solubilizing bacteria is a promising approach for increasing K availability in soils cultivation of more K demand crops (Zahar *et al*., 1984; Vandevivaea *et al*., 1994 and Barker *et al*., 1998). Several microorganisms such as *Aspergillus niger*, *Bacillus extroquens* and *Clostridium pasteurianum* were found to be able to grow on muscovite, biotite, orthoclase, microcline and micas under *in vitro* conditions (Reitmeir, 1951).

Phosphate solubilizing microorganisms (10% of total soil microorganisms), include a large number of soil micro-flora can solubilize inorganic phosphate (including soil phosphate) with the production of inorganic (carbonic and sulfuric) and organic (citric, butyric, oxalic, malonic, lactic etc.) acids and phosphatase enzyme (Whitelaw *et al.*, 1997 and Sundara *et al*., 2001). The activities of such microorganisms are affected by different soil parameters including soil fertility, temperature, moisture, organic matter and soil physical properties (Kim *et al*., 1998).

The mechanism of mineral phosphate solubilization by PSB strains is associated with the release of low molecular weight organic acids (Goldstein, 1995 and Kim *et al*., 1997), which through their hydroxyl and carboxyl groups chelate the cations bound to phosphate, there by converting it into soluble forms (Kpomblekou and Tabatabai, 1994). However, P-solubilization is a complex phenomenon, which depends on many factors such as nutritional, physiological and growth conditions of the culture (Reyes *et al*., 1999). There is experimental evidence to support the role of organic acids in mineral phosphate solubilization (Halder *et al*., 1990).

Maize is (*Zea mays* L.) is the third most important cereals next to rice and wheat in the world as well in India, contributing about 20 per cent share of worlds total cereal production. Maize is being consumed both as food and fodder crop and also required by various industries in India. At present, about 35 per cent of the maize produce is used for human consumption, 25 per cent each in poultry feed and cattle feed and 15 per cent in food processing like corn flackes, pop corn etc.

Maize is known as “Queen of cereal” because of its high production potential and wider adoptability. In world, maize occupies an area of 163.9 million ha with the production of 832 million tones and productivity of 5080 kg per ha. In India, maize is grown over an area of 8.55 million ha with the production of 21.73 million tones and a productivity is 2540 kg per ha. In Karnataka, it cultivated in an area of 13.6 lakh ha with the production of 40.9 lakh tones and productivity of 3018 kg per ha (Anon, 2012). Maize is an exhaustive crop and utilize more nutrients from the soil for growth and development. Among all the cereal, it require high nitrogen (150 kg/ha), phosphorus (75 kg/ha) and potash (37.5 kg/ha) of recommended dose. Solubilization of insoluble minerals by bacteria helps to uptake and utilization of nutrient from the soil.

Hence, the present investigation was undertaken to study the bacteria solubilizing both potassium and phosphorus and their effect on Maize (*Zea mays* L.) with the following objectives.

1. Isolation and characterization of bacteria solubilizing both potassium and phosphorus from rhizosphere soils of different crops from different locations.
2. Screening and selection of efficient isolates of bacteria solubilizing both potassium and phosphorus.
3. To evaluate efficient bacterial isolates solubilizing both potassium as well as phosphorus on growth and yield of maize under green house condition.

REVIEW OF LITERATURE

The use of plant growth promoting rhizobacteria (PGPR), including phosphate and potassium solubilizing bacteria (PSB and KSB) as bio fertilizers was suggested as a sustainable solution to improve plant nutrient and production. Phosphate and potassium are major essential macronutrients for plant growth and development and soluble P and K fertilizers are commonly applied to replace removed minerals and to optimize yield. When phosphate is added into soils as a fertilizer in relatively soluble and plant available forms, it is easily converted into insoluble complexes with calcium carbonate, aluminium and iron oxides and crystalline and amorphous aluminum silicate. Consequently, to achieve optimum crop yields, soluble phosphate fertilizers have to be applied at high rates which cause environmental and economic problems. On the other hand, K deficiencies become problem because K decreases easily in soils due to crop uptake, runoff, leaching and soil erosion. Direct application of rock phosphate and potassium minerals may be agronomically more useful and environmentally more feasible than soluble P and K. Rock phosphate and potassium minerals are cheaper sources of P and K. However, most of them are not readily available to a plant because the minerals are release nutrients slowly and their use as fertilizer often causes insignificant increase in crop yield.

2.1 Potassium in soil

The total K is rather poorly correlated with available K and is rarely used to describe K fertility status of a soil. The immediate source of K for plants is small amount available in the soil solution. The concentration of potassium in the soil solution was range from 1 to 2 per cent and K was the non-exchangeable and soil mineral fraction will be drawn upon. Supply of K to the plants depends directly on the K concentration in soil solution and indirectly on soil minerals (Sparks and Huang, 1985).

The potassium content of Indian soils varies from less than 0.5 per cent to 3.00 per cent. The average total potassium content of these soils is 1.52 per cent (Mengel and Kirkby, 1987). Potassium content of Indian soils had traditionally been considered as adequate. In the recent years, the importance of K and the need for its continuous optimal availability for the better crop production is felt as long before symptoms of K deficiency became visible and severe losses in terms of yield and quality had been caused to crop and produce (Khawilkar and Ramteke, 1993).

In mineral soils, K occur in the form of silicate minerals *viz.*, muscovite, orthoclase, biotite, feldspar, illite, mica, vermiculite, smectite and so on. The total pool of soil K is extremely complex and this could be solubilized by bacteria through production of acids and it will be available for plant uptake (Ullaman *et al.*, 1996).

2.2 Phosphorus in soil

Total P in Indian soils range from 100 ppm to over 2000 ppm phosphorus (460 to 9200 kg/ha of plough layer). However, total P is rather poorly correlated with available P and is rarely used to describe P fertility status of a soil. The immediate source of P for plants is the small amount that is in the soil solution. The concentration of P in the soil solution is the order of 0.1 to 1 ppm. As this is removed, the equilibrium is disturbed and P in the labile fraction will be drawn upon. The supply of P to the plant depends directly on the concentration of P in soil solution and indirectly on soil (Larsen, 1967).

The total amount of phosphorus in the earth's crust is of the order of 10^{15} metric tons. It exists mainly as apatites, like flour-, chloro-, hydroxyl-, and carbonate apatites. There are 200 forms of phosphorus minerals occurring in nature. The rock phosphates, which are high in carbonate apatite are most commonly mined as fertilizer source (Paul and Clark, 1989).

Phosphorus in the soil is most immobile, inaccessible and unavailable of all nutrient elements. These characteristics cause wide spread deficiency of P for agricultural production (Holford, 1997). Low phosphorus availability of many tropical and subtropical soils in combination with insufficient P fertilizer application has been identified as one of the major factors responsible for low production on small farms (Kretzchmar *et al.*, 1991).

In acidic soils, phosphorus occurs in various forms of aluminium (Al) and iron (Fe) phosphates. In neutral and alkaline soils, it is more likely to occur as calcium (Ca) and magnesium (Mg) phosphate and adsorbed on surface of Ca and Mg carbonates respectively. The total 'pool' of soil P is extremely complex and no single component of it can be identified as 'plant available' P (Holford, 1997).

2.3 Mineral potassium and phosphate solubilization potential by single bacteria

Hu *et al*. (2006) reported that two phosphate- and potassium-solubilizing strains (KNP413 and KNP414) were isolated from the soil of Tianmu Mountain, Zhejiang Province (China) and they were phenotypically and phylogenetically characterized. Both isolates effectively dissolved mineral phosphate and potassium, while strain KNP414 showed higher dissolution capacity even than *Bacillus mucilaginosus* AS1.153, the inoculant of potassium fertilizer widely used in China. When grown on Aleksandrov medium, both strains were rod-shaped spore-formers with a large capsule and they formed slimy and translucent colonies. The DNA G+C contents were 57.7 mol per cent for strain KNP413 and 56.1 mol per cent for strain KNP414. Strain KNP413 shared a 16S rRNA gene sequence with similarity of more than 99.1 per cent with strain KNP414 and *Bacillus mucilaginosus* strains HSCC 1605 and YNUC0001, and a 94.6 per cent similarity with *Bacillus mucilaginosus* VKMB-1480D, the type strain of *Bacillus mucilaginosus*. Strains KNP413 and KNP414 together with other *Bacillus mucilaginosus* were clustered with *Paenibacillus* strains in a group. The use of a specific PCR primer PAEN515F designed for differentiating the genus *Paenibacillus* from other members of the *Bacillaceae* showed that strains KNP413 and KNP414 had the same amplified 16S rRNA gene fragment (0.9-kb) as members of the genus *Paenibacillus.*

2.4 Occurrence and distribution of potassium solubilization bacteria (KSB)

Microbial inoculants that are able to dissolve potassium from mineral and rocks, have influence on plant growth and have both economic and environmental advantage. The first evidence of microbial involvement in solubilization of rock potassium was shown by Muntz (1890).

Several microorganisms like *Aspergillus niger*, *Bacillus extroquens* and *Clostridium pasteurianum* were found to grow on muscovite, biotite, orthoclase microclase and mica under *in vitro* (Reitemeir, 1951).

The silicate solubilizing bacteria *B. mucilaginosus* sub sp. *siliceus* liberates potassium from feldspar and aluminosilicates (Norkina and Pumpyanskaya, 1956). The microorganism like bacteria, fungi and actinomycetes were colonized even on the surface of mountain rocks (Gromov, 1957). Duff and Webley (1959) reported silicate dissolving action of a Gram negative bacteria *Erwinia, B. herbicola* or with *Pseudomonas* strains.

Webley *et al.* (1960) demonstrated that the silicaceous materials in rocks can be attacked through the products of metabolism of microorganisms. Heinen (1960) studied and identify the ability of *Bacillus caldolyticus* and *Proteus mirabium* to grow and solubilize quarts. Aleksandrov *et al.* (1967) isolated different bacterial species like silicate bacteria which found to dissolve potassium, silicates and aluminium from insoluble minerals.

Liu (2001) isolated silicates bacteria *B. mucilaginosus* CS 1 and CS 2 from soil and exhibited inhibitory activity on the growth of Gram negative bacteria *Escherichia coli* and they identified strain CS 1 as *B. mucilaginosus.* Lin *et al.* (2002) reported that the *B. mucilaginosus* dissolved the silicates, colonizes and develop in rhizosphere as well as non-rhizosphere soil.

Raj (2004) identified silicate solubilizing bacteria from rice ecosystem (SSB) in a medium containing 0.25 per cent insoluble magnesium tri silicate and also reported that *Bacillus* sp. found to solubilize silicate minerals more efficiently under *in vitro* conditions.

Potassium solubilizing rhizobacteria were isolated from the roots of wheat crop by the use of specific potassium bearing minerals and their effect was studied by Mikhailouskaya and Tchernysh (2005). Badar (2006) reported that the bacteria capable of dissolving silicate minerals from field spar samples.

Sugumaran and Janarthanam (2007) isolated K solubilizing bacteria from soil, rocks and minerals samples *viz.*, orthoclase and muscovite mica. Among the isolates *B. mucilaginosus* solubilized more potassium by producing slime in muscovite mica.

2.5 Occurrence and distribution of phosphate solubilizing microorganisms in soil

Kucey *et al.* (1989) reported that the P solubilizing bacteria and fungal population in various cultivated and Virgin Alberta soil. Phosphate solubilizing microorganisms have been found in almost all soils that was tested, although their populations vary with different soils, climate and history. Salih *et al.* (1989) reported isolation of phosphate dissolving fungi from the rhizosphere of different plant *viz.*, egg plant and cucumber.

The microbial involvement in solubilization of inorganic P was first observed by Stalstrom (1903). Sackeet *et al.* (1908) gave conclusive evidence to show that soil bacteria dissolved rock phosphates, bone meal and di and tri calcium phosphate.

Sperberg (1957) reported that PSM in the rhizosphere of subterranean clover (*Trifolium subterraneum* L.), rye grass (*Loliumperenne*. L.) and wheat (*Triticum aestivum* L.) constituted 26 to 39 per cent of microbial population. Swaby and Sperber (1958) reported that the PSM are plenty in soil, but they were lacking in food materials to stimulate them to solubilize the unavailable P.

Smith *et al.* (1961) found that *Bacillus megaterium* var. *phosphaticum* readily decomposed glycerophosphate. Bardiya (1970) isolated several yeast, fungi and bacteria from rhizosphere of leguminous crops and soils in rock phosphate deposit area, which were found to solubilize low grade rock phosphate, *Pseudomonas striata* being the most efficient in solubilization. Raj (1980) enumerated the PSB in four different soil types and observed the population ranging from 0.11×10^5 to 5.86×10^5 colony forming units per gram or ml dry weight of soil.

Illmer and Schinner (1995) isolated *Penicillium* spp. and *Pseudomonas* spp. having high ability to solubilize inorganic P like hydroxyl apatite, calcium hydrogen phosphates dehydrate from forest soils. Rajan *et al*. (1996) studied the phosphate rock for direct application to soils.

2.6 Mineral potassium solubilizing potential of bacteria

Many of the indigenous soil microorganisms have the potential to absorb and mobilize the fixed form of nutrient from trace mineral sources. Silicate bacteria were found to dissolve potassium, silica and aluminium from insoluble minerals and they are known to liberate phosphoric acids that solubilize apatite and release available form of nutrients from apatite (Heinen, 1960).

Duff *et al.* (1963) isolated 2 keto-gluconicacid producing Gram-negative bacteria *Pseudomonas fluorescence* (Strain No. 2062) and reported that the isolated bacteria had the capacity of 17 per cent to dissolve the resistant natural phosphate and silicates in the soil.

Sheng and Huang (2002) reported that potassium release from minerals was affected by pH, dissolved oxygen and strains used. The content of potassium in solution inoculated with bacteria was increased by 84.8 to 127.9 per cent compared with the control. The extent of potassium solubilization by *B. edaphicus* in the liquid media and reported better growth on illite than feldspar (Sheng and He, 2006).

Badar (2006) studied the extent of potassium and phosphorus solubilization by silicate solubilizing, bacteria it ranged from 4.90 mg/l at pH 6.5 to 8.0 and the potassium solubilization by *B. mucilaginosus* isolated from soil, rock and mineral samples recorded 4.29 mg/l release of potassium in media supplemented with muscovite mica (Sugumaran and Janarthanam, 2007).

The potassium releasing characteristics of a bacteria from different minerals using soil column experiment. Potassium release affected by pH aerobic conditions and soil mineral properties. More K was produced in a more aerobic condition than less aerobic condition. The release of potassium was in the order of illite, feldspar and muscovite (Sheng, 2006 and Badar, 2006).

2.7 Mechanisms of potassium solubilization

Argelis *et al.* (1993) reported on weathering mechanism carried out by *Penicillium frequentans* and *Clasdosporium, Cladosporoides* on unaltered sand stone, granite and lime stone. They also reported that both fungal species isolated have the capacity to produce large amounts of oxalic, citric and gluconic acids in broth culture that cause extensive deterioration of clay silicates, mica and feldspar from both sand stone and granite and also of calcite and dolomite from lime stone. Finally they concluded that the filamentous fungi are able to cause an extensive weathering of stone due to organic acid excretion.

The organic compounds produced by microorganisms such as acetate, citrate and oxalate could increase mineral dissolution rates in laboratory experiments by Friedrich *et al.* (1991) and Styriakova *et al.* (2003) and in the soil by Sheng *et al.* (2003) and Badar *et al.* (2006).

Sheng and He (2006) reported that solubilization of illite and feldspar by microorganism is due to the production of organic acids like oxalic acid and tartaric acids and also due to production of capsular polysaccharides which helps in dissolution of minerals to release potassium.

And decomposition of silic at eminerals by *B. mucilaginosus* due to production of oxalate, citrate and the extent of which polysaccharides absorbed organic acids decomposes minerals (Liu *et al.*, 2006).

Priyanka and Sindhu (2013) studied on bacterial isolates were obtained from wheat rhizosphere on modified Aleksandrov medium containing mica powder as potassium source. Twenty bacterial strains, among 137 cultures tested, showed significant potassium solubilization on mica powder supplemented plates and the amount of K released by different strains varied from 15 to 48 mg/l. In glucose amended medium broth, bacterial strains WPS73 and NNY43 caused 41.0 and 48.0 mg mg/l of K solubilization. Bacterial strain WPS73 caused maximum solubilization (49.0 mg/l) at 25°C whereas bacterial strain NNY43 caused maximum solubilization at 30°C.

Javad *et al*. (2013) studied the isolation, screening, and characterization of six isolates of K solubilizing bacteria (KSB) from some Iranian soils. The ability of all isolates were tested in three treatments including acid-leached soil, biotite and muscovite by analyzing the soluble K content after 5 days of incubation at 28 ± 2°C. Identification and phylogenetic analyses were also carried out by morphological, biochemical, and 16S rDNA analyses. Among the six efficient isolates, five isolates belonged to *Bacillus megaterium* (JK3, JK4, JK5, JK6 and JK7), while isolate JK2 belonged to *Arthrobacter* sp. Here in, isolate JK2 had lower potential for K solubilization (910 mg kg^{-1}) compared with other isolates in acid-leached soils. The six bacterial strains showed higher solubilized K in biotite treatment than other two treatments. It could be concluded that the isolates belong to *B. megaterium* were the most efficient KSB under *in vitro* condition.

2.8 Mineral phosphate solubilization (MPS)

Microorganisms in the soil play a pivotal role in solubilizing insoluble phosphorus and make it available to plant. The first evidence for the involvement of microorganisms in inorganic phosphate solubilization was given by Stalstorm (1903). Sackeet *et al.* (1908) showed the participation of bacteria in dissolving insoluble sources like rock phosphate, bonemeal, dicalcium phosphate and tricalcium phosphate and called for more research on the role of microbes in making P available to crop.

Gerretsen (1957) attempted to elucidate the mechanism by which bacteria dissolve mineral phosphates. Sperberg (1957) attempted to elucidate the mechanism by which bacteria dissolve mineral phosphates and identified organic acids produced in the culture as the sole responsible factor and demonstrated that organisms able to solubilize P were present in higher proportions in the rhizosphere than in nearby non rhizosphere soil (Sperberg, 1957). This phenomenon, exhibited by the microbes is known as mineral phosphate solubilization (MPS) (Goldstein, 1986).

Various microorganisms including fungi are known to solubilize different types of insoluble phosphates that occur in almost all soils, although their populations vary with different soil types, climatic conditions and crop history. These microorganisms also bring about changes in soil reaction in the soil micro environment leading to the availability of inorganic phosphate sources. The different forms of insoluble phosphates like calcium phosphate, iron phosphate and aluminium phosphate in soil react with the metabolic byproducts released by soil microorganisms, there by reducing inorganic phosphate fixation to a remarkable degree and increase its direct availability.

Hilda and Reynaldo (1999) used phosphate solubilizing bacteria as inoculants which simultaneously increased P uptake by the plant and crop yield. Strains from the genera *Pseudomonas, Bacillus* and *Rhizobium* are among the most powerful phosphate solubilizers. The principle mechanism for mineral phosphate solubilization is the production of organic acids and acid phosphatases. Genetic manipulation of phosphate-solubilizing bacteria to improve their ability to increase the plant growth may include cloning genes involved in both mineral and organic phosphate solubilization.

2.9 Mechanism of mineral phosphate solubilization

It is an important to know the actual mechanism of MPS by microorganisms for the future utilization such as genetic transfer to higher crop plants of this invisible, ecofriendly and miniature industrial process. The mechanism of MPS had been a subject of analysis through research for a long time and still is a matter of curiosity. The important mechanisms so far hypothesized have been reviewed here under.

Mineral phosphate solubilization activity occurs as a consequence of microbial nitrate production and CO_2 formation (Hopkins and Whiting, 1916), sulfur oxidation (Rudolfs, 1922).

The mechanism is the production of H_2S which react with ferric phosphate to yield ferrous sulphate with concomitant release of phosphate (Swaby and Sperberg, 1957).

Chien (1979) reported that the dissolution of P from North Carolina phosphate rock increased by addition of urea in acid soils. Urea hydrolyses the organic matter of the soil and that the products of hydrolysis chelate Ca^{2+} ions there by release the phosphorus from the rocks. Ammonium sulphate and potassium chloride on the other hand increased the phosphorus-absorption capacity of the soil there by reducing the water soluble phosphorus.

These process result in the formation of inorganic acids like sulphuric acid (Sperber, 1958), nitric acid and carbonic acid (Vazquez *et al.*, 2000). However, their effectiveness has been less accepted than the concept of involvement of organic acids in solubilization (Kim *et al.*, 2003).

Chien (1979) reported that increasing $CaCl_2$ concentration decreased P solubility from the rock phosphates. Wilson and Ellis (1984) also showed decrease in solubility of rock phosphate with increasing Ca^{2+} activity in soil solution. Acidification did result due to respiratory H2CO3 production as postulated by Jurinak *et al.* (1986) or due to H^+ excretion originating from NH_4 assimilation as proposed by Parks *et al.* (1990) and could be the alternative mechanisims of PO_4 solubilization.

An HPLC analysis of the culture solution of *Pseudumona* spp., in contrast to the expectation, did not detect any organic acid while solubilization occurred (Illmer and Schinner, 1995).

Sode *et al.* (1995) reported that PQQ-GDH over expression was utilized by phosphotransferase system (PTS). Goldstein (1995) suggested that extracellular oxidation pathway may play an essential role in soils where calcium phosphates provide a significant pool of unavailable mineral phosphorus. Krishnaraj *et al.* (1998) had proposed a model highlighting the importance of protons that are pumped out of the cell to be the major factor responsible for phosphate solubilization. Here, direct role of organic or inorganic acids have been ruled out.

It is cleared that, phosphate solubilization by PSMs is through different mechanisms and there is considerable variation among the organisms in this respect. Each organism can act in one or more than one way to bring about the solubilization of insoluble phosphate. Though, it is very difficult to pin point a single mechanism involved in production of organic acids and consequent pH reduction appears to be of great importance.

Chen *et al.* (2006) isolated, screened and characterize the 36 strains of phosphate solubilizing bacteria (PSB) from Central Taiwan. Mineral phosphate solubilizing activities of all isolates were tested on tricalcium phosphate medium by analyzing the soluble P content after 72 h of incubation at 30 ^{o}C. Identification and phylogenetic analysis of 36 isolates were carried out by 16S rDNA sequencing. Ten isolates belonged to genus *Bacillus*, nine to genus *Rhodococcus*, seven to genus *Arthrobacter,* six to genus *Serratia* and one each to genera *Chryseobacterium*, *Delftia, Gordonia* and *Phyllobacterium.* In addition, four strains namely, *Arthrobacter ureafaciens, Phyllobacterium myrsinacearum, Rhodococcus erythropolis* and *Delftia* sp. HPLC analysis detected eight different kinds of organic acids, namely: citric acid, gluconic acid, lactic acid, succinic acid, propionic acid and three unknown organic acids from the cultures of these isolates.

2.10 Effect of biofertilizer on the soil enzyme activity

The soil enzymes are remarkable molecules that show a high degree of specificity in catalyzing biological reactions. The various activities associated with biotic and abiotic components contribute to overall soil enzyme activities.

The enzymes produced by proliferating microorganisms mediate many processes occurring in soil. The variation in the microbial population might results in an alteration of the enzyme activity. The enzymes have biological significance as they participate in the biological cycling of mineral elements. They play an important role in the decomposition of organic residues and transformation of some of the mineral compounds (Kiss *et al.*, 1975). Some of the important soil enzymes are dehydrogenase, urease and phosphatase.

Singaram and Kamalakumari (1995) in a long term experiment studied six soil enzymes related to carbon, nitrogen and phosphorus cycling. The enzyme such as amylase, catalase, cellulase, dehydrogenase, phosphatase and urease were found to be superior in the FYM treatment.

Manjunath (2006) observed a marked increase in dehydrogenase activity in the soil of organic farms than that of conventional farms in the selected major cropping system *viz.*, cotton, sugarcane, jowar and vine yard.

Ponmurugan and Gopi (2006) conducted an experiment to enumerate the population density of phosphobacteria in the rhizosphere soils of brinjal, chilly, cotton, green gram, groundnut, maize, paddy, ragi, sorghum and turmeric using Ketznelson and Bose medium following dilution plate technique. They isolated phosphobacteria from these soils and isolated strains were inoculated in specific media containing specific substrates to produce growth regulating substances such as IAA and GA3 and phosphatase enzyme. The result showed that the population levels of phosphobacteria were higher in the rhizosphere soil of groundnut plant. Further, all the strains of phosphobacteria were able to produce phytohormones and phosphatase enzyme under *in vitro* conditions.

Kondapanaidu (2008) observed significant difference between different treatments with respect to dehydrogenase activity in soil after the harvest of chilli. The treatment given with 50 per cent RDN through chemical fertilizer + 50 per cent of recommended N through vermicompost + biofertilizer + panchagavya showed the highest enzyme activity.

2.11 Effect of inoculation of phosphate solublizers on plant growth and yield

For the first time, Geresten (1957) demonstrated the increased uptake of P and grain yield due to inoculation of seedlings with PSM. Sundara Roa *et al.* (1963) reported that the seed inoculation with *Bacillus megaterium* increased the uptake of PO4 from both soil and fertilizer P sources. Since, the beneficial effect of inoculation of soil with PSM along with application of insoluble forms of P sources such as rock phosphate, tricalcium phosphate and bone meal has been reported is plant species (Ahmed and Jha, 1977 and Loheuarete and Berthelin, 1988).

Ahmed and Jha (1977) reported the increase in P uptake and yield by gram due to inoculation with *Bacillus megaterium* and *B. circulans*. They also isolated hydroxyl apatite and rock phosphate solubilizing microorganisms from Bihar soils. Nair and Subba Rao (1977) reported that the incidence of PSM and available P in different rhizosphere soils of coconut and cacoa were directly related.

Basavanna (1980) reported that the phosphorus concentration, total phosphorus uptake and the dry matter of maize plant increased on application of rock phosphate with P solubilizing bacteria and fungi. Raj (1980) compared the efficacy of autotropic and heterotropic bacteria to solubilize P, using cowpea as an indicator plant and rock phosphate as source of P and observed that P availability to plants increased to a greater extent when soil was inoculated with heterotrophic bacteria than that with autotrophic bacteria.

Raj *et al.* (1981) conducted a greenhouse experiment and observed, increased P uptake and plant height by finger millet and also increased P availability in soil by inoculating the crop with *Bacillus circulans* and applying 32 P labeled super phosphate and tricalcium phosphate. Increased P uptake and plant growth has been reported in calcareous soil inoculated with phosphate solubilizing microorganisms (Khalafallah *et al.*, 1982).

Kundu and Gaur (1984) reported that by inoculation of rice seedlings with mixed cultures of *Azotobacter chroococcum*, *Pseudomonas striata* and *Aspergillus awamori was* increased in uptake of N, P, grain and straw yield under green house condition.

Insoluble inorganic phosphate compounds are made available to the plants when PSM alone was inoculated into soil. Phosphorus solubilization in soil under green house or field conditions is much more difficult to prove than solubilization of P in solution culture, but due to the addition of PSM to soil several studies shown that there is a plant growth responses (Kucey *et al.*, 1989).

The fungi *Aspergillus niger* solubilize all the insoluble phosphate substances (bearing tricalcium phosphate) better than bacteria (Gaind and Gaur, 1990). Mohad *et al.* (1991) reported that the P solubilizing cultures (*Pseudomonas striata and Bacillus polymixa)* significantly increase shoot P concentration, plant height, dry biomass, seed yield and oil content of sunflower grown in soil inoculated with *Glomus fasciculatum* and *Pseudomonas striata* a phosphate solubilizing bacterium.

Fankem *et al* (2008) conducted experiment in strains on PGPR activity of *Pseudomonas*. Among all the bacteria tested, three *P. fluorescens* strains (CB501, CD511 and CE509) were selected. On agar plates, two strains (CB501 and CE509) showed an ability to solubilize the three phosphate types ($Ca_3 (PO_4)_2$, $AlPO_4 \cdot H_2O$ or $FePO_4 \cdot 2H_2O$). A green house trial was conducted using *Zea mays* L., the results obtained using 5 parameters including grain yield and P uptake, revealed that strain CB501 was the best plant growth promoter.

Monica *et al.* (2010) collected and studied the diversity of culturable PSB from acid soils of the Northeast of Argentina. Assays in growth medium supplemented with tricalcium phosphate revealed different phosphorus solubilization activity and temporal patterns of solubilization. The isolates were grouped according to their REP fingerprinting profiles and phylogenetically classified by 16S rDNA and biochemical analyses. These isolates were assigned to the genera *Enterobacter, Pantoea, Pseudomonas, Acinetobacter, Burkholderia*, and *Exiguobacterium*. Four isolates showing high phosphorus solubilizing activity in *in vitro* assays were inoculated on common beans (*Phaseolus vulgaris*), some of them promoted plant growth, increased photosynthesis, P and N content of leaves.

Praveen *et al.* (2012) studied and evaluate the ability of P-solubilizing fungi and phosphorus levels on growth, yield and nutrient content in maize. The field experiment was conducted to test the effect of P-solubilizing fungus *Penicillium bilaji* and *Penicillium* spp. Higher growth and yield of maize were achieved when P-solubilizing fungi treated along with 100 per cent of RD P_2O_5 application compared to 0 and 50 per cent. It is concluded that single and dual inoculation along with P-fertilizer gave 20 to 23 per cent higher maize yield over control.

2.12 Effect of inoculation of potash solubilizes on plant growth and yield

The first report on increase in yield of maize and wheat by application of organo minerals with siliate solubilizing bacteria was done by Aleksandrov (1967). Khudsen *et al.* (1982) isolated potassium solubilizing bacteria from rock samples showed higher activity in potassium release from acid leached soil and improving greengram seedling growth. Phosphorus solubilizing bacteria and silicate bacteria play important role in plant nutrition through the increase in P and K uptake by plant.

Zahar *et al.* (1984) studied the effect of soil inoculation of the silicate bacteria. *Bacillus cirulans* on the release of K and Si from different minerals and in different soil resulted that the bacteria could persist for a long time where high population density could be detected after 14 month particularly in soils containing higher levels of organic matter and observed an increased yield in rice due to silicate solubilizing bacteria (Muralikannan, 1996).

Sheng and Huang (2002) reported that the effect of potassic bacteria on sorghum resulted in increase biomass and contents of P and K in plants than the control. Park *et al.* (2003) reported that bacterial inoculation could improve phosphorus and potassium availability in the soil by producing organic acid and other chemicals by stimulating growth and mineral uptake of tobacco plants. The effect of KSB (*Bacillus* sp.) on grain yield, plant silica content of rice and available silica in soil (Raj, 2004).

Sheng *et al.* (2003) worked on potassium releasing bacterial strain *B. edaphicus* for plant-growth promoting effect and nutrient uptake on cotton and rape seed in K deficient soil pot experiments resulted increased root and shoot growth and potassium content was increased by 30 and 26 per cent respectively and in chilli crop increased biomass and K uptake due to inoculation of potash solubilizer.

Mikhailouskaya and Tcherhysh (2005) reported on effect of inoculation of K mobilizing bacteria on several eroded soils which were comparable with yields on moderately eroded soil without bacterial inoculation, resulted increased wheat yield upto 1.04 t/ha. Sheng (2006) studied the effect of inoculation of KSB *Bacillus edaphicus* chilli and cotton which resulted in increased P and K content and plant biomass.

Sheng and He (2006) recorded an increased root and shoot growth and also showed significant higher N, P and K content of wheat plant components due to inoculation of *B. edaphicus* grown in a yellow brown soil that had low available K. In field experiment recorded increased yield in tomato crop due to inoculation of silicate dissolving bacteria *B. cereus* as bioinoculant along with feldspar and rice straw on K releasing capacity (Badar, 2006).

Sugumaran and Janarthanam (2007) recorded increase in the dry matter by 25 per cent and oil content by 35.4 per cent of groundnut plant and available P and K was increased from 6.24 and 9.28 mg/kg and 86.57 to 99.60 mg/kg, respectively in soil due to inoculation of *B. mucilaginosus* (KSB) compared to uninoculated control.

Archana *et al.* (2008) conducted an experiment to study the effect of potassium solubilizing bacteria on growth and yield of maize. Efficient K solubilizing bacteria *Bacillus* spp. were used and the result showed that there was a further increase in growth, nutrition and yield of maize. The results indicated that all the inoculated bacterial isolates increased plant growth, nutrient uptake and yield component of maize plant significantly over absolute fertilizer control.

Basak and Biswas (2009) studied the dynamics of K release from waste mica inoculated with potassium solubilizing microorganism (*Bacillus mucilaginosus*) and to investigate its effectiveness as potassic-fertilizer using sudan grass (*Sorghum vulgare* var *sudanensis)* as test crop grown under two Alfisols. Results revealed that application of mica significantly enhanced biomass yield, uptake and per cent K recoveries by sudan grass than control (no-K). Biomass yield, uptake and per cent K recoveries increased further when mica was inoculated with bacterial strain in both the soils than uninoculated mica. Alfisol from Hazaribag recorded higher yield, uptake and K recoveries than Alfisol from Bhubaneswar. The dynamics of K in soils indicated that K was released from mica to water-soluble and exchangeable pools of K due to inoculation of mica with *Bacillus mucilaginosus* in both the soils.

Sangeeth *et al.* (2012) studied the potassium solubilizing bacteria, on the basis of biochemical and 16S rDNA sequence analysis, the bacterium was identified as *Paenibacillus glucanolyticus* strain IISRBK2. The strain was also evaluated for plant growth and potassium uptake of black pepper in soil artificially treated with 0.5, 1 and 1.5 g K kg^{-1} of soil in the form of wood ash. Inoculation with strain *P. glucanolyticus* was found to increase tissue dry mass (ranging from 37.0 to 68.3 per cent) of black pepper in 1g K kg^{-1}wood ash amended soil and K uptake in live bacterium inoculated black pepper plants increased by 125 to 184 per cent compared to uninoculated control.

Bagyalakshmi *et al*. (2012) conducted experiment on the efficacy of indigenous potassium solubilizing bacteria (KSB) in combination with various dosages of potash fertilizers along with recommended dose of N and P fertilizers in tea plants. Soil and leaf samples were drawn from the respective plots and they were subjected for the analysis of various parameters related to nutrients and quality aspects. Among various treatments, plants treated with 100:100:75 kg of NPK with KSB concentration formulation was found to be the best in terms of high chlorophyll, carotenoid, N, P and K contents in the crop shoots followed by other treatments. Potassium content in soil and also in crop shoots was greatly improved due to the application of KSB along with possible reduced doses of potash source.

2.13 Effect of co-inoculation of potash and phosphate solubilizers on plant growth and yield

Lin *et al.* (2002) recorded an increase in biomass by 25 per cent and K and P uptake were more than 50 per cent in tomato plant due to inoculation of silicate dissolving bacteria (*B. mucilaginosus*) than the non inoculation. Thus, there is a potential in applying RCBC13 for improving K and P nutrition. Effect of plant growth promoting rhizobacteria (PGPR) including phosphate and potash solubilizing bacteria (PSB and KSB) as biofertilizers as a sustainable solution to improve plant nutrient status and production (Vessey, 2003)

Wu *et al.* (2005) found inoculation of K *(B. mucilaginosus*) along with P solubilizer (*B. megaterium*) and N- fixer (*Azotobacter chroccoccum*) increased the growth, nutrient uptake significantly in maize crop and also improved soil properties such as organic matter content and total N in soil.

Ramarethinam and Chandra (2005) in a field experiment recorded increased brinjal yield, plant height and K uptake compared to control due to inoculation of potash solubilizing bacteria *(Frateuria aurantia*).

Han *et al.* (2006) evaluated the potential of PSB and KSB inoculated in nutrient limited soil planted with pepper and cucumber. Results showed that co-inoculation of PSB and KSB showed high P and K content and plant growth compared to control. Han and Lee (2005) found that the co-inoculation of PSB and KSB in combination with direct application of rock P and K material into the soil resulted increased N, P and K uptake, photosynthesis and the yield of eggplant grown on P and K limited soil.

Supanjani *et al.* (2006) reported that integration of P and K rocks with inoculation of phosphorus and potassium solubilizing bacteria increased P availability from 12 to 21 per cent and availability from 13 to 15 per cent. Improved in photosynthesis and leaf area by 16 to 35 per cent as compared to. The treatment with P and K rocks and P and K solubilizing bacterial strain were sustainable alternative to the use of chemical fertilizer.

Badar *et al.* (2006) studied on the effect of inoculation combined with K and P bearing minerals on sorghum plants and reported increased in dry matter yield and P and K uptake in three different soils *viz*. clay.

Sandy and calcareous soils increase in dry matter by 48, 65 and 58 per cent, P uptake by 71, 110 and 116 per cent, K uptake by 41, 93 and 79 per cent and improved fertility through inoculation of PSB. The increased rice grain yield in a field experiment due to effect of silicate solubilizing bacteria recorded 5218 kg/ha grain yield than control 4419 kg/ha (Balasubramanian and Subramanian, 2006).

The potential PSB, *B. megaterium* variety *phosphaticum* and KSB, *B. mucilaginosus* were evaluated in pepper and cucumber crops. The outcome of the experiment showed that rock phosphate and potassium applied either singly or in combination don't significantly enhance availability of soil phosphorus and potassium indicating their unsuitability for direct application and co-incubation of PSB and KSB resulted in consistently higher P and K available than in the control (Vassilev *et al.,* 2006).

Biraj and Dipak (2010) studied the seffect of co-inoculation of potassium solubilizing (*Bacillus mucilaginosus*) and N fixing (*Azotobacter chroococcum* A-41) bacteria on solubilization of waste mica (a potassium-bearing mineral) and their effects on growth promotion and nutrient uptake by a forage crop of sudan grass (*Sorghum vulgare* Pers.) in a Typic Haplustalf. The co-inoculation of these two microorganisms resulted in the highest biomass production and nutrient acquisition.

MATERIAL AND METHODS

The present investigation on "Studies on bacteria solubilizing both potassium and phosphorus and their effect on maize (*Zea mays* L.)" was carried out at the Department of Agricultural Microbiology, University of Agricultural Sciences, and Dharwad. The materials used in the study, the pro11cedures and the techniques which were adopted are detailed in this chapter.

3.1 Isolation of bacteria solubilizing both potassium and phosphorus

3.1.1 Collection of soil samples

The rhizosphere soil of different crop plants of sorghum, maize, chilli, cotton and banana were collected from Dharwad, Haveri and Davanagere districts. Recorded longitude and latitude of place, the samples were brought in polythene bags.

3.1.2 Isolation and purification of bacteria solubilizing both potassium and phosphorus

Potassium as well as phosphorus solubilizing bacteria were isolated from different rhizosphere soil. Each soil sample five gram added to 25 ml of liquid modified Aleksandrov's medium (Hu *et al.*, 2006) and shaken for 48 h at 150 rpm at 30 ^{0}C. and 10^{-6} dilution series were made. Dilutions were plated onto the same medium and incubated at 30 ^{0}C for 24 h. The colonies on the 10^{-6} dilution plate were picked and grown in the same liquid medium for 48 h at 150 rpm at 30 ^{0}C and then re-isolated by streaking on fresh plates. The colonies were selected based on colony colour and morphology from these plates.

3.2 Identification and characterization of the bacterial isolates

All the selected isolates were examined for the colony morphology, cell shape, Gram reaction and ability to form spores as per the standard procedures given by Barthalomew and Mittewer (1950) and Anonymous (1957).

3.2.1 Biochemical characterization

The biochemical characterization of the isolates was essentially carried out as per the procedures outlined by Cappuccino and Sherman (1992). The tests conducted are detailed below.

3.2.1.1 Starch hydrolysis (Eckford, 1927)

The ability of the isolates to hydrolyse starch was examined, the petriplates containing starch agar were inoculated with test cultures and incubated at 30°C for three days. After incubation the plates were flooded with Lugol's iodine solution and allowed to stand for 15 to 20 min. The clear zone around the colony was considered as positive for the test.

3.2.1.2 Casein hydrolysis (Seeley and Vandemark, 1970)

The plates containing skim milk agar was streaked with test cultures and incubated at 30°C for one week. The clear zone around the colony against a black background after incubation was taken as positive for casein hydrolysis.

3.2.1.4 Acid and gas production (Seeley and Vandemark, 1970)

The isolates were tested for acid and gas production by inoculating five ml of pre-sterilized glucose broth medium in test tubes containing Durham's tube and bromocresol purple (15 ml/l of 0.04 per cent solution) as pH indicator. The tubes were incubated for seven days at 30°C. Accumulation of gas in the Durham's tube was taken as positive for gas production and the change in colour of medium from purple to yellow was taken as positive for acid production.

3.2.1.4 Catalase test (Blazevic and Ederer, 1975)

The nutrient agar slants were inoculated with test organisms and were incubated at 30°C for 24 h. After incubation the tubes were flooded with one ml of hydrogen peroxide (3%) and observed for production of gas bubbles. The occurrence of gas bubbles was scored positive for catalase activity.

3.2.1.5 Hydrogen sulphide production (Cowan and Steel, 1970)

The bacterial isolates were inoculated to test tubes containing five ml of sterile medium and incubated at room temperature 28°C. The test tubes were observed for H_2S production. The formation of black ring in the medium was taken as positive for H_2S production.

3.2.1.6 Urease test (James and Natalie Sherman, 1992)

The bacterial isolates were tested for urease activity by inoculating the cultures to five ml of pre-sterilized urea broth containing phenol red as pH indicator. The tubes were incubated for 24 to 48 hours at 30°C. Formation of deep pink colour was taken as positive for urease activity.

3.2.1.7 Gelatin liquefaction (Blazevic and Ederer, 1975)

To the pre-sterilized nutrient gelation deep tubes, the test cultures were inoculated and tubes were incubated at 28±2°C for 24 h. Following this, the tubes were kept in a refrigerator at 4° for 30 min. The tubes with cultures that remained liquefied were taken as positive and those that solidified on refrigeration were taken as negative for the test.

3.2.1.8 Oxidase test (Cappuccino and Sherman, 1996)

To the trypticase soyagar plates, overnight culture of the test isolate was spotted and the plates were incubated for 24 h at 28±2°C. After incubation, two to three drops of tetramethyl phenylenediamine dihydrochloride was added to the surface of the growth of test organism. The colour change to maroon was taken as oxidase positive.

3.2.1.9 Denitrification test

The nitrate broth tubes with inverted Durham's tube inside were inoculated with the overnight grown culture of the test organism and that were incubated for two weeks at 25°C. After one week of incubation the inverted Durham's tube were observed for the accumulation of gas.

3.2.1.10 Methyl red test (Seeley and Vandemark, 1981)

The test culture containing MR-VP broth were sterilized and inoculated with the test cultures. The tubes were incubated at 28±2°C for 48 h. After incubation five drops of methyl red indicator was added to each tube and gently shaken. The production of red colour was taken as positive for the test and production of yellow colour was taken as negative for the test.

3.2.1.11 Voger – Proskauer test (Seeley and Vandemark, 1981)

To the pre-sterilized tubes containing MR-VP broth test cultures were inoculated. The tubes were incubated for 48 h at 37°C. After incubation ten drops of Barritt's reagent A was added and gently shaken followed by addition of ten drops of Baritt's reagent B. The development of rose colour in the broth was taken as positive for the test.

3.2.1.12 Growth at 7 per cent NaCl

The tubes of nutrient broth 3 ml/tube containing seven per cent sodium chloride were inoculated with a loop full culture of the test isolates grown overnight in nutrient broth. The tubes were incubated at 28±2°C and the growth was observed after 24 h in terms of turbidity which was taken as positive for the test.

3.3 Screening of isolates for mineral potassium and phosphorus solubilization

3.3.1 Quantitative estimation of K and P released from insoluble K and P bearing mineral

The isolates showing zone of solubilziation on Aleksandrov agar were further examined for their ability to release K and P from broth (supplemented with 0.1 per cent muscovite mica and 0.5 per cent Tri calcium phosphate). One ml of overnight culture of each isolate was inoculated to 25 ml of modified Aleksandrov broth (Hu *et al.*, 2006) in nine replicates. All the inoculated flasks were incubated for two weeks at 28±2°C. The amount of K and P released in the broth was estimated at 5, 10 and 15 days of incubation from triplicate flasks at each stage in comparison with a set of uninoculated controls. The broth cultures were centrifuged at 10,000 rpm for 10 min in the microcentrifuge to separate the supernatant from the cell growth and insoluble potassium and phosphate.

3.3.1.1 Available K

The available K content in the supernatant was determined by flame photometry (Sugumaran and Janarthanam, 2007).

One ml of the culture supernatant was taken in a 50 ml volumetric flask and the volume was made to 50 ml with distilled water and mixed thoroughly. After that the solution was fed to flame photometer and K content was determined.

Simultaneously, a standard curve was prepared using various concentrations by dilutions of 100 ppm KCl solution. Amount of potassium solubilized by the isolates was calculated from the standard curve.

Preparation of standard curve (K)

Potassium chloride was dried at 60°C and 1.908 g of it was dissolved in distilled water and made up the volume to one liter. Ten ml of this was diluted further to 100 ml with distilled water to obtain 100 ppm solution and used for preparation of standards 0, 2, 4, 6, 8 and 10 ppm. These standards were fed to flame photometer to obtain K standard curve.

3.3.1.2 Available P

The available P content in the supernatant was estimated by phosphomolybdic blue color method of (Jackson, 1973).

Reagents used

Chloromolybdic acid

The chloromolybdic acid reagent was prepared by dissolving 7.5 g of ammonium molybdate in 150 ml distilled water to which 162 ml of concentrated HCl was added. The volume was made upto one liter with distilled water.

Chlorostannous acid

The chlorostannous acid reagent was prepared by dissolving 25 g of $SnCl_2.2H_2O$ in 100 ml concentrated HCl and making the volume to one liter with distilled water.

Both the reagents were stored in amber colored bottles in a refrigerator.

Procedure

One ml of the culture supernatant was taken in a 50 ml volumetric flasks to which 10 ml of chloromolybdic acid was added and mixed thoroughly. The volume was made upto approximately three fourth with distilled water and 0.25 ml chlorostannous acid was added to it. Immediately, the volume was made to 50 ml with distilled water and mixed thoroughly. After 15 min, the blue color developed was read in spectrophotometer at 610 nm using a reagent blank.

Simultaneously, a standard curve was prepared using various concentrations of standard 2 ppm KH_2PO_4 solution. The amount of phosphorus solubilized by the isolates was calculated from the standard curve.

Preparation of standard curve (P)

Potassium dihydrogen phosphate was dried at 40°C and 0.2195 g of it was dissolved in 400 ml distilled water. Twenty five ml of 7 N H_2SO_4 was added to it and volume was made upto one liter with distilled water and mixed thoroughly. Twenty ml of this was diluted further to 500 ml with distilled water to obtain two ppm solution and used for preparation of standard curve.

3.3.2 Production of growth promoting substances by the isolates

The isolates were subjected to qualitative analysis for the production of IAA (Bric *et al.,* 1991) and GA (Brown and Burlingham, 1968).

Luria agar supplemented with sodium dodecyl sulphate (0.06%) and glycerol (1%) was prepared and plated. The surface area of the agar medium was divided into squares of 2 cm x 2 cm by marking on the bottom of each plate. The overnight cultures of each isolates were grown on Luria agar was spotted with sterile tooth pick in each square. The spotted plates were overlaid immediately with sterile disc of Whatman No. 1 filter paper. Plates were incubated until the colonies reached the size of 0.5 to 2.0 mm in diameter. After an appropriate incubation period, the filter paper discs were removed from the plates and treated with Salkowaski's reagent (2% of 0.5 M $FeCl_3$ in 35% perchloric acid) by soaking in a petridish containing the reagent. The reaction was allowed to proceed until adequate colour was developed.

Bacteria producing IAA were identified by the formation of characteristic red halo around the colony on the filter paper. The paper discs after treatment with Salkowaski's reagent were viewed under UV light. The spots giving typical green fluorescence were taken as positive for GA production.

The isolates showing IAA and GA production were further examined for the amount of IAA and GA production as detailed below.

3.3.2.1 Quantitative estimation of IAA and GA extraction

The overnight cultures of the isolates which showed the production of IAA and GA subjected to quantitative estimation by inoculated to 50 ml of sterilized Czapeck's solution and incubated at 37°C for seven day in dark condition. After incubation, the cultures were centrifuged at 6000 rpm for 20 min. The supernatant was collected in a conical flask and used for estimation of IAA and GA.

3.3.2.2 Quantitative estimation of IAA (Gordon and Paleg, 1957)

Twenty five ml of the supernatant was collected and the pH was adjusted to 2.8 using 1N HCl in a 100 ml conical flask. Equal volume of diethyl ether was added to it and incubated in dark for four hours. Extraction of IAA was done at 4°C in a separating funnel using diethyl ether. The organic phase was discarded and the solvent phase was pooled and evaporated to dryness. To the dried material, two ml of methanol was added, pooled and the IAA present in the methanol extract was determined using the method of Gordon and Paleg (1957).

Added to 0.5 ml of methanol, 1.5 ml of distilled water and four ml of Sapler's reagent (1 ml of 0.5 M $FeCl_3$ in 50 ml of 35% perchloric acid) and incubated in dark for one hour. The intensity of pink colour development was read at 535 nm in a UV-visible spectrophotometer. From the standard curve prepared with known concentrations of IAA, the quantity of IAA in the culture filtrate was determined and expressed as µg/25 ml of the medium.

3.3.2.3 Quantitative estimation of GA (Paleg, 1965)

Twenty five ml of the culture filtrate was taken in a test tube to which two ml of zinc acetate was added. After two minutes, two ml of potassium ferrocyanide was added and centrifuged at 1000 rpm for 15 minutes. To five ml of this supernatant was added five ml of 30 per cent HCl and incubated at 20°C for 75 min. The blank sample was treated with five per cent of HCl and the absorbance of samples as well as blank was measured at 254 nm in a UV-visible spectrophotometer. The amount of GA present in the extract was calculated from the standard curve and expressed as µg/25 ml of the medium. The standard curves of IAA and GA were prepared by using graded concentrations of IAA and GA3.

3.4 Green house evaluation of efficient bacteria solubilizing both potassium and phosphorus (K-P SB) for growth, nutrient and yield of maize plant

A pot culture experiment was conducted using eight efficient bacteria solubilizing both potassium and phosphorus in comparison with local strain obtained from Department of Agricultural Microbiology, UAS Dharwad, to study their performance in enhancing the growth, yield, P and K content of maize plant as detailed below.

3.4.1 Treatments

The treatments for pot culture experiment were presented in Table 1. Fifteen treatments each with six replication were designed, three replications were used to record observation on plant growth parameters after 30, 60 days and at harvest of plant growth and three replication were used to record observation on dry biomass and yield at harvest.

3.4.2 Soil type

The medium black soil collected from Main Agricultural Research Station, University of Agricultural Sciences, Dharwad was mixed with FYM (150 g/pot) and recommended dose of fertilizer was applied according to the treatments and filled into the earthen pots of 30 cm diameter at the rate of 15 kg/pot. The soil was mixed with sand in the ratio of 10:1.

3.4.3 Soil Analysis

The soil was analyzed for available nitrogen content by Kjeldhal method (Jackson, 1973) and organic carbon content by wet oxidation method (Jackson, 1973). The pH of the soil was determined in 1:2.5 soil solution using a digital pH meter. The available phosphorus content was determined by Olsen's method (Olsen *et al.*, 1954) and the available potassium by flame photometer method (Stanford and English, 1949). The properties of the soil are presented in Table 2.

Table 1: Details of the treatment used for pot culture experiment

Tr No.	Treatments
T_1	Control (no inoculation and no fertilizer)
T_2	Recommended Dose of Fertilizer
T_3	Recommended Dose of potash with rock phosphate
T4	Recommended Dose of phosphorus with Mica
T5	Local strain of *Bacillus* (KSB-11) with RP and Mica
T6	Local strain of *Pseudomonas striata* (PSB 98 (2))with RP and Mica
T7	Co-inoculation of local strain of KSB-11 and PSB 98(2) with RP and Mica
T_8	Isolate K-PSB 2 with RP and Mica
T_9	Isolate K-PSB 20 with RP and Mica
T_{10}	Isolate K-PSB 21 with RP and Mica
T_{11}	Isolate K-PSB 28with RP and Mica
T_{12}	Isolate K-PSB 32 with RP and Mica
T_{13}	Isolate K-PSB 39 with RP and Mica
T_{14}	Isolate K-PSB 36 with RP and Mica
T_{15}	Isolate K-PSB 50 with RP and Mica

- Nitrogen – was applied to all the treatment at recommended dose of fertilizer
- Phosphorous applied in the form of RP (rock phosphate) 'P' content 35 per cent
- Potash applied in the form of Mica (muscovite mica) 'K' content 25 per cent
- KSB- Potassium solubilizing bacteria,
- PSB- Phosphorus solubilizing bacteria.

3.4.4 Seed

Maize (*Zea mays* L) seeds of hybrid 900-M Gold obtain from Monsanto Company were used as trails.

Fertilizers

The recommended dose of fertilizers of maize was 150:75:37.5 kg NPK per hectare. P in the form of rock phosphate, N in the form of urea and K in the form of mica were added according to the treatment schedule.

3.4.5 Seed treatment and sowing

Maize seeds were inoculated following the method of Weller and Cook (1983). The strains selected for the treatments were grown on medium for 48 h. Growth was scraped and thoroughly mixed with sterile carboxy methyl cellulose (CMC) (1%) suspension. Maize seeds were surface sterilized with sodium hypochlorite (4%) for five min and then thoroughly rinsed twice with sterile water. The seeds were then placed in CMC based culture suspension and air dried overnight by placing in a laminar air flow chamber.

3.4.6 Experiment Details

Location	: MARS Dharwad
Season	: *Rabi*-2012-13
Crop	: Maize
Variety	: 900-M Gold
Total number of treatments	: 15
Number of replication	: 3
Design	: Complete Randomize Design (CRD)

3.4.7 Sowing and maintenance

The inoculated seeds were sown in pots at 4 seeds per pot. After germination, thinning was done to retain one plant in each pot. The pots were watered regularly to maintain optimum moisture and other routine care was taken to protect the plants from pest and diseases.

3.4.8 Observation

The plant growth parameters were recorded at 30, 60 Days after sowing (DAS) and at harvest

3.5 Plant growth parameters

3.5.1 Plant height

The plant height was measured at 30, 60 DAS and at harvest from the base of the plant to the base of fully opened top leaf and expressed in centimeters.

3.5.2 Number of leaves per plant

The number of leaves per plant were counted and recorded at 30, 60 DAS and at harvest.

3.5.3 Girth of the stem

The girth of the stem was measured at 30, 60 DAS and at harvest of plant growth. The circumference was measured at the centre of the plant in centimeters and was taken as girth of the stem in centimeter.

3.5.4 Root length

The root length was recorded at 30, 60 DAS and at harvest by uprooting the plant and measuring the length from tip of the longest root to the neck region and expressed in centimeters.

3.5.5 Dry matter content

The dry matter content of maize plant was recorded at 30, 60 DAS and at harvesting. The uprooted plant, root and shoot portions were separated and air dried separately in an oven at 60°C to constant weight. The shoot and root dry weight were recorded and expressed in g per plant.

Table 2: Chemical-physico and biological properties of soil used in pot culture experiment

I	**Chemical prosperities**		
1	pH	7.3	pH meter (Jackson, 1967)
2	Electrical conductivity (dS/m)	0.28	EC bridge (Jackson, 1973)
3	Organic carbon (%)	0.47	Walkely and Black's wet Oxidation method (Jackson, 1967)
4	Available nitrogen (kg/ha)	192	Alkaline permanganate method (Subbaiah and Asija, 1966)
5	Available P_2O_5 (kg/ha)	26.7	Olsen's method (Muhr *et al*., 1965)
6	Available K_2O (kg/ha)	141	Flame photometer method (Jackson, 1967)
II	**Initial microbial population before sowing**		
1	Bacteria (cfu/g dry soil)	9.1×10^6	Dilution plate tcchnique
2	Fungi (cfu/g dry soil)	6.8×10^3	Dilution plate tcchnique
3	Actinomycetes (cfu/g dry soil)	3.8×10^3	Dilution plate tcchnique
4	PSB (cfu/g dry soil)	2.8×10^4	Dilution plate tcchnique
5	KSB (cfu/g dry soil)	2.7×10^4	Dilution plate tcchnique

3.6 Yield parameters

3.6.1 Number of cobs per plant

The number of cob in each treatment was recorded by direct counting.

3.6.2 Cob weight (g)

The weight of cob from each plant in each treatment was recorded and expressed as gram per plant.

3.6.3 Grain weight (g)

The grains from the cob of each plant were separated manually, grain weight was recorded for each treatment and expressed as grams per plant.

3.6.4 Test weight (100 seed weight)

The weight of 100 grains randomly selected from each plant was recorded for each treatment and expressed in grams.

3.7 Chemical analysis of plant

3.7.1 Nutrient uptake studies

The plant samples collected at harvest were used for chemical analysis. The dried samples were ground in a Willey mill and passed through 40 mesh sieve. The ground material was collected in butter paper bags and used for chemical analysis. The total nitrogen in the plant samples was determined by micro-Kjeldahl method. The phosphorus content in the plant samples was determined by Vanadomolybdate phosphoric yellow color method. The potassium content in the plant samples were determined by Flame photometer method (Jackson, 1973).

3.7.2 Estimation of chlorophyll

The chlorophyll content was measured by using a SPAD (Soil Plant Analysis Device) meter at different stages of growth by selecting four leaves randomly at the centre of the leaves and the average worked out.

3.7.2 Enzyme activity

The soil sample were collected by completely uprooting the plants from each replicate pot at 30, 60 DAS and at harvest and used for determination of enzyme activities.

3.7.3 Dehydrogenase activity

The enzyme assay involves colorimetric determination of 2, 3, 5 triphenylfaormazone (TPF) produced by reduction of 2, 3, 5-triphenyl tetrazolium chloride by soil microorganisms. Ten gram of soil samples was used to estimate this enzyme activity. The reduced product, TPF was extracted by methanol and its concentration was measured using a spectrophotometer at 485 nm. The enzyme activity was expressed as µg of TPF produced per gram of soil when incubated for 24 h at 37°C (Casida *et al*., 1964).

The standard graph of different concentrations of TTC was prepared in methanol to include 0, 5, 10, 20, 30 and 40 µg TPF per ml.

3.7.4 Phosphatase activity

The alkaline phosphatase activity was measured by estimating concentration of P-nitrophenol ashydrolysed product of the substrate P-nitrophenyl phosphate (PNP). One gram of soil sample was used to estimate the activity of alkaline phosphatase. The enzyme activity was expressed as µg of P-nitrophenyl phosphate hydrolyzed per gram of soil per h at 37± 8°C (Evazi and Tabatabai, 1979).

The standard graph of different concentrations of P-nitrophenol solution was prepared to include 0, 10, 20, 30, 40 and 50 µg per ml. the intensity of colour was read in spectrophotometer at 420 nm against a blank.

3.8 Enumeration of rhizosphere microbial population

The representative rhizosphere soil of maize were collected from each replicate pot. The isolation and enumeration of free living nitrogen fixers, phosphate solubilizing microorganisms and potassium solubilizing bacteria.

From rhizosphere soil of maize was done by using serial dilution and standard plate count technique using Norris agar for free-living nitrogen fixers (Norris, 1959), Pikovskaya's agar for phosphate solubilizers (Pikovskaya, 1948) and potassium solubilizer in Aleksandrov medium (Hu *et al.,* 2006).

Ten grams of freshly collected and air dried soil sample was transferred to 90 ml sterile water blank and mixed well. One ml of this suspension was transferred to 9 ml sterile water blank and subsequent dilution were prepared up to 10^{-4} in the same manner. The dilution used were 10^{-4} for isolation of P- and K-solubilizer and free-living N-fixers. One ml of suspension from each appropriate dilution was transferred aseptically to sterile Petri plates and suitable molten agar medium cooled to 38°C was transferred into the Petri plates. The plates were gently rotated in clockwise and anticlock-wise directions for uniform distribution of soil suspension. The medium was allowed to solidify and the plates were incubated in an inverted position at 30°C in an incubator for 4 days and the number of colony forming units (CFU) was recorded. The counts were expressed as CFU per gram of soil.

3.9 Statistical analysis of the data

The data obtained from the experiment were subjected to statistical analysis by Completely Randomized Design. Interpretation of the data was carried out in accordance with Panse and Sukhatme (1985). The levels of significance used in the F and't' test was P=0.01. The critical difference values were calculated wherever the 'f' test values were significant.

EXPERIMENTAL RESULTS

In this study, the attempts were made to isolate potassium as well as phosphorus solubilizing bacteria from rhizosphere soil of different crop plants around Dharwad, Haveri and Davanagere districts. The isolates were examined for their ability to solubilize insoluble potassic and phosphorus minerals. The selected isolates were characterized and tentatively identified upto genus level based on morphological and biochemical properties. The efficient both K-P solubilizings were further subjected for their ability to solubilization K and P from potassium mineral (mica) and phosphorus mineral tri calcium phosphate (TCP), mechanisms involved in K and P solubilization and production of plant growth promoting substance and for other beneficial traits. Highly efficient K-P solubilizing strains were also tested for their influence on growth and nutrient uptake of maize plant under pot culture conditions. The results obtained in these studies are presented here under.

4.2 Isolation of bacteria solubilizing both potassium and phosphorus from rhizosphere soils of different crops

The rhizosphere soil samples of different crops were collected and used for the isolation of bacteria solubilizing both potassium and phosphorus (K-PSB) as described in material and method. The details of the place of soil sample collected and the crops from whose rhizosphere the K-PSB were isolated are furnished in Table 3. Out of them total 50 K-PSB isolates selected 30 were from Dharwad, 10 each from Haveri and Davanagere district from rhizosphere of sorghum, maize, chilli, cotton and banana. These isolates were purified and maintained for further use.

4.2.1 Identification of K-PSB isolates

All the selected isolates of K-PSB were identified upto genus level based on their morphological and biochemical characters and the results are presented in Table 4. Among 50 isolates 39 were Gram positive; rod shape belongs to genera *Bacillus*. But 11 isolates were Gram negative; rod shape belongs to genera *Pseudomonas*.

4.2 Bacteria solubilizing both potassium and phosphorus minerals

The qualitative analyses of the isolates for both K-P solubilization are presented in Table 5. All the isolates were examined for their ability to solubilize muscovite mica and tri-calcium phosphate (TCP) on agar media supplemented with mica (0.2%) and TCP (0.5%).

The diameter of zone of solubilization formed by the isolates ranged from 3.0 to 11.5 mm at 72 h after incubation. Among the isolates K-PSB 32 recorded maximum solubilization (11.5 mm in diameter) followed by K-PSB 36 (11.0 mm) and K-PSB 20, K-PSB 35, K-PSB 49 and K-PSB 50 (10.0 mm). However, the isolate K-PSB 45 showed the least solubilization zone of 3.0 mm diameter.

4.3 Quantitative estimation of K and P solubilizing activity of K-PSB isolates

The amount of K and P released from muscovite mica and tri-calcium phosphate in a modified Alexandrov broth by the isolates were studied at 5, 10 and 15 days after incubation (DAI) (Table 6). The results were indicated that, amount of K and P released from the different minerals mica and TCP. All the strain increased with increase in the incubation time and maximum at 15 DAI.

The K released from mica by strain at 15 DAI ranged from 2.36 to 29.83 µg/ml and P released from tri-calcium phosphate (TCP) ranged from 3.44 to 14.25 per cent.

Among the isolates K-PSB 32 released maximum amount of K from mica and P from TCP were 29.83 µg/ml and 14.25 per cent , respectively followed by K-PSB 21, 28.74 µg/ml and 13.50 per cent and K-PSB 36 (27.76 µg/ml and 13.66 per cent) these isolates were significantly superior over all other isolates.

4.4 Production of plant growth promoting substance by the isolates

All the isolates were examined for the production of IAA and GA on Luria's Agar supplemented with SDS (0.01%) and glycerol (1%). Based on the development of red colour on the filter paper or green fluorescence under UV light. All the 50 isolates were positive for IAA and GA production (Table 7). All the isolates were earlier identified as K and P solubilizers. Based on solubilization and quantification estimation, selected isolates were further subjected to determination of the IAA and GA.

Table 3: Details of places and crop plants used for isolation of bacteria solubilizing both potassium and phosphorus

Sl. No.	Place	District	Soil type	Crop	Code no of Isolates	GPS values		
						Latitude (N)	Longitude (E)	Elevation (m)
1	Chitanahalli	Davanagere	Black soil	Maize	K-PSB1	14^0 29' 57.6"	75^0 58' 87.1"	2017
2	Kondaji	Davanagere	Black soil	Banana	K-PSB2	14^0 31' 30.3"	75^0 51' 51.0"	1880
3	Sattur	Davanagere	Red soil	Cotton	K-PSB3	14^0 33' 87.4"	75^0 57' 70.0"	2002
4	Sattur	Davanagere	Red soil	Cotton	K-PSB4	14^0 25' 56.2"	75^0 20' 26.3"	1982
5	Maganahalli	Davanagere	Black soil	Maize	K-PSB5	14^0 32' 86.8"	75^0 56' 10.1"	1930
6	Ambruth Nagar	Davanagere	Red soil	Cotton	K-PSB6	14^0 30' 99.2"	75^0 55' 76.1"	1935
7	Maganahalli	Davanagere	Red soil	Chilli	K-PSB7	14^0 29' 63.7"	75^0 55' 87.8"	1907
8	Yele-Bethur	Davanagere	Black soil	Maize	K-PSB8	14^0 29' 82.2"	75^0 55' 88.8"	1920
9	Kadaji (DTC)	Davanagere	Black soil	Cotton	K-PSB9	14^0 30' 82.5"	75^0 56' 11.7"	1956
10	Kadaji (DTC)	Davanagere	Black soil	Banana	K-PSB10	14^0 31' 00.2"	75^0 56' 15.4"	1926
11	ARS Hanumanamatti	Haveri	Red soil	Maize	K-PSB11	14^0 39' 68.9"	75^0 33' 55.6"	2089
12	ARS Hanumanamatti	Haveri	Red soil	Chilli	K-PSB12	14^0 39' 75.0"	75^0 33' 62.8"	2020
13	ARS Hanumanamatti	Haveri	Red soil	Cotton	K-PSB13	14^0 39' 81.2"	75^0 33' 71.5"	2010
14	ARS Hanumanamatti	Haveri	Red soil	Cotton	K-PSB14	14^0 38' 21.2"	75^0 34' 89.6"	1929
15	Haveri	Haveri	Black Soil	Chilli	K-PSB15	14^0 39' 85.0"	75^0 33' 68.0"	1966

16	Motebennur	Haveri	Black Soil	Maize	K-PSB16	14^0 40' 14.3"	75^0 33' 49.7"	2050
17	Karkal	Haveri	Red soil	Banana	K-PSB17	14^0 40' 68.0"	75^0 32' 88.7"	1988
18	Baydgi	Haveri	Black Soil	Chilli	K-PSB18	14^0 42' 04.6"	75^0 31' 32.7"	2106
19	Ranebennur	Haveri	Red soil	Chilli	K-PSB19	14^0 42' 97.9"	75^0 29' 32.2"	2013
20	Ranebennur	Haveri	Black Soil	Maize	K-PSB20	14^0 43' 47.5"	75^0 28' 58.4"	1987
21	ARS Hebbali	Dharwad	Black soil	Cotton	K-PSB 21	15^0 27' 76.9"	75^0 02' 64.2"	2350
22	ARS Hebbali	Dharwad	Black soil	Cotton	K-PSB 22	15^0 27' 74.5"	75^0 02' 61.5"	2315

Contd…

23	ARS Hebbali	Dharwad	Black soil	Maize	K-PSB 23	15^0 27' 70.7"	75^0 02' 68.8"	2238
24	ARS Hebbali	Dharwad	Black soil	Cotton	K-PSB 24	15^0 27' 74.9"	75^0 02' 87.1"	2235
25	ARS Hebbali	Dharwad	Black soil	Cotton	K-PSB 25	15^0 27' 80.2"	75^0 02' 68.8"	2223
26	ARS Hebbali	Dharwad	Black soil	Banana	K-PSB 26	15^0 27' 42.1"	74^0 56' 08.2"	2397
27	ARS Hebbali	Dharwad	Red soil	Banana	K-PSB 27	15^0 27' 40.4"	74^0 56' 12.3"	2410
28	ARS Hebbali	Dharwad	Red soil	Chilli	K-PSB 28	15^0 27' 37.3"	74^0 56' 08.2"	2408
29	ARS Hebbali	Dharwad	Black soil	Maize	K-PSB 29	15^0 26' 14.4"	75^0 54' 82.6"	2289
30	ARS Hebbali	Dharwad	Black soil	Banana	K-PSB 30	15^0 26' 10.1"	74^0 54' 77.1"	2241
31	ARS Mugad	Dharwad	Black soil	Maize	K-PSB 31	15^0 26' 14.4"	74^0 54' 82.6"	2289
32	ARS Mugad	Dharwad	Black soil	Maize	K-PSB 32	15^0 26' 16.2"	74^0 54' 86.0"	2305
33	ARS Mugad	Dharwad	Black soil	Cotton	K-PSB 33	15^0 26' 10.1"	74^0 54' 77.1"	2241

34	ARS Mugad	Dharwad	Black soil	Banana	K-PSB 34	15^0 26' 07.8"	74^0 54' 71.3"	2236
35	ARS Mugad	Dharwad	Red soil	Maize	K-PSB 35	15^0 26' 21.5"	75^0 54' 85.0"	2261
36	ARS Mugad	Dharwad	Red soil	Maize	K-PSB 36	15^0 26' 43.0"	74^0 54' 76.0"	2273
37	ARS Mugad	Dharwad	Black soil	Maize	K-PSB 37	15^0 27' 03.0"	74^0 54' 12.4"	2370
38	ARS Mugad	Dharwad	Black soil	Banana	K-PSB 38	15^0 27' 42.1"	74^0 56' 08.2"	2397
39	ARS Mugad	Dharwad	Black soil	Banana	K-PSB 39	15^0 27' 37.3"	74^0 56' 08.7"	2408
40	ARS Mugad	Dharwad	Black soil	Cotton	K-PSB 40	15^0 25' 36.9"	74^0 56' 09.3"	2422
41	MARS Dharwad	Dharwad	Red soil	Maize	K-PSB 41	15^0 29' 46.0"	74^0 58' 60.9"	2251
42	MARS Dharwad	Dharwad	Red sfoil	Maize	K-PSB 42	15^0 29' 44.7"	74^0 58' 49.0"	2253
43	MARS Dharwad	Dharwad	Red soil	Banana	K-PSB 43	15^0 29' 58.7"	74^0 58' 87.1"	2259
44	MARS Dharwad	Dharwad	Black soil	Maize	K-PSB 44	15^0 29' 80.4"	75^0 51' 51.0"	2289
45	MARS Dharwad	Dharwad	Black soil	Chilli	K-PSB 45	15^0 30' 13.7"	75^0 57' 70.0"	2256
46	MARS Dharwad	Dharwad	Black soil	Chilli	K-PSB 46	15^0 29' 92.1"	75^0 20' 26.3"	2274
47	MARS Dharwad	Dharwad	Black soil	Chilli	K-PSB 47	15^0 30' 01.5"	75^0 56' 10.1"	2290
48	MARS Dharwad	Dharwad	Red soil	Maize	K-PSB 48	15^0 29' 76.4"	75^0 55' 76.1"	2320
49	MARS Dharwad	Dharwad	Red soil	Banana	K-PSB 49	15^0 29' 54.1"	75^0 55' 87.8"	2329
50	MARS Dharwad	Dharwad	Black soil	Banana	K-PSB 50	15^0 29' 54.9"	75^0 55' 88.8"	2326

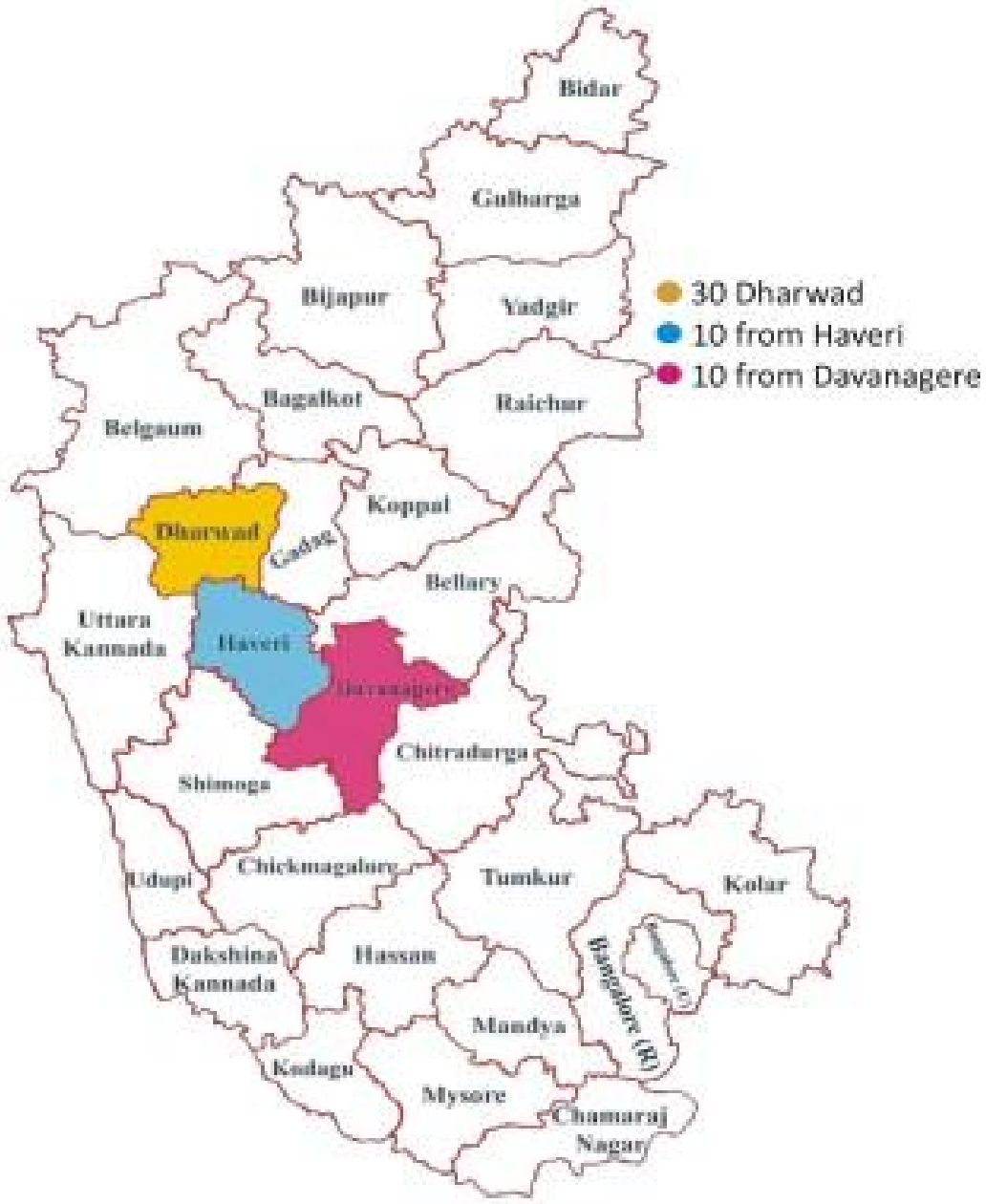


Plate 1 : Soil sample collected Districts

Plate 1: Soil sample collected Districts

Table 4: Morphological and biochemical characteristics of bacterial isolates solubilizing both potassium and phosphorus

Sl No	Code No. of the Isolates	Morphological characters		spore formation	Biochemical test													Tentatively identified probable genus
		Colony characters	Gram reaction & cell shape		1	2	3	4	5	6	7	8	9	10	11	12	13	
1	K-PSB1	Whitish, rough transparent	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	*Bacillus*
2	K-PSB2	White, smooth, slimy , spreading	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	*Bacillus*
3	K-PSB3	Greyish white, smooth, widely spreading	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	*Bacillus*
4	K-PSB4	Medium, shiny, white slimy	-	-	-	-	+	-	+	+	-	-	-	-	+	-	-	*Pseudomonas*
5	K-PSB5	Large smooth, opaque creamy white flat surface	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	*Bacillus*
6	K-PSB6	White, raised circular	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	*Bacillus*
7	K-PSB7	Whitish, white transparent	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	*Bacillus*
8	K-PSB8	White, smooth widely spreading	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	*Bacillus*
9	K-PSB9	Creamy white, smooth widely spreading, large size	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	*Bacillus*
10	K-PSB10	Creamy white, smooth, raised, large	-	-	-	-	+	-	+	+	-	-	-	-	+	-	-	*Pseudomonas*
11	K-PSB11	Creamy white small	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	*Bacillus*
12	K-PSB12	White, rough surface round	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	*Bacillus*

13	K-PSB13	White smooth, small raised	-	-	-	-	+	-	+	+	-	-	-	-	+	-	-	*Pseudomonas*
14	K-PSB14	White, smooth circular, opaque	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	*Bacillus*
15	K-PSB15	White, smooth widely spreading	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	*Bacillus*

Contd…

16	K-PSB16	Creamy white, slimy, small raised	-	-	-	-	+	-	+	+	-	-	-	-	+	-	-	*Pseudomonas*
17	K-PSB17	White smooth slimy large	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	*Bacillus*
18	K-PSB18	Creamy white irregular, opaque shape	-	-	-	-	+	-	+	+	-	-	-	-	+	-	-	*Pseudomonas*
19	K-PSB19	White smooth slimy large	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	*Bacillus*
20	K-PSB20	Greyish white smooth widely spreading	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	*Bacillus*
21	K-PSB 21	Greyish white, smooth, widely spreading	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	*Bacillus*
22	K-PSB 22	Lave smooth, opaque creamy white flat surface	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	*Bacillus*
23	K-PSB 23	White, raised circular	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	*Bacillus*
24	K-PSB 24	Creamy, smooth, raised, large shape	-	-	-	-	+	-	+	+	-	-	-	-	+	-	-	*Pseudomonas*
25	K-PSB 25	Whitish, rough transparent	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	*Bacillus*
26	K-PSB 26	White, smooth widely spreading	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	*Bacillus*
27	K-PSB 27	Creamy white smooth, small raised	-	-	-	-	+	-	+	+	-	-	-	-	+	-	-	*Pseudomonas*
28	K-PSB 28	Creamy white, smooth widely	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	*Bacillus*

		spreading,															
29	K-PSB 29	Creamy white small	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	*Bacillus*
30	K-PSB 30	White, rough surface round	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	*Bacillus*
31	K-PSB 31	White, smooth circular, opaque	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	*Bacillus*
32	K-PSB 32	White smooth slimy large	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	*Bacillus*
33	K-PSB 33	White smooth slimy large	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	*Bacillus*
34	K-PSB 34	Greyish white, smooth, widely spreading	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	*Bacillus*
35	K-PSB 35	White, smooth, rised colony	-	-	-	-	+	-	+	+	-	-	-	-	+	-	-	*Pseudomonas*

Contd…

36	K-PSB 36	Whitish, rough transparent	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	*Bacillus*
37	K-PSB 37	Creamy, smooth, raised, large	-	-	-	-	+	+	-	+	-	-	-	-	+	-	-	*Pseudomonas*
38	K-PSB 38	Whitish, rough transparent	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	*Bacillus*
39	K-PSB 39	Whitish, rough transparent	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	*Bacillus*
40	K-PSB 40	White raised slimy	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	*Bacillus*
41	K-PSB 41	White raised slimy, large	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	*Bacillus*
42	K-PSB 42	Creamy white small, slimy	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	*Bacillus*
43	K-PSB 43	White, raised, circular	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	*Bacillus*
44	K-PSB 44	Creamy white, smooth, large	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	*Bacillus*
45	K-PSB 45	Creamy white, smooth, large	-	-	-	-	+	+	-	+	-	-	-	-	+	-	-	*Pseudomona*

																		s
46	K-PSB 46	Creamy white small	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	*Bacillus*
47	K-PSB 47	Creamy white large, circular	-	-	-	-	+	+	-	+	-	-	-	-	+	-	-	*Pseudomonas*
48	K-PSB 48	White, circular slimy	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	*Bacillus*
49	K-PSB 49	Whit circular slimy, round	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	*Bacillus*
50	K-PSB 50	White raised slimy	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	*Bacillus*

1 – Starch hydrolysis 2 – Casein hydrolysis 3 – Urea hydrolysis 4 – Gelatin liquefaction

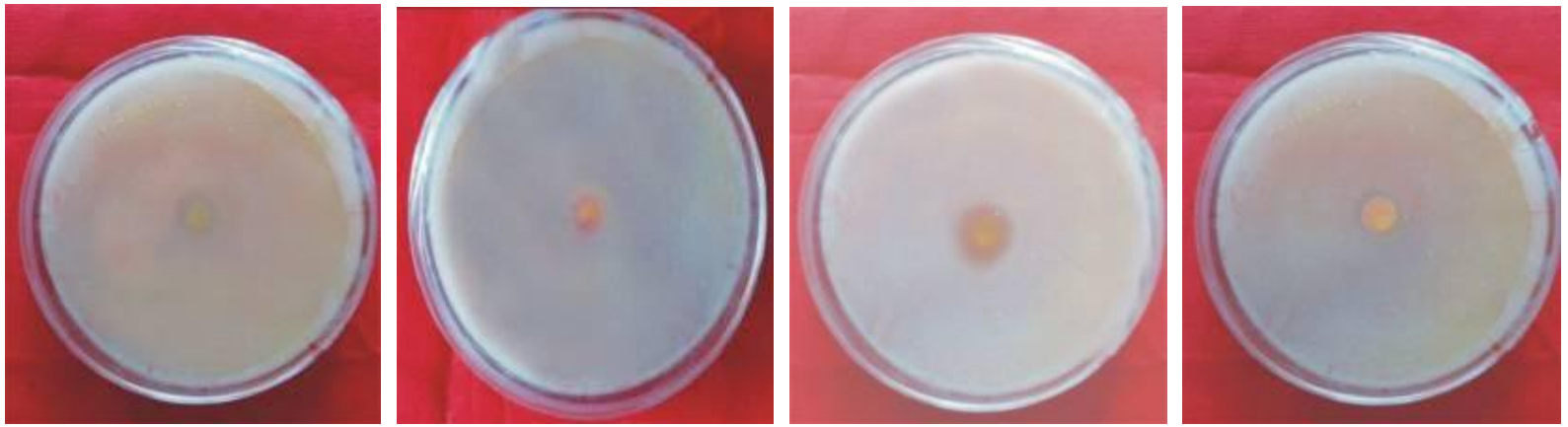
5 – Catalase test 6 – Acid production 7 – Nitrate reduction test 8 – Methyle red test

9 – Growth at 7% NaCl 10 – H_2S production 11 – Gas production 12 – Citrate utilization

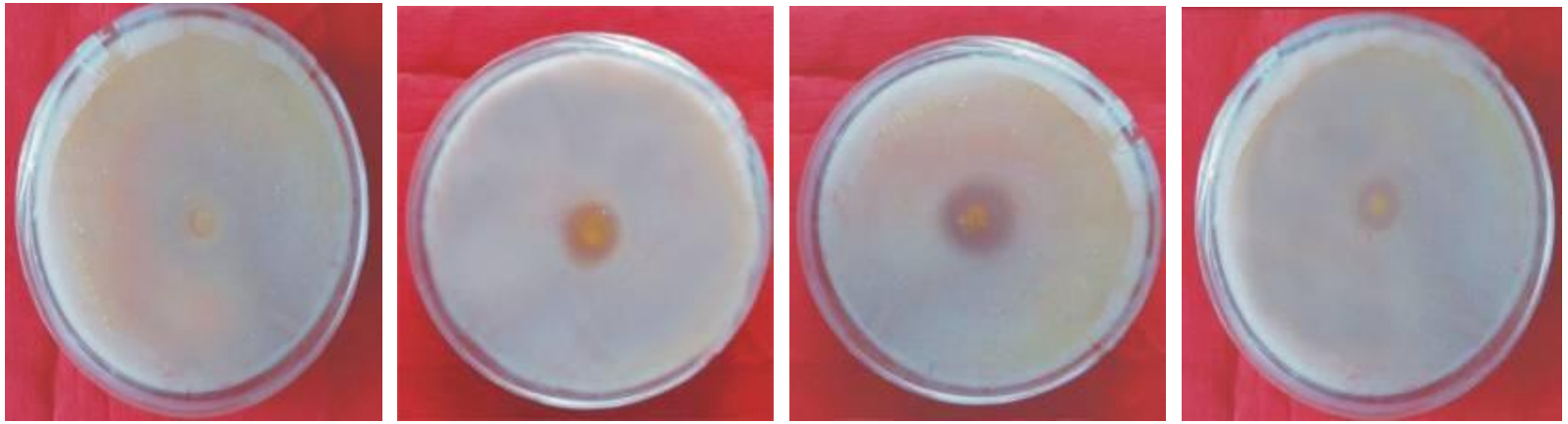
13 – V. P. text +: Positive, - : Negative

Table 5: Zone of solubilization by K-PSB isolates at three days of incubation

Sl No	Isolate no	Zone of solubilization (Dia in mm)
1	K-PSB 1	4.0
2	K-PSB 2	9.5
3	K-PSB 3	6.5
4	K-PSB 4	6.0
5	K-PSB 5	7.2
6	K-PSB 6	7.0
7	K-PSB 7	5.2
8	K-PSB 8	8.0
9	K-PSB 9	5.2
10	K-PSB 10	7.1
11	K-PSB 11	5.0
12	K-PSB 12	7.2
13	K-PSB 13	6.0
14	K-PSB 14	7.0
15	K-PSB 15	6.2
16	K-PSB 16	5.0
17	K-PSB 17	4.2
18	K-PSB 18	5.5
19	K-PSB 19	6.2
20	K-PSB 20	10.0
21	K-PSB 21	9.2
22	K-PSB 22	8.1
23	K-PSB 23	6.2
24	K-PSB 24	9.1
25	K-PSB 25	7.1
26	K-PSB 26	7.4
27	K-PSB 27	4.5
28	K-PSB 28	8.2
29	K-PSB 29	10.0
30	K-PSB 30	4.5
31	K-PSB 31	6.0
32	K-PSB 32	9.5
33	K-PSB 33	7.0
34	K-PSB 34	5.4
35	K-PSB 35	10.0
36	K-PSB 36	11.0
37	K-PSB 37	7.2
38	K-PSB 38	7.0
39	K-PSB 39	11.5
40	K-PSB 40	6.2
41	K-PSB 41	6.8
42	K-PSB 42	7.2
43	K-PSB 43	5.2
44	K-PSB 44	4.2
45	K-PSB 45	3.0
46	K-PSB 46	5.2
47	K-PSB 47	5.5
48	K-PSB 48	7.0
49	K-PSB 49	10.0
50	K-PSB 50	10.0



K-PSB 2 K-PSB 20 K-PSB 21 K-PSB 28



K-PSB 32 K-PSB 36 K-PSB 39 K-PSB 50

Plate 2: Zone of solubilization K-PSB isolates at 3 Days incubation

Table 6: Release of K^+ ion and Pi from mica and TCP by bacteria solubilizing both potassium and phosphorus at different incubation times

Sl. No.	Isolate no	5 DAI		10 DAI		15 DAI	
		Potassium (ppm)	Phosphorus (%)	Potassium (ppm)	Phosphorus (%)	Potassium (ppm)	Phosphorus (%)
1	K-PSB 1	5.09	2.38	7.28	3.38	9.76	5.39
2	K-PSB 2	7.54	4.72	16.50	9.48	23.87	12.05
3	K-PSB 3	3.35	3.14	4.51	3.65	6.04	4.39
4	K-PSB 4	1.48	5.38	2.96	6.39	4.33	8.69
5	K-PSB 5	2.85	2.15	5.53	5.49	6.63	7.27
6	K-PSB 6	3.60	1.27	4.68	3.45	7.38	5.55
7	K-PSB 7	4.17	1.28	7.77	4.73	9.90	6.54
8	K-PSB 8	2.56	3.39	4.89	5.50	7.51	6.59
9	K-PSB 9	1.53	6.34	2.52	9.93	3.55	10.84
10	K-PSB 10	2.35	7.17	3.53	9.39	4.46	9.22
11	K-PSB 11	6.71	1.38	10.26	3.48	11.71	5.49
12	K-PSB 12	5.58	2.59	7.38	3.46	9.53	4.45
13	K-PSB 13	1.92	6.54	3.39	7.36	5.93	8.87
14	K-PSB 14	2.32	4.43	4.50	5.63	7.48	6.47
15	K-PSB 15	3.35	1.38	5.54	3.40	7.66	4.48
16	K-PSB 16	1.87	5.30	2.63	6.46	4.66	8.60

17	K-PSB 17	1.00	3.34	1.56	5.29	3.49	7.36
18	K-PSB 18	1.28	7.02	2.40	7.66	5.48	9.07
19	K-PSB 19	4.65	3.43	5.78	4.45	7.35	6.19
20	K-PSB 20	8.01	4.95	18.50	10.49	26.83	14.13
21	K-PSB 21	7.09	6.35	17.55	9.63	28.74	13.50
22	K-PSB 22	5.62	0.72	9.49	2.58	8.05	3.46
23	K-PSB 23	3.57	4.78	6.17	5.42	8.41	6.56

Contd…

24	K-PSB 24	1.68	5.45	2.44	7.45	3.67	9.37
25	K-PSB 25	6.30	1.30	7.19	2.76	9.60	4.46
26	K-PSB 26	3.85	3.46	5.50	4.20	8.35	6.39
27	K-PSB 27	1.83	5.56	2.61	6.41	4.42	7.63
28	K-PSB 28	7.36	7.04	15.09	9.46	26.15	13.93
29	K-PSB 29	4.41	2.60	5.81	3.32	7.46	5.57
30	K-PSB 30	8.31	1.51	10.80	2.28	13.36	3.44
31	K-PSB 31	8.08	0.68	9.42	3.69	11.76	5.55
32	K-PSB 32	7.95	6.60	20.17	7.45	29.83	14.25
33	K-PSB 33	3.59	2.51	5.38	3.67	8.34	5.57
34	K-PSB 34	1.62	5.44	2.83	6.64	4.62	7.34

35	K-PSB 35	2.64	6.38	3.47	7.37	5.39	7.39
36	K-PSB 36	6.77	4.51	17.55	9.10	27.76	13.66
37	K-PSB 37	2.45	5.84	3.51	7.63	5.42	10.59
38	K-PSB 38	4.13	3.61	5.35	6.40	7.80	7.59
39	K-PSB 39	9.39	6.38	21.27	8.57	27.74	12.36
40	K-PSB 40	3.52	4.34	5.36	6.19	7.42	6.59
41	K-PSB 41	4.83	5.49	7.32	6.19	8.53	7.53
42	K-PSB 42	1.40	5.47	2.55	7.50	3.72	8.28
43	K-PSB 43	0.91	3.56	1.35	4.59	2.36	5.53
44	K-PSB 44	3.67	2.60	4.53	3.80	6.44	5.24
45	K-PSB 45	0.71	6.05	1.57	7.57	3.94	8.55
46	K-PSB 46	2.85	3.52	3.59	5.47	5.54	6.49
47	K-PSB 47	1.62	7.34	1.61	8.54	3.56	9.46
48	K-PSB 48	2.49	3.37	3.59	5.55	5.63	7.47
49	K-PSB 49	3.31	2.68	4.43	6.55	7.49	8.35
50	K-PSB 50	6.08	4.83	13.02	12.31	23.87	14.10
	S.Em±	0.15	0.15	0.17	0.15	0.49	0.16
	CD@1%	0.55	0.57	0.62	0.55	1.82	0.61

Table 7: Qualitative testing of production of plant growth promoting substance by bacteria solubilizing both potassium and phosphorus

Sl. No.	Isolate no	Indole acetic acid (IAA)	Gibberllic acid(GA)
1	K-PSB 1	+	+
2	K-PSB 2	+	+
3	K-PSB 3	+	+
4	K-PSB 4	+	+
5	K-PSB 5	+	+
6	K-PSB 6	+	+
7	K-PSB 7	+	+
8	K-PSB 8	+	+
9	K-PSB 9	+	+
10	K-PSB 10	+	+
11	K-PSB 11	+	+
12	K-PSB 12	+	+
13	K-PSB 13	+	+
14	K-PSB 14	+	+
15	K-PSB 15	+	+
16	K-PSB 16	+	+
17	K-PSB 17	+	+
18	K-PSB 18	+	+
19	K-PSB 19	+	+
20	K-PSB 20	+	+
21	K-PSB 21	+	+
22	K-PSB 22	+	+
23	K-PSB 23	+	+
24	K-PSB 24	+	+
25	K-PSB 25	+	+
26	K-PSB 26	+	+
27	K-PSB 27	+	+
28	K-PSB 28	+	+
29	K-PSB 29	+	+
30	K-PSB 30	+	+

31	K-PSB 31	+	+
32	K-PSB 32	+	+
33	K-PSB 33	+	+
34	K-PSB 34	+	+
35	K-PSB 35	+	+
36	K-PSB 36	+	+
37	K-PSB 37	+	+
38	K-PSB 38	+	+
39	K-PSB 39	+	+
40	K-PSB 40	+	+
41	K-PSB 41	+	+
42	K-PSB 42	+	+
43	K-PSB 43	+	+
44	K-PSB 44	+	+
45	K-PSB 45	+	+
46	K-PSB 46	+	+
47	K-PSB 47	+	+
48	K-PSB 48	+	+
49	K-PSB 49	+	+
50	K-PSB 50	+	+

4.5 Quantitative determination of IAA and GA produced by the K-PSB isolates

The amount of IAA and GA produced by the selected nine isolates were determined at 10 DAI and the results are presented in Table 8. The amount of IAA produced by different strains ranged from 3.38 to 8.90 µg/25ml broth. Among the isolates examined K-PSB 50 was found to produce the high amount of IAA 8.9 µg/25ml broth, which was on par with K-PSB 32 (8.41 µg/25ml broth), while five isolates showed more than 5 µg/25ml broth and four isolates produced 3.5 µg/25ml broth of IAA.

The amount of GA produced by the strain ranged from 1.27 to 3.67 µg/25ml broth (Table 8). Among the isolates K-PSB 50 produced the highest amount of GA 3.67 µg/25ml broth, which was on par with K-PSB 32 (3.48 µg/25ml broth), while seven isolates produced more than 2 µg/25ml broth and two isolates produced less than 2 µg/25ml broth.

4.6 Effect of inoculation of bacteria solubilizing both potassium and phosphorus on growth, yield and nutrient content of maize plants

To study the effect of inoculation of eight efficient isolates bacteria solubilizing both potassium and phosphorus on growth, yield and nutrient content of maize plant. A pot culture experiment was conducted and the results were recorded at 30, 60 DAS and at harvest.

4.6.1 Root length (cm)

There is a significant difference in the root length of maize plants was observed at 30, 60 days of plant growth and at harvest due to various inoculation treatments and insoluble form of minerals and fertilizer application Table 9.

The results obtained by treatment receiving inoculation of *Bacillus* isolateK-PSB 32 with rock phosphate and mica recorded at 30, 60 DAS and at harvest significantly maximum root length 15.83, 52.67 and 65.53 cm, respectively and was on par with the treatment co-inoculated local strains KSB11 and PSB98 (2) with rock phosphate and mica were 14.90, 50.33 and 64.23 cm, respectively and treatment with Recommended Dose Fertilizer (RDF) (14.80, 49.67 and 63.93 cm, respectively). However, all the other inoculated isolates showed no significant increase in root growth and were isolates superior over control.

4.6.2 Shoot length (cm)

All the inoculated with bacteria solubilizing both potassium and phosphorus increased the shoot length of maize plant significantly over control Table 9.

At 30, 60 DAS and at harvest the treatment inoculation of *Bacillus* isolate K-PSB 50 with rock phosphate and mica recorded significantly maximum shoot length were 41.90, 105.33 and 125.53 cm, respectively and was on par with treatment co-inoculated local strains of *Bacillus* (KSB11) and *Pseudomonas striata* (PSB98 (2)) with rock phosphate and mica (40.53, 104.00 and 122.50 cm, respectively) and treatment Recommended Dose of Fertilizer. However, all the other inoculated isolates showed no significant increase in shoot length and superior over control.

4.6.3 Stem girth (cm)

At 30, 60 DAS and at harvest the treatment inoculation of *Bacillus* isolate K-PSB 50 with rock phosphate and mica recorded significantly maximum stem girth (Table 10) were 4.70, 9.63 and 12.33 cm, respectively and was on par with treatment co-inoculated local strains of *Bacillus* and *P. striata* with rock phosphate and mica 4.23, 9.03 and 12.17 cm, respectively and treatment RDF (4.13, 8.80 and11.80 cm, respectively). All the inoculated bacterial isolates significantly increased stem girth over control.

4.6.4 Number of leaves per plant

All the inoculated bacteria increased the number of leaves in maize plant significantly superior over control (Table 10). Among all inoculated treatments highest number of leaves was recorded with treatment receiving *Bacillus* strain K-PSB 50 with rock phosphate and mica significantly maximum higher number of leaves at 30, 60 DAS and at harvest 6.67, 11.33 and 9.67, respectively and was on par with treatment co-inoculated local strains of *Bacillus* and *P. striata* with rock phosphate and mica (6.33, 11.00 and 9.33, respectively). There was no significant difference between the treatments recorded.

Table 8: Quantification of IAA and GA by selected bacterial isolates solubilizing both potassium and phosphorus

Sl. No	Isolates	IAA (µg/25ml broth)	GA (µg/ 25ml broth)
1	Isolate K-PSB 2	4.50	2.72
2	Isolate K-PSB 20	2.43	1.27
3	Isolate K-PSB 11	4.53	1.57
4	Isolate K-PSB 21	3.38	2.75
5	Isolate K-PSB 28	7.53	2.55
6	Isolate K-PSB 32	8.48	3.48
7	Isolate K-PSB 36	6.43	2.87
8	Isolate K-PSB 39	5.53	2.65
9	Isolate K-PSB 50	8.90	3.67
	S.Em±	0.15	0.12
	CD@1%	0.53	0.39

Table 9: Effect of bacteria solubilizing both potassium and phosphorus on root and shoot length of maize at 30, 60 DAS and at harvest

Tr. No.	Treatment	Root length (cm)			Shoot length (cm)		
		30DAS	60DAS	At harvest	30DAS	60DAS	At harvest
T1	Control (no inoculation and no fertilizer)	10.00	29.67	53.40	28.77	81.00	96.50
T2	Recommended Dose of Fertilizer	14.80	49.67	63.93	40.30	103.57	122.53
T3	Recommended Dose of potash with rock phosphate	12.83	37.33	56.33	33.67	95.00	103.33
T4	Recommended Dose of phosphorus with Mica	12.07	32.33	58.73	32.00	94.50	103.43
T5	Local strain of *Bacillus* (KSB-11) with RP and Mica	12.10	32.00	59.03	31.97	91.50	110.17
T6	Local strain of *Pseudomonas striata* (PSB 98 (2)) with RP and Mica	12.33	37.00	60.63	33.33	100.30	117.03
T7	Co-inoculation of local strain of KSB-11 and PSB 98(2) with RP and Mica	14.90	50.33	64.23	40.53	104.00	122.50
T8	Isolate K-PSB 2 with RP and Mica	10.43	44.33	63.43	36.07	98.83	115.83
T9	Isolate K-PSB 20 with RP and Mica	13.17	38.67	63.17	38.50	97.53	116.10
T10	Isolate K-PSB 21 with RP and Mica	13.40	49.00	63.53	38.83	99.57	116.93
T11	Isolate K-PSB 28with RP and Mica	13.50	35.00	59.30	38.33	94.63	113.00
T12	Isolate K-PSB 32 with RP and Mica	15.83	52.67	65.53	38.60	98.73	122.43
T13	Isolate K-PSB 36 with RP and Mica	13.17	45.67	62.70	38.63	101.67	117.73
T14	Isolate K-PSB 39 with RP and Mica	12.67	44.67	62.70	36.43	102.00	114.20
T15	Isolate K-PSB 50 with RP and Mica	13.83	48.33	63.27	41.90	105.33	125.53
	S.Em ±	0.71	0.97	0.48	0.85	0.74	1.07
	CD @1%	2.36	3.24	1.55	2.71	2.38	3.30

Plate 3: General view of pot culture experimental setup (at 30 DAS)

Plate 4: General view of pot culture experimental setup (at maturity)

Table 10: Effect of bacteria solubilizing both potassium and phosphorus on stem girth and number of leaves of maize at 30, 60 DAS and at harvest

Tr. No.	Treatment	Stem girth (cm)			No. of leaves/plant		
		30DAS	60DAS	At harvest	30DAS	60DAS	At harvest
T1	Control (no inoculation and no fertilizer)	2.37	6.23	8.73	5.00	7.33	7.33
T2	Recommended Dose of Fertilizer	4.13	8.80	11.80	6.33	11.00	9.33
T3	Recommended Dose of potash with rock phosphate	3.30	7.40	9.33	5.00	9.33	7.67
T4	Recommended Dose of phosphorus with Mica	3.23	7.73	9.70	5.33	9.33	7.67
T5	Local strain of *Bacillus* (KSB-11) with RP and Mica	3.87	8.43	10.43	5.67	9.00	8.33
T6	Local strain of *Pseudomonas striata* (PSB 98 (2)) with RP and Mica	3.87	7.37	10.77	5.67	9.00	8.67
T7	Co-inoculation of local strain of KSB-11 and PSB 98(2) with RP and Mica	4.23	9.03	12.17	6.33	11.00	9.33
T8	Isolate K-PSB 2 with RP and Mica	3.47	8.40	10.57	5.67	9.67	8.67
T9	Isolate K-PSB 20 with RP and Mica	3.83	7.30	10.00	5.33	10.00	8.00
T10	Isolate K-PSB 21 with RP and Mica	3.40	7.47	10.30	5.33	10.33	7.67
T11	Isolate K-PSB 28 with RP and Mica	3.53	8.53	9.90	6.00	10.67	8.00
T12	Isolate K-PSB 32 with RP and Mica	3.37	8.57	10.57	6.00	10.33	9.00
T13	Isolate K-PSB 36 with RP and Mica	3.50	8.33	10.43	6.33	9.67	8.33
T14	Isolate K-PSB 39 with RP and Mica	3.53	8.92	11.37	5.33	9.67	8.67
T15	Isolate K-PSB 50 with RP and Mica	4.70	9.63	12.33	6.67	11.33	9.67
	S.Em ±	0.20	0.14	0.16	0.34	0.25	0.37
	CD @1%	0.66	0.49	0.56	1.10	0.78	1.18

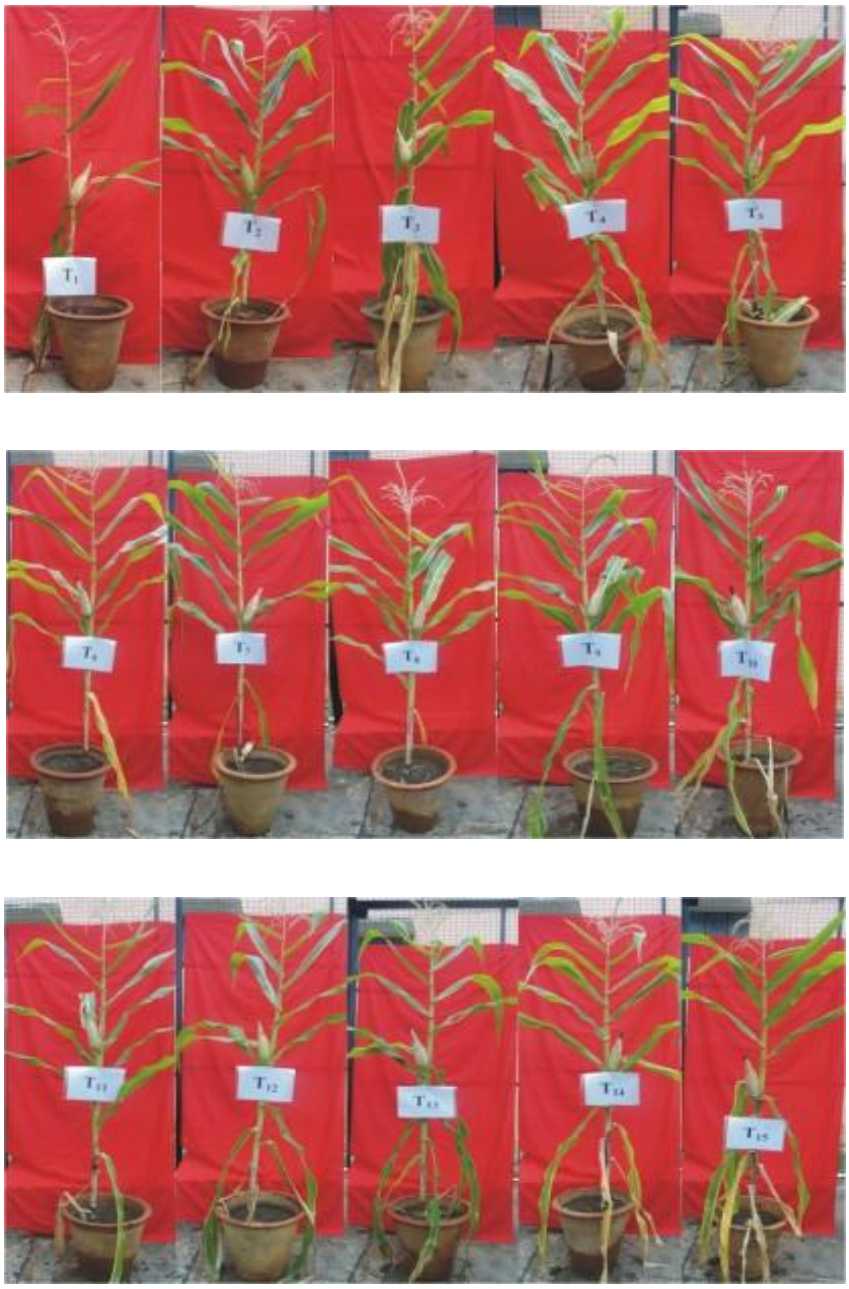


Plate 5: Effect of inoculation of bacteria solubilizing both potassium and phosphrous at 90 DAS

4.6.5 Chlorophyll content

The data on chlorophyll content of maize plant at 30, 60 DAS and at harvest as influenced by inoculation of bacteria solubilizing both potassium and phosphorus (Table 11).

All inoculated bacteria enhanced the chlorophyll content significantly over control. Among all the inoculated isolates K-PSB 50 with rock phosphate and mica treatment recorded maximum chlorophyll content at 30, 60 DAS and at harvest (42.50, 44.27 and 43.43 SPAD value, respectively). There was no significant difference between the treatment recorded and were isolates superior over control.

4.6.6 Dry matter production

The data on dry matter of shoot and root production by maize plants at 30, 60 DAS and at harvesting

4.6.7 Shoot dry matter

All the inoculated treatments recorded significantly higher shoot dry matter over control (Table 12, 13 and 14).

At 30, 60 DAS and at harvest treatment receiving inoculation of K-PSB 32 with rock phosphate and mica recorded significantly maximum shoot weight were 8.97, 39.49 and 221.20 g/plant, respectively and was on par with treatment co-inoculated local strains of *Bacillus* and *P. striata* with rock phosphate and mica (8.89, 38.39 and 220.60 g/plant), the other inoculated isolates superior over control.

4.6.8 Root dry matter

All the inoculated treatments recorded significantly higher root dry matter over control (Table: 12, 13 and 14).

At 30 DAS treatment receiving inoculation of K-PSB 50 with rock phosphate and mica recorded significantly maximum root weight were 3.99 g/plant on par with treatment co-inoculated local strains of *Bacillus* and *P. striata* with rock phosphate and mica (3.85 g/plant).

At 60 DAS and at harvest treatment receiving inoculation of K-PSB 32 with rock phosphate and mica recorded significantly maximum root weight were 4.71 and 14.50 g/plant and was on par with treatment co-inoculated local strains of *Bacillus* and *P. striata* with rock phosphate and mica (4.31 and 13.60 g/plant). The all other inoculated isolates superior over control.

4.6.9 Total dry matter

All the inoculated treatments recorded significantly higher total dry matter over the uninoculated fertilizer control (Table 12, 13 and 14) fig 1.

At 30, 60 DAS and at harvest the treatment inoculation of isolate K-PSB 32 with rock phosphate and mica recorded significantly heights total dry matter were 12.80, 44.2 and 235.7 g/plant, respectively and was on par with treatment with co-inoculated local strains of *Bacillus* and *P. striata* with rock phosphate and mica (12.44, 43.70 and 2334.27 g/plant, respectively) and was inoculated isolates superior over control.

4.7 Yield and yield parameters

The data on yield and yield parameters of maize plant as influenced by inoculation of potassium as well as phosphorus solubilizing bacteria (Table: 15) fig 2.

4.7.1 Cob weight (g)

Among the inoculated treatments isolate K-PSB 50 with rock phosphate and mica recorded significantly heights cob weight were 114.90 g/plant and was on par with the treatment co-inoculated local strains of *Bacillus* and *P. striata* with rock phosphate and mica (113.40 g/plant) and was all treatment significantly higher cob weight over control.

4.7.2 Grain yield (g per plant)

The grain yield of maize plant was increased significantly with the treatment receiving inoculation of K-PSB 50 with rock phosphate and mica.

Table 11: Effect of bacteria solubilizing both potassium and phosphorus on chlorophyll content of maize at 30, 60 and 90 DAS.

Tr. No.	Treatment	Chlorophyll content (SPAD value)		
		30DAS	60DAS	90 DAS
T1	Control (no inoculation and no fertilizer)	30.90	34.23	33.90
T2	Recommended Dose of Fertilizer	41.00	42.60	42.30
T3	Recommended Dose of potash with rock phosphate	36.07	40.10	38.67
T4	Recommended Dose of phosphorus with Mica	35.90	39.53	37.13
T5	Local strain of *Bacillus* (KSB-11) with RP and Mica	38.27	41.07	40.97
T6	Local strain of *Pseudomonas striata* (PSB 98 (2)) with RP and Mica	38.30	40.33	38.07
T7	Co-inoculation of local strain of KSB-11 and PSB 98(2) with RP and Mica	41.87	43.07	42.37
T8	Isolate K-PSB 2 with RP and Mica	39.10	40.40	38.70
T9	Isolate K-PSB 20 with RP and Mica	38.20	41.97	39.93
T10	Isolate K-PSB 21 with RP and Mica	39.47	40.10	37.20
T11	Isolate K-PSB 28with RP and Mica	39.87	41.97	39.87
T12	Isolate K-PSB 32 with RP and Mica	39.30	41.73	40.33
T13	Isolate K-PSB 36 with RP and Mica	39.80	42.10	40.47
T14	Isolate K-PSB 39 with RP and Mica	39.50	41.70	40.03
T15	Isolate K-PSB 50 with RP and Mica	42.50	44.27	43.43
	S Em ±	0.49	0.91	0.39
	CD @1%	1.60	2.86	1.25

Table 12: Effect of bacteria solubilizing both potassium and phosphorus on dry matter content (g/plant) of maize at 30 DAS

Tr. No.	Treatment	Shoot weight (g/plant)	Root weight (g/plant)	Total dry matter (g/plant)
T1	Control (no inoculation and no fertilizer)	3.69	1.61	5.57
T2	Recommended Dose of Fertilizer	8.68	3.68	12.37
T3	Recommended Dose of potash with rock phosphate	5.11	2.33	7.40
T4	Recommended Dose of phosphorus with Mica	4.98	2.35	7.21
T5	Local strain of *Bacillus* (KSB-11) with RP and Mica	6.03	2.65	8.55
T6	Local strain of *Pseudomonas striata* (PSB 98 (2)) with RP and Mica	4.55	3.18	8.26
T7	Co-inoculation of local strain of KSB-11 and PSB 98(2) with RP and Mica	8.89	3.85	12.44
T8	Isolate K-PSB 2 with RP and Mica	6.04	2.38	8.30
T9	Isolate K-PSB 20 with RP and Mica	6.28	2.29	8.43
T10	Isolate K-PSB 21 with RP and Mica	6.06	2.74	8.81
T11	Isolate K-PSB 28with RP and Mica	6.11	3.07	9.26
T12	Isolate K-PSB 32 with RP and Mica	8.97	3.39	12.80
T13	Isolate K-PSB 36 with RP and Mica	6.98	3.43	10.35
T14	Isolate K-PSB 39 with RP and Mica	6.05	2.74	8.69
T15	Isolate K-PSB 50 with RP and Mica	7.32	3.99	10.35
	S.Em ±	0.03	0.07	0.15
	CD @1%	0.12	0.26	0.52

Table 13: Effect of bacteria solubilizing both potassium and phosphorus on dry matter content (g/plant) of maize at 60 DAS

Tr. No.	Treatment	Shoot weight (g/plant)	Root weight (g/plant)	Total dry matter (g/plant)
T1	Control (no inoculation and no fertilizer)	10.04	2.06	12.10
T2	Recommended Dose of Fertilizer	38.50	3.30	41.80
T3	Recommended Dose of potash with rock phosphate	14.69	3.51	18.20
T4	Recommended Dose of phosphorus with Mica	15.47	3.23	18.70
T5	Local strain of *Bacillus* (KSB-11) with RP and Mica	24.99	2.51	27.50
T6	Local strain of *Pseudomonas striata* (PSB 98 (2))with RP and Mica	28.07	3.03	31.10
T7	Co-inoculation of local strain of KSB-11 and PSB 98(2) with RP and Mica	39.39	4.31	43.70
T8	Isolate K-PSB 2 with RP and Mica	38.42	2.98	41.40
T9	Isolate K-PSB 20 with RP and Mica	20.09	3.11	23.20
T10	Isolate K-PSB 21 with RP and Mica	32.48	3.12	35.60
T11	Isolate K-PSB 28with RP and Mica	22.37	3.33	25.70
T12	Isolate K-PSB 32 with RP and Mica	39.49	4.71	44.20
T13	Isolate K-PSB 36 with RP and Mica	36.84	2.83	39.69
T14	Isolate K-PSB 39 with RP and Mica	38.34	3.06	41.40
T15	Isolate K-PSB 50 with RP and Mica	38.80	3.31	42.10
	S Em ±	0.10	0.09	0.18
	CD @1%	0.36	0.33	0.60

Table 14: Effect of bacteria solubilizing both potassium and phosphorus on dry matter content (g/plant) of maize at harvest

Tr. No.	Treatment	Shoot weight (g/plant)	Root weight (g/plant)	Total dry matter (g/plant)
T1	Control (no inoculation and no fertilizer)	148.00	8.60	156.60
T2	Recommended Dose of Fertilizer	219.70	13.30	233.00
T3	Recommended Dose of potash with rock phosphate	188.20	9.60	197.80
T4	Recommended Dose of phosphorus with Mica	186.60	9.30	195.90
T5	Local strain of *Bacillus* (KSB-11) with RP and Mica	197.62	10.90	208.57
T6	Local strain of *Pseudomonas striata* (PSB 98 (2))with RP and Mica	207.50	13.10	220.60
T7	Co-inoculation of local strain of KSB-11 and PSB 98(2) with RP and Mica	220.60	13.60	234.27
T8	Isolate K-PSB 2 with RP and Mica	219.10	11.80	230.90
T9	Isolate K-PSB 20 with RP and Mica	215.51	12.37	227.90
T10	Isolate K-PSB 21 with RP and Mica	200.90	10.30	211.20
T11	Isolate K-PSB 28with RP and Mica	198.10	11.53	209.70
T12	Isolate K-PSB 32 with RP and Mica	221.20	14.50	235.70
T13	Isolate K-PSB 36 with RP and Mica	206.30	12.60	218.90
T14	Isolate K-PSB 39 with RP and Mica	204.70	13.60	218.30
T15	Isolate K-PSB 50 with RP and Mica	219.00	13.37	232.37
	S.Em ±	0.58	0.22	0.40
	CD @1%	1.94	0.76	1.42

LEGEND

T_1	Control (no inoculation and no fertilizer)
T_2	Recommended Dose of Fertilizer
T_3	Recommended Dose of potash with rock phosphate (RP)
T4	Recommended Dose of phosphorus with Mica
T5	Local strain of *Bacillus* (KSB-11) with RP and Mica
T6	Local strain of *Pseudomonas striata* (PSB 98 (2)) with RP and Mica
T7	Co-inoculation of local strain of KSB-11 and PSB 98(2) with RP and Mica
T_8	Isolate K-PSB 2 with RP and Mica
T_9	Isolate K-PSB 20 with RP and Mica
T_{10}	Isolate K-PSB 21 with RP and Mica
T_{11}	Isolate K-PSB 28 with RP and Mica
T_{12}	Isolate K-PSB 32 with RP and Mica
T_{13}	Isolate K-PSB 39 with RP and Mica
T_{14}	Isolate K-PSB 36 with RP and Mica
T_{15}	Isolate K-PSB 50 with RP and Mica

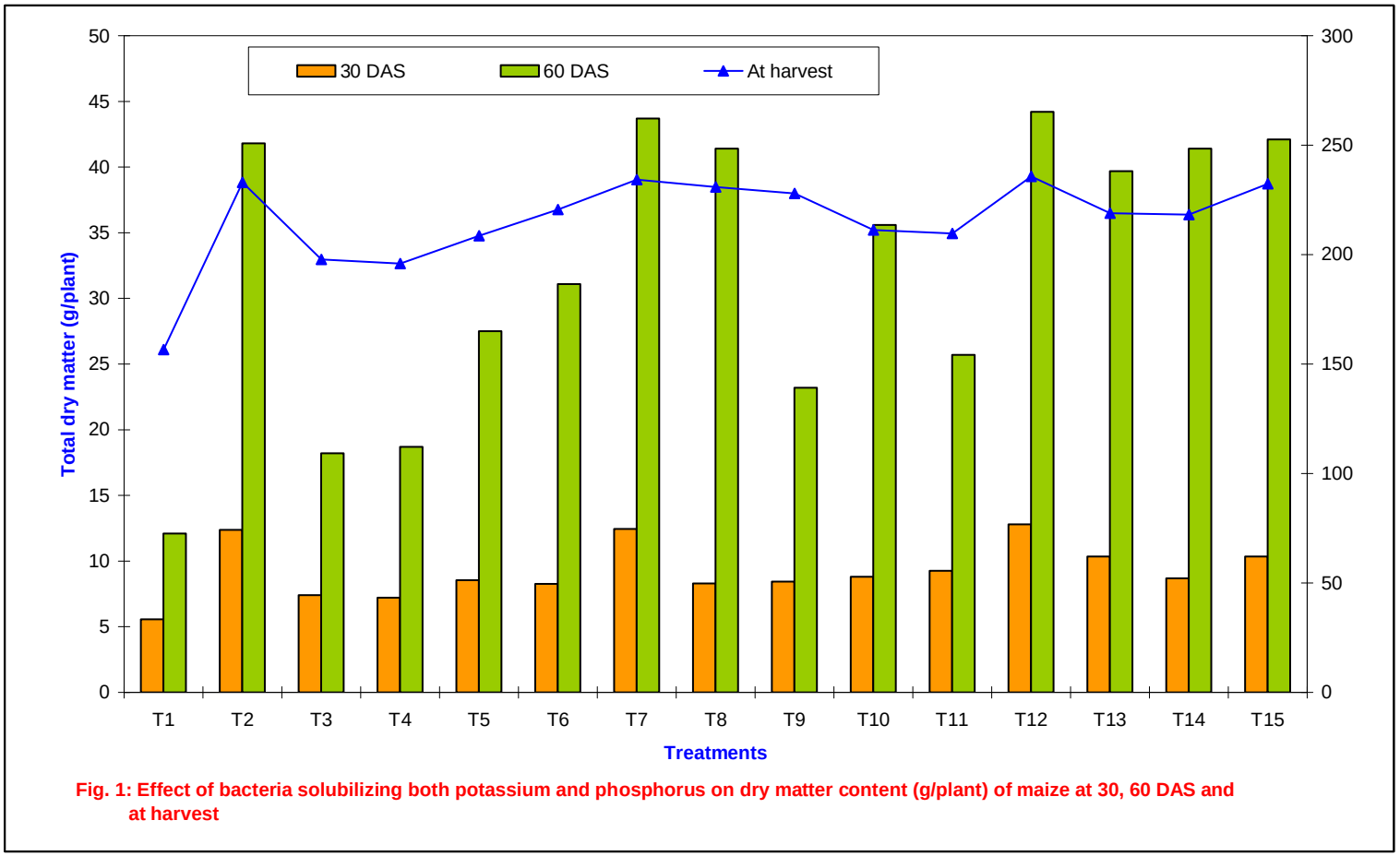


Fig. 1: Effect of bacteria solubilizing both potassium and phosphorus on dry matter content (g/plant) of maize at 30, 60 DAS and at harvest

Fig. 1: Effect of bacteria solubilizing both potassium and phosphorus on dry matter content (g/plant) of maize at 30, 60 DAS and

Table 15: Effect of bacteria solubilizing both potassium and phosphorus on yield and yield parameters of maize plants at harvest

Tr. No.	Treatment	Cob weight (g/plant)	Grain yield (g/plant)	Test weight (g/ 100grain)
T1	Control (no inoculation and no fertilizer)	75.40	23.80	19.63
T2	Recommended Dose of Fertilizer	112.60	51.03	26.33
T3	Recommended Dose of potash with rock phosphate	88.60	36.70	21.87
T4	Recommended Dose of phosphorus with Mica	85.40	38.57	22.57
T5	Local strain of *Bacillus* (KSB-11) with RP and Mica	93.50	32.03	26.60
T6	Local strain of *Pseudomonas striata* (PSB 98 (2))with RP and Mica	101.40	44.60	25.63
T7	Co-inoculation of local strain of KSB-11 and PSB 98(2) with RP and Mica	113.40	52.80	28.03
T8	Isolate K-PSB 2 with RP and Mica	109.80	45.93	23.50
T9	Isolate K-PSB 20 with RP and Mica	106.70	41.87	26.53
T10	Isolate K-PSB 21 with RP and Mica	91.30	39.60	25.93
T11	Isolate K-PSB 28with RP and Mica	96.40	37.77	26.83
T12	Isolate K-PSB 32 with RP and Mica	112.47	50.93	27.77
T13	Isolate K-PSB 36 with RP and Mica	105.80	46.50	24.23
T14	Isolate K-PSB 39 with RP and Mica	103.00	42.53	25.57
T15	Isolate K-PSB 50 with RP and Mica	114.90	52.93	28.63
	S.Em ±	0.23	0.19	0.21
	CD @1%	0.78	0.72	0.75

LEGEND

T_1	Control (no inoculation and no fertilizer)
T_2	Recommended Dose of Fertilizer
T_3	Recommended Dose of potash with rock phosphate (RP)
T4	Recommended Dose of phosphorus with Mica
T5	Local strain of *Bacillus* (KSB-11) with RP and Mica
T6	Local strain of *Pseudomonas striata* (PSB 98 (2)) with RP and Mica
T7	Co-inoculation of local strain of KSB-11 and PSB 98(2) with RP and Mica
T_8	Isolate K-PSB 2 with RP and Mica
T_9	Isolate K-PSB 20 with RP and Mica
T_{10}	Isolate K-PSB 21 with RP and Mica
T_{11}	Isolate K-PSB 28 with RP and Mica
T_{12}	Isolate K-PSB 32 with RP and Mica
T_{13}	Isolate K-PSB 39 with RP and Mica
T_{14}	Isolate K-PSB 36 with RP and Mica
T_{15}	Isolate K-PSB 50 with RP and Mica

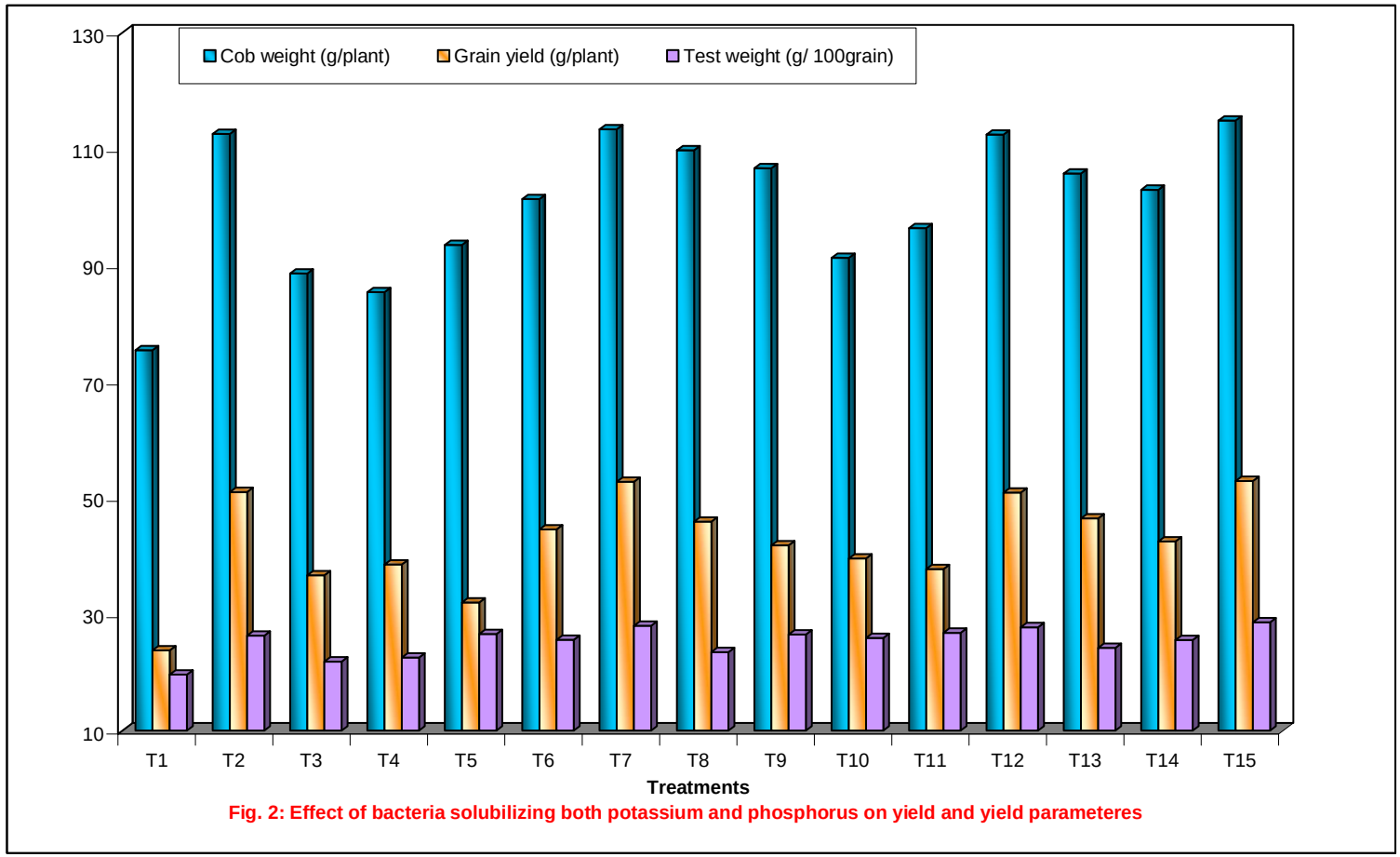


Fig. 2: Effect of bacteria solubilizing both potassium and phosphorus on yield and yield parameteres

Fig. 2: Effect of bacteria solubilizing both potassium and phosphorus on yield and yield parameteres

Were 52.93 g/plant and was on par with the treatment co-inoculated local strains of *Bacillus* and *P. striata* with rock phosphate and mica (52.80 g/plant) (Table 15).

4.7.3 Hundred grain weight

Hundred grain weight was found to be increased significantly with the treatment receiving inoculation of K-PSB 50 with rock phosphate and mica (28.63 g/100 grain) and was on par with treatment with co-inoculated local strains of *Bacillus* and *P. striata* with rock phosphate and mica (28.03 g/100grain) and K-PSB 32 with rock phosphate and mica (27.77 g/100 grain). All the treatment was significantly higher grain weight over control (Table 15).

4.7.4 Nutrient concentration of plant (%)

The data on plant nutrient concentration is given in (Table 16, 17 and 18)

4.7.5 Nitrogen concentration (%)

At 30 DAS N concentration of plant was significantly highest in inoculation of K-PSB 32 with rock phosphate and mica (2.94 %) and was on par with the treatment co-inoculated local strains of *Bacillus* and *P. striata* with rock phosphate and mica (2.89 %) and K-PSB 50. Same trend was recorded at 60 DAS and at harvest. However, all the treatment was higher N concentration over control (Table 16) fig 3.

4.7.6 Phosphorus concentration (%)

All the inoculated treatments recorded significantly higher phosphorus concentration over control (Table 17) fig 4.

At 30 DAS significantly highest P concentration was recorded isolate K-PSB 28 with rock phosphate and mica (0.41%) and was on par with treatment Control with RDF (0.40%). and was at 30 DAS inoculated isolates superior each other.

At 60 DAS and at harvest significantly higher P concentration recorded inoculation of isolate K-PSB 32 with rock phosphate and mica (0.50 and 0.45 per cent, respectively) and all other isolates were on par with each other.

4.7.7 Potassium concentration (%)

All the inoculated treatments recorded significantly higher potassium concentration over control (Table 18) fig 5.

At 30, 60 DAS and at harvest significantly higher K concentration recorded inoculation of K-PSB 32 with rock phosphate and mica were 3.18, 4.10 and 2.75 per cent, receptively and was on par with the treatment co-inoculated local strains of *Bacillus* and *P. striata* with rock phosphate and Mica (2.92, 3.85 and 2.68 per cent, receptively) and treatment RDF and was significantly isolates superior over control.

4.8 Enzymatic activity in soil

The data recorded on enzymatic activities in soil viz., dehydrogenase (µg TPF/g/day) and phosphatase (µg PNP/g/h) at 30, 60 DAS and at harvesting is presented in Table 19 and 20.

All the inoculated treatments recorded significantly higher dehydrogenase and phosphatase over control.

In case dehydrogenase activity at 30, 60 DAS and at harvest inoculation of isolate K-PSB 32 with rock phosphate and mica recorded significantly heights dehydrogenase activity were 3.83, 6.50 and 4.63 µg TPF/g/day, respectively and on par with the treatment co-inoculated local strains of *Bacillus* and *P. striata* with rock phosphate and mica 3.43, 6.40 and 4.33 µg TPF/g/day, respectively. K-PSB 50, 3.45, 5.20 and 4.27 µg TPF/g/day, respectively and treatment RDF (3.23.,5.30 and 4.18 µg TPF/g/day, respectively) (Table: 19) fig 6.

In case of phosphatase activity at 30, 60 DAS and at harvest inoculation of isolate K-PSB 32 with rock phosphate and mica recorded significantly heights phosphatase activity (16.47, 20.40 and 17.53 µg PNP/g/day, respectively) and was on par with the treatment co-inoculated local strains of *Bacillus* and *P. striata* with rock phosphate and mica (15.53, 19.53 and 17.20 µg PNP/g/day, respectively) (Table 20) fig 7.

Table16: Effect of bacteria solubilizing both potassium and phosphorus on plant nitrogen concentration (%)

Sl.No.	Treatment	N concentration (%)		
		30 DAS	60 DAS	At harvest
T1	Control (no inoculation and no fertilizer)	1.16	1.36	0.85
T2	Recommended Dose of Fertilizer	2.76	3.65	2.37
T3	Recommended Dose of potash with rock phosphate	1.36	1.75	1.55
T4	Recommended Dose of phosphorus with Mica	1.55	1.94	1.47
T5	Local strain of *Bacillus* (KSB-11) with RP and Mica	2.25	2.85	2.35
T6	Local strain of *Pseudomonas striata* (PSB 98 (2))with RP and Mica	2.19	3.27	2.03
T7	Co-inoculation of local strain of KSB-11 and PSB 98(2) with RP and Mica	2.89	3.75	2.55
T8	Isolate K-PSB 2 with RP and Mica	2.65	3.47	2.45
T9	Isolate K-PSB 20 with RP and Mica	2.54	3.51	2.33
T10	Isolate K-PSB 21 with RP and Mica	2.26	3.17	2.20
T11	Isolate K-PSB 28with RP and Mica	2.55	3.55	2.46
T12	Isolate K-PSB 32 with RP and Mica	2.94	3.85	2.69
T13	Isolate K-PSB 36 with RP and Mica	2.46	3.48	2.36
T14	Isolate K-PSB 39 with RP and Mica	2.36	3.41	2.25
T15	Isolate K-PSB 50 with RP and Mica	2.85	3.66	2.47
	S.Em ±	0.02	0.02	0.04
	CD @1%	0.06	0.08	0.14

LEGEND

T_1	Control (no inoculation and no fertilizer)
T_2	Recommended Dose of Fertilizer
T_3	Recommended Dose of potash with rock phosphate (RP)
T4	Recommended Dose of phosphorus with Mica
T5	Local strain of *Bacillus* (KSB-11) with RP and Mica
T6	Local strain of *Pseudomonas striata* (PSB 98 (2)) with RP and Mica
T7	Co-inoculation of local strain of KSB-11 and PSB 98(2) with RP and Mica
T_8	Isolate K-PSB 2 with RP and Mica
T_9	Isolate K-PSB 20 with RP and Mica
T_{10}	Isolate K-PSB 21 with RP and Mica
T_{11}	Isolate K-PSB 28 with RP and Mica
T_{12}	Isolate K-PSB 32 with RP and Mica
T_{13}	Isolate K-PSB 39 with RP and Mica
T_{14}	Isolate K-PSB 36 with RP and Mica
T_{15}	Isolate K-PSB 50 with RP and Mica

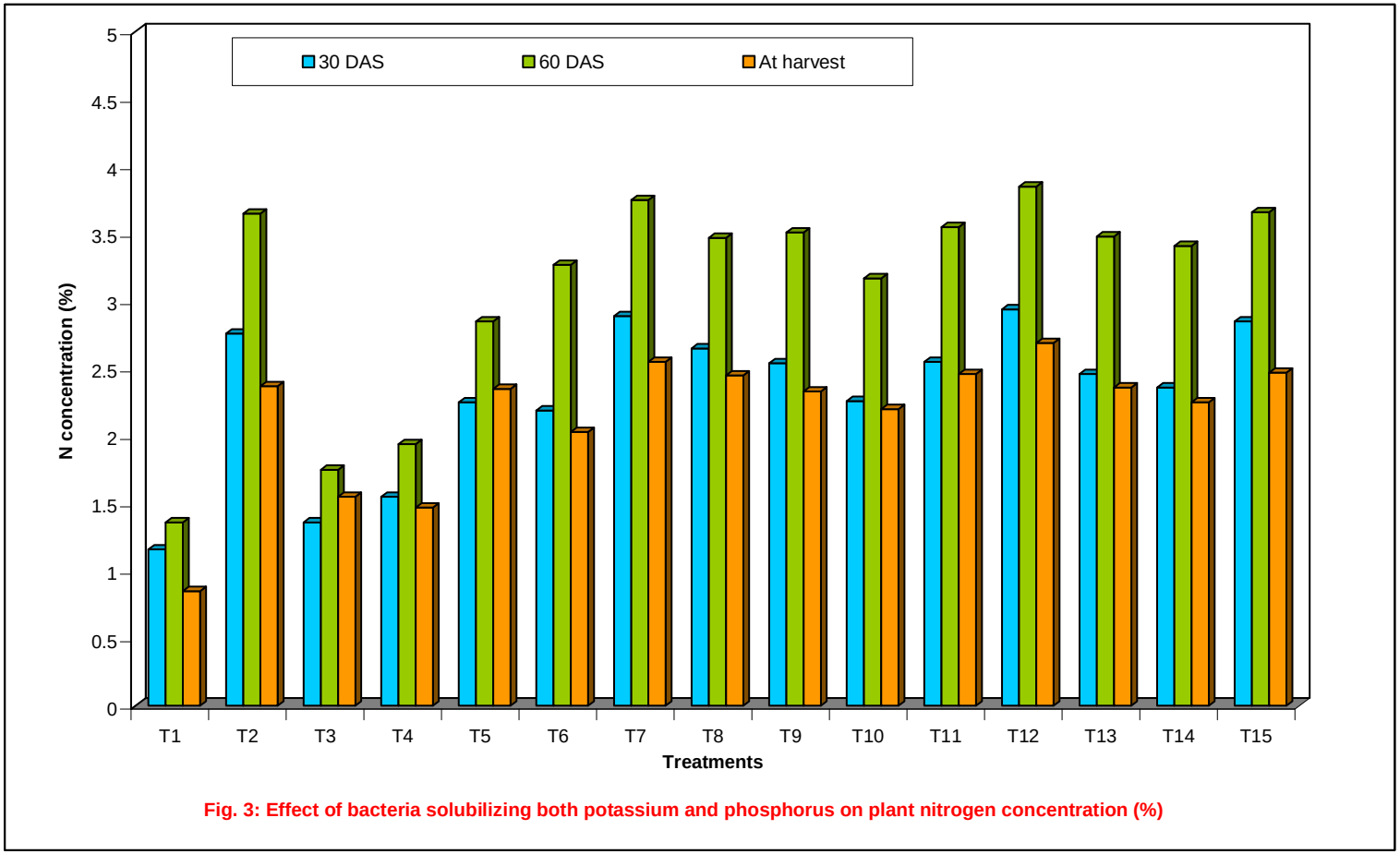


Fig. 3: Effect of bacteria solubilizing both potassium and phosphorus on plant nitrogen concentration (%)

Fig. 3: Effect of bacteria solubilizing both potassium and phosphorus on plant nitrogen concentration (%)

Table 17: Effect of bacteria solubilizing both potassium and phosphorus on plant phosphorus concentration (%)

Sl.No.	Treatment	P concentration (%)		
		30 DAS	60 DAS	AT Harvest
T1	Control (no inoculation and no fertilizer)	0.15	0.28	0.23
T2	Recommended Dose of Fertilizer	0.40	0.45	0.42
T3	Recommended Dose of potash with rock phosphate	0.25	0.34	0.34
T4	Recommended Dose of phosphorus with Mica	0.25	0.40	0.36
T5	Local strain of *Bacillus* (KSB-11) with RP and Mica	0.26	0.40	0.30
T6	Local strain of *Pseudomonas striata* (PSB 98 (2))with RP and Mica	0.34	0.46	0.40
T7	Co-inoculation of local strain of KSB-11 and PSB 98(2) with RP and Mica	0.39	0.46	0.43
T8	Isolate K-PSB 2 with RP and Mica	0.33	0.38	0.36
T9	Isolate K-PSB 20 with RP and Mica	0.34	0.38	0.37
T10	Isolate K-PSB 21 with RP and Mica	0.33	0.43	0.33
T11	Isolate K-PSB 28with RP and Mica	0.41	0.44	0.36
T12	Isolate K-PSB 32 with RP and Mica	0.38	0.50	0.45
T13	Isolate K-PSB 36 with RP and Mica	0.35	0.37	0.34
T14	Isolate K-PSB 39 with RP and Mica	0.28	0.37	0.29
T15	Isolate K-PSB 50 with RP and Mica	0.37	0.42	0.40
	S.Em ±	0.013	0.014	0.011
	CD @1%	0.042	0.042	0.038

LEGEND

T_1	Control (no inoculation and no fertilizer)
T_2	Recommended Dose of Fertilizer
T_3	Recommended Dose of potash with rock phosphate (RP)
T4	Recommended Dose of phosphorus with Mica
T5	Local strain of *Bacillus* (KSB-11) with RP and Mica
T6	Local strain of *Pseudomonas striata* (PSB 98 (2)) with RP and Mica
T7	Co-inoculation of local strain of KSB-11 and PSB 98(2) with RP and Mica
T_8	Isolate K-PSB 2 with RP and Mica
T_9	Isolate K-PSB 20 with RP and Mica
T_{10}	Isolate K-PSB 21 with RP and Mica
T_{11}	Isolate K-PSB 28 with RP and Mica
T_{12}	Isolate K-PSB 32 with RP and Mica
T_{13}	Isolate K-PSB 39 with RP and Mica
T_{14}	Isolate K-PSB 36 with RP and Mica
T_{15}	Isolate K-PSB 50 with RP and Mica

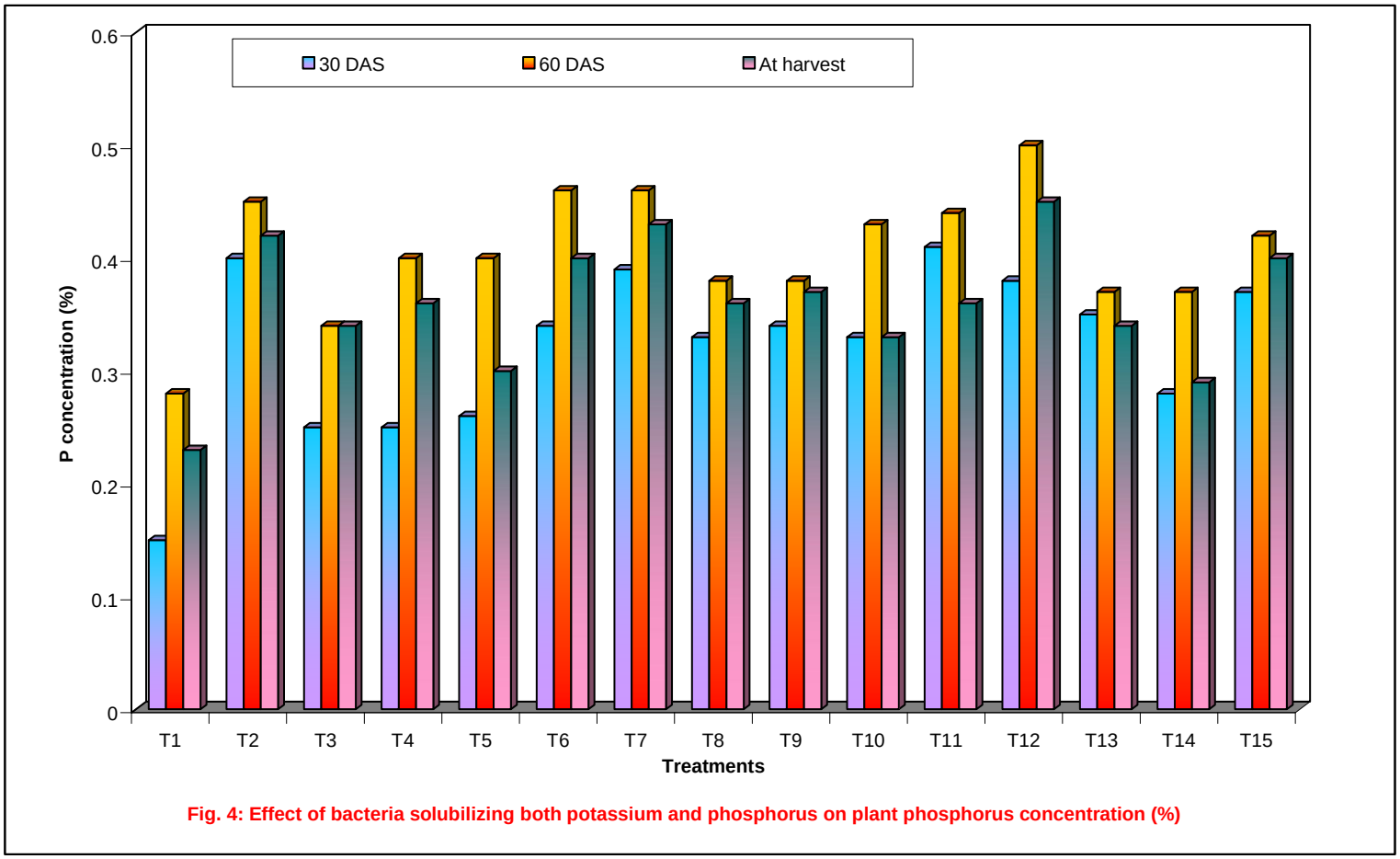


Fig. 4: Effect of bacteria solubilizing both potassium and phosphorus on plant phosphorus concentration (%)

Fig. 4: Effect of bacteria solubilizing both potassium and phosphorus on plant phosphorus concentration (%)

Table 18: Effect of bacteria solubilizing both potassium and phosphorus on Potassium concentration (%)

Sl.No.	Treatment	K concentration (%)		
		30 DAS	60 DAS	AT Harvest
T1	Control (no inoculation and no fertilizer)	1.09	1.81	1.12
T2	Recommended Dose of Fertilizer	2.90	3.80	2.60
T3	Recommended Dose of potash with rock phosphate	2.22	2.22	1.28
T4	Recommended Dose of phosphorus with Mica	1.33	2.25	1.15
T5	Local strain of *Bacillus* (KSB-11) with RP and Mica	2.77	3.45	2.33
T6	Local strain of *Pseudomonas striata* (PSB 98 (2))with RP and Mica	1.73	2.74	1.77
T7	Co-inoculation of local strain of KSB-11 and PSB 98(2) with RP and Mica	2.92	3.85	2.68
T8	Isolate K-PSB 2 with RP and Mica	2.73	3.10	2.27
T9	Isolate K-PSB 20 with RP and Mica	2.30	3.23	2.47
T10	Isolate K-PSB 21 with RP and Mica	2.50	3.60	2.33
T11	Isolate K-PSB 28with RP and Mica	2.24	3.45	1.73
T12	Isolate K-PSB 32 with RP and Mica	3.18	4.10	2.75
T13	Isolate K-PSB 36 with RP and Mica	2.45	3.60	2.32
T14	Isolate K-PSB 39 with RP and Mica	2.75	3.57	2.31
T15	Isolate K-PSB 50 with RP and Mica	2.78	3.67	2.35
	S.Em ±	0.08	0.12	0.07
	CD @1%	0.30	0.41	0.28

LEGEND

T_1	Control (no inoculation and no fertilizer)
T_2	Recommended Dose of Fertilizer
T_3	Recommended Dose of potash with rock phosphate (RP)
T4	Recommended Dose of phosphorus with Mica
T5	Local strain of *Bacillus* (KSB-11) with RP and Mica
T6	Local strain of *Pseudomonas striata* (PSB 98 (2)) with RP and Mica
T7	Co-inoculation of local strain of KSB-11 and PSB 98(2) with RP and Mica
T_8	Isolate K-PSB 2 with RP and Mica
T_9	Isolate K-PSB 20 with RP and Mica
T_{10}	Isolate K-PSB 21 with RP and Mica
T_{11}	Isolate K-PSB 28 with RP and Mica
T_{12}	Isolate K-PSB 32 with RP and Mica
T_{13}	Isolate K-PSB 39 with RP and Mica
T_{14}	Isolate K-PSB 36 with RP and Mica
T_{15}	Isolate K-PSB 50 with RP and Mica

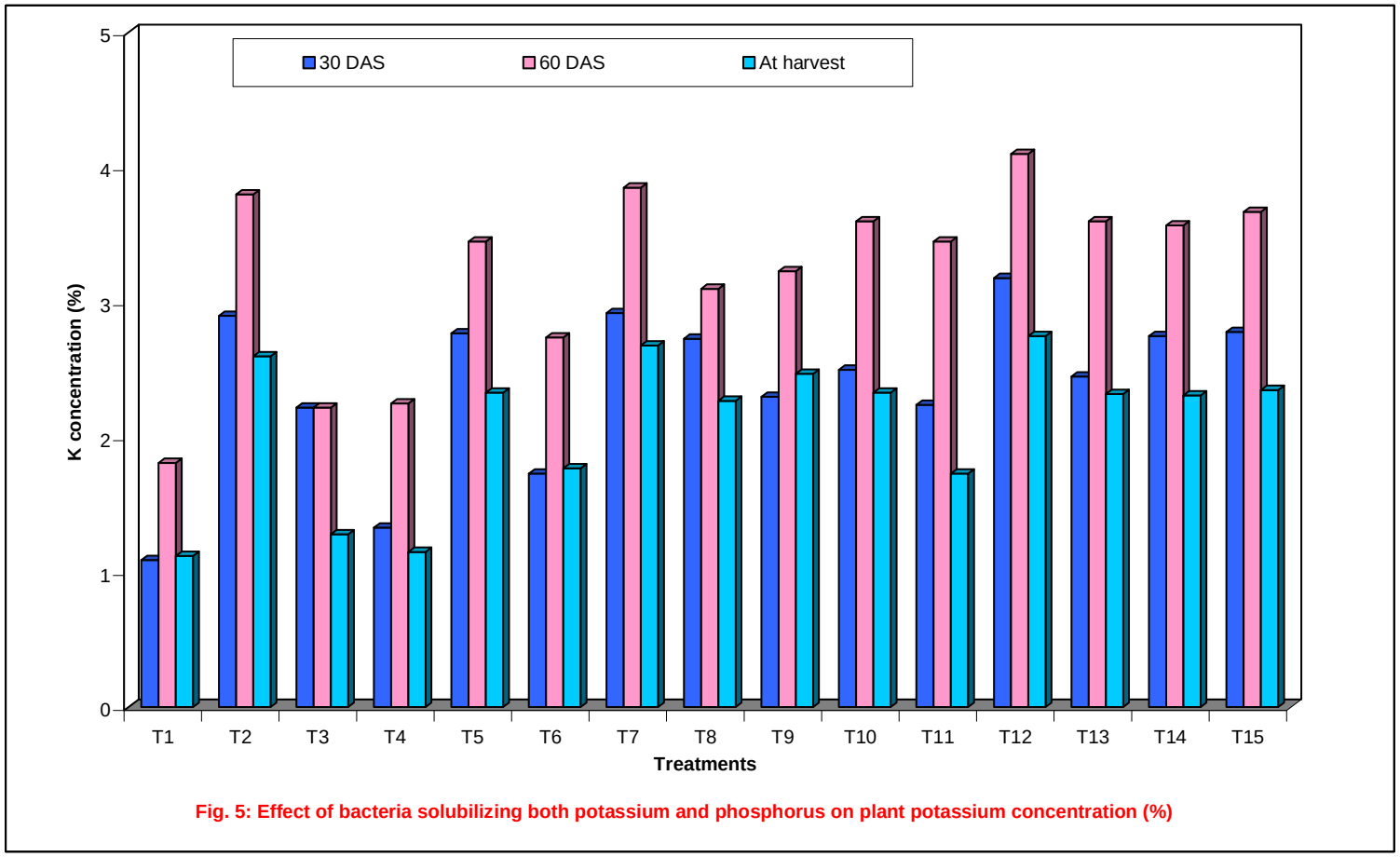


Fig. 5: Effect of bacteria solubilizing both potassium and phosphorus on plant potassium concentration (%)

Fig. 5: Effect of bacteria solubilizing both potassium and phosphorus on plant potassium concentration (%)

Table 19: Effect of bacteria solubilizing both potassium and phosphorus on dehydrogenase activity (µg TPF/g soil/day) of soil

Sl.No.	Treatment	30 DAS	60 DAS	At harvest
T1	Control (no inoculation and no fertilizer)	0.79	2.17	1.43
T2	Recommended Dose of Fertilizer	3.23	5.30	4.18
T3	Recommended Dose of potash with rock phosphate	2.27	3.43	2.82
T4	Recommended Dose of phosphorus with Mica	2.13	3.97	3.50
T5	Local strain of *Bacillus* (KSB-11) with RP and Mica	3.15	4.33	3.37
T6	Local strain of *Pseudomonas striata* (PSB 98 (2))with RP and Mica	3.04	4.13	3.50
T7	Co-inoculation of local strain of KSB-11 and PSB 98(2) with RP and Mica	3.43	6.40	4.33
T8	Isolate K-PSB 2 with RP and Mica	3.31	4.70	3.57
T9	Isolate K-PSB 20 with RP and Mica	3.27	4.27	3.77
T10	Isolate K-PSB 21 with RP and Mica	2.55	3.32	2.47
T11	Isolate K-PSB 28with RP and Mica	3.40	4.73	3.80
T12	Isolate K-PSB 32 with RP and Mica	3.83	6.50	4.63
T13	Isolate K-PSB 36 with RP and Mica	3.13	4.80	2.93
T14	Isolate K-PSB 39 with RP and Mica	3.03	4.60	3.30
T15	Isolate K-PSB 50 with RP and Mica	3.45	5.20	4.27
	S.Em ±	0.10	0.06	0.09
	CD @1%	0.38	0.22	0.31

LEGEND

T_1	Control (no inoculation and no fertilizer)
T_2	Recommended Dose of Fertilizer
T_3	Recommended Dose of potash with rock phosphate (RP)
T4	Recommended Dose of phosphorus with Mica
T5	Local strain of *Bacillus* (KSB-11) with RP and Mica
T6	Local strain of *Pseudomonas striata* (PSB 98 (2)) with RP and Mica
T7	Co-inoculation of local strain of KSB-11 and PSB 98(2) with RP and Mica
T_8	Isolate K-PSB 2 with RP and Mica
T_9	Isolate K-PSB 20 with RP and Mica
T_{10}	Isolate K-PSB 21 with RP and Mica
T_{11}	Isolate K-PSB 28 with RP and Mica
T_{12}	Isolate K-PSB 32 with RP and Mica
T_{13}	Isolate K-PSB 39 with RP and Mica
T_{14}	Isolate K-PSB 36 with RP and Mica
T_{15}	Isolate K-PSB 50 with RP and Mica

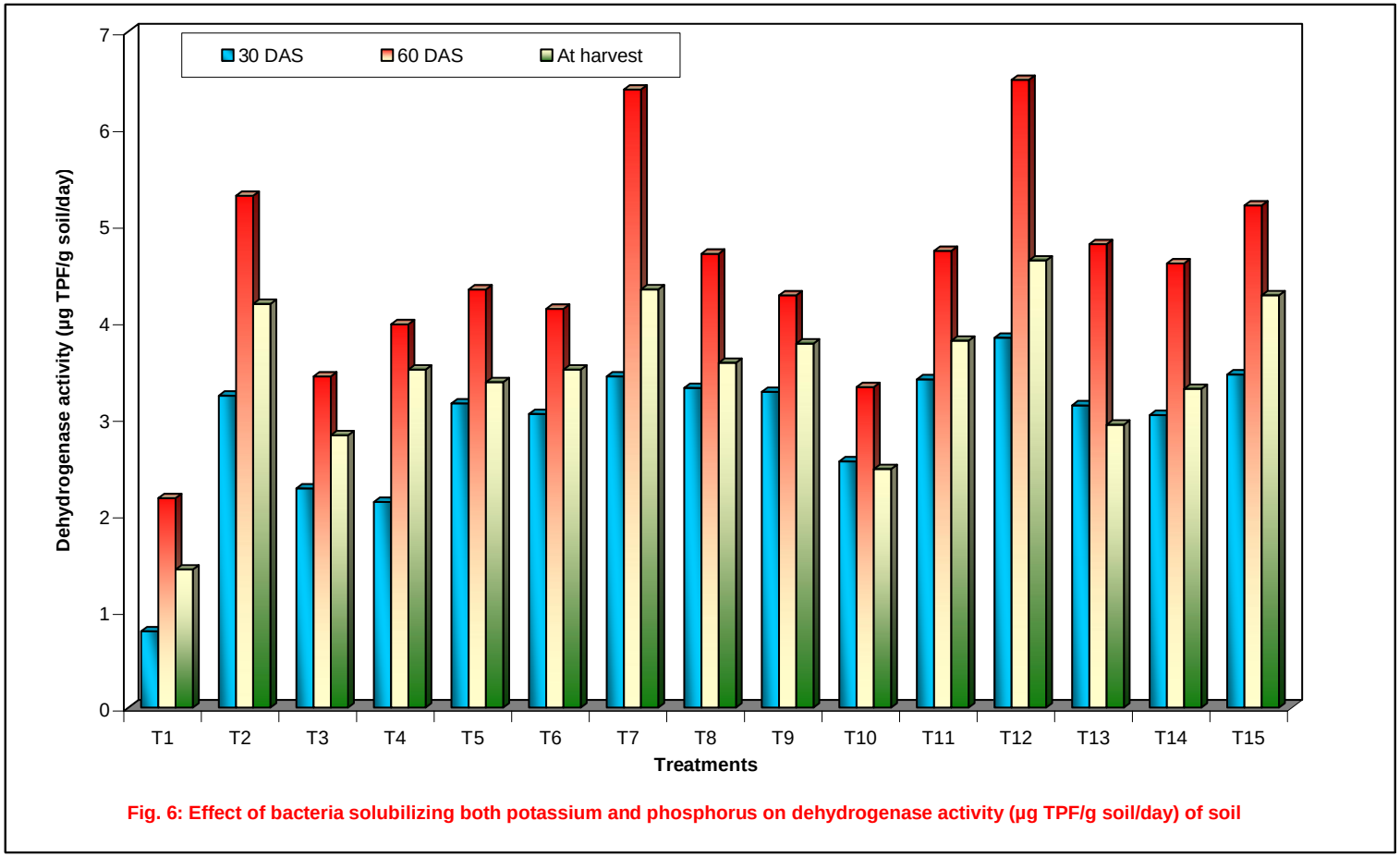


Fig. 6: Effect of bacteria solubilizing both potassium and phosphorus on dehydrogenase activity (µg TPF/g soil/day) of soil

Fig. 6: Effect of bacteria solubilizing both potassium and phosphorus on dehydrogenase activity (µg TPF/g soil/day) of soil

4.9 Beneficial microbial population

The results were recorded on the microbial population (rhizosphere soil) *viz*., potassium solubilizing microorganisms (KSB), phosphorus solubilizing microorganisms (PSB) and free-living nitrogen fixers (FNF) at 30, 60 DAS and at harvest (Tables: 21, 22 and 23).

4.9.1 Beneficial microbial population of rhizosphere PSB, KSB and free living nitrogen fixers (cfu/g of soil).

In case of nitrogen fixing bacteria at 30, 60 DAS and at harvest significantly maximum microbial population observed inoculation of K-PSB 50 with rock phosphate and mica were 11.73 ,27.20 and 16.67 cfu x 10^4/g soil and was on par with all other inoculated isolates. However, all inoculated isolates were superior over control.

In case of PSB at 30 DAS significantly maximum microbial population observed inoculation of K-PSB 32 with Rock phosphate and mica (14.50 cfu x 10^4/g soil). At 60 DAS and at harvest significantly maximum microbial population recorded inoculation of K-PSB 50 with rock phosphate and mica (25.60 and 18.73 cfu x 10^4/g soil) and was on par with other inoculated isolates. However, all inoculated isolates were superior over control.

In case of KSB at 30, 60 DAS and at harvest significantly maximum microbial population observed inoculation of K-PSB 50 (9.43, 19.03 and 13.63 cfu x 10^4/g soil) and was on par with other inoculated isolates. However, all inoculated isolates were superior over control.

The treatments with inoculation of bacteria solubilizing both potassium and phosphorus did not differ significantly and were on par with each other .There was increase in microbial population at 60 DAS but the decrease in population observed at harvest.

Table 20: Effect of bacteria solubilizing both potassium and phosphorus on phosphatase activity (µg PNP/g soil/day) of soil

Sl.No.	Treatment	30 DAS	60 DAS	At harvest
T1	Control (no inoculation and no fertilizer)	7.33	10.37	9.53
T2	Recommended Dose of Fertilizer	14.13	17.50	16.50
T3	Recommended Dose of potash with rock phosphate	8.50	11.47	10.43
T4	Recommended Dose of phosphorus with Mica	8.90	12.37	11.63
T5	Local strain of *Bacillus* (KSB-11) with RP and Mica	12.87	15.50	12.57
T6	Local strain of *Pseudomonas striata* (PSB 98 (2))with RP and Mica	13.47	16.73	14.47
T7	Co-inoculation of local strain of KSB-11 and PSB 98(2) with RP and Mica	15.53	19.53	17.20
T8	Isolate K-PSB 2 with RP and Mica	14.60	17.40	16.60
T9	Isolate K-PSB 20 with RP and Mica	12.70	17.57	14.70
T10	Isolate K-PSB 21 with RP and Mica	13.90	16.53	15.83
T11	Isolate K-PSB 28with RP and Mica	13.10	17.47	14.57
T12	Isolate K-PSB 32 with RP and Mica	16.47	20.40	17.53
T13	Isolate K-PSB 36 with RP and Mica	13.80	18.43	14.60
T14	Isolate K-PSB 39 with RP and Mica	15.27	18.30	15.53
T15	Isolate K-PSB 50 with RP and Mica	15.40	19.50	16.47
	S.Em ±	0.30	0.15	0.21
	CD @1%	1.01	0.58	0.71

LEGEND

T_1	Control (no inoculation and no fertilizer)
T_2	Recommended Dose of Fertilizer
T_3	Recommended Dose of potash with rock phosphate (RP)
T4	Recommended Dose of phosphorus with Mica
T5	Local strain of *Bacillus* (KSB-11) with RP and Mica
T6	Local strain of *Pseudomonas striata* (PSB 98 (2)) with RP and Mica
T7	Co-inoculation of local strain of KSB-11 and PSB 98(2) with RP and Mica
T_8	Isolate K-PSB 2 with RP and Mica
T_9	Isolate K-PSB 20 with RP and Mica
T_{10}	Isolate K-PSB 21 with RP and Mica
T_{11}	Isolate K-PSB 28 with RP and Mica
T_{12}	Isolate K-PSB 32 with RP and Mica
T_{13}	Isolate K-PSB 39 with RP and Mica
T_{14}	Isolate K-PSB 36 with RP and Mica
T_{15}	Isolate K-PSB 50 with RP and Mica

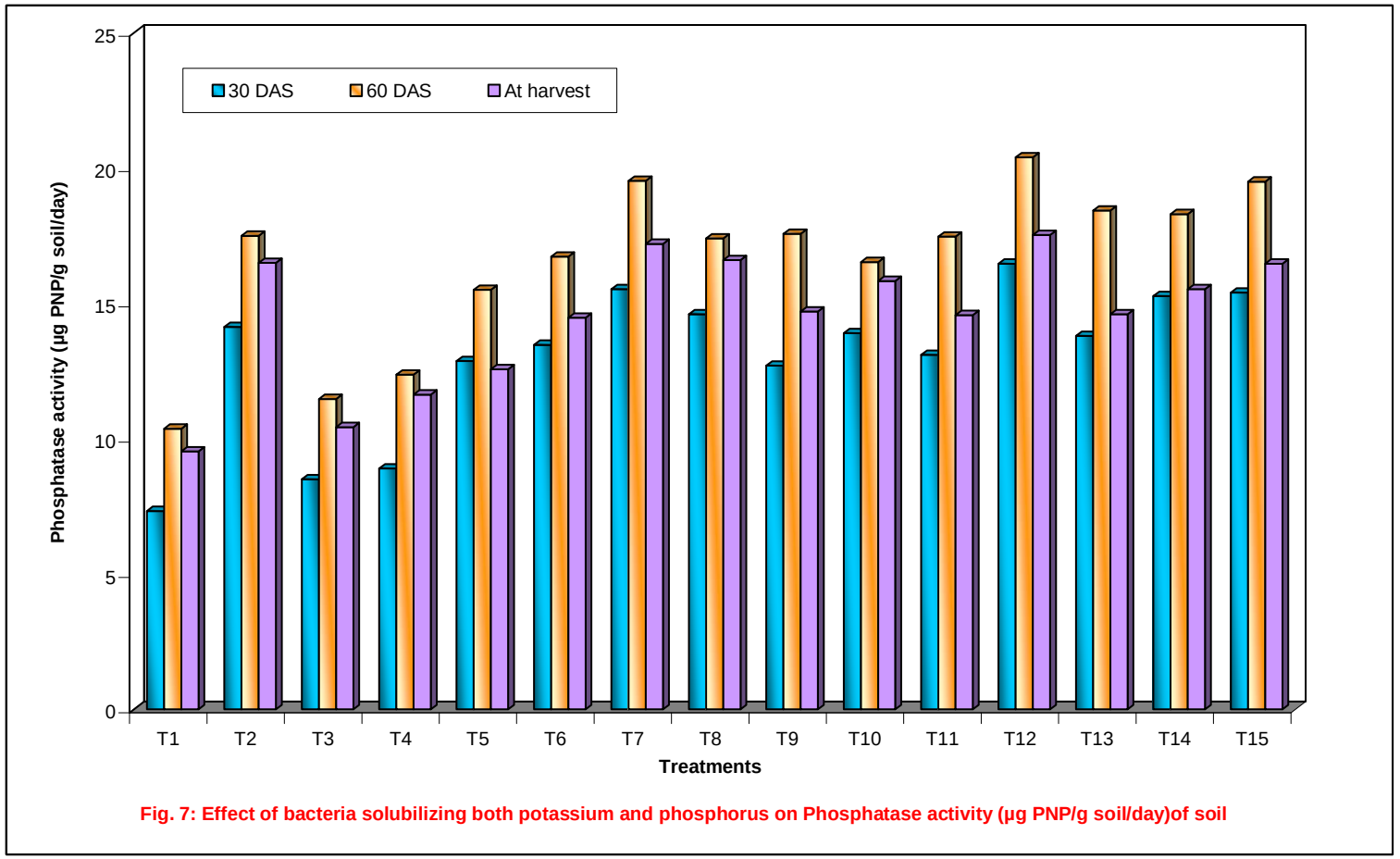


Fig. 7: Effect of bacteria solubilizing both potassium and phosphorus on Phosphatase activity (µg PNP/g soil/day)of soil

Fig. 7: Effect of bacteria solubilizing both potassium and phosphorus on Phosphatase activity (µg PNP/g soil/day)of soil

Table 21: Population of nitrogen fixers in the rhizosphere of maize influenced by bacteria strains solubilizing both potassium and phosphorus at 30, 60 DAS and at harvest

Sl.No.	Treatment	Nitrogen fixers population (cfu X 10^4 /g soil)		
		30 DAS	60 DAS	At harvest
T1	Control (no inoculation and no fertilizer)	5.97	12.57	8.57
T2	Recommended Dose of Fertilizer	8.97	25.87	15.53
T3	Recommended Dose of potash with rock phosphate	8.10	18.40	11.50
T4	Recommended Dose of phosphorus with Mica	7.77	20.53	12.77
T5	Local strain of *Bacillus* (KSB-11) with RP and Mica	8.60	23.47	12.77
T6	Local strain of *Pseudomonas striata* (PSB 98 (2))with RP and Mica	9.00	23.53	14.33
T7	Co-inoculation of local strain of KSB-11 and PSB 98(2) with RP and Mica	10.87	26.03	16.40
T8	Isolate K-PSB 2 with RP and Mica	9.53	23.40	13.47
T9	Isolate K-PSB 20 with RP and Mica	10.50	23.60	13.40
T10	Isolate K-PSB 21 with RP and Mica	10.27	24.43	15.57
T11	Isolate K-PSB 28with RP and Mica	9.83	24.97	15.40
T12	Isolate K-PSB 32 with RP and Mica	10.50	22.47	16.03
T13	Isolate K-PSB 36 with RP and Mica	10.90	21.70	13.07
T14	Isolate K-PSB 39 with RP and Mica	10.83	22.60	14.77
T15	Isolate K-PSB 50 with RP and Mica	11.73	27.20	16.67
	S.Em ±	0.45	0.29	0.19
	CD @1%	1.51	0.94	0.67

Table 22: Population of PSB in the rhizosphere of maize influenced by potassium bacteria strains solubilizing both potassium and phosphorus at 30, 60 DAS and at harvest

Sl.No.	Treatment	PSB population (cfu X 10^4/g soil)		
		30 DAS	60 DAS	At harvest
T1	Control (no inoculation and no fertilizer)	6.80	11.53	7.67
T2	Recommended Dose of Fertilizer	11.53	18.80	15.60
T3	Recommended Dose of potash with rock phosphate	8.47	11.70	9.70
T4	Recommended Dose of phosphorus with Mica	7.00	11.93	10.60
T5	Local strain of *Bacillus* (KSB-11) with RP and Mica	12.17	16.57	12.80
T6	Local strain of *Pseudomonas striata* (PSB 98 (2))with RP and Mica	12.53	22.77	15.40
T7	Co-inoculation of local strain of KSB-11 and PSB 98(2) with RP and Mica	14.20	24.77	18.50
T8	Isolate K-PSB 2 with RP and Mica	13.27	23.03	17.40
T9	Isolate K-PSB 20 with RP and Mica	12.07	22.43	13.57
T10	Isolate K-PSB 21 with RP and Mica	12.70	22.53	15.73
T11	Isolate K-PSB 28with RP and Mica	13.03	24.63	14.70
T12	Isolate K-PSB 32 with RP and Mica	14.50	24.63	16.53
T13	Isolate K-PSB 36 with RP and Mica	13.73	22.37	18.63
T14	Isolate K-PSB 39 with RP and Mica	13.57	22.70	17.43
T15	Isolate K-PSB 50 with RP and Mica	14.47	25.60	18.73
	S.Em ±	0.24	0.21	0.11
	CD @1%	0.81	0.73	0.41

Table 23: Population of KSB in the rhizosphere of maize influenced by bacteria strains solubilizing both potassium and phosphorus at 30, 60 DAS and at harvest

Sl.No.	Treatment	KSB population (cfu X 10^4 /g soil)		
		30 DAS	60 DAS	At harvest
T1	Control (no inoculation and no fertilizer)	3.17	5.87	5.53
T2	Recommended Dose of Fertilizer	7.07	13.63	10.57
T3	Recommended Dose of potash with rock phosphate	6.53	12.80	9.43
T4	Recommended Dose of phosphorus with Mica	5.30	10.70	7.50
T5	Local strain of *Bacillus* (KSB-11) with RP and Mica	8.57	18.40	12.43
T6	Local strain of *Pseudomonas striata* (PSB 98 (2))with RP and Mica	8.47	17.40	10.23
T7	Co-inoculation of local strain of KSB-11 and PSB 98(2) with RP and Mica	9.20	18.73	13.43
T8	Isolate K-PSB 2 with RP and Mica	8.63	16.47	9.87
T9	Isolate K-PSB 20 with RP and Mica	7.33	15.53	10.40
T10	Isolate K-PSB 21 with RP and Mica	8.73	18.53	12.67
T11	Isolate K-PSB 28with RP and Mica	8.50	16.63	10.60
T12	Isolate K-PSB 32 with RP and Mica	9.13	18.30	13.31
T13	Isolate K-PSB 36 with RP and Mica	8.60	18.57	12.60
T14	Isolate K-PSB 39 with RP and Mica	8.77	17.53	11.73
T15	Isolate K-PSB 50 with RP and Mica	9.43	19.03	13.63
	S.Em ±	0.15	0.14	0.15
	CD @1%	0.54	0.50	0.51

DISCUSSION

Microorganisms play a key role in the field of agriculture by converting the unavailable form of nutrient to available form there by increasing its availability in soil and enhancing agricultural production.

Soil is the dynamic ecosystems that harbour many micro-organisms which are closely related to the plants. Microorganisms play their role in two ways one as pathogens causing diseases and the other as beneficial ones such as biological control agents, nutrient mobilizers and solubilizes. Our interest was to elucidate the mechanisms involved in solubilization of some of the fixed nutrients especially P and K for the agriculture use.

The potassium solubilization ability of the bacteria could be attributed to the production of protons, organic acids, siderophores, exopolysaccharides, and organic ligands (Groudev, 1987; Grayston *et al*., 1997; Welch *et al*., 1999; Liermann *et al*., 2000 and Rogers and Bennett, 2004).

The phosphorus solubilization bacteria have been ability to converts insoluble rock phosphate into soluble and available forms for plant growth (Nahas *et al*., 1990 and Bojinova *et al*., 1997). This conversation is through acidification, chelation and exchange reaction (Gerke, 1992) and produced in the periplasm, strong organic acids (Aleksandrov, 1967).

The numerous microorganisms particularly those associated with plant roots have the ability to increase plant growth and productivity (Cheng *et al*., 1986). However, certain groups of microorganisms can directly or indirectly transform rocks and minerals in quantities large enough to influence the geological distributions. These transformations include enzymatic oxidation, reduction reactions, formation of chelates and complexes with protein, amino-acids, organic acids etc. (Henderson and Duff, 1963).

On this context, that investigations were carried out on the isolation, identification and characterization of soil bacteria and their ability to mineral solubilization, others beneficial traits and their influence on growth and nutrient uptake of maize plants. The results obtained on this investigation are discussed in this chapter.

5.1 Isolation and characterization of bacteria solubilizing both potassium and phosphorus from different crop plants

The isolation of microorganisms involve in solubilization of insoluble mineral nutrients is more in the rhizosphere soil of many crop plants, with this view different bacterial isolates were isolated from the rhizosphere soils of different crop plants from the place around Dharwad, Haveri and Davanagere districts. It was interesting to note that ability of bacterial isolates from the rhizosphere soils were able to grow and solubilize the medium containing fixed insoluble form of nutrients.

The results were agree with the findings of Norkina and Pumpynaskaya (1956) isolated two strains of *Bacillus* sp. and *Pseudomonas* from rhizosphere soils of various crop plants as mineral potassium soluiblizers. In support of Gaur *et al*. (1973) and Duff *et al*. (1963) isolated 2-keto gluconic acid producing *Pseudomonas* strain capable of solubilizing many minerals like quartz, silicates and phlogopite. Similarly (Fredrich *et al*., 1991) isolated different groups of microorganisms like *Bacillus mucilaginosus* and *Thiobacillus thioxidans* capable of solubilizing silicates.

The results of this study, supported by Christophe *et al*. (2006), they isolated many of the plant root associated bacteria contributing to mineral weathering and demonstrated that *Burkholderia glathei* PMLI (12) significantly increased biotite weathering.

In the present study, the results of all the experiments concludes that *Bacillus* sp. was potential in solubilizing the potassium and phosphate mineral. The results are in agree with findings of Hu *et al*. (2006), they isolated two phosphate and potassium solubilizing *Bacillus* sp. from the soils in the modified Aleksandrov medium of containing phosphorite and potassium minerals like kaolinite and potassium feldspar.

5.2 Characterization of bacterial isolates for release of mineral nutrients from their fixed source

Based on colony morphological and biochemical characters the organisms were tentatively identified as *Bacillus* sp. and *Pseudomonas* sp. then the organisms were inoculated to Aleksandrov broth containing fixed mineral sources.

Similarly, Fan and Yan (2006) isolated and characterized bacteria *Bacillus mucilaginosus* capable of solubilizing two potassium bearing minerals like feldspar and illite. The probable mechanism of action as mentioned by these scientists for solubilization of potassium bearing minerals was by the action of organic acids like oxalic acid and capsular polysaccharides.

The morphological characterization revealed that all bacterial isolates solubilizing both potassium and phosphorus were Gram positive short to long rods with spore production, but differed in their physiology and nutrition. Avakyan *et al*. (1984), Webley *et al*. (1960) and Purushotham *et al*. (1974) reported *Bacillus mucilaginosus* solubilized insoluble silicates.

All the above mentioned reviewed will strongly support the present study carried out to isolate the bacterial strains like *Bacillus* sp. and *Pseudomonas* sp. capable to solubilizing potassium and phosphate mineral. Hence, these isolates were selected for further experiments.

All the fifty bacterial strains solubilizing both potassium and phosphorus were further screened for their ability to solubilize potassium and phosphate mineral like muscovite mica and tri-calcium phosphate in agar and broth medium. The zone of solubilization by all the mineral potassium solubilization strains ranges from 3.0 to 11.5 mm at 72 h after incubation (HAI). Such observation were made earlier that among the K and P bearing silicate minerals mica and TCP was found to be solubilize readily (Tandon and Sekhon, 1988; Sugumaran and Janarthanam, 2007; Mikhailouskaya and Tehernysh, 2005).

The bacterial isolates solubilizing both potassium and phosphorus were subsequently tested for the ability to release K and P from muscovite mica and TCP in the external broth. The amount of K released from muscovite mica ranged from 2.36 µg/ml to 29.83 µg/ml and P released from TCP ranged from 3.44 to 14.25 per cent, respectively. Among the isolates *Bacillus* species K-PSB 32 showed the higher K and P releaser from the insoluble K and P mineral source used. The findings are in agree with the findings of Hu and Boyer (1996) reported that *Bacillus megaterium* was capable of solubilizing mica in appreciable amounts. The different efficiency of bacteria to solubilize insoluble form potassium and phosphate could be due difference in their ability to release organic acids (Sheng and He., 2006 and Liu *et al.,* 2006).

The results also indicated greater variation between the isolates to solubilize the same or different source of insoluble potassium minerals (Mikhailouskaya and Tehernysh, 2005; Liu *et al*., 2006 and Hu *et al*., 2006). Observed variation in the amount of potassium solubilization by the strains of same species of *Bacillus* and *Pseudomonas.*

In contrast with the above, Hu *et al*. (2006) reported that *B. megatherium* and *B. mucilaginosus* were capable of solubilizing both rock phosphate and potassium. They also reported that co-inoculation of these two *Bacillus* sp. were potential in solubilizing potassium rocks. The present study, indicated that *Bacillus* sp. was also capable of releasing some amount of phosphorus from TCP (14.25%) but was comparatively very less and the results compare well with the observations of (Badar, 2006). Greater releases of K from muscovite have been documented by *B. muciloginosus* (Sugumaran and Janarthanam, 2007).

Potassium as well as phosphorus solubilizing bacteria were also examined for production of IAA and GA. Only selected isolates were studied in quantification of IAA and GA in the range of 3.38 to 8.90 µg/25 ml broth and 1.27 to 3.67 µg/25 ml broth, respectively. Sheng and Huang (2001) reported growth enhancement of *Bacillus* may also relate to its ability to produce hormones.

5.3 Effect of inoculation of bacteria solubilizing both potassium and phosphorus on growth and yield of maize plant

5.3.1 Growth parameters

The inoculation of bacteria solubilizing both potassium and phosphorus had significant influence on different plant growth parameters.

Based on the efficiency of both K-P solubilization, the eight selected bacterial isolates were further examined for their performance to enhance growth, nutrient uptake and yield components of maize plant over control.

The root length of maize plants was increased in Treatment 12 (T_{12}) at different growth stage of plant 30, 60 DAS and at harvest (15.83, 52.67 and 45.33 cm, respectively) due to inoculation of bacteria solubilizing both potassium and phosphorus K-PSB 32 was higher the root length and was on par with co-inoculated local strains of *Bacillus* sp. and *P. striata* and treatment with RDF.

Similarly increases in the plant root length due to inoculation of *B. edaphicus* potassium solubilizing bacteria had been reported by Sheng and He (2006), in maize (Wu *et al*., 2005), in brinjal (Ramarethinam and Chandra, 2005).

Similar findings were also noticed by Berthelin and Leyval (1982) using the mycorrhizal fungi for the solubilization of mica. Finally, they had concluded the weathering of biotite mica resulted in increase of potassium that had direct impact on maize. Igual *et al.* (2001) used phosphate solubilizing bacteria to increase potassium uptake by the test plants.

The plant height of maize was increased in Treatment 15 (T_{15}) at different growth stages of plant 30, 60 DAS and at harvest (41.90, 105.33 and125.53 cm, respectively) due to inoculation of potassium as well as phosphorus solubilizing bacteria K-PSB 50 and was on par with co-inoculation of local strains and treatment RDF. However, all the inoculated isolates superior over control.

Similarly, Sheng *et al* (2003) reported plant growth promoting effect and nutrient uptake of K releasing strain *Bacillus edaphicus* NBT on cotton and rape. The application of biofertilizer containing N fixer (*Azotobacter chroococum*), P solubilizer (*Bacillus megaterium*) and K solubilizes (*Bacillus mucilaginosus*) and AM fungi (*Glomus mosseae*) significant increased the growth of maize.

5.3.2 Dry matter production

In accordance with the root and shoot growth and dry matter content in root and shoot as well as total dry matter content of plant was also enhanced due to the inoculation of bacterial isolates solubilizing both potassium and phosphorus over control.

The total dry matter content of maize plant increased due to inoculation of K-PSB 32 at different plant growth stage it recorded higher dry matter content at 30, 60 DAS and at harvest (12.80, 44.20 and 235.70 g/plant, respectively) and was on par with co-inoculation of local strains *Bacillus* sp. and *P. striata* and treatment with RDF.

However, all inoculated isolates superior over control. Our results agree with those already reported (Shen *et al.,* 2005 and Wu *et al*., 2005) significantly increase in biomass yield due to inoculation of mica with *B. mucilaginosus* may be attributed to mobilization of K from waste mica because of secretion of organic acids by bacterial strains, which in turn increased the biomass yield.

The results are in agreement with those of Badar (2006) who obtained 58 per cent increased dry matter content of sorghum plants due to inoculation of silicate dissolving bacteria. Similar results on the enhanced dry matter content of ground nut (Sugumaran and Janarthnam, 2007), wheat (Mikhailouskya and Tcherhsh, 2005), brinjal (Nayak, 2001), chilli (Ramarethinam *et al*., 2005), tomoto (Li, 2002), pepper and cucumber (Han *et al*., 2006).

Similarly, Lin (2002) recorded increase in the biomass by 125 per cent effect of plant growth promoting rhizobacteria (PGPS) including phosphate and potash solubilizing bacteria (PSB and KSB) as biofertilizer as a sustainable solution improve plant nutrient and production (Vessey , 2003).

5.3.3 Yield and yield parameters

The yield attributing characters like cob weight, grain yield and test weight of maize were significantly enhanced in the treatments receiving inoculation of bacteria solubilizing both potassium and phosphorus strains of K-PSB 50 and K-PSB 32. Further recorded an increase in the grain yield per plant by 52.93 and 50.93 g/plant over control. Similarly, inoculation of potassium solubilizing bacteria *Bacillus mucilaginosus* had been reported to significantly increasing the yield of maize (Alexandrov, 1967), sorghum (Vintikova, 1964), wheat (Muralikaman, 1996), tomoto (Kalaiselvi, 1999), brinjal (Zhang *et al.,* (2004) and chilli (Supanjani *et al.,* 2006).

5.4 Population of KSB, PSB and free living nitrogen fixers

In the present investigation, treatment inoculation bacteria isolates of solubilizing both potassium and phosphorus had significantly influenced by the KSB, PSB and free living nitrogen fixers population was significantly superior over all other inoculated isolates and was significantly low population control treatment.

The are mainly due to growth hormone production and may be due to release of organic acid and the result are comparable with those of Hu *et al*. (2002).

5.4.1 Effect of inoculation of bacteria solubilizing both potassium and phosphorus on the nutrient of maize plant

In the present study, the nutrient concentration *viz*.. NPK in plant were significantly highest with the inoculation of potassium as well as phosphorus solubilizing bacteria as compared to the control. Maximum nutrient content reported K-PSB 32 followed by K-PSB 50 and treatment RDF showed higher nutrient content in all the N, P and K uptake, this is in conformity with the finding of Supanjani *et al*., (2006).

Han and Lee (2005) reported the synergistic effect of soil fertilized with rock phosphate and potassium minerals and co–inoculation with phosphate solubilizing bacteria *Bacillus megaterium* and potassium solubilizing bacteria *B. mucilaginosus* KCTC 3870 on the improvement of P and K uptake by eggplant grown under limited P and K soil in greenhouse.

5.5 Enzyme activity

It is well known that enzyme plays a key role in transformation, recycling and availability of plant nutrients in soil. They are likely to be influenced by fertilizer and manures. Various enzyme activities were found to be maximum in treatment receiving inoculation of K-P solubilizing bacteria.

In the present investigation, there was increase in dehydrogenase and phosphatase activity in treatment of inoculation of K-PSB 32 followed by K-PSB 50 and control with RDF.

During the entire cropping period, the enzyme activity increased initially at 30 DAS and then declined with the age of the crop. These observations are in accordance with the findings of Singaram and Kamalakumari (1995) in where they also noticed similar trend in phosphatase activity in maize rhizosphere. The variation in the urease and phosphatase were little influenced by different inoculation treatments. More than the microbial population, the enzyme activities are regulated by the soil characters like organic carbon, pH and nutrient status (Nagaraja *et al.*, 1998).

SUMMARY AND CONCLUSIONS

Potassium availability to crop plants in soil is generally low since nearly 98 per cent of total K in soil is in mineral forms. Further fixation of added P in soil reduces the efficiency of applied K fertilizer since a large quantity become unavailable to plants.

Solubilization of soil minerals, by fungi and bacteria are well established. However, less information is available on K-P solubilizing bacteria and their impact on growth and development of crop plants. In this context, attempts were made to isolate bacteria solubilizing both potassium and phosphorus from different rhizosphere soil samples of crop plants. The efficiency of the isolates to solubilize insoluble potassium and phosphorus mineral, production of plant growth promoting substance and other agronomically beneficial traits were studied under laboratory and pot culture condition. The *in vitro* efficient bacterial strains of solubilizing both potassium and phosphorus were further tested for their effects on growth, nutrient uptake and yield of maize plants under green house condition.

The rhizosphere soil samples of different crops were used in the study for isolation of bacteria solubilizing both potassium and phosphorus. The total 50 bacteria solubilizing both potassium and phosphorus isolates were isolated on Aleksandrov's media supplemented with mica and tri-calcium phosphate (TCP) as a potassium and phosphorus source respectively.

All bacterial cultures were identified upto genus level and were found to belong to the genera *Bacillus* and *Pseudomonas*. All the isolates were able to solubilize patassium and phosphorus minerals (mica and TCP) under *in vitro* condition. The amount of K released by the isolates ranged from 2.36 to 29.83 µg/ml and P released ranged from 3.4 to 14.25 per cent. Among isolate K-PSB 32 showed maximum solubilization (29.83 µg/ml and 14.25 per cent of K and P, respectively) followed by K-PSB 21 (28.74 µg/ml and 13.50 per cent of K and P, respectively).

All the isolates were tested for production of plant growth promoting substance. The amount of IAA produced by the strain ranged from 3.38 to 8.90 µg/25 ml broths and that of GA ranged from 1.27 to 3.67 µg/25 ml broth.

The analysis of spatial release of K and P by the isolates in general has shown an increase in the amount of K and P released into mica and TCP broth with increasing period of incubation.

Eight of these efficient K-P solubilizing bacteria, *Bacillus species* (K-PSB 2, K-PSB 20, K-PSB 21, K-PSB 28, K-PSB 32, K-PSB 36, K-PSB 39 and K-PSB 50) were further examined for their influence on the growth nutrient uptake and yield of maize plants under green house condition. The plant growth increased at different growth stages at 30, 60 DAS and at harvest due to inoculation of isolate K-PSB 50. maximum root length were recorded (15.83, 52.67 and 65.53 cm, respectively), shoot length (41.90, 105.33 and 125.53 cm. respectively) and stem girth recorded (4.70, 9.63 and 12.53 cm, respectively) the dry matter content on shoot, root and total plant recorded at 30, 60 DAS and at harvest, isolate K-PSB 32 showed maximum total dry matter (12.80, 44.2 and 235.7 cm, respectively) and yield and yield parameters highest cob weight (114.90 g/plant) and grain yield (52.93 g/plant) recorded on treatment inoculated with strain K-PSB 50.

The nutrient concentration increased due to inoculation of bacteria solubilizing both potassium and phosphorus isolates. Inoculation of K-PSB 32 The plant nitrogen concentration at 30, 60 DAS and at harvest, recorded maximum of 2.94, 3.85 and 2.69 per cent, respectively and phosphorus concentration of 0.41, 0.50 and 0.45 per cent, respectively. Potassium concentration of 3.18, 4.10 and 2.75 per cent, respectively.

The results indicated that, all the inoculated bacterial isolates solubilizing both potassium and phosphorus resulted in increased plant growth, nutrient uptake (K and P) and yield component of maize plant significantly over uninoculated fertilizer control.

Conclusions

1. Isolate and characterized the bacteria solubilizing both potassium and phosphorus from rhizosphere soils of different crops.
2. The inoculation of bacteria solubilizing both potassium and phosphorus improved dry matter content in maize plants.
3. The plant nutrient concentration (N, P and K) was highest with the inoculation of K-PSB isolates.
4. There was significant improvement in the growth and yield with the inoculation of K-PSB isolates

as compared to control and reference strain inoculated single.

5. There was significant improvement in soil biological indicator such as microbial population and enzyme activity due to inoculation of isolates K-PSB.

Future line of work

- Isolation and chareteration of potential bacteria strains solubilizing both potassium and phosphorus from different Agro-ecological Zones and Cropping system.
- Evaluation of bacterial isolates solubilizing both potassium and phosphorus for other crops under natural field condition.
- Molecular characterization of bacteria solubilizing both potassium and phosphorus isolates.
- Compatibility study with other PGPR and Bio-control organism.

REFERENCES

Ahmed, N. and Jha, K. K., 1977, Effect of inoculation with phosphate solubilizing organisms on the yield and P uptake of gram, *J.Indian Soc. Soil*, 25 : 391-393.

Aleksandrov, V. G., Blagodyr, R. N. and Live, I. P., 1967, Liberation of phosphoric acid from apatite by silicate bacteria. *Mikrobiyolzh*. (Kiev), 29 : 111-114.

Anonymous, 1957, Manual of microbiological method. McGraw Hill Book Company Inc., New York, p.127.

Anonymous, 2012, Agricultural statistics at a glance, published by ministry of Agriculture, Govt. of India.

Archana, D. S., Savalgi, V. P. and Alagawadi, A. R., 2008, Effect of potassium solubilizing bacteria on growth and yield of maize. *Soil Biol. Ecol.,* 28(1-2) : 9-18.

Argelis, D. T., Gonzala, D. A., Vizcaino, C. andGartia, M. T., 1993, Biochemical mechanism of stone alteration carried out by filamentous fungi living in monuments. *Biogeo. Chem*., 19 : 129-147.

Avakyan, Z. A., Belkanova, G. T. and Karavaiko and Piskunov, V. P., 1984, Silicon compounds in solution during bacterial quartz degradation. *Mikrobiol*., 54: 301-307.

Badar, M. A., 2006, Efficiency of K- feldspar combined with organic material and silicate dissolving bacteria on tomato yield*. J. App. Sci. Res*., 2(12) : 1191-1198.

Badar, M.A., Shafei, A.M. and Sharaf, S.H. El-Deen, 2006, The dissolution of K and phosphorus bearing minerals by silicate dissolving bacteria and their effect on sorghum growth. *Res. J. Agril. Biolog. Sci.*, 2 : 5-11.

Bagyalakshmi, B., Ponmurugan, P. and Marimuthu, S., 2012, Influence of potassium solubilizing bacteria on crop productivity and quality of tea (*Camellia sinensis*). *African J. Agril. Res.,* 7(30): 4250-4259.

Balasubramaniam, P. and Subramanian, S., 2006, Assement of soil test based potassium requirement for low land rice in udic haplustalf under the influence of silicon fertilization, Tamil Nadu Agric., Kumulur, Truchirapalli, pp. 621-712.

Bardiya, M. C., 1970, Studies on microbiological solubilization of rockphosphate. *Ph. D. Thesis,* P. G. School, IARI, New Delhi, p. 12.

Barker, W. W., Welch, S. A., Chu, S., Banfield, J. F., 1998, Experimental observations of the effects of bacteria on aluminosilicate weathering. *Am Mineral*, 83 :1551–1563.

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

Basak, B. B. and Biswas, D. R., 2009, Influence of potassium solubilizing microorganism (*Bacillus mucilaginosus*) and waste mica on potassium uptake dynamics by sudan grass (*Sorghum vulgare* Pers.) grown under two Alfisols. *Plant Soil,* 317:235–255.

Basak, B. B. and Biswas, D. R., 2010, Coinoculation of potassium solubilizing and nitrogen fixing bacteria on solubilization of waste mica and their effect on growth promotion and nutrient acquisition by a forage crop. *Biol. Fertility Soils*, 46 : 641-648.

Basavanna, G. R. M., 1980, Nutritional and growth characteristics of some phosphorus solubilizing microorganisms and their effect on plant growth. *M. Sc. Thesis,* Univ. Agric. Sci., Bangalore, Karnataka (India).

Bennett, P. C., Choi, W. J. and Rogera, J. R., 1998, Microbial destruction of feldspars. *Mineral Mngt.*, 8(62A): 149–150.

Berthelin, J. and Leyval, C., 1982, Ability of symbiotic and non-symbiotic rhizospheric microflora of maize (*Zea mays* L.) to weather micas and to promote plant growth and plant nutrition. *Plant Soil,* 68: 369-377

Biraj, B. B. and Dipak, D. B., 2010, Co-inoculation of potassium solubilizing and nitrogen fixing bacteria on solubilization of waste mica and their effect on growth promotion and nutrient acquisition by a forage crop. *Biol Fertil Soils*, 46 :641–648.

Blazevic, D. J. and Ederer, G. M., 1975, Principles of biochemical tests in diagnostic microbiology, Wiley and Company, New York, pp.13-45.

Bojinova, D., Velora, R., Grancharov, I. and Zhelevs, S., 1997, The bioconversion of tunisian phosphorite using *Aspergillus niger* Nutr*. Cyc. Agro. Ecosyst*., 47:227-232.

Bric, J. M., Bostock, R. M. and Silverstone, S. E., 1991, Rapid in situ assay for indole acetic acid production by bacteria immobilized on a nitro cellulose membrane. *Appl. Environ. Microbiol*., 57: 535-538.

Brown, M. E. and Burlingham, S. K., 1968, Production of plant growth substances by *Azotobacter chroococcum*. *J. Gen. Microbiol*., 53: 135-144.

Cappuccino, J. G. and Sherman, N., 1992, Microbiology : A laboratory manual. The Benjamin/Comings Publishing Company, Inc., California.

Cappuccino, J.G. and Sherman, N., 1996, In: Microbiology: A laboratory manual. The Benjamin/Cunnings Publishing Company Inc. (4th Edition), Melopark, California.

Casida, L. E., Klein, D.A. and Snatoro, T., 1964, Soil dehydrogenase activity. *Soil Sci*., 98: 371-376.

Chen, Y. P., Rekha, P. D., Arun, A. B., Shen, F. T., Lai, W. A. and Young , C. C., 2006, Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. *Appl. Soil Ecol.,* 8 (34) : 33–41

Cheng, Y. C., Barker, R., Kleifeld, O. and Chet, I., 1986, Increased growth of plants in the presence of biological control agents *Trichoderma harzianum*. *Plant Dis.,* 70: 145-148.

Chien, S. H., 1979, Dissolution of phosphate rock in acid soil as influenced by nitrogen and potassium fertilizers. *Soil Sci.,* 127 : 371-376.

Christophe, C., Turpault, M.P. and Freyklett, P., 2006, Root associated bacteria contribute to mineral weathering and to mineral nutrition in trees and budgeting analysis. *Appl. Environ. Microbiol*., 72 (2): 1258-1266.

Cowan, S. T. and Steel, K. J., 1970, Manual for the identification of medical bacterial. Lowe and Brydon, London, p.30.

Duff, R. B. and Webley, D. M., 1959, 2- ketogluteric acid and natural chelator produced by soil bacterial*. Chem. India*, . 1376-1377.

Duff, R. B., Webley, D. M. andSott, R. O., 1963, Solubilization of minerals and related materials by 2 ketogluconic acid producing bacteria. *Soil Sci*., 5 : 105-114.

Eckford, M. D., 1927, Thermophillic bacteria in milk. *American J. Hyg.*, 7: 200-201.

Evazi, Z. and Tabatabai, M. A. 1979, Phosphatese in soil. *Soil Biol. Biochem*., 9:167-172.

Fang, S. X. and Yan, L. H., 2006, Solubilization of potassium bearing minerals by wild type strain of *Bacillus edaphicus* and its mutants and increased potassium by wheat. *Can. J. Microbiol*., 52: 66-72.

Fankem, H., Ngo, N. L., Deubel, A., Quinn, J., Merbach, W., Etoa, F. X. and Nwaga, D., 2008, Solubilization of inorganic phosphates and plant growth promotion by strains of *Pseudomonas fluorescens* isolated from acidic soils of Cameroon: *African J. Microbiol. Res.,* 6 (2) : 171-178.

Friedrich, S., Platonova, N. P., Karavaiko, G. I., Stichel, E. and Glombitza, F., 1991, Chemical and microbiological solubilization of silicates. *Acta Biotech.,* 11(3):187–196.

Gaind, S. A. and Gaur, A. C., 1990, Influence of temperature on the efficiency of phosphate solubilizing microorganism. *Indian J. Microbiol*., 30 : 314-317.

Gaur, A. C., Madan, M. and Ostwal, K. P., 1973, Solubilization of phosphatic compounds by native microflora of rock phosphates. *Indian J. Exp. Biol.,* 11: 427-429.

Gerke, L., 1992, Phosphate, aluminum and iron in the soil solution of three different soil in reaction to varying concentrations of citric acid. *Z. PfL. Ernahr. Bodenkde*, 155:17-22.

Gerretsen, F. C., 1957, The influence of microorganisms on the phosphate intake by the plant. *Plant Soil*, 1 : 51-81.

Ghosh, A. B. and Hasan, R., 1980, *Soil Fertility Map of India*, ICAR, New Delhi.

Goldstein, A. H., 1986, Bacterial solubilization of mineral phosphates : Historical perspective and future prospects. *The American J. Alternate. Agric.,* 1: 51-57.

Goldstein, A. H., 1994, Involvement of the quino protein glucose dehydrogenase in the solubilizing of exogeneous mineral phosphates by Gram-negative bacteria. In phosphate in microorganisms : cellular and molecular biology. *Cell Mol. Biol*., Eds., pp. 197-203.

Goldstein, A. H., 1995, Recent progress in understanding the molecular genetics and biochemistry of calcium phosphate solubilization by Gram-negative bacteria. *Biol. Agric. Hort*., 12: 185–193.

Gordon, S. A. and Paleg, L. G., 1957, Quantitative measurement of indole acetic acid. *Plant Physiol*., 10: 37-48.

Grayston, S. J., Vaughan, D., Jones, D., 1997, Rhizosphere carbon flow in trees, in comparison with annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability. *Appl Soil Ecol*., 5(1):29–56.

Gromov, B. V., 1957, The microflora of rocks and primitive soil in some northern regions of the USSR. *Mikrobiologiya,* 26 : 52-54.

Groudev, S. N., 1987, Use of heterotrophic microorganisms in mineral biotechnology. *Acta Biotech*., 7 (4):299–306.

Halder, A. K., Mishra, A. K., Bhattacharya, P. and Chakrabarthy, P. K., 1990, Solubilization of rock phosphate by *Rhizobium* and *Bradyrhizobium*. *J. Gen. Appl. Microbiol*., 36 : 81–92.

Han, H. S. and Lee, K. D., 2005, Phosphate and potassium solubilizing bacteria effect on mineral uptake, soil availability and growth of eggplant. *Res. J. Agri. Biol. Sci.,*1 :176–180.

Han, H. S., Supanjani and Lee, K. D., 2006, Effect of co-inoculation with phosphate and potassium solubilizing bacteria a mineral uptake and growth of pepper and cucumber. *Plant Soil Envrion*., 52(3) : 130-136.

Heinen, W., 1960, Silicon metabolism in microorganisms. *Arkiv Mikrobiol*, 37:199- 210.

Henderson, M. E. K. and Duff, R. B., 1963, The release of metallic and silicate ions from minerals and soils by fungal activity. *J. Soil Sci*., 14: 236-246.

Hilda, R. and Reynaldo, F., 1999, Phosphate solubilizing bacteria and their role in plant growth promotion: *Biotechnol. Adv.,* 17 : 319–339

Holford, I. C. R., 1997, Soil phosphorus : Its measurement and its uptake by plants. *Aust. J. Soil Res.,* 35 : 227- 39.

Hopkins, C. G. and Whiting, A. L., 1916, Soil bacteria and phosphates III. *Agric. Exptl. Stat. Bull.,* 190 : 395-406.

Hu, X. and Boyer, G. L., 1996, Siderophore mediated aluminium uptake by Bacillus megatherium ATCC 19213. *Appl. Environ. Microbiol*., 62: 4044-4048.

Hu, X.F., Chen, J. and Guo, J. F., 2006, Two phosphate and potassium solubilizing bacteria isolated from Tiannu mountain, Zhejiang, China. *World J. Micro. Biotech.,* 22: 983-990.

Igugla, M. J., Valerde, A., Cervanters, E. and Velazuez, E., 2001, Phosphate solubilizing bacteria as inoculants for agriculture : use of updated molecular techniques in their study. *Agromie*, 21: 561-568.

Illmer, P. and Schinner, F., 1995, Solubilization of inorganic calcium phosphate solubilization mechanisms. *Soil Biol. Biochem.,* 27 : 257-263.

Jackson, M. L., 1973, *Soil Chemical Analysis*, Prentice Hall of India (P.) Ltd., New Delhi.

James, G. C. and Natalie Sherman, 1992, Microbiology and laboratory manual, rockland community college, suffern, New York, Third Edition. The Benjamin/ Cummings publishing Co. Inc., Redwood, City, California.

Javad, K. Z., Nasser, A., Shahin, O., Mostafa, E. and Ali, A., 2013, Isolation and characterization of potassium solubilizing bacteria in some Iranian soils: *Archives Agron. Soil Sci.*, 10 : 1080-1095.

Jurinak, J. J., Dudley, L. M., Allen, M. F. and Knight, W. G., 1986, The role of calcium oxalate in the availability of phosphorus in soils of semi arid region, a thermodynamic study. *Soil Sci.,* 142 : 255- 261.

Kalaiselvi, P., 1999, Influence of silicate solubilizing bacteria on dissolution of silicate and potassium in rice soil added with graded levels of potassium and rice residues. *M. Sc. (Agri.) Thesis*, Tamil Nadu Agril. Univ., Coimbatore (India).

Khalafallh, M. A., Saber, M. S. M. and Abel-El, Maksound, H. K., 1982, Influence of phosphate dissolving bacteria on the efficiency of superphosphate in calcareous soil cultivated with viciafaba *Z. pflangernachr, Bodenkel*, pp. 145-159.

Khanwilkar, S. A. and Ramteke, J. R., 1993, Response of applied K in cereals in Maharashtra. *J. Agri.,* 28: 84-96.

Khudsen, D., Peterson, G. A. and Prov, P. F., 1982, Lithium, sodium and potassium. In : Page A. l. (Ed.) *Methods of Soil Analysis Part 2 – Agronomy Monograph 9*, 2nd Edn. ASA and SSSA Madison, WI.

Kim, K. Y., McDonald, G. A. and Jordan, D., 1998, Effect of phosphate solubilizing bacteria and vesicular-arbuscular mycorrhizae on tomato growth and soil microbial activity. *Biol. Fert. Soil,* 26:79-87.

Kim, K.Y., Jordan, D. and Krishnan, H.B., 1997. Rahnella aqualitis, a bacterim isolated from soybean rhizosphere, can solubilize hydroxyapatite. *FEMS Microbiol. Lett*., 153 : 273–277.

Kim, T., Jung. W., Lee. B., Yoneyama. T., Kim. H., Kim. K., 2003, P effects on N uptake and remobilization during regrowth of Italian ryegrass (*Lolium mutiflorum*). *Environ. Exptl. Bot*., 50 : 233-242.

Kiss, S., Dragan, B. M. andRadulescu, D., 1975, Biological significance of enzyme accumulated in soils. *Adv. Agron*., 27 : 25-27.

Kondapanaidu D. A., 2008, Effect of INM on yield, nutrient uptake and quality of chilli in a vertisol. *M. Sc (Agri) Thesis*, Univ. Agric. Sci. Dharwad, Karnataka (India).

Kpomblekou, K. and Tabatabai, M. A., 1994, Effect of organic acids on release of phosphorus from phosphate rocks. *Soil Sci*., 158 : 442– 453.

Kretzschamr, R. M., Hafner, H. H, Bationo, A. and Marschner, R., 1991, Long and short term effect of crop residues on aluminium toxicity, phosphorus availability and growth of pearlmillet in an acid sandy soil*. Plant Soil*, 136 : 215-223.

Krishnaraj, P. U., Khanuja, S. P. S. and Sadasivam, K. V., 1998, Mineral phosphate solubilization (MPS) and *mps* genes- components in eco-friendly Pfertilization. *Abstracts of Indo US Workshop on Application of Biotechnology for Clean Environment and Energy*. National institute of Advanced Studies, Bangalore, August 5-8,1998, p.27.

Kucey, R. M. N., Janzen, H. H. and Leggett, M. E., 1989, Microbially mediated increased in plant available phosphorus. *Adv. Agron*., 42 : 198-228.

Kundu, B. S. and Gaur, A. C., 1984, Rice response to inoculation with N fixing and psolubilizing microorganisms. *Plant Soil*, 79 : 227-234.

Larsen, S., 1967, Soil phosphorus. *Adv. Agron.,* 19 : 115- 120.

Li, Y.F., 2002, The characteristics and function of silicate dissolving bacteria fertilizer. *Soil and Ferti.*, 2: 48-49.

Liermann, L. J., Kalinowski, B. E., Brantley, S. L., Ferry, J. G., 2000, Role of bacterial siderophores in dissolution of hornblende. *Geochim Cosmochi Acta.* 64(4):587–602.

Lin, Q. M., Rao, Z. h., Sun, Y. X., and Xing, L. I., 2002, Identification and practical application of silicate- dissolving bacteria. *Agric. Sci. China*, 1 : 81-85.

Liu, D., Lian, B. and Dong, H., 2012, Isolation of *Paenibacillus* sp. and assessment of its potential for enhancing mineral weathering. *Geomicrobiol. J.*, 29 : 413-421.

Liu, G. Y., 2001, Screening of silicate bacteria with potassium releasing and antagonistical activity. *Chinese J. Appl. Environ. Biol*., 7(1) : 66-68.

Liu, W., Xu, X., Wu, S., Yang, Q., Luo, Y. and Christie, P., 2006, Decomposition of silicate minerals by *Bacillus mucilaginosus* in liquid culture. *Environ. Geochem. Health*, 28 : 133- 140.

Loheuarete, F. and Berthelin, J., 1988, Effect of phosphate solubilizing bacteria on maize growth and root exudates over four levels of labile phosphorus. *Plant Soil*, 105 : 11-17.

Manjunath, B., 2006, Impact of farmers organic farming practices on soil properties in northern dry zone of Karnataka. *M. Sc (Agri.) Thesis*, Univ. Agric. Sci. Dharwad, Karnataka (India).

Mengel, K. and Kirkby, E. A., 1987, Principles of Plant Nutrition, *International Potash Inst. Bern, Switzerland*, pp. 200-210.

Mikhailouskaya, N. and Tcherhysh, A., 2005, K-mobilizing bacteria and their effect on wheat yield. *Latnian J. Agron*., 8 : 154-157.

Mohad, S. R., Gupta, D. N. and Chevan, A. S., 1991, Enrichment of P availability and P uptake in rice by P- solubilizing culture. *J. Maharashtra Agri. Univ*., 14 : 178-181.

Mónica, M. C., Pedro, A. S., Luis, A. M. and Mario, A.O., 2010, Comparison of *in vitro* solubilization activity of diversephosphate-solubilizing bacteria native to acid soil and their ability to promote *Phaseolus vulgaris* growth: *Biol. Ferti. Soils,* 46: 727–738

Muntz, A., 1890, Sur la decomposition des rock formation de la terrabale. *Compt. Rend. Akad. Sci*., Paris, 110 : 1370-1372.

Muralikannan, M., 1996, Biodisolution of silicate, phosphate and potassium by silicate solubilizing bacteria in rice ecosystem. *M. Sc. (Agri.) Thesis*, Tamil Nadu Agric. Univ., Combatore (India).

Nagaraja, M. S., Ramakrishna, Parama, V. R and Siddaramappa, R., 1998, Effect of atrazine urea- N-mineralization and activity of some soil enzymes. *Indian J. Agron.*, 46 : 189-192.

Nahas, E., Banzatto, D. A., Assis, L. C., 1990, Fluorapatite solubilization by *Aspergillus niger* in Vinasse medium. *Soil. Biol. Biochem*, 22:1097-1101.

Nair, S. K. and Subba Rao, N. S., 1977, Distribution and activity of phosphate solubilizing microorganisms in the rhizosphere of coconut and cacoa. *J. Plantation Crops*, 5 : 67-70.

Nayak, B., 2001, Uptake of potash by different plants with the use of potash mobilizing bacteria (*Fraleuria Aurania*). *M. Sc. (Agri.) Thesis*, Univ. Agril. Sci., Bhubaneshwar.

Norkina, S. P. and Pumpyansakya, L. V., 1956, Certain properties of silicate bacteria. *Crop Sci., Soc. Japan*, 28 : 35-40.

Norris, J. R., 1959, Isolation and identification of isolates *Azotobacter. Lab. Practices*, 8: 239-240.

Olsen, S. R., Cole, C. V., Watanabe, F. S. and Dean, L. A., 1954, Estimation of available phosphorus in soils by extraction with sodium bicarboanate, *US Dept. Agri. Circ.,* 939, Washington, D.C.

Paleg, L. G., 1965, Physiological effects of gibberellins. *Annu. Rev. Pl. Physiol*., 16: 291-322.

Panse, V. G. and Sukhatme, P. V., 1985, Statistical methods for Agriculture workers, ICAR, Pubilication New Delhi, p.359.

Park, M., Singvilay, D., Seok, Y., Chung, J., Ahn, K. and Sat, 2003, Effect of phosphate solubilizing fungi on 'P' uptake and growth of tobacco with rock phosphate. *Appl. Soil Korean J. Soil. Sci. fertile*., 36 : 233-238.

Parks, E. J., Olsen, G. J., Brinckman, F. E. and Baldi, F., 1990, Characterization by high performance liquid chromatography (HPLC) of the solubilization of phosphorus in ore by a fungus. *J. Indust. Microbiol.,* 9 : 183- 190.

Paul, E. A. and Clark, F. E., 1989, Phosphorus transformations in soil. In : *Soil Microbiology and Biochemistry*, Academic Press Inc., California, pp. 224- 234.

Pikovskaya, R. I., 1948, Mobilization of phosphates in soil in connection with the vital activities of some microbial species. *Mikrobiol.*, 17: 362-370.

Ponmurugan, P. and Gopi, C., 2006, Distribution pattern and screening of phosphate solubilizing bacterial isolated from different food and forage crops. *J. Agron.,* 5 (4) : 600-604.

Praveen, M. P., Kuligod, V. B., Hebsur, N. S., Patil, C. R. and Kulkarni, G. N., 2012, Effect of phosphate solubilizing fungi and phosphorus levels on growth, yield and nutrient content in maize (*Zea mays* L.): *Karnataka J. Agri. Sci.,* 25 (1) : 58-62.

Priyanka, P. and Sindhu, S. S., 2013, Potassium solubilization by rhizosphere bacteria and influence of nutritional and environmental conditions. *J. Microbiol. Res.*, 3 (1): 25-31.

Purushothaman, D. A., Chandramohan and Natarajan, R., 1974, distribution of silicate dissolving bacteria in velar estury. *Curr. Sci*., 15: 282-283.

Raj, J., 1980, Role of microorganisms in the release of soil phosphate to plant. *Ph. D. Thesis,* Univ. Agric. Sci., Bangalore, Karnataka (India).

Raj, J., Bagyaraj, D. J. and Manjunath, A., 1981, Influence of soil inoculation with vesicular arbuscular mycorrhizal fungus and a phosphate dissolving bacterium on plant growth and 32 P uptake. *Soil. Biol. Biochem*., 13 : 105-108.

Raj, S. A., 2004, Solubilization on a silicate and concurrent release of phosphorus and potassium in rice ecosystem. In: Biofertilizer technology for rice based cropping system, pp. 372-378.

Rajan, S. S. S., Watkinson, J. H., Sinclair, A. G., 1996, Phosphate rock for direct application to soils. *Adv. Agron*., 57 : 77-159.

Ramarethinam, S. and Chandra, K., 2005, Studies on the effect of potash solubilizing/ mobilizing bacteria *Fratewicia Aurania* on brinjal growth and yield. *Pestol.,* 11 : 35-39.

Reitemeir, R. F., 1951, Soil potassium. In : *Advances in Agronomy, American Society of Agronomy*, Vol. iii (Ed.) Norman, A. G., Academic Press, Int. Publ. New York, pp. 113-164.

Reyes, I., Bernier, L., Simard, R. and Antoun, H., 1999. Effect of nitrogen source on solubilization of different inorganic phosphates by an isolate of *Pencillium rugulosum* and two UV-induced mutants. *FEMS Microbiol*. *Ecol*., 28 : 281–290.

Rogers, J. R. and Bennett, P. C., 2004, Mineral stimulation of subsurface microorganisms: release of limiting nutrients from silicates. *Chem. Geol*., 203 (1–2):91–108.

Rudolfs, W., 1922, Influence of sulfur oxidation upon growth of soybeans and its effect on bacterial flora of soil. *Soil Sci.,* 14 : 247- 262.

Sackeet, W. G., Pattern, A. J. and Brown, C. W., 1908, The solvent action of soil bacteria upon the insoluble phosphates of rawboke and natural rockphosphate. *Zblt. Bakt*., 28 : 688.

Salih, H. M., Yahye, A. L. and Abdnl Rah, B. H., 1989, Availability of phosphorus in a calcareous soil treated with rock phosphate or superphosphate as effected by phosphate dissolving fungi. *Plant Soil*, 120 : 181-185.

Sangeeth, K. P., Bhai, S. R. and Srinivasan, V., 2012, *Paenibacillus glucanolyticus*, a promising potassium solubilizing bacterium isolated from black pepper (*Piper nigrum* L.) rhizosphere. *J. Spices Aromat. Crops* 21 (2) : 118–124

Seeley, H. W. and Vandemark, P. J., 1970, Microbes in action : A laboratory manual of microbiology, D. P. Tarapo Revale Sons and Company Ltd., Bombay, pp.86-95.

Seeley, H. W. and Vandemark, P. S., 1981, Microbes in action – A laboratory manual for microbiology, Freeman and Company, San Francisco, USA, p.388.

Sheng, X. F. and He, L.Y., 2006, Solubilization of potassium bearing minerals by a wild type strain of *Bacillus edaphicus* and its mutants and increased potassium uptake by wheat. *Can J. Microbiol.*, 52 : 66-72.

Sheng, X. F. and Huang, W. Y., 2001, Mechanism of potassium release from feldspar affected by the strain NBT of silicate bacterium. *Acta. Pedol. Sin*., 39 : 863- 871.

Sheng, X. F. and Huang, W. Y., 2002, Study on the conditions of potassium release by strain NBT of silicate bacteria scientia. *Agricultura- Sinica*, 35(6): 673-677.

Sheng, X. F., 2006, Growth promotion and increased potassium uptake of cotton and rape seed by a potassium releasing strain of *Bacillus edaphicus*. *Soil Biol. Biochem*., 37 (1) : 1918-1922.

Sheng, X. F., Xia, J. J and Cheng, J., 2003, Mutagenesis of the *Bacillus edaphicus* strain NBT and its effect on growth of chilli and cotton. *Agric. Sci., China*., 32 : 258-265.

Singaram, P. and Kamalakumari, K., 1995, Long term effect of FYM and fertilizers on enzyme dynamics of soil. *J. Indian Soc. Soil Sci*., 43: 378-381.

Singh, G., Biswas, D. R. and Marwah, T. S., 2010, Mobilization of potassium from waste mica by plant growth promoting rhizobacteria and its assimilation by maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.). *J. Plant Nutrit.*, 33 : 1236-1251.

Smith, J. H., Allison, and Soulides, D. A., 1961, Evaluation of *Phosphobacteria* as a soil inoculant. *Proc. Soil Sci. Soc Amer*., 25 : 109-111.

Sode, Koji, Sayaka, Sungimoto, Hika Ustnsheand Waksto Tsugawa, 1995, Effect of PQQ glucose dehydrogenase over expression in *Escherichia coli* on sugar dependent respiration. *J. Biotechnol.,* 43 : 41-44.

Sparks, D. L. and Haung, P. M., 1985, Physical chemistry of soil potassium. *In potassium in Agriculture* (Ed.) Munson, R. D. *American Soc. Agron*. *J*., pp. 201-276.

Sperberg, J. I., 1957, Solubilization of mineral phosphate by soil bacteria. *Nature*, 180 : 994-995.

Stalstorm, V. A., 1903, Beitrang Zurkennturs der Einwiskingsterilea and in ha hung botinblichenorganischenstrolteamtdiltoslichkeit der phosphorsen des tricalcium phosphate. *Zentralbatt fur Bakteriologie*, 11 : 724- 732.

Stanford, P. and English, W. L., 1949, Use of frame photometer in rapid soil tests for potassium and calcium. *Agron., J.,* 41: 446-447.

Styriakova, I., Styriak, I., Hradil, D. and Bezdicka, P., 2003, The release of iron bearing minerals and dissolution of feldspar by heterophic bacteria of *Bacillus* species. *Ceramic. Silicaty,* 47(1) : 20-26.

Subbaiah, B.V. and Asija, G.L., 1966, A rapid procedure for the estimation of available nitrogen in soils. *Curr. Sci.,* 25: 259-260.

Sugumaran, P. and Janarthanam, B., 2007, Solubilization of potassium containing minerals by bacteria and their effect on plant growth. *World J. Agric. Sci*., 3(3) : 350- 355.

Sundara Rao, W. V. B., Bajpai, P. D., Sharma, J. P. and Subbaiah, B. V., 1963, Solubilization of phosphates by phosphate solubilizing organisms using 32 P as tracer and influence of seed bacterization on the uptake by the crop. *Indian Soc. Soil Sci*., 11 : 209-218.

Sundara, B., Natarayan, V. and Hari, K., 2001, Influence of phosphorus solubilising bacteria on soil available P-status and sugarcane development on a tropical vertisol. *Proc. Soc. Sugar. Technol.,* 24:47-51.

Supanjani, Han, H. S., Jung, S. J., and Lee, K. D., 2006, Rock phosphate, potassium and rock solubilizing bacteria as alternative sustainable fertilizers. *Agro. Sustain. Devel.,* 26 : 233-240.

Swaby, R. J., and Sperber, J. I. 1958, Phosphate dissolving microorganisms in the rhizosphere of legumes. *Aust. J. Agri. Res*., 9 : 778-781.

Tandon, H. L. S. and Sekhon, G. S., 1988, Potassium Research and Agricultural Production in India, Fertilizer development and consultation organization, New Delhi, p.144.

Ullaman, W. J., Kirchman, D. L. and Welch, W. A., 1996. Laboratory evidence by microbially mediated silicate mineral dissolution in nature. *Chem. Geol*., 132 : 11-17.

Vandevivaea, P., Welch, S. A., Ullman, W. J. and Kirchman, D. L., 1994, Enhanced dissolution of silicate minerals by bacteria at near-neutral pH. *Microb. Ecol*., 27(3):241–251.

Vassilev, N., Medina, A., Azcon, R. and Vassilev, M., 2006, Microbial solubilization of rock phosphate as media containing agro industrial wastes and effect of the resulting product on plant growth and phosphorus uptake. *Plant Soil*. 287 : 77-84.

Vazquez, P., Holguin, G., Puente, M. E., Lopez-Cortes, A. And Bhashan, Y., 2000, Phosphate solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastel lagoon. *Biol. Fert. Soil.,* 30 : 460-468.

Vessey, K. J., 2003, Plant growth promotion rhizobacteria as biofertilizer. *Plant Soil*., 25 : 557-586.

Vintikova, H., 1964, A contribution to study on efficiency of silicate bacteria. *Rostl Vyn*., 31: 1219-1228.

Webley, D. M., Duff, R. B., Mitchell, W. A., 1960, A plate method for studying the break down of synthetic and natural silicates by soil bacteria. *Nature*, 188: 766-767.

Welch, S. A., Barker, W. W., Banfield, J. F., 1999, Microbial extracellular polysaccharides and plagioclase dissolution. *Geochim Cosmochi Acta*., 63(9):1405–1419.

Weller, D. M. and Cook, R. J., 1983, Supression of stalk rot of wheat by seed treatments with flurescent pseudomonas. *Phytopathology*, 73: 463-469.

Whitelaw, M. A., Harden, T. Y. and Bender, G. L., 1997, Plant growth promotion of wheat inoculated with *Penicillium radicum* sp. Nov. *Australian J. Soil Res.,* 38: 291-300.

Wilson, M. A. and Ellis, B. G., 1984, Influence of calcium solution activity and surface area on the solubility of selected rok phosphates. *Soil Sci.*, 138 : 354-359.

Wu, S. C., Cao, Z. H., Li, Z. G., Cheung, K. C. and Wong, M. H., 2005, Effect of biofertilizer containing N-fixer, P and K solubilizers and AM-fungi on maize growth. *Geoderma* 125 : 155- 166.

Zahar, M. K., Monib, M., Abdel-Al ShI and Heggo, A., 1984, Significance of soil inoculation with silicate bacteria. *Zentralbl Mikrobiol*., 139(5):349–357.

Zhang, C.J., Tu, G.Q. and Cheng, C.J., 2004, Study on potassium dissolving ability of silicate bacteria. *Shaguan College J.*, 26: 1209-1216.

Appendix I: Media composition

1. Modified Aleksandrov Medium (Hu *et al*., 2006)

Glucose	: 5.0 g
Magnesium sulphate	: 0.005 g
$FeCl_3$	: 0.1g
Calcium carbonate	: 0.1 g
Calcium phosphate	: 2.0 g
Potassium aluminum silicate	: 2.0 g
Tri calcium phosphate	: 5.0 g
Mica	: 2.0 g
Yeast extracts	: 2.0 g
Agar	: 20.0 g
Distilled water	: 1000 ml

2. Aleksandrov Medium (Hu *et al*., 2006)

Glucose	: 5.0 g Magnesium
sulphate	: 0.005 g
$FeCl_3$	: 0.1g
Calcium carbonate	: 2.0g
Potassium mineral	: 2.0 g
Calcium phosphate	: 2.0 g
Distilled water	: 1000 ml

3. Pikovskay's media (Pikovskaya, 1948)

Glucose	: 10 g
$Ca_3 (PO_4)_2$	: 5 g
$(NH_4)_2SO_4$	: 0.5 g
KCl	: 0.2 g
$MgSO_4$	: 0.1
$MnSO_4$	: Trace
$FeSO_4$	: Trace
Yeast extract	: 0.5
NaCl	: 0.1
Agar	: 20 g
Distilled water	: 1000 ml

4. **Noris - N - free media (Norris, 1959)**

$_2HPO_4$	: 1 g
$MgSO_4$	: 0.2 g
$CaCO_3$	: 1 g
NaCl	: 0.2 g
Glucose	: 10.0 g
Agar	: 18.0 g
Distilled water	: 1000 ml

5. Lauria agar Medium (Sambrook *et al*., 1989)

Tryptone	: 10.0 g
Yeast extract	: 10.0 g
Sodium chloride	: 5.0 g
Agar	: 18.0 g
Distilled water	: 1000 ml
pH	: 7.2

6. Nutrient Agar (Anon, 1957)

Peptone	: 5.0 g
Beef Extract	: 3.0 g
Sodium chloride	: 5.0 g
Agar	: 18.0 g
Distilled water	: 1000 ml
pH	: 18.0 g

7. Martin's Rose Bengal Agar (Martin, 1950)

Dextrose	: 10.0 g
Peptone	: 5.0 g
KH_2PO_4	: 1.0 g
$MgSO_4.7H_2O$	: 0.5 g
Rose Bengal	: (1 part in 30,000 parts of medium)
Agar	: 18.0 g
Streptomycin	: 30 mg
Distilled Water	: 1000 ml
pH	: 6.8

8. Kuster's agar (Kuster and Williams, 1964)

Starch	: 10.0 g
Sodium chloride	: 2.0 g
Calcium carbonate	: 0.02 g
Casein	: 0.3 g
KH_2PO_4	: 2.0 g
$MgSO_4.7H_2O$	: 0.5 g
Agar	: 18.0 g
Distilled water	: 1000 ml
pH	: 7.1- 7.8

9. Starch agar (Eckford, 1927)

Peptone	: 5.0 g
Beef extract	: 3.0 g
Starch	: 10 ml
Distilled water	: 1000 ml
Agar	: 18
pH	: 7.2

10. Czapeck's Solution (Mahadevan and Sridhar, 1984)

$NaNO_3$	:2.00 g
K_2HPO_4	: 1.00 g
$MgSO_4.7H_2O$	: 0.50 g
KCl	: 0.50 g
$FeSO_4.7H_2O$	: 0.01 g
Sucrose	: 30.00 g
Yeast extract	: 1.00 g
Distilled water	: 1000 ml

11. Nutrient gelatin (Cappuccino and Sherman, 1992)

Peptone	: 5.00 g
Beef extract	: 3.00 g
Gelatin	: 120.0 g
Distilled water-	: 1000 ml
pH	: 6.8

12. Skim milk agar

Skim milk powder	: 100.0 g
Peptone	: 5.0 g
Agar	: 15.0 g
pH	: 7.2

13. MR-VP broth (pH 6.9)

Peptone	: 7.0 g
Dextrose	: 5.0 g
Potassium phosphate	: 5.0 g

14. Luria agar

Tryptone	: 10.0 g
Yeast extract	: 5.0 g
Sodium chloride	: 5.0 g

Agar	: 18.0 g
Distilled water	: 1000 ml
pH	: 7.2

Appendix II: Biochemical tests

Gelatin Liquefaction

The gelatin liquefaction ability of bacterial isolates was examined by the procedure of Blazevic and Ederer (1975). Plates of gelatin agar in triplicates inoculated with cultures in one spot were incubated at 30°C for three days. After incubation, the plates were flooded with 12 per cent $HgCl_2$ solution and allowed to stand for 20 minutes and observed for clear zone around the growth of organism to indicate gelatin liquefaction.

Hydrolysis of starch

The ability of the isolates to hydrolyse starch was examined by the procedure of Eckford (1927).Triplicate plates of starch agar were inoculated with test cultures and incubated at 30° C for three days. After incubation, the plates were flooded with Lugol's iodine solution, allowed to stand for 15 -30 minutes and observed for clear zone around the colony to indicate hydrolysis of starch. Starch agar was prepared by suspending one gram of starch powder in 10 ml of cold distilled water, mixed with 90 ml nutrient agar and autoclaved bat 121°C for 20 minutes.

Casein hydrolysis (Seeley and Vandemark, 1970)

Triplicate plates of skim milk agar streaked with test cultures were incubated at 30°C for one week and then observed for clear zones around the colony against a background. Skim milk agar was prepared by suspending 10 grams of skim milk powder in 100 ml distilled water and later heated ,cooled and then mixed with 900 ml sterilized nutrient medium before pouring into the plates.

Acid and Gas production (Seeley and Vandemark, 1970)

Bacterial isolates were tested for acid and gas production by inoculating to five ml of pre-sterilized glucose broth medium in test5 tubes containing Durham's tube and bromo cresol purple (15 ml/L of 0.04% Solution) as pH indicator. The tubes were incubated for seven days at 30°C. The accumulation of gas in the Durham's tube was taken as positive for gas production and change in colour of medium to yellow was taken as positive for acid production.

Catalase test (Blazevic and Ederer, 1975)

Nutrient slants were inoculated with test organisms and were incubated at 30°C for 24 hours. After incubation, the tubes were flooded with one ml of three per cent hydrogen peroxide and observed for gas bubbles. The occurrences of gas bubbles was scored positive for catalase.

Hydrogen sulphide producton (Cowan and Steel, 1970)

Bacterial isolates were inoculated to test tubes containing 5 ml of sterile H_2S medium and incubated at 28±2°C for 3 to 5 days. Then the tubes were examined for H_2S production. The formation of black ring in the test tube was taken as positive for H_2S production.

STUDIES ON BACTERIA SOLUBILIZING BOTH POTASSIUM AND PHOSPHOROUS AND THEIR EFFECT ON MAIZE (*Zea mays* .L)

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ABSTRACT

Bacteria solubilizing both potassium and phosphorus isolated by soil samples collected from different rhizosphere and crops from Dharwad, Haveri and Davanagere districts of Karnataka. The total 50 bacterial isolates were tested for their potassium and phosphrous solubilization and characterized upto genus level based on morphological and biochemical characters.

In vitro evaluation of potassium and phosphorus solubilization by bacteria tested at different days after incubation (DAI). The maximum solubalization of K and P observed at 15 DAI ranges from 2.36 to 29.83 µg/ml and 3.44 to 14.25 per cent respectively. All the isolates were tested for beneficial traits like production of growth promotion substance and the amount of IAA and GA produced by the isolates ranged from 3.38 to 8.90 µg/25ml and 1.27 to 3.67 µg/25ml respectively.

The efficient eight isolates of *Bacillus* species which solubilize both potassium and phosphorus was examined for their influence on growth, yield and nutrient content of maize plant under green house condition. All the inoculated treatment with bacteria were recorded maximum dry matter content at 30, 60, and at harvest recorded in isoaltes K-PSB 32 with rock phosphate and mica were 12.80, 44.2 and 235.7 g/plant, respectively. The yield components as compared with uninoculated fertilizer control, the isolate K-PSB 50 with rock phosphate and mica were recorded the heights cob weight 144.9 g/plant and the highest grain yield 52.93 g/plant and other parameters, followed by isolate K-PSB 32 with rock phosphate and mica.

From the present study, it was concluded the bacterial isolates K-PSB 50 and K-PSB 32 were efficient K-PSB isolates. They capable to solubilize both K and P from the mineral source of mica and tricalcium phosphate respectively under *in vitro* condition. They produced plant growth promoting substances such as IAA and GA resulting in increased biomass, total dry matter and cob yield of maize plant.