CHAPTER - V

DISCUSSION

Legume plants through the root nodule symbiosis with *Rhizobium* offer a practical and economical means of utilizing the vast untapped source of atmosphere nitrogen. *Rhizobium* biofertilizers offer best hope and scope to reduce the cost of inorganic fertilizers. It is need to understand that the bioferfilizers can only supplement the inorganic nitrogen. They can neither form an alternate source of inorganic fertilizers nor can replace the same in the wider perspective of sustaining the yielding potential in the present day situation. The beneficial effects of *Rhizobium* inoculation to various leguminous crop plants have been investigated by several workers (Maria *et al*., 1989; Dakora and felix, 1995; Thakare *et al*., 1999; Singh *et al*., 2011; Qureshi *et al*., 2014). Efficient mutant strains of Rhizobium spp was derived by using chemical muteagens such as NTG (N-methyl-N-nitro-nitrosoguanidine), EMS (Ethylmethane sulphonate) *etc.* (Singh *et al*., 1992; Allan, 1989;).

In the present study, the twenty four locations studied for the nodulation were Pichavaram, Singarakuppam, C. Mutlur, Mudasal odai, Thillai vidangal, Killai, Vallam padugai, Parangipettai, Kodiyampalaiyam, Pinathur, Puthuchathiram, Periyappatu of cuddalore district. Pandaravadi, Thiruvadudurai, Sirkazhi, Senniyalore, Thirukkuvalai, Nagapattinam, Thiruvalangadu, Vedaranyam, Kuthalam, Tharangapadi, Palaiyur and
Palayakoodalore of Nagapattinam district of Tamil Nadu, to study their efficiency for establishing symbiosis with the blackgram, variety ADT.3.

The nodulation was found to be good (16.2 to 21.6 plant\(^{-1}\)) in plants collected at Killai, Pinnathur, Nagapattinam, and C. Mutlur, moderate (12.0 to 15.0 plant\(^{-1}\)) in plants collected at Sirkazhi, Palaiyur, Pichavaram, Kodiypalaiyam, Thirukkuvalai, Vedaranyam, Mudasalodai, Pandaravadi, Thiruvadudurai, Palayakoodalore, Senniyalore, Periyappattu, Tharangampadi, Thillaividangal, Thiruvalangadu, Singarakuppm, Kuthalam, Vallam padugai, and Parangipettai. The lowest nodule number was recorded (9.9 plants\(^{-1}\)) in plants collected at Puthuchathiram.

Variation in the natural nodulation in rice fallow blackgram at different locations of South Arcot District was observed by Prasad (1983), for rice fallow. These results showed the need for improving the nodulation of blackgram by suitable management including seed inoculation with efficient strains to increase the growth and yield.

Blackgram soil samples collected from different locations of these two Districts \textit{viz.}, Cuddalore and Nagapattinam belonged to 3 textural groups \textit{viz.}, Sandy clay, Sandy loam and clay loam. of the 24 samples, analysed 1 was clay loam, 3 was sandy clay and 20 was sandy loam. The texture of the soil influenced the root nodulation of blackgram.

In general, the physio-chemical properties of the soils do not have
any correlation with number of nodules of blackgram plants grown in Cuddalore and Nagapattinam districts of Tamil Nadu. However, a positive correlation between organic matter content and root nodulation; between organic matter content and rhizobial population; between pH and root nodulation between pH and rhizobial population was recorded. The role of organic matter content on the heterophoric mode of nutrition of various soil bacteria including *Rhizobium* is well documented (Jackson, 1991).

The wide variations in rhizobial cell counts and nodule mass in beans are related to variation in physio-chemical conditions of the soil (Vincent, 1965 and Malder *et al.*, 1969). In the present study, Soil organic carbon contents were ranged from 0.35 to 0.97 percent, soil pH ranged from 7.2 to 8.60 and EC ranged from 0.40 to 0.89 dsm⁻¹.

Further soil analysis revealed that the available nitrogen content ranged from 56.18 to 148.82 kg/ha and the phosphorous content ranged from 11.59 to 21.20 kg/ha, while the potassium content ranged from 110.11 to 210.84 kg/ha.

Among the twenty four samples analyzed for N, P and K, Killai was found to be good in available N, P, and K (148.82 kg/ha, 21.20 kg/ha, 210.84 kg/ha) respectively.

The soil collected at Killai possessed the maximum number of 114 x 10⁶ cells g⁻¹ of moisture free soil. The native rhizobial population was
categorized as high ranging from \((77 \text{ to } 114 \times 10^6 \text{ g}^{-1})\) in the soils collected from Killai, Pinathur, and C.Multlur, average \((20 \text{ to } 65\times 10^6 \text{ g}^{-1})\) in the soils collected from Vallam padugai, Nagapattinam, Sirkazhi, Pichavaram, Palaiyur, Parangipettai, and low \((1 \text{ to } 12\times 10^6 \text{ g}^{-1})\) in the soils collected from Thiruvalangadu, Vedaranyam, Kuthalam, Pandaravadi, Mudasal odai, Thiruvadudurai, Puthuchathiram, Palayakoodalore, Thillai vidangal, Tharangapadi, Kodiyyamalaiyam, Singarakuppam, Periyapattu, Senniyalore and Thirukkuvalai were recorded. This location had the least natural nodulation and also showed lower native rhizobial population. The present study clearly established the existence of a parallelism between the native soil population of rhizobial and natural nodulation. It also revealed that the pH and EC are relatively more important critical factors in determining the natural nodulation and native rhizobial soil population.

In the present study, twenty four rhizobial cultures were isolated from blackgram plants collected from twenty four different locations of Cuddalore and Nagapattinam districts of Tamil Nadu. The rhizobial isolates obtained from Pichavaram, Singarakuppam, C. Muttur, Mudasal odai, Thillai vidangal, Killai, Vallam padugai, Parangipettai, Kodiyyamalaiyam, Pinathur, Puthuchathiram, Periyapattu, Pandaravadi, Thiruvadudurai, Sirkazhi, Senniyalore, Thirukkuvalai, Nagapattinam, Thiruvalangadu, Vedaranyam, Kuthalam, Tharangapadi, Palaiyur and Palayakoodalore were designated as BGR CN-1, BGR CN-2, BGR CN-3, BGR CN-4, BGR CN-5, BGR CN-6, BGR CN-7, BGR CN-8,
BGR CN-9, BGR CN-10, BGR CN-11, BGR CN-12, BGR CN-13, BGR CN-14, BGR CN-15, BGR CN-16, BGR CN-17, BGR CN-18, BGR CN-19, BGR CN-20, BGR CN-21, BGR CN-22, BGR CN-23, and BGR CN-24 respectively.

All the twenty four culture were authenticated as *Rhizobium leguminosorum bixax phaseoli* as Dobbeleare (1999) reported that Bacterial IAA would stimulate plant root proliferation and stimulation of lateral root and root hair development.

Bhat and carlson (1992) analysed the effects of different pH levels on the production of IAA by *Rhizobium leguminosarum biovar trifolii* was studied and found that IAA synthesis was greatest at 7.0 and an acidic (or) alkaline medium blocked IAA production. In pot culture experiments, the inoculation of *Rhizobium leguminosarum biovar Phaseol* stimulated IAA production and increased content of plants.

In the present study, All the twenty four rhizobial isolates produced IAA in tryptophane supplemented yeast extract mannitol broth and the quantity ranged from 1.89 to 6.31 µg ml\(^{-1}\) of the culture medium. The isolate BGR CN-6 produced maximum IAA of 6.31µg ml\(^{-1}\) which also possessed the potential for higher rate of root colonization and nodule development. Most *Rhizobium* species have been shown to produce IAA (Ahemad and Khan, 2012b,f; Ahemad and Khan, 2011e, j).However, supplementation of culture media with tryptophan increases the IAA production by most of the rhizobacteria (Spaepen and Vanderleyden, 2011).
Bacterial polysaccharides are necessary for functioning of *Rhizobium*-legume symbiosis. Exopolysaccharides (EPS), Lipo polysaccharides (LPS), Capsular polysaccharides and cyclic (1-2) glycon play an essential role in the formation of the infection thread and in nodule development (Kannerberg and Brewin 1994; Lugtenberg and Kamilova, 2009; Ahemad and Khan 2012a).

In the current study, The rhizobial isolates produced exopolysaccharides (EPS) in varying quantities from 16.35 to 361.50 µg ml\(^{-1}\) in yeast extract mannitol broth. The isolate, BGR CN-6 produced the maximum EPS of 361.50 µg ml\(^{-1}\). Interestingly this isolate was also efficient in IAA production.

Nitrogen fixing organisms are generally categorized as (a) symbiotic N\(_2\) fixing bacteria including members of the family rhizobiaceae which forms symbiosis with leguminous plants (e.g. rhizobia) (Ahemad and Khan, 2012d; Zahran, 2001)

The nitrogen fixing efficiency of the *Rhizobium* isolates is an attribute for selecting strains for crop improvement programme (Kirichenko and Malichenko 2000).

Success in obtaining high N-fixation by the *Rhizobium-legume* symbiosis depends on a series of determining factors.
i. Effectiveness and efficiency of the *Rhizobium* strain present in the soil in relation to the species and varieties of legumes.

ii. Competitive ability of the introduced rhizobia in relation to native rhizobial population,

iii. Ability of the host to supply its microsymbiont with its nutritional needs,


The present results revealed that there was a wide variation in the nitrogen fixing efficiency of 24 isolates. The strain BGR CN-6 recorded the highest nodule ARA of 204.58 n moles C$_2$H$_4$ formed h$^{-1}$ g$^{-1}$.

Several workers have reported earlier on the beneficial effects of rhizobial inoculation on legumes (Rangaswamy 1975; Law and Strijdom 1975; Bagyaraj and Hegde 1978). The *Rhizobium leguminosarum biovar phaseoli* inoculation of seeds of Blackgram with different strains of *Rhizobium* viz., BGR CN-1, BGR CN-2, BGR CN-3, BGR CN-4, BGR CN-5, BGR CN-6, BGR CN-7, BGR CN-8, BGR CN-9, BGR CN-10, BGR CN-11, BGR CN-12, BGR CN-13, BGR CN-14, BGR CN-15, BGR CN-16, BGR CN-17, BGR CN-18, BGR CN-19, BGR CN-20, BGR CN-21, BGR CN-22, BGR CN-23, and BGR CN-24 increased the growth, dry weight, nodulation, total nitrogen and leghaemoglobin content of nodules in blackgram plants.
The factors influencing the nitrogen fixation in Rhizobium-Legume symbiosis has been studied by many workers. In the present study, the isolate BGR CN-6 was found to be the most efficient one among the twenty four isolates of Rhizobium legumisarum biovar. phaseoli in increasing the growth, dry weight, nodulation nitrogen content and leghaemoglobin content of the nodules of the blackgram plants. The increase in growth on 45 DAS in terms of height was 60.13, dryweight was upto 68.31; and nitrogen content was upto 73.16 per cent in plants raised from seeds inoculated with BGR CN-6, these results accordance with (Jahanian et al., 2012).

Siderophore production by rhizobia and diazotrophs has been of special significance due to its prominent role of in chelating and marking it available for nitrogen fixing process (Guerinot, 1991). Among the three types of siderophores, it was observed that all the isolates produced only phenolate type of siderophore. Similar kind of results were observed in Pseudomonas putida, Azospirillum, Azotobacter (Jahanian et al., 2012; Tian et al., 2009) Agrobacterium tumefaciens (Ong et al., 1979) and cowpea rhizobia (Modi et al., 1985) produced catechol like siderophores. The hydroxymate or salicylate type siderphores were not produced by any of the isolated studied.

The bacterial strains possessing intrinsic antibiotic resistance (IAR) are reported to possess better competitive survival. This character of the nodule bacteria could be exploited for monitoring their population in soil
and to determine the percentage of nodules occupancy by inoculated culture, blackgram *Rhizobium* BGR CN-6 was found to tolerate upto 200ppm of kanomycin and rifampicin and upto 300ppm of streptomycin and penicillin on the other hand, it is sensitive to gentamycin. Same kind of were observed in *Azospirillum brasilense, Bacillus pantothenicus*, previously by (Thakuria *et al*. 2004).

It is reported that the isolate producing wet type of colonies are sensitive to gentamycin, while the dry type of colony producing strains are sensitive to penicillin rifampicin, streptomycin and kanomycin (Sinclair and Eaglesham, 1984). The gentamycin sensitive isolate BGR CN-6 obtained in the present study produced wet type colony confirming with the above findings. No correlations between degree of resistance and colony morphology observed in *R.phaseoli* strains (Beyon and josey, 1980) similarly no correlation between resistance to antibiotic and symbiotic effectiveness in cowpea was noted (Hagedorn,1979). The antibiotic resistant strains generally retain the properties of the parent strain and are readily recoverable from soil and particularly useful for enhancing the nodulation of *P.vulgaris* under kenyan conditions (Karanja and Wood 1998).

Microorganisms (viruses, bacteria, and fungi etc.), are resistance to stress from polyaromatic hydrocarbons, heavy metals, radiation, wounding, insect predation, high salt concentration, draft, extremes of temperature, high light intensity, and flooding (Glick, 2012; Lugtenberg and Kamilova, 2009).
Feyez et al., (1990) demonstrated that *B. japonicum* strains were some that stimulated when 100 and 250 meq/l of the sodium chloride was added to YEM medium. Further increase in the salt concentration seriously reduced growth and proliferation of *Rhizobium* and complete mortality was observed at 1000 meq NaCl. The high sensitivity to salinity will certainly reflect on the establishment of effective symbiosis with Bean grown in saline soils. The limitation on the growth of isolates is most probably due to high salinity level of the medium (Young 1986).

Strains of *R.leguminosarum* have been reported to be tolerant to NaCl concentrations upto 250 mM in broth culture (Breedveld et al., 1991). *Rhizobium* strains of *Vigna unguiculata* were tolerant to NaCl upto 5.5 percent which is equivalent to about 450 mM (Mpepereki et al., 1997).

The Fast growing strains belonging to *Rhizobium* are comparatively more sensitive to salt than *Brady rhizobium* strains (Subba Rao et al., 1972).

Many species of bacteria adapted to saline conditions by the intracellular accumulation of low molecular weight organic solutes called osmolytes (Csonka and Hanson 1991). *Glycine betaine* (N, N, N-trimethyl glycine betaine) is a very powerful osmo protectant in *Sino rhizobium meliloti*, the root symbiont of crop, alfalfa. Indeed exogenisously supplied glycine betaine strongly stimulated the growth of the free living bacteria and enhances the nitrogen fixation activity in nodulated alfalfa seedlings.
subjected to salt stress. This bacterium uses glycine betaine as osmoprotectant and growth substrate at high formalities. Interestingly, the catabolism of glycine betaine predominates over its accumulation in stressed cells (Roland Jalibart and Mohamed Jeber, 1997).

An another osmoprotectant, ectoine was as effective as glycine betaine in improving the growth of *R. meliloti* under adverse (0.5 M NaCl) osmotic conditions (Talibart *et al.*, 1994).

The effect of different levels of salt concentration on the adsorption and root colonization of blackgram roots by the rhizobial strains was studied. The adsorption of rhizobial cells and their colonization in the rhizosphere were considerably affected by the increase in the concentration of NaCl. Saline stress affected both adsorption and exchange of *A. brasilense* to maize and wheat roots (Jofre *et al.*, 1998). The reduction in the adsorption of *A. brasilense* under saline stress is related to the disappearance of 100 KD a protein in their outer membrane. The decrease in the anchorage was explained with the changes in the levels as EPS, glucons, and LPS of the cells exposed to salt stress. The EPS production was reduced by 64 percent in cells exposed to salt stress.

Osmotic stress (stock) results in the formation of specific proteins of bacteria. The Na\(^+\), K\(^+\), and Mg\(^{2+}\) concentrations are increased in cells of cowpea *Rhizobium* under salt stress (Zahran and sprent, 1986). The rhizobial
cells responded to high salt stress by changing their morphology. The cells appeared as spiral (or) filament like structures and the cell size is greatly expanded. The *Rhizobium* strains that are best and able to form effective symbiosis with their host legumes at high salinity levels are not necessarily derived from saline soils (Subba Rao *et al.*, 1990).

Strains of *Achromobacter piechaudii* have been reported to be tolerant to NaCl concentrations upto 172 mM in broth culture (Mayak *et al.*, 2004).

The strains BGR CN-6, BGR CN-10, were found to growth upto 2.5 percent and the isolate BGR CN-6 alone was able to grown upto 3.0 percent. The salt tolerant strains BGR CN-6 was isolated from Killai.

The sodium chloride (NaCl) at different concentrations reduced EPS and IAA production in proportion to their levels. At 6.31 percent no detectable EPS and IAA produced except the isolate BGR CN-6 which produced 5.00 µg ml$^{-1}$ of IAA and 361.50 µg ml$^{-1}$ of EPS.

The EPS production in highly influenced by salt. In the presence of 0.3M NaCl, *R.meliloti* showed decrease in mucoidy, 40 percent less EPS production probability the expression of genes responsible for EPS is repressed by salt concentrations. The low level expression of galactoglucon production genes under salt conditions correlated with the decrease in mucoidy and EPS production in NaCl containing media (Javierlloret *et al.*, 1998).
The synthesis pattern of lipo polysaccharide from various species of *Rhizobium* was modified by salt. The length of side chains increased changing the surface antigenic polysaccharide and EPS by salt stress. EPS found very important for the development of root nodules (Bhat and carlson, 1992).

At 3.0 percent NaCl concentration, no detectable EPS produced by the isolate BGR CN-3, BGR CN-10, BGR CN-14, which might be correlated to non expression of galactoglucon producing genes. However, the isolate BGR CN-6 managed to produce comparatively appreciable amount of EPS by switching over to alternate pathway.

In the present study the two rhizobial strains obtained by selection for salt tolerance was subjected to treatment with chemical (N-methyl-N-nitro-N-nitroso guanidine). The mutagenesis can be used to add desirable characters to an already at proved effective inoculant strain (Drake 1970). In general the mutation frequency varied with mutagen concentration and the nature of bacterial species subjected to treatment. The isolates BGR CN-6 showed highest mutation frequency followed by BGR CN-10. In general there was a reduction in mutation frequency at the higher concentrations of tested. The surviving population decreased with the increase in NTG concentration.

The reversion frequency of salt tolerant mutants developed by NTG varied with mutagen concentration. The percentage reversion frequency was
minimum at 150 ppm. Tilak et al., (1994) tried colchicine for inducing mutation in *A.pinnata* at five different concentrations *viz.*, 0.5, 1.0, 1.5, 2.0, and 2.5 percent. He found that 0.5 and 1.0 percent induced mutations and higher levels were found to be toxic. The lower levels recorded less mutagenic effect. The reversion frequency was also found to be higher at 1.0 level.

The mutant of Blackgram rhizobia BGR CN-6M showed better tolerance to different salts *viz.*, Na$_2$So$_4$, Nacl, MgSo$_4$, Mgcl$_2$, K$_2$So$_4$, Kcl, and Cacl$_2$ tested at varied concentrations. At 3.0 percent concentration, the mutant isolate BGR CN-6M alone was able to produce growth.

The chickpea *Rhizobium* at 3.5 percent concentration showed inhibition with the salts of chloride ions. On the other hand the growth appeared at 3.5 per cent concentration with sulphates showing the chloride ions were more toxic than sulphate ions (Elskeikh and wood 1989).

Balasubramanian and Prabakaran (1979) developed 1000 ppm streptomycin resistant mutant from an efficient parent strain *Rhizobium* sp. associated with cowpea. The mutant performed better than parent strain broth in sterilized as well as normal soil. Antibiotic concentration gradient was used to characterize several cultures of *R.phaseoli* and *Rhizobium spp.* based on the intrinsic resistance (Bromfield *et al.*, 1986). The wild parent BGR CN-6S, and the reference strain BGR-AU-1 and mutant BGR CN-6M
possessed same level of antibiotic tolerance \textit{i.e} upto 200ppm for Kanomycin, 300ppm for streptomycin, and 200ppm for rifampicin. Interestingly the level of tolerance to penicillin was 500ppm for BGR 2M and on the other hand it was 300ppm for BGR CN-6S, BGR-AU - 1 recorded the lowest level of tolerance to various antibiotics tested. Mistra and Bhattacharyya (1994) indicated that the antibiotic resistance effect of \textit{Rhizobium leguminosarum} strains showed sensitiveness at higher dose of axomycillin, pencillin, colacicillin, norbactin, and oxytetracycline while at lower dose found to be tolerant to different antibiotics. Based on the result obtained from the present study, the IAR patterns of the blackgram rhizobial isolates were both strain specific, and antibiotic specific. The IAR technique found sensitive enough to discriminate between rhizobial isolates of the same legume inhabiting different habitats. The antibiotic resistance IAR of the fast growing rhizobia were different from those of slow growing isolates (Subramanian and Babu 1993).

Most \textit{Rhizobium} species have been shown to produce IAA. (Prinsen,1991). Microbially released IAA could play a role in rhizobial-plant symbiosis and demonstrated to be nod inducers. The flavonoids also stimulate the production of IAA by \textit{Rhizobium}.

In the present study, sodium chloride at different concentrations reduced the EPS and IAA production in proportion of their levels. At 3.0 percent no detectable IAA and EPS produced except the mutant strain BGR
CN-6M which recorded 5.00 µgml\(^{-1}\) of IAA and 292.00 µgml\(^{-1}\) of EPS. It has been confirmed that elevated levels of IAA in nodules are derived from a mutant of *Brady Rhizobium japonicum* strains which produce 30-fold more IAA than the wild type strain (Kosenko 1989) The mutants exhibited greater nitrogenase activity and released greater quantities of extracellular nitrogenase, Sarcted ammonia, indole acetic acid, like substances and amino acids. Bacteroides of plants inoculated with IAA over producing mutant *B.japonicum* strains recorded high amounts of IAA than that of bacteroids of wild type strains.

In the present study, inhibitory effect on the salt tolerant mutant BGR CN-6M was lesser with sulphate ions than that of chloride ions. The inhibitory effect of different salts lies not only with concentration (osmotic pressure) but also with the type of ions presents (Botsford 1984). The root adsorption of halo tolerant mutant cells was considerably affected by the concentrations as well as type of salts tested. The increase in salt concentration decreased the number of cell adhesion. The salt tolerance mutant BGR CN-6M was more competitive than parent strain and reference strain and higher number cells adhered to the root.

Root exudates serves as a nutrient source or stimulant for microorganisms and playa significant role in plant microbe interactions. (Lee and Gakinss, 1982). In the present study, blackgram root exudates were collected, fractionated and constituents separated, the cationic fractions of
blackgram root exudates contained seven amino acids, namely alanine, aspartic acid, glutamic acid, glycine, valine, proline, and serine. The occurrence of ten amino acids from the root exudates of Cicer and screen amino acids from Eleusine were reported (Rai 1991).

The separation of anionic fraction revealed the presence of five organic acids namely citric, gluconic, malic, oxalic, and succinic acids. Malic acid was formed to be more predominant in the root exudates of C₄ plants, (Kloss 1984) whereas, wheat and rice root exudates are C₃ plants, rich in oxalic acid followed by citric acid (Boureau, 1977). In the root exudate of blackgram citric acid was found to be in more predominant (12.10 (µg) plant⁻¹) followed by oxalic, malic, succinic, and gluconic acids in black gram root exudates.

The separation of neutral fraction of blackgram root exudate revealed the presence of five sugars namely glucose, fructose, maltose, ribose and arabinitol. Exudation of five sugars from cicer roots and four sugars from Eleusine roots (Rai 1991) were reported earlier.

Heinrich and Hess (1985) reported the active secretion of sucrose by wheat roots and its subsequent cleavage to glucose and fructose. In blackgram root exudates, the absence of sucrose may perhaps be due to cleavage by root invertase activity in the present study.
The chemotaxis of salt tolerant *Bradyrhizium* towards root exudates has been studied (Soby and Bergnaan, (1983) and Napoles *et al.*, (1998). In the present study the effect of various fractions of blackgram root exudates, *viz.*, cationic, anionic and neutral fractions and their combinations on chemotaxis of blackgram strains BGR CN-6M, BGR CN-10M, BGR CN-6S, BGR CN-10S and reference strain BGR-AU-1 was studied.

The chemotactic response to recombined anionic + cationic + neutral fractions was found to be more than crude root exudate. Among the individual fractions, anionic fraction of blackgram root exudate elicited more attraction zone followed by cationic and neutral fractions. The chemotaxis assay carried out in channeled chamber also showed that the recombined fractions of the root exudates produced higher chemotactic ratio than the crude root exudates.

The cationic fraction elicited more response in the case of cicer rhizobia followed by neutral and anionic fraction. Gulati (1978) the studied on the chemotaxis of *Azospirillum* towards root exudate of finger millet under salt stress condition revealed that the more chemotactic response towards organic acids was found be higher than other fractions. In the present study, the texture of the belonged to normal and salt affected soil used for the study sandy clay, the pH, EC, and ESP of the normal soil was 7.0, 1.0 dsm\(^{-1}\), and 11.0 percent respectively which the salt affected soil recorded 8.6, pH 0.89 dsm\(^{-1}\)EC, 12.4 percent ESP respectively.
The reduction in the growth of *Medicago sativa* was noted at pH 8.0. indicated that strains of *Rhizobium meliloti* are particularly affected by soil pH and identified as the major limiting factor in determining their number in soil. Rao and Johri (1989) studied chemotaxis of *pseudomonas* towards the root exudates of soybean.

The seed inoculation of blackgram rhizobial isolates significantly increased plant growth nodulation nodule ARA, leghaemoglobin and nitrogen content of blackgram plants in both normal and problem soil of Cuddalore and Nagapattinam districts. The performance of BGM CN-6M in saline soil was significantly were than the reference strain. The increase in growth observed with BGR-AU-1 inoculated blackgram was not significant and found to be on par with control. On the other hand, the performance of mutants and the reference strains was found to be on par in normal soil of course increase in glusters was significant.

The inoculation of *Rhizobium leghaemoglobin* biovar, *trifoli* BGR-2M mutant strains increased the growth, nodulation, nodule mass, nodule ARA, nodule leghaemoglobin, N.content and yield of leguminous plant. In saline soil, whereas the reference strain could not. The intracellular concentration of Na⁺ reached values up to 800mM suggesting that metabolism is not affected by rather high concentration of salt. The glycine betaine as a is a very powerful osmo protestant that strongly stimulates the growth of the free-living bacterium and enhance the nitrogen fixing activity
Rao (1995). In the present study, the inherent effectiveness of a strain seems more related to its salinity tolerance, cultural characteristics (m) and ecological origin. A number of incipient nodules noticed under salinity stress did not differentiate further on removal of stress showing the irreversibility of salinity induced nodule damage, although salinity does not affect colonization of roots by rhizobia.

The mutants of *Rhizobium japonicum* was developed by the chemical mutagen N-methyl-N-nitro-N nitrosoguanidine (NTG) were reported to be more efficient than that of wild type.

Only the *Rhizobium* strains which are tolerant to salinity can forms effective symbiosis under salinity and improve the plant growth. The mutant strain of pea rhizobia showed better competitive saprophytic ability than the wild strain in salt affected soil (Manju bajaj *et al*, 1999). The present results also revealed that the mutant strain BGR CN-6M possessed more competitive ability than other rhizobial strains both in normal and Problem soils of cuddalore and Nagapattinam districts of Tamil Nadu.

In blackgram plants inoculated with salt tolerant mutant the ureide metabolism was more pronounced and recorded more Ureides (allantoin) and Ureide assimilating enzyme like Xanthine oxidase, Uricase, allantoinase, allantoicase and where than uninoculated control as well as referenced strain inoculated blackgram. A positive correlation between leghaemoglobin
content (Dakora and Felix 1995), nitrogenase activity and ureide concentration in Xylem sap was reported. Avline et al., (1995) proposed that, Ureides assay as a suitable measure to assess nitrogen fixation in blackgram - Rhizobium symbiosis.

Agricultural pesticides commonly have side effects on the non target microorganisms. Surprisingly, a negligible amount of research has been carried out in the past on the effects of chemicals on nodulation of blackgram. In this study, the effect of different pesticides like furadan, endosulphan and monocrotophos on rhizobial population, nodulation, and nodule ARA of blackgram plants was tested. The pesticides showed inhibitory effect on the rhizobial population and nitrogen fixation of blackgram plants. Among the pesticides tested, monocrotophos exhibited more toxicity to Rhizobium than the endosulphan and Furadan.

The organochlorine and thiodan inhibited growth and nitrogen fixation of Azospirillum lipoferum. The active ingredient of the insecticide was non-specifically bound to protein and mainly absorbed on the cell envelope (Buff et al., 1992) In contradictory to this, some of the insecticides like carbofuran and diflubenzuron have showed increased effect on growth, nitrogen fixation and amount of ATP in cells. (Sanchez et al., 1994) showing their stimulatory effect.

Among the different fungicide tested, thiram was found to be more toxic than either capton (or) macozeb. These results of Gallori et al. (1991)
showed captan and thiram were more toxic to diazotrophs and the cell growth and nitrogen fixation than other.

The application of herbicides such as alachlor, chloramben, and nitrofen, significantly reduced the rhizobial population and biological nitrogen fixation in the rhizosphere of blackgram plants. The inhibitory effect of different herbicides namely 2, 4, thiobencarb and alachlor was reported by many workers (Martinez Toledo et al., 1990; Rivarola et al., 1992; Salmeron et al., 1991). Interestingly, the rhizosphere population of rhizobia in the salt tolerant mutant BGR CN-6M inoculated blackgram was not significantly reduced although the reduction was significant in amplification of the adaptive its expression in the soil environment.

The maximum benefits of BNF system could be achieved by removing the constrains associated with symbiosis. The use of agrochemicals like herbicides, fungicides, and insecticides constitute a potential hazard for establishment and functioning of the \( \text{N}_2 \) fixing root nodules (Lindstrom, 1989). The application of the herbicides dinoseb to red clover in the field reduced the levels of nitrogenase activity of plants.

Recommended field rates and higher rates of captan application reduced nodulation and \( \text{N}_2 \) fixation in \( T.\text{repens} \). The harmful effect of thiram and captan on nodulation and \( \text{N}_2 \) fixation of several grain and forage legumes were studied by Graham et al. (1986).
The mutants showed 1.1 to 1.9% higher protein content without any significant change in amino acid composition. The inoculation of stress tolerant mutant (salt stress) blackgram rhizobia BGR CN-6M at graded N levels namely 50% of recommended N (10 kg ha\(^{-1}\)), 75% recommended N (15 kg ha\(^{-1}\)) and 100% recommended N (20 kg ha\(^{-1}\)) increased the growth and yield of blackgram Var.ADT-3 grown under field conditions in salt affected soil as rain fed crop. The inoculation of BGR CN-6M (Mutant) at moderate levels (50%) of recommended N produced maximum effect on crop than at higher levels (75% and 100%) of application.

Inoculation of blackgram rhizobial mutant BGR CN-6M increased the vigour the increased shoot and root length observed in BGR CN-6M inoculated blackgram could be explained with the phytohormones produced. The increased root and shoot length observed in this *Azospirillum* inoculated sunflower was found to be on par with culture filtrate sprayed as well as IAA sprayed plants indicating the major role of phytohormone production of the inoculated bacterium (Okon and Venderleyden, 1997).

Significant increase in plant height and dry matter production have been achieved in saline soil as a result of *Rhizobium leuminosarum biovar phaseoli* BGR CN-6M mutant inoculation. The inoculation effect was more pronounced with 50% of recommended N than at 75% and 100% lends. The increase in plant height and dry matter production is accomplished by the increase root volume, root length and more number of lateral roots and also
by increased mineral uptake besides increased nitrogen fixation. Increased root biomass of the inoculated plant could be explained for the higher nutrient uptake and concomitant increased yield. The inoculation of *Rhizobium* significantly enhanced the plant height (cm) and dry matter accumulation of blackgram at various stages of crop growth. (Shivash Sharma and Sharma (2011).

The yield parameters of blackgram such as number of branches/plant$^{-1}$, no. of pods/plant$^{-1}$, no.of clusters plant$^{-1}$, Test weight, grain yield and protein content were significantly increased in the mutant inoculated blackgram in salt affected soil over the uninoculated control mutants. The mutants developed by using EMS, NTG and colchicine registered higher protein content, chlorophyll content and higher activity of ammonia assimilating enzymes. The incorporation as biofertilizer to rice resulted in higher N content, N uptake, and higher nitrogen accumulation in soil than wild parents. (Kumar, 1994).

Blackgram corp response to BGR CN-6M mutant was more pronounced at 50% nitrogen and found to be on par with the effect produced at 100% N alone. Response to Rhizobia declined with increase in N levels from 50 to 100 per cent recommended N. The better performance of *Rhizobium* with moderate doses of combined nitrogen is attributed to the congenial environment and ideal condition for the growth and multiplication as well as functioning of the bacterium in the rhizosphere.
The mutant BGR CN-6M inoculation established effective symbiosis with blackgram under stress condition, and recorded better yield at moderate levels of N. The beneficial effect is not only increased over all yield of blackgram but also the improved quality of the grain. Further the saving of 50% inorganic 'N' reduced the cost on fertilizer input. The overall benefit will help to sustain the blackgram yield under salt stress with sizable reduction in the nutrient input cost.

The mutant BGR CN-6M has several characteristics, which demonstrate its rhizocompetence and survival at stress conditions when compared to their parents, which includes the ability for its higher amounts of secretion of IAA, higher synthesis of exopolysaccharides, better root adherence, better nodulation, high nitrogen fixation, and better colonization. All these attributes explained for their performance under stress condition. The blackgram mutant BGR CN-6M can be used as a bioinoculant with all potential for increasing the growth and yield of blackgram saline soils under semiarid and rainfed conditions.