LITERATURE REVIEW

The increasing cost of chemical fertilizers and energy crisis have drawn attention of scientists world over to the renewable resources and microbial processes such as nitrogen fixation, phosphate mobilization, cellulose degradation etc., to supplement essential nutrient needs of crop plants. Inoculation of seeds or soil with nitrogen fixing and phosphate mobilizing microorganisms change the microbial population of rhizosphere, consequently affecting the plant growth.

Bacterial inoculants prepared from Rhizobium, Azotabacter, Azospirillum and phosphate-mobilizing microorganisms have been used extensively and a vast literature on the beneficial effects of these microorganisms on crop plants is available (Deka and Kaketi 1996; Srivastava and Siddique 1992; Caballero et al., 1992 etc.). This review would focus mainly on the beneficial aspects of nitrogen fixing microorganisms and phosphate mobilizers i.e. phosphate solubilizers and arbuscular mycorrhizal (AM) fungi, their interactions and interactive effects on crop plants.

NITROGEN-FIXING MICROORGANISMS

**Rhizobium spp. (Symbiotic nitrogen fixer)**

Hellriegel and Wilfarth (1888) described the unique role of root-nodule bacteria in fixing atmospheric nitrogen in leguminous plants. Considerable information on the fundamental and applied aspects of this
problem have accumulated. *Rhizobium*, the microsymbiont in legume-
*Rhizobium* symbiosis was first isolated by Beijerinck, (1888) and its
production for agricultural purposes has now attained industrial dimensions
in many countries.

There is a voluminous literature on the beneficial effects of *Rhizobium*
inoculation. Earlier, this has been reviewed by several workers (Fred et al.,
1932; Date 1977; Davis et al., 1984). In recent studies, Ghosh (1989)
recorded increased dry matter and yield of mungbean inoculated with
*Rhizobium* sp. and 40 kg P/ha. Similar observations were made by
Kothari and Saraf (1990) and Deka and Kakati (1996). Jadhav et al.
(1990) studied different methods of application of *Rhizobium* culture on
growth and yield of groundnut. They showed that slurry (liquid) application
of *Rhizobium* sp. was most effective in increasing dry pod yield, number
of nodules, dry weight of nodules, dry weight of plants and percentage
germination along with compost. Patil et al. (1990) reported improved
germination of groundnut seeds by the treatment of *Rhizobium* sp. with
a pesticide thiram. Gunjal and Patil (1990) studied the effect of *Rhizobium*
inoculation with split doses of nitrogenous fertilizer applied at the time
of sowing and flowering on the nodulation, dinitrogen fixation and yield
of groundnut under field conditions. *Rhizobium* inoculation with split
application of 10 kg N/ha, each at the time of sowing and flowering
produced the highest dry weight of nodules, nitrogen content in shoot and
root and dry pod yield as compared to application of full dose of nitrogen
at the time of sowing or flowering. Their observations also indicated the suppressive effects of full dose of nitrogenous fertilizer at the time of sowing or flowering on nodulation and dinitrogen fixation. Mandhare and Patil (1990) observed that inoculation of *Rhizobium* strains in french bean improved the grain yield, dry matter weight, number of nodules, organic carbon and total nitrogen. Also the highest yield, organic carbon and total nitrogen was recorded in multistrain treatment. Response of four pea varieties to seed inoculation with three rhizobial strains under field condition showed that application of two strains viz., Pea 2 and Pea 12 improved nodulation, shoot dry weight and yield of peas (Wange, 1990). Emmimath (1990) assessed the influence of different levels of phosphorus on nodulation, dry matter and yield of bengalgram. The application of 40 and 50 kg P$_2$O$_5$/ha reduced the nodule number, nodule weight, dry matter and grain yield. However, lower dose of phosphatic fertiliser at 30 kg P$_2$O$_5$/ha was found to be beneficial. Yadav *et al.* (1992) observed an increase in nodule number, nitrogenase activity and seed and straw yield in greengram treated with *Rhizobium* sp. and enriched compost. Similar observations were made by Rajput *et al.* (1993).

Wange and Patil (1996) conducted a field experiment in medium black soil to evolve best cultivar and *Rhizobium* strain combination in respect to nodulation, vegetative growth and yield of the pigeonpea crop over recommended dose of fertilizer nitrogen. Rhizobial strains R1 and R3 performed significantly well in improving nodulation, plant height, shoot dry weight, pod number and grain yield. Further, strain R3 performed well
with cv. V1 and U3, whereas, strain R1 with cv. V2 which indicated that strains of pigeonpea rhizobia respond differently with different cultivars of same host species.

**Azotobacter (Free-living nitrogen fixer)**

*Azotobacter*, a non-symbiotic nitrogen-fixing bacterium was isolated and described for the first time by Beijerinck (1901). Mishustin and Shilnikova (1969) showed an increase of at least 7 to 12 percent in the yield of different crops due to inoculation with *Azotobacter*. In a study, the contribution of atmospheric nitrogen was determined in the total nitrogen of grains due to the inoculation of barley seedlings with a strain of *Azotobacter* possessing an affinity for the barley rhizoplane. A quantitative method of isotopic dilution was used following the application of labelled ammonium nitrate. An increase in the nitrogen content of the grains as a result of biological nitrogen fixation and reduced consumption of nitrogen from fertilizers and the soil were recorded (Troitskaya and Troitskii, 1988). Rubiak et al. (1989), however, found no activity of different strains of *Azotobacter chroococcum* isolated from root system of sorghum, when root segments were tested for nitrogen fixation using the acetylene reduction assay. Srivastava and Siddique (1992) studied the effect of *Azotobacter* culture and phosphorus level on growth and yield of sorghum. Sorghum seeds were inoculated with *Azotobacter* and sown in plots fertilized with 0, 20, 40 or 60 kg P\textsubscript{2}O\textsubscript{5}/ha before sowing. Application of phosphorus (P) increased grain yields from 2.79 t/ha with
20 kg P₂O₅ to 4.05 t/ha with 60 kg P₂O₅/ha whereas seed inoculation with **Azotobacter** sp. increased grain yield in unfertilized plots to 2.85 t/ha. The highest yield of 4.98 t/ha was recorded with seed inoculation and the highest fertilizer rate.

In a field experiment, in 1987-89, wheat seed inoculation with **Azotobacter chroococcum** (Mal 103) and its mutants resistant to methyl ammonium chloride (Mac 3, Mac 27) and methyl alanine (Mal 3) produced more grain yield than the uninoculated control. Application of 60 kg Na/ha + seed inoculation with Mal 3 or Mal 103 produced similar grain yield (4.30 and 4.08 t/ha, respectively) to application of 120 kg N only (4.45 t/ha) (Lakshminarayan et al., 1992). Similar responses to seed inoculation and N application were observed by others also (Vireshwar et al., 1993; Gill et al., 1993; Shivankar et al., 1993 and Prabhjeet et al., 1994). Jana and Mishra (1994) reported that best nitrogen fixation by **Azotobacter chroococcum** B12 was found at pH 7.0 with low concentration of nitrogen salts after 30 h incubation at 28°C. This strain showed tolerance against salinity, metals and antibiotics. The pesticide Rogor inhibited growth and nitrogen fixation at a very low concentration.

**Azospirillum (Associative nitrogen-fixer)**

The first species of **Azospirillum** was isolated by Beijerinck (1925) from nitrogen poor sandy soil in the Netherlands and was originally named **Spirillum lipoferum**. The bacterium was later isolated from soil (Schroder, 1932) and from dried seaweed in Indonesia (Becking, 1963) and as a
phyllosphere bacterium of tropical plants (Dobereiner et al. 1976; Becking, 1982). The genus was renamed as Azospirillum with two species. A. brasilense and A. lipoforum by Tarrand et al. (1978). Kapulnik et al. (1981) investigated the potential of Azospirillum brasilense to enhance development and increase growth of several grasses, wheat and sorghum as nitrogen-fixing microorganism. In both sterilized and unsterilized systems, heading and flowering occurred earlier in the plants inoculated with the bacterium, as compared to the uninoculated ones. Total shoot and root weights, total N-contents, plant height and leaf length were significantly increased. Kalininskaya (1990) observed the influence of combined nitrogen on azospirilla in pure culture, in association of rice plants and in soil under rice. In pure culture, complete inhibition of nitrogen fixation occurred at nitrogen concentrations of 56-70 mg/litre and > 210 mg/litre, for NH₄-N and NO₃-N, respectively. In field experiments, nitrogen application of 120-240 kg/ha (urea) has no adverse effect on growth of nitrogen-fixing microorganisms and did not inhibit nitrogen fixation in the soil. In pot experiments, Azospirillum sp. and Azotobacter sp. were the most sensitive diazotrophs to high doses of nitrogen fertilizers.

Kravchenko and Makarova (1990) recorded the nitrogenase activity of streptomycin resistant mutant of Azospirillum brasilense in a dernopodzolic soil (pH 6.2) after its application to the soil in combination with plant remains of pea and wheat plants. Their numbers declined for the first three weeks and then stabilised. In soils with plant remains, the
numbers of A. brasilense decreased less than the control soils. An increase in nitrogen fixation was also recorded in soils inoculated with Azospirillum sp. along with plant remains.

Caballero et al. (1992) reported significant increase in grain yield of wheat, ranging from 23 to 63% due to the inoculation of Azospirillum sp. in 1986 and from 29 to 43% in 1987. The increase in yield was further enhanced by the treatment of Azospirillum and nitrogen fertilizers @ 0, 60, 90, 120 Kg/ha.

PHOSPHATE MOBILIZING MICROORGANISMS

Although solubilization of insoluble phosphates was known as early as 1903 by Stalstrom (1903) and Sackett et al. (1908), a systematic approach in this regard was made by Pikovskaya (1948) who showed dissolution of tricalcium phosphate (TCP) by pure culture of 'bacterium P'. Since then, there have been several reports on the solubilization of insoluble phosphate by microorganisms (Mishustin and Nau-mova, 1956).

Phosphate mobilization in soil

Rose (1957) suggested that phosphate solubilizing capacity of different soils may be tested by inoculating liquid medium with soil suspension which may give an idea regarding soil’s total activity of phosphate solubilizing microbes. Chandrasekaran (1969) found higher solubilization of inorganic phosphate by four rhizosphere soils as compared to non-rhizosphere soils. Yin (1988) isolated phosphate dissolving
microorganisms including bacteria, actinomycetes and fungi from alkaline, saline, chernozems, yellow brown earth, albic soils, terrarossared earths and latosols collected from different parts of China. The highest number of phosphate dissolving microorganisms was in chernozems (48.9 million/g soil), and the lowest in alkaline soils (20 thousand/g soil). Among the 265 strains of phosphate dissolving bacteria, Bacillus megaterium, Arthrobacter spp., Flavobacter spp., Erwinia spp. and Pseudomonas spp. showed phosphate solubilizing activity of 2-30 mg/g soil. Bacillus polymyxa, B. alcaligenes and B. subtilis showed phosphate solubilizing activity of 20-25 mg/g soil whereas Bacillus cereus and B. sphaericus had a P. solubilizing activity of 15 to 20 mg/g soil. Bacillus firmus, however, exhibited lowest phosphate solubilizing activity of 2-5 mg/g soil.

Sattar and Gaur (1989) isolated phosphate dissolving microorganisms from rhizosphere soil of rice (Oryza sativa L.), jute (Corchorus spp.) and dhaincha (Sesbania spp.). The two groups of isolates identified as Pseudomonas aeruginosa and Bacillus megaterium differed in morphological and biochemical characters. Yahya and Al-Azawi (1989) enumerated phosphate solubilizing bacteria (PSB) in 52 soil samples collected from agricultural areas at Baghdad. More than 90% of the samples were inhabited with indigenous PSB. The number varied and ranged from 0.012-28.4 x 10^4 cells per gram soil. The correlations between PSB counts and electrical conductivity, available phosphorus, cation exchange capacity, soil moisture, organic matter and pH were insignificant. Both abundance and numbers of PSB were more pronounced
in descending order under vegetables, legumes, grasses, cereal and orchard trees. Martinez et al. (1990) found a greater variation in the population of phosphate solubilizing microorganisms collected from rhizosphere soil of different plants. The highest number of PSM was reported in rhizosphere of sugarcane. Numbers of PSM capable of dissolving either Ca, Fe, or Al phosphates were related to the amount of that form of phosphate present in the soil. *Bacillus* spp. and *Pseudomonas* spp. were the main bacteria. *Penicillium* spp. and *Aspergillus* spp. the main fungi and *Streptomyces* spp. the only actinomycetes isolated from the soils. The efficiency of bacterial and fungal strains in phosphate solubilization varied greatly with the form of inorganic phosphate, but bacteria were most efficient in all cases. Patgiri and Bezbaruah (1990) reported that, of the 46 strains of aerobic heterotrophic bacteria isolated from the soils in Assam, only *Bacillus subtilis*, *B. licheniformis*, *B. cereus*, *Pseudomonas* sp. and *P. putrificans* possessed significant phosphate solubilizing and phosphatase activity. Inoculation of soil with these strains increased the available phosphate content of the soil during a 22-day period in the presence and absence of leaf litter or super phosphate.

Kekhainova and Taleva (1990) observed that phosphate solubilizing microorganisms in the sunflower rhizosphere were stimulated by nitrogenous fertilizer on a grey forest soil (pH 4.9-5.3) but depressed on a leached chernozem (pH 6.0). Rhizosphere microorganisms were most abundant at the blossoming (flowering) stage. Reyes-de-Alvarez (1991) determined
microbial population in two soils from Venezuela in potato dextrose yeast (Y) and mineral agar media with three sources of phosphorus; CaHPO$_4$ (CaP), Navy rock phosphate (NP) and Monte Fresco-Rock phosphate (MFP). Fungal populations were higher in CaP and MFP than in Y and NP. Bacterial populations in all media did not differ significantly. CaHPO$_4$ solubilizing microorganisms consisted of 45% fungi and only 3% bacteria. Selective behaviour of the microflora towards different phosphate sources occurred. Yadav and Singh (1991) found little variation in number of phosphate solubilizing microorganisms in soils of sugarcane belt in North Bihar. Populations were dependent on the organic carbon contents of the soils. Evaluation of the predominant phosphorus solubilizers, *Bacillus cereus*, *Aspergillus niger*, *Penicillium digitatum* and *Bacillus megaterium* indicated that fungi were more efficient in red loam soil (Haplustalf), while in calcareous soil (calcifluvent) and in liquid medium, *B. megaterium* was more efficient. Phosphorus release by all the organisms was associated with reduction in pH of the medium. Solubilizing effect of *Bacillus megaterium* was progressively enhanced by increasing glucose concentration (0.5 to 2.0%) in the medium, but with rock phosphate such enhancement was not observed beyond 0.5%. Rokede and Patil (1992) suggested that the phosphate dissolving microorganisms were generally isolated on Pikovskaya agar medium. The phosphate solubilizing fungal population occurred more in acidic than neutral soils while the bacterial population was found more in alkaline soils. Among the fungal genera, *Aspergillus* and *Penicillium*, and among the bacterial genera, *Bacillus*
and *Pseudomonas* were the predominant phosphate solubilizers. Phosphate solubilizers varied in their phosphate solubilizing ability.

Luo *et al.* (1993) isolated phosphate solubilizing bacteria from red soil of China under long term rice and dry land crop rotations. The soil pH was not significantly related to phosphate solubilization but there was a highly significant relationship between the total amount of succinic, oxalic, acetic and malonic acids produced by bacteria and the amount of phosphates solubilized from ALPO$_4$, FePO$_4$ and Ca$_3$(PO$_4$)$_2$, indicating the phosphate solubilization resulted not only from H*, but from the chelating effects of organic acids. Among different isolates, *Enterobacter aerogenes* was the most effective tricalcium phosphate (TCP) and rock phosphate (RP) solubilizer (liberated 80.7 and 20.6 mg P$_2$O$_5$ respectively). In the presence of TCP, all sugars (arabinose, fructose, galactose, sorbitol, mannitol, xylose, sucrose, maltose, lactose) except xylose showed a positive effect on phosphate solubilization (PS) activity. But in the presence of RP, all sugars showed a negative effect on PS activity. All the test nitrogen sources proved inferior to the ammonium sulphate regarding solubilization of TCP and RP. The rise in PS activity with TCP and RP was slow and steady with a rise in EDTA concentration from 0-6 mg/ml of the medium, acidic pH of the medium supported PS activity (Jugnu *et al.* 1993).

Nahas *et al.* (1994) worked on the occurrence of phosphate solubilizing and acid and alkaline phosphatase producing bacteria and
fungi from thirteen different soil types and vegetation. Total bacteria and fungi ranged from 3.7 to 142.6x10^5 and 2.3 to 57.7x10^3/g soil, respectively, predominating in crop soils. Of these, 7.1-55.6% of bacteria solubilized phosphatase, 9.3-44.6% produced acid phosphatase and 7.4-47.3% produced alkaline phosphatase. Fourteen bacteria and ten fungi showed a high ability to produce soluble phosphate in liquid medium. Also, the fungi presented more acid phosphate activity than the bacteria and conversely, the bacteria more alkaline phosphatase activity than the fungi.

Varsha et al. (1994) isolated phosphate solubilizing fungi like Aspergillus sp., Penicillium sp. and Rhizopus sp. from the rhizosphere of 24 crops, compost and garden soil using tricalcium phosphate in Pikovskaya's medium. Aspergillus aculeatus was found to be best solubilizer of tricalcium phosphate (94%), dicalcium phosphate (54.5%). Aluminium phosphate was, however, solubilized best by Aspergillus niger (36.8%). Singhal et al. (1994) reported the solubilization of 5 types of rock phosphates by Aspergillus japonicus and A. foetidus at pH 8 and pH 9. Solubilization was higher in the presence of pyrite than in control lacking either pyrite or fungal inoculum. Both the species of Aspergillus were found to be good pyrite solubilizers and could grow over a wide pH range. Solubilization of the rock phosphate was the result of organic acid release and pyrite oxidation. Varsha et al. (1995) observed inorganic phosphate solubilization by some yeasts; Saccharomyces cerevisiae (local isolate), S. cerevisiae ATCC 9896 and Rhodotorula minuta NCIM 3359. R. minuta NCIM 3359 and S. cerevisiae ATCC 9896, solubilized 42.10
mg P₂O₅ on 15th day and 30.10 mg P₂O₅ on 3rd day from tricalcium phosphate respectively. Senegal rock phosphate was solubilized maximally by *R. minuta* (5.45 mg P₂O₅ and *S. cerevisiae* (8.88 mg P₂O₅) on 5th day of growth. Illmer *et al.* (1995) reported that *Aspergillus niger*, *Penicillium simplicisimum*, *Pseudomonas* sp. (P118/89) and *Penicillium aurantiogriseum* were very effective in solubilizing hardly soluble AlPO₄. *A. niger* produced citrate, oxalate and gluconate, whereas the other species did not produce any organic acids in detectable amounts. This indicated that the production of organic acid is an important solubilization mechanism of AlPO₄ but not the only effective one. Organic acids alone were able to solubilize AlPO₄ to a certain extent, although they were less effective compared to biotic leaching. Vora and Shelat (1996) observed above 15% solubilization of the applied phosphate source by the different isolates from soils of Gujarat.

**CROP RESPONSE TO PHOSPHATE SOLUBILIZERS**

Since the first report by Gerretsen (1948) on the beneficial effects of phosphate solubilizing microorganisms, there have been many reports on the increased phosphorus uptake and yield of different crops due to inoculation with phosphate mobilizing or solubilizing microorganisms. Asea *et al.* (1988) demonstrated that when calcareous chernozemic soil was inoculated with *Penicillium bilaii*, plant dry matter yield of wheat increased by 16%, total plant phosphorus uptake by 14% and proportion of P derived from native P sources by 11% even in the presence of added
rock phosphate. Domey et al. (1988) observed that phosphate solubilizing bacteria capable of improving phosphorus nutrition of plants, stimulated growth of wheat plants under conditions of phosphorus deficiency. No correlation was observed between phosphate solubilizing ability of isolates and their effects on the plant growth. Mohod et al. (1989) found that the use of phosphate mobilizing culture alone or in combination with phosphatic fertilizers increased the root cation exchange capacity, available phosphorus and phosphorus uptake by the rice plants. The culture increased the phosphorus release efficiency of rock phosphate and made it equivalent to single super phosphate with respect to available phosphorus in soil. Salih et al. (1989) evaluated phosphate dissolution ability of *Penicillium* spp. and *Aspergillus* spp. in a calcareous soil treated with rock phosphate or superphosphate and subsequent uptake of phosphorus by sorghum. *Penicillium* spp. were found to be more effective in increasing available phosphorus in the soil treated with rock phosphate or super phosphate than *Aspergillus* spp. The dry matter production and phosphorus uptake by the plants inoculated with these fungi were greater in soil treated with rock phosphate than with super phosphate. Banik et al. (1989), however, suggested that rock phosphate with or without bacterial inoculation, can serve as a substitute for single superphosphate in jute cultivation without adverse effect on quality or yield of fibre. Manjunatha and Devi (1990) observed increased phosphorus uptake in groundnut plants inoculated with phosphate solubilizing bacterium, *Pseudomonas striata* along with phosphatic fertilizer @ 75 and 50 kg P$_2$O$_5$/ha and farm yard manure @
10 t/ha. Siddaramappa et al. (1991) found that application of Udaipur rock phosphate supplemented with combinations of farm yard manure, green leaf manure, biogas spent slurry and phosphate solubilizing \textit{Pseudomonas striata} and \textit{Aspergillus awamori} increased phosphate solubilization in soil and phosphorus uptake by the rice crop. Gaind and Gaur (1991) reported improved nodulation, available $P_2O_5$ content of soil, root and shoot biomass, straw and grain yield and nitrogen and phosphorus uptake by the mung bean plants upon inoculation with thermotolerant species of phosphate solubilizing \textit{Bacillus subtilis}, \textit{B. circulans} and \textit{Aspergillus niger}. Similar observations were recorded by Satpul and Kapoor (1992). Dubey and Billore (1992) showed an increase in the yields of cereals, legumes, potatoes and other field crops after inoculation with rock phosphate and phosphate solubilizing microorganisms, \textit{Bacillus megaterium}, \textit{Pseudomonas striata} and \textit{Aspergillus awamori}. The use of low grade rock phosphate was recommended for both neutral and alkaline soils with phosphate solubilizing microbial inoculants. Datta et al. (1992) observed that rice seedlings treated with phosphate dissolving bacterium \textit{Bacillus firmus} alone did not significantly affect grain yield but in combination with 5t/ha poultry manure + 21.8 kg $P_2O_5$/ha rock phosphate and 21.8 kg $P_2O_5$/ha single super phosphate, grain yield (4.17 t/ha) was increased as compared to control (3.17 t/ha). Effect of inoculation with phosphate solubilizing bacterium \textit{Bacillus firmus}, irrigation schedules and phosphorus levels on lentil cv. JL-1 was observed in field trials during the winter seasons of 1985/86 and 1986/87 by Rathore et al. (1992).
Highest yields were obtained with irrigation at both branching and pod formation (1.25 t/ha) or with 60 kg P₂O₅/ha (1.2 t/ha). Seed inoculation significantly increased yields over uninoculated controls. Tomar et al. (1993) inoculated black gram (Vigna mungo cv. T9) with 0 or 10 g phosphate solubilizing bacteria/kg seeds and given 0, 20, 40, 60 or 80 kg P₂O₅/ha. Seed yield increased with increasing rates of upto 60 kg P₂O₅/ha and was further increased by bacterial inoculation. Tiwari et al. (1993) found highest mean grain yield of 56.33 g/pot (9 plants) of wheat when inoculated with Pseudomonas striata supplemented with 5 or 10 t rice straw/ha + 60 or 120 kg N/ha + 60 kg P₂O₅/ha as Mussoorie rock phosphate and single superphosphate (1:1). The lowest yield of 3.81 g was recorded with 60 kg N + 60 kg P + 5 t rice straw. Soil application of malathion caused a significant increase in dry matter content and P-uptake of pot grown greengram (Vigna radiata) inoculated with Pseudomonas striata or Aspergillus awamori (Krishnamurthy and Alagawadi, 1994).

Dubey (1996) in a number of field trials carried out during 1991 and 1992 with soybean under rainfed conditions on vertisol found greater yield and phosphorus content in the plants treated with Pseudomonas striata and rock phosphate than the plants treated with Pseudomonas striata and single super phosphate.

**ARBUSCULAR MYCORRHIZAL (AM) FUNGI AND CROPS**

The mycorrhizal association with plant root system has recently
assumed importance in the mineral nutrition of plants particularly with respect to phosphorus. Marks and Kozlowski (1973) reviewed the reports of the conspicuous association of these fungi with root system of woody plants which exerted considerable beneficial effect on the growth of roots and uptake of nutrients. Arbuscular mycorrhizal (AM) fungi (Vesicular arbuscular mycorrhizal (VAM) fungi) are known to infect a wide range of crops such as cereals, grasses, pulses, cotton, tobacco, potatoes, sugarcane, tomatoes and a number of orchids and plantation crops (Gerdemann, 1968; Mosse, 1973; Kruckelmann, 1975; Tinker, 1975).

The effect of plant species on vesicular arbuscular mycorrhizal (VAM) fungal spore production was compared using corn, bahiagrass, soybean and sudangrass as the host crop. The VAM endophytes were *Glomus claroideum*, *G. etunicatum*, *G. mosseae*, *G. macrocarpum* and *Gigaspora margarita*. At 14 weeks after planting, spore production by *G. claroideum*, *G. etunicatum*, *G. mosseae* and *G. macrocarpum* was greater with bahiagrass than with corn or sudangrass. There was no difference between bahiagrass or sudangrass with *G. margarita*. Soybean was not a suitable host plant for VAM spore increase (Struble and Skipper, 1988). Rajapakse et al. (1989) determined the response of four cultivars of cowpea (*Vigna unguiculata*), varying in nitrogen fixation capacity, to inoculation with *Glomus fasciculatum* at four levels of added phosphorus in the rooting medium. Root colonization was negatively correlated with phosphorus content of growing medium and shoot P-concentration. Geetakumari et al. (1990) investigated the effect of *Glomus fasciculatum* on finger millet
with or without organic amendment. The mycorrhizal fungus and the farm yard manure were beneficial for improving the growth of finger millet as the grain and straw yields increased. Nagahashi et al. (1996) observed the effect of solution phosphorus (P) concentration upon growth of pregerminated spores of VAM fungus Gigaspora margarita. The number of branches and the total hyphal length were both significantly inhibited at 10 mM phosphorus. In addition, germinated spores exposed to exudates produced by roots of Daucus carota grown in the presence of P showed significantly less hyphal branching than those exposed to exudates produced by P-stressed roots. Their observations supported the view that high phosphorus concentration inhibits mycorrhizal formation (Hepper, 1983; Thomson et. al. 1991). Schreiner et al. (1997) conducted a pot study on soybean plants grown in P-fertilized (-P) or low-P soil (-P) or P-soil colonized by one of the AM fungi Glomus etunicatum, G. mosseae or Gigaspora rosea. Dry weights of the AM plants, as a group, were halfway between the dry weights of the +P and -P plants, but within the AM group, G. mosseae plants had the highest pod dryweights and pod/stem and root/stern ratio and the lowest specific root lengths, while plants colonized with G. etunicatum had high stem dry weights and were highly nodulated. Correlations between plants and soil traits indicated that interactions within the plant soil systems were mediated by the AM fungi.

**Interactions between nitrogen fixers and phosphate mobilizers**

Nitrogen-fixing microorganisms benefit the plants by converting
atmospheric nitrogen in utilizable forms and phosphate mobilizing microorganisms render insoluble forms of phosphorus available to plants. The need of two major nutrients thus can be met by the inoculation with the two groups of microorganisms. In this context dual inoculation of both the groups of microorganisms can be considered and would be highly beneficial provided their interaction is synergistic.

Phosphorus is a key element and is an essential requirement in biological nitrogen fixation. Esposito and Wilson (1956) observed a slow growth and decreased nitrogen fixation by *Azotobacter* sp. in culture medium devoid of phosphorus. On the other hand, the growth and nitrogen fixation by *Azotobacter* sp. was stimulated by phosphatic fertilizers (Ernandes, 1958; Deglyaneva, 1959 and Brown et al. 1962). Pavlovich (1959) observed an adverse effect of *Bacillus subtilis*, *B. mesentericus* and beneficial effect of *Bacillus megaterium* on *Azotobacter* growth. Panosyan et al. (1962) reported that *Bacillus subtilis*, *B. megaterium*, *Pseudomonas* spp. and many species of actinomycetes stimulated growth and nitrogen fixation by *Azotobacter*.

Combined inoculation of *Rhizobium* and phosphate solubilizing *Pseudomonas striata* or *Bacillus polymyxa* with or without added fertilizers on chickpea yield and nutrient content was studied under greenhouse conditions. *Rhizobium* inoculation alone increased nodulation and nitrogenase activity, whereas the phosphate solubilizers increased the available phosphorus content of the soil. The combined inoculation
increased nodulation, available phosphorus of soil as well as dry matter of the plants, grain yield and P and N uptake by the plants. The inoculation effects, however, were more pronounced in the presence of added fertilizers (Alagawadi and Gaur, 1988). Alagawadi and Gaur (1988) also studied interaction between *Azospirillum brasilense* and phosphate solubilizing *Pseudomonas striata* and *Bacillus polymyxa* and their influence on yield, nutrient uptake and acetylene reduction activity of sorghum in a sandy loam alluvial soil under greenhouse conditions. Significant increase in the yield level of sorghum occurred due to combined inoculation over single inoculation indicating a positive interaction between the two groups of bacteria. Further increase in yield was recorded by applying 40 kg N/ha as urea and 60 kg P$_2$O$_5$/ha as rock phosphate.

In an attempt to supply mixed inoculant to the farmers, Sarojini et al. (1989) observed the interaction effect of free-living nitrogen-fixing bacterium *Azotobacter* and phosphate solubilizing *Pseudomonas striata* on their growth pattern. No antagonistic behaviour of one organism towards other was noted. Phosphate solubilization was observed by the mixed culture suggesting that they could be used as a mixed microbial inoculant.

Sarojini and Mathur (1990) showed the effect of *in situ* incorporation of paddy straw along with mixed microbial inoculants, *Azotobacter* sp. and *Pseudomonas striata* on the yield of wheat crop in alluvial sandy loam soil. Seed bacterization with these microorganisms along with 60 kg N/
ha and 60 kg P$_2$O$_5$/ha produced a significant effect on the yield of wheat crop grown in paddy straw amended soil.

In a pot experiment, lentil cv Giza 9 seeds were inoculated with *Rhizobium leguminosarum* along with increasing doses (50, 100, 200, 400 kg/feddan (1 feddan = 0.42 ha) of rock phosphate with or without a 1:1 mixture of elemental S and rock phosphate in the presence or absence of phosphate dissolving bacteria. Plant dry weight and N, P, Fe, Zn, Mn and Cu uptake increased with rock phosphate, sulphur and phosphate dissolving bacteria compared with untreated control. Dry matter yield and nutrient uptake was slightly higher with sulphur application (Saber and Kabesh, 1990).

Mahgoub *et al.* (1991) reported that maize cv. Giza 2 plants receiving *Azospirillum lipoferum* treatment alone or in combination with phosphate solubilizing bacteria along with nitrogenous fertilizer showed increased grain yield in comparison to those plants which received only microbial inoculation treatment.

Gururaj and Mallikarjunaiah (1994) studied the effect of *Azotobacter* spp. and phosphate solubilizing fungi *Penicillium* spp. and seedling growth of sunflower. Increased seed germination and length of plumule/radicle were recorded with all *Azotobacter* cultures except one culture which reduced radicle length by 13.6% compared with the control. All fungal cultures greatly reduced seed germination and seedling growth. A combination of *A. chroococcum* GA-1 and GA-3 with *Penicillium* HF-
4 and HF-5 and Aspergillus GF-1 and GF-2 increased radicle and plumule length but the remaining culture combinations decreased radicle/plumule length. Rice et al. (1995) co-cultured Rhizobium meliloti and a phosphorus solubilizing fungus Penicillium bilaii in sterile peat. A combined inoculant was prepared by mixing equal proportions of yeast extract mannitol broth cultures of both organisms with sterile peat (gamma-irradiated). The cured inoculant contained 6.1x10³ Rhizobium CFU/g and 1.1 x 10⁷ Penicillium CFU/g. Addition of 26 mg/ml sucrose to yeast extract mannitol broth resulted in increased population of both organisms. A common production and delivery system of both organisms was thus found to be possible by the co-culturing of both the organisms in sterile peat.

Belimov et al. (1995) carried out pot experiments to investigate the effect of inoculation with pure and mixed cultures of Azospirillum lipoferum 137, Arthrobacter mysorens 7 and the phosphate solubilizing strain Agrobacterium radiobacter 10 on growth and mineral nutrition of two barley cultivars. A significant positive effect on grain yield of the two barley cultivars was obtained after inoculation with mixture of A. lipoferum 137 + A. radiobacter 10 and A. lipoferum 137 + A. mysorens 7 only. The acetylene reduction activity on roots or in batch culture was significantly higher when A. lipoferum 137 and A. radiobacter 10 was used together. Using N¹⁵ isotope dilution technique it was established that these mixed cultures significantly increased the accumulation of nitrogen fertilizer in the plants. The strain A. radiobacter 10 promoted a better accumulation of phosphorus fertilizer by plants and A. mysorens increased
the total phosphorus content in plant tissues. Maximum positive effect of joint inoculation on plant development was observed when the combined nitrogen in soil was in short supply. It was concluded that inoculation with bacterial mixtures provided a more balanced nutrition for the plants and the improvement of root uptake of nitrogen and phosphorus was the major mechanism of interaction between plants and bacteria. Field experiments confirmed the assertion that mixed inoculation increased the grain yield and nitrogen nutrition of plants as compared with single inoculation.

An increase in mungbean yield and groundnut yield was observed by the inoculation of *Rhizobium* spp. and phosphate solubilizing bacteria along with phosphatic fertilizers (Rasal, 1996 and Balamurugan and Gunasekaran, 1996, Khan et al., 1997 and Khan et al., 1998).

**Interaction between nitrogen fixing microorganisms and AM fungi**

Satizabal and Saif (1987) inoculated *Centrosema macrocarpum* plants with *Rhizobium* strains and mycorrhizal fungi *Glomus manihotis* or *Acaulospora longula*. Highest yield of dry matter, mineral absorption, nodulation and infection by VAM fungi was recorded in dual inoculation with *Rhizobium* and *Glomus* sp. A small application of N-fertilizer was advised at the time of sowing. Cruz et al. (1988) studied effectiveness of four VAM fungi and *Rhizobium* in promoting growth of three legume trees in P-deficient soil. *Glomus fasciculatum* + *Rhizobium* and *Gigaspora margarita* + *Rhizobium* were most effective for *Acacia mangium* and *Albizia falcata* (syn. *Paraserianther falcata*), *Scutellospora persica*
Rhizobium, Gigaspora margarita + Rhizobium and Glomus fasciculatum + Rhizobium were most effective for Acacia auriculiformis. Consistently poor growth was attained by seedlings inoculated with Sclerocystis clavispora + Rhizobium, Rhizobium alone, or by uninoculated seedlings. Badr-El-Din and Moawad (1988) observed the combined effect of vesicular arbuscular mycorrhizae (VAM) and Rhizobium spp. on the cold season legumes, lentil and fababean and the summer legume, soybean in soil with low number of indigenous VA mycorrhizal spores. VA mycorrhizal inoculation increased nodulation of these legumes. Inoculation with Rhizobium alone significantly increased plant dry weight and N content of lentil and fababean as well as seed yield of soybean. The dual inoculation with both rhizobia and mycorrhizae induced more significant increase in plant dry weight, N and P content of lentil and fababean as well as seed yield of soybean, than inoculation with either VA mycorrhizae or Rhizobium alone. Lee (1988) reported that inoculation of Alnus firma seedlings with Rhizobium and Glomus sp. showed increased growth as compared with uninoculated control.

In field experiments, Young et al. (1988) observed that inoculation of soybean with Rhizobium alone significantly increased nodulation, nodule weight and nitrogenase activity of nodules and affected yields in the range of 13% to +134%. Inoculation with VAM fungi alone did not have significant effect on nodulation and nitrogenase activity. Mixed inoculation with Rhizobium and mycorrhiza affected yields in the range of 8% to 145%.
Inoculation of tomatoes with *A. chroococcum* enhanced root infection by *Glomus fasciculatum*, stimulated the plant growth and resulted in increased shoot nitrogen, Ca, Mg, K compared with the uninoculated plants (El Shanshoury *et al.*, 1989). Shivaram and Rai (1990) inoculated a bufel grass/siratro (*Cenchrus ciliaris/Macropitalium atropurpureum*) pasture with *Rhizobium* and VAM inoculation grown in greenhouse conditions. The associative effect of microorganisms was studied in terms of forage yield, accumulation of N and P in the fodder tissue and also competition of rhizobia by ELISA. Combined inoculation resulted in a significant increase in drymatter production (19.6 g), nitrogen uptake (360.9 mg) and phosphorus uptake (67.3 mg). Mycorrhizal plants showed highest 32P activity. Khasa *et al.* (1990) observed increased biomass of *Leucaena leucocephala*, *Phaseolus vulgaris* and soybean, rice, onion and cowpea due to the inoculation with *Glomus* sp. and *Rhizobium* spp. Similar observations were also made by Mallesha *et al.* (1992) and Widiastuti and Toruan (1992). Inoculation of *Glomus vesiculiferum*, *G. clarum* with or without *Rhizobium* TAL 1145 into fumigated or non-fumigated nursery soil sown with *Leucaena leucocephala* seeds resulted in enhanced nursery and plantation growth. Thiagarajan *et al.* (1992) suggested that the appropriate pairing of rhizobial strain and VAM fungus should be used for field inoculation. Dual inoculation of cowpea with *Rhizobium* JRC14 and *Glomus pallidum* increased mycorrhizal infection, pod yield, nodule formation and N and P contents of the plants.
Biro et al. (1993) reported that the degradation of soils by mining decreased rhizobial and mycorrhizal populations resulting in a relatively low number of spontaneous Rhizobium nodules but insufficient to influence the yield of pea grown in mine spoils. Inoculation with an effective strain of Rhizobium leguminosarum bv. viciae, however, enhanced dry matter production. Although the frequency of mycorrhizal infection was also greater after rhizobial inoculation, no positive correlation existed between dry matter production and percentage of mycorrhizal infection. Spoils treated with lignite, straw and sewage sludge had high levels of spontaneous mycorrhizal populations, indicating that organic materials had a favourable effect on the recultivation processes and fertility of mine spoils.

Verma et al. (1994) studied inoculation effect of Rhizobium sp. and Glomus mosseae on the growth and biomass production of Acacia nilotica. Inoculation of Rhizobium sp., VAM fungi and application of nitrogen and phosphorus singly or in different combinations enhanced the growth and biomass production compared with uninoculated seedlings. Maximum seedling volume was found in seedlings inoculated with G. mosseae and Rhizobium in combination, and in the Rhizobium + phosphorus combination, followed by the mixed inoculation of VAM fungi. The results suggested that inoculation of VAM fungi and Rhizobium, singly or in combination is equally effective or more beneficial than use of chemical fertilizers in raising seedlings of Acacia nilotica. In a pot experiment, Phaseolus vulgaris seeds were inoculated at sowing with Rhizobium phaseoli strain 127 K 44 in the presence and absence of soil
inoculation with vesicular arbuscular mycorrhizal fungus (*Glomus* spp.). Plants inoculated with both symbionts formed nodules in large clusters, as opposed to the single or linear groups of nodules that formed on plants inoculated only with *R. phaseoli* (Baird and Caruso, 1994). Elgala *et al.* (1995) found improved plant growth and increased P, N and Zn contents in wheat and maize plants inoculated with *Azotobacter chroococcum* and VA mycorrhizal spores isolated from onion fields in sandy soils, along with rock phosphate or superphosphate. They concluded that with microbial inoculation, rock phosphate could be used as a cheap source of phosphorus in alkaline soils and combined inoculation could reduce the rate of fertilizer required to maintain high productivity. Barea *et al.* (1996) studied the impact of genetically modified *Rhizobium meliloti* strain on development and function of arbuscular mycorrhiza, root morphology, nutrient uptake and biomass accumulation in *Medicago sativa*. No adverse effect of *Rhizobium* on mycorrhizal fungus *Glomus mosseae* was observed. Inoculation with *Rhizobium* strain greatly increased the number of mycorrhizal entry points in the alfalfa root system. Mycorrhizal development and good quality of nodulation coincided with increased biomass and nutrient uptake by the plants. An improved AM formation and the quality of nodulation first increased nutrient uptake, which could induce changes in root morphology.

The effect of inoculation with four arbuscular mycorrhizal fungi (*Glomus clarum*, *G. etunicatum*, *G. manhotis*, *Gigaspora margarita*) either individually or in a mixture, and with *Rhizobium leguminosarum*
bv. *Phaseoli* was determined in a pot experiment on the growth, mineral nutrition and contribution of biologically fixed N\(_2\), to three nodulating beans. Capacity of the different AM fungi to infect and colonize the roots of different bean varieties differed significantly. Inoculation with AM fungi significantly increased production of dry matter by 8-23% and the concentration of phosphorus in plants by 160-335%. A strong positive correlation of percentage root infection by AM fungi and plant phosphorus concentration and accumulation was observed which suggested that the observed inoculation responses were the result of improved P acquisition by the mycorrhizal roots.

**Interaction among phosphate mobilizing microorganisms**

Plants inoculated with phosphate solubilizing microorganisms and mycorrhizal fungi exhibited pronounced increase in phosphorus uptake in comparison with uninoculated plants grown in phosphorus deficient soil (Azcon *et al.* 1976; Barea *et al.* 1975). Piccini and Azcon (1987) reported that utilization of Bayovar rock phosphate phosphorus by *Medicago sativa* was increased by inoculation with phosphate solubilizing bacteria and *Glomus* sp. Paulino and Azcon (1987) evaluated response of *Centrosema pubescens* to inoculation with *Glomus* sp. and phosphate solubilizing bacteria in a medium amended with two rock phosphates. The mycorrhizal plants showed more efficient nutrient assimilation, biomass production, nodulation and nitrogenase activity than uninoculated plants. Effects of VA symbiosis were enhanced in the presence of phosphate solubilizing
bacteria. The solubilization of two rock phosphates was increased by the dual inoculation. Sattar and Gaur (1989) inoculated lentil cv. L 59 with arbuscular mycorrhiza and 4 phosphate dissolving microorganisms with increasing doses (0, 45, 90, kg P₂O₅/ha) of rock phosphate. Maximum grain yield, hay yield and phosphorus uptake (3.20 g/pot, 6.74 g/pot and 48.33 mg P₂O₅/pot respectively) was recorded in plants treated with *Pseudomonas striata* and *Glomus* sp. The response of wheat var. BD 2204 to phosphate solubilizing microorganisms and *Glomus fasciculatum* were investigated in the presence and absence of rock phosphate. In case of grain and straw production, *Aspergillus awamori*, a phosphate solubilizing microorganism performed better than all cultures. the increase being 22 and 17% over uninoculated control. *Pseudomonas striata* recorded 41% higher phosphate uptake when applied with *Glomus* sp. (Sattar and Gaur, 1989). Pathiratna et al. (1990) showed dual inoculation of the annual grass *Pennisetum* sp. and a perennial legume *Pueraria* sp. with mycorrhizal fungi *Glomus fasciculatum* and phosphate solubilizing microorganism, stimulated significantly the growth and uptake of phosphorus from soil enriched with poorly soluble apatite.

Leyval et al. (1990) demonstrated that mineral transformations in rhizosphere soil of maize, pine and beach, inoculated with mycorrhizal fungus *Laccaria laecata* and phosphate solubilizing bacteria *Agrobacterium* sp. were more significant as compared to rhizosphere of uninoculated plants. A synergistic interaction was observed when chilli (*Capsicum annum*) plants were inoculated with *Glomus* spp. and *Pseudomonas*
*striata* in presence of rock phosphate. Dual inoculation resulted in increased shoot dry mass, P content and fruit yield of chilli plants (Sreenivasa and Krishnaraj, 1992).

Associative effect of phosphate solubilizing bacteria and VAM fungi on phosphatase activity in soil and nutrient uptake by the maize plants was examined by Heggo and Barakah (1993). The plants with dual inoculation showed increased plant dry weight and N, P, K, Ca, Zn, Mn contents. The application of rock phosphate or superphosphate decreased the phosphatase activity in soil while VAM fungi increased the phosphatase activity.

**Interaction among nitrogen-fixing microorganisms**

Interactions among nitrogen-fixing microorganisms and their interactive effects have been studied in a number of investigations. Rai and Gaur (1988) observed significant increase in grain and straw yield and nitrogen uptake of wheat plants treated with *Azotobacter* sp. and *Azospirillum* sp. Similar observations were made by Fayez (1990) and Zambre and Konde (1990). In a pot experiment, Pahwa (1990) inoculated *Centrosema pubescens* seeds with *Rhizobium* strain TAL-655, *Azotobacter chroococcum* strain ICM-2001 or *A. chroococcum* strain S-3 singly or in all possible combinations. Greater dry matter yields were observed from seeds inoculation with *Rhizobium* + *A. chroococcum* ICM 2001, and *Rhizobium* + *A. chroococcum* ICM2001 + S-3 compared with the control. The *Azotobacter* treatment did not increase higher dry matter
significantly. Nodule number/plant, crop yield and nitrogen uptake were related to dry matter yield. Kothari and Saraf (1990) found that seed inoculation of *Vigna radiata* with *Rhizobium* and *Azotobacter chroococcum* was superior to inoculation with *Rhizobium* and *Azospirillum brasilense* or *Rhizobium* alone in increasing dry matter accumulation in different plant parts and seed yield compared with uninoculated seeds. Partitioning of dry matter in different plant parts (leaves, stalks, pod, husk, seeds) at harvest was affected by phosphorus rates but not by seed inoculation. Rasal and Paril (1993) obtained increased percentage germination of sorghum seeds due to combined inoculation of *Azotobacter* sp. and *Azospirillum* sp. Yadav *et al.* (1994) found increased nodulation, nitrogenase activity and plant growth of chickpea due to the inoculation of *Rhizobium* and *Azotobacter* strain Mac 27.

**Interaction between nitrogen fixers, phosphate solubilizers and AM fungi**

Singh and Singh (1993) investigated the associative effect of *Bradyrhizobium japonicum*, vesicular arbuscular mycorrhiza and phosphate solubilizing microbes on soybean in a mollisol. Inoculation with endophyte alone resulted in 70% root colonization. Addition of rock phosphate or inoculation with phosphate solubilizing bacteria except *Bacillus polymyxa* stimulated root infection of native as well as introduced VAM endophytes. Application of rock phosphate with triple inoculation significantly increased grain yield, nodulation, nitrogen uptake and available soil phosphorus.
Bethlenfalvay (1994) demonstrated the impact of *Glomus mosseae*, *Bacillus* sp. and *Rhizobium* sp. and on plant growth and soil aggregation under *Pisum sativum* cultivation. Complex interaction was found among the three microorganisms. *Bacillus* sp. antagonized the effect of vesicular arbuscular mycorrhizal fungi and *Rhizobium* sp. inoculation on plant growth. The-VAM-*Rhizobium* combination significantly promoted soil aggregation which was slightly attenuated by inoculation with *Bacillus* sp.

The present review has shown that the beneficial aspects of single and dual inoculation of nitrogen fixing and phosphate mobilizing microorganisms on crop plants have received adequate attention of researchers. But very few reports are available on the effects of combined inoculation of crop plants with nitrogen fixing microorganisms and phosphate solubilizing microorganisms in the presence of AM fungus. Therefore, the information generated through this study may be useful and interesting for further investigations.