DISCUSSION

The present study of the rhizobia from wild legumes was undertaken on account of the fact that N-fixation by the soil bacterium Rhizobium in symbiosis with legumes is of considerable importance in agriculture. The earlier studies by various scientists indicate that rhizobia from different wild legumes can be a better source for the preparation of commercial inoculum (Allen and Hamatova, 1973; Oblisami, 1973; Dye, 1979; Rangaswami and Oblisami, 1962; Basak and Goyal, 1980). The summary of the nodulation survey data from all over the world by Allen and Allen (1981) shows that at species level only 15% of the family Leguminosae have been examined, of which 91% have been recorded with nodules. (Out of 19,700 species of Leguminosae, only 3,108 species have been examined till now).

In India, literature survey shows that only 125 species of legumes belonging to 38 genera have been studied and many of the species remained to be unexplored. In the present investigation a total of 42 species were examined for nodulation from the region of Marathwada. All the species belonged to the family Papilionoideae except one (Cassia sp.) that belonged to Caesalpinoideae. Of total 42 species 13 (Alysicarpus rugosus, Crotolaria linifolia, Eleotris monophylla, Olricidia sepium, Indigofera astragalina, I. echinata, I. trita, Macuna pruriens,
*Sebания bispinosa, S. sesban, Tephrosia hirta, T. pumila, Uneria rufescens* have been explored for the first time in India whereas 8 species (*Alysicarpus rugosus* var. *styracifolius, A. rugosus* var. *heyneanus, Crotolaria naikiana, C. notonii, I. duthiei, I. glandulosa* var. *glandulosa, I. glandulosa* var. *sykesii*) for the first time in the world.

Number of nodules varied in different species studied. Maximum numbers were seen on *Sebания bispinosa* in natural habitat. Attachment and colour of nodule also varied according to the species. Largest nodules were seen in *Canavalia gladiata* while the smallest on the roots of *Alysicarpus rugosus* var. *heyneanus*. *Allen and Allen (1981)* described eight types of nodules based upon the shape. Accordingly, in the present investigation globose, corolloid, elongate and kidney shaped nodules were seen on the wild legumes collected throughout Marathwada region. Shape of the nodules remained same within the genus. For instance all the species of *Alysicarpus* had globose nodules while all the species of *Tephrosia* had elongate nodules. Kidney shaped nodules were seen in *Uneria rufescens*, but in the genus having large number of species, shape of the nodules varied. In *Indigofera astragalina, I. cordifolia* and *I. echinata, I. linifolia* nodules were globose with smooth and rough surface while *I. duthiei* and *I. trita* had elongate nodules. This indicates that shape and attachment
of nodules to the host is a specific character and not the
generic character.

Sixty strains of *Rhizobium* were isolated from the
nodules of 42 legumes using standard methods described by
studies were also done by the standard procedures.

Methodology is of great importance while evaluating N-
fixation during the host *Rhizobium* symbiosis. Cross
inoculation studies were carried out using Leonard bottle
jar technique which is the most acceptable method in order
to study the rhizobial infection in sterile conditions.
Various methods (laboratory and field methods by direct and
indirect means) have been discovered by different authors
specified in the field (Bergersen, 1980). Acetylene
reduction for nitrogenase activity has become more popular
(Hardey *et al.*, 1973). But this could not be done due to
lack of laboratory facilities. Nodule number and nitrogen
content were taken as criteria for N-fixation in the
present study. Analysis of total nitrogen in DM was done
by Microkjeldahl technique (Parker, 1961). Comparison of
total N in DM of inoculated plant with uninoculated control
and N-supplemented control in search of effective strain
have been suggested by Vincent (1970). Haydock *et al.* (1980)
examined statistical relation between N-percent (Y) and DM
per plant (X) and the data showed that most effective
Rhizobium strains can be selected on the basis of DM yields of the whole plant or plant top only without the need for N-analysis. Therefore, N-analysis was not carried out in few experiments.

**Morphology and Biochemical Characteristics**

All the strains were gram negative measuring 0.5-1.0 x 1.3-3.0 μ. Colonies were colourless to milky with gum. Strains were both the slow and fast growing. Moreover, Congo red was absorbed by the rhizobial isolates in the present investigations. All these morphological characters of the cells and colonies of all the strains confirmed to *Rhizobium* (Kleczkowska et al., 1968; Jordan and Allen, 1974; Rangaswami and Oblisamy, 1962; Graham and Parker, 1964; Basak and Goyal, 1980).

Isolates of *Rhizobium* from wild legumes showed normal growth at 1.6 to 2.0 % NaCl. Graham and Parker (1964) and Basak and Goyal (1980) reported that most of the *Rhizobium* strains could tolerate up to 2.0 % NaCl concentration, but in the present study Igs-136(b) from *I. glandulosa* var. *sykesii* was found to grow up to 3.0 % NaCl concentration. Similar observations have also been made by some workers (Yadav and Vyas, 1971; Subba Rao et al., 1975; Pillai and Sen, 1973) in gram, groundnut, cowpea, gaur, lucerne and *Dolichos lablab* *Rhizobium*. Moreover, 13 isolates from the
Indigofera spp., Smithia conferta and Eleiottia monophylla were stimulated at 0.01 % NaCl concentration in the medium. Basak and Goyal (1980) also reported the increased growth of rhizobial isolates from legume trees at 0.2 to 1.2 % salt concentration in the medium.

None of the strains gave positive reaction for gelatin liquifaction and indole production. Strain MP-13 from Macuna pruriens showed positive reaction for MR-test while 9 strains from other wild legumes performed starch hydrolysis and 11 produced H₂S. Production of NH₃ and reduction of nitrate was shown by majority of the strains. 35 strains formed serum zone; 14 and 15 of them gave acid and alkaline reaction respectively. The biochemical data in the present study support the findings of Graham and Parker (1964) and Graham (1976) that biochemical characteristics of rhizobial strains from different legumes or from different groups do not have any advantage in the current classification. Characteristic feature of cowpea group rhizobia producing alkalinity, however, was shown by certain strains in this investigations. Similarly, strains, those reduced nitrate were more effective for nodulation and N-fixation in groundnut. Muthusamy et al. (1973) also observed that nitrate reducing strains were found to be highly efficient in nodulation and nitrogen fixation in groundnut. Strains from Indigofera spp. were slow growers
indicating their affinity with cowpea group as suggested by Graham (1976).

Variability in the utilization of carbohydrates among the rhizobial strains from different legumes was seen. Most of the strains utilized all the eleven carbohydrates studied. However, Igg-65 did not utilize glucose, fructose and lactose while Igg-68 did not utilize arabinose. Strain Id-104 could not grow on raffinose whereas Ia-104 on five carbon sources. Mannitol was most favourable for the growth of all rhizobial strains studied. The results confirm the earlier findings of Rangaswami and Oblisami (1962) and Basak and Goyal (1980b) in case of the rhizobial isolates from 15 legumes and 19 tree legumes. They found that all the rhizobia showed more preference for arabinose followed by other carbohydrates. In this investigation also arabinose, mannose, xylose, galactose, sucrose and maltose gave profuse growth of rhizobial isolates. Graham and Parker (1964) were unable to show any relationship between the rhizobial groups and utilization of carbohydrates. Raffinose was utilized in different degrees by the rhizobia of all the cross inoculation group including Agrobacterium sp.

During the earlier studies on rhizobia, it became apparent that the ability of a particular organism to pass from one plant to another was limited to a certain group of
plants. This resulted in the establishment of a series of plant groups known as Cross Inoculation Groups (Fred et al., 1932). In the present investigation host plants such as *Medicago sativa*, *Phaseolus vulgaris*, *Trifolium alexandrinum*, *Pisum sativum*, *Glycine max*, *Vigna sinensis* and *Arachis hypogaea* were used in order to allocate the rhizobial isolate to a particular group. Accordingly, cross inoculation tests with rhizobial strains revealed that majority of them have affinity to *Vigna sinensis* and *Arachis hypogaea*. Out of 71 strains, 57 nodulated *Vigna sinensis* and 44 nodulated groundnut indicating that they belong to cowpea group. 38% strains belong to *R. japonicum* while some of them can be grouped under *R. trifolii*, *R. leguminosarum*, *R. meliloti* and *R. phaseoli*. Infectivity of the *Rhizobium* to a particular host(s) is attributed to many reasons. One of the reason is the specificity of the *Rhizobium* towards legume host which is due to its selective attachment to legume lectins (Vance, 1983; Dazzo and Hubbel, 1975). The present study also indicated that some strains nodulated more than one host of different group(s). Thus Id-21 from *Indigofera duthiei* although nodulated *Vigna sinensis* and *Arachis hypogaea* also nodulated *Medicago sativa*, *Phaseolus vulgaris*, *Pisum sativum* and *Glycine max*; Id-24 nodulated *P. vulgaris*, *Glycine max*, *Vigna sinensis* and *Arachis hypogaea*; SB-35 from *Sesbania bispinosa* nodulated *Medicago sativa*, *Glycine max* and also *Arachis*
hypogaea; Co-57 from Crotalaria orixensis nodulated Glycine max, Vigna sinensis and Arachis hypogaea; Igg-65 and Igg-71 from Indigofera glandulosa var. glandulosa nodulated Phaseolus vulgaris, Glycine max, Vigna sinensis and Arachis hypogaea; Ie-108(a) from Indigofera echinata nodulated Trifolium alexandrinum, Glycine max, Vigna sinensis and Arachis hypogaea; Cl-114 from Crotalaria linifolia nodulated Glycine max, Vigna sinensis and Arachis hypogaea; Av-121 from Alysicarpus vaginalis again nodulated G. max, V. sinensis and A. hypogaea. These results agree with the invalidity of cross inoculation group concept (Wilson, 1944; Nutman, 1965; Norris, 1965; Graham, 1964, 1976). These results obtained in the present study are also in agreement with those of Sears and Carroll (1927), Johnson and Allen (1952), Bhide (1956), Rangaswami and Oblisami (1962), Basak and Goyal (1980) who also noted that cowpea group plants and soyabean are nodulated by the isolates from different non-cultivated legumes. It appears that these strains from wild legumes in the present investigation may be included in promiscus cowpea type (Fredy 1932). However, Brackwell (1981) adds that although it is an unsatisfactory basis for taxonomic classification of the rhizobia, it is still used and will probably remain in use until an acceptable alternative is proposed. It provides accurate guidelines for the selection of effective inoculum strains and aim of the present study also was explored the
possibility of the utilization of rhizobial strains from wild legumes of this region – Marathwada – for groundnut crop.

**Efficacy of rhizobial isolates against groundnut**

Of the 65 strains from wild legumes, 34 nodulated and groundnut SB-11 were efficient in nitrogen fixation when studied *in vitro*. Variation in their efficacy of course was seen. 22 strains were more effective. *Rhizobium* *Ie-108(a)* and *Ie-108(b)* were most effective in both the sterile and natural soil. Interestingly, maximum strains isolated from the *Indigofera* spp. were more efficient in the nodulation and N-fixation in groundnut SB-11. Nodulation of legume crops by the rhizobial isolates from different non-cultivated legumes have been reported by many workers (Allen and Allen, 1936, 1939; Bowen, 1956; Dye, 1979; Carroll, 1934; Basak and Goyal, 1980). Oblisami (1973) found that the rhizobial strains from *Cassia mimosoides* were most efficient on *Vigna sinensis*, *Phaseolus mungo* and *P. aureus*. Similarly, Rangaswami and Oblisami (1962) noted that isolates from *Sesbania speciosa*, *Crotalaria retusa* and *Clitoria* were most effective on soyabean. Rhizobial isolates from tree legumes were noted to be effective on green and black gram (Basak and Goyal, 1980). Using rhizobial strains from wild legumes, particularly *Indigofera* spp. in the present findings
suggest that they can be placed under cowpea group. Non-nodulation of groundnut by isolates from other legumes might be due to their adaptive differences (Vance, 1983).

Field trials with six rhizobial isolates from Indigofera spp. and Smithia sp. indicated that all the strains were effective; strains Iea-108(a) and Igg-72 gave higher pod yields than others. Increase in the yield of legume crop due to rhizobial inoculation have been reported by many workers (Date, 1975; Ayanaba, 1977; Sundara Rao et al., 1975; Subba Rao, 1976; Gangawane and Datar, 1977; Shinde, 1981; Vaishya and Gajendragadkar, 1982). In case of groundnut, there are reports that Rhizobium inoculation increases pod yield (Loutfi et al., 1966; Hamdi, 1976; Stoddard, 1978; Ratner et al., 1979; Wyness et al., 1980; Sen and Weaver, 1980, 1981; Graham and Donawa, 1982; Nambiar and Ravishankar, 1983; Nambiar and Datt, 1983). The sources of Rhizobium were either from the groundnut nodules or culture collection. In the present investigation strains from the Indigofera spp. or Smithia spp. proved to be better for effective nodulation and N-fixation in groundnut for the first time. Simultaneously, nutrient status of the soil was changed due to rhizobial inoculation. N and P content slightly increased while K reduced. Increase in the N content of soil due to inoculation of groundnut Rhizobium has also been reported by Muthusamy et al. (1973). They
that increase in the nitrogen content of soil is due to nitrogen fixing capacity of \textit{Rhizobium}. Depletion of certain nutrients from the soil by groundnut is also known (Seshadri, 1962). Inoculation of \textit{Rhizobium} in the red laterite was found to be more effective than in the black cotton soil. Competition between the native \textit{Rhizobium} and inoculant \textit{Rhizobium} may be the main reason as nodulation in the uninoculated control was more in the black cotton soil than in the red laterite soil (Date and Brockwell, 1978). Subba Rao (1976) and Nambiar and Dart (1982) also observed that nodulation and yield of groundnut pods varied at different places in India in the All India Coordinated trials. As regards rotation of groundnut with jowar, inoculation of \textit{Rhizobium} resulted in the increase of nodulation and yield of groundnut pods. Growing of groundnut over groundnut or cotton did not have any effect. This is in accord with the earlier finding that inoculation with effective \textit{Rhizobium} strains increase in the fields where groundnut had not been previously grown. (Seeger, 1961; Pettit et al., 1975; Burton, 1976). There are also few reports as the effect of \textit{Rhizobium} inoculation on groundnut where groundnut had been previously grown. Thus, in U.S.A. inoculation did not increase pod yields (Nambiar and Dart, 1982). This might be attributed to the direct or indirect influence of soil microorganism on nodulation of groundnut by the \textit{Rhizobium} strain used in this study. Crop rotation
is known to exert influence on soil microorganisms indirectly through the plants (Nash and Synder, 1965; Gangawane and Deshpande, 1975).

**Effect of soil types**

The inoculant strains of *Rhizobium* do not always give the expected nitrogen fixation in different soils. This is probably due to the predominance of native rhizobia over the inoculant strains. In the present studies three soil types (black cotton, red laterite and clay soil) were taken in order to see the nodulation and N-fixation in groundnut. Black cotton soil supported more growth and N-fixation without inoculation of rhizobial strain. But red laterite soil was more favourable than other soils for the nodulation, growth and nodulation inoculated with *Rhizobium* Ie-108(a). Edaphic and microbiological factors might be responsible for this. Ghai *et al.* (1982) showed that cowpea strains MNH, M-20 and RM-6 were effective nodulators in loamy sand soil while RM-6 and RM-5 in loamy clay soil. They advocate that competitive ability with native *Rhizobium* of the strains in a particular soil decides the performance of the effective strain. In addition, soil microflora (bacteria, Actinomycetes, fungi, algae) may play important role in nodulation of a legume. Variation in rhizosphere mycoflora of groundnut in different soils has been indicated by Gangawane (1972). It can be suggested that *Rhizobium* Ie-108(a) may be best competitor in red laterite soil.
Effect of pesticides on Rhizobium and nodulations

Successful eradication of pests and diseases of groundnut by the use of pesticides (fungicides, insecticides, antibiotics, herbicides) is known. Some pesticidal compounds toxicity to soil microorganisms including Rhizobium in vitro has been observed (Subba Rao, 1977; Oblisami et al., 1973; Wainright, 1977; Singh and Agnihotri, 1980; Gaur, 1980; Heininen et al., 1982; Gangawane et al., 1979; Gangawane and Saler, 1974). Growth of the Rhizobium Ie-108(a) on agar was most inhibited by fungicides in the present study. These results accord with Suillia and Anusaya (1980) who also noted the inhibitory effects of ceresan, blitox, bavistin, and PCNB on four strains of groundnut Rhizobium in vitro. Cuprex, parasan, maneb and zineb were more toxic than bavistin and aliette and others. The antibacterial activity of cationic surfactants against gram negative bacteria is well established (Bater et al., 1941; Fisher, 1976). Other fungicides are also known to be inhibitory against different rhizobia (Afifi et al., 1969; Kecakes and Vincent, 1969a; Kecskes, 1970; Daitloff, 1970a; Gilberg, 1971; Sud and Gupta, 1972; Ganguli et al., 1975; Fisher and Clifton, 1976). In case of groundnut Rhizobium, Gangawane et al. (1979) noted the inhibitory activity of nine 4-Aroyl, 3,5-diaryl pyrazole compounds. Changes in the membrane permeability and inhibition of respiration in the bacterium in the
presence of fungicides is probably the main reason of toxicity as has been suggested by Forsyth (1964) and Fisher et al. (1978).

*In vivo* studies indicated the reduction of nodulation in groundnut is due to fungicides at higher concentration. Cuprez did not exert any effect while toxicity of bavistin and difolatan and thiram was negligible. Seed dressing with benlate and thiram, however, did not affect the nodulation; aliette and benlate on the contrary stimulated DM yield of plants. Some reports that there is no correlation between the results *in vitro* and *in vivo*. Thiram was toxic to *Rhizobium leguminosarum in vitro* but did not affect the nodulation of *Vicia sativum in vivo* (Kecskes and Vincent, 1969b). Similarly, captan has no effect on nodulation of this crop (Krataka and Ujevic, 1970). Fisher et al. (1978) noted that hyamine 3500, the most inhibitory compound to *Rhizobium trifolii in vitro*, had no effect on fixation *in vivo*. Factors such as ease of penetration and partition characteristics which favour the efficacy of the fungicides used in this study are probably responsible for their inhibitory or stimulatory action on *Rhizobium* within the groundnut root nodule. According to Mosse (1964) cell wall of the normal mature bacterioids of *R. trifolii* is in direct contact with the host membrane. He however, observed that bacterial cell wall had contacted so that it no longer filled the space enclosed by the host's
membrane envelop in clover nodule grown in Triton X 45 and PP-222 treated soils. There might also have been the effect of the fungicides as adhesion of the bacterial cells to the root hair and thereby affecting the infection process. These reasons are probably sufficient to explain the reduction of nodulation in groundnut by the use of fungicides. Stimulatory effects of fungicides on nodulations of groundnut are already known (Mukewar and Bhide, 1969, 1970; Delala and Lodha, 1976). Increased nodulation and root and shoot growth by bavistin in the present study might be attributed to its stimulatory activity on groundnut.

Antibiotics such as Erythromycin, Penicillin, Streptopenicillin, Aureofungin, Terramycin, Ampicillin, Chloramycetin and Soframycin were proved to be toxic to the Rhizobium Ie-108(a) in vitro; Penicillin and Streptopenicillin were more toxic than others (Vintika and Vintikova, 1958; Vincent, 1977). Antibacterial action of these antibiotics except Aureofungin against gram negative bacteria including Rhizobium is well known. Mukewar and Bhide (1970), however, noted the stimulatory action of Aureofungin on groundnut nodulation in soil. Insecticides such as Rogor, Cyathion, Hexasulfan, Phendal and DDT were inhibitory to Rhizobium Ie-101(a), Ie-108(a) and Ie-108(b) at higher concentrations. Many workers reported inhibition of the growth of different rhizobia by various insecticidal compounds (Salem, 1971; Shin-Chsiang Lin et al., 1972;
Oblisami et al., 1973; Salama et al., 1973; Nirmal et al., 1977; Tu, 1977). DDT was less harmful on Rhizobium Ie-108(b). Parek and Gaur (1970) noted no harmful effect of DDT on the Rhizobium of green gram. The possible explanation for the inhibitory action of these compounds on rhizobia may accord with (as given for the fungicides) such as alteration of permeability of the cell membrane, respiration and other metabolic processes in the bacterial cell.

Herbicides such as 2,4-D, tolkan and slam F-34 were not toxic to Rhizobium Ie-108(a) even at 50-5200 µg/ml level in vitro. This is in accordance with Grossbard (1970) who also found that there is no effect of asulam on Rhizobium trifolii. Kaszubiak (1966) also showed non-toxic effect of halogenated aliphatics, TCA and dalapon on Rhizobium spp. even at 20,000 µg/ml concentration. Inhibitory effects of 2,4-D on different rhizobia, however, have been shown by Gaur and Mishra (1972) and Kao and Wang (1981) in pure cultures.

The inhibitory nature of the pesticides on the Rhizobium in the present findings indicate that (1) seed treatment should be done with more number of bacterial cells so as to insure sufficient load of Rhizobium to compensate the harmful effect of pesticidal compound (Oblisami et al., 1973) and (2) separation of the pesticide from the inoculum
by applying a polyvinyl acetate coating to seed after pesticidal treatment and prior to rhizobial inoculation. Anderson (1978) advocates that doses of pesticides 10 to 100 times more than that of the normal recommended doses are sufficient for an investigation into pesticidal effects on soil microorganisms. In the light of this explanation the doses used in the present investigation may not be harmful at the field level or this much concentration of pesticidal residue may not reach into the soil when applied at recommended doses. However, more studies are needed to establish the magnitude of the problem under natural soil conditions as has very well been stated by Oblisami et al. (1973).

Mineral Nutrition and Nodulation

According to Edwards (1977) the amount of nitrogen fixed by the legume - Rhizobium systems is influenced by chemical factors of the environment which may affect the host plant per se, the Rhizobium per se and the development and effective functioning of nodule. Effect of mineral elements on the nodulation of groundnut by Rhizobium Ie-108(a) was studied in the present investigation. Micronutrients are essential for the growth of groundnut in very minute quantities. Efforts were made to arrange nutrient solution devoid of an element in control by the use of double distilled water. Micronutrients such as Mn,
Mo, Co, Bo, Zn and Mg stimulated nodulation and N-fixation in the groundnut inoculated with rhizobial isolate Ie-108(a) at the optimum concentration. The optimum concentrations, however, differed according to the micro-nutrient. Thus 0.25 μg/ml of Mn, 0.5 μg/ml of Mo, 0.084 μg/ml of Co, 0.5 μg/ml of Bo, 0.5 μg/ml of Zn, 0.03 μg/ml of Mg stimulated the nodulation and N-fixation in the present study. Cu on the other hand inhibited nodulation. The present results are in agreement with Arunachalam et al. (1978) who also noted that trace elements such as Co (0.5 ppm), Mn (1.0 ppm), Zn (1.0 ppm), Mo (1.0 ppm) are stimulatory to groundnut Rhizobium in vitro. They also noted that Cu is inhibitory at higher concentrations (more than 5 ppm) to groundnut Rhizobium. Vincent (1954) stated that Cu might be toxic to rhizobial growth if direct contact between the bacterium and the element is encountered and this view supports the present finding.

There are reports that trace elements other than Cu are stimulatory to N-fixation in different legumes (Holding and Lowe, 1971; Norris, 1959; Mulder et al., 1959; Parker and Harris, 1977; Kier et al., 1983; Brenchley and Thornton, 1925; Shukla and Yadav, 1982; Mukewar et al. 1982). Studies by Sharma and Ghonsikar (1976) however indicated that application of Mo to Rhizobium inoculated cowpea did not improve nitrogen levels in nodules. Increase in the
nodulation and nitrogen fixation with trace elements in the groundnut might be attributed to an increase in the haemoglobin concentration in nodules and increased plant growth resulting into enhanced activity of Rhizobium in the present investigation.

P and K increased nodulation and N-fixation in Rhizobium Ie-108(a) inoculated groundnut while N was detrimental for nodulation although it favoured shoot and root growth. Nitrogen sources are reported widely to decrease the nodulation of different legumes and to reduce N$_2$ fixation (Dixon, 1969; Dart, 1974; Lie, 1974; Rabie and Kumazawa, 1979; Streetar, 1981; Eaglesham et al., 1982, 1983; Evans, 1982; Ram et al., 1983). The results in the present investigation also confirm the earlier reports of Ball et al. (1983). In case of N, they noted that ammonium nitrate increased plant weight but reduced N$_2$ fixation in groundnut. Phosphorus is known to directly limit nodulation and N$_2$ fixation directly (Munns, 1977) and there are many reports that phosphorus fertilization improve nodulation in different legumes (Kaushik and Singh, 1969; Gates, 1974; De Mooy, 1965; Shukla and Yadav, 1982). In case of groundnut, Shivshanker et al. (1982) showed that nodulation, leghaemoglobin, N$_2$ fixation and DM are increased with the increased levels of P. Similar interpretation can be given for K. It is also known that low potassium
availability limits the $N_2$ fixation in legumes (Blaser and Brady, 1950; Jones et al., 1977; Christopher and Roskosk, 1983) and present findings support the need of K for groundnut–Rhizobium symbiosis.

**Interaction between rhizosphere microflora and Rhizobium:**

The rhizosphere and soil microflora was estimated employing dilution plate method. The dilution plate method have been used by many investigators but it is criticised by some scientists (Parkinson, 1967) on the grounds that it exaggerates the importance of sporulating fungi whereas many fungi are known to occur as vegetative forms in the rhizosphere (Agnihotrudu, 1955; Parkinson, 1957). However, it is still most convenient method for comparing results obtained from a large number of estimations and for the isolation and identification of fungal taxa. The purpose of the present investigation was to isolate and identify the fungal taxa in the rhizosphere and soil of groundnut at seedling stage. Rhizoplane and root nodule fungi were noted using serial root washing (Harley and Waid, 1955).

Quantitative and qualitative variations were observed in the mycoflora in the rhizosphere and soil. Rhizosphere effect was found to be more than one showing the influence of root on the organisms. This is in agreement with the similar reports by Rao (1962) and Gangawane and Deshpande.
(1973) in case of groundnut. Many investigators had noted that maximum rhizosphere influence with the initial vegetative development (Starkey, 1929a; Joffe, 1969). 15 species of fungal species were recorded from the rhizosphere and/or from the soil of groundnut. Various number of species have been recorded by different workers from the rhizosphere of groundnut. Joffe (1969) recorded 157 species while Rao (1962) noted only 16 species. Variety of the plant, nature of soil as well as the method and media employed are the responsible factor for this. Presence of more Aspergillus species can be explained on the basis of their heavily sporulating habit and their capacity to produce antibiotics. Rhizoctonia bataticola, a potential pathogen on the rhizoplane and on the root nodules is of economic importance.

Twenty nine fungal species were recorded from the rhizosphere of 10 weeds, although variation in the number of species was observed. Nature of root exudates may account for this. It is well established that plant root exudates play an important role in stimulating microorganisms in the rhizosphere (Rovira, 1965b, Yousef and Mankarios, 1969; Gangawane and Deshpande, 1973).

In vitro studies on the effect of rhizosphere mycoflora of groundnut and certain weeds showed that majority of the
species had neutral effect on the *Rhizobium* Ie-108(a) while few such as *Aspergillus nidulans*, *Fusarium semitectum* and *Trichoderma* sp. had stimulatory effect in the present studies. Lim (1961) noted that *Paecilomyces* sp. had stimulatory effect on the *Rhizobium*. Sethi and Subba Rao (1968) also noted stimulatory effect of *Paecilomyces* sp., *Penicillium* sp., *Phoma* sp. and *Rhizopus* sp. on the *Rhizobium trifolii*, *R. phaseoli*, *R. leguminosarum*, *R. japonicum*, *R. meliloti* and cowpea rhizobia *in vitro*. These organisms may increase infection process of *Rhizobium* on the roots of groundnut and needs further investigation. Gupta (1974) found that *Tetracoccusporium paxinum* enhance nodulation in *Trigonella foenum-graecum*.

Some Actinomycetes isolates from the rhizosphere of groundnut and weeds on the contrary were antagonistic to *Rhizobium* strains when tested *in vitro*. The failure of nodulation in certain parts of western Australia has been attributed to the presence of antagonistic microorganisms (Hely et al., 1957; Smith and Holland, 1958). Suppression of nodulation by Actinomycetes is also noted by other workers. Wieringa (1963) isolated a number of Actinomycetes antagonistic to *R. trifolii*. Oblisami and Rangaswami (1967) found three species of *Streptomycyes* antagonistic to *R. meliloti*, *R. leguminosarum*, *R. phaseoli* and *R. japonicum* in sterile soil, while Jayasheela et al. (1982) also noted
antagonistic property of Actinomycetes against \textit{R. japonicum} on the plates. Actinomycetes Eg-5 antagonistic to \textit{Rhizobium in vitro}, however, did not show any adverse effect on the nodulation of groundnut \textit{in situ} in the present investigation. Altered root metabolism may be responsible for this.

A pathogenic strain of \textit{Rhizoctonia bataticola} was isolated from infected groundnut plant. Pathogenic nature of this organism causing dry root rot of groundnut is known (Chohan, 1979). \textit{Rhizoctonia bataticola} reduced growth of rhizobial strains, it also decreased nodulation and growth of groundnut. Interaction between nodulation and certain fungi including \textit{Rhizoctonia} spp. have been studied by some workers. Purkayastha \textit{et al.} (1981) noted the reduction in disease severity of charcoal rot of soybean inoculated with \textit{Rhizobium}. On the other hand Patil \textit{et al.} (1982) noted that \textit{Aspergillus niger} reduced the growth of \textit{Rhizobium in vitro} and also nodulation in groundnut \textit{in vivo}. Moreover, Kush (1982) also showed that inoculation of \textit{Rhizoctonia bataticola} and \textit{Rhizobium} together in green gram significantly reduced nodule number and nitrogen activity per plant. Antagonistic interactions in the host cells is suggested for this detrimental effect of the pathogen on nodulation.

Actinomycetes Eg-5 and Eg-10 were found to be antagonistic to \textit{Rhizoctonia bataticola} on the plates. Many
antagonistic microorganisms are known to exist in soil and many of them are found to be the best tool for biological control of *Rhizoctonia bataticola* and other pathogens (Brina, 1951; Vittal Rao and Rao, 1966; Kaufman and Williams, 1968). The inhibition of *R. bataticola* in the present study may be due to the antagonistic principle of Actinomycetes. The *Rhizobium* and *R. bataticola* when inoculated together with Actinomycetes *Eg-5*, therefore, increased growth and nodulation. This might be mainly due to the suppression of the growth of *R. bataticola* in the rhizosphere of groundnut by the Actinomycetes. Percentage infection of *R. bataticola* on the roots was also reduced. Biological control of *R. bataticola* without suppression of nodulation in groundnut can therefore be achieved. However, further soil trials are warranted in this connection.

**Whey irrigation, rhizosphere microflora and nodulation**

Whey is the product of leaf protein extraction and may be used for irrigation purpose. It is known that whey amounts about 40% of soluble carbohydrates, 10-20% crude protein and 20% inorganic matters. There are also other organic components like vitamins, growth promoting substances etc. The 'whey' might have supported the growth and multiplication of *Rhizobium* in the rhizosphere of groundnut. Infection process consequently might have been also improved leading to the more nodulation. This was
of course seen at optimum dilution (1:1) of whey where more nodulation and maximum growth of the plants was seen in the present investigation. Inhibition of *Azotobacter* in both the rhizosphere and soil is attributed to increase of more nitrogen in soil due to whey. Inhibition of growth of *Azotobacter* and nitrogenase activity in the presence of nitrogen is known (Baldensperger, 1980).

Some fungal species dominated in the rhizosphere of 'whey'-irrigated plants. Increased competition among microorganisms for the food material from whey might have allowed some fungi to dominate while others to be suppress. Some species of aspergilli are known to solubilize the different elements in the rhizosphere of plants. Increase of *Aspergillus niger* in the rhizosphere due to whey is therefore of practical importance as it may help in S and P nutrition of plants. Thus utilization of whey for irrigation purpose prove to be very useful not only in improving the plant growth directly but it also has bearings to improve soil fertility through the application of diluted whey to the groundnut crop.