5 DISCUSSION

5.1 Distribution and Isolation of VAM

VAM association in *P. phaseoloides* and its role in biological nitrogen fixation is well established (Waidhyanatha *et al.*, 1979). However, there exists wide variation in the degree of VAM colonisation in roots and distribution of VAM spores in soil (Hayman, 1978). In the present study also such variations are recorded. A positive correlation was observed with respect to soil pH and VAM spore count. Palakkad soil having a pH of 5.8 contained maximum count of VAM spores while Kottayam soil the pH of which is 4.5 had the minimum spore count. A number of factors are considered to affect the population of VAM fungi in soil (Kruckelmann, 1975; Tarja Lehto, 1994) of which soil pH is believed to be most important factor (Bethlenfalvay, 1992). Kruckelmann (1975) also found that VAM spore population was more influenced by soil pH than by any other factors as observed in the present study.

All the 16 soil samples showed considerable variation in the species distribution of VAM fungi. *Glomus* spp. was comparatively more in all the soils. Rekharani and Mukerji (1987) while studying the species distribution of VAM fungi found that Indian soils are rich in *Glomus* spp. Soil moisture and temperature are also reported to determine the population densities of different species. *Glomus* spp., in general, prefer low temperature (18° to 20°C), soil moisture upto -31 bars. But *Gigaspora* spp. prefer a soil temperature of 25-35°C and affected even at -10 bars soil moisture (Koske, 1981). Low temperature and high moisture
prevailing in soils of rubber fields could be attributed to the difference in the population of *Glomus* spp. and *Gigaspora* spp.

*Leguminous cover crops play a vital role in Hevea rubber cultivation due to their multifarious beneficial activities* (Potty *et al.*, 1980). *P. phaseoloides* is the most popular crop in rubber plantation and its efficiency depends on the presence of optimum level of nutrients (Mathew *et al.*, 1978) and efficient prokaryotic microsymbiont, the *Bradyrhizobium* sp. VAM is another partner of the tripartite association in leguminous plants and it is considered to be essential for maximizing biological nitrogen fixation (Crush, 1974; Hayman and Day, 1978). VAM association in *P. phaseoloides* is well established and the latter fails to establish in the absence of compatible fungal partner (Waidyanatha *et al.*, 1979). It emphasises the need for investigating the distribution of VAM and using the efficient ones for maximum beneficial activity in *P. phaseoloides* which contributes substantially to the productivity.

5.2 **Effect of Inoculation of Different VAM Isolates on P. phaseoloides**

Comparative evaluation of the efficiency of different VAM fungi on *P. phaseoloides* revealed that all the VAM fungi promoted the growth of this cover crop and some species of VAM were comparatively more efficient in increasing growth and nutrient contents like NPK in tissues under sterile as well as unsterile conditions. The plants grown under sterile condition responded well to the VAM inoculation. In the absence of VAM the growth was stunted proving the role and importance of VAM in the establishment of *P. phaseoloides*. The failure of higher plants to grow normally in the absence of VAM was well established in many crops (Gerdemann, 1975; Hayman, 1978) especially under sterile condition. Compared to
unsterile condition, plants grown under sterile condition did not show much growth improvement irrespective of VAM species used. This might be due to better establishment of mycorrhizae in unsterilised soil. Soil bacteria are reported to influence the germination of spores of *G. epigaeus* and thereby increase VAM colonisation and growth of plants (Daniels and Trappe, 1980). Mosse (1981) also reported that in sterilised soil the native microflora has been destroyed and hence unfavourable for the germination of mycorrhizal spores and infection which is in confirmity with the findings of this study. Pot culture experiments conducted earlier in many crops using sterilised soils (Mosse and Hayman, 1971; Gerdemann, 1975) showed that VA mycorrhizal inoculation can drastically improve plant growth. Plant improvement under unsterile condition as observed in the present study was also reported by Mosse and Hayman (1971) and Bagyaraj and Manjunath (1980). Out of 11 selected VAM fungi, *G. fasciculatum* registered maximum beneficial effect on *P. phaseoloides* followed by *A. laevis*. The superiority of *G. fasciculatum* over other species in improving plant growth was well established (Kormanik et al., 1982; Habte and Aziz, 1985). Under field conditions, VAM development is influenced by VAM species, plant under consideration and edaphic and environmental factors. VAM species vary considerably in their efficiency to infect and influence plant growth (Carling and Brown, 1980). In nature, a root system is typically colonized by more than one fungal species. Host response also differs with fungal species (Carling and Brown, 1980; Wilson, 1988) and with geographic isolate within a species (Bethlenfalvay et al., 1989). The response range may also be due to changes in efficiency of different endophytes during the growing season (Daft et al., 1981), to varying uptake of or exclusion capabilities of different fungi for different elements (Menge et al., 1982; Victoria et al., 1993) or even a change in soil environment itself during the season (Bazin et al., 1990).
VAM inoculated plants, in general, and those inoculated with *G. fasciculatum* and *A. laevis* in particular show better growth parameters, nutrient uptake as well as higher biochemical constituents. The mechanism of improved plant growth caused by mycorrhizal inoculation has been investigated by many workers. Greater soil exploration by mycorrhizal roots as a means of increasing phosphate uptake is well established and the same phenomenon is also observed in the present study. They also improve the uptake of elements like Zn, Cu, S, etc. The other beneficial effects are their role in biological control of root pathogen, biological nitrogen fixation, hormone production and greater ability to withstand stress (Varma, 1979; Bagyaraj, 1984; Cooper, 1984). All these factors cumulatively influence growth of plants upon inoculation with selected mycorrhizal endophyte which vary from nothing to three-fold (Ruehle and Marx, 1979) and confirms the results obtained in this study.

The existence of a positive relationship between biomass production and per cent colonisation as observed in the present study, tends to uphold observations of many scientists that VA mycorrhizal colonisation enhances the plant growth through increased P uptake (Gerdemann, 1975; Hayman and Mosse, 1971; Tinker, 1975). The growth of *P. phaseoloides* is directly related to root colonisation, mineral uptake, nitrogen fixation and biochemical constituents. Plant macronutrients N, P and K have profound influence on the physiological activities and biomass production. In leguminous plants like *P. phaseoloides*, phosphates, potassium and trace elements contribute to the nodulation and nitrogen fixation by *Bradyrhizobium* sp. (Gibson, 1976; Munns and Mosse, 1980). The substantial increase in VA mycorrhizal colonisation and nodule production in *P. phaseoloides* ultimately contributed to enhanced N content leading to increased plant growth. Similar enhancement in nodule and biomass was observed by Bagyaraj *et al.* (1979) in *Glycine max* inoculated with *Rhizobium* sp. and VAM fungi.
Physiological, chemical and physical mechanism of P uptake by VAM fungi were postulated, of which the most valid one is the hypothesis that hyphae ramifying into the rhizosphere from infected roots increase the effective P absorbing surface of the root by exploring larger volume of soil. It has been thought that this additional surface area and the distribution of P absorbing sites on the hyphae in the soil might wholly account for the superior absorbing capabilities of efficient VA mycorrhizal roots. Recent studies with tomato roots show that VAM root not only have more P absorbing sites, but also these sites on mycorrhizal roots have a greater affinity for P (Cress et al., 1979). The difference in P uptake among different VAM fungi colonising *P. phaseoloides* observed in the present study might be due to the difference in root colonisation capability, mycelial extension and P absorbing sites.

All VAM fungi used in this study enhanced the concentration of sugars, amino nitrogen and phenols in *P. phaseoloides*. *G. fasciculatum* inoculated plants registered the maximum levels of these components in the tissues. Next to N, P is considered to be an essential nutrient and it plays a major role in the physiological activities of plants. Phosphate occupies a key position in the energy transfer (ATP-ADP) and promotes various activities in plants. The enhanced levels of sugars, amino nitrogen and phenols could be attributed to higher levels of P uptake as noticed in the present study. More the P uptake more is the quantity of various biochemical constituents. P uptake also indirectly increases the amino nitrogen level by augmenting the extent of nodulation and nitrogen fixation (Subba Rao and Krishna, 1988). The increased levels of sugars in tissue might be due to enhanced photosynthetic rate as well as larger area of leaf exposed to sunlight as evidenced by increased biomass and rapid growth in *G. fasciculatum* inoculated plants. This
finding is in conformity with that of Nemac and Guy (1982) who observed enhanced level of sugars upon VAM infestation while Young et al. (1972) recorded increased amino acids.

Phenol is another component, the quantity of which is influenced by VAM inoculation and the maximum was registered in G. fasciculatum inoculated plants. Enhanced phenol levels upon VAM colonisation as observed in the present study was also recorded earlier by Krishna (1981). Phenols that impart defence mechanism against the invasion of plant pathogens are synthesized by various biochemical pathways; sugars and amino acids serve as precursors for phenols synthesis via shikimic acid and acetate pathways (Stafford, 1974).

Photosynthesis is the primary physiological activity of plants and is influenced by many factors. Nitrogen either biologically fixed at nodule site or applied as manure/fertiliser ultimately contribute to increased photosynthetic rate (Lugg and Sinclair, 1981). In the present study, VAM inoculation caused substantial increase in chlorophyll content as well as the rate of carbon assimilation which could be attributed to the enhanced nitrogen in tissues under the influence of VAM. Similar observations were also made by Pang and Paul (1980) and Kucey and Paul (1982). P. phaseoloides inoculated with VAM, in general, and G. fasciculatum and A. laevis in particular promoted the absorption of potassium. In succession with nitrogen and phosphorus, potassium is given its full recognition as a fertiliser element. Evans and Sorger (1966) reported that potassium is the only univalent cation generally indispensable for all living organisms and it is involved in the activation of a number of enzymes. Potassium has a major role on photosynthesis which has been established with a wide range of higher plants (Moss
and Peaslee, 1965; Peaslee and Moss, 1966; Cooper et al., 1967; Hartt and Burr, 1967). Obviously, the increased biomass upon VAM inoculation especially *G. fasciculatum* and *A. laevis* could be due to the enhanced level of chlorophyll and photosynthetic rate. *G. fasciculatum* inoculated plants having maximum photosynthetic activity are also rich in soluble sugars and amino acids indicating that such compounds originated from photosynthesis. These results of the present study clearly indicate the usefulness of VAM in *P. phaseoloides* establishment in rubber plantations.

The present study emphasises the need for the use of selected VAM fungi as there exists wide variations among different VAM isolates in influencing the growth of *P. phaseoloides*. Two isolates showing maximum beneficial effect in *P. phaseoloides* i.e., *G. fasciculatum* and *A. laevis* are considered for detailed studies of agronomical importance.

5.3 Effect of Inoculation of *G. fasciculatum* and *A. laevis* on Root Colonisation, Growth and Some Biochemical Constituents of *P. phaseoloides* at Different Intervals after Planting

It has been well established that VAM inoculation leads to enhanced root colonisation, growth, nodulation and nitrogen fixation in *P. phaseoloides* (Waidyanatha et al., 1979). In the present study, VAM inoculation progressively increased root colonisation upto 50 days and throughout the experiment the infection per cent of VAM inoculated plants are higher than the uninoculated plants which get infected by native VAM fungi. Root colonisation irrespective of VAM inoculation steadily increased upto 50th day. Hayman (1970) and Sullia and Chandranath (1991) reported a steady increase of root colonisation by VAM fungi upto the end of the growing season in many cultivated legumes. However, a lag phase in root
colonisation and a three phase infection system was recognised by Sutton (1973) and Saif (1977). Sutton (1973) also noted an initial lag phase of 20-25 days in the case of field grown *Phaseolus* beans and soybean and such phenomenon was attributed to rapid growth of the seedlings and the time required for spore germination, germtube growth and penetration of the host plant root. In the second phase, lasting 30-35 days, extensive mycorrhizal development coincided with most shoot growth and copious spread of external mycelium leading to multiple infections which are in conformity with the result of the present study. It seems likely that for the symbiosis to be established, molecular signaling events must precede to various physiological and anatomical changes in both the symbiosis during initial changes upto 10th day.

Growth and uptake of P and K in *P. phaseoloides* increased with age of the seedlings. Nodulation was recorded from 20th day of growth and increased progressively. Stimulation of the activity of *Rhizobium* which depended on adequate supply and uptake of P and VAM infestation was well established (Smith and Daft, 1977; Smith et al., 1979). It is possible for the existence of such influence of P on *Bradyrhizobium* sp. in the nodulation of *P. phaseoloides*. Concomitant with the increase in nodulation there was an increase in the nitrogenase activity and nitrogen content in VAM inoculated *P. phaseoloides* plants which confirm the findings of Smith and Daft (1977) and Kucey and Paul (1982) who also made such observations.

VAM association not only augmented absorption of nutrients but also lead to considerable difference in biochemical constituents (Gianinazzi and Gianinazzi, 1984) of plants due to changes in the mineral nutrients as well as symbiosis. Important biochemical constituents influenced by VAM association are carbohydrates (Nemac and Guy, 1982), amino acids (Krishna and Bagyaraj, 1983).
and phenols (Krishna, 1981; Morandi et al., 1984; Lakshmanan, 1987). In the present study, phenols, sugars and amino nitrogen content of VAM inoculated plants were more than the uninoculated control plants from the 20th day of inoculation and confirms the above findings. Invariably all the three treatments registered lower levels of phenols, sugars and amino nitrogen in the 20th day samples. The explanation for such phenomenon could be depletion of stored nutrients in the cotyledons during the initial establishment of seedlings (Bewley and Black, 1978; Mayer and Poljakoff Mayber, 1975) and switch over to the self reliance on photosynthesis. Noggle and Fritz (1986) also reported such decreased carbohydrate levels before the plants are supported by leaf photosynthesis and the uptake of water and inorganic solutes from soil.

Apart from this hypothesis for reduced sugars, amino acids and phenols, it can also be attributed to enhanced respiration (Hayman, 1982) due to VAM infection process. Increased respiratory activity of higher plants due to injury by pathogens or saprophytes is a common physiological phenomenon in higher plants (Goodman et al., 1967; Brenneman and Black, 1979) and such activity takes place at the expense of energy reserves like sugars and amino acids (Akazawa and Uritani, 1962). Utilisation of simple carbohydrate and amino acids by VAM fungi seems to be yet another passive action for reduced level of sugars and amino acids on 20th day, the active stage of symbiosis (Bevege et al., 1975). After 20 days of infection the level of sugars, starch, amino nitrogen and phenols showed an ascending trend irrespective of VAM inoculation. Similar trends were also made by Sutton (1973) and he hypothesised that such change was due to most shoot growth and copious spread of external mycelium leading to multiple infection. Young et al. (1972) observed an enhanced amino acid content in corn plants due to increased VAM infestation. Leguminous plants exhibit tripartite symbiotic association involving
VAM fungi and *Rhizobium*, a unique phenomenon which results in enhanced nitrogen fixation (Mane *et al.*, 1993). Nitrogen is the precursor for amino nitrogen and atmospheric nitrogen fixed by *Bradyrhizobium* sp. as evidenced by escalated nitrogenase activity and total nitrogen level in tissues contributed to amino nitrogen pool of *P. phaseoloides*.

The increased level of sugars, starch and amino nitrogen would be contributing to the formation of phenolics in *P. phaseoloides* as suggested by Neish (1964). Phenols are also synthesised in plants at the expense of amino acids, tyrosine and phenylalanine by the enzyme tyrosine ammonia lyase (TAL) and phenylalanine ammonia lyase (PAL) (Vance *et al.*, 1980) and forms yet another precursor for phenols in plants.

### 5.4 Effect of Different Levels of Rock Phosphate and Inoculation of *G. fasciculatum* and *A. laevis* on VAM Colonisation, Nutrient Content and Growth of *P. phaseoloides*

Phosphorus is one of the essential nutrients of plants and is doubly essential for leguminous plants as it is required for nitrogen fixation in large amounts (Munns and Mosse, 1980). One of the functions of VAM in plants is the absorption of soil P. Colonisation of plant roots by VAM fungi has been studied in relation to P nutrition (Bagyaraj and Sreeramulu, 1982). Such studies have clearly indicated that there is an inverse relation between root colonisation and P content in soil (Graham *et al.*, 1981; Schwab *et al.*, 1983). In the present study, increased application of rock phosphate beyond 50 per cent recommended level considerably reduced VAM colonisation. Baylis (1967), Mosse (1971), Azcon *et al.* (1978) and Miranda *et al.* (1989) have in fact shown that high P levels decreased and finally eliminated mycorrhizal infection from soil which are in confirmity with the present findings.
High soil P levels are known to result in root P concentration that may inhibit mycorrhizae and reduce external hyphae of VAM in soil (Sanders, 1975). Other workers (Abbott and Robson, 1977; Same et al., 1983; Abbott et al., 1984; Miranda et al., 1989) observed a stimulating effect of low soil P application on root colonisation and they have suggested that the growth of VAM fungi may be influenced by the supply of P in soils naturally very deficient in this nutrient.

Spores, the infective propagules production is as important as root colonisation. VAM spore population in the present study, irrespective of VAM species, increased with the increase in root infection. Studies conducted in other crops in Australia and elsewhere (Mosse and Bowen, 1968) add additional support to the present observations.

Inoculation of *P. phaseoloides* with *A. laevis* and *G. fasciculatum*, significantly increased dry weight of shoot, nodule weight and nitrogenase activity in the absence of added phosphate and application of rock phosphate at 50 and 100 per cent of recommended dose. So also in the case of N, P and K. At all levels of rock phosphate addition, *G. fasciculatum* showed better growth, nodulation, nitrogenase activity and nutrient content than *A. laevis*. The species difference of VAM fungi in promoting the growth of plants at different levels of P is not uncommon. Plenchette et al. (1981) clearly proved the VAM species variation on growth of plants raised under different levels of P. The growth, nodulation and nutrient content of *P. phaseoloides* inoculated with VAM fungi at 50 per cent and 100 per cent of recommended dose of rock phosphate was almost same. Such observations with respect to growth and nutrient uptake especially P, K, Zn and Cu due to mycorrhizal inoculation at different levels of P application has been reported
by earlier workers (Hayman, 1980; Govinda Rao et al., 1983; Chandrashekara et al., 1995).

The degree of response of *P. phaseoloides* to VAM inoculation at 100 per cent recommended level of P was less when compared to 50 per cent P application. The decreased response to VAM inoculation at higher levels of P is an important point to be taken into consideration. Earlier studies also showed that plants infected with VAM are known to be more effective in the uptake of P from rock phosphate and soil low in available P than the uninoculated plants (Mosse, 1973). There is evidence that P from poorly soluble rock phosphate may be available only to mycorrhizal plants (Powell and Daniel, 1978). This is attributed to an increased effective surface area of P absorption in VAM which compensates for the low mobility of P in soil (Tinker, 1975). A factor undoubtedly important in fungal efficiency, but we do not measure, is the amount or intensity of hyphae distributed in the soil mix. Its external hyphae are correlated with internal hyphae (Tisdall and Oades, 1979), the difference among fungus species may be due to difference in volume of soil explored, since for the low P treatment, root colonisation was correlated with plant growth. Zones of P deficiency is estimated to be 1-2 mm around a root, would have taken place at low P, and mycorrhizal fungi may explore soil at least 8 mm distant from the roots (Rhodes and Gerdemann, 1975). This distance may vary if different fungal species produce different amounts of hyphae.

It is also observed in the present study that plants inoculated with *A. laevis* and *G. fasciculatum* showed enhanced nitrogenase activity and N content. *P. phaseoloides* being a legume, fixes atmospheric nitrogen in association with *Bradyrhizobium* sp. (Kothandaraman et al., 1993). Biological nitrogen fixation is an energy consuming process and hence require more P. On an average 16 moles of
ATP are required for every mole of N fixed. The reduced rate of nitrogen fixation due to low level of P is well illustrated in S. guyanensis grown in very low phosphate (2 ppm Olsen P level) soil from Brazilian cerrado where the combination of G. fasciculatum and rock phosphate greatly increased nodulation and N\textsubscript{2} fixation in addition to P uptake and growth (Mosse et al., 1976). This increased P uptake by VAM stimulated the activity of Bradyrhizobium which is well known to depend on an adequate supply of P. Studies with lucerne showed that the effects of mycorrhizae on nodulation and nitrogenase activity preceded those on growth (Smith and Daft, 1977; Smith et al., 1979).

Taking into consideration the performance of different isolates in the pot culture studies and giving importance to the growth, biomass production, nodulation and nitrogenase activity, nutrient content and the extent of P fertiliser could be saved, the isolate of G. fasciculatum appears to be the most promising mycorrhizal fungus for P. phaseoloides.

5.5 Impact of Azotobacter sp., Beijerinckia sp. and B. circulans on Root Colonisation by G. fasciculatum and Growth, Nutrient Content and Rhizosphere Microbial Population of P. phaseoloides

It has been proved beyond doubt that VAM association of higher plants modify the saprophytic and pathogenic microflora (Tinker, 1982). VAM colonized roots have intimate contact with surrounding soil and subject to interaction leading to physicochemical reactions of soil, microbiological activities including plant pathogens (Sutton and Sheppard, 1976; Tisdall, 1991). Foster and Nicolson (1981) analysed microbial composition of soil aggregates in VAM inoculated soils and identified a range of fungi, bacteria, actinomycetes and algae including cyanobacteria.
In the present study inoculation of *Azotobacter* sp., *Beijerinckia* sp. and *B. circulans* with and without *G. fasciculatum* significantly increased root colonisation. Enhanced VAM colonisation of *P. phaseoloides* resulted in increased uptake of P and K, growth improvement, nodulation and nitrogen fixation and the importance of these activities are discussed earlier in Chapter 5.2.

In the absence of VAM inoculation *Azotobacter* sp., *Beijerinckia* sp. and *B. circulans* increased the natural VAM infestation. Under natural condition, several events take place in VAM colonisation including VAM spore germination, growth of mycelia through the soil, stimulation and attachment of infective hyphae to the root, penetration of the root, establishment of biotrophy and secondary hyphal streak outside and inside the root. Non-symbiotic nitrogen fixing bacteria and phosphate solubilising bacteria are reported to interact favourably with VAM fungi, either native or introduced at one or more of these stages (Bagyaraj and Menge, 1978; Mohandas, 1987; Brown and Carr, 1984). Such favourable activity of beneficial bacteria and VAM fungi were investigated in depth by Tilak et al. (1987a and 1987b). They isolated *Azospirillum* spp. from surface disinfected mycorrhizal roots of onion and from surface disinfected spores of *G. fasciculatum* and other species of *Glomus*. The increased VAM infestation in *P. phaseoloides* might also occur due to the production of plant hormones by free living N₂ fixers. The involvement of plant hormones, especially cytokinin in mycorrhizal development has been suggested by many workers (Allen et al., 1980, 1982; Barea and Azcon-Aguilar, 1982) and the stimulation of VAM infection in *P. phaseoloides* and is growth by free living nitrogen fixers could involve hormonal interaction.
The enhanced VAM infestation due to bacterisation in control plants having native population of VAM fungi in tomato and alfalfa was reported by Azcon et al. (1978) which confirms the observation made in this study.

Dual inoculation of *P. phaseoloides* with *G. fasciculatum* and any one of the beneficial bacteria i.e., *Azotobacter* sp., *Beijerinckia* sp. and *B. circulans* registered higher rate of root colonisation and subsequent beneficial action than the corresponding bacterial inoculation treatment without VAM. It proves beyond doubt the synergistic action of these microorganisms with VAM in improving plant growth. Similar interactions have also been observed between *A. paspali* and VAM fungi in *Paspalum* (Barea et al., 1973) and between *A. chroococcum* and *G. fasciculatum* in *Festuca arundinacea* (Ho and Trappe, 1979) and lettuce (Brown and Carr, 1980). Manjunath et al. (1981) conducted a triple interaction study between free living nitrogen fixing bacterium *Beijerinckia mobilis* phosphate solubilising fungus *Aspergillus niger* and *G. fasciculatum* and found a synergistic beneficial effect on the growth of onion with all the three microorganisms.

The increased root colonisation by dual inoculation of VAM and phosphobacterium as observed in the present study has already been established by Barea et al. (1975), Pathiratna et al. (1990), Heggo and Barakah (1993) and Singh and Singh (1993). Barea et al. (1976) demonstrated that a very high proportion of the phosphate solubilising bacteria tested, produced IAA, GA₃ and cytokinin and they are considered to augment root colonisation by VAM fungi. It is needless to find reasons for the augmented growth, nodulation, nitrogen fixation and nutrient uptake in *P. phaseoloides* with more root colonisation by VAM under the influence of *Azotobacter* sp., *Beijerinckia* sp. and *B. circulans* as these activities were directly proportional to VAM colonisation. The increased growth, nodulation, nitrogen fixation and nutrient uptake by *P. phaseoloides* inoculated with VAM and beneficial
bacteria might be through increased VAM formation and direct action of these bacteria by nitrogen fixation, phosphate solubilisation and production of growth promoting substances.

Both _Azotobacter_ sp. and _Beijerinckia_ sp. inoculation with and without _G. fasciculatum_ significantly increased growth, nodulation, nitrogen fixation and nutrient uptake. These two bacteria are well known non-symbiotic nitrogen fixers which improve plant growth (Bagyaraj, 1984). Bagyaraj and Menge (1978) studied the interaction between _A. chroococcum_ and VAM fungus _G. fasciculatum_ in tomato and found a synergistic effect on plant growth and nitrogen fixation. Such phenomenon might also have taken place in _P. phaseoloides_ inoculated with _G. fasciculatum_ and non symbiotic nitrogen fixing bacteria. The beneficial effect on plant growth by free living N₂ fixing organisms was attributed to hormone production, rather than, or in addition to nitrogen fixation as suggested by Bagyaraj and Menge (1978).

Among the various combinations, _G. fasciculatum_ and phosphobacteria combination was found to be sound and improve the growth, nodulation, nitrogen fixation and nutrient uptake in plants as evidenced from the results of the present study. Raj _et al._ (1981) also reported similar effects while studying the dual inoculation of _G. fasciculatum_ and phosphobacteria in finger millet. They also proved that the phosphate solubilising bacteria rendered more P soluble while VAM enhanced P uptake and concluded that with combined inoculation there was a synergistic effect on P supply and plant growth.

Dual inoculation with _G. fasciculatum_ and phosphobacteria in _P. phaseoloides_ resulted in more N content than the plants receiving _G. fasciculatum_ and either of _Azotobacter_ sp. or _Beijerinckia_ sp. The augmented N content in the tissues of these plants did not coincide with the trend in nitrogenase activity. The
enhanced level of N might be due to increased absorption of N by the ramification of extramatrical mycelia as evidenced by increased root colonisation in this treatment. VAM usually increase the growth of plants solely by enhancing nutrient uptake (Abbott and Robson, 1984) by increasing site of nutrient absorption (Cooper, 1984). He also reported that nitrogen absorption by plants is directly related to VAM colonisation. The results of the present study is in line with these earlier findings. The phosphobacteria also reported to produce hormones (Barea et al., 1976) and vitamins (Baya et al., 1981) and such compounds might also contributed to the growth P. phaseoloides and subsequent physiological activity.

Corresponding to the increased VAM infestation and growth of P. phaseoloides under the influence of different beneficial soil bacteria there was an increase in the rhizosphere population of saprophytic bacteria, fungi and non-symbiotic N₂ fixers and the maximum being in dual inoculation. Such increase in microbial population is well established (Bagyaraj and Menge, 1978; Secilia and Bagyaraj, 1987) and the physiology of host plant is reported to be responsible for such changes. Rambelli (1973) and Linderman (1988) postulated that the photosynthetic rate increases and the partitioning of photosynthate to shoot and root changes. The nutritional status of the host tissue changes in response to altered uptake of minerals from the soil and this in turn can change structural and biochemical aspects of root cells that can alter membrane permeability and the quality and quantity of root exudation. Altered exudation changes the composition of microorganisms in the rhizosphere now appropriately called mycorrhizosphere.

Azotobacter spp. population is less in acidic rubber growing soils. Alexander (1967) also observed the absence or reduced population of Azotobacter spp. in soils of low pH. But upon its inoculation there was $86 \times 10^2$ bacterial cells
per gram of soil. In the presence of VAM fungi 23 per cent increase of *Azotobacter* spp. population was recorded and this shows selective compatibility of *G. fasciculatum* and *Azotobacter* sp. and has much agronomic importance in acid soil in which the population of latter was less. Population of *Beijerinckia* spp. in acid soil is also poor (Kothandaraman, 1979) as seen in the present study. But there is positive response upon inoculation with this bacteria and phosphobacteria with and without *G. fasciculatum*. However in the presence of *G. fasciculatum*, *Beijerinckia* sp. recorded maximum count. Endomycorrhizae in the roots are reported to have direct effect on the exudation of substances from the roots which in turn affect the distribution of microorganisms in the rhizosphere (Mukerji and Subba Rao, 1982). The root exudates contain numerous substances including sugars (Subba Rao, 1977). Kothandaraman (1979) observed enhanced growth of *Beijerinckia* spp. when simple sugars were made available. The observed increase in *Beijerinckia* spp. population could be due to the increase of sugars in the root exudates of *G. fasciculatum* inoculated *P. phaseoloides*.

Increase in phosphobacterial count upon VAM colonisation in association with phosphobacteria as observed in the present study was reported by Barea *et al.* (1975). The superiority of phosphobacteria over others in promoting the growth of *P. phaseoloides* might be due to their unique property of solubilisation of bound phosphate in acidic soil, the vital nutrient for nodulation, nitrogen fixation and growth.

Though there was considerable changes in the population of bacteria and fungi upon root colonisation of *P. phaseoloides* with *G. fasciculatum* with and without beneficial bacteria, the population of actinomycetes were unaltered. VAM fungi and soil actinomycetes are generally have an antagonistic effect on each other,
each suppressing the growth and multiplication of other in the rhizosphere (Krishna et al., 1982). The unaltered and lesser population of actinomycetes might be due to the influence of VAM present in soil and introduced *G. fasciculatum*.

5.6 Screening of Host Plants for Mass Multiplication of VAM

Mass production of inoculum of VAM appears to be a bottleneck for detailed investigation of these fungi in promoting plant growth and field application. Since VAM fungi are obligate symbionts, they need living host for proliferation and the magnitude of multiplication differ with the plants under consideration. In the present study good root colonisation and VAM spore count in soil-vermiculite mixture was recorded when *S. bicolor* was used as host plant. Compared to other host plants tested *S. bicolor* produced more root biomass. High susceptibility of *S. bicolor* to VAM colonisation as well as the light weight of vermiculite soil mixture prove to be a better means of mass production of inoculum for various studies and distribution for farmers. Hayman (1982) also found such effect and included *S. bicolor* as one of the host plant for VAM bio-fertiliser production.

In the light of the results obtained from the present study, it is concluded that the growth and nutrient content of *P. phaseoloides* could be enhanced by dual inoculation with *G. fasciculatum* and either of *Azotobacter* sp., *Beijerinckia* sp. or phosphobacteria. This study also indicated the possibility of substantial reduction in the use of rock phosphate. *S. bicolor* is found to a better host plant for mass multiplication of *G. fasciculatum*. 