2. REVIEW OF LITERATURE
Phosphorus is one of the major nutrients limiting plant growth and next only to nitrogen in terms of nutrient requirement. It is also an important component of DNA and RNA. Many microorganisms are potential solubilizers of bound phosphates in soil. Thus microorganisms play a significant role in the transformation of this essential element.

2.1 Phosphorus in soils

A major part (98-99%) of phosphorus in soils is associated with primary or secondary minerals and soil organic matter. Microorganisms (1-2%) and soluble phosphorus (0.01%) serve as insignificant reservoirs of phosphorus in soil. Phosphorus compounds exist in soils as both organic and inorganic forms. The organic phosphates are composed of nucleic acids, phospholipids and phytin and are derived from plants and microorganisms. Most of the inorganic phosphorus compounds in soils are composed of those containing calcium and those containing iron and aluminium.

2.2 Phosphate-solubilizing microorganisms in various soil types

There are several reports on isolation of phosphate-solubilizing microorganisms from diverse sources (Gaur, 1990). Soil serves as a good habitat for most of the phosphate-solubilizing microorganisms. The physical and chemical characteristics of soil affect the composition of microbial populations both qualitatively and quantitatively. Many reports are available on phosphate-solubilizing microorganisms in different types of soils. The occurrence and numbers of phosphate-solubilizing organisms in soils have been reviewed (Kucey et al., 1989;
Phosphate-solubilizing microorganisms have been found in almost all soils tested although the numbers vary with the soil climate and history (Sperber, 1958; Katznelson and Bose, 1959; Katznelson et al., 1962; Chhonkar and Subba Rao, 1967; Agnihotri, 1970; Banik and Dey, 1982; Kucey, 1983; Thomas et al., 1985; Thomas and Shantaram, 1986).

solubilizing microorganisms were isolated from an alluvial soil (Banik and Dey, 1982). Kucey (1983) estimated phosphate-solubilizing bacteria and fungi from southern Alberto soil samples collected from seventeen different sites. Molla et al. (1984) studied mineralisation of organic phosphates in soil. Phosphate-solubilizing microorganisms in coconut plantation soils were isolated by Thomas et al. (1985). Thomas and Shantaram (1986) also studied phosphate-solubilizing organisms from coconut plantation soils of Kerala. Likewise, Patgiri and Bezbaruah (1990) surveyed tea soils in Assam for phosphate-solubilizing microorganisms. Rajarathinam et al. (1995) screened soils of Kamarajar district in Tamil Nadu for phosphate-solubilizing microorganisms. Occurrence of phosphate-solubilizing bacteria in soils of five districts of Marathwada region was reported by Bilolikar et al. (1996). Johri et al. (1999) reported the occurrence of phosphorus-solubilizing bacteria in alkaline soils. The importance of soil microorganisms in mobilising soil phosphorus for utilization by plants was emphasized (Richardson, 2001, 2002).

### 2.3 Microorganisms involved in solubilization of phosphorus

Many bacteria, fungi and a few actinomycetes are potential solubilizers of bound phosphates in soil thus playing an important role in soil solubilizing phosphorus and making it available to plants. According to Subba Rao (1993), information available from different sources indicate that 13 genera of bacteria, 19 genera of fungi and one genus of actinomycete are involved in phosphate solubilization.

Phosphate-solubilizing microbial populations are influenced by the physical and chemical characteristics of soils. Earnest (1923) isolated 28 species of fungi
representing 12 genera from five soils. Among them, *Aspergillus fumigatus*, *A. flavus*, *Penicillium luteum*, *P. funiculosum* and *Cladosporium herbarum* exhibited phosphorus-solubilizing ability from rock phosphate. Menkina (1950) isolated new strains of *Bacillus megaterium* var. *serratia* and *B. megaterium* var. *phosphaticum* which were able to liberate inorganic phosphorus from organic compounds. Moreau (1959) observed that liberation of phosphorus from glycerophosphate or tricalcium phosphate was mainly by bacteria and also by some fungi isolated from three forest soils. Addition of glucose and lecithin to the soil increased the population of phosphate-solubilizing bacteria in soil. Das (1963) tested 18 fungi isolated from paddy fields for their ability to utilize insoluble phosphates. Only three of them (*Aspergillus niger*, *Penicillium* sp. and *Sclerotium rolfsii*) exhibited phosphate-solubilizing ability. Mikovski (1964) found that organic compounds were mainly decomposed by bacteria like *Bacillus*, *Pseudomonas* and *Micrococcus* while *Penicillium*, *Aspergillus* and *Trichoderma* decomposed tricalcium phosphate. Medina (1968) observed higher number of bacteria and low populations of fungi and actinomycetes as solubilizers of phosphorus. But the actinomycetes were found to be high phosphorus solubilizers. Vidyasekaran *et al.* (1973) isolated three bacteria viz., *Bacillus polymyxa*, *B. circulans* and *Pseudomonas striata* and one fungus, *Aspergillus awamori* which were found to be effective solubilizers of tricalcium phosphate. Bardiya and Gaur (1974) preferred isolation of rock phosphate solubilizers for they also solubilize different forms of insoluble phosphates. Arora and Gaur (1979) demonstrated phosphorus-solubilizing activity by *Pseudomonas striata* and *Aspergillus awamori*. Phosphate-solubilizing bacteria and fungi constituted 0.5 and
0.1% respectively of the general soil microbial populations in Prairie soils with the bacteria outnumbering the fungi by 2 to 150 fold (Kucey, 1983). Similarly, Banik and Dey (1982) observed that phosphate-solubilizing bacteria outnumbered phosphate-solubilizing actinomycetes and fungi in Indian soils by 3 and 50 fold respectively. Banik and Dey (1983) isolated 8 bacteria, 2 actinomycetes and 6 fungi which are capable of solubilizing tricalcium phosphate to a higher degree. However, Thomas et al. (1985) isolated more phosphate-solubilizing fungi from alluvial, laterite and clayey soils than from sandy soils in coconut plantations in Kerala. Further, Thomas and Shantaram (1986) observed lesser populations of phosphorus-solubilizing microorganisms in clayey soils than in alluvial and sandy soils in coconut plantations of Kerala. The phosphorus-solubilizing bacteria isolated belong to genera like *Pseudomonas, Micrococcus, Bacillus, Corynebacterium* and *Alcaligenes*. Al-ghazali et al. (1986) demonstrated phosphate-solubilizing ability of bacteria like *Enterobacter, Pseudomonas* and *Aeromonas* isolated from Alkhair river sediments. Ali et al. (1986) reported the superiority of *Bacillus, Agrobacterium, Aspergillus, Penicillium* and *Streptomyces* over all other cultures in phosphate solubilization. Of the 46 strains of aerobic heterotrophic bacteria isolated from tea soils in Assam, only *Bacillus subtilis, B. licheniformis, B. cereus, Pseudomonas* sp. and *P. putrefaciens* possessed significant phosphate-solubilizing and phosphatase activity (Patgiri and Bezbaruah, 1990). *Bacillus megaterium, B. polymyxa* and *Pseudomonas stutzeri* were the most efficient among the phosphate-solubilizing bacteria isolated from the soils of Kamarajar district in Tamil Nadu and glucose was the best carbon source for solubilization of phosphorus (Rajarathinam et al., 1995).
2.4 Solubilization of phosphorus by microorganisms

Microorganisms are involved in a range of processes that affect the transformation of soil phosphorus and are thus an integral component of soil phosphorus cycle. In particular, soil microorganisms are effective in releasing phosphorus through solubilization and mineralization (Richardson, 2002). The concept of using soil microorganisms to improve mobilization of poorly available forms of soil phosphorus is not new. More than 50 years ago, Gerretsen (1948) first demonstrated that pure cultures of soil bacteria could increase phosphorus nutrition of plants through increased solubility of calcium phosphates. Many microorganisms have the potential to bring the phosphates into solution form. This attribute is not rare since 10 to 50% of the bacterial isolates tested are capable of solubilizing calcium phosphates (Rokade and Patil, 1992). Species of *Pseudomonas*, *Bacillus*, *Micrococcus*, *Flavobacterium*, *Penicillium*, *Aspergillus* and *Sclerotium* and others are involved in this process.

*Bacillus subtilis*, *B. megaterium*, *B. mesentericus* and *B. mycoides* carried out solubilization of phosphates from different insoluble phosphates (Sen and Paul, 1957). Muramtsev (1958) observed that microorganisms can dissolve calcium phosphate in acid, neutral and alkaline culture media. *In vitro* screening of several forest nursery seed bed fungi revealed that *Penicillium* sp., *Aspergillus niger*, *A. flavus*, *Fusarium oxysporum*, *Sclerotium rolfsii* and *Cylindrocladium* were effective in dissolving phosphorus in tricalcium phosphate, hydroxyapatite and fluoroapatite (Agnihotri, 1970). Likewise, *in vitro* experiments carried out by Gostkowska (1976) with 73 different strains of *Rhizobium* demonstrated that *Rhizobium meliloti*, *R. lupini* and
*R. leguminosarum* were capable of dissolving tricalcium phosphate and ferrous phosphate. Fernandez *et al.* (1987) observed mineralization of organic phosphorus into available form by strains of *Bacillus cereus*. Shingte *et al.* (1987) isolated 21 fungal, 9 *Rhizobium* and 4 *Azotobacter* cultures for phosphorus-solubilizing ability. They observed that *Aspergillus awamori* was superior to all other fungal cultures. Similarly, *Rhizobium* spp. from berseem, mung, soybean and lucerne were superior to french bean and groundnut rhizobia.

### 2.5 Extent of solubilization of phosphorus by microorganisms

Several studies were conducted to determine the extent of phosphorus solubilization by different types of microorganisms including bacteria and fungi. Although bacteria have been used in the commercial preparations of phosphate dissolving cultures to improve growth of plants, fungi seem to be better agents than bacteria (Rokade and Patil, 1992). Experiments with cultures of many bacteria and fungi such as *Bacillus*, *Pseudomonas*, *Aspergillus* and *Penicillium* showed that they are potential solubilizers of bound phosphates. But they vary in their efficiency to dissolve tricalcium phosphate.

Of 149 cultures of soil fungi tested by Mehta and Bhide (1970), 42 cultures showed potential to solubilize tricalcium phosphate ranging from 22 to 98.2%. Among the fungi tested, *Penicillium* sp., *Aspergillus fumigatus*, *A. niger*, *Pythium* sp., *Curvularia interseminata*, *C. lunata*, *Chaetomium fumiti* and *Humicola* spp. were more efficient in solubilizing phosphorus. Similarly, Ostwal and Bhide (1972) observed tricalcium phosphate solubilization of 13 to 58% in liquid medium and 8 to 37% in soil. *Pseudomonas putida* exhibited higher solubilization (58%) in liquid
medium when compared to soil (37%). Reichlova (1972) reported release of 13-16% of phosphorus after 55 h by strains of *Rhizobium japonicum* isolated from soybean nodules. Arora and Gaur (1979) observed that *Aspergillus awamori, Pseudomonas striata* and *Bacillus polymyxa* solubilized hydroxy apatite by 11.9, 10.2 and 6.6% respectively.

### 2.6 Phosphate-solubilizing bacteria as inoculants

Both bacteria and fungi secrete organic acids into the soil and lower the pH in their vicinity to bring about solubilization of bound phosphates (Sundara Rao and Sinha, 1963). Phosphate-solubilizing microorganisms could therefore play important roles in environments where phosphorus is not readily available as in soils of low pH (Kannaiyan, 2003). A substantial number of bacterial species, mostly associated with rhizosphere, may exert beneficial effect on plant growth (Glick, 1995). This group of bacteria has been termed “plant growth promoting rhizobacteria” or PGPR (Kloepper and Schroth, 1978). Among them, some phosphate-solubilizing bacteria have already been used as commercial biofertilizers for agricultural improvement (Subba Rao, 1993; Rodriguez and Fraga, 1999). Although plant growth promoting rhizobacteria occur in soil, usually their numbers are not high enough to compete with other bacteria commonly established in the rhizosphere. Therefore, for agronomic utility, inoculation of plants by target microorganisms at much higher concentration than those normally found in soil is necessary to take advantage of their beneficial properties for enhancing yields by plants (Igual *et al.*, 2001, 2002).

Beneficial effects of inoculation with phosphorus-solubilizing microorganisms to many crop plants have been described (Subba Rao, 1993; Tomar *et al.*, 1996;
Rhizobia are, perhaps, the most promising group of phosphate-solubilizing bacteria on account of their ability to fix nitrogen and capacity of some strains for solubilization of phosphorus (Halder et al., 1990; Halder and Chakrabarty, 1993). It has been reported that phosphate-solubilizing strains of *Rhizobium* and *Bradyrhizobium* increase growth and phosphorus content of leguminous as well as non-leguminous plants (Chabot et al., 1996; Antoun et al., 1998; Chabot et al., 1998; Peix et al., 2001). Synergistic interactions between vesicular arbuscular mycorrhizae and phosphate-solubilizing bacteria have been reported (Ray et al., 1981; Piccini and Azcon, 1987; Frey-Klett et al., 1997; Toro et al., 1997; 1998; Kim et al., 1998). Similarly, plant growth can be increased by dual inoculation with *Azospirillum* (Alagawadi and Gaur, 1992; Belimov et al., 1995) or *Azotobacter* (Monib et al., 1984; Kundu and Gaur, 1984). Inoculation with mixed cultures such as *Pseudomonas striata* and *Aspergillus awamori* increased the yield of cotton more than with either strain alone (Kundu and Gaur, 1980a). Similar increase in yield was observed after inoculation with peat based cultures of *Bacillus polymyxa* and *Pseudomonas striata* (Kundu and Gaur, 1980b). Phosphate-solubilizing microorganisms when inoculated with rock phosphate increased rice yields (Gaur and Ostwal, 1972; Anthoni Raj et al., 1994; Thamizvendan and Subramanian, 2000). Inoculation of *Vigna uniguiculata* seeds with either rock phosphate or a combination of rock phosphate and single super phosphate and *Aspergillus awamori* resulted in increased yields (Nagaraju and Nanjundappa, 1996). Similarly, concomitant inoculation of sunflower with phosphorus-solubilizing microorganisms along with rock phosphate increased grain yield (Andhani Gowda et al., 1998).