Indian soils are poor in nitrogen, and about 46 percent of them are low, 52 percent are medium and only 2 percent are high in its phosphorus content (Ghosh and Hasan, 1979). Hence, to meet the crop requirement of nitrogen and phosphorus, chemical fertilizers are added to these soils for getting optimum yields. Though chemical fertilizers have amply proved their eminent role in modern agriculture, the escalating costs, the energy crisis and the environmental hazards caused by them have made the researchers to think in other directions where nutritional requirements of the crops are met without such problems. In this direction, partial success has been achieved by using the renewable resources through microbiological processes such as biological nitrogen fixation, organic matter decomposition, microbial phosphate mobilization, etc., by which a substantial part of the chemical fertilizers can be saved.

The nitrogen contribution from "Legume Rhizobium symbiosis", which is in the forefront of the modern agriculture (Khurana and Dudeja, 1981; Kothari and Saraf, 1990; Jadhav et al., 1990; Deka and Kaketi 1996 etc.), and from nonsymbiotic (associative and free-living) microorganisms (Rubaiak et al., 1989; Srivastava and Siddique, 1992; Jana and Mishra, 1994; Caballero et al., 1992 and Lakshminarayana, 1993) have proved their worth in nitrogen nutrition of the crop plants. Similarly the use of AM fungi have been shown to possess proven ability in increasing the phosphorus uptake of the plant by
developing symbiotic association with plant roots (Gerdemann, 1968; Gerdemann, 1975; Mose, 1973; Marschner and Dell, 1994; Ibijbijen et al., 1996 and Schreiner et al., 1997). But AM fungi do not contribute to the solubilization of insoluble phosphates like rock phosphate which is a very cheap source of phosphorus rather it helps in absorption of soluble phosphorus by the roots of the plants (Kucey et al., 1989). On the other hand, some soil microbes are known to solubilize the inorganic phosphates and make it available to the plants and other microorganisms growing in the vicinity (Gaur et al., 1980, Kucey, 1983; Nahas et al., 1994; Illmer and Schinner, 1995a and b; Premono et al., 1994; Khan et al., 1997 and Vassileva et al., 1998). Application of rock phosphate and increasing its solubilization by means of inoculation of soil with phosphate mobilizing microorganisms appears more appealing because of the low costs of rock phosphate in many countries.

Inoculating the crops with either nitrogen fixing microorganisms or phosphate mobilizers with rock phosphate can supply either N or P according to their specific functions. But the crops do need both the nutrients in enough quantities for better productivity. Such microorganisms may be present in the soil naturally or can be added to the soils in agricultural fields. Inoculation with nitrogen fixers and phosphate mobilizers simultaneously, may supply both the nutrients to the crops if the two groups interact with each other favourably. Interaction of two groups of beneficial organisms such as nitrogen fixers and AM fungi (Lee, 1988; Shivaram et al., 1988; Shivaram
and Rai, 1990; Mallesha et al., 1992; Singh, 1995 and Barea et al., 1996), phosphate solubilizers and AM fungi (Leyval, 1990; Sreenivasa and Krishnaraj, 1992 and Hego and Barakah, 1993 and Omar 1998), two groups of nitrogen fixers (El Mokadem et al., 1989; Pahwa, 1990; Kothari and Saraf, 1990, and Yadav et al., 1994) and nitrogen fixers and phosphate mobilizers (Alagawadi and Gaur, 1988; Sarojini et al., 1989 and Belimov et al., 1995) are known. But the information on the interaction of nitrogen fixers, phosphate solubilizers and AM fungi and their effect on associating crop plants, particularly under Indian conditions, are very meagre. The present study has focussed this aspects of the microbial transformations in the soil. To achieve this objective, interaction of *Rhizobium* spp. or *Azotobacter* sp. (nitrogen fixers) with *Pseudomonas striata* (phosphate mobilizer) in liquid culture and interaction of the nitrogen fixers (*Rhizobium* sp., *Bradyrhizobium* sp., *Azotobacter* sp.) with phosphate mobilizers (*Pseudomonas striata* or *Penicillium variable*) with, or without application of arbuscular mycorrhizal (AM) fungus (*Glomus fasciculatum*) and its influence on growth and yield of the crops have been investigated.

**Isolation and screening of phosphate solubilizing microorganisms:**

Microorganisms isolated from the soil of Aligarh District differed markedly in phosphate solubilizing activity. Among the plants inoculated in the study, chickpea rhizosphere soil contained maximum number of phosphate solubilizing microorganisms followed by mustard and rice. Similar observations were made by Sankaram
(1960) and Mahmoud et al. (1973) who reported the occurrence of greatest number of phosphate dissolving organisms in the legume rhizosphere. However, Paul (1966) and Taha et al. (1969) isolated phosphate solubilizing Bacillus megaterium from cereals. Nikitin (1959) isolated Pseudomonas sp. from maize. Duff et al. (1963) isolated Pseudomonas fluorescens from oat rhizosphere and Vora and Shelat (1996) isolated a number of phosphate solubilizing microorganisms from rhizosphere soil of different crops. Among 700 soil samples collected at Aligarh, only 15 samples were found to contain tricalcium phosphate solubilizing microorganisms. The solubilization of tricalcium phosphate by different microorganisms have been documented by many workers (Louw and Webley, 1959; Ahmad and Jha, 1968; Kneuer, 1968; Sethi and Subba Rao, 1968; Bajpai and Sundara Rao, 1971; Pamela et al. 1982; Varsha et al. 1994 and Nahas et al. 1994). However, rock phosphate was less vulnerable to attack by the isolated organisms. Out of 20 bacterial and fungal isolates, only 60% of them solubilized rock phosphate as compared to solubilization of tricalcium phosphate. The solubilization of rock phosphate by various microorganisms has been observed by Meyer and Konig, (1960); Bardiya and Gaur, (1972); Arora and Gaur, (1979); Gaur (1986); Rokade and Patil, (1992); Varsha et al. (1995) and Vassileva et al. (1998). In general, the fungal isolates showed better efficiency in solubilization of different insoluble phosphates. This finding is in agreement with the findings of Ortuno et al. (1978) and Arora and Gaur, (1979).
The microbiological solubilization of different phosphate sources resulted in the change in pH of the culture medium and a positive correlation was noted between the amount of phosphate solubilized and the reduction of pH of the medium towards acidic range. The solubilization of insoluble phosphate and decrease in pH may positively be due to the production of organic acids as reported by a number of workers (Gerretsen, 1948; Louw and Webley, 1959; Bardiya and Gaur, 1972; IImmer et al. (1995).

In liquid culture medium interaction of *Rhizobium* sp., *Bradyrhizobium* sp. and *Pseudomonas striata* showed definite beneficial effects on the growth of *Rhizobium* sp. or *Bradyrhizobium* sp. when the medium lacked available phosphorus. When grown alone, *Rhizobium* sp. or *Bradyrhizobium* sp. demonstrated very poor growth in same medium. A scanty growth of *Rhizobium* sp. or *Bradyrhizobium* sp. in medium devoid of available phosphorus is quite understandable as the organism does not solubilize insoluble phosphates. Similar observations were made by Esposito and Wilson (1956) with *Azotobacter* sp. which showed slow growth and decreased nitrogen fixation in culture medium devoid of available phosphorus. The stimulated growth and nitrogen fixation in the presence of phosphatic fertilizers were demonstrated by Ernandes, 1958 and Brown et al., 1962. The population of *P. striata* in combined inoculation after 72 h of incubation was slightly lower than in single inoculation probably due to depletion of nutrients. It showed however, higher cell counts at 48 h. Interaction of *Azotobacter chroococcum* and *P. striata* showed similar trend in the modified medium, devoid of available
phosphorus. *A. chroococcum* population was very high when the organisms was grown in association with phosphate solubilizers. It was probably not due to the availability of phosphorus alone but other factors such as production of vitamins (Baya *et al.*, 1981) and growth promoting substances (Barea *et al.*, 1975) by the phosphate solubilizing bacteria, as the population of *A. chroococcum* in combined inoculation treatments exceeded the population in that of Jensen’s medium containing available phosphate. Similarly the population of phosphate solubilizing, *P. striata* was also stimulated when grown in association with *A. chroococcum*.

In the present study, it was also observed that solubilization of tricalcium phosphate (TCP) by *P. striata* was augmented when it were grown in combination either with *Rhizobium* sp. or *Bradyrhizobium* sp. or with *A. chroococcum*. This observation is contrary to the observations of Kundu and Gaur (1981) who recorded a decrease in the net amount of phosphate solubilized in combined inoculation of *Azotobacter* sp. and *P. striata*. A significant increase in nitrogen fixation by *A. chroococcum* in the presence of *P. striata* further confirms their synergistic interaction. This increase in nitrogen fixation can be attributed to the oxygen depletion effect by the phosphate solubilizer which might have created a favourable condition for the growth and nitrogen fixation by *A. chroococcum*. Such stimulation of growth and nitrogen fixation was seen in *Azotobacter* sp. by Panosyan *et al.* (1962) when the organism was associated with phosphate solubilizing *Bacillus subtilis*, *B. megaterium*, *Pseudomonas* sp. and several actinomycetes.
Interaction of nitrogen fixers and phosphate mobilizers in sterile soil and its effect on crop productivity:

The results obtained on the associative effect of nitrogen fixers and phosphate solubilizers in culture medium prompted to study the interaction of nitrogen fixers and phosphate solubilizers in soil in the presence or absence of arbuscular mycorrhizal fungus *Glomus fasciculatum*. The two phosphate solubilizers i.e. *P. striata* and *P. variable* and *G. fasciculatum* have shown stimulatory effect on the nodulation in both the legumes i.e. chickpea and greengram. In triple inoculation with *Rhizobium* sp. + *P. striata* and *G. fasciculatum* the dry weight of the nodules was significantly greater than in the dual inoculation with *Rhizobium* sp. and *P. striata* and other treatments including control. Such an effect can be attributed to the increased supply of phosphorus to the host plant or due to the production of plant hormones by the organisms. The supply of phosphorus to the host plants influences the nodulation and nitrogen fixation in higher plants (Schreven, 1958). Application of phosphatic fertilizers in the presence of *Rhizobium* spp. has been reported to increase the nodule dry weight of greengram (Kothari and Saraf, 1990). The applied rock phosphate must have been solubilized by the inoculated phosphate solubilizers (Kavimandan and Gaur, 1971) thereby providing the plant with available phosphorus whose deficiency otherwise might have led to poor nodulation and nitrogen fixation. A further increase in nodulation with the application of *Glomus fasciculatum* can be attributed to the better absorption of available phosphorus by the roots of host plants (Kucey *et al.*, 1989). Also
It has been recognized that the growth promoting substances produced by bacteria improve the plant growth (Brown, 1974) and the plant hormones also play a role in the infection mechanisms of *Rhizobium* sp. (Nutman, 1965).

The dry matter production was recorded maximum in both the legumes in the treatments receiving all the three microorganisms viz. *Rhizobium* sp. or *Bradyrhizobium* sp., *Pseudomonas striata* and *Glomus fasciculatum*. The dual inoculation with *Rhizobium* sp. or *Bradyrhizobium* sp. and *Pseudomonas striata* or *Penicillium variable* showed nearly similar dry matter productions. Similar results have been obtained by Algawadi and Gaur (1988) who observed significant increase in the yield and nutrient uptake of chickpea inoculated with *Rhizobium* sp. and *Bacillus polymyxa*, a phosphate solubilizing bacterium. The maximum grain yield was recorded in the treatment with *Bradyrhizobium* sp. or *Rhizobium* sp. + *Pseudomonas striata* + *Glomus fasciculatum* followed by the treatments of *Rhizobium* sp. or *Bradyrhizobium* sp. with *P. striata* or *P. variable*. The yield response to combined inoculation with *Rhizobium* sp. or *Bradyrhizobium* sp., phosphate solubilizing fungus *P. variable* and *Glomus fasciculatum* was inferior to the treatment with *Rhizobium* sp. or *Bradyrhizobium* sp. and *Pseudomonas striata*. Increase in the yield due to mixed inoculation of *Rhizobium* sp. and *Bacillus megaterium* with superphosphate and rock phosphate in mungbean and soybean (Bhatnagar *et al.*, 1979) and due to combined inoculation of *Rhizobium* sp. and *Azotobacter* sp. in chickpea,
soybean and mungbean (Rawat and Sanoria, 1976) are known. The increase in the yields are attributed not only due to the improved nutritional conditions but also due to the prolonged survival of *Rhizobium* sp. in the large amounts of polysaccharide gums (Jauhri *et al.*, 1979) or due to growth promoting substances (Azcon and Barea, 1978) and a non-extractable protein produced by *Azotobacter vinelandii* (Burns *et al.*, 1981).

Nitrogen and phosphorus uptakes by chickpea and greengram plants at 45 and 90 days and at 35 and 60 days respectively were highest in combined inoculation with *Rhizobium* sp. or *Bradyrhizobium* sp. + *Pseudomonas striata*. and mycorrhizal fungus *G. fasciculatum* followed by dual inoculation with *Rhizobium* sp. or *Bradyrhizobium* sp. and phosphate solubilizing bacteria or phosphobacteria. N and P uptakes by the plants inoculated with *Rhizobium* sp. or *Bradyrhizobium* sp. and phosphobacteria and *Rhizobium* sp. or *Bradyrhizobium* sp. and phosphofungi were at par between themselves and significantly superior over control. Increased N uptake by inoculation of *Rhizobium* sp. in chickpea, groundnut and greengram was observed by Patil and Moniz, (1974), Gunjal and Patil (1990); Jadhav *et al.*, (1990) and Yadav *et al.* (1992) and increased P uptake due to inoculation of phosphate solubilizing bacteria in wheat (Gaur *et al.*, 1980 and Gaur 1985), in barley (El-Din and Baber, 1983), in *Vicia faba* (Khalafallah *et al.*, 1982), in groundnut (Salih *et al.*, 1989). in rice (Mohod, 1989) and in mungbean (Gaind and Gaur ,1991). Increased N and P uptake due to combined
inoculation of *Rhizobium* sp. and phosphobacteria (*Pseudomonas* sp.) in *Medicago sativa* (Azcon and Barea, 1978), in clover (Delorenzini *et al.*, 1979) in chickpea (Algawadi and Gaur, 1988) and in groundnut (Balamurugan and Gunasekaran, 1996) is also known. Significant increase in uptake of both P and N in chickpea due to the combined inoculation of *Rhizobium* sp., phosphobacteria or phosphate solubilizing bacteria and *Glomus* sp. was recorded by Poi *et al.* (1989). In the present study better establishment of introduced phosphate solubilizing microorganisms was shown in combined inoculation with *Rhizobium* sp. or *Bradyrhizobium* sp. as the counts of phosphate solubilizing bacterium or fungus were found higher in treatments with *Bradyrhizobium* sp. or *Rhizobium* sp. in rhizosphere soil of chickpea and greengram compared with single inoculation and control. Again the combined inoculation showed higher phosphate solubilizing microorganism counts than single inoculation which were further enhanced by the addition of *Glomus fasciculatum*. This increase may be attributed to associative effect of microorganisms as Ocampo *et al.* (1975) observed increased number of *Azotobacter* and phosphobacteria in the rhizosphere of lavender plants. Kundu and Gaur, (1984) also observed associative effect of *Azotobacter* sp. and phosphobacteria (*P. striata* and *Bacillus polymyxa*) in the rhizosphere of cotton, wheat and rice when both the organisms were inoculated together.

Apart from increasing the dry weight, uptake of N and P and yield of the crops, inoculation of *Rhizobium* sp. in chickpea and
greengram resulted in increased N content of soil. Inoculation with that of phosphate mobilizing microorganisms also resulted in increased available P content in soil. This increase was, however, more pronounced in combined inoculation particularly in the presence of *Glomus fasciculatum*. This may have occurred as a result of increased phosphate solubilizing bacterial population which was recorded highest along with the *G.fasciculatum*. Wani *et al.* (1978) reported significantly higher available P$_2$O$_5$ content in soil after the harvest of chickpea crop inoculated with phosphate solubilizing *P.striata, A.awamori* or *P.digitatum*. Sattar and Gaur (1989) also observed increased available phosphorus in the rhizosphere of rice, wheat and lentil inoculated with *Pseudomonas striata* in combination with rock phosphate. Similar observations were made by Salih, (1989), Gaind and Gaur (1991) and Patil *et al.* (1990). It is well established fact that the legume crops improve the nitrogen status of the soil (Subba Rao, 1975). The percentage of root infection and number of mycorrhizal spores were greater in the presence of phosphate solubilizing bacteria than with phosphate solubilizing fungi. The increased mycorrhizal infection can be attributed due to stimulatory effect of growth promoting substances produced by the bacteria which in turn increase root formation, plant growth, spore germination and infection of VA mycorrhizae (Brown, 1972; Azcon and Barea, 1978 and Azcon *et al.*, 1976). However, the results have clearly shown the benefits of using nitrogen fixing *Rhizobium* sp. or *Bradyrhizobium* sp., phosphate solubilizing *P.striata* and
arbustular mycorrhizal fungus *G. fasciculatum* in augmenting the yield and nutrient uptake by the crop.

**Interaction of *Azotobacter chroococcum* and phosphate mobilizing microorganisms and its effect on crop productivity**

The wheat crop inoculated with *A. chroococcum* showed significant increase in dry matter yield compared to control. Kundu and Gaur, (1980) and Rai and Gaur, (1988) observed increased wheat yield due to *A.chroococcum* inoculation. The combined inoculation of *A.chroococcum* with *P.striata* enhanced the dry matter yield which was further augmented in the presence of *G. fasciculatum*. The dual inoculation of *A.chroococcum* and *Azospirillum brasilense* increased the root biomass of rice seedlings (Dewan and Subba Rao, 1979), dry matter yield of sorghum and maize (Tilak et al., 1982) and dry matter of wheat (Zambre and Konde, 1990 and Rabie et al., 1995). The associative or combined effect of *A.chroococcum* and phosphate mobilizing microorganisms in increasing the yield of various crops has been observed by several workers (Kundu and Gaur, 1982 and Sarojini and Mathur, 1990). The beneficial effects of dual inoculation of *Azotobacter* sp. and VAM fungus on pasture and forage crops have also been reported (Hernandez et al., 1994). The grain and straw yields of wheat inoculated with *A. chroococcum* and *Pseudomonas striata* were significantly superior over single inoculation. The increased yields can be attributed due to *N*₂ fixation by *A.chroococcum* and phosphate solubilization by *P.striata* (Gaur and Ostwal, 1972. Gaur, 1985) and to the growth promoting substances produced by these organisms (Barea et al., 1976). In both the cases, the
associative effect of *A.chroococcum* and *P.striata* was more prominent in the presence of *G. fasciculatum*. Like dry matter production grain and straw yield were also maximum in triple inoculation with *A. chroococcum, P. striata* and *G. fasciculatum* followed by the dual inoculation with *A. chroococcum* and *P.striata* and *A.chroococcum* with *P.variable*. However, in the triple inoculation with *A. chroococcum, P.variable* and *G.fasciculatum*, the yields of grain and straw were at par with the dual inoculation of *A. chroococcum* and phosphate solubilizing *P.striata* or *P.variable* which could possibly be attributed to the slightly negative interaction of phosphate solubilizing fungus *P.variable* and arbuscular mycorrhizal fungus *G. fasciculatum* or competition between the two fungi. This was also observed by Sattar (1982). The treatments with *A.chroococcum + P. Striata* and *A.chroococcum + P.variable* were at par with each other. Similar trends were noted in nitrogen and phosphorus uptake by the wheat plants as were seen in grain and straw yield i.e. the maximum N and P uptake was observed in triple inoculation with *A. chroococcum, P.striata* and *G. fasciculatum* followed by dual inoculation with *A.chroococcum* with *P.striata*. In combined inoculation, N and P uptake by the crops were significantly higher, even in single inoculation treatment compared to control. Single inoculation of *A.chroococcum* has been shown to increase the N uptake (Kundu and Gaur 1980 and Troitskaya and Troitskii, 1988) while single inoculation of phosphate solubilizing bacteria is reported to increase the P uptake (Khalafallah *et al.*, 1982; Gaur, 1985; Salih *et al.*, 1989; Mohod *et al.*, 1989; Manjunatha and Devi, 1990 and
Gaur and Gaind, 1991) in various crops. The dual inoculation of *A. chroococcum* and *Glomous* sp. produced plants having higher P and N content due to improvement in absorptive area of the root system (Harnandez et al., 1994). Combined inoculation of free-living nitrogen fixing *A. chroococcum* and phosphate solubilizing *P. striata* are also reported to have increased, N and P contents of wheat and other crops (Kundu and Gaur, 1984). The population of *A. chroococcum* and phosphate solubilizing *P. striata* or *P. variable* in wheat rhizosphere gave evidence for the associative effect when inoculated together, as the population of both the microorganisms were higher in combined inoculation and this effect was more pronounced in the presence of *Glomus fasciculatum*. Increase in the population of *A. chroococcum* was recorded in the barley rhizosphere by Ramacle (1966) when the crop was inoculated with both *A. chroococcum* and *Bacillus* sp. Similarly, Rubenchick et al. (1958) reported increased *A. chroococcum* population in the rhizosphere of wheat, oat and barley inoculated with *A. chroococcum* and *B. megaterium* compared to single inoculation with *A. chroococcum*. They also observed enhanced spore germination of *Bacillus* sp. in the presence of *A. chroococcum*. Ocampo et al. (1975) also reported greater number of *Azotobacter* sp. and phosphate solubilizing bacteria *Pseudomonas* sp., *Agrobacterium* sp. and *Bacillus* sp. in the lavender rhizosphere while Kundu and Gaur (1980a) observed synergistic interaction of *Azotobacter* sp. and phosphate solubilizing microorganism like *Aspergillus awamori* in the rhizosphere of cotton inoculated with both the organisms together.
In the present study, inoculation of *P. striata* with or without *G. fasciculatum* has resulted in increased content of available P$_2$O$_5$ in the soil. *P. striata* which is capable of solubilizing insoluble rock phosphate (Gaur 1972, Yadav and Singh 1991 and Goenadi and Saraswati, 1993) must have increased the available P when rock phosphate was added. In the absence of rock phosphate, they must have released native phosphorus (Bajpai and Sundra Rao, 1971). Increase in available soil phosphorus due to inoculation of *P. striata* has been reported by Shinde and Patil (1985). The available P content in soil, without microbial inoculation, after harvest of crop was least which may be due to crop uptake or chemical fixation of phosphate. However, the nitrogen content of soil was not altered markedly except with all the three microorganisms viz. *A. chroococcum*, *P. striata* and *G. fasciculatum* where there was a marginal increase in the total N content of soil which can be attributed to the nitrogen fixing activity of *A. chroococcum*.

The percentage of roots infection by the AM fungus *G. fasciculatum* and spore number were recorded maximum in the presence of *A. chroococcum* and *P. striata* followed by *A. chroococcum* and *P. variable* inoculation. These findings are in confirmity with those obtained by Singh and Singh (1993).

The present study has demonstrated synergistic interaction between nitrogen fixing and phosphate mobilizing microorganisms in *vitro* as well as in soil supplemented with rockphosphate as a phosphorus source. A favourable effect of this interaction has been
evident on the productivity of chickpea, greengram and wheat crops which are commonly grown in India. In any soil poor in phosphorus and nitrogen content, the introduction of such standard strains of nitrogen fixing and phosphate mobilizing microorganisms may prove fruitful for these crops and possibly for other crops as well.